Host Responses to Presentation of Particulate Virulence Factors of Bacteria and Parasites

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न हि ज्ञानेन सदृशं पवित्रमिह विद्यते ।
तत् स्वयं योगसंसिद्धः कालेनात्मनि विन्दति ॥

Bhagavad Gita, Chapter 4, verse 38

Summary

Virulence factors decorate the surface of pathogens or are secreted as vesicular components of various shapes and sizes, thereby functioning as particulates and mediating diverse host responses. The knowledge of such particulate virulence factors and the host responses they mediate can be exploited in the development of disease intervention strategies like vaccines and therapeutics.

Polysaccharides form an important class of virulence factors in bacteria like *Streptococcus pneumoniae, Haemophilus influenza b* and *Neisseria meningitidis*. Vaccine development against polysaccharide antigens is often challenging due to poor immunogenicity, absence of T cell recruitment in the case of polysaccharide vaccines and variables like the nature of the carbohydrate antigen, the carrier protein, the conjugation chemistry and the type of adjuvant used in the case of glycoconjugate vaccines. In the first part of this thesis (Chapter 3), a vaccine delivery system was developed to address the shortcomings of existing glycoconjugate vaccines. Capsular polysaccharide-3 (CPS-3) was used as the antigen and the well-characterized glycolipid α-GalCer (KRN 7000) was used as the adjuvant. Multiple cmbinations of CPS-3 and α-GalCer were formulated, including their co-encapsulation into either nano- or micrometer sized particles of polylactic acid. Incorporation of antigen and adjuvant in these particles was qualitatively and quantitatively assessed using biochemical and biophysical techniques. The particles were found to induce an immune response in mice, highlighting the immunogenic properties of the formulation. A physical mixture of CPS3 and α-GalCer as well as a CPS3 polysaccharide-protein conjugate used as controls produced comparable antibody titers that were lower than that obtained using the commercially available vaccine Prevnar 13®. Importantly, upon challenge with CPS-3 antigen, the particulate and soluble formulations, including Prevnar 13® mice developed a hyporesponsiveness phenotype except for the mice immunized with the physical CPS-3/α-GalCer mixture. Several studies have highlighted the hyporesponsiveness observed against the serotype-3 capsular polysaccharide of *S. pneumoniae* and this could be a serious drawback of the commercial vaccine. Using glycan microarray analysis, the antisera from Prevnar13 and physical CPS-3/α-GalCer mixture immunized groups correlated with a differential recognition of synthetic CPS-3 substructures highlighting the possible influence of the polysaccharide size on immune response. Thus, co-administration of α-GalCer in a physical mixture with CPS-3 leads to an altered recongition of certain epitopes and can be used to overcome hyporesponsiveness. The results obtained here have to be further validated using the other vaccine serotypes of *S. pneumoniae*. These results combined with the chemical

synthesis of carbohydrate antigens indicate a promising future for the use of synthetic glycolipids as vaccine adjuvants. This would assist in the rational design of highly controlled vaccines enhancing the efficacy and minimizing formulation inconsistencies. The polymeric particulates of polylactic acid presented in this thesis, have a potential future application as excellent drypowder multicomponent vaccine formulation comprising of numerous antigens with well-defined adjuvants formulated on a single carrier platform. The packaging of the PLA based vaccine formulation into inhalers increases the shelf life and also encourages the needle-free self administration of the vaccine through the intranasal routes.

Extracellular vesicles in the form of exosomes, ectosomes, microvesicles and microparticles are gaining importance as modulators of both systemic and cellular host responses. Unicellular pathogens like *Trypanosama cruzi, Leishmania donovani, Cryptococcous neoformans*, *and Plasmodium falciparum* are known to secrete such extracellular vesicles that can act as carriers of virulence factors. In the second part of this thesis (Chapter 4), the ability of *Toxoplasma gondii* to secrete/shed such extracellular vesicles and possible effector functions mediated was investigated. The study describes the isolation and characterization of Exosome Like Vesicles (ELVs) from the peritoneal fluid of *Toxoplasma*-infected mice and *in vitro* cultured tachyzoites. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements showed that the isolated ELVs had a uniform size of 80-100 nm. Proteomic analysis revealed several parasitic proteins such as SAG-1, SAG-2, SAG-3, GRA-3, GRA-6, GRA-7, GRA-12, Myosin, Actin, ROP-2, ROP-4, ROP-8, MIC-1, MIC-2, MIC-3, AMA-1, HSP-70 and HSP-90. Flow cytometry studies also showed differential expression of parasite glycolipids, glycosylphosphatidylinositol (GPI) anchors on these ELVs. The proteins and glycolipid reported here are immunodominant epitopes important for both diagnosis and immunoprophylatics. The ELVs, in the presence of an external inducer, showed enhancement in apoptosis induction of uninfected macrophages. The particulate nature of the ELVs comprising of several important parasitic factors might exert an effect on distant cell populations and modulate the host responses to favor parasite growth. The observations reported here draws attention to the previously unnoticed role of *Toxoplama* ELVs. The role of ELVs in the context of *in vivo Toxoplasma* infection needs further investigation and the results might give useful insights for designing reliable prevention strategies against toxoplasmosis.

Zusammenfassung

Virulenzfaktoren finden sich meist auf der Oberfläche von Pathogenen, können aber auch in Form von Vesikeln unterschiedlicher Form und Größe sezerniert werden. Dabei rufen sie durch ihre partikulären Eigenschaften verschiedenartige Reaktionen im Wirt hervor. Die Untersuchung dieser Partikel und der ausgelösten Reaktionen des Wirtes können entscheidende Hinweise bei der Bekämpfung von Krankheiten liefern, zum Beispiel bei der Entwicklung von Impfstoffen oder Therapeutika.

Während Polysaccharide bedeutende Virulenzfaktoren in bakteriellen Erregern wie *Streptococcus pneumoniae*, *Haemophilus influenzae* b und *Neisseria meningitidis* sind, gestaltet sich die Entwicklung von Polysaccharid-basierten Impfstoffen oft schwierig. Dies lässt sich im Falle von Polysaccharidimpfstoffen auf die geringe Immunogenität und das Fehlen einer T-Zell-vermittelten Immunantwort zurückführen, bei Glykokonjugatimpfstoffen dagegen auf die Wahl der richtigen Kombination aus Kohlenhydratantigen, Trägerprotein, Konjugationschemie und Art des Adjuvans. Der erste Teil dieser Arbeit (Kapitel 3) beschreibt die Entwicklung eines Formulierungssystems für Impfstoffe, um die Schwachstellen bestehender Glykokonjugatimpfstoffe zu umgehen. Als Antigen wurde das Kapselpolysaccharid von *S. pneumonia*e Serotyp 3 (CPS-3), als Adjuvans das gut charakterisierte Glykolipid α-GalCer (KRN 7000) gewählt. Es wurden Formulierungen verschiedener Kombinationen aus CPS-3 und α-GalCer hergestellt, darunter die Verkapselung beider Komponenten in mikro- oder nanometergroßen Partikeln aus Polymilchsäure. Der Einschluss von Antigen und Adjuvans wurde dabei mit Hilfe biochemischer und biophysischer Methoden qualitativ und quantitativ verfolgt. Die Partikel induzierten eine Immunantwort in Mäusen, was die immunogenen Eigenschaften der Formulierung bestätigt. Eine physikalische Mischung aus CPS-3 und α-GalCer und ein CPS-3-Polysaccharid-Protein-Konjugat erzeugten vergleichbare Antikörperspiegel, die jedoch geringer waren als die durch den kommerziell erhältlichen Impfstoff Prevnar13® hervorgerufenen. Bemerkenswerterweise wurde bei Verabreichung von CPS-3 zur Simulation einer natürlichen Infektion in mit allen partikulären und löslichen Formulierungen – einschließlich Prevnar13 – behandelten Mäusen ein hyporesponsiver Phänotyp beobachtet, mit Ausnahme der physikalischen CPS-3/α-GalCer-Mischung. Diese induzierte Hyporesponsivität ist eine bekannte Eigenschaft von *S. pneumoniae* CPS-3 und könnte ein entscheidender Nachteil des kommerziell erhältlichen Impfstoffes sein. Mittels Glykan-Mikroarrayanalyse stellte sich heraus, dass die Antiseren der mit Prevnar13 und der physikalischen CPS-3/α-GalCer-Mischung immunisierten Mäuse sich

durch unterschiedliche Erkennungsmuster synthetischer CPS-3-Substrukturen auszeichneten. Dies weist auf einen möglichen Zusammenhang zwischen Immunantwort und Größe des Polysaccharids hin. Durch die Applikation einer physikalischen Mischung aus α-GalCer und CPS-3 lässt sich also eine Veränderung des Erkennungsmusters der Kohlenhydratepitope hervorrufen, was mit ausbleibender Hyporesponsivität einhergeht. Die hier gezeigten Ergebnisse sollen im nächsten Schritt mittels weiterer *S. pneumoniae*-Serotypen auf Allgemeingültigkeit überprüft werden. In Kombination mit dem Fortschritt in der Synthese von kleinstmöglichen Kohlenhydratantigenen könnte mit den gewonnenen Erkenntnissen ein großer Schritt hin zu gezielt und kontrolliert hergestellten Impfstoffen mit stark verbesserten Wirkungsprofilen getätigt werden, indem Unzulänglichkeiten durch die Formulierung schlecht charakterisierter Inhaltsstoffe ausgeschlossen werden. Die hier präsentierten Polymilchsäurepartikel könnten in der Herstellung von Trockenpulverimpfstoffen Anwendung finden, die potentiell mehrere Antigene in Kombination mit gut charakterisierten Adjuvantien auf einer einzigen Trägerplattform präsentieren. Durch die gegenüber proteinbasierten Impfstoffen erhöhte Haltbarkeit wird außerdem eine intranasale Applikation in Inhalatoren ermöglicht, was ein großer Fortschritt im Zuge der Entwicklung spritzenfreier Impfstoffe wäre.

Extrazelluläre Vesikel in Form von Exosomen, Ektosomen, Mikrovesikeln und Mikropartikeln gewinnen zunehmend an Bedeutung, weil sie Reaktionen im Wirt auf sowohl systemischer als auch zellulärer Ebene beeinflussen können. Einzellige Pathogene wie *Trypanosama cruzi, Leishmania donovani, Cryptococcous neoformans* und *Plasmodium falciparum* sezernieren solche Vesikel, die als Träger von Virulenzfaktoren fungieren können. Im zweiten Teil dieser Arbeit (Kapitel 4) wurde die Fähigkeit von *Toxoplasma gondii* untersucht, extrazelluläre Vesikel freizusetzen. Beschrieben ist die Isolierung und Charakterisierung von Exosom-ähnlichen Vesikeln (ELVs) aus der peritonealen Flüssigkeit *Toxoplasma*-infizierter Mäuse sowie aus *in vitro* kultivierten Tachyzoiten. Messungen mittels Transmissionselektronenmikroskopie (TEM) und dynamischer Lichtstreuung (DLS) zeigten, dass die ELVs eine gleichmäßige Größe von 80-100 nm haben. Durch Analyse des ELV-Proteoms wurden verschiedene Proteine des Parasiten wie SAG-1, SAG-2, SAG-3, GRA-3, GRA-6, GRA-7, GRA-12, Myosin, Aktin, ROP-2, ROP-4, ROP-8, MIC-1, MIC-2, MIC-3, AMA-1, HSP-70 und HSP-90 identifiziert. Glykosylphosphatidylinositolanker (GPI), parasitäre Glykolipide, wurden mittels Durchflusszytometrie auf den ELVs gefunden. Die hier beschriebenen Proteine und Glykolipide sind immundominante Epitope, die sowohl für die Diagnostik als auch für die Immunprophylaxe eine wichtige Rolle spielen. Darüber hinaus

bewirkten die ELVs eine Verstärkung der Apoptoseauslösung durch einen externen Stimulus in nicht infizierten Makrophagen. Durch die partikulären Eigenschaften der ELVs und die Anwesenheit diverser wichtiger parasitärer Faktoren könnten die Vesikel Einfluss auf räumlich entfernte Zellpopulationen nehmen und die angeborene Immunantwort des Wirtes zu Gunsten des Parasites modifizieren. Die hier beschriebenen Entdeckungen deuten auf eine bisher unbekannte Funktion der ELVs von *Toxoplasma* hin, die eine bedeutende Rolle im Zusammenhang mit Infektionen spielen kann und weitergehender Untersuchungen bedarf. Somit lassen sich möglicherweise wichtige Aufschlüsse in der Entwicklung verlässlicher Präventionsstrategien gegen Toxoplasmose gewinnen.

Abbreveations

APC	Antigen-presenting cell
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
CD	Cluster of differentiation
CPS	Capsular polysaccharide
CRM_{197}	*Corynebacterium diphtheriae* mutant CRM_{197}
CWPS	Cell-wall polysaccharide
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DLS	Dynamic light scattering
DSAP	Disuccinimido adipate
DTT	Dithiothreitol
EAP	External aqueous phase
ECL	Enhanced chemiluminescence
EDC	*N*-ethyl-*N*'-(diethylaminopropyl)-carbodiimide
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ELV	Exosome like vesicle
FACS	Fluorescent-activated cell sorting
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
GPI	Glycosylphosphatidylinositol
GSL	Glycosphingolipid
HPAEC	High Pressure Anion Exchange Chromatography
HRP	Horseradish peroxidase
IAP	Internal aqueous phase
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
i-NKT	Invarient natural killer T cell
i.p.	Intraperitoneal
kDa	KiloDalton

MALDI-TOF-MS	Matrix assisted laser desorption/ ionization time-of-flight mass spectrometry
MHC-I/II	Major histocompatibility complex class I/II
mRNA	Messenger RNA
Mw	Molecular weight
NHS	N-hydroxysuccinimide
NK	Natural killer cell
NKT	Natural killer T cell
OD x	Optical density (x = wavelength in nm)
OP	Organic phase
PAD	Pulsed amperometric detection
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PBS-T	Phosphate buffered saline with 0.1% Tween-20
PE	Phycoerythrin
PLA	Polylactic acid
PRR	Pattern-recognition receptor
PVA	Polyvinyl alcohol
RNA	Ribonnucleic acid
rpm	Revolutions per minute
RT	Room temperature
s.c.	Subcutaneous
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy
TAE	Tris-acetate-EDTA
TCR	T cell receptor
TFA	Trifluoroacetic acid
T_H	T helper cell
TLR	Toll-like receptor
TMB	3,3’,5,5’- Tetramethylbenzidine
TNF	Tumor necrosis factor

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1. Introduction

1.1 Infection and Immunity

1.1.1 Infection

Infection is the invasion or acquisition by one organism or cell type, the host, by another organism (microbe) resulting in diametrically opposite outcomes. Either the death of the host, elimination of the microbe or the co-existence of both the host and the microbe can be the result [1]. Humans as part of the evolutionary process act as a natural reservoir and by virtue of their ability to traverse into different ecological niches have evolved to acquire or be infected by various organisms ranging from viruses, bacteria, parasites and even multicellular organisms like worms. Hence, microbial infection or acquisition can lead to elimination, commensalism, colonization, disease and latency [1, 2]. The infecting microbe can either benefit the host or simply co-exist, commonly constituting the microbiome with the net interaction resulting in a healthy state. However, in an opposite disease state, the normal microbiome can be detrimental to the host either due to the infection by a new organism, or by the alteration of an already commensal organism leading to detrimental effect of the host resulting in damage or death of the host with the microbe being termed as a pathogen. Hence, not all infections constitute disease and not all microbes constitute a pathogen but the classification is dependent on the net interaction between the infected host and the invading microbe [3].

1.1.2 Virulence and Virulence Factors

The infecting organisms are broadly classified as pathogenic and non-pathogenic based on their disease causing ability and destruction of the host. This disease causing ability is thought to be solely pathogen centric, mediated by certain components present or released by the pathogens termed virulence factors [4]. However, the ability of certain commensal bacteria to evolve from non-pathogens to later become pathogenic on account of antibiotic therapy, chronic infections or immunosuppression focused the attention on the host responses towards the infecting organisms [5]. Consequently, infections are classified either as primary infections or opportunistic infections based on the host cell response and damage incurred following the infection. In both cases, the outcome of host cell damage is attributed to a concerted action of the microbe or microbial components on the host and the responses

produced by the host against the invading microbe or the microbial component [3, 5]. Hence, host resistance forms an important barrier in defining the interaction between the hosts and invading microbe. In humans, the mucosal surfaces of gastro-intestinal and nasopharangeal surfaces provide the first line of resistance against infectious agents and define a niche for the invading microbes where they might exist as commensals. Depending on the weakened host-responses, these commensals might breach the mucosal barriers and interact with the systemic circulation and hence form a devastating host response leading to damage or death of the host [6, 7]. Some of the virulence factors can be involved in the infection process itself like adhesins, or can provide protection to the microbe from the host like capsules of gram positive bacteria, or can release certain components like exotoxins damaging the host cell, or factors that modulate the apoptosis leading to reduced host response [5]. The end result might be either destruction of the microbe or an increased host response leading to the destruction of the host

1.1.3 Immunity

The host-pathogen interaction often leads to an optimal host response/resistance resulting in the destruction or minimizing the effects of the pathogenesis, benefiting the host or might lead to an overwhelming or no response leading to the destruction of the host. The host response is brought about by complex, collective and concerted defense reactions termed immunity. Immunity is a defense reaction mediated by the body against as invading pathogen or certain components of the pathogen aimed at maintaining the integrity of the host. Immunity can be broadly classified into innate and adaptive mechanisms that are again dependent on the extent of the interaction between the host and pathogen. The innate immunity is characterized by the first line instant defense mechanisms of the host that can be mediated by physical barriers such as mucous, epithelial layers of skin and gut. The physical barriers minimize the interaction of the infecting microbe/pathogen and remove them from the host surfaces. Upon breaching the physical barriers, the pathogens are exposed to the innate immune cells like macrophages that recognize the microbe via pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). The recognition of the pathogen (PAMP and DAMP) might lead to a host response characterized by inflammation mediated by soluble host factors like cytokines that further recruit other innate cells like neutrophils and soluble factors like acute phase proteins and complement that contain the infection and mark the microbe for further destruction [8, 9]. The innate immune response

prompts the adaptive immunity that is characterized by a slower and specific response against certain components of the microbe called as antigens that might or might not be the same as that recognized by the innate immune system. Adaptive immunity is activated and controlled by the antigen presenting cells (APC) such as macrophages, B cells and dendritic cells [10]. The APCs process and present the antigens on either MHC I or MHC II molecules and thereby activate the adaptive immune cells, the B- and T-lymphocytes which define the humoral and cellular arms of adaptive immunity. The B-lymphocytes differentiate into memory B cells and plasma cells which produce specific effector molecules called antibodies against the antigen of the extracellular microbes. The antibodies bind the surface antigens of the microbe and mark them for endocytosis by a process known as opsonization. The T lymphocytes on the other hand differentiate into cytotoxic (T_C) and helper (T_H) T cells with the cytotoxic T cells directly destroying the intracellular microbes like viruses and phagocytized bacteria by recognizing the antigens presented on MHC I molecules. The helper T_H cell recognizes the antigen presented on MHC II of an APC and gets activated to produce cytokines that help in B cell differentiation or activation of other cells such as natural killer cells (NK). The concerted action between innate and adaptive responses of the immune system can provide temporary or permanent protection against an invading pathogen.

1.1.4 Vaccines

Vaccines are a preferred intervention strategy aimed at eliciting a protective immune response against an invading pathogen or its virulence factors aimed at protecting the host from further infections (prophylactic vaccines) or curing the outcome of an infection (therapeutic vaccines). Based on the type of pathogen, its interaction with the host and the nature of the virulence factors (antigen), different types of vaccines have been used (Table 1). The first form of vaccines were largely empirical and consisted of the whole pathogens, attenuated or killed by chemical treatment which produced a robust immune response and conferred long lasting protection [11]. Subsequent identification of new virulent factors like toxins resulted in the specific production of vaccines against the inactivated toxin (toxoid). However, the major drawback of the attenuated vaccines was the reactivation of the pathogen back to the virulent form combined with the non-specific responses resulting in deleterious side effects. The advent of DNA technology combined with cell culture techniques

Vaccine Type	Examples
Whole Pathogen Vaccines	
Live (Attenuated)	Varicella (chickenpox) Influenza, Rotavirus, Polio (OPV).
Killed/ Inactivated	Polio (IPV) Hepatitis A
Toxoid-Antitoxoid Vaccines (Inactivated Toxin)	Tetanus, Diphtheria
Subunit Vaccines	
Protein	Hepatitis-B, Pertussis
Polysaccharide	*Neisseria meningitidis* *Streptococcus pneumoniae*
Conjugate Vaccines	*Neisseria meningitidis* *Haemophilius influenzae* *Streptococcus pneumoniae*

Table 1. Examples of vaccines targeting different diseases

led to the phase of rational design of vaccines. Efforts were made to refine the antigenic determinant by using cell culture and recombinant protein engineering techniques that led to the development of subunit protein vaccines [12]. Although tremendous progress was made with protein and peptide based vaccines, a major bottleneck was the mutability of certain pathogens that led to large changes in the antigenic determinants. An important drawback of subunit vaccines was the loss of immunogenicity due to over refinement of the antigen. This lack of immunogenicity was compensated by immunostimulatory compounds called adjuvants that provided help in restoring the immunogenicity. The concept of adjuvants became central to vaccine research. The presence of polysaccharides and high level of posttranslational modification on certain gram positive bacteria and other pathogens resulted in the emergence of a novel form of glycan based vaccines which included either polysaccharides or shorter oligosaccharide antigenic structures combined with proteins forming the conjugate vaccines [12].

1.1.5 Adjuvants and Vaccine Delivery Systems

Adjuvants are substances which, when used in conjunction with an antigen, enhance the immunogenicity of the antigen leading to a productive immune response [13]. It is beyond doubt that the live or attenuated whole organisms retaining their external morphology in addition with inherent natural adjuvants (bacterial flagelin, viral dsRNA) are superior inducers of adaptive immunity. The live/attenuated whole organisms concentrate the antigens and maximize the interaction with the different cells of the immune system mimicking a natural infection scenario leading to a robust immune response [14-17]. On the other hand, the sub-unit vaccines even though antigenic, fail to induce a protective immune response due to their reduced immunogenicity partly due to the loss of danger associated molecular pattern, reduced interaction with the immune system and dilution of the antigen [15, 18]. Hence, adjuvants improve the immunogenicity of the antigen by enhancing the magnitude and speed of the immune response, stimulating CTL responses, modulating antibody diversity, affinity and isotype distribution and finally minimize the dose of the antigen [15, 19, 20]. One of the most important properties of adjuvants are their ability to concentrate the otherwise soluble antigens producing a depot effect maximizing the concentration of the antigen at the site of injection and increasing the interaction with the cells of the adaptive immunity [18]. It is also evident that the adjuvants provide an additive effect and work best when co-delivered with the antigen to produce a productive response. Hence most of the adjuvants also work as delivery systems and the concept of adjuvants and delivery systems have been interchangeably used in the context of vaccination [21]. Hence, as seen in Figure 1, adjuvants are broadly classified into particulate or non-particulate adjuvants [19].

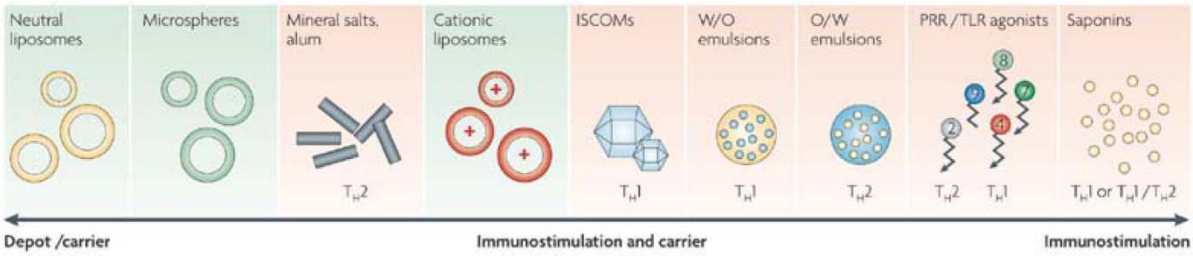

Figure 1. Vaccine adjuvants. Particulate and non-particulate vaccine adjuvants functioning either as depot/carriers or as immunostimulants adopted from [22].

The particulate adjuvants also function as delivery systems that include emulsions (MF 59, Freunds adjuvant), mineral salts (Alum $Al(OH)_3$), liposomes, virus-like particles (VLP), immune stimulating complexes (ISCOMS of saponins) and nano/microparticles of chitosan

and polylactic acid (PLA) [23-27]. The non-particulate adjuvants include individual compounds like lipopolysaccharides (LPS), flagelins, unmethylated CpG DNA, β-glucan, double stranded RNA and endogenous therapeutics like cytokines that have intrinsic immune modulatory properties [20]. However, as the co-delivery of the antigen and adjuvant is prerequisite for maximal response, most non-particulate adjuvants work best when associated with a particulate adjuvant.

Adjuvants are also classified based on their interaction with the immune cells and the modes whereby they modulate the immune response into type A, B or C [22]. Most of the non-particulate adjuvants are either type A (PAMPs which target the TLR) or type-C (endogenous adjuvants like cytokines and glycolipids) that modulate the immune response either towards a T_H1 cell mediated response to combat intracellular pathogens and cancer cells or a T_H2 antibody response directed towards extracellular pathogens or a mixed T_H1/ T_H2 response. The particulate delivery systems (liposomes and PLA particles) mainly function to enhance the antigen presentation to the T cells in a MHC dependent pathway and are classified as type-B adjuvants [19]. However, the co-delivery of a type A or C adjuvant class with the type-B adjuvant or delivery system enhances the potency of the vaccine/antigen in a synergistic manner [28-30] .

Although different compounds and delivery systems show promising adjuvant properties, the aluminum based system (Alum) is the only human approved adjuvant that acts by forming emulsions, concentrating and adsorbing the antigens with negligible side effects [31, 32]. However, alum has its own shortcomings as it poorly adsorbs some negatively charged polysaccharide antigens, general requirement for repeated booster doses, inability to elicit an IgA mucosal responses and inability to elicit a cytotoxic T cell (CTL) responses [31]. The field of adjuvants and delivery systems is an area of intense research and several new adjuvant formulations are currently being explored as summarized in Table 2.

Particulate Adjuvants	
Adjuvant Name	**Properties/ Remarks**
Alum based [$Al(PO)_4$, $Al(OH)_3$, $AlK(SO)_4$]	Most used; weak
Mineral salts (Ca, Fe and Zr)	Better tolerated than alum
Tensioactive compounds (Quil A, QS21)	Glycosides from steroids, plant extract or only fractions, too toxic for human
Emulsions (oil in water or vice versa, MF59, Adjuvant 65 , Montanide, Freunds)	Depot effect, in general too toxic for routine human prophylactic vaccine
Liposomes	Low stability, manufacturing? Quality?
Polymeric microspheres (poly lactide, poly lactide-co-glycolide)	Depot effect, dry powder formulations, good encapsulation of hydrophobic and hydrophillic antigens, too many variables
Non-Particulate Adjuvants	
Adjuvant Name	**Properties/ Remarks**
Bacteria-derived (peptidoglycan, LPS, flagellin, trehalose, DNA (CpG motifs))	Good stimulation of immune response, too toxic
Cytokines (IFN-γ, GM-CSF)	Potential in DNA vaccines
Glycolipids, CD1d ligands, (α-galactosylceramide/KRN-7000)	Specific i-NKT cell adjuvants, encapsulation and co-delivery needed
Carbohydrates (**Inulin**, glucans, dextrans, lentinans, glucomannans and galactomannans)	Good stimulation of immune response, less toxic, ongoing research

Table 2. Vaccine adjuvants. Various particulate and non-particulate substances used as vaccine adjuvants with their associated advantages and disadvantages [26, 33, 34].

1.1.5.1 Biodegradable Poly Lactic acid (PLA) as Particulate Vaccine Delivery System and Adjuvant

Polylactic acid (PLA) and poly lactide-co-glycolide (PLGA) are condensation polymers of hydroxyacid monomers, D-lactic, L-lactic, and/or glycolic acid. They form excellent biodegradable and biocompatible scaffolds and can be fabricated into various shapes and size and are completely inert with excellent bio safety records. They have been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMEA) for use in humans and veterinary use and have served as excellent scaffolds for various biomedical applications like sutures, delivery matrices, orthopedic fixtures and medical implants [35-37].

Polylactic acid polymers have also been fabricated as excellent controlled release systems for the delivery of various hydrophilic and hydrophobic drugs [38, 39]. The fabrication of the particles is achieved by water in oil (w/o) single emulsion or water in oil in water (w/o/w) double emulsion either by solvent evaporation or solvent diffusion technique. The polymer is generally dissolved in the organic solvent (oil phase) and the drug/antigen

depending on the solubility is either dissolved in the oil or water phase. Apart from this, the PLA/PLGA particles are used as vaccine delivery systems where various antigens ranging from proteins, peptides, lipids and polysaccharides have been encapsulated and delivered [40]. An important feature of the particulate systems is the ability to custom fabricate particles depending on the physio-chemical nature of the drug/antigen combination with the type of polymer used which impacts the drug/antigen release. Generally, PLGA or PLA have been used for encapsulation of antigens due to their homogenous distribution on the matrix [41]. The PLA polymer is more hydrophobic than the PLGA polymer. The hydrophobicity has a great impact on the degradation of the polymer by hydrolysis. The PLGA polymers are more prone to degradation than PLA [42]. The degradation of the polymer generally follows an initial burst release on contact with the aqueous environment where the antigens adsorbed on the surface are initially released within a few hours to days [43-45]. This initial phase is followed by a delayed degradation of the remaining particle that can take place in few weeks to months or up to a year depending on the properties of the polymer used, the porosity of the particles with PLGA polymers degrading faster compared to PLA polymers [46, 47]. The above mentioned physio-chemical properties of PLA polymers enables the fabrication of a control release systems either for a fast or sustained delivery of the antigen. A second important feature of the polymeric system is that different sized particles can be readily formulated in the micrometer range of 1-30 microns [48, 49]. Further control of various parameters like emulsion ratio, the power employed for dispersion, the surfactant and the evaporation technique used results in sub-micron particles up to <200 nm [50-52]. The above features of the polymeric particles combined with the different size have a direct impact on their function as vaccine adjuvants by increasing the efficiency of antigen presentation and maximizing the interaction with immune cells like phagocytes and dendritic cells. PLA by themselves are inert and do not cause unwanted immune responses but can cause a very mild inflammation. This inflammatory response is thought to further aid the adjuvant nature of the particles [22, 53, 54]. The size differences of the particles can further fine tune the immune response as they interact differently with cells of the immune system. The particles undergo uptake either through phagocytosis (1-10 μm), micropinocytosis (0.5-5 μm) or clathrin mediated endocytosis (< 500 nm) [55-57]. It has been shown that there is about a six fold difference in the uptake of nanoparticles as compared to the 10 μm particles by weight and by number, this difference is about 700,000 times higher [58]. During the course of immunization, nanoparticles are efficiently taken up by a cell or permeate the biological barrier and reach the bloodstream from where they are taken up by the mononuclear

phagocytic system [59, 60]. However, microparticles are not so efficiently up-taken by the cells and hence are retained at the point of injection where they continuously release the antigen in a pulsating manner and function as antigenic depots [61] . In this regard, PLA particles have been shown to serve as excellent single point vaccine delivery systems, displaying long lasting immunological memory after one year of immunization with protection against challenge as compared to traditional vaccination protocols that involve a priming dose followed by several repeated booster doses [62, 63].

Another advantage of the PLA particulate system based on size is efficient presentation of the antigen both to the MHC-I and MHC-II pathways promoting both cellular and humoral immune responses. PLGA particles in the 2-8 μm range are shown to be potent inducers of antibodies and this has been attributed to the depot effect of the microparticles, promoting an up regulation of MHC class II pathway with the secretion of the cytokine IL-4 skewing the immune response towards a T_H2 type characterized by antibodies [64-66]. On the other hand, nanoparticles as compared to microparticles have been shown to induce higher levels of IFN-γ with up-regulation of the MHC class I molecule skewing the immune response towards a T_H1 type [67]. Co-encapsulation of the PLA particles with a T_H1 adjuvant like CPG ODN or MPLA can promote a mixed T_H1/ T_H2 response characterized by both cell mediated CD8 and CD4 Humoral immunity [68-72]. Hence, polylactide based biodegradable particles can be fabricated with tailor made specificities to elicit a response either against an intracellular pathogen by a T_H1 response or an extracellular pathogen by a T_H2 response.

Most of the commercial sub-unit vaccines are marketed as suspensions in a liquid state that need constant refrigeration to maintain the quality of the vaccine. Polymeric PLA particles can be lyophilized, aerosolized and stored as dry powder formulations without damaging the antigen. Such formulations have bright future applications in low income countries where the refrigeration costs of maintaining a vaccine are very high.

1.1.5.2 NKT Cells and Glycolipids Adjuvants

1.1.5.2.1 NKT Cells

Natural Killer T (NKT) cells are specialized sub set of lymphocytes that combine the cytotoxic activity of Natural killer (NK) cell and the immune regulatory properties of T cells and hence bridge both the innate and adaptive immune responses [73]. The NKT cells were defined based on two independent discoveries. i) a T cell subset having a NK cell receptor NK 1.1(CD 161) [74] and ii) a subset of T cells expressing a invariant T cell receptor (TCR) α-chain composed of Vα14-Jα18 (combined with semi-invariant β-chain Vβ8.2, Vβ7 or Vβ6 in mouse) or Vα24-Jα18 (combined with Vβ11 in humans) as compared to the classical CD4 CD8 T cells [75, 76]. A contrasting feature of the NKT cell TCR as compared to the TCRs of CD4 and CD8 T cells is their recognition of lipid antigens presented on non-classical MHC- I like membrane bound glycoprotein CD1d that are mainly expressed on B cells, macrophages, dendritic cells and epithelial cells [77, 78]. CD1d is the only mouse isoform present compared to humans that have five isoforms (CD1a-e) [79, 80]. The CD1 isoforms are thought to traffic the different endocytic compartments with CD1a mainly present in early endosomes whereas CD1b and CD1d are localized to the late endosomes where maximum microbial lipids are accumulated during infections [81-83]. NKT cells are further classified as type I or type II NKT cells based on their unique response towards glycolipid antigens presented on CD1d and are hence known to be CD1d restricted [84]. Type I NKT cells are known as invariant NKT cells (iNKT) based on the limited diversity of their TCR in specifically recognizing the glycolipid antigen α-galactosylceramide (α-GalCer) presented on CD1d [85-87]. Type II NKT (non Vα14 and Vα24) have diverse TCR that are not activated by α-GalCer but recognize sulfatide and non-lipidic small molecules in a CD1d dependent manner [88, 89].

1.1.5.2.2 Glycolipid Antigens as iNKT Cell Adjuvants

Invariant natural killer T (iNKT) cells get activated by their TCRs that recognize self and foreign lipids antigens presented on CD1d molecule of an APC. The extent of iNKT cell activation is dependent on the nature of the glycolipid antigen with few glycolipids (α-GalCer) functioning as potent iNKT cell ligands. However, the iNKT cells can also be activated through the cytokine IL-12 produced by an APC through pattern recognition receptor (PRR) activation in which case the glycolipid can be a weak antigen. The latter is

particularly true in the case of few exogenous glycolipid antigens of certain bacteria. In either case, the activation of the iNKT cells by the potent agonist glycolipid antigen leads to rapid release in copious amounts of cytokines [90]. The cytokines can be pro-inflammatory like IL-2 and IFN-γ that mediate a T_H1 type response against bacteria, viruses and parasites or a T_H2 type response mediated by IL-4, IL-10, GMCSF etc or a mixed T_H1/ T_H2 type response [91-93]. As seen in Figure 2, the iNKT cells interact directly or indirectly with innate immune cells like DC, macrophage, neutrophils and also with cells of the adaptive immune system like B cells and T cells directly or through the activation of DC. Dendritic cells (DC), like iNKT cells play an important role at the interface between innate and adaptive immunity [94]. Dendritic cells constitutively express CD1d and are involved in the presentation of intravenously delivered α-GalCer resulting in *in vivo* activation of splenic iNKT cells [95-97].

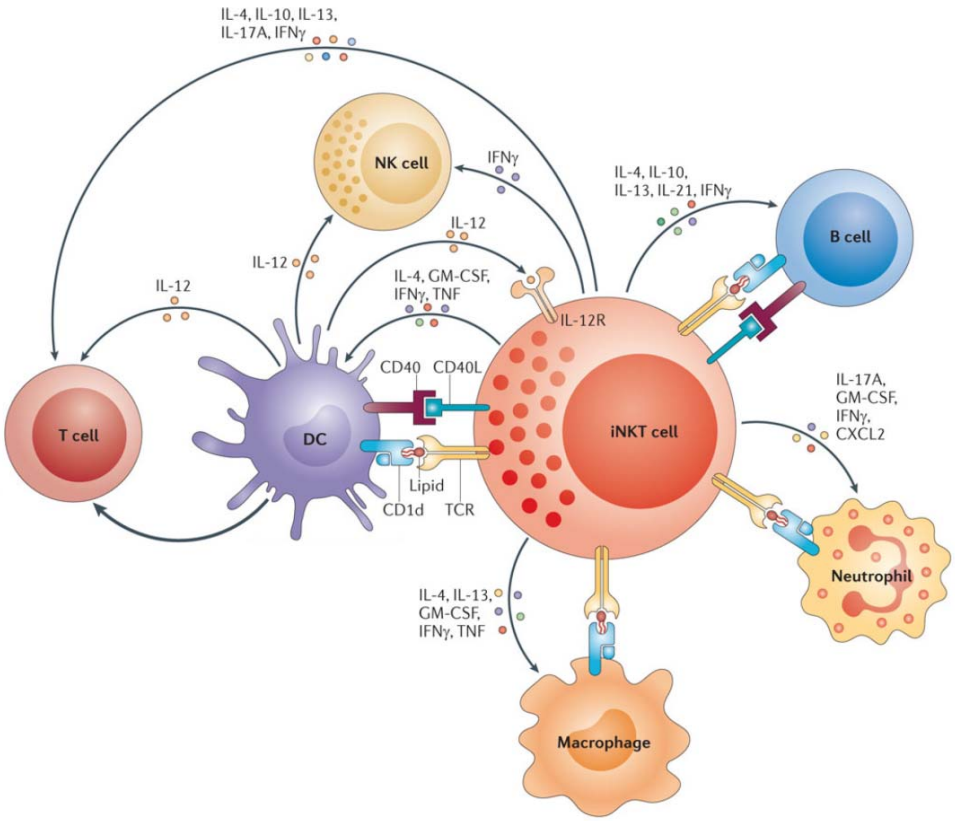

Figure 2. iNKT cell activation and interaction with different leukocytes. The CD1d dependent presentation of glycolipids by an APC like macrophage, dendritic cell (DC), B cell or neutrophil leads to activation of the iNKT cell. The activated iNKT cells produce the T cell cytokines like IL-4, IFN-γ, granulocyte-macrophage colony stimulating factor (GM-CSF) that cause the reciprocal activation of the APC leading to various innate and adaptive immune activation like either B cell maturation, T cell activation by DC and microbial killing by macrophages and DC. The iNKT cell cytokine IFN-γ and IL-12 from DC might also transactivate natural killer (NK) cells mediating a microbicidal activity. Figure adopted from [98].

Similarly, in the lungs, pulmonary DCs can take up glycolipid antigens and present it to iNKT cells or transmigrate into draining lymph nodes and present the antigen to conventional T cells in case of protein [99]. In the infection scenario, DCs, the professional antigen presenting cells can get activated via the PRR mediated pathway and produce the cytokine IL-12. The production of the cytokine IL-12 leads to the up-regulation of antigenic glycolipids or the presentation of microbial glycolipids on CD1 molecules [98, 100]. The subsequent cognate recognition of this DC-CD1-iNKT leads to further secretion of IL-12 that binds to the IL-12 receptor on iNKT cells, to activate them to produce IFN-γ [95, 101, 102]. The IFN-γ production brought about by activation of iNKT cells leads to transactivation of NK cells that are involved in immune surveillance and mediate an antitumor effect [103-106].

Besides DCs, B cells that are also APCs can present antigenic lipids leading to activation of iNKT cells. It has been shown that B cell receptor (BCR) mediated uptake of CD1d restricted antigens was effective in enhancing B cell responses in vivo [107]. B cell modulation has been achieved by synthesis of an antigen linked to iNKT cell agonist [107, 108]. This cognate iNKT-B cell interaction followed by the release of iNKT cytokines IL-4 induces B cell maturation leading to higher antibody titers, affinity maturation, antibody class-switching and expansion of memory B cell pools enhancing specific antibody responses [109-111].

This direct and reciprocal activation of the different cells specifically modulates both the innate and adaptive immune responses and hence iNKT cells along with well-defined glycolipids can function as vaccine adjuvants.

1.1.5.2.3 α-Galactosyl Ceramide and Its Analogs

α-Galactosylceramide (α-GalCer) was one of the first, specific and most potent iNKT cell glycolipid antigen identified [112]. It was originally isolated from marine sponges and belongs to the family of ‘agelasphin’ glycolipids that contain saccharides that are α- or β-linked to a phytosphingosine containing ceramide backbone [113]. Several structure activity relationship studies further refined the structure resulting in the commercial, fully synthetic, potent iNKT antigen KRN 7000 [114, 115]. However, α-GalCer is a marine sponge glycolipid with an α-linked anomeric sugar that is not common to mammals and hence cannot be a natural iNKT cell ligand. α-GalCer potently activates iNKT cells producing IFN-γ and IL-4

resulting in a mixed T_H1/T_H2 cytokine response mediating both immunostimulatory and immunosuppressive functions, not beneficial for specific treatments in case of cancer or diabetes [116]. The potent activity and massive amount of cytokine released by α-GalCer activation leads to iNKT cell inactivation and anergy leading to hyporesponsiveness towards subsequent administration of α-GalCer [117]. Considerable efforts were spent in the identification of various exogenous and endogenous glycolipid ligands of iNKT cells.

1.1.5.2.4 Bacterial Glycolipids

One of the first discoveries of an exogenous iNKT cell glycolipid ligand was from the bacterial species *Sphingomonadaceae,* gram negative bacteria that contain glycosylceramides in their cell wall instead of LPS [118-120]. Subsequent isolation and purification of the glycolipid fractions reported two classes of glycolipids, both having a ceramide containing sphingosine lipid but differing in their carbohydrate content. The first class termed GSL-1 contained α-glucuronic acid and the second class GSL-1' contained galacturonic acid as the sugar [121-123]. Although the chemically synthesized GSL-1 showed an iNKTcell activation potential, it was however low as compared to that of α-GalCer [124]. This differential activation potential was attributed to the stereochemistry at the C-4 position that differs between a glucose and galactose residues [125]. Hence, the difference in the terminal sugar combined with the length of the glycolipid tail influences the antigenicity of the glycolipid ligand**.**

A second class of exogenous iNKT cell glycolipids were reported from the pathogenic bacterium, *Borrelia burgdorferi,* the causative agent of Lyme disease and the bacterium, *Streptococcus pneumoniae causing* pneumococcal pneumonia. The analysis of *B. burgdorferi* glycolipids revealed the presence of galactose as the terminal sugar but with a diacylglycerol (DAG) containing lipid tail as compared to ceramide containing α-GalCer [126]. The chemically synthesized structure BbGL-II showed iNKT activation potential and was the first reported non-glycosphingolipid iNKT cell antigen [126]. However, the analysis of the *Streptococcus pneumoniae* glycolipids revealed a DAG glycolipid but containing a α-glucosyl sugar moiety capable of stimulating iNKT cells to the same level as BbGL-II [127]. A striking difference between the ceramide containing α-GalCer and the DAG containing glycolipids in that the ceramide lipids show potent activity with only galactose terminated sugar moieties

whereas the DAG containing glycolipids show similar activation potential with both galactose and glucose as the terminal sugar. Hence the discovery of the galactosyl and glucosyl DAG strengthened the role of iNKT cells in recognizing bacterial pathogens and the different effector functions they mediate.

Apart from the bacterial species, various other exogenous and endogenous iNKT cell glycolipid antigens have been identified like cholesterol esters of *Helicobacter pylori* [128], lipopeptidophospoglycans from *Leishmania* [129], phosphatidylinositolmannoside from mycobacterium [130] and endogenous self-lipids like isoglobotrihexosylceramide (iGB3) [131] and plasmalogen lysophosphatidylethanolamine [132]. The effect of the glycolipid tail and the attached sugar in modulating different levels of iNKT cell stimulation have been exploited by synthesizing various α-GalCer analogues to be used in immunotherapy which can skew the immune response specifically towards a T_H1 or T_H2 response.

2. Materials and Methods

2.1 Materials

2.1.1 Instruments and Implements

Analytical balance	Mettler Toledo, Columbus, OH, USA
Autoclave	Laboclav, steriltechnik AG, Detzel Schloss, Germany
CarboPac PA 20 column	Dionex, Thermo Scientific, Rockford, USA
Cell counter chamber	Neubauer, Marienfeld, Lauda Koenigshofen, Germany
Centrifuges	5810R & 5417R, Eppendorf, Wesseling-Berzdorf, Germany
Confocal microscope	Zeiss LSM 700, Oberkochen, Germany
Electrophoresis system	Biorad, Munich, Germany
ELISA plate reader	Infinite M200, Tecan, Crailsheim, Germany
Flow cytometer	FACS Canto II, BD Pharmingen, Heidelberg, Germany
Freezer	Liebherr, Ahrensfelde, Germany
Heating Block	ThermomixerComfort,Eppendorf,WesselingBerzdorf,Germany
Homogenizer	IKA® T-10 Basic and T-18 Digital ULTRA TURRAX® Werke GmbH, Germany
Homogenizer probe	S 10 N-5G and S 18 N-10G IKA® Werke GmbH, Germany
HPLC	ICS 5000, Dionex, Thermo Scientific, Rockford, USA
Incubator for cell culture	NuAire, Plymouth, USA
Magnetic stirrer	MR Hei-Tec, Heidolph, Schwabach, Germany
Microarray printer	SciFlexarrayer, Scienion, Berlin, Germany
Fluorescent scanner	Genepix 4300A, Molecular Devices, Sunnyvale, CA, USA
Light Microscopes	Hund, Wilovert, Buckinghamshire, UK
Multichannel pipette	Eppendorf, Wesseling-Berzdorf, Germany
Oven	Binder, Tuttlingen, Germany
pH meter	Mettler Toledo, Columbus, OH, USA
Pipettes	Gilson Inc, Middleton, USA
Refrigerator	Liebherr, Ahrensfelde, Germany
Shaker	Neolab, Heidelberg, Germany
Sonicator	Bransons, Emerson Electric Co, St. Louis, USA
Sterile bench	Herasafe KS, Thermo Scientific, Bonn, Germany
Superose 12 column	GE Healthcare, Uppsala, Sweden
Ultra centrifuge	Optima™ L-90K, Beckman Coulter, GmbH, Krefeld, Germany
Ultra centrifuge bottles	Type 70-Ti & SW 40- Ti Beckman Coulter, GmbH, Krefeld, Germany
Vortexer	Vortex Gene, Scientific Industries, Bohemia, NY, USA
Water bath	Memmert, Schwabach, Germany
Water deionizer	Integra UV Plus, Neolab, Heidelberg, Germany
Zetasizer μv	Malvern Instruments Ltd, Malvern, UK
Mastersizer 2000	Malvern Instruments Ltd, Malvern, UK

2.1.2 Consumables

Amicon® Ultra-4 Centrifugal Filters	Millipore, Bedford, MA, USA
Boiling tubes	Thermo Scientific, Bremen, Germany
Cell culture flasks	Corning, Corning, NY, USA
Cell culture plates	Corning, Corning, NY, USA
Cell strainer (40 µm)	Thermo Scientific, Bremen, Germany
Centricon® Centrifugal Filters	Millipore, Bedford, MA, USA
Combitips	Eppendorf, Wesseling-Berzdorf, Germany
Cryotubes	Corning, Corning, NY, USA
ELISA plates (Nunc)	Thermo Scientific, Bremen, Germany
FACS tubes	Sarstedt, Nuremburg, Germany
Polypropelene tubes (15 ml)	Corning, Corning, NY, USA
Polypropelene tubes (50 ml)	Corning, Corning, NY, USA
Microscope slide	Sarstedt, Nuremburg, Germany
Needles	B. Braun, Melsungen, Germany
Nitrocellulose membrane	GE Healthcare, Waukesha, USA
Pasteur pipettes	Roth, Karlsruhe, Germany
Petri dishes	Corning, Corning, NY, USA
Sterile flasks	Corning, Corning, NY, USA
Sterile filters	Roth, Karlsruhe, Germany
Serological pipettes (5, 10, 25 ml)	Corning, Corning, NY, USA
Syringes	B. Braun, Melsungen, Germany
Eppendorf tubes	Eppendorf, Wesseling-Berzdorf, Germany

2.1.3 Chemicals, Polymers and Special Reagents

All chemicals used in this study were of high purity and were supplied by Sigma Aldrich (Munich, Germany), Roth (Karlsruhe, Germany) and AppliChem GmbH (Darmstadt, Germany). The polymer Polylactic acid (PLA) RESOMER, R-203 S (0.25-0.35 dL/g) was from Evonik Industries AG, Germany and PURASORB PDL 05 Poly-DL-lactide) (0.49 dL/g) was a kind gift from Purac Biochem, Netherlands. The Glycosphingolipid (α-GalCer/ KRN-7000) was from Cayman Chemicals USA. *Streptococcous pneumoninae* capsular polysaccharide (CPS) and cell-wall polysaccharide (CWPS) was from STATENS serum institute, Denmark.

2.1.4 Mice, Cell Lines and Parasite Strains

Mice strain

C57BL/6	Charles River, Sulzfeld, Germany; Max Planck Institute for Infection Biology, Berlin, Germany

Cell lines

Macrophage cell line (RAW 264.7)	ATCC TIB-71
Human foreskin fibroblast (HFF)	ATCC CRL-1635
African Green Monkey Kidney Cells (Vero)	ATCC CCL-81

Parasite strains

Toxoplasma gondii	Type 1	(RH)	virulent
	Type 2	(PTG)	avirulent

2.1.5 Antibodies, Reagents and Kits

2.1.5.1 Antibodies

Primary antibodies

Goat anti-DT (CRM_{197}) IgG	Thermo Scientific, Bremen, Germany
Rabbit polyclonal CPS-3 antisera	STATENS serum institute, Denmark.
Mouse monoclonal anti CPS-3 IgG (5F6)	Prof. Liise-anne Pirofski, Yeshiva University, USA
Mouse anti-galactocerebroside IgG (mGalC)	Millipore, Temecula, CA, USA
Mouse anti-SAG1 (P-30) IgG	Leica Biosystems Newcastel Ltd, UK
Mouse anti-*Toxoplasma* GPI-A IgG (T3 3F12)	Dr. Nahid Azzouz
Mouse anti-*Toxoplasma* GPI-B IgG (T5 4E10)	Dr. Nahid Azzouz

Secondary Antibodies

Goat anti-mouse IgG (H+L)-FITC	Sigma-Aldrich, St. Louis, MO, USA
Goat anti-mouse IgG-AF 647	Invitrogen, Carlsbad, CA, USA
Goat anti-mouse IgG-AF 635	Invitrogen, Carlsbad, CA, USA
Goat anti-rabbit IgG-488	Abcam plc, Cambridge, UK
Rabbit anti-mouse IgG-HRP	Sigma-Aldrich, St. Louis, MO, USA
Mouse anti-rabbit IgG-HRP	Sigma-Aldrich, St. Louis, MO, USA
Rabbit anti-goat IgG-HRP	Sigma-Aldrich, St. Louis, MO, USA

2.1.5.2 Reagents and Kits

Acrylamide/ bis-acrylamide (29:1)	AppliChem GmbH, Darmstadt, Germany.
Alcian blue 8GX	Sigma-Aldrich, St. Louis, MO, USA
Aldehyde/Sulfate Latex Beads (5 µm)	Life technologies, Darmstad, Germany
Allumium phosphate adjuvant (Alum)	Adju-Phos®, Brenntag Biosector, Denmark
Amicon centrifugal filters	Sigma-Aldrich, St. Louis, MO, USA
Ammonium persulfate (APS)	Sigma-Aldrich, St. Louis, MO, USA
Annexin V Apoptosis Detection Kit	BD Pharmingen, Heidelberg, Germany
Avidin-HRP	PeproTech, Rocky Hill, NJ, USA
BCA Protein Assay Kit	Thermo Scientific, Rockford, USA
Bovine serum albumin (BSA)	Biomol, Hamburg, Germany
Coomassie Protein Assay	Biorad, Munich, Germany
CRM_{197} Protein	Phenex Inc, San Diego, USA
Coumarin-6	Polysciences GmbH, Eppelheim, Germany
DTT	Promega, Madison, WI, USA
ECL Western Blot Detection Reagent	GE Healthcare, Uppsala, Sweden
ELISA Kit IL-2, IL-4, IFN-γ	Thermo Scientific, Rockford, USA
Milk powder	Sigma-Aldrich, St. Louis, MO, USA
Protein molecular weight ladder	Biorad, Munich, Germany
Streptavidin-HRP	PeproTech, Rocky Hill, NJ, USA
TEMED	AppliChem GmbH, Darmstadt, Germany
Tween-20	Sigma-Aldrich, St. Louis, MO, USA

2.1.5.3 Biochemistry Reagents

Stock solutions

Ammonium persulfate (APS)

10% in H_2O

Lectin binding buffer

10 mM HEPES (11.9 g)
1 mM MgCl2 (5 mL from 1M MgCl2)
1 mM CaCl2 (5 mL from 1M aCl2)
Volume adjusted to 500 ml with water

SDS-PAGE running buffer (10X)

30.3 g Tris base
144 g Glycine
Volume adjusted to 1 L with water

Western blot transfer buffer (1X)

100 ml running buffer (10X)
150 ml Methanol
Volume adjusted to 1 L with water

Coomassie stain

2.5 gms Coomassie brilliant blue R-250
1 L methanol in acetic acid in water (50:10:40)

Alcian blue stain

2 gms Alcian blue
1 L ethanol in acetic acid in water (20:3:67)
Filter through paper filters

Coomassie destaining solution

Methanol: Acetic acid: Water (50:10:40)

Alcian blue destaining solution

7% acetic acid in H_2O

2.1.6 Materials for Cell Biology Methods

2.1.6.1 Media components

DMEM (with 4.5 g/L Glucose, without L-glutamine and sodium pyruvate)	PAN Biotech, Aidenbach, Germany
Penicillin 10000 U/mL, Streptomycin 10 mg/mL (Pen/Strep)	PAN Biotech, Aidenbach, Germany
Stable glutamine 200 mM	PAN Biotech, Aidenbach, Germany
Sodium Pyruvate 100 mM	PAN Biotech, Aidenbach, Germany
β- Mercaptoethanol (β- Me) 50 mM	PAN Biotech, Aidenbach, Germany
Fetal calf serum (FCS)	PAN Biotech, Aidenbach, Germany

Complete DMEM

DMEM	500 ml
FCS	10%
Glutamine	1%
Pen/ Strep	1%
Sodium pyruvate	1%
β- Mercaptoethanol	0.1 %

2.1.6.2 Buffers

Erythrocyte lysis buffer (ACK)

0.1 M Tris-HCl (pH 7.5)	10%
0.16 M NH_4Cl	90%

Buffers for microscopy

Cell fixation buffer

4% paraformaldehyde (PFA) in PBS, pH- 7.4

PFA quenching buffer

100 mM Glycine in PBS, pH-7.4

Cell permiabilization buffer

0.1 % Tween-20 in PBS, pH- 7.4

Cell extricating buffer

0.25 % Trypsin/ 0.02 % EDTA, pH- 7.4

ELISA coating buffer		ELISA blocking buffer and diluent
$NaCO_3/NA_2HCO_3$	pH 9.4	1% BSA in PBS
PBS	pH 7.4	

ELISA washing buffer

0.1% Tween-20 in PBS

2.2 Methods

2.2.1 Vaccine Delivery Systems

2.2.1.1 Fabrication of PLA Nano (NP) and Micro Particles (MP)

Polylactic acid (PLA) nano (< 500 nm) and microparticles (2-8 μm) were fabricated using a water/oil/water (W_1 /O / W_2) double emulsion solvent evaporation technique as depicted in Figure 3. A two-step approach was employed where 100 μL of the internal aqueous phase (IAP or W1) (2.5% BSA in 10% sucrose) was emulsified into 2 mL of the organic phase (OP or O) (50 mg/ ml PLA-PDL 50, 0.49 dL/ g in Dichloromethane) in a 10 mL glass vial using a sonicator fitted with a micro tip (output control-4, 40 W, 1 min, 4^0C) resulting in the primary emulsion (W_1 /O). The composition of the primary emulsion was essentially the same for both nano and microparticles. For nanoparticle formulations, the primary emulsion was immediately emulsified drop wise into 8 mL external aqueous phase (EAP or W_2) in a 50 ml polypropelene tube (Falcon) containing 2% Low molecular weight Polyvinylalcohol- PVA (Mw. 13-23K, 87-89% hydrolysis in 10% sucrose) using a sonicator fitted with a microtip (output control-5, 50 W, 2 min, 4^0C). Microparticles were fabricated by emulsifying the primary emulsion, to the external aqueous phase (EAP or W_2 8 mL (2% Polyvinylalcohol-PVA, Mw. 31-50K, 98-99 % hydrolysis in 10% sucrose) by using a homogenizer with S 18 N-10G probe (10000 rpm, 8 min) resulting in the secondary emulsion (W_1 /O / W_2). The resulting secondary emulsion was poured into a 25 mL glass beaker, covered with an aluminum foil punched with holes and subjected to solvent evaporation and particle precipitation at 400 rpm for 6-8 h under a sterile bench. The resulting nano and microparticles were collected by centrifugation at 13000 x g for 15 min, washed with nanopure water and lyophilized for 48-72 h to obtain a fine power of PLA nano or microparticles. The vials were hermetically sealed and stored at 4^0 C till further use.

2.2.1.2 Fabrication of 6-Coumarin Loaded Fluorescent PLA Nano- and Microparticles.

For the fabrication of fluorescent PLA nano and microparticles, 100 μL of the fluorescent dye 6-coumarin (1 mg/mL in dichloromethane) was added to the polymer containing organic phase (OP) during particle preparation. All other parameters were the same as described in method 2.2.1.1.

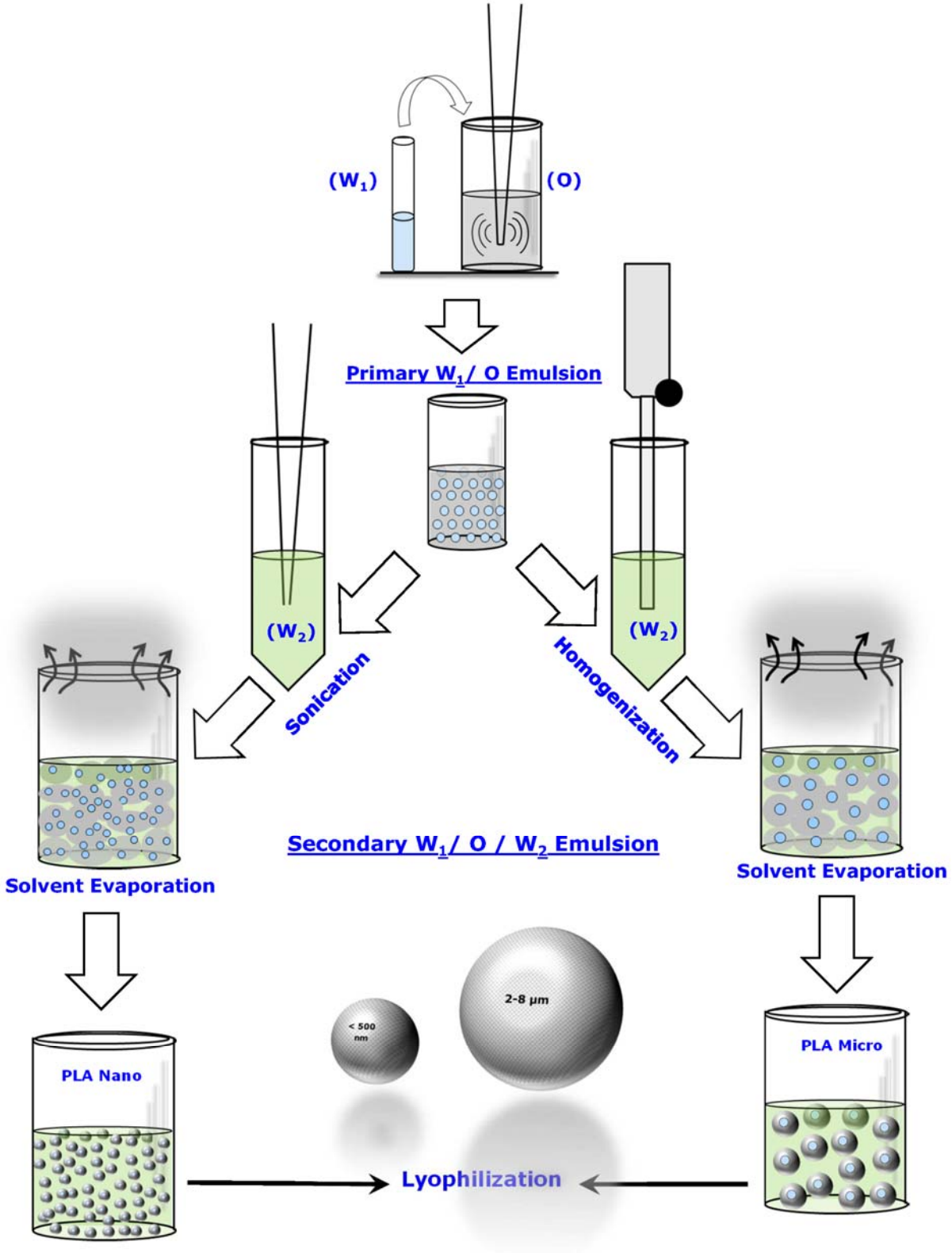

Figure 3. Schematic of PLA nano and microparticle fabrication process.

2.2.1.3 Formulation of Antigen Loaded PLA Nano and Microparticles

2.2.1.3.1 Depolymerization of Polysaccharide Antigen

The *Streptococcous pneumoninae* capsular polysaccharide antigen CPS-3 (10 mg) was dissolved in 10 mL of nanopure water, stabilized for 2 h by intermittent sonication in a sonicator water bath and transferred into a 25 mL two mouthed V bottom flask. Depolymerization/ size reduction was carried out using a Branson sonicator fitted with a microtip probe at 30 W power for 30 min-1 h under a stream of argon. The size reduction was monitored using SDS-PAGE/ Dynamic light scattering techniques. The size reduced polysaccharide solution was centrifuged at 20000 x g for 15 min on a table-top centrifuge, lyophilized and reconstituted with nanopure water and stored as 10 mg/mL aliquots until further use.

2.2.1.3.2 Glycolipid Solubility

The glycosphingolipid (GSL) α-GalCer (KRN-7000) from here on mentioned as GSL was solubilized in 3:1 chloroform in methanol, dried under a stream of N_2 and stored as 1 mg aliquots at -20^0 C till further use. Depending on the end use, the aliquots were reconstituted either in 1:1 chloroform in methanol for particle preparation, DMSO for *in vitro* stimulation experiments or in PBS with sonication for *in vivo* experiments.

2.2.1.4 Formulation of PLA Nano (NP) and Microparticles (MP) Co-encapsulating Glycolipid and Polysaccharide Antigens

Polylacticacid (PLA) nano and microparticles coencapsulating the glycolipid and polysaccharide antigens in different combinations were formulated using the water/oil/water (W_1 /O / W_2) double emulsion solvent evaporation technique as described in Methods 2.1.1.1. Four types of particles were formulated consisting of Dummy, GSL only, CPS-3 only and CPS3- GSL (Figure 4). Formulation parameters for primary emulsion were essentially the same for both nano and microparticles. Briefly, 100 µL of the internal aqueous phase (IAP) with/ without the size reduced CPS-3 polysaccharide was emulsified into the organic phase (OP) with/ without the glycolipid GSL using the parameters as described in method 2.1.1.1.

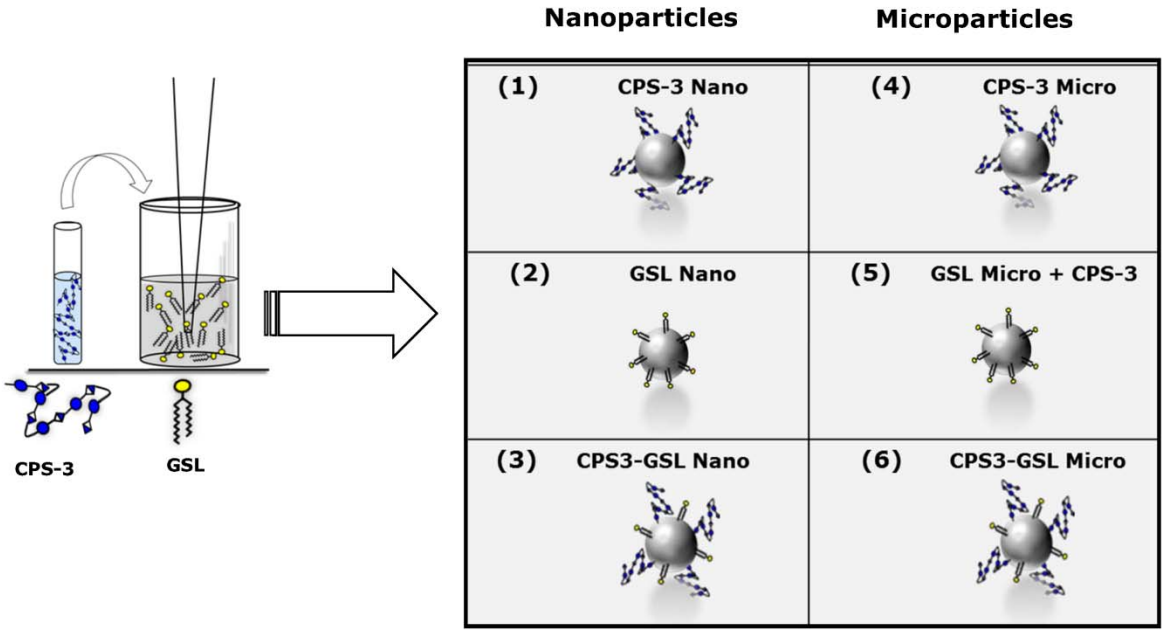

Figure 4. Schematic representation of various particulate formulations of CPS-3 and GSL. The CPS-3 polysaccharide and GSL were formulated in different combinations resulting in the following particulate antigens (1) Nanoparticles containing only CPS-3. (2) Nanoparticles containing only GSL. (3) Nanoparticles co-encapsulating both CPS3 and GSL. (4) Microparticles containing only CPS-3. (5) Microparticles containing only GSL. (6) Microparticles co-encapsulating both CPS3 and GSL

2.2.1.4 Preparation of CRM_{197}- CPS3 Protein Glycoconjugate

The capsular polysaccharide-3 antigen was chemically conjugated to the immunogenic protein CRM_{197} by periodate oxidation followed by reductive amination and amide bond formation via an activated NHS ester.

2.2.1.4.1 Treatment of Polysaccharide

2.2.1.4.2 Periodate Oxidation

The size reduced CPS-3 (from method 2.2.1.3.1) was reacted with sodium periodate ($NaIO_4$) to generate additional aldehyde groups as shown in Figure 5.

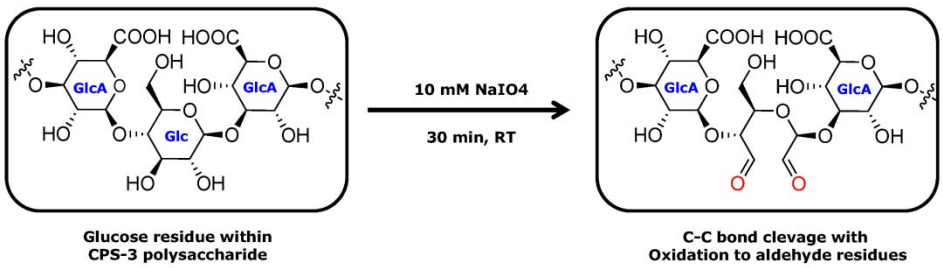

Figure 5. Schematic of the periodate oxidation reaction of CPS-3 resulting in formation of reactive aldehyde groups.

Sodium periodate cleaves the carbon-carbon bond between two adjacent hydroxyls in a carbohydrate and oxidizes the hydroxyl groups to reactive aldehyde groups. The generated aldehyde groups can be used to introduce amine groups for further conjugation procedures. The size reduced CPS-3 (2 mg) was volume adjusted to 750 µL with 0.1 M sodium phosphate buffer pH 7.4 and 250 µL of a 10 mg/mL (46 mM) stock of $NaIO_4$ in water was added to get a final concentration of 10 mM of $NaIO_4$. Oxidation was carried out for 30 min at RT after which 100 µL of 100 % Glycerol was added and further incubated for 15 min to quench the reaction. Excess $NaIO_4$ was removed by washing four times with water using a 3 kDa MWCO concentrator. Then the oxidized polysaccharide was collected in a fresh microfuge tube and subjected to reductive amination reaction.

2.2.1.4.3 Introduction of Primary Amino Groups by Reductive Amination

The periodate treated CPS-3 was subjected to reductive amination reaction to incorporate amine groups ($-NH_2$) using the homobifunctional cross-linker adipic acid dihydrazide (ADH) as shown in Figure 6. The periodate oxidized CPS-3 was incubated for 48 h at 30^0 C in 1 mL of 100 mM sodium phosphate buffer pH 7.4 containing 20 mg adipic acid dihydrazide (ADH) & 10 mg sodium cyanoborohydride ($NaBH_3CN$). The aminated polysaccharide was washed five times with nanopure water using a 3 KDa MWCO concentrator to remove the unreacted ADH and lyophilized as 1 mg aliquots.

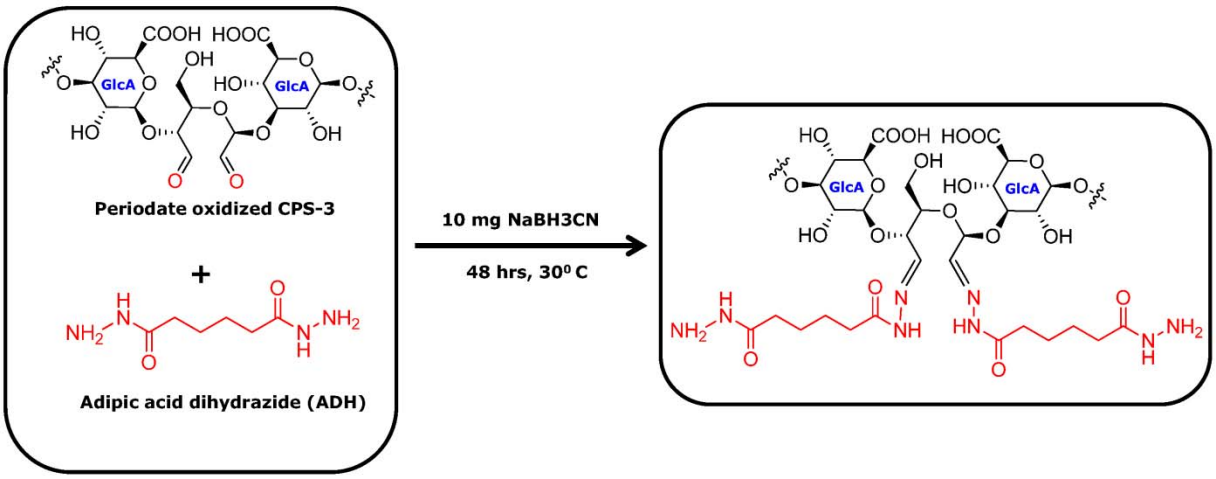

Figure 6. Schematic of the reductive amination reaction reaction of the periodate oxidized CPS-3 in presence of adipic acid dihydrazine resulting in formation of aminated CPS-3 polysaccharide.

2.2.1.4.4 Conjugation of Aminated CPS3 with CRM $_{197}$ Carrier Protein

The CPS-3 polysaccharide with the newly formed amine group from the previous step was conjugated to the carrier protein CRM_{197} using a disuccinimido adipate (DSAP) linker (Figure 7). The DSAP linker contains an amine-reactive *N*-hydroxysuccinimide (NHS) ester located on both ends of the 6-carbon spacer arm that can react with primary amines at pH 7-9 to form stable amide bonds. The DSAP linker was used to conjugate the amino-linked CPS-3 polysaccharide to the primary amines of the side chain of lysine residues on the surface of the CRM_{197} protein. The linker 20 mg was dissolved in 200 µL DMSO followed by activation with 10 µL triethylamine. The aminated CPS-3 polysaccharide (1 mg) was dissolved in 9:1 DMSO:H_2O and was added drop wise to the linker solution kept on a magnetic stirrer and allowed to react for 2 h at RT. After the incubation time, 250-500 µL phosphate buffer was added and the unreacted DSAP linker was extracted out by adding 10 mL of chloroform in 15 mL polypropylene tube followed by centrifugation at 3000 × g for 3 min. The aqueous phase of the chloroform extraction step was collected in a fresh tube and the extraction was repeated twice. This aqueous phase now containing the CPS-3 polysaccharide with the bound DSAP linker was added drop wise to solution of 500 µg CRM_{197} protein in 500 µL sodium phosphate buffer pH 7.4 and incubated overnight at RT with stirring on a magnetic stirrer. The conjugates were washed with sodium phosphate buffer pH 7.4 and concentrated using a 10 kDa MWCO concentrator and stored in phosphate buffered saline (PBS) at 4^0C.

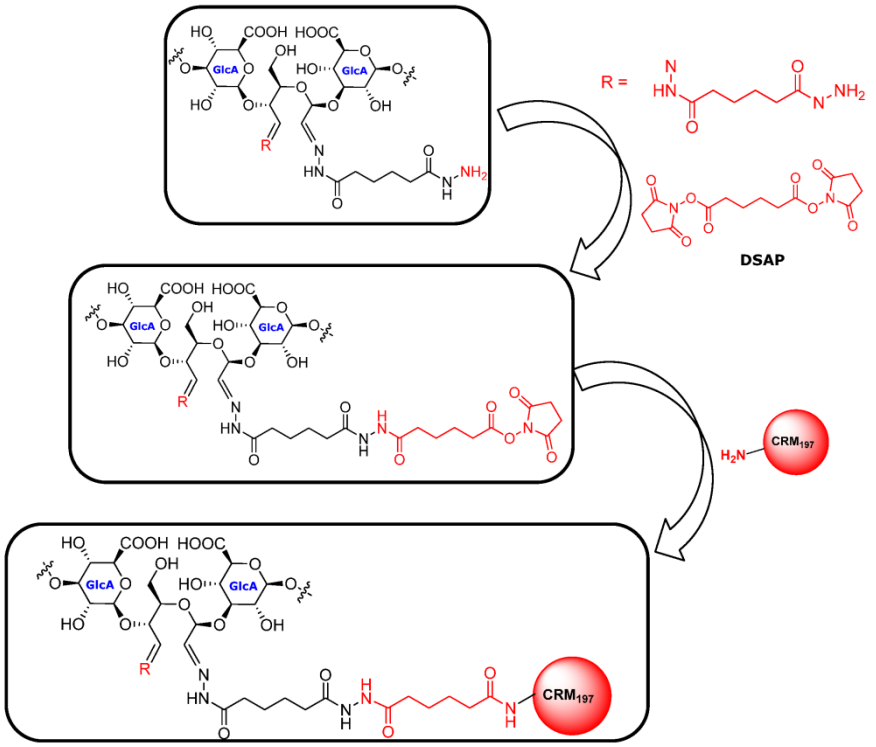

Figure 7. Schematic of conjugation procedure where the aminated CPS-3 polysaccharide was conjugated to the carrier protein CRM_{197} using the DSAP linker.

2.2.2 Analytical Methods

2.2.2.1 Dynamic Light Scattering (DLS) Analysis

Size distribution of ELVs or nanoparticles was analyzed by dynamic light scattering (DLS) batch measurement technique using the Zetasizer μV nano (Malvern). Vesicles (10 μL) or 1 mg/mL nanoparticle suspension (10 μL) was diluted in 1 mL PBS or water and three measurements were performed at 25^0 C, each measurement comprised of 15 scans for duration of 14 min. Microparticle size distribution was analyzed using a Mastersizer with each measurement performed in triplicates with a size range of 0.02-2000 μm.

2.2.2.2 Electron Microscopy and Immune Gold Labeling

For cryo-EM analysis, a droplet of 3.5 μL ELVs was applied on a freshly glow-discharged R1.2/1.3 Quantifoil™ grid (Quantifoil Micro Tools GmbH, Jena, Germany) without additional carbon support film and plunge-frozen in liquid ethane using a Vitrobot™ plunger (FEI, Eindhoven, Netherlands). Samples were screened using a Tecnai Spirit transmission electron microscope (FEI) operated at 120 kV. Images were recorded using a 2kx2k Eagle CCD camera (FEI) at a nominal magnification of 42.000x applying a defocus of about -2 μm.

Immune gold labelling was done by first fixing the ELVs in 4% paraformaldehyde (PFA)-PBS solution for 20 min followed by quenching with a solution of 0.1 M Glycine for an additional 20 min. The ELVs (10 μL) were adsorbed on freshly glow-discharged R2/4 Quantifoil™ grid (Quantifoil Micro Tools GmbH, Jena, Germany) for 20 min at RT. The grids were washed in PBS and incubated with the corresponding primary antibody for 45 min. After washing four times with phosphate buffered saline + 0.1% Tween-20 (PBS-T), detection was done using goat anti-mouse 10 nm gold conjugate IgG incubated for 30 min. The grids were washed with PBS-T followed by 1 wash with PBS and negatively stained with 2% uranylacetate solution (50 μL) for 15 s. Samples were screened using a Philips CM100 transmission electron microscope operated at 100 kV equipped with a 1kx1k F114 Fastcan CCD camera (TVIPS).

2.2.2.3 Protein Concentration Determination

Colorimetric estimation of proteins was carried out using the coomassie G-250 based Bradford method or by the copper-based method of BCA as per manufacturer's instructions. In both cases, the concentration was derived from a standard curve generated using the protein BSA, expressed as μg/ mL of protein.

2.2.2.4 SDS-PAGE and Western Blot

Samples were mixed with loading buffer and heated at 95º C for 15 min in case of proteins or incubated for 15 min at RT for polysaccharides and loaded onto polyacrylamide gels of different percentages depending on the sample. Electrophoresis was carried out at 130 V, 25 mA for 90 min. The gels were washed three times in nanopure water and stained either with coomassie brilliant blue for proteins (15 min) or with Alcian blue stain for polysaccharides (30 min) and destained to get a clear back ground.

Western blot analysis was performed in a tank blot system by electrophoretically transferring the SDS-PAGE resolved proteins and polysaccharides prior to staining from the polyacrylamide gels onto a nitrocellulose membrane. Protein transfer was done at 25 V, 250 mA for 2 h. The nitrocellulose membrane was washed twice with phosphate buffered saline + 0.1% Tween-20 (PBS-T) and blocked with a solution of 5% non-fat dried milk powder dissolved in PBS-T for 2 h at RT or overnight at 4^0C. Subsequent incubation with the desired primary antibody or serum was carried out at room temperature for 90 min followed by washings with PBS-T and incubation with a HRP conjugated secondary antibody for 60 min at RT. The blots were developed using the ECL plus reagent as per manufacturer instructions and images were acquired using a LAS 5000 system.

2.2.2.5 Proteomic Analysis of Microvesicles

Tryptic digestion of the coomassie stained protein bands was performed as reported previously [133] with minor modifications. Briefly, protein bands were excised manually using a clean scalpel and transferred to clean microfuge tubes from Eppendorf. The gel pieces were destained with 50% acetonitrile, 100 mM ammonium bicarbonate, dried using a speed vac concentrator, and digested overnight at 37° C using 50 ng of trypsin under basic conditions.

All mass spectrometric analyses were performed on an AmaZon ETD Speed ion trap massspectrometer (Bruker Daltonics, Bremen, Germany) coupled to an Ultimate 3000 UHPLC system (Dionex, Thermo Fisher Scientific, Germany). The instrument was operated in the positive ion mode with the experimental parameters as follows: capillary exit voltage, 4500 V; with maximum accumulation time of 50 ms with ICC ON. In MS experiments, a mass range of *m/z* 400-1500 was scanned and for MS/MS experiments, a mass range of *m/z* 100–1500 was scanned. The width of isolation was set to 2.2 and the fragmentation amplitude was set from 30-200% in the 'smart fragmentation' mode

The mass spectrometer was set up to perform CID fragmentation on the selected precursors in positive mode. An m/z range from 400-1500 Da was scanned for precursor scanning. The three most intense signals in every MS scan were selected for MS/MS experiments, and just signals with a charge state ≥2+ were selected for MS/MS. For MS/MS experiments, a m/z range from 100-1500 Da was scanned. Both, MS precursor and MS/MS scans were recorded in the instrument's "enhanced resolution mode".Tryptic digested peptides were reconstituted in 0.1% formic acid before online LC separation by reversed phase chromatography (ProteCol™ Capillary Column, SGE, Ringwood, Vic, Australia, 300 µm x 10 mm [precolumn] and 300 µm x 150 mm [analytical column]). The samples were injected onto the precolumn in 100% solvent A (0.1% formic acid) at the flow rate of 8 µL/min and washing the precolumn for 5 min in buffer A eluted unbound components. The analytical column was equilibrated in 2% solvent B (acetonitrile with 0.1% formic acid) and a linear gradient using an increasing solvent B concentration was applied at the flow rate of 5 µL/min as follows: linear increase of buffer B from 2 to 30% (from 5 min to 61 min), further increase to 60% (61 to 76 min), followed by a steep increase to 90% (76 to 77 min). The column was held at 90 % B for 5 min (77 to 82 min) before re-equilibrating the analytical column in 2% solvent B. Meanwhile the precolumn was re-equilibrated in 100% solvent A before injection of the next sample.

Data analysis and protein identification was performed using MASCOT 2.3 (MatrixScience, UK) with the following search parameters: Cysteine as carbamidomethyl was set as fixed modification, deamidation (Asn/Gln) and oxidation (Met) were set as variable modifications. Up to one missed tryptic cleavage was allowed. Peptide tolerance (both MS and MS/MS) was set at ±0.2 Da. The data was searched against NCBI protein database.

2.2.2.6 Flow Cytometry

Flow cytometry analysis was carried out using a FACS Canto^{TM} II instrument from BD bioscience. ELVs and glycolipid (GPI) were analysed by immobilizing approximately 5 µg of vesicles or glycolipid on 10 µL of a 4 µm diameter aldehyde/ sulphate latex beads overnight at 4^0C. The beads were quenched with 0.1 M glycine-PBS for 20 min at RT. In case of PLA particles, a known amount of particles was weighed and washed three times with water. The aldehyde latex and PLA particles were blocked with 1% BSA-PBS for 60 min at RT. Incubations with the corresponding antibodies diluted in 1% BSA-PBS was carried out at RT for 90 min followed by washings with PBS-T. Detection was done using antibodies conjugated with a fluorescent probe and for lectin staining; the fluorescently labelled Lectin was incubated in Lectin binding buffer for 90 min. A total of 50000 events were recorded for each sample and data was analyzed using FlowJo 7.6.5 software (Treestar Inc., Ashland, OR, USA).

2.2.2.7 Confocal Microscopy Analysis of Antigen Loaded PLA Nano and Microparticles

The encapsulation of GSL and CPS-3on the PLA particles was checked by confocal microscopy analysis. Briefly, 10 mg of the GSL-CPS3 particles were washed three times with nanopure water and incubated with the corresponding primary antibodies for 90 min at RT. For CPS-3, the rabbit polyclonal antibody was used and for GSL, the mouse monoclonal galactocerebroside antibody (mGalC) was used. Incubations were carried out in 0.1% BSA/ PBS after which, the particles were washed three times with PBS and incubated with the corresponding secondary antibody conjugated with a fluorescence probe for 45 min at RT. The particles were washed three times with PBS and images were acquired using a confocal microscope (LSM-700 Carl Zeiss AG).

2.2.2.8 Estimation of Encapsulated Antigens from PLA Nano and Microparticles

2.2.2.8.1 Particle Lysis Protocol

In order to estimate the loading and encapsulation efficiency of the antigens, the PLA particles were lysed using strong alkaline conditions. Briefly, 5 mg each of the different particle types Dummy, GSL, CPS-3, and CPS3-GSL were washed three times with 2 mL of nanopure water and particles were pelleted by centrifugation at 15000 x g for 10 min. The particle pellet was resuspended in 250 µL of 0.5 N NaOH and incubated overnight at room temperature (RT).

The particle suspension proceeded from turbid to clear by the end of the incubation time indicating complete particle lysis. The particle lysate was neutralized with 112 µL of 1N HCl solution.

2.2.2.8.2 Flochs Extraction

The CPS-3 polysaccharide and GSL were extracted from the particle lysate by phase partitioning using chloroform or chloroform in methanol depending on the antigen. For extraction of CPS-3 polysaccharide, 600 µL of chloroform was added to the particle lysate, vortexed vigorously and centrifuged at 14000 rpm for 5 min. The upper aqueous phase containing the polysaccharide was carefully collected into a separate microfuge tube, leaving behind approximately 100 µl of the aqueous phase. The extraction was repeated with an additional 400 µl of nanopure water and the aqueous phases from the two extractions were pooled and centrifuged at 13000 rpm for 5 min to pellet the residual chloroform. The contents were transferred into a fresh microfuge tube, leaving behind any chloroform pellet and incubated at 37^0 C for 30 min to further evaporate trace amounts of chloroform. The volume was noted down and readjusted with 10 X PBS to obtain the final polysaccharide extract in 1X PBS.

The GSL was retrieved from the residual chloroform phase (also containing approx. 100 µL of residual aqueous phase) after the Flochs extraction step. Briefly, 200 µL of methanol was added to the 600 µL of chloroform phase to get chloroform:methanol ratio of 3:1. After vortexing and centrifugation at 15000 x g for 5 min, the lower chloroform:methanol phase was carefully collected using a 1 mL syringe and transferred into a clean boiling tube. The contents were completely air dried using a stream of nitrogen and used for analysis or capped tightly and stored at -20^0 C till further use.

2.2.2.9 MALDI-TOF-MS

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis was performed to analyse the GSL released from hydrolyzed PLA particles in the chloroform fraction of the Flochs extract. MALDI-TOF-MS analysis was performed on an Autoflex™ Speed mass spectrometer equipped with a Smartbeam™ II laser optics running at 1000 Hz. The instrument was controlled by the FlexControl 3.3 software. The air dried chloroform phase was resuspended in 100 µL of nanopure water and a volume (5 µL) was mixed with an equal volume of the matrix (saturated solution of 2, 5

dihydroxybenzoicacid/ 50% acetonitrile containing 0.1% TFA). The sample-matrix mixture (1µL) was spotted onto a 384-spot MALDI target (600 µm polished steel) and allowed to dry at room temperature. Internal calibration was performed using a standard calibration mix and spectra were acquired in reflector- negative ion mode within the mass range of *m/z* 700 to *m/z* 1500 da. After analysis, the acquired spectra were baseline corrected and smoothed using with the software Flex Analysis.

2.2.2.10 HPAEC-PAD Quantification of α-GalCer (GSL) from PLA Nano and Microparticles

The quantification and the percent encapsulation efficiency (% EE) of GSL on the PLA nano and microparticles was evaluated by acid hydrolysis followed by monosaccharide quantification using High Pressure Anion Exchange Chromatography (HPAEC) method coupled with Pulsed Amperometric Detection (PAD). A sample (5 mg) of each particle type was hydrolyzed and phase-partioned according to the method described in section 2.2.2.8 to release the encapsulated antigens. The aqueous phase of the phase-partioned particle lysate containing the polysaccharide (CPS-3) and the organic phase containing the glycolipid (α-GalCer) was dried under a stream of nitrogen and processed for acid hydrolysis [134]. Acid hydrolysis was carried out using 2M TFA at 120^0C for 2 h after which the samples were dried using a speed-Vac concentrator. For hydrolysis of the polysaccharide CPS-3 containing glucuronic acid residues, an additional step of methanolysis was performed prior to TFA hydrolysis with 2N methanolic-HCl at 80^0C for 24 h. The sample was dried under a stream of nitrogen followed by TFA hydrolysis. After the removal of TFA, the samples were reconstituted in 500 µL of nanopure water and filtered using a 0.45 µm HPLC-syringe filter and 10 µl of the samples were injected onto a Carbo-Pac PA-20 column (ThermoFischer) fitted with a Carbo-Pac PA-20 guard column which was connected to a Dionex ICS 5000 HPLC system. Seperations were done at a flow rate of 0.5 mL/min with an isocratic elution of 10 mM NaOH for 15 min, followed by a washing step with 200 mM NaOH for 5 min followed by regeneration with 10 mM NaOH for 20 min for analysis of neutral monosaccharides (galactose content of GSL containing samples). For uronic acid containing acidic monosaccharides, a combination of isocratic and gradient elution was used with elution of 10 mM NaOH for 16 min, followed by a washing step with 200 mM NaOH for 5 min, followed by second elution with linear gradient of 500 mM NaOAc in 100 mM NaOH reaching 60% in 15 min followed by a washing step with 200 mM NaOH for 10 min and regeneration with 10 mM NaOH for 20 min. The chromatography was monitored by pulsed amperometric detection with a gold working electrode and a Ag/ AgCl reference electrode by

selecting the quadrapule-potential waveform set for carbohydrates. GSL was quantified by comparing the galactose content with a standard curve of galactose in the range of 25-0.6 µM treated under the same conditions as the samples. The chromatograms were processed using the Chromeleon® 7 software.

2.2.2.11 Size Exclusion Chromatography (SEC) of CRM_{197}-CPS3 Protein Glycoconjugate

The CRM_{197}-CPS3 protein-polysaccharide conjugate was analyzed and purified using size exclusion chromatography performed on a Dionex ICS 5000 HPLC system equipped with a photodiode array detector (PAD) consisting of a deuterium and tungsten lamp. Chromatography was performed in phosphate buffered saline using the Superose 12 10/300 GL column (GE Healthcare) with a flow rate of 0.4 mL/min. A sample (100 µL) containing 200 µg sample was injected with duration of 70 min per run. A 50 µg/ 100 µL standard solution of CRM_{197} was used as a standard to compare the molecular weight increase of the conjugates. Fractions were manually collected and analyzed by SDS-PAGE and western blot. The chromatographic data was processed using the Chromeleon® 7 software.

2.2.2.12 Glycan Microarray

Glycan arrays were used to determine the binding preferences of sera towards the different sub-structures of CPS-3. For this purpose, the following synthetic sub-structures of CPS-3 were synthesized by Dr. Sharavathi Parameshwarappa and Dr. Subramanian Govindan and these structures were printed on a microarray slide by Mr. Andreas Geissner following standard protocols established in the lab with the printing format shown in Figure 8.

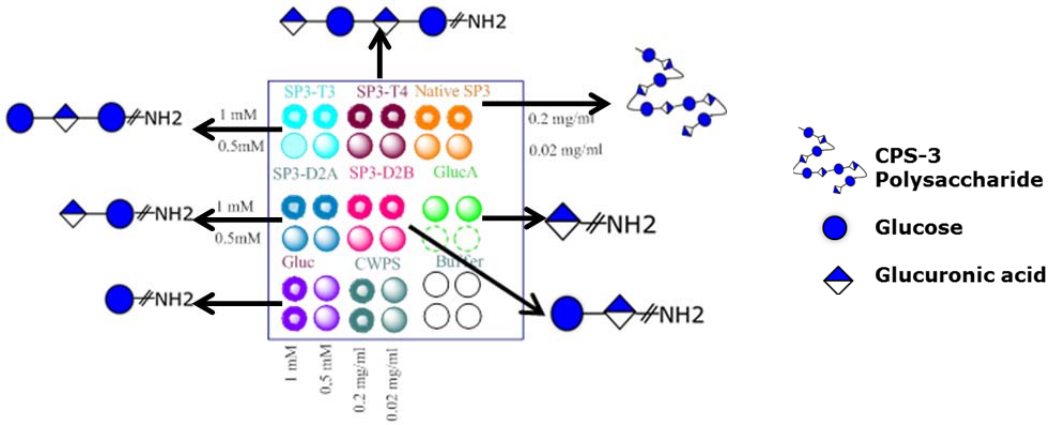

Figure 8. Microarray slide layout used for screening of CPS3 immunized sera. Various synthetic sub structures of CPS-3 made of repeating units of glucose (blue circles) and glucuronic acid (rhomboids) residues were printed in two concentrations. Native CPS3 (orange circles) and cell wall polysaccharide (CWPS) (green circles) were also printed.

The CPS-3 polysaccharide and the substructures in two different concentrations were dissolved in the printing buffer (50 mM sodium phosphate, pH 8.5). These individual solutions were spotted on a CodeLink NHS activated glass slides (Surmodics) using a S3 piezoelectric microarray printer (Scienion) equipped with a type 4 coated nozzle. The spotting chamber was constantly kept at 65% relative humidity. Post-printing, the slides were incubated over night at room temperature in a humidity saturated chamber and the remaining reactive groups on the slide surface were quenched by incubation with 50 mM sodium phosphate, 100 mM ethanolamine pH 9.0 at room temperature for 1 h. Slides were subsequently washed three times for 5 min with water, dried by centrifugation at 300 x g for 5 min (CombiSlide system, Eppendorf) and stored at 4 °C until further use.

Prior to the start of the screening procedure, a 64 well incubation gasket (FlexWell 64 grid, Grace BioLabs) was attached to the slide and well secured with clips. The primary antibody/ sera were diluted in 1% BSA-PBS solution and 30 µL were dispensed into each well in duplicates and incubated for 1 h at RT in a humid chamber. Following the incubation step, the wells were washed three times for 5 min with PBS containing 0.1% Tween-20 (PBST) by adding 50 µL to each well. The detection antibody dilutions were prepared in 1% BSA-PBS and 30 µL dispensed into each well and incubated at RT in the dark for 45 min. The slides were washed twice for 5 min with PBS-T, once with PBS, rinsed with water and dried by centrifugation. Fluorescence read out was measured with a GenePix 4300A microarray reader (Molecular Devices) and the individual spots were analyzed using GenePix Pro 7 (Molecular Devices) using the mean fluorescence intensity. Spot diameter was kept identical for all samples and the mean of four spots from duplicate wells were used when quantifying the results.

2.2.3 Cell Biology Methods

2.2.3.1 Cell Culture

All cell lines and parasites were grown in complete DMEM medium supplemented with 10% foetal calf serum (FCS). Human Foreskin Fibrablasts (HFF) cells were grown to 80-90% confluence in 75 cm^2 sterile cell culture flasks after which sub-culturing was done by dislodging the cells with trypsin-EDTA (0.25% trypsin, 0.02% EDTA) solution. The spent medium was discarded prior to adding 5 mL of trypsin-EDTA solution and the flasks were incubated at 37^0 C for 5 min. The cells were mechanically dislodged by tapping and the reaction was stopped by adding 5 mL of complete DMEM medium. The cells were recovered by centrifugation at 300 x g for 10 min and typically one 75 cm^2 flask was split in two 300 cm^2 flasks that reached 90%

confluence after about 5 days. African Green Monkey Kidney (VERO) cells were also propagated under similar growth conditions but were harvested using a cell scraper.

2.2.3.2 Cultivation of *Toxoplasma gondii*

The mice virulent strain of *Toxoplasma gondii* Type-I (RH) and the avirulent strain Type-II (PTG) were propagated in Human Foresikn Fibroblast (HFF) cells or African Green Monkey Kidney (VERO) cells grown to 80-90% confluence in complete DMEM medium. The parasites were propagated in 75 cm^2 flasks for routine maintenance and in 6 x 300 cm^2 flasks to achieve high density of approximately 10^{10} parasites.

2.2.3.3 Isolation and Purification of ELVs

ELVs were isolated from the spent medium of approximately 10^{10} tachyzoites propagated *in vitro* for 6 h in 25 mL of DMEM medium or from the peritoneal lavage of mice infected with tachyzoites using differential centrifugation approach [135]. In both cases, the production medium or peritoneal lavage was subjected to low speed centrifugation at 300 x g for 15 min, 2000 x g for 30 min and 15,000 x g for 30 mins at 4^0 C. The supernatant of the 15,000 x g centrifugation step was subjected to two rounds of high speed centrifugations at 110,000 x g for 90 min at 4^0 C using a 70-ti fixed angle rotor and on subsequent washing of the pellet with 10 mL PBS the second centrifugation was carried out in a SW-40 swing bucket rotor to obtain the final pellet of ELVs. In an alternate protocol, the 15000 x g centrifugation step was replaced by a filtration step using 0.45 μm and 0.2 μm pore size syringe filters followed by two rounds of centrifugation at 110,000 x g as above. For some applications, after the first centrifugation step at 110,000 x g, the pellet was centrifuged on a 30% cushion of sucrose to remove the nonspecific proteins and the vesicle containing sucrose fraction was diluted with PBS and further centrifuged at 110,000 x g for 90 min to obtain the final pellet of ELVs which were resuspended in 50-100 μL of PBS and the protein content estimated using the Bradford assay.

Starting with 10^{10} parasites and with a production time of 6 h, approximately 7-10 μg ELVs were obtained vesicles as measured by protein content.

2.2.3.4 Isolation of Glycosylphosphatidylinositol anchors (GPI) from *Toxoplasma gondii*

Frozen parasite pellets were thawed, resuspended in PBS, and pooled before centrifugation in a glass tube at 2000 x g for 15 min at 4^0C. The parasite pellet was resuspended in chloroform:methanol 1:1 (4 mL) using a Pasteur pipette in a sonication water bath. The resulting solution was centrifuged at 2000 x g for 15 min at 4^0C and the supernatant was transferred to a new glass tube and the remaining pellet was now resuspended in 4 mL chloroform:methanol:water (10:10:3). After another round of centrifugation, both supernatants were pooled in a V-bottom flask and the solvent was removed using a rotary evaporator to complete dryness forming a thin film of lipids. The content of the flask was resuspended in 4 mL of water-saturated 1-butanol on a sonication water bath and subsequently, 3 mL water was added, and the solution was thoroughly mixed and centrifuged at 2000 x g for 15 min at 4^0C. The supernatant (butanol phase) was transferred into a fresh glass tube of known weight, and 4 mL water-saturated 1-butanol was added to the lower phase followed by another round of centrifugation. The two upper phase/ butanol phase were pooled and flushed with a stream of nitrogen to evaporate the butanol leading to the precipitation of the glycolipid (GPI). The contents were periodically centrifuged at 2000 x g for 15 min at 4^0C to obtain a white pellet comprising the GPI. Approximately 200-300 µL of the remaining butanol was pipetted out and the glycolipid pellet was completely dried using a stream of nitrogen.

2.2.3.5 Enzyme-linked Immunosorbent Assay (ELISA)

Cytokine quantification (IL-2, IL-4 and IFN-γ) was performed using an indirect sandwich ELISA format according to the manufactures instructions (BD Bioscience). The plates were coated with 100 µL of the corresponding capture antibodies at 1 µg/ml PBS and incubated overnight at 4^0C followed by three washes with PBS-T. The wells were blocked with 300 µL of blocking buffer for 2 h at room temperature followed by incubation for 90 min with 100 µL of the samples or 100 µL of standards (IL-2, IL-4 and IFN-γ) in reagent diluent. The plates were washed five times with PBS-T and incubated for 1 h at RT with 100 µL of corresponding biotinylated detection antibody diluted 1:400, 1:400, 1:250 (IL-2, IL-4 and IFN-γ) along with the Streptavidin HRP conjugate at 1:250 dilution. Following incubations, the plates were washed seven times with PBS-T and developed with 100 µL TMB substrate reagent for 30 min at RT in dark. The reaction was stopped by the addition of 2% H_2SO_4 (50 µL) and the absorbance was measured at 450 nm with a correction wavelength at 570 nm. The unknown concentrations were extrapolated from the standard curves.

To quantify CPS-3, the plates were coated with 100 µL of the monoclonal antibody (mAB5F6, a kind gift from Prof. Liise-anne Pirofski) at 1 µg/mL in PBS and incubated overnight at 4^0C followed by three washes with PBS-T. The wells were blocked with 300 µL of blocking buffer for 2 h at room temperature followed by incubation for 2 h with 100 µl of the samples or 100 µL of standards. The samples included the aqueous phase of the different particle lysates subjected to flochs extraction and the standard included the CPS-3 polysaccharide diluted over a concentration range of 30-0.62 µg/mL prepared in reagent diluent. After the incubation time, the plate was washed five times with PBS-T and incubated with rabbit polyclonal CPS-3 antisera diluted 1:2000 in reagent diluent and incubated for 1 h at RT. The plate was washed five times with PBS-T and incubated with goat anti rabbit-HRP conjugate diluted 1:2500 in reagent diluent and incubated for 1 h at RT. After the incubation time, plates were washed seven times with PBS-T and developed with 100 µL of TMB substrate reagent for 30 min at RT in dark. The reaction was stopped by the addition of 2% H_2SO_4 (50 µl) and absorbance was measured at 450 nm with a correction wavelength at 570 nm. The unknown concentrations were extrapolated from the standard curves.

To evaluate the antibody response (titers), the plates were coated with 1 µg CPS-3/PBS and incubated overnight at 4^0C followed by three washes with PBS-T. The plates were blocked with 300 µl of 10% FCS solution for 2 h at RT. The plates were probed with 100 µL of serum diluted in reagent diluent and incubated for 2 h at RT. Prior to incubations, the sera were pre-adsorbed with 10 µg/mL of cell-wall polysaccharide (CWPS) incorporated directly in the reagent diluent (1% BSA-PBS). Following incubations with the serum, the plates were washed five times with PBS-T and incubated with 100 µL of anti-mouse IgG HRP diluted 1:10000 and incubated for 1 h at RT. The plates were washed seven times with PBS-T and developed with 100 µL of TMB substrate reagent for 30 min at RT in dark. The reaction was stopped by the addition of 2 % H_2SO_4 (50 µl) and absorbance was measured at 450 nm. The absorbance values were normalized by subtracting the absorbance values of each group with their respective pre-immune sera and these normalized values were used.

2.2.3.6 Cellular Uptake of PLA Nano- and Microparticles

Uptake studies were performed to check for the uptake of PLA nano and microparticles by antigen presenting cells (APC). For this analysis, RAW 264.7 macrophage cells were plated at $1x10^5$ cells/ well in a 8-chamber slide and incubated with the fluorescent 6-coumarin PLA nano and microparticles at a concentration of 50 µg/ mL for 12 h. Confocal microscopy was performed to evaluate the uptake of particles by the RAW macrophages.

2.2.3.7 Splenocyte Isolation

Spleens were retrieved from the sacrificed C57BL/6 mice and washed twice in PBS. Splenocyte cell suspension was prepared by crushing the spleen in a 10 cm cell culture dish containing 10 mL of PBS through a 40 µm cell strainer using a 10 mL syringe plunger. Splenocytes were pelleted at 300 x g for 10 min at 4^0C and resuspended in 3 mL of ACK buffer for 10 min at RT for RBC lysis. The reaction was stopped by adding 10 mL of PBS and the pellet was washed twice with PBS. The splenocytes obtained were counted using Trypan blue to exclude the dead cells. A total of $6x10^7$ cells were obtained /spleen.

2.2.3.8 Isolation and Production of Bone Marrow Dendritic Cells

For the production of dendritic cells, bone marrow from the tibiae and femurs of C57BL/6 mice was flushed into incomplete DMEM medium and pipetted vigorously to disentangle the cells. The cells, $1x10^6$/ mL were plated in a 6 well dish with 4 mL/well of cell suspension in DMEM media containing 1ng/mL GM-CSF. The plates were incubated in a 37^0C incubator with 5 % CO_2 and media changed on day 3, 5, and 7. On day 8, the majority of the dendritic cells was harvested after staining for the surface marker CD 11c+ by flow cytometry.

2.2.3.9 *In vitro* Stimulation of Splenocytes by PLA Nano and Microparticles

Splenocytes were seeded in a 96 well flat bottom plate containing 200 µL complete DMEM medium at a concentration of 5×10^5 cells/well. The cells were stimulated either with 100 ng/mL soluble GSL or 50 µg/mL of particulate (nano and micro) GSL for 48 h after which the supernatant was retrieved and checked for the cytokines IL-2, 1L-4 & IFN-γ using a sandwich ELISA format.

2.2.3.10 Dendritic Cell Loading and Splenocyte Activation

In an alternative method of splenocyte activation, bone marrow-dendritic cells (BM-DC) were first seeded at 1 x 10^5 cells/ well in a 96 well flat bottom plate along with 100 ng/ mL soluble GSL or 50 µg/mL particulate GSL (nano and micro). Incubations were carried on for 6 h to facilitate DC mediated uptake and presentation of GSL both from the soluble and particulate forms. After the incubation time, DCs were washed three times with PBS followed by incubation with splenocyte suspension (5 x 10^5 cells/well) for an additional 24 h after which the supernatants were collected and checked for the T-cell cytokine IL-2 using ELISA.

2.2.3.11 Apoptosis Studies in RAW 264.7 Macrophages

For analysis of apoptosis, RAW 264.7 macrophages (1x10^5 cells/500 µL) were incubated in complete DMEM medium followed by addition of 2 µg of RH and PTG ELVs. PBS was used as negative control and all samples were incubated for 6 h at 37 °C. After the incubation time, the samples were treated with or without 50 U/ mL of IFN-γ and were further incubated for 12 h at 37 °C. After incubation, the cells were detached using Trypsin/EDTA solution. The apoptotic cells were detected using flow cytometry by performing Annexin staining using the AnnexinV-APC Apoptosis Detection Kit.

2.2.4 *In vivo* Studies

2.2.4.1 Ethics Statement

Animal experiments were performed in strict accordance with the German regulations of the Society for Laboratory Animal Science and the European Health Law of the Federation of Laboratory Animal Science Associations. The protocol was approved by the Landesamt für Gesundheit und Soziales Berlin (Permit No. G0104/13). All efforts were made to minimize suffering.

2.2.4.2 Immunization

Female C57BL/6 mice 6-8 weeks old were housed in separate cages with three mice per cage. Each sample group consisted of six mice/ group. A one prime two boost regime was followed as shown in Figure 9A followed by challenge with 5 µg of capsular polysaccharide-3 (CPS-3). Mice were immunized by s.c injection with 100 µL of PBS or various particle suspensions and protein conjugates containing CPS-3 (2 µg) and GSL (1 µg). The Prevnar®

group was immunized with 1/5th concentration (400 ng equivalent of CPS-3) as shown in Figure 9B. The CRM_{197}-CPS3 conjugates were adsorbed with alum (1:1 V/V) for 1 h, volume adjusted with PBS to 600 µL (equivalent to 100 µL/ mice) and incubated overnight at 4^{0}C on a tumbler. Prior to immunization, mice were weighed and serum collected.

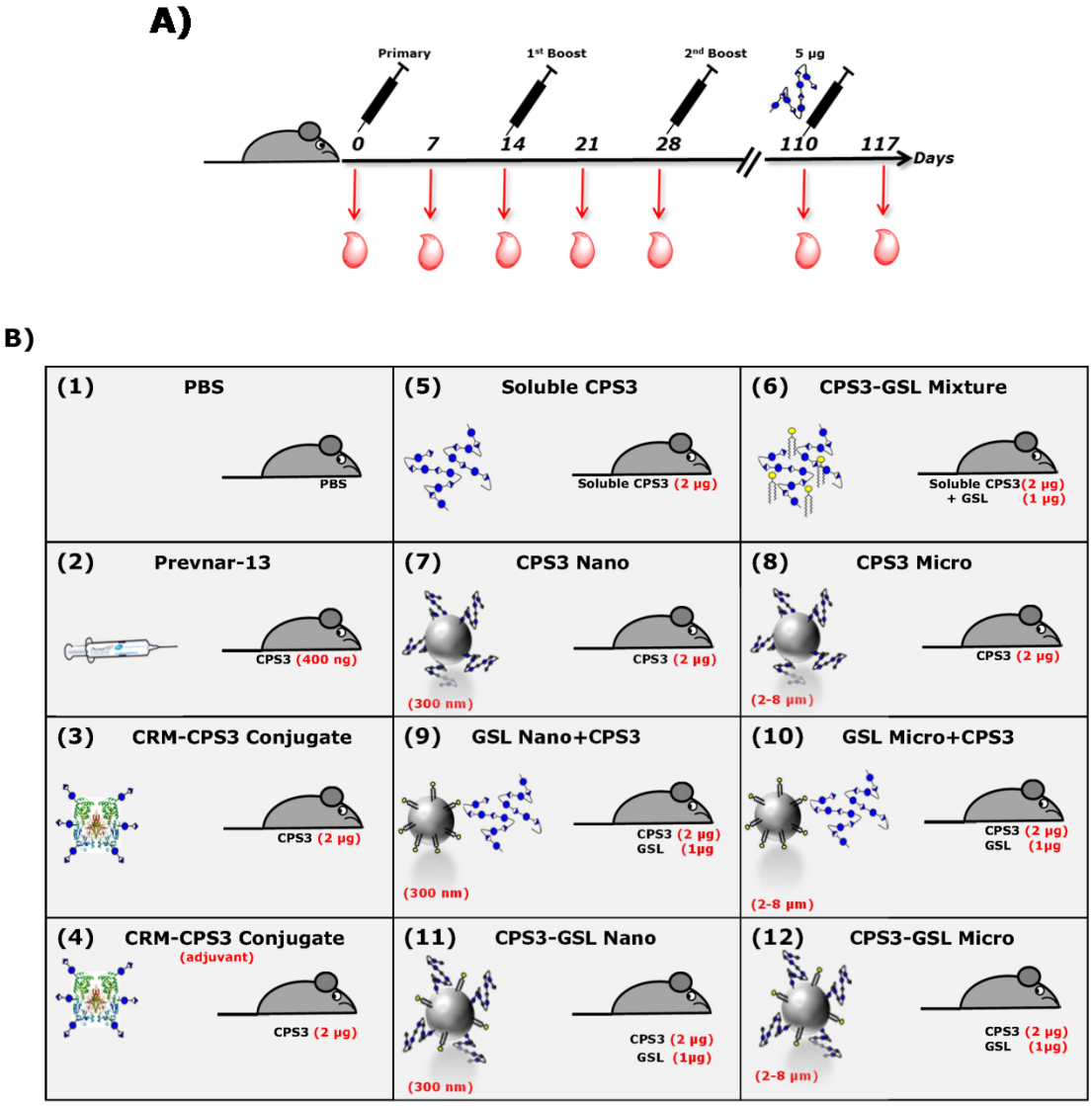

Figure 9. (**A**) Schematic representation of the immunization schedule. (**B**) The various experimental groups immunized with different formulations of CPS-3 antigen.

2.2.4.3 Serum Collection

For analysis of antibody titers in sera, blood was retrieved from the mouse tail vein. Blood was incubated at RT for 30 min, before centrifugation at 10000 rpm, RT for 10 min. Sera were pipetted into new Eppendorf tubes and stored at -20 ºC until further use.

3. Fabrication, Characterization and Immunological Evaluation of Polymeric Particles for the Delivery of Polysaccharide-Glycolipid Combination Vaccines

The following chapter describes the fabrication, characterization and immunological evaluation of a dry powder particulate formulation of polylactic acid (PLA) as a vaccine delivery system. The study focuses on T cell independent antigens (TI) and in particular the capsular polysaccharides of *Streptococcus pneumoniae.* Glycolipids demonstrate potent T cell adjuvant properties by activation of iNKT cells in a CD1d dependent manner. The glycosphingolipid α-galactosylceramide (α-GalCer/ KRN 7000) is a commercial glycolipid with standalone iNKT activation potential. This study aims to utilize the unique properties of the PLA based particulate system to co-deliver the TI antigen, in this case the capsular polysaccharide of *Streptococcus pneumoniae* serotype-3 (CPS-3) along with the iNKT cell glycolipid adjuvant α-galactosylceramide (α-GalCer/ KRN 7000) to the same B cell. The uptake and presentation of glycolipid by the CPS-3 specific B cell would result in cognate interaction and activation of iNKT cells leading to recruitment of iNKT cell help for B cells. Such a help will promote B cell differentiation and immunological memory. The CPS-3 and GSL antigens were presented on the PLA particulate system in different combinations and sizes ranging from <500 nm to 2-8 μm. The particulate formulations of the CPS-3 antigen and GSL adjuvant was compared with the CRM_{197} protein conjugate of the polysaccharide CPS-3 with alum as an adjuvant. The entire system was compared to the commercial vaccine Prevnar 13®.

3.1 Introduction

3.1.1 Carbohydrate Antigens

Carbohydrates (glycans) decorate on the surface of almost all cells and organisms. They exist as very well defined conjugates with specific linkages either with proteins forming glycoproteins or with lipids forming glycolipids. Carbohydrates are present on the surface of a wide range of pathogens like viruses (HIV-GP120), bacteria (LPS and various capsular polysaccharide structures), fungus (Cryptococcus β-glucan and Candida β-mannan), parasites (*Plasmodium* glycosylphosphatidylinositol) and serve as important virulence factors. Carbohydrates/ glycans like globohexaosylceramide (Globo H), fucosyl GM1, Thomsen–Friedenreich (TF) and 2-6-α-N-acetylgalactosamine (Tn), sialyl Tn and polysialic acid (PSA) are also important disease biomarkers found on the surface of various malignant tissues. Hence carbohydrates, either as monosaccharide, oligosaccharide or polysaccharide antigens form important targets of intervention strategies [136].

3.1.2 *Streptococcous pneumoniae*

Of the many pathogenic microbes infecting humans, bacterial infections inflict one of the highest human miseries in the form of deaths and economic burden [137]. Pneumonia caused by *Streptococcus pneumoniae*, a gram positive diplococci, lancet shaped, ubiquitous, extracellular respiratory bacteria is one of the most commonly acquired respiratory infections affecting all age groups across geographical locations [138]. Humans are the natural carriers and the bacteria habitat the upper mucosal surfaces and airway epithelia especially the nasopharyngeal regions of the respiratory tract. Although a commensal bacteria, *Streptococcus pneumoniae* can become an opportunistic pathogen depending on age, immunosuppression, genetic background, and outcome of other diseases leading to invasive pneumococcal disease (IPD) characterized by pneumonia, meningitis, otitis media, and bacteremia [138]. IPD causes the worst form of infections killing approximately 1.6 million people per year globally of which 8–12% are children below 6 years of age with an underdeveloped immune system and an equally high number of elderly with a weakened immune system or people having immune deficient syndrome on account of AIDS or Cancer worldwide [139-141].

3.1.3 *Pneumococcal* Virulence Factors

Streptococcous pneumoniae as all gram positive bacteria is an encapsulated pathogen normally carried in the upper respiratory tract with asymptomatic infections. Occasionally the bacteria can transcend to the lower respiratory tract where it colonizes the lung epithelia leading to direct interaction with the innate defense mechanisms of the host leading to elimination of the bacteria or establishment of a successful colonization. The outcome of this host-pathogen interaction leads to an inflammation that along with bacterial colonization of lungs, leads to pneumonia that can further transcend into the bacteria invading and causing systemic infections resulting in bacteremia leading to meningitidis [138]. The surface components of the bacteria are mainly evolved to assist the bacteria in infection colonization, invasion and evasion of the host responses. The virulence factors can be summarized based on the four important steps of infection, colonization and invasion (Figure 10).

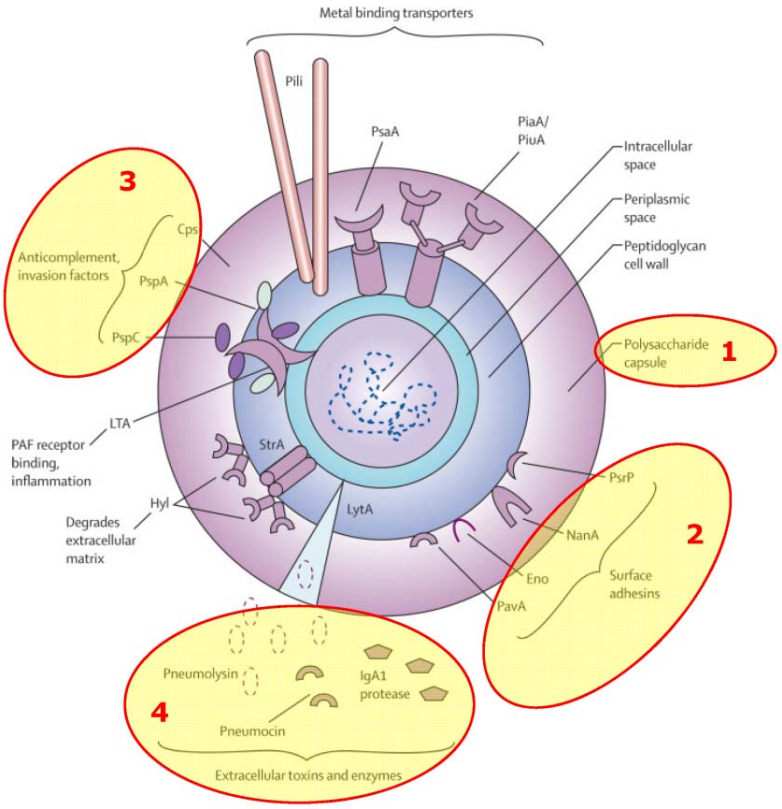

Figure 10. Virulence factors of *S. pneumoniae*. The various proteins and polysaccharides used by *S. pneuomoniae* during the different phases and steps of establishing a successful infection and colonization. The four important groups are 1. the capsular polysaccharide that provides protection from the mucus in the nasopharyngeal region and from the immune cells during the planktonic invasive form in circulation. 2. A group of adhesines that mediate the binding to the host airway epithelia, 3. A group of proteins and enzymes that degrade the complement factors and also aid in attachment and 4. A group of secretory proteins that cause host cell damage. Figure adopted and modified from [142] .

The bacteria are protected by a thick outer capsule made of polysaccharides that offers the first stage of protection to the bacteria against the deposition of complement factors, avoiding entrapment and ejection by the mucous, preventing engulfment by host phagocytes both in the colonizing phase in the lungs and the invasive phase during circulation. The second set of virulence factors consists of a set of proteins that function as adhesins to establish contact with the lung cell epithelia and also favor the invasion process. Some of the adhesins and associated proteins are hyaluronate lyase that degrades the extracellular matrix and neuraminidase that functions by removing the terminal sialic acids on host glycopeptides and mucins to expose the binding sites on the host surface [143]. The proteins pneumococcal adhesion and virulence-A (PavA) and enolase are two lectins that directly bind to the host surfaces through fibronectin and plasminogen [144, 145]. Having established a successful and strong contact on the host cell epithelia, the bacteria protects itself from various host responses using proteins like pneumococcal surface protein (PspA and C) which bind complement control protein factor H, block the fixation complement factor C3 and also inhibits the binding of C3b to factor B protecting the bacteria from complement mediated lysis. The bacteria also secrete a protease that degrades the Ig A, the essential immunoglobin of mucosal immunity. The expression of the protein LytA the lysis of the bacteria releasing the cell wall components comprising of techoic acid and the toxin pneumolysin, a pore forming peptide that mediates cell lysis creating an intense inflammatory reaction enabling the bacteria to invade into the systemic circulation. Once in circulation, the bacteria overexpress the polysaccharide capsule in order to protect itself from the immune system and mask the various proteins on its surface. The exposure of the bacteria to the two immunological niches of lung tissue and circulation influences the expression of one or the other component benefitting the bacteria to adapt to these host environments.

3.1.4 Pneumococcal Capsular Polysaccharide

The pioneering work of Heidelberger & Avery in 1923 established the classification of one of the important virulence factors of *S.pneumoniae* as polysaccharides [146, 147]. The surface of *Streptococcus pneumoniae* is decorated with a thick layer of repeating glycans units linked through different linkages forming the capsular polysaccharide (CPS). The repeating unit can be as simple as a disaccharide and in some cases can even be an octasaccharide as big as $0.5\text{-}2\text{x}10^6$ Da measuring about 200-400 nm and can account for half the dry weight of the bacteria [148, 149]. The capsular polysaccharide forms one of the most important

virulence factors in both the commensal and invasive forms of the pneumococcal infections and is directly dependent on the thickness of the capsule [150]. The capsular polysaccharide (CPS) prevents opsonophagocytosis of the bacteria, helps in colonization, renders the bacteria resistant to mechanical clearance by the mucous, restricts autolysis and reduces exposure to antibiotics [151]. During an infection event which is primarily through the nasopharyngeal region, the first line of defense the bacteria encounters is the mucous that can entrap and eject the bacteria. The presence of the polysaccharide capsule, which is highly negatively charged, repels the binding of the mucous and facilitates the adsorption of the bacteria on the lung epithelia. However, the colonization of the bacteria on the lung epithelia is not favored by the capsule as it obstructs binding of certain bacterial adhesins on the host cell. Hence the bacteria down regulate the polysaccharide capsular content to enhance colonization and growth on the

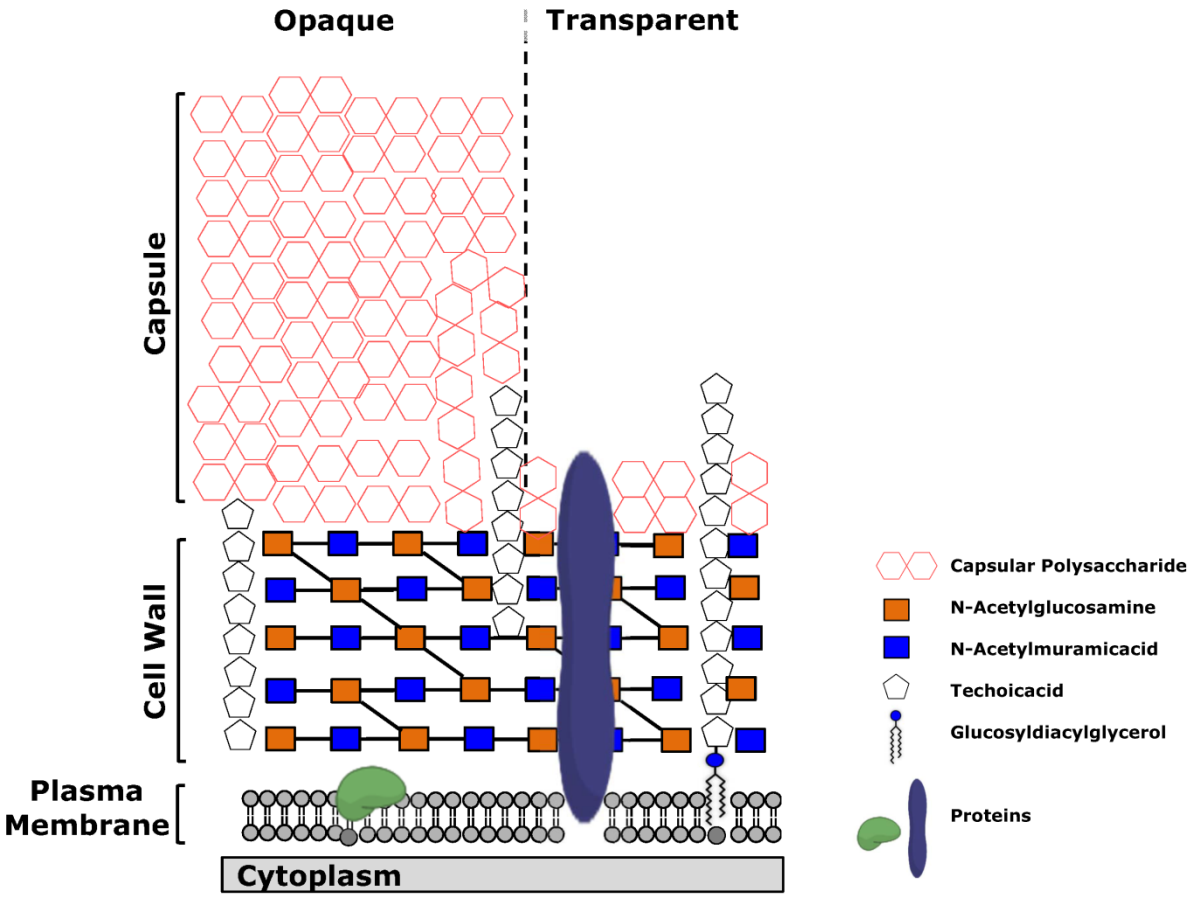

Figure 11. Cross-section of *S. pneumoniae* showing the three cell boundaries. i) The polysaccharide capsule showing phase variation between rough (left half) or smooth (right half) defining the different growth phases. **ii)** The middle cell-wall consisting of highly cross linked *N*-Acetylglucosamine and *N*-acetylmuramicacids, several techoic acid polymers and protein virulence factors. **iii)** The plasma membrane, consisting of several membrane associated proteins and the lipotechoic acid residues (LTA) linked through a glucosyldiacylglycerol (Glc-DAG) moiety.

lung epithelia. During a successful invasion event, the bacteria now become exposed to a different immunological niche where they encounter specific antibodies, complement factors and phagocytic cells that can kill the bacteria as part of the host response. In the planktonic, invasive form, the bacteria overexpress its capsule many fold to protect against the host response. The bacteria exist in two forms, a transparent form found in lung colonization characterized by a low polysaccharide capsule and an opaque invasive form characterized by a thick polysaccharide capsule [152].

The biosynthesis of CPS is mediated by the transcription of several genes located on the *cps* locus flanked by a 1, 6-α-glucosidase gene *dexB* and the oligopeptide ABC transporter gene *aliA* [153, 154]. *The cps locus contain several glycosyl transferases* producing various glycans where in the Wzx/ Wzy flippase-polymerase dependent pathway is used to produce the polysaccharide or the locus can possess a single transferase where the synthase pathway is used to produce the simpler polysaccharide [155, 156]. Hence based on the number/unique combination of genes coding for the transferases, *S. pneumoniae* have capsules made up of varied combinations of different glycans resulting in >90 different serotypes having different composition of glycans, 20 of which are known to cause serious infections in humans. Out of the >90 serogroups, except for serotype 3 which is made of alternating units of glucose and glucuronic acid, the rest are synthesized using the Wzx/ Wzy pathway. Each serotype possesses only a unique combination of *cps* genes or alleles at any given time producing a specific type of capsule. However, the *cps* locus is prone to variability either by slipped-strand mispairing resulting in gene truncation or by high genetic transformability resulting in recombination events resulting in replacement of either a part or the entire *cps* locus with the homologous region from a donor strain resulting in serotype/ capsule switching [157-159]. However, no transcriptional elements of the *cps* promoter which is thought to be involved in the transcriptional and translational control of CPS production between the colonizing and invasive forms of the bacteria are identified to date [160].

3.1.4.1 *Streptococcus pneumoniae* Serotype-3 Capsular Polysaccharide (CPS-3)

The capsular polysaccharide of *S.pneumoniae* serotype-3 is made up of simple disaccharide repeating units [⟶3)-β-D-GlcA-(1⟶4)-β-D-Glc-(1⟶] terminating either with a glucose or glucuronic acid residue (Figure 12) [161, 162] .

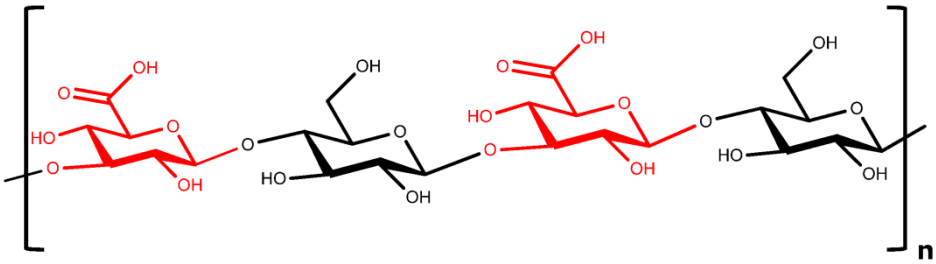

Figure 12. Chemical structure of Capsular polysaccharide-3 (CPS-3). The repeating unit of CPS-3 indicated by glucurinic acid (red) and glucose (black). n≈37 repeating units [163].

As opposed to the other serotypes, the CPS of serotype-3 is linear and is synthesized using the synthase based pathway [154]. This result in a large molecular weight polymer of approximately $0.14x10^5$ Da [163] . *S.pneumoniae* serotype-3 is also known to possess one of the largest capsules measuring 400 nm which is not covalently bound to the preceding cell wall [164]. The capsular polysaccharide of *S.pneumoniae* serotype-3 is implicated as one of the leading factors for the high incidence of pneumococcal infection acute otis media (AOM) in children [164].

3.1.5 Vaccines Against Capsular Polysaccharide

The capsular polysaccharides are the largest surface exposed virulence factors not just in *Streptococcus pneumonia* but also in other encapsulated bacteria like *Haemophilus influenza* type b, *Neisseria meningitidis* and hence are prime targets of vaccine development. The emergence of antibiotic resistance in bacteria has provided a large emphasis towards the development of polysaccharide based vaccines. Not surprisingly, vaccine development against carbohydrate/polysaccharide antigens are not straightforward because of the strain dependent variability of the antigens, similarity of the antigen with host structures, variable host factors like age and finally poor immunogenicity of the antigen itself. Based on these factors, only two broad classes of carbohydrate based vaccines exist i) capsular polysaccharide vaccines and ii) glycoconjugate vaccines.

3.1.5.1 Capsular Polysaccharide Vaccines

Based on the discovery of Heidelberger and Avery, *S. pneumonia* was the first polysaccharide antigen known to induce an immune response and protection against pneumococcal pneumonia [165]. This discovery further led to the development of a 14-valent polysaccharide vaccine that was later expanded to include the current formulation of 23 different disease causing serotypes of *S. pneumoniae* commercially called PPSV-Pneumovax® 23. The inception of this vaccine resulted in a great decrease in the incidence of *pneumococcal* infections offering protection to more than 90% of isolates responsible for invasive pneumococcal pneumonia. Unfortunately, the vaccine failed to offer protection in neonates, immunocompromised individuals and elderly. This failure was mainly attributed to the mechanism of immune activation mediated by carbohydrates that are T independent (TI) antigens and do not require a cognate antigen specific B cell and T cell interaction to produce an immune response as in case of protein antigens [166]. The TI antigens are further classified into TI-1 and TI-2 antigens [167, 168]. TI-1 antigens like bacterial lipopolysaccharide (LPS) act as B cell mitogens capable of providing intrinsic signals inducing proliferation and differentiation of both *naïve* and mature B cells [166, 169]. On the other hand, TI-2 antigens like capsular polysaccharides are large repetitive glycan structures and are poorly degraded *in vivo* with no capacity to intrinsically activate B cells [170, 171]. TI-2 antigens like polysaccharides mainly bind to the antigen specific B cell receptors (BCR) that are surface IgG molecules, and cause their activation by cross linking the receptor resulting in the producing plasma cells and IgM antibodies as shown in Figure 13 [166, 172].

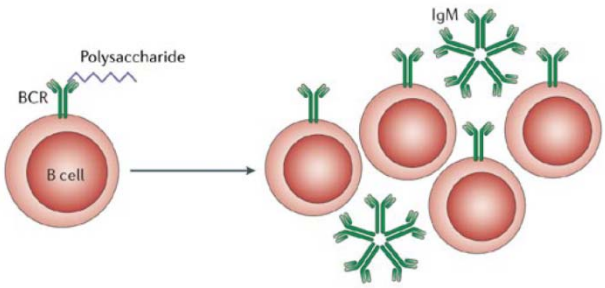

Figure 13. Polysaccharide activation of B cells through a B cell receptor (IgG) leading to clonal expansion and production of IgM class of antibodies with no immunological memory. Figure adopted from [172].

TI-2 antigens mainly activate mature B cells and the outcome of such an interaction produces primarily IgM antibodies that are short lived, and in the absence of T cell help produces no isotype class-switch, affinity maturation and immunological memory. B cells mature late in children and fade away in elderly with advancement of age. The vaccine is not effective in children below two years of age with underdeveloped B cell responses and in elderly due to a weakened immune system. Polysaccharide vaccines only provide systemic protection against invasive pneumococcal infections but do not prevent the colonization and carriage of the bacteria in the nasopharyngeal regions[173]. This combined with the poor population coverage of the vaccine further contributes to the spread of the bacteria. Indeed, due to the cost benefits, the vaccine is still a success in elderly that have to be revaccinated every five years [174, 175].

3.1.5.2 Glycoconjugate Vaccine

The pioneering studies by Avery & Goebel in 1929 demonstrated that non-immunogenic sugars can be made immunogenic by conjugating to a protein moiety to produce sugar/polysaccharide specific immune response [176]**.** This concept was later on exploited to address the T-independent behavior of polysaccharide antigens by chemically conjugating the polysaccharide fragment to a carrier protein making it a T dependent (TD) antigen and the first glycoconjugate vaccine for human use was developed in 1980 [177]. The conjugate vaccines were also extended to pneumococcal antigens with the development of a 7-valent conjugate vaccine which was later expanded to include the current formulation of 13 different disease causing serotypes commercially called Prevnar-13®. The 7 valent conjugate vaccine provided excellent protection in infants and children and was >90% effective in preventing invasive pneumococcal disease for the vaccine included serotypes [178]. The vaccine was also efficacious in reducing the carriage of the bacteria and was superior to the PPSV in this regard. The proposed mechanism and superiority of the PCV was thought to be mediated by the carrier protein having a T cell epitope which when internalized by an antigenic specific B cell would be processed and presented on a MHC class II molecule. The subsequent activation of the B cell receptor by the sugar on the conjugate and the recognition of the MHC restricted carrier peptide by a T cell results in the activation of antigen specific B cell by the T cell stimulatory cytokine release. This cascade induces the B cells to undergo differentiation into memory cells and antibody producing plasma cells with affinity maturation and class switching producing IgG class of specific antibodies (Figure 14).

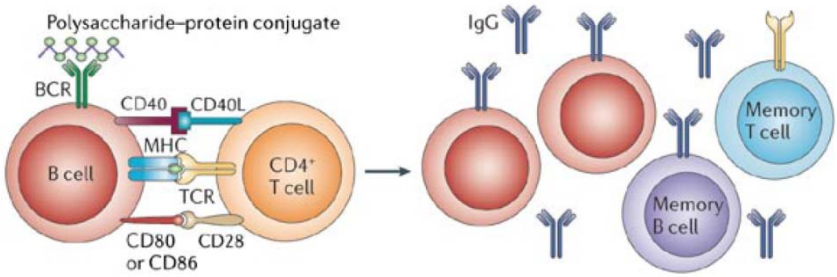

Figure 14. Mechanism of B cell activation by PCV. The recognition of the PCV by the polysaccharide specific BCR leads to B cell activation and internalization of the PCV. The PCV is processed and the carrier protein having T cell epitope is presented on MHC molecules. Recognition of the peptide MHC fragment by the T cell along with co-receptor ligation of CD40-40L leads to production of T cell cytokines which induce B cell differentiation producing high affinity IgG antibody producing plasma cells and memory T and B cells. Figure adopted from [172].

There are certain limitations for conjugate vaccines as compared to PPSVs. The PCVs are limited in serotype inclusion of 13 CPS types as compared to the PPSVs that include 23 serogroups and provide no comparable benefit in adults as compared to PPSV [179, 180]. Capsular polysaccharides are chemically unreactive and hence have to be modified to create reactive functional groups to facilitate its conjugation to the protein carriers [181]. The chemical modification of polysaccharides often results in the loss of immunogenicity towards the targeted epitope [181]. Formulation parameters for each individual serotype vary in terms of carrier protein used, linkage type of the polysaccharide, size of the polysaccharide and the overall polysaccharide immunogenicity resulting in higher cost of producing the PCV as compared to the PPSV [181]. Although the PCV provide excellent protection against the vaccine included serotypes of *S.pneumoniae*, there has been an increase in the incidence of mid ear infections (acute otis media- AOM) caused by serogroups of pneumonia not included in the PCV [182]. This has been due to the nasopharyngeal carriage and serotype replacement by non-vaccine serotype of *S.pneumoniae* leading to serious concerns [183, 184]. Additional ways of inducing TD immunity towards a polysaccharide antigen have been considered including polysaccharide peptide mimics and DNA vaccines. This area of research is definitely intense and promising.

3.1.6 Nasopharyngeal Carriage of *Streptococcus pneumoniae* and Natural Immunity

Humans are the natural carriers of *S.pneumoniae* with the nasopharyngeal region being the natural habitat. An important observation was the presence of serotype specific natural antibodies of IgG isotype directed against the capsular polysaccharide in individuals with history of pneumococcal carriage [185]. Although, the levels of antibodies were different for different serotypes, depending on the immunogenic property of the polysaccharide, the pneumococcal carriage definitely provided protection and reduced the risk of carriage [186]. Polysaccharides are known TI antigens, incapable of producing IgG type antibodies. Infection of mice with polysaccharide encapsulated bacteria produced serotype specific IgG indicating some form of T cell help which were largely CD4+ type [187]. This T cell help was thought to be mediated by the various immunogenic proteins such as PspA, PsaA, pneumolysin that are associated below the capsule [187]. However, it has been reported that the level of polysaccharide IgG antibodies were always higher and there was no change in the level of the antibodies against the bacterial proteins PspA or PsaA indicating that apart for the proteins other factors could also be involved in mediating this T cell help [188]. This observation coincides with the independent discovery of a specific subset of invariant Natural Killer like T cells (iNKT) and the subsequent discovery of their corresponding glycolipid ligand α-glucosyl diacylglycerol (α-Glc DAG). α-Glc DAG are components of the bacterial cell wall that narrow the gap between T-dependent and independent antigens in the context of pneumococcal natural infection. The use of an iNKT cell adjuvant glycolipid combined with a TI antigen to raise polysaccharide specific immune responses mimics the natural infection scenario. This combination could also address the shortcomings associated with using the protein conjugate to raise a TD response and more pneumococcal serotypes could be included in a vaccination regime. Co-delivering the glycolipid adjuvant and the polysaccharide antigen with the aim of eliciting a protective mucosal and systemic immunity could serve as a novel vaccination platform against pneumococcal infections.

3.1.7 Mucosal Delivery of Polysaccharide Antigens

An important aspect of vaccination against infectious organisms is the route, site of infection and the corresponding mode of vaccine delivery. In the case of pneumococcal infections, this is the mucosal surfaces of the respiratory tract especially the nasopharyngeal region. Mucosal immunization of experimental animals with whole cell pneumococci has

shown protection against infection and colonization [189]. Mucosal immunization can be beneficial as it elicits both local and systemic immunity [190]. It can be advantageous as the local secretory immunoglobulin-A (sIgA) has been shown to prevent both bacterial colonization at the mucosal surfaces and invasion into the systemic circulation [191, 192]. The mucosal immune system develops earlier in infants and lasts longer in the elderly and is beneficial for AIDS patients as HIV-infected individuals can develop normal mucosal antibody responses even in late clinical phases [193-195]. However, targeting the mucosal immune system is limited in success as it presents numerous hurdles, including diversity in mucosal surface structure, complexity in immune cell interaction and limitations in experimental methodology. Mucosal immunity can be elicited by oral, nasal or pulmonary delivery of antigens. Unfortunately, in these cases the traditional delivery of antigens in a soluble form is not the optimal delivery system. Targeting the mucosal surfaces pertaining to the packing and presentation of the antigen along with novel adjuvant activities has to be pursued [196]. These include particulate formulations like liposomes and polymeric particles of biodegradable polymers (polylactic acid) that package the antigen in a multivalent fashion with an appropriate size resulting in pathogen mimicry [56]. The particulate nature of these antigens also confers an adjuvant like property, protects against antigen degradation and assists in the controlled release of antigen at a particular site [197]. Many antigens have been formulated into liposomes, and oral delivery of these antigens to target the mucosal surfaces have been tried, but with little success. Oral immunization has been limited because of inefficient antigen uptake, induction of tolerance, and proteolytic degradation of the antigens before they reach the immune cells [198]. Liposomes are also limited by their instability during storage, limited shelf-life, and the difficulty in producing a sterile product [56].

Pulmonary vaccine delivery is becoming an attractive mode of immunization, because of the high vascularization in the pulmonary area, the absence of acidity, lack of enzymes, and a thin epithelial layer that allows for low doses of antigen to be administered [199, 200]. However, delivering of antigens to deep lung tissues is challenging and requires a delivery system with a mass median aerodynamic size between 1 and 5 μm to deposit the antigen on the alveolar region [201, 202]. Polylactic acid and other biodegradable polymers have shown great promise in this regard with practical ease of fabrication and aerosolization into particles of different sizes [202]. An important advantage of aerosolization is that the antigens can be formulated into very stable dry powders and packed in inhalers [203]. This formulation enhances the stability of the antigen, improves the retention time on the mucosal surfaces,

facilitates enhanced uptake and presentation by alveolar APC like macrophages [204, 205]. An important feature of drypowder formulations is that it removes the need of cold storage of the vaccine formulation thereby encouraging self-administration and reducing the costs involved in maintenance of health care volunteers.

The infection biology of *S.pneumoniae* summarizes certain key points beneficial in vaccine development. The T cell help mediated by iNKT- α-GalCer- CD-1d interaction opens a new area where iNKT cell ligands, mainly α-GalCer lipids can be used as novel adjuvants. Co-delivering a TI antigen, in this case the pneumococcal capsular polysaccharide (CPS-3) along with an iNKT ligand (α-GalCer) in a particulate form through the natural route of infection can serve as a rational approach of vaccination against *Streptococcus pneumoniae* infections.

3.2 Results and Discussion

3.2.1 Glycolipid Antigens Show Immune Stimulatory Properties with Adjuvant Activity

Glycolipid antigens, especially the glycosphingolipids (GSL) and glycosyldiacylglycerols (Glc-DAG) have shown promising T cell adjuvant properties with respect to the activation of iNKT cells through a CD1d dependent pathway [120, 206]. Polysaccharide antigens activate antigen specific B cells in a T cell independent (TI) manner by cross linking their BCR. Hence, the co-delivery of an iNKT cell agonist like GSL may provide iNKT cell help converting a TI CPS-3 antigen into a T cell dependent (TD) antigen. The immune stimulatory potential and adjuvant property of α-GalCer (KRN 7000) referred to as GSL (glycosphingolipid) from here on was checked by injecting it into mice along with the CPS-3 antigen as a physical mixture. The adjuvant activity of GSL was compared to the commercial vaccine Prevenar-13® that was injected in the presence of alum. The capsular polysaccharides of pneumococcus are very large polymers and hence form very viscous solutions. It is also known that *Streptococcus pneumoniae* serotype-3 produces one of the largest capsules. The capsular polysaccharide (CPS-3) was sonicated for approximately 1h to partially depolymerize and size reduced the polysaccharide to render it less viscous. The size reduction was monitored by SDS-PAGE followed by Alcian blue staining. Although the staining of the CPS-3 on the gel was limited, there was a visible size reduction of the polysaccharide seen in lane 2 (Figure 15A) as compared to the native polysaccharide (lane 1). This reduced staining efficiency might be due to the negative charge of the CPS-3 polysaccharide that consists of glucose and glucuronic acid repeating units.

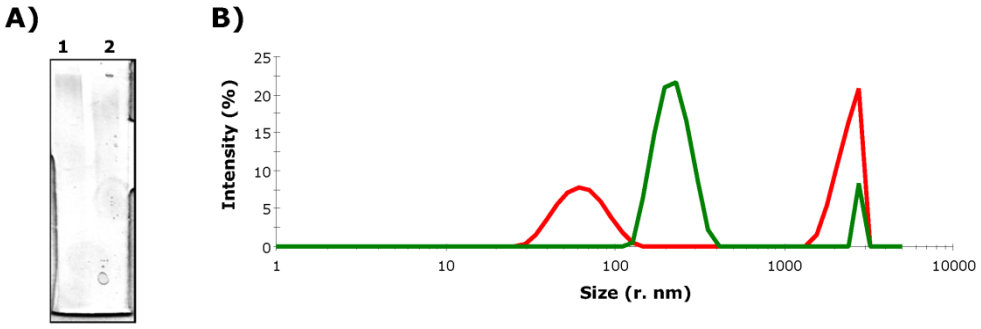

Figure 15. Size reduction of CPS-3 polysaccharide. (**A**) Profile of native and size reduced CPS-3 polysaccharide run on a 10% SDS-PAGE stained with alcian blue (1. Native polysaccharide (50 µg), 2. Size-reduced polysaccharide (50 µg)). (**B**) Reduction of CPS-3(50 µg) hydrodynamic size (native-red) and (size-reduced-green) analyzed by dynamic light scattering technique.

The size reduction was further analyzed by a more sensitive technique of Dynamic Light Scattering (DLS) which gives information of the size of particulates between 1 nm-1 µm based on the light scattering. The native polysaccharide (in red) had two light scattering intensity peaks, one in 100 nm low molecular weight range and the other in the 1000 nm high molecular weight range (Figure 15B). On sonication, the scattering intensity of the high molecular weight peak drastically decreased with an increase in scattering intensity of the peak at the 500 nm range indicating size/molecular weight reduction (Fig 3.1B, in green). This size reduced polysaccharide was used for all further experiments.

3.2.1.1 Immunization Studies to Evaluate CPS-3 Specific Immune Responses of Mice Immunized with Glycosphingolipid (GSL) and Alum as Adjuvant.

The ability of the size reduced CPS-3 antigen to elicit a polysaccharide specific IgG response was evaluated by immunizing C57BL/6 mice (n=6) through the sub-cutaneous route. Different formulations were prepared either with or without GSL for soluble CPS-3 (Table 3). The commercial vaccine Prevnar® served as a positive control and phosphate buffered saline (PBS) as the negative control. All formulations were normalized to an injection volume of 100 µL per mouse and immunizations followed a prime-boost regime (Figure 16A). The sera collected on day 21 were analyzed for polysaccharide specific IgG antibodies by ELISA using CPS-3 polysaccharide as coating antigen. The IgG titers of the different experimental groups were represented by plotting the absorbance values at 450 nm after subtracting their

	Formulation	Antigen	Adjuvant	CPS-3 Dose
1	PBS	-	-	-
2	Soluble CPS-3	CPS-3	-	2 µg/ 100 µL/ mouse
3	CPS3-GSL Physical Mixture (PM)	CPS-3	GSL (α-GalCer) 1 µg	2 µg/ 100 µL/ mouse
4	Prevnar 13®	Capsular Polysaccharide (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	Alum ($AlPO_4$)	0,4 µg/ 100 µL/ mouse

Table 3. Formulations used for evaluating adjuvant potential of GSL and Alum.

corresponding pre-immune (day 0) values. The experimental group immunized with the CPS-3 polysaccharide antigen (blue square) didnot elicit a specific IgG response on day 21 and the mean serum titers were comparable to the control group immunized with PBS (black circles) (Figure 16B). This clearly demonstrates the T-independent behavior of the polysaccharide antigen and the inability of the B cells to produce IgG class of antibodies. However, there was a significant increase in the CPS-3 specific IgG titers when the polysaccharide (CPS-3) and the iNKT cell adjuvant (GSL) were co-delivered in form of a physical mixture (orange diamond). These findings clearly demonstrate the role played by the glycolipid GSL in assisting the B cells to produce IgG type antibodies. However, the Prevnar® group with only 1/5th dose of CPS-3 immunized produced superior IgG response with mean titers significantly higher than the group that received CPS3-GSL physical mixture (CPS3-GSL PM).

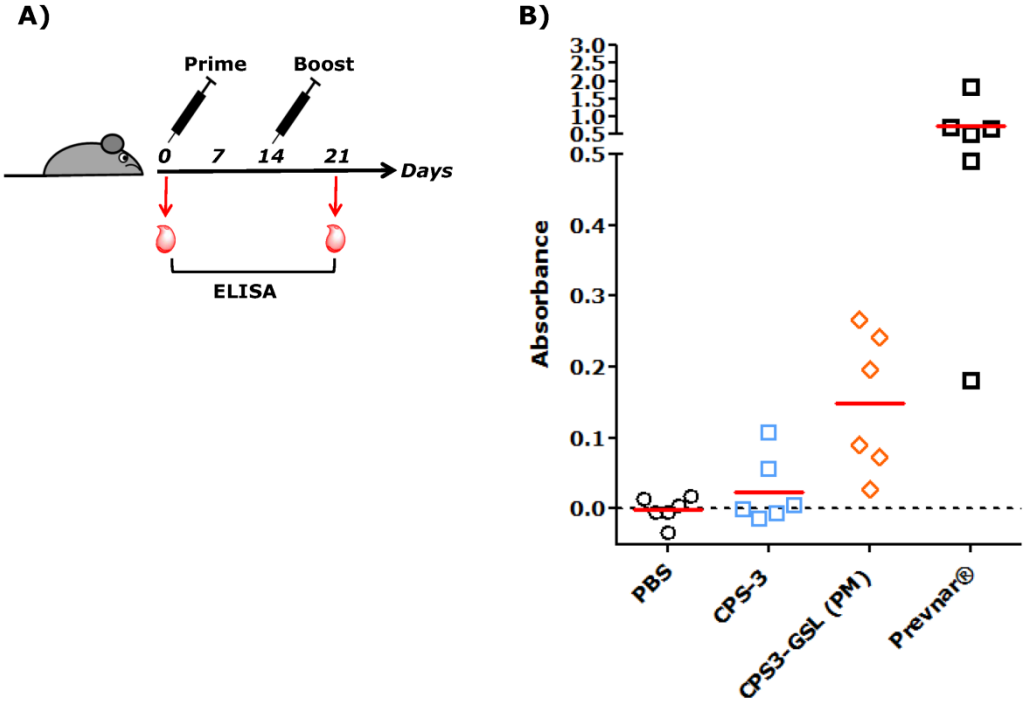

Figure 16. Evaluation of serum antibodies from mice immunized with CPS-3 polysaccharide antigen. (**A**) C57BL/6 mice were primed at day zero followed by a boost-immunization at day fourteen with the formulations listed in table 3.1. Sera 50 µL was collected both prior and after immunization and analyzed by ELISA. (**B**) ELISA titers of sera collected on day twenty one expressed as OD 450 absorbance values after subtracting the pre-immune values of corresponding experimental groups. Sera were diluted 200 X with 1% BSA-PBS and pre-incubate with 10 µg/mL cell-wall polysaccharide. 100 µL of the diluted pre-incubated sera was added per well of the microtiter plate which was coated with 1 µg of CPS-3 polysaccharide. Following washings, the plates were incubated with a HRP conjugated mouse secondary antibody diluted 1:10000 and developed using the chromogenic substrate TMB. Absorbance was measured at 450 nm and the data were plotted using the graphpad prism software with the red horizontal lines representing the mean OD values of the experimental groups.

Two important factors may have influenced the outcome. i) The conjugation of CPS-3 to the carrier protein (CRM_{197}) in the Prevnar® formulation represents a particulate scaffold where the carrier protein apart from providing T cell epitopes functions as a delivery system concentrating the antigen. ii) The presence of the adjuvant (alum) which forms emulsions further increase the local concentration of the antigen improving the efficiency of the delivery and enhancing the overall immune response. Therefore, the delivery of CPS-3 and GSL as an abstract physical mixture (PM) might have diluted the local concentration of the antigen (CPS-3) and the glycolipid adjuvant (GSL) leading to a reduced immune response. It also known that co-delivering both the antigen and the adjuvant to the same B cell increases the efficiency of the immune response. Hence, it was envisaged that co-delivering the above antigens (CPS-3 and GSL) on a common scaffold, might improve the immunization efficiency resulting in an enhanced polysaccharide specific IgG response. Considering the physiochemical property and solubility of the hydrophilic (CPS-3) and the hydrophobic (GSL) antigens, it is quite challenging to present them on a common delivery platform. Thus, I decided to fabricate polymeric particles of polylactic acid (PLA) which are reported to be excellent systems for co-encapsulation of hydrophilic and hydrophobic compounds. It has been shown that *S. pneumoniae* has a size of approximately 2 μm but can also form long chains and colonies reaching up to 10 μm. The average size of the aluminum adjuvant gels (Alum) is below 10 μm with an average size of 3 μm [207]. PLA particles of different sizes in the sub-micron range and micrometer range (< 10 μm) promote different types (T_H1/T_H2) of immune response. Considering these properties, PLA particles of two different size ranges < 500 nm and 2-8 μm were fabricated to study the outcome of the immune response on the size dependent presentation of the antigens CPS-3 and GSL.

3.2.2 Fabrication and Characterization of Polylactic Acid (PLA) Nano- and Microparticles

Polymeric particles of polylactide or polylactide co-glycolide have been used as efficient systems for encapsulating and delivering various hydrophilic and hydrophobic components. Besides the physiochemical properties of the ligands (polysaccharide and glycolipid antigen), the PLA polymer used can influence the final fabrication of the particles and impact the encapsulation efficiency, release kinetics and finally the size of the particles. The optimal process parameters for the fabrication of PLA nano and microparticles particles were first standardized.

3.2.2.1 Fabrication of PLA Microparticles

Microparticles were fabricated using the double emulsion solvent evaporation technique [208, 209]. Three fabrication conditions were tried using the low molecular weight polymer of Poly-D/L-Lactide (inherent viscocity 0.25-0.35 dL/g) resulting in the particle types (1, 2 and 3) as shown in Table 4. The constituents of the IAP, OP and EAP were kept constant but the energy input in making the secondary emulsion was varied. This was accomplished using a handheld homogenizer with an analogous power output (Power 1-6) reaching speeds of 8-20K rpm. The size and the distribution of the particles (types-1, 2 & 3) were first analyzed using the mastersizer. As seen in Table 4, the particles showed a volume averaged size D_v (0.5) of 7.5, 4.5 and 2.6 µm with varying span values indicating the heterogeneity of the particles.

Primary Emulsion				Secondary Emulsion		Power	Size D_v (0.5)	Span
Fabrication Condition (Particle Type)	IAP (W1)	OP (O)	IAP:OP (W1:O)	EAP (W2)	W1:O:W2			
	Poly Lactide- R-203 S (Inherent viscosity 0.25-0.35 dL/g)							
1	50 µL	2 mL (50 mg)	1: 40	8 mL (1%)	1:4	3	7.5 µm	4.1
2	50 µL	2 mL (50 mg)	1: 40	8 mL (1 %)	1:4	4	4.5 µm	1.3
3	50 µL	2 mL (50 mg)	1: 40	8 mL (1 %)	1:4	6	2.6 µm	0.9
	Poly-D/L-Lactide PDL-50 (Inherent viscosity 0.49 dL/g)							
5	50 µL	2 mL (100mg)	1: 40	8 mL (2 %)	1:4	10 K rpm	5.5 µm	1.4
6	100 µL	2 mL (100mg)	1: 20	8 mL (2 %)	1:4	10 K rpm	5.5 µm	1.09

Table 4. Process parameters used for the fabrication of PLA microparticles. The various parameters used in fabricating the microparticle (type-6) highlighted in red. IAP= Internal Aqueous Phase, OP= Organic Phase and EAP= External Aqueous Phase.

The particle type-1 had a very broad size distribution ranging from of 2-33 µm and was outside the intended size range selected for my studies. However, the type-2 and type-3 microparticles had very narrow span values with a distribution of 2-8 µm (type-2) and 1.5-4

µm (type-3) particles. Although the microparticle (type-2) had an optimal size range intended for the current studies, they were very unstable and rapidly lysed when dispersed in an aqueous solvent like water or PBS. The lysis may be due to the low molecular weight of the polymer that is known to degrade rapidly. Hence, in order to increase the stability of the particles, high molecular weight PLA polymer (0.49 dL/g) was used. Apart from the molecular weight of PLA, the concentration of the polymer was also changed to increase the particle yield. A homogenizer with an accurate digital power output was used to better control the secondary emulsion parameters. The new fabrication condition resulted in microparticles (particle type-5) with a volume averaged size D_v (0.5) of 5.5 µm and a distribution of 2-10 µm (Table 4). In order to incorporate higher amounts of hydrophilic antigens, the volume of the IAP was increased to 100 µL (fabrication condition/particle type-6) keeping all other parameters same as that of particle type-5. The microparticles (type-6) resulted in very stable particles having a smooth surface morphology as analyzed by scanning electron microscopy (SEM) (Figure 17A). The particles were very homogenous with a volume averaged size D_v (0.5) of 5.5 µm and a distribution of 2-8 µm as analyzed by mastersizer (Figure 17B). This condition was chosen for the formulation of all other microparticle types.

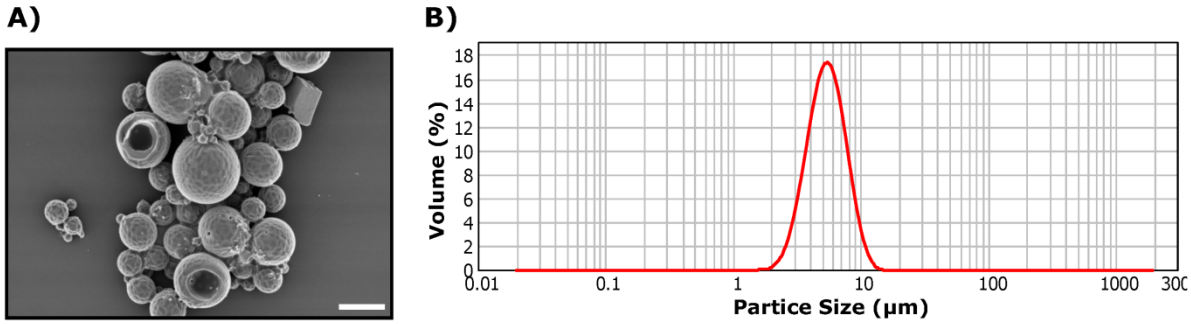

Figure 17. Characterization of PLA microparticles. (**A)** Scanning electron micrograph of the microparticle (type-6) produced using the fabrication condition-6. (**B)** Size analysis of the microparticles (type-6) produced using the fabrication condition-6. Scale bar represents 5 µm.

3.2.2.2 Fabrication of PLA Nanoparticles

The fabrication of nanoparticles smaller than 500 nm was achieved using the fabrication parameters detailed in Table 5. The polymer of choice was Poly-D/L-lactide with inherent viscosity of 0.49 dL/g. The nanoparticle fabrication condition-1 was the same as that used for the fabrication of microparticle (fabrication condition-5, Table 4) except for the power input that was 21K rpm for 10 min. The size and polydispersity of nanoparticle type-1 was analyzed by DLS batch measurement technique. The particles showed a size of > 1000 nm with a high polydispersity index (PDI) of 0.7 as compared to a monodisperse system that should have a PDI value close to 0 and intercept close to 1. Therefore, in subsequent fabrication conditions (2-5), sonication was chosen for the preparation of the secondary emulsion along with varying the other parameters such as power input (Watt), time, and the concentration of the emulsifier/ stabilizer (polyvinyl alcohol-PVA) as in Table 5. Using conditions 2-5, nanoparticles with a size average of below 500 nm with narrow distribution were not obtained. The PVA used in the EAP plays a critical role in the formation and stabilization of the secondary emulsion (W1/O: W2) influencing the particle size. The use of

Polymer	Primary Emulsion				Secondary Emulsion		Power	Z-Average	PDI	Intercept
	Fabrication Condition	IAP (W1)	OP (O)	IAP:OP (W1:O)	EAP (W2)	W1:O:W2				
Poly –D/L-Lactide (PDL) Inherent viscosity 0.49dL/ g	1	50 µL	2 mL (100 mg)	1: 40	8 mL (2 %)	1:4	21 K RPM	1150 nm	0.727	0.318
	2	50 µL	2 mL (100 mg)	1: 40	8 mL (1%)	1:4	40 W	729 nm	0.596	0.771
	3	50 µL	2 mL (100 mg)	1: 40	8 mL (1 %)	1:4	50 W	473 nm	0.302	0.778
	4	50 µL	2 mL (50 mg)	1: 40	8 mL (2 %)	1:4	50 W	400 nm	0.49	0.804
	5	50 µL	2 mL (100 mg)	1: 40	16 mL (2 %)	1:8	50 W	533 nm	0.40	0.870
	6	50 µL	2 mL (100 mg)	1: 40	8 mL (2 %) Low M.Wt 13-23 KD, 83-87 % hydrolysis	1:4	50 W	377,5 nm	0.195	0.844
	7	100 µL	2 mL (100 mg)	1: 20	8 mL (2 %) Low M.Wt	1:4	50 W	377,7 nm	0.133	0.848

Table 5. Process parameters used for the fabrication of PLA nanoparticles. The various parameters used in fabricating nanoparticle (type-6) highlighted in red. IAP= Internal Aqueous Phase, OP= Organic Phase and EAP= External Aqueous Phase.

low molecular weight PVA resulted in lower particle size compared to the high molecular weight compound [210]. Fabrication condition 6 was tried using PVA of molecular weight (18-23 kDa) as reported in Table 5. The resulting particles had an average size of 377 nm with a PDI value of 0.195 as analyzed by DLS. In order to incorporate a higher concentration of hydrophilic components (antigens), fabrication condition-7 (particle type-7) was used where the volume of IAP was increased to 100 µL keeping all other parameters the same as that of fabrication condition-6. The nanoparticle (type-7) had a smooth surface morphology as analyzed by transmission electron microscopy (TEM) (Figure 18A) with an average size of 377 nm with PDI and intercept values of 0.13 and 0.8 indicating very narrow distribution (Figure 18B). This condition was chosen for the formulation of all other nanoparticle types.

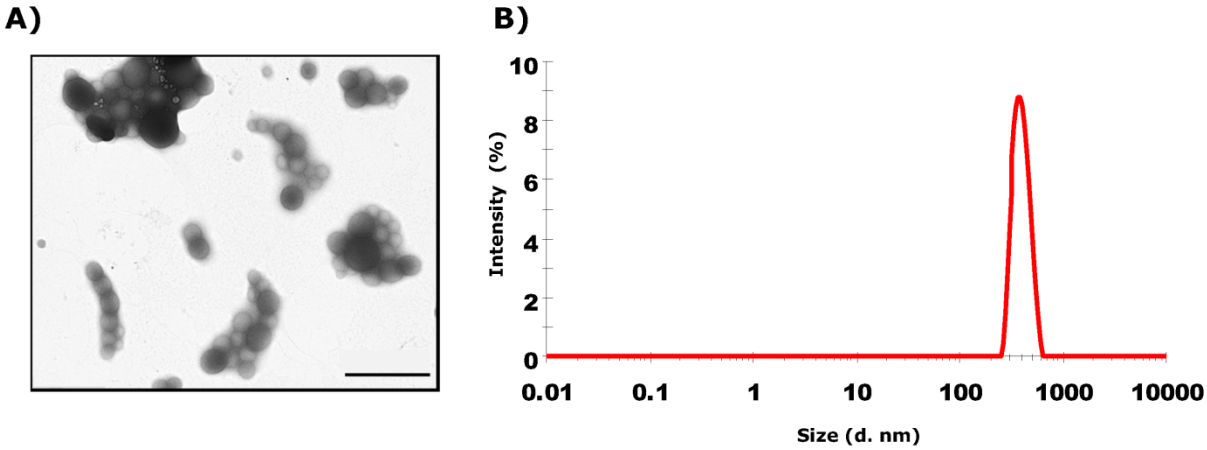

Figure 18. Characterization of PLA nanoparticles. (**A)** Electron micrographs of PLA nanoparticles (type-7) as observed by transmission electron microscopy (TEM). (**B**) Nanoparticle (type-7) size and polydispersity measured by dynamic light scattering (DLS). Scale bar represents 1000 nm.

3.2.3 Fabrication of PLA Nano- and Microparticles Encapsulating Capsular Polysaccharide-3 (CPS-3) Antigen

The presentation of the soluble TI polysaccharide antigens on PLA polymeric particle has been shown to greatly enhance the immune response by producing protective polysaccharide specific IgG antibodies [209, 211]. Both nano and microparticle formulations enhanced the IgG response with nanoparticle formulations producing high titer antibodies providing protective immune response irrespective of the antigen used [209, 211]. It was planned to encapsulate the size-reduced CPS-3 antigen on PLA particles in the size range of <500 nm (nano) and 2-8 µm (micrometer) to provide not only information on the particulate presentation of CPS-3 but also the size effect. The process parameters established for the fabrication of nano- and microparticles were directly used for the microencapsulation of the CPS-3 antigen by emulsifying 500 µg of the size-reduced polysaccharide. Besides the particles encapsulating CPS-3, corresponding control particles (dummy) without the antigens were also prepared (Figure 21).

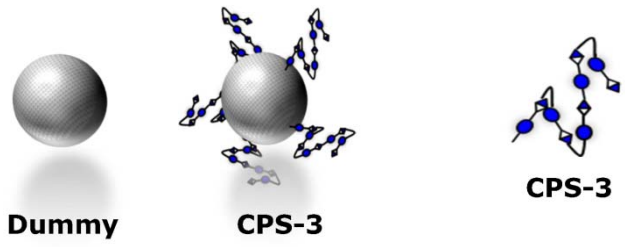

Figure 19. Schematic representation of polysaccharide encapsulated PLA nano- and microparticles.

The encapsulation and specific surface localization of the CPS-3 antigen on the particles was confirmed by flow cytometry and confocal microscopy analysis. Both the dummy and polysaccharide (CPS-3) encapsulated particles were incubated with the rabbit anti-CPS-3 polyclonal serum followed by detection using a FITC conjugated secondary antibody. The analysis was performed on microparticles considering their size and ease of handling in flow cytometry experiments as compared to the nanoparticle formulations. Compared to the dummy particles (grey histograms) the CPS-3 loaded particles showed a positive shift (green histograms) in flow cytometry experiments indicating the encapsulation of the CPS-3 on the microparticles (Figure 20A). Confocal microscopy analysis (Figure 20B) revealed the specific surface localization of CPS-3 on the polysaccharide encapsulated particles with aggregation (upper panel) as compared to the dummy particles that did not aggregate nor stain with the antisera (lower panel). The surface localization of the CPS-3

antigen is an important feature considering that polysaccharide specific immune responses are primarily mediated by cross-linking of the B cell receptor.

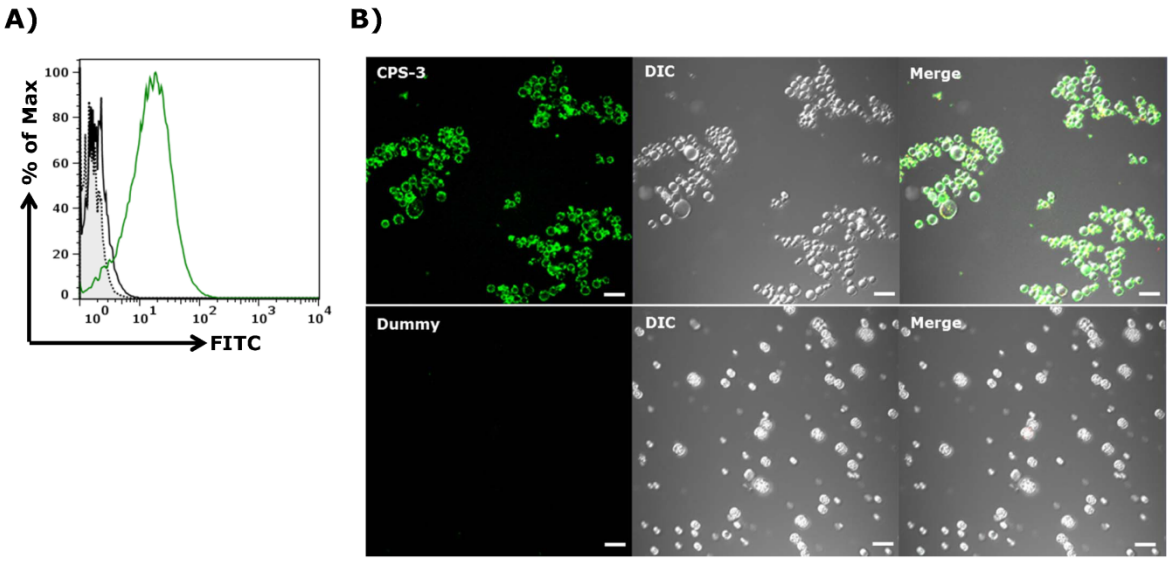

Figure 20. Characterization of polysaccharide (CPS-3) encapsulating PLA microparticles. (**A**) Histogram representation of CPS-3 encapsulation analyzed by flow cytometry using the rabbit polyclonal CPS-3 antisera (1:300) probed with a FITC conjugated secondary detection antibody (1:500) (green histogram). Corresponding controls included the dummy particles with no CPS-3 encapsulation stained with primary and secondary antibodies (black histogram). The unlabeled dummy beads are shown as tinted histograms. (**B**) Confocal laser scanning micrograph where the specific surface localization of CPS-3on microparticle was analyzed using rabbit polyclonal CPS-3 antisera probed with a FITC conjugated secondary detection antibody (upper panel). Corresponding controls included the dummy particles with no CPS-3 encapsulation but stained with primary and secondary antibodies (lower panel) showing the surface localization of CPS-3 on the microparticles. The green color indicates CPS-3 on the PLA microparticles with DIC indicating the phase contrast image of the particle morphology. Scale bar represents 10 µm.

3.2.3.1 Immunization Studies to Evaluate Polysaccharide Specific Immune Response of Mice Immunized with CPS-3 Encapsulated PLA Nano- and Microparticles

The ability of the CPS-3 encapsulated nano- and microparticles to elicit a polysaccharide specific IgG response was evaluated by immunizing C57BL/6 mice (n=6) subcutaneously. Different soluble and particulate formulations were prepared along with PBS as negative control and the commercial vaccine Prevnar® as a positive control (Table 6). The polysaccharide content and percent encapsulation of the nano- and microparticle formulations

	Formulation	Antigen	Size	Adjuvant	CPS-3 Dose	CPS-3 Load	%EE CPS-3
1	PBS	-	-	-	-	-	-
2	Soluble CPS-3	CPS-3	-	-	2 µg/ 100 µL/ mouse	-	-
3	CPS-3 Nanoparticles	CPS-3	300-500 nm	-	2 µg/ 100 µL/ mouse	1.2 µg/mg particles	24
4	CPS-3 Microparticles	CPS-3	2-8 µm	-	2 µg/ 100 µL/ mouse	1.0 µg/mg particles	20
5	Prevnar 13®	CPS- (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14,18C, 19A, 19F, 23F)	-	Alum ($AlPO_4$)	0,4 µg/ 100 µL/ mouse	-	-

Table 6. Details of the various soluble and particulate formulations of CPS-3 used for immunization.

were 1.2 µg and 1 µg/mg per mg of particles with a encapsulation efficiency (%EE) of 24% and 20% respectively. CPS-3 estimation was performed by quantitative ELISA technique using a standard curve (CPS-3) after lysing and extracting the polysaccharide from the particles. Attempts were also made to quantify the extracted polysaccharides by acid hydrolysis and analyzing the monosaccharides glucose and glucuronic acid content by high pressure anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). However, incomplete hydrolysis of CPS-3 did not yield the individual monosaccharide constituents glucose and glucuronic acid due to the inefficiency of acid hydrolysis procedure to cleave β (1→4) linkages. All formulations were normalized to an

injection volume of 100 µL per mouse containing a polysaccharide concentration of 2 µg except the Prevnar® group that received 400 ng of CPS-3.

The mice were immunized following a prime-boost regime (Figure 21A) and sera were collected on day 21 and analyzed for polysaccharide specific IgG antibodies by ELISA using 1 µg CPS-3 polysaccharide as coating antigen. The IgG titers were represented by plotting the absorbance values at 450 nm of the different experimental groups after subtracting their corresponding pre-immune (day zero) titers. The group immunized with soluble CPS-3 antigen (blue square) failed to elicit specific IgG response at day 21 with the mean serum titers comparable to the control PBS group (black circles) Figure 21B. However, the nano- and microparticle formulations of CPS-3 (orange circles and blue filled squares) elicited similar IgG titers indicating no major difference between different sized formulations.

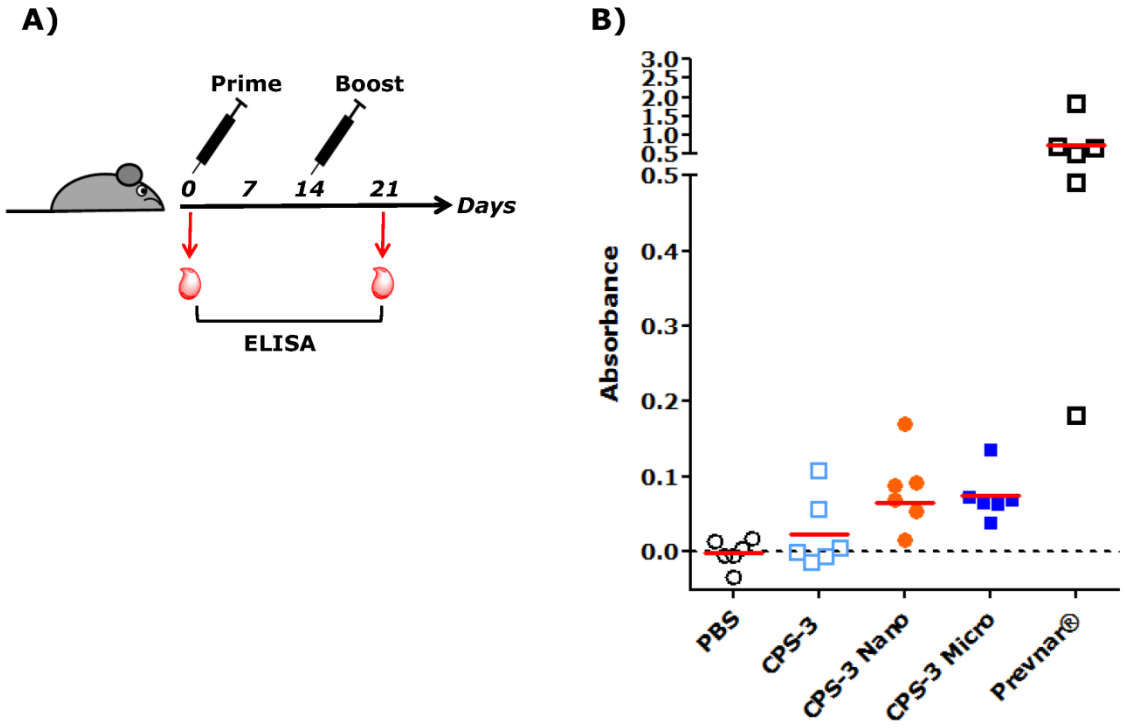

Figure 21. Evaluation of serum antibodies from mice immunized with CPS-3 encapsulated PLA nano and microparticles. (**A**) C57BL/6 mice were primed at day zero followed by a boost-immunization at day fourteen with the formulations listed in table 3.1. Sera 50 µL was collected both prior and after immunization and analyzed by ELISA. (**B**) ELISA titers of sera collected on day twenty one expressed as OD 450 absorbance values after subtracting from the pre-immune values of corresponding experimental groups. Sera were diluted 200 X with 1% BSA-PBS and pre-incubate with 10 µg/mL cell-wall polysaccharide. 100 µL of the diluted pre-incubated sera was added per well of the micro titer plate which was coated with 1 µg of CPS-3 polysaccharide. Following washings, the plates were incubated with a HRP conjugated mouse secondary antibody diluted 1:10000 and developed using the chromogenic substrate TMB. Absorbance was measured at 450 nm and the data were plotted using the graphpad prism software with the red horizontal lines representing the mean OD values of the experimental groups.

However, the IgG titers from the particulate formulations were marginally higher than the soluble CPS-3 formulation indicating a subtle effect of the particulate presentation of the antigen (CPS-3 polysaccharide). However, the commercial vaccine (black squares) produced IgG titers that were substantially higher than the particulate formulations. It is evident from the above experiments that apart from the particulate presentation of the antigen, the role of T cell activation and help is equally important in eliciting a robust IgG response against a TI polysaccharide antigen. This T cell help in the commercial vaccine is primarily mediated by the carrier protein CRM_{197}. Although attempts have been made to co-encapsulate carrier proteins having T cell epitopes with TI antigens on the PLA particles, the results have not been promising. This problem is partly due to the denaturation of the protein during the fabrication process. To overcome this challenge, it was envisaged to enhance the polysaccharide specific IgG response in a T cell dependent manner by inducing the specific activation of iNKT cells. Therefore, the PLA particles were encapsulated with both the CPS-3 antigen and the glycolipid ligand GSL (α-GalCer).

3.2.4 Fabrication of PLA Nano- and Microparticles Co-Encapsulating Capsular Polysaccharide-3 (CPS-3) and Glycosphingolipid (GSL) Antigen

The PLA particles encapsulating GSL and CPS-3 or co-encapsulating both antigens (Dummy, CPS-3, GSL and CPS3-GSL) were fabricated in the nano and micrometer range as shown in Figure 22.

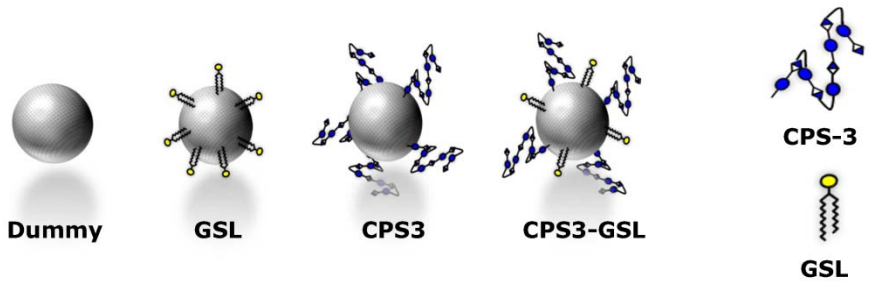

Figure 22. Schematic representation of different combinations of GSL and CPS-3 particles. Dummy [GSL(-)/CPS-3(-)], GSL [GSL(+)/CPS-3(-)], CPS-3 [GSL(-)/CPS-3(+)] and CPS3-GSL [GSL(+)/CPS-3(+)]. Particles were prepared both in the nanometer (<500 nm) and micrometer range (2-8 μm).

The encapsulation and specific surface localization of GSL and CPS-3 antigen on the mono- or co-encapsulated particles was confirmed by flow cytometry and confocal microscopy analysis. Both the dummy and GSL encapsulated particles were incubated with the FITC

conjugated galactose binding lectin (RCA-120) or the galactocerebrocide binding antibody (mGalC) followed by detection using a FITC conjugated secondary antibody. For the analysis

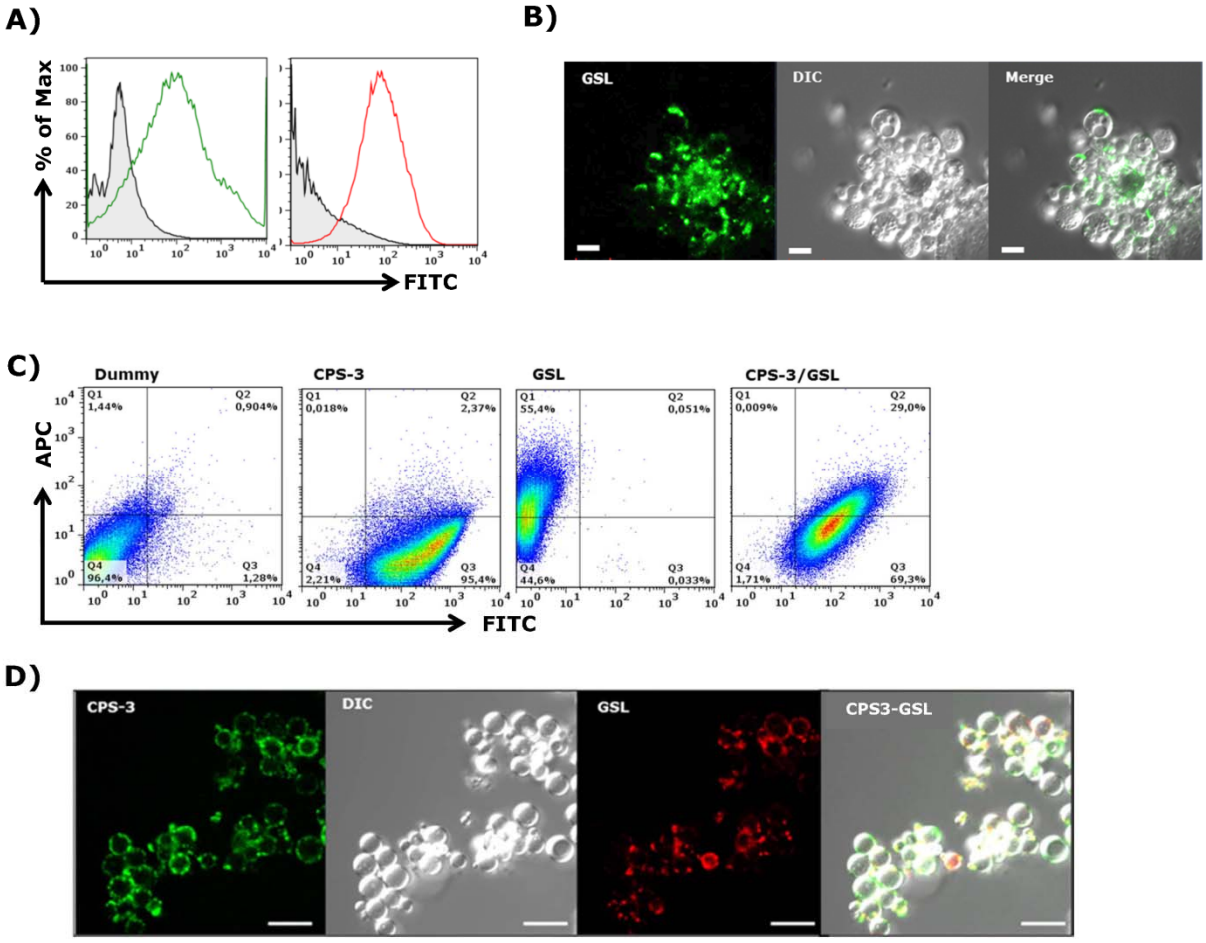

Figure 23. Characterization of CPS-3 and GSL co-encapsulating PLA microparticles. (**A**) Histogram representation of GSL encapsulation analyzed by flow cytometry using the FITC labelled lectin RCA-120 (1:500) (left panel, green histogram) and the mouse monoclonal galactocerebrocide antibody (mGalC) (1:500) probed with a FITC conjugated secondary detection antibody (1:500) (right panel, red histogram). Corresponding controls included dummy particles with no GSL encapsulation but stained with the lectin RCA-120 and antibody mGalC (tinted histogram). (**B**) Specific surface localization of GSL on microparticles analyzed by confocal laser scanning microscopy using the mouse monoclonal galactocerebrocide antibody (mGalC) probed with a FITC conjugated secondary detection antibody. Scale bars represent 10 µm. (**C**) Quadrant representation of microparticles dummy, CPS-3, GSL and CPS3-GSL, simultaneously analyzed by flow cytometry using the rabbit polyclonal CPS-3 antisera (1:300) and the mouse monoclonal antibody mGalC**,** probed with the corresponding FITC and PE conjugated secondary detection antibody (1:500). (**D**) Specific surface co-localization of CPS-3 and GSL on microparticles co-encapsulating both the antigens analyzed by confocal laser scanning microscopy using rabbit polyclonal CPS-3 antisera (1:300) and the mouse monoclonal antibody mGalC**,** probed with the corresponding FITC and PE conjugated secondary detection antibody (1:500). The green color indicates CPS-3 and the red color indicated GSL on the PLA microparticles with DIC indicating the phase contrast image of the particle morphology. Scale bars represent 10 µm.

of polysaccharide (CPS-3) and GSL co-encapsulated formulations, the particles were incubated with the polyclonal CPS-3 specific anti-serum from rabbit or the galactocerebrocide binding antibody from mice (mGalC) followed by detection using a FITC conjugated and PE conjugated secondary antibodies respectively. The analysis was performed on microparticles considering their size and ease of handling in flow cytometry experiments when compared to the nanoparticle formulations. Compared to the dummy particles (grey histograms) the GSL loaded particles showed a positive shift both when probed with the lectin RCA-120 (green histograms) and also with the galactocerebroside antibody (red histogram) indicating the encapsulation of the GSL on the microparticles (Figure 23A). Confocal microscopy analysis (Figure 23B) revealed the specific surface localization of GSL on the particles with visible aggregation. The co-encapsulation of CPS-3 and GSL was confirmed by flow cytometry by comparing to the dummy or mono encapsulated particles of CPS-3 and GSL (Figure 23C). Confocal microscopy analysis (Figure 23D) further confirmed the specific surface localization of both CPS-3 (green) and GSL (red) on the same PLA particles.

3.2.4.1 Quantification and Encapsulation Efficiency of CPS-3 and GSL

The quantification of GSL and CPS-3 content of the vaccine formulations is important to define the antigen dose. Although the total carbohydrate content can be estimated by various methods, the presence of contaminants or any other carbohydrate containing excipients such as the lyoprotectant (sucrose) used in the formulation process may lead to erroneous values. In order to estimate both the CPS-3 and GSL antigens seperately, particles were lysed with NaOH and the lysate subjected to phase partitioning between the organic (chloroform in methanol 1:1) and water phases to obtain the polysaccharide (CPS-3) in the (upper) aqueous phase and the GSL in the (lower) organic phase. The extraction efficiency was monitored by analyzing the CPS-3 in the aqueous phase by ELISA and the GSL containing organic phase by MALDI. Analysis of the aqueous phase by ELISA showed the presence of CPS-3 only in the mono and double encapsulated formulations (CPS-3 and CPS3-GSL) with comparable absorbance values indicating identical levels of encapsulation and extraction of CPS-3 in these particle types (Figure 24A). The antigen load and percent encapsulation efficiency of CPS-3 in the co-encapsulated formulations, as estimated by quantitative ELISA was 1 µg and 20% per mg of PLA nano and microparticle formulations respectively.

The organic phase of the particle lysate was subjected to MALDI analysis for determination of the GSL content. The organic phase of the GSL encapsulated particle lysate showed a characteristic peak at *m/z* 880 Da (Figure 24B, bottom panel) corresponding to molar mass of GSL standard (top panel). The analysis also confirms the stability of GSL and purity of particle preparation as no other contaminating peaks were detected. The dummy particles particles (Figure 24B, middle panel) showed no characteristic peaks in the mass range indicating no interference from the polymer scaffold (PLA) that further confirms the peak at 880 Da to be specifically that of GSL. To quantitate GSL, several techniques of GC-MS and fluorescence associated HPLC have been reported that are prone to sample loss

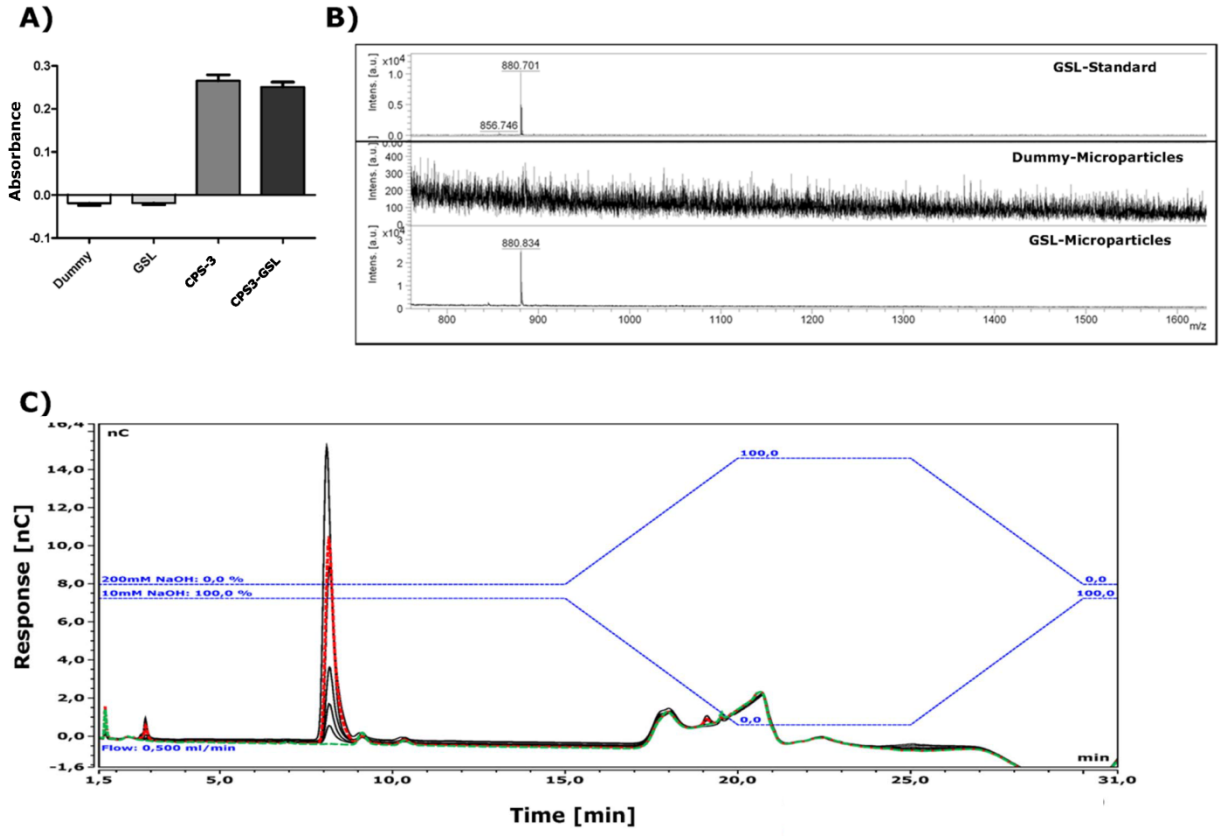

Figure 24. Quantification of CPS-3 and GSL from co-encapsulated particles. (**A**) Analysis of CPS-3 in the aqueous phase of the particle types (Dummy, GSL, CPS-3 and CPS-3-GSL) after lysis and phase partioning using sandwitch ELISA were the mouse-monoclonal antibody CPS-3-5F6 (1mg/ml) diluted to 1:1000 was used as the capture antibody followed by incubation with the rabbit polyclonal antisera. Detection was done using HRP conjugated secondary antibody and developed using the chromogenic substrate TMB. Data is represented as absorbance values at 450 nm plotted using the Graphpad prism software. (**B**) Analysis of GSL in the lower organic phase of the particle types dummy (middle panel) and GSL (lower panel) after lysis and phase portioning using MALDI analysis. The soluble GSL (top panel) served as a positive control. (**C**) Acid hydrolysis and analysis of GSL (Galactose content) in the lower organic phase of the particle types CPS-3(green) and GSL (red) compared with a Galactose standard (black) by HPAEC.

during the processing. Hence, the more reliable technique of monosaccharide analysis using High Pressure Anion Exchange Chromatography (HPAEC) with a pulsed amperometric detection using a gold electrode was used. The organic phase obtained from the various particle types, were dried and subjected to acid hydrolysis using 2 M trifluoroacetic acid (TFA) to cleave the galactose sugar from the ceramide lipid part of GSL. The TFA released galactose (red chromatogram) was estimated by comparing to a standard curve of galactose processed in a similar manner (Figure 24C, black chromatogram). It is also evident that the organic phase of the CPS-3 encapsulated particles (green chromatogram) stays at baseline and does not show any peaks indicative of galactose or any other monosaccharides. This observation further indicates the purity of the preparation and the efficiency of extraction of CPS-3 from the organic phase with respect to the CPS-3 encapsulated particles. The concentration of GSL in the mono encapsulated formulation was 0.9 and 0.64 μg /mg of PLA nano- and microparticles with a encapsulation efficiency of 30% and 21% respectively. In the CPS3-GSL co-encapsulated formulation, the concentration of GSL was 0.9 and 0.94 μg/mg of PLA nano- and microparticles with a encapsulation efficiency of 30% and 31% respectively.

3.2.4.2 Splenocyte Activation by Soluble and Particulate GSL

The biological activity of GSL (α-GalCer) is characterized by the specific activation of iNKT cells through the cognate recognition and binding of the glycolipid ligand presented on a CD1d molecule of an APC through the invariant T cell receptor (iTCR).The glycolipid can be presented on a CD1d molecule of an APC by the uptake and processing of a particulate antigen or by direct loading in the case of soluble antigens (GSL). It has been shown that GSL when presented in a particulate manner enhances the iNKT activation potential many fold as compared to its activity in the soluble form [212, 213]. The activation of iNKT cells by GSL when presented in a soluble and particulate form in different size ranges (nano and microparticles) was investigated. Since PLA particles are negatively charged, it was first decided to check the uptake of the nano- and micro particles by the macrophage cell line (RAW 264.7). The dye 6-coumarin was used for the fabrication of fluorescent PLA nano- (Figure 25A, left panel) and microparticles (Figure 25A, right panel). The fluorescent nano and microparticles (50 μg/mL) were incubated with the macrophage cell line RAW 264.7 cells for 12h, washed and imaged using confocal microscope. The particles were non-refractory to the cell and there was uptake of both the nano- (Figure 25B, top panel) and microparticles (Figure 25B, bottom panel). Although the cells were incubated with equal

amounts of particles by weight, the nanoparticles showed higher uptake as compared to microparticles due to the higher size to volume ratio of the nanoparticles compared to the microparticles.

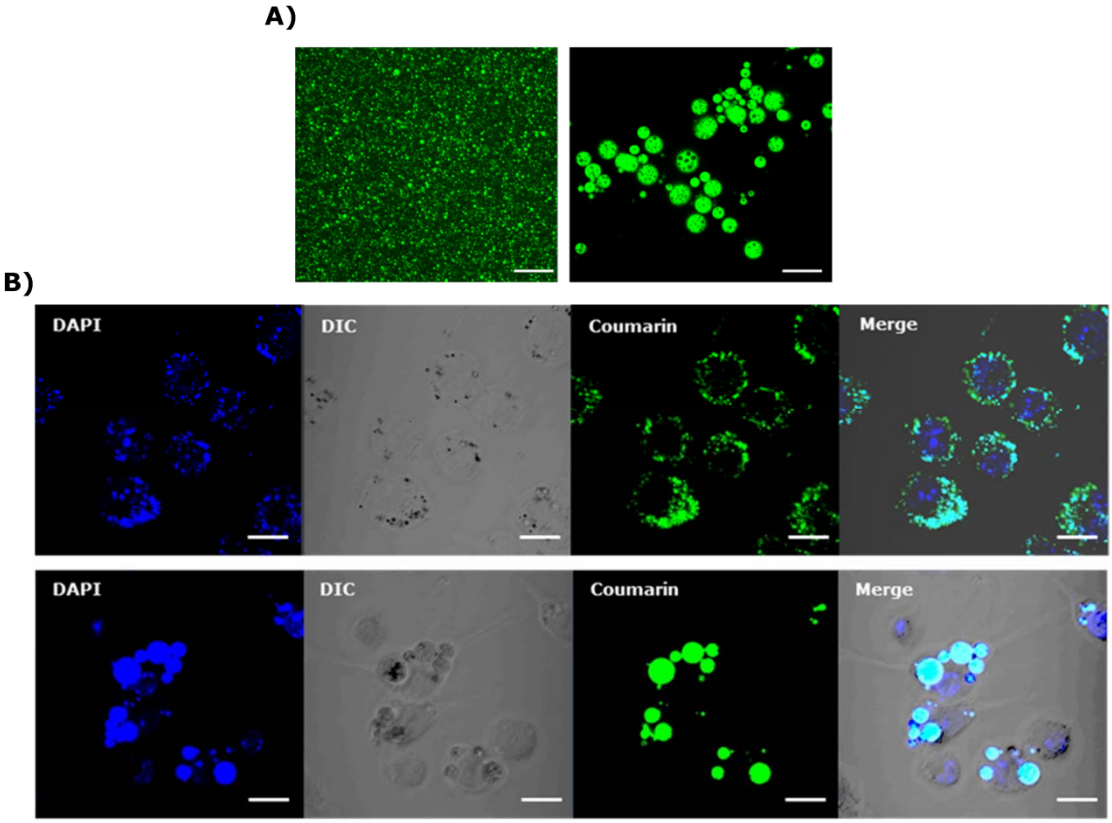

Figure 25. Uptake analyses of 6-coumarin loaded PLA nano and microparticles. (**A)** 6-coumarin loaded fluorescent PLA nano (left panel) and microparticles (right panel) analyzed by confocal laser scanning microscopy. Scale bars represent 10 µm. (**B)** Uptake analysis of 6-coumarin loaded PLA nano (top panel) and microparticles (bottom panel) 50 µg/mL incubated with RAW 264.7 macrophage ($1x10^5$ cells) for 12h and analyzed by confocal laser scanning microscopy. Blue color indicates the cell nucleus stained with DAPI (cross talk of 6-coumarin in the DAPI channel is visible) with the DIC indicating the phase contrast image to view the external cell morphology and green color indicating the fluorescent PLA nano and microparticles. The merged image clearly differentiates the cell nucleus from the fluorescent PLA particles. Scale bars represent 10 µm.

Next, a splenocyte assay, one of the most commonly used ex vivo assay to test the iNKT activation by glycolipids (GSL) was performed. Splenocyte cell suspension prepared from C57Bl/6 mice was incubated with soluble and particulate GSL for 48h and the supernatant was analyzed by ELISA. To analyze the uptake and presentation of the GSL by an APC, the soluble and particulate formulations were first incubated for 6h with BM-DC isolated from C57Bl/6 mice followed by co-culture with splenocyte cell suspension. Compared to the mock treated cells, the GSL containing samples showed better activation in

terms of IL-2 release shown as increase in absorbance values at 450 nm (Figure 26A and B). In samples incubated with the dummy particles having no GSL encapsulation, there was no IL-2 release or the levels were comparable to that of the mock treated cells indicating the specific activation of iNKT cells by GSL and not due to other contaminants in the sample. The low background activity of the Dummy particles further show that PLA by themselves are quite inert and do not induce non-specific activation of iNKT cells.

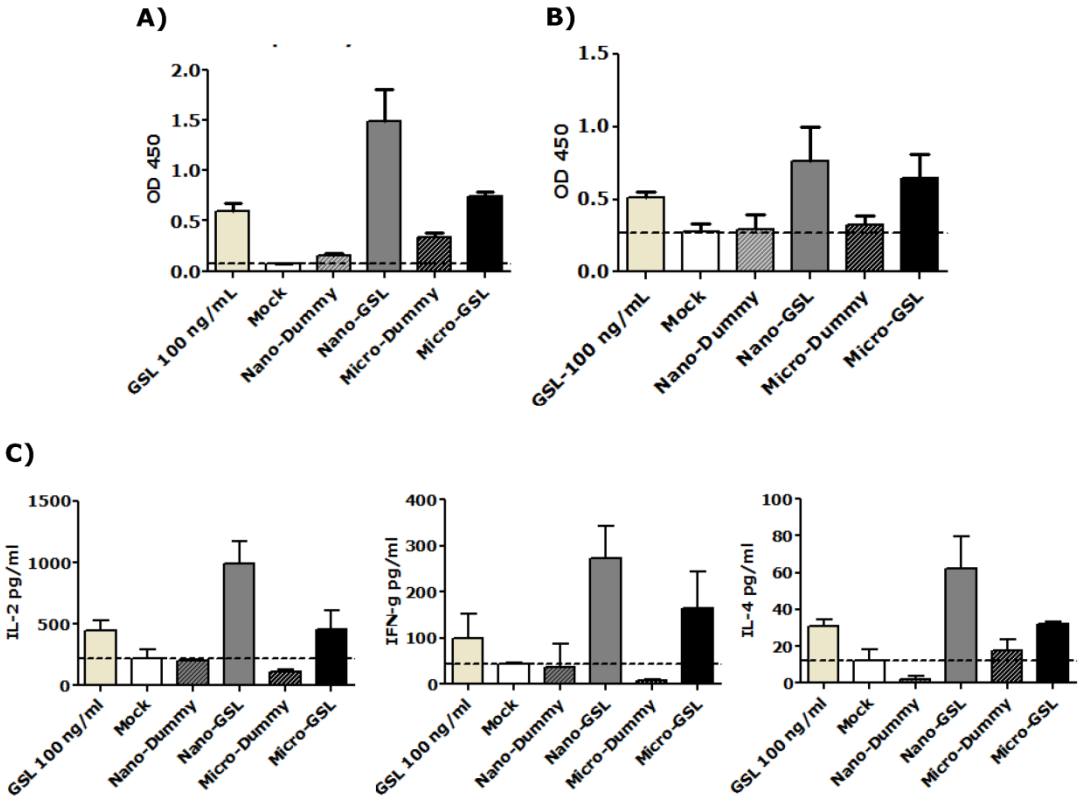

Figure 26. Splenocyte activation assay. (**A**) Splenocyte single cell suspension ($5x10^5$ cells) was incubated with soluble and particulate GSL formulations for 48h and supernatants were analyzed for cytokine IL-2 represented as OD 450 absorbance. (**B**) BM-Dendritic cells from C57BL6/J mice ($1x10^5$ cells) was incubated with the soluble and particulate GSL formulations for 6h, washed with PBS and co-cultured with $5x10^5$ splenocytes for 24h and supernatants were analyzed for cytokine IL-2 represented as OD 450 absorbance. (**C**) Splenocyte single cell suspension ($5x10^5$ cells) incubated with soluble and particulate GSL formulations for 48h and the amount of cytokines IL-2, IL-4 and IFN-γ in the supernatant was quantified by ELISA and the values are represented as pg/mL of individual cytokines. Soluble GSL 100 ng/mL (Yellow bars), GSL loaded nano (greybars) and microparticles (black bars) 50-60 µg/mL normalized to GSL content of 50 ng/mL. Dummy nano (grey bar horizontal lines) and microparticles (black bar horizontal lines) 50-60 µg/mL. Mock PBS (white bar) corresponding to volume of soluble GSL.

GSL (α-GalCer) is a potent iNKT cell ligand with a standalone activity as compared to other glycolipid antigens, characterized by the production of both T_H1/T_H2 T cell cytokines IFN-γ and IL-4 along with the production of specific T cell cytokine IL-2. In order to evaluate

and estimate the T_H1/T_H2 responses, the soluble and particulate nano- and microparticle GSL formulations were incubated for 48h with splenocyte cell suspension prepared from C57Bl/6 mice and the cytokines (IFN-γ, IL-4 and IL-2) in the supernatant was analyzed by ELISA. Both the soluble (Figure 26C, panel 1) (yellow bar) and particulate (grey and black bar) GSL formulations produced the cytokine IL-2 higher than the mock treated samples (clear bar) indicating specific T cell activation. The same was true in the case of the T_H1/T_H2 cytokines with both the soluble and particulate GSL formulations producing more IFN-γ than IL-4 (Figure 26C, panel 2 & 3). In all cases, the response of the dummy particle formulations without any GSL encapsulation was comparable to that of the mock indicating the inert nature of the particles further demonstrating the specific activation of iNKT cells by GSL antigen. In all cases, the nanoparticle GSL formulations demonstrated a higher activation potential with enhanced secretion of the cytokines IL-2, IFN-γ, and IL-4 as compared to the microparticles. The higher activation potential of the nanoparticle formulations could be due to the higher surface loading of the nanoparticles with GSL and the superior uptake (Figure 25B). It is worth to note that the nanoparticle formulations with only half the amount of GSL encapsulated (50 μg/mL) produced twice the amount of cytokines compared to the soluble GSL formulations. Hence, the above experiments indicate that the microencapsulation of GSL into PLA particles can be used as particulate T cell adjuvants to activate iNKT cells which provide help to a B cell activated by a TI antigen like polysaccharide.

3.2.4.3 *In vivo* Immunogenicity Evaluation of CPS-3 and GSL Co-encapsulated PLA Nano- and Microparticles

The particulate presentation of the glycolipid (GSL) antigen shows superior T cell adjuvant properties via iNKT cell activation. The incorporation of GSL in the particulate delivery of the TI polysaccharide antigen (CPS-3) would promote the specific IgG (CPS-3) response in a T cell (iNKT) dependent manner as compared to particles immunized with CPS-3 alone. Immunogenicity was evaluated by immunizing C57BL/6 mice (n=6) with the various particulate and soluble formulations of CPS-3 and GSL through the sub-cutaneous route as shown in Table 7 along with PBS as negative control. The CPS3-GSL physical mixture (PM) served as control to evaluate the importance of delivering both the CPS-3 and GSL antigens in a particulate form. Nano- and microparticles where only GSL was encapsulated were used to show the importance of co-encapsulation and presentation of both the antigens (CPS-3 and GSL) on the same particle. In these GSL encapsulated control particle group, the CPS-3

antigen (2 µg) was mixed just prior to immunizing the mice. The adjuvant property of GSL across all the formulations was evaluated by comparing it with the experimental group that was immunized with only soluble CPS-3 antigen. The mice were immunized following a

	Formulation	Size	GSL DOSE	CPS-3 Dose	CPS-3 Load	%EE CPS-3	GSL Load	%EE GSL
1	PBS	-	-	-	-	-	-	-
2	Soluble CPS-3	-	-	2 µg/ 100 µL/ mouse	-	-	-	-
3	CPS3-GSL Physical mixture	-	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse	-	-	-	-
4	GSL Nanoparticles + Soluble CPS-3	300-500 nm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse	-	-	0.9 µg/mg particles	30
5	GSL Microparticles + Soluble CPS-3	2-8 µm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse	-	-	0.64 µg/mg particles	21.3
6	GSL-CPS3 Nanoparticles	300-500 nm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse	1 µg/mg particles	20	0.9 µg/mg particles	30
7	GSL-CPS3 Microparticles	2-8 µm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse	1 µg/mg particles	20	0.94 µg/mg particles	31.3

Table 7. Formulations used for immunization of GSL encapsulation or CPS3-GSL co-encapsulation of PLA nano- and microparticles.

prime-boost regime as shown in Figure 27A and sera were collected on day 21 and analyzed for CPS-3 specific IgG antibodies by ELISA. The IgG titers were represented by plotting the absorbance values at 450 nm of the different experimental groups after subtracting their corresponding day zero pre-immune titers. The experimental group immunized with only soluble CPS-3 antigen (Figure 27B) (blue squares) failed to elicit a polysaccharide specific IgG response as compared to the group immunized with a mixture of CPS-3 and GSL (PM) indicating the importance of iNKT T cell help (orange diamonds). The GSL encapsulated nanoparticle (filled purple squares) and microparticle (filled green inverted triangles) formulations mixed with the soluble CPS-3 antigen also failed to elicit an IgG response. The titers were comparable to that of the soluble CPS-3 and PBS control group possibly due to the dilution of the CPS-3 antigen at the injection site. However, the CPS-3 and GSL co-encapsulated nano- (purple open squares) and microparticles (green inverted open triangles)

showed a positive trend in the increase of IgG and the mean titers were above the control formulations of CPS-3 (blue squares) and GSL only particles. This further highlights the

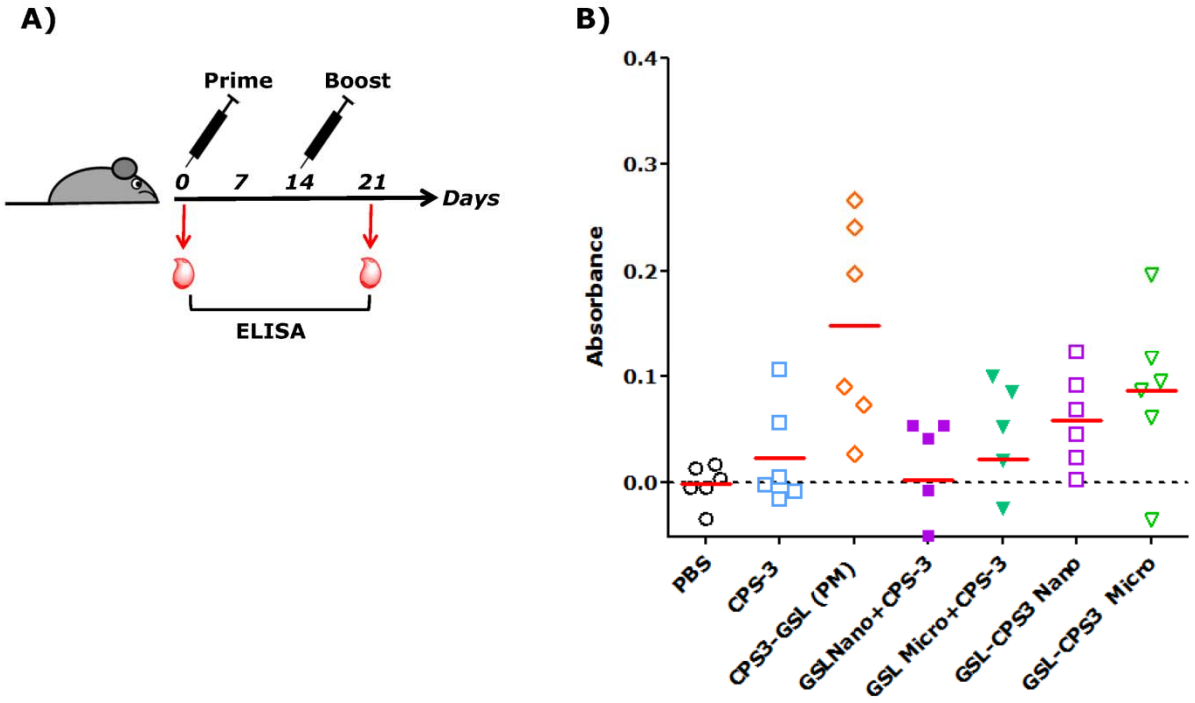

Figure 27. Evaluations of serum antibodies from mice immunized with CPS-3-GSL co-encapsulating PLA nano and microparticles. (**A**) C57BL/6 mice were primed at day zero followed by a boost-immunization at day fourteen with the formulations listed in Table 3.1. Sera (50 µL) were collected both prior and after immunization and analyzed by ELISA. (**B**) ELISA titers of sera collected on day twenty one expressed as OD 450 absorbance values after subtracting from the pre-immune values of corresponding experimental groups. Sera were diluted 200 X with 1% BSA-PBS and pre-incubate with 10 µg/ml cell-wall polysaccharide. The diluted pre-incubated sera (100 µL) was added per well of the microtiter plate that was coated with 1 µg of CPS-3 polysaccharide. Following washings, the plates were incubated with a HRP conjugated mouse secondary antibody diluted 1:10000 and developed using the chromogenic substrate TMB. Absorbance was measured at 450 nm and the data were plotted using the Graphpad prism software with the red horizontal lines representing the mean OD values of the experimental groups.

importance of co-encapsulating both the B cell cross linking (CPS-3) and iNKT stimulating (GSL) antigens on the same polymeric particle and the role of GSL in enhancing the polysaccharide specific IgG response. However, the particulate presentation of the CPS-3 and GSL antigens yielded no drastic increase of antibody titers when compared to the experimental group immunized with the soluble physical mixture of CPS-3 and GSL. The reasons for the lower particulate response might be due to factors such as release kinetics of the particles, route of immunization and the polysaccharide size. The preferred route of immunization for the particulate formulations is by intra muscular (i.m) injection as compared to sub-cutaneous (s.c) injections used in this study. The i.m injection ensures rapid dispersion

and dissemination of the particles to the various immune rich tissues and organs like spleen and lymph node increasing the amplitude of the immune response [214, 215] . Particulate formulations are generally immunized as single point injections and the antigen release is thought to proceed via a rapid burst phase priming the immune system followed by a slow release phase giving a depot effect that shapes the immunological memory. On the other hand, the superior IgG response produced by the physical mixtures might be due to hydrophobic GSL forming micells when mixed with the hydrophilic CPS-3 antigen. The micells of the PM could have provided a particulate effect by concentrating the antigens (CPS-3 and GSL) at the point of delivery leading to enhanced processing and presentation of the GSL leading to superior and faster IgG response. Hence, it was decided to prolong the exposure time of the mice with the particulate and soluble antigen up to 110 days followed by immunization with CPS-3 to determine the a memory recall responses.

3.2.5 Influences of Polysaccharide Size on the Immune Response

3.2.5.1 Preparation of CRM_{197}-CPS3 Protein Conjugate

Apart from the route of immunization and release kinetics of the polymeric particles, polysaccharide size can play an important role in determining the outcome of the immune response. This was evident in Section 3.2.1.1 where the Prevnar® formulation at $1/5^{th}$ the antigen concentration produced superior IgG titers compared to the PM. Apart from differences in the carrier (CRM_{197} vs PM) and adjuvant (alum vs GSL) the size of the CPS-3 polysaccharide may play an important role. The information on the specific size of the polysaccharide used in the commercial vaccine Prevnar® is not available due to patent restrictions. Presumably, shorter polysaccharide repeating units might have been used as compared to the PM formulations that comprise high molecular weight polysaccharide (CPS-3). This becomes an impeding factor when the CPS-3 antigen is normalized by weight for immunization studies. As T independent antigens like carbohydrates mainly function by cross linking the BCR, a high molecular weight polysaccharide (low molarity) encounters and cross links very few BCR as compared to the low molecular weight oligosaccharide (high molarity) of the same antigen that can crosslink large number of BCR leading to an enhanced immune response. Hence, the size reduced polysaccharide (CPS-3) was conjugated to the carrier protein CRM_{197} to investigate the effect of CPS-3 size on the outcome of immune response when using the same carrier protein and adjuvant (CRM_{197} and Alum). The CRM_{197}-CPS3

protein conjugate was also used to compare against the physical mixture group which utilizes the same polysaccharide but different adjuvant systems (Alum and GSL).

The conjugation was performed as described in the methods section (2.2.1.4) and monitored using SDS-PAGE followed by coomassie staining to check for molecular weight increase. There was a significant increase in the molecular weight of the protein (Figure 28, panel A) in lane 3 (conjugate) especially in the 75-250 kDa regions as compared to the CRM standard (lane 2). To corroborate that the molecular weight increase of the proteins was due to conjugation of the polysaccharide, western blot analyses was performed and probed using antibodies against the protein CRM_{197} and against the CPS-3 polysaccharide. Both the standard CRM_{197} (Figure 28, panel B lane 4) and the conjugate (lane 5) stained positive when probed with an anti-CRM_{197} antibody matching the coomassie profile. However, as seen in Figure 28, panel C (lane 7), the CPS-3 antisera only stained the higher molecular weight bands (above 75 kDa) but not the predominant band corresponding to 75 kDa on panel B (indicated by star). The 75 kDa band may have originated from the cleavage of DSAP linker used to conjugate the polysaccharide and protein. This band stained only with antibodies against CRM but not with the CPS-3 antisera. Hence, it was decided to purify the CRM-CPS3

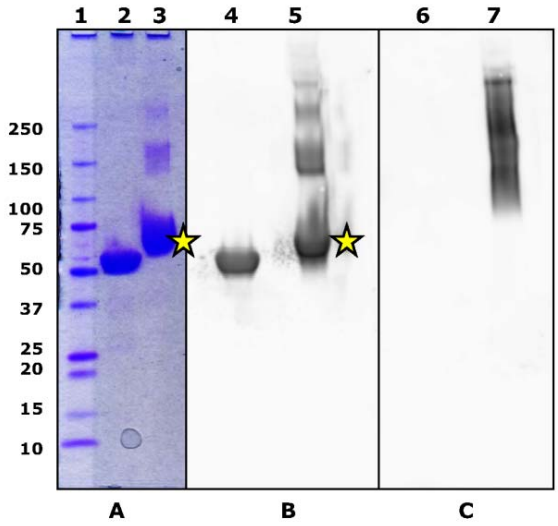

Fig 28. SDS-PAGE and western blot analysis of CRM_{197}-CPS3 conjugates. Panel A. Coomassie stained profile of CRM_{197}-CPS3 conjugate resolved on a 4-15% polyacrylamide gel, **Panel B.** The presence of CRM protein in the CRM_{197}-CPS3 conjugate analyzed by western blot using the anti-DT/CRM polyclonal antibody (1:1000) and probed with a HRP conjugated secondary antibody (1:5000) and **Panel C**. The presence of CPS-3 in the CRM_{197}-CPS3 conjugate analyzed by western blot using the anti-CPS3 polyclonal antibody (1:2500) and probed with a HRP conjugated secondary antibody (1:5000), Where 1. Protein ladder ranging from 250-10 kDa M.Wt, 2, 4, 6. CRM_{197} standard (5 µg) and 3, 5, 7. CRM_{197}-CPS3 conjugates (10 µg).

conjugates by size exclusion chromatography (SEC) in order to enrich the bands at molecular weight 100 kDa and above which stained positive with both anti-CRM_{197} and anti-CPS3 antibodies. The SEC profile of the conjugates revealed at least three species which eluted between 18-30 min (Figure 29A, blue trace) as compared to the CRM protein standard (black trace) that eluted as a very sharp peak at 32 min. The elution profile of the conjugate was similar to the coomassie stained gel (Figure 28, panel A). The peak eluting at 30 min preceding the CRM protein standard (32 min) may be the 75 kDa linker-associated CRM fraction. The elution's from 18-28 min were termed as fraction 1 (Fr-1, red legend) which would primarily contain the CRM- CPS3 conjugate and the remaining elution from 29-31 min was collected as fraction 2 (Fr-2, green legend) comprising the linker-associated CRM fraction. The SEC purified fractions were resolved using PAGE. In the coomassie stained gel (Figure 29B), most of the CRM_{197}-CPS3 species in the 100-250 kDa region eluted in fraction 1 (lane 3) but the linker-associated CRM species were equally distributed both in lane 3 and lane 4 around the 75 kDa region. Therefore, contrary to the earlier assumption, a fairly large

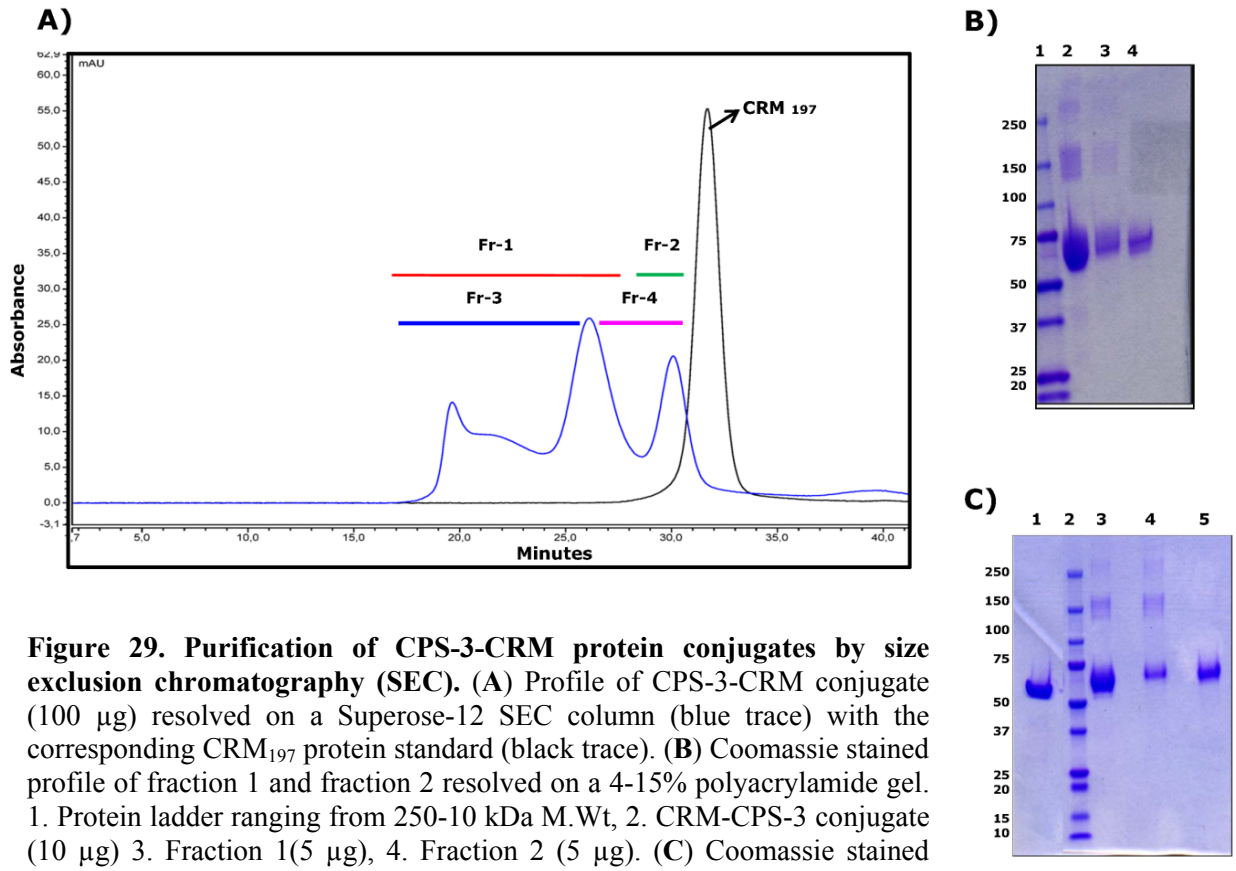

Figure 29. Purification of CPS-3-CRM protein conjugates by size exclusion chromatography (SEC). (**A**) Profile of CPS-3-CRM conjugate (100 µg) resolved on a Superose-12 SEC column (blue trace) with the corresponding CRM_{197} protein standard (black trace). (**B**) Coomassie stained profile of fraction 1 and fraction 2 resolved on a 4-15% polyacrylamide gel. 1. Protein ladder ranging from 250-10 kDa M.Wt, 2. CRM-CPS-3 conjugate (10 µg) 3. Fraction 1(5 µg), 4. Fraction 2 (5 µg). (**C**) Coomassie stained profile of fraction 3 and fraction 4 resolved on a 4-15% polyacrylamide gel. 1. CRM197 standard (5 µg), 2. Protein ladder ranging from 250-10 kDa M.Wt, 3. CRM-CPS-3 conjugate (10 µg) 4. Fraction 3 (5 µg), 5. Fraction 4 (5 µg).

amount of linker-associated CRM $_{197}$ was also present in the second peak eluting between 25-28 min and exclusion of this peak resulted in a major loss of the CRM_{197}-CPS3 conjugate. Hence, in a subsequent injection, the elutions from 18-26 min were collected and termed as fraction 3 (Fr-3, blue legend) while the remaining elutions from 26-31 min formed fraction-4 (Fr-4, pink legend). As seen in the coomassie stained gel Figure 29C, most of the CRM_{197}-CPS3 species in the 100-250 kDa region eluted in fraction 3 (lane 4) and majority of the linker-associated CRM species eluted in fraction 4 (lane 5) around the 75 kDa region. However, the linker-associated CRM species could not be completely separated from fraction 3 (lane 4) but there was substantial improvement in the purification compared to the un-purified conjugate (lane 3). This was the best compromise that could be achieved without a major loss of conjugate. The protein content of the purified CRM_{197}-CPS3 conjugate was determined by BCA analysis and the content of the polysaccharide was determined by quantitative ELISA. The poor separation/ resolution of the conjugates might be due to the random cross-linking of a polysaccharide to the carrier protein resulting in an ill-defined matrix-like structure as compared to conjugation with oligosaccharides of defined sequence length that result in well-defined radial particulate structures.

3.2.5.2 Immunization Studies to Evaluate Polysaccharide (CPS-3) Specific Immune Responses from Mice Immunized with Glycosphingolipid (GSL) and Alum Adjuvant

The ability of the size reduced CPS-3 polysaccharide antigen and its CRM conjugate to elicit a polysaccharide specific IgG response was evaluated by immunizing C57BL/6 mice (n=6) sub-cutaneously. Different formulations were prepared either with or without GSL (for soluble CPS-3) and with or without Alum (for CRM_{197}-CPS3 conjugate) along with a control that included only phosphate buffered saline (PBS) Table 8. The influence of polysaccharide size on the immunogenicity was evaluated by comparing the preperations against the commercial vaccine Prevnar®. All formulations were normalized to an injection volume of 100 µL per mouse. The mice were immunized following a prime-boost regime as

	Formulation	Antigen	Adjuvant	CPS-3 Dose
1	PBS	-	-	-
2	CPS-3	CPS-3	-	2 µg/ 100 µL/ mouse
3	CPS3-GSL Physical Mixture (PM)	CPS-3	GSL (α-GalCer) 1 µg	2 µg/ 100 µL/ mouse
4	CRM-CPS3 Conjugate	CPS-3	-	2 µg/ 100 µL/ mouse
5	CRM-CPS3 Conjugate (Alum)	CPS-3	Alum ($AlPO_4$)	2 µg/ 100 µL/ mouse
6	Prevnar 13®	CPS- (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	Alum ($AlPO_4$)	0,4 µg/ 100 µL/ mouse

Table 8. Details of the various soluble CPS-3 formulations (Alum/GSL) that were used for immunizations.

shown in Figure 30A and sera were collected on day 21 and analyzed for polysaccharide specific IgG antibodies by binding to ELISA plates coated with 1 µg CPS-3 polysaccharide. The IgG titers were represented by plotting the absorbance values at 450 nm of the different groups after subtracting their corresponding pre-immune (day 0) values. The experimental group immunized with only the CPS-3 polysaccharide antigen (Figure 30B,blue square) failed to elicit a CPS-3 specific IgG response on day 21with the mean serum IgG titers comparable

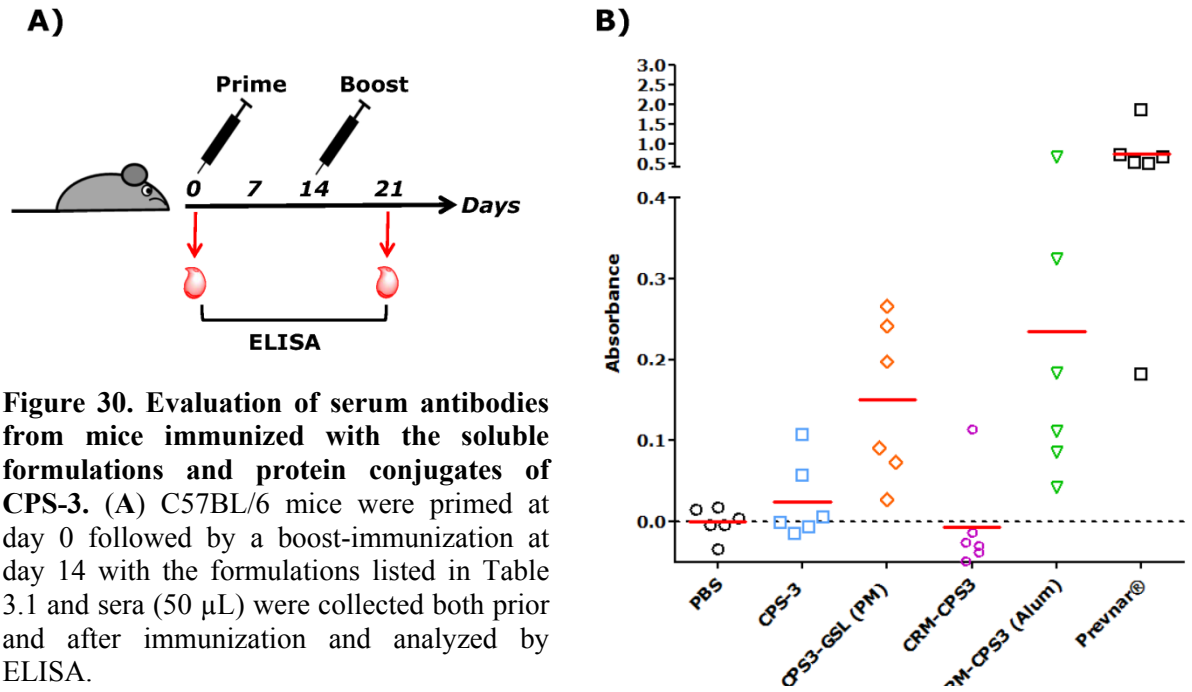

Figure 30. Evaluation of serum antibodies from mice immunized with the soluble formulations and protein conjugates of CPS-3. (**A**) C57BL/6 mice were primed at day 0 followed by a boost-immunization at day 14 with the formulations listed in Table 3.1 and sera (50 µL) were collected both prior and after immunization and analyzed by ELISA.

(**B**) ELISA titers of sera collected on day 21 expressed as OD 450 absorbance values after subtracting from the pre-immune values of corresponding experimental groups. Sera were diluted 200 X with 1% BSA-PBS and pre-incubate with 10 µg/mL cell-wall polysaccharide. The diluted pre-incubated sera (100 µL) was added per well of the microtiter plate which was coated with 1 µg of CPS-3 polysaccharide. Following washings, the plates were incubated with a HRP conjugated mouse secondary antibody diluted to 1:10000 and developed using the chromogenic substrate TMB. Absorbance was measured at 450 nm and the data were plotted using the graphpad prism software with the red horizontal lines representing the mean OD values of the experimental groups.

to the control group immunized with PBS (black circles). This observation clearly demonstrates the TI behavior of the polysaccharide antigen and the inability of the polysaccharide specific B cell to undergo class switching in the absence of T cell help. However, comparable IgG titers were observed when the polysaccharide was injected using T cell adjuvants either as physical mixture (CPS3-GSL, orange diamonds) or CRM protein conjugate with alum as adjuvant (inverted green triangles). However, the antibody titers of CRM_{197}-CPS3 conjugate immunized without the adjuvant alum (purple circles) was similar to that of the PBS and soluble CPS-3 groups. These findings indicate the absolute necessity of an adjuvant and more specifically a T cell adjuvant to produce polysaccharide specific IgG antibodies. The influence of the size on the outcome of the immune response was evident as the commercial vaccine containing oligosaccharide repeating units produced superior IgG titers in comparison to the in-house prepared CRM_{197}-CPS3 conjugate. Hence, the high molecular weight CPS-3 produced comparable IgG titers when immunized either as PM or CRM conjugates irrespective of the adjuvant (alum or GSL) indicating the importance of polysaccharide size when the antigen is normalized by weight.

3.2.6 Mice Immunized with Particulate Formulations and Challenged with CPS-3 Polysaccharide Show Hyporesponsiveness and No Immunological Memory

In order to improve the IgG titers and facilitate the long lasting memory responses, all groups of mice were boosted on day 28 with the formulations listed in Table 9.

	Formulation	Size	GSL DOSE	CPS-3 Dose
1	PBS	-	-	-
2	Soluble CPS-3	-	-	2 µg/ 100 µL/ mouse
3	CPS3-GSL Physical mixture (PM)	-	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse
4	CRM-CPS3 Conjugate	-	-	2 µg/ 100 µL/ mouse
5	CRM-CPS3 Conjugate (Alum)	-	-	2 µg/ 100 µL/ mouse
6	Prevnar® 13	-	-	0,4 µg/ 100 µL/ mouse
7	CPS-3 Nanoparticles	300-500 nm	-	2 µg/ 100 µL/ mouse
8	CPS-3 Microparticles	2-8 µm	-	2 µg/ 100 µL/ mouse
9	GSL Nanoparticles + Soluble CPS-3	300-500 nm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse
10	GSL Microparticles + Soluble CPS-3	2-8 µm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse
11	GSL-CPS3 Nanoparticles	300-500 nm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse
12	GSL-CPS3 Microparticles	2-8 µm	1 µg/ 100 µL/ mouse	2 µg/ 100 µL/ mouse

Table 9. Details of the various soluble and particulate formulations of CPS-3 (Alum/GSL) used for day 28 boost immunization.

The mice were maintained for a total of 82 days to facilitate the uptake and processing of the particulate antigens (Figure 31A). On day 110, the sera were collected and all groups were boosted with 5 µg of the CPS-3 polysaccharide and memory responses were analyzed on day 117. There was no improvement in IgG titers on day 110 (blue square) in mice immunized with the CPS-3 nano and microparticle formulations as compared to day 21 (green circles) [Figure 31B]. However, in the experimental groups immunized with particulate formulations co-encapsulating both GSL and CPS-3, there was a minor increment in the IgG

titers on day 110 (Figure 31C, blue square). This was true only for the nanoparticle formulations but the titers of the microparticle formulations were similar to or lower than that of the day 21 values (green circles). However, the day 117 titers post CPS-3 challenge (orange triangles) across all groups and particulate formulations showed a hyporesponsive trend with the IgG titers falling below the day 21(green circle) levels.

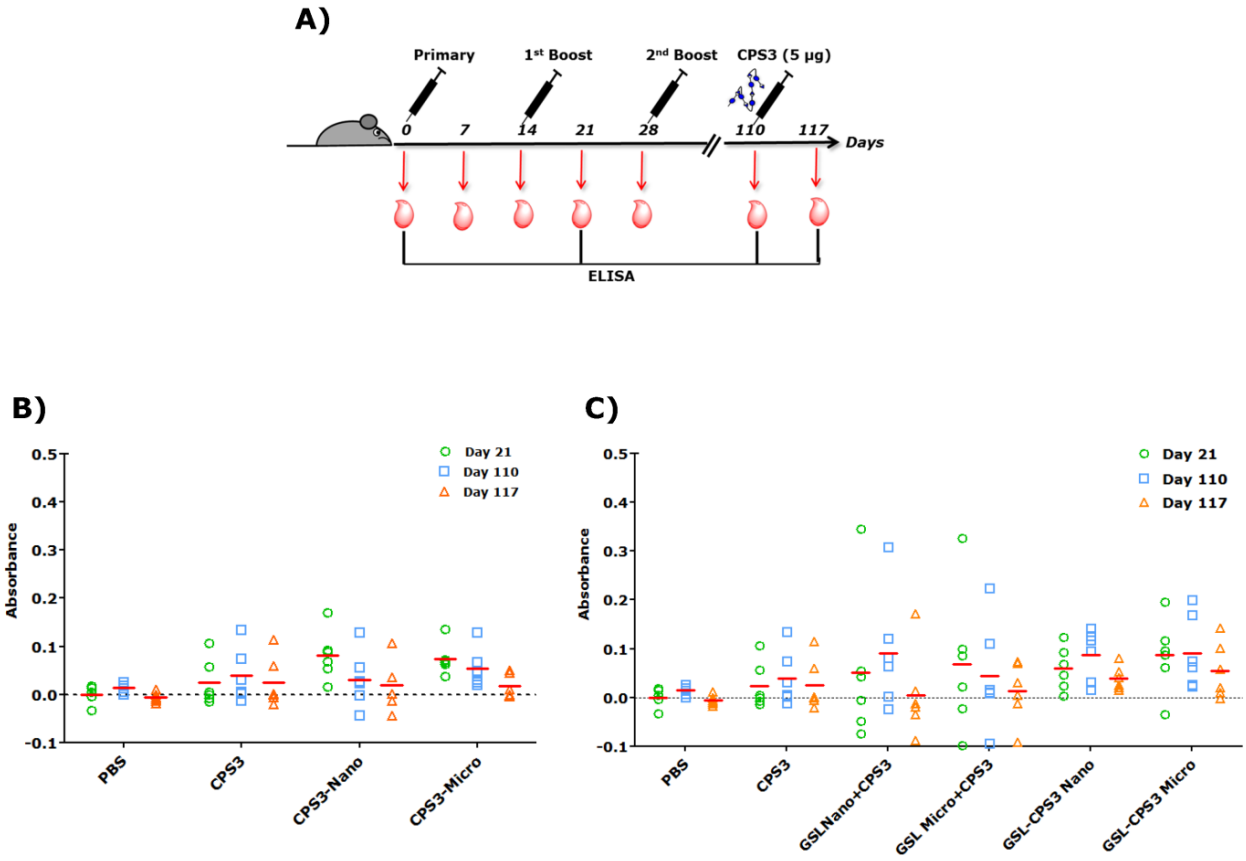

Figure 31. Evaluation of memory recall response of mice immunized with particulate formulations and challenged with CPS-3 polysaccharide. (**A**) C57BL/6 mice boosted on day 28 with particulate formulations of CPS-3 and GSL followed by challenge with 5 µg CPS-3 polysaccharide on day hundred ten followed by and analysis of the boosting response on day hundred seventeen. Sera 50 µL was collected both prior to and after challenge. (**B**) ELISA titers of sera from CPS-3 PLA nano and microparticles immunized groups collected on day (21, 110 and 117). (**C**) ELISA titers of sera from GSL PLA and CPS-3-GSL PLA nano and microparticles immunized groups collected on day (21, 110 and 117). The titers are expressed as OD 450 absorbance values after subtracting from the pre-immune values of corresponding experimental groups. Sera were diluted 200 X with 1% BSA-PBS and pre-incubate with 10 µg/mL cell-wall polysaccharide. Diluted pre-incubated sera (100 µL) was added per well of the microtiter plate which was coated with 1 µg of CPS-3 polysaccharide. Following washings, the plates were incubated with a HRP conjugated mouse secondary antibody diluted to 1:10000 and developed using the chromogenic substrate TMB. Absorbance was measured at 450 nm and the data were plotted using the graphpad prism software with the red horizontal lines representing the mean OD values of the experimental groups.

3.2.6.1 GSL Rescues the Polysaccharide Induced Hyporesponsiveness in Soluble Formulation

Hyporesponsiveness is the inability to mount a booster immune response equal to or higher than the post primary levels. In case of carbohydrate based vaccines, hyporesponsiveness was thought to be mediated by repeated exposure to the polysaccharide antigen leading to T independent immune activation with no replenishment of memory B cell pools. However, hyporesponsivness was also reported in the case of polysaccharide conjugate vaccines that induce immunity in a T cell dependent manner. An important observation was the direct correlation of conjugate vaccine induced hyporesponsiveness against *S.pneumoniae* serotype-3 (CPS-3) in children leading to incidence of acute otis media (AOM) infections. In this thesis, as hyporesponsiveness was observed in the particulate formulations immunized with or without GSL, I investigated if the same was true with the various soluble formulations, most importantly the CRM conjugate of CPS-3 (both in house and commercial vaccine). Hence, the sera collected at different time points [day 0, 21, 110 and 117 (Figure 32A)] were analyzed by ELISA for increase in polysaccharide (CPS-3) specific memory IgG response on boosting with CPS-3 antigen. Mice immunized with the plain CPS-3 polysaccharide (Figure 32B, blue line) mounted no significant IgG response as the levels were comparable to the PBS group (black line). Comparing the adjuvant formulations, the day 21

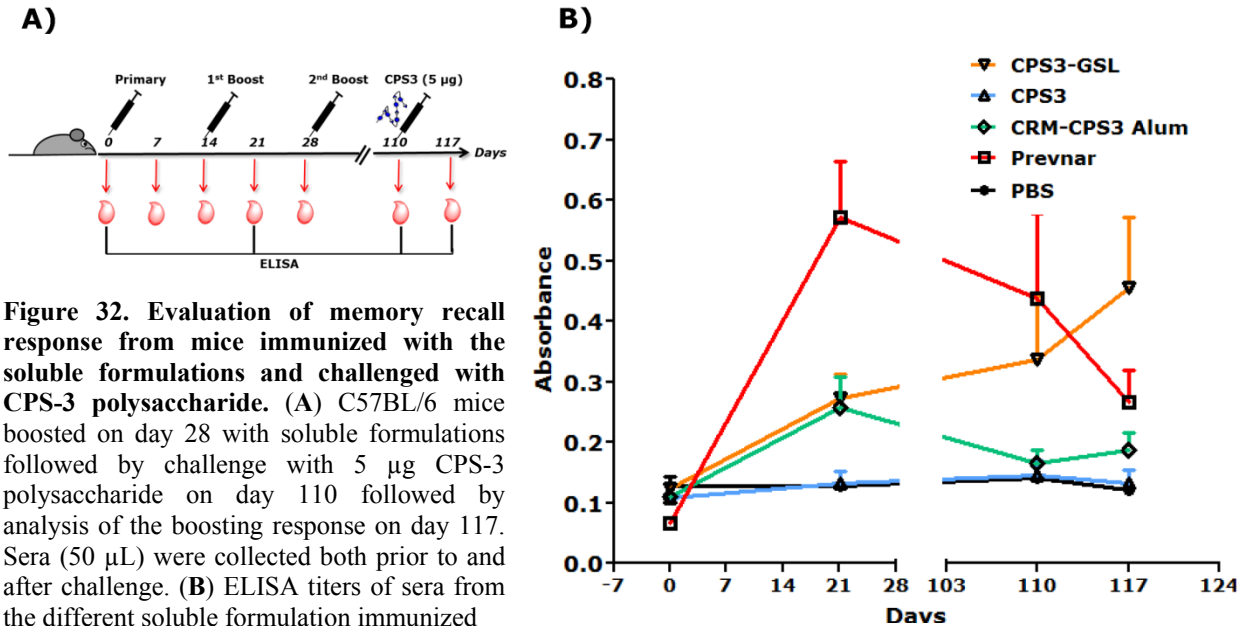

Figure 32. Evaluation of memory recall response from mice immunized with the soluble formulations and challenged with CPS-3 polysaccharide. (**A**) C57BL/6 mice boosted on day 28 with soluble formulations followed by challenge with 5 µg CPS-3 polysaccharide on day 110 followed by analysis of the boosting response on day 117. Sera (50 µL) were collected both prior to and after challenge. (**B**) ELISA titers of sera from the different soluble formulation immunized groups collected on day (21, 110 and 117). The titers are expressed as OD 450 absorbance values after subtracting from the pre-immune values of corresponding experimental groups. Sera were diluted 200 X with 1% BSA-PBS and pre-incubate with 10 µg/mL cell-wall polysaccharide. Diluted pre-incubated sera (100 µL) was added per well of the microtiter plate which was coated with 1 µg of CPS-3 polysaccharide, detected with a HRP conjugated mouse secondary antibody diluted to 1:10000 and developed using TMB. Absorbance was measured at 450 nm and the data were plotted using the graphpad prism software.

mean IgG titers were in agreement with the previous results obtained. The mean IgG titers of CRM_{197}-CPS3 (alum) and CPS3-GSL (PM) at day 21 were comparable in magnitude (green and orange lines) and that of the commercial vaccine (red lines) was the highest. However, there was a drastic decrease in the IgG titers of the protein conjugates CRM_{197}-CPS3 and Prevnar® (green and red lines) on day 110 (pre CPS-3 boost). This decrease was expected as the antibody responses would have subsided during the course of the 82 days the mice were rested. This observation was however in contrast to the experimental group immunized with CPS3-GSL (PM). On day 110, sera of the CPS3-GSL (PM) group showed an increasing trend of IgG titers as compared to the day 21 values (orange line). The titers were further boosted when the mice were challenged with the CPS-3 polysaccharide and the sera analyzed on day 117. In comparison, the commercial Prevnar® formulations showed a further decrease in IgG titers compared to day 110, clearly indicating polysaccharide induced hyporesponsiveness. The CRM_{197}-CPS3 conjugate prepared in-house (green line) showed no increase in titers and the values at day 117 were similar to that of day 110. Hence, although the mice demonstrated a robust booster response at day 21 when immunized with commercial Prevnar® vaccine, there was a hyporesponsive trend on subsequent immunization with the polysaccharide at day 117. It can be concluded cautiously that the hyporesponsiveness induced by polysaccharide vaccines can be rescued by using the iNKT adjuvant GSL (α-GalCer). Incorporating GSL into a commercial polysaccharide vaccine may be beneficial in eliciting a TD response, with no associated hyporesponsiveness. This would encourage the shift from using a protein conjugate vaccine thereby minimizing the vaccination cost and increasing the sequence coverage.

3.2.6.2 Glycan Array Analysis Reveals Different Epitope Recognition

Mapping of the minimal epitope required for the generation of a productive immune response is one of the critical aspects in the design of vaccines against polysaccharide antigens. In case of *S. pneumoniae* capsular polysaccharides, this can be a simple di-saccharide repeating unit as in serotype-3 or up to an octasaccharide. Attempts to generate the minimal repeating unit from the natural polysaccharides often results in heterogeneous mixtures of varying chain lengths that are difficult to purify and characterize. The chemical synthesis of very pure sequence defined oligosaccharide structures combined with the microarray platform provides a powerful tool for carbohydrate vaccine research. Utilizing this, the sera generated against the various soluble formulations collected on days 21, 110 and 117 were screened on the microarray platform. Different synthetic CPS-3 constructs varying

in chain length and combinations of glucose (blue circles) and glucuronic acid (blue-white diamonds) along with the natural CPS-3 and cell wall polysaccharide (CWPS) (Figure 33A) were printed as different sub-blocks numbered 1-8 (Figure 33B). The day 21 sera from the CPS-3 immunized mice produced low IgG titers which bound weakly to the CPS-3 (polysaccharide) antigen (sub-block 3) (Figure 33C). Although weak IgG binding to the tri-

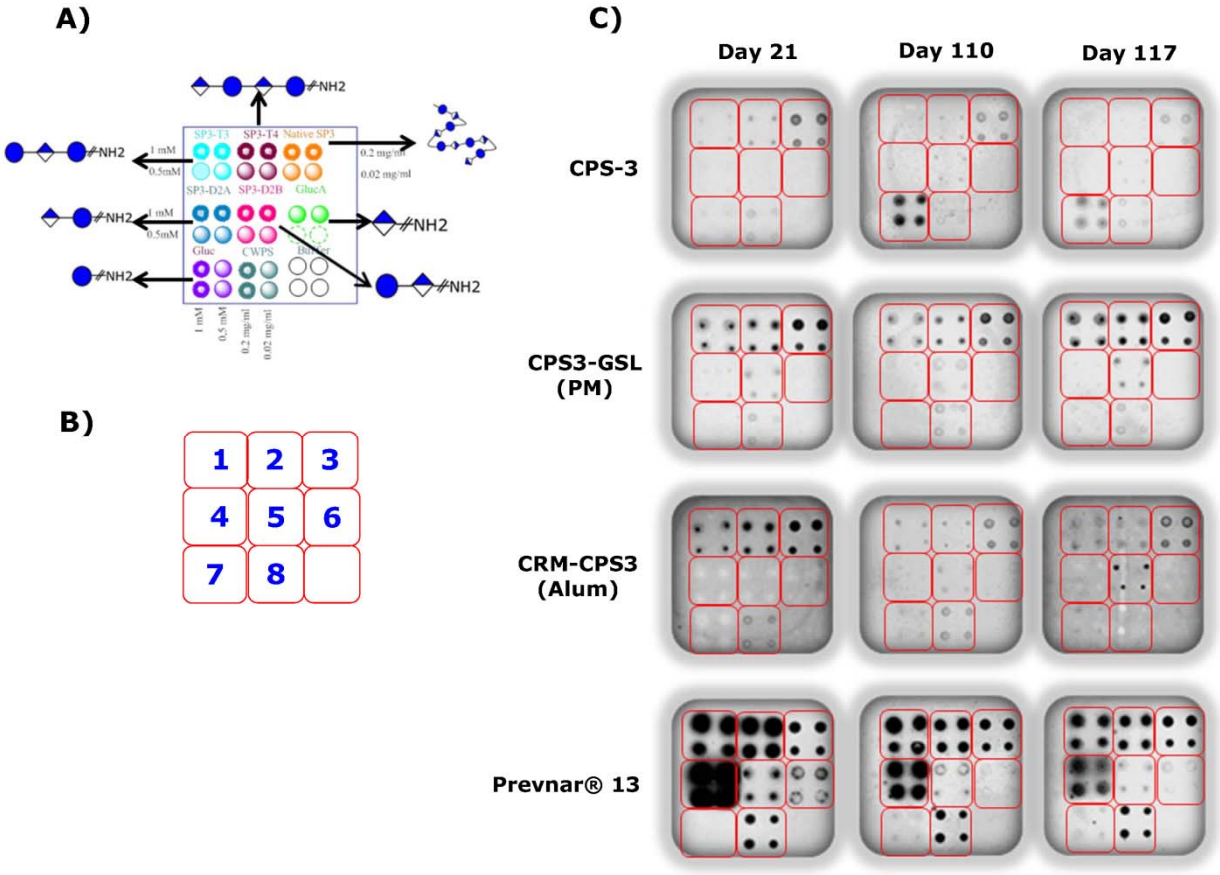

Figure 33. Glycan array analysis of various CPS-3 soluble formulations immunized sera. (**A**) Glycan array block depicting the printing pattern of the various CPS-3 synthetic constructs the natural capsular polysaccharide-3 and the cell wall polysaccharide (CWPS). (**B**) The different sub-blocks numbered 1-8 on which the antigens were printed with each sub-block containing two concentrations of the antigen. Sub-block 3, the natural CPS-3(orange spots) and sub-block 8 the CWPS (green spots) was printed in two concentrations of 0.2 and 0.02 mg/mL. All other synthetic constructs sub-block1; Glc-GlcA-Glc-NH_2 (indigo spots), sub-block 2; GlcA-Glc-GlcA-Glc-NH_2 (magenta spots), sub-block 4; GlcA-Glc-NH_2 (blue spots), sub-block 5; Glc- GlcA-NH_2 (pink spots), sub-block 6; GlcA-NH_2 (green spots), sub-block 7; Glc-NH_2 (purple spots) were printed in two concentrations of 1 and 0.5 mM except sub-block-6 which contained only spots of 1 mM concentration. (**C**) Sera (day 21, 110 and 117) from the different experimental groups immunized with various soluble formulations was diluted 200 X with 1% BSA-PBS and incubated for 1h. The spots were detected using an Alexafluor-635 conjugated mouse secondary antibody diluted 400X and incubated for 1h.

and tetrasaccharide constructs (sub-block 1 and 2) were observed, these eventually disappeared by day 110 and 117. In contrast, the group immunized with the PM and CRM_{197}-CPS3 (alum) showed strong binding in the order CPS-3 (polysaccharide antigen) (block 3) > tertrasaccharide (block 2) > trisaccharide (sub-block 1) for the day 21 sera. However in case of CRM_{197}-CPS3 (alum), binding eventually decreased by day 117 to levels lower than the day 21 values corroborating the hyporesponsiveness observed due to polysaccharide immunization. In case of the CPS3-GSL (PM), the day 110 sera showed a marginal decrease in the intensity of binding (sub-block 3, 2, 1) which on polysaccharide challenge got restored to levels observed at day 21.

In case of the commercial vaccine Prevnar®, the day 21 sera apart from recognizing the tri-, tetra- and natural polysaccharide (sub-blocks 1, 2 and 3) also bound the di- and monosaccharide epitopes (sub blocks 4, 5 and 6). It has been shown that acid hydrolysis and enzymatic treatment of the CPS-3 polysaccharide yields shorter oligosaccharide fragments terminating either with a glucose or glucuronic acid residue [161, 162]. The glucuronic acid structures were shown to react strongly with the antisera as compared to the glucose [216]. This is evident in the day 21 sera that bound strongly to the glucuronic acid terminated structure (sub-block 4) as compared to the glucose terminated structure (sub-block 5). The additional epitopes recognized in the Prevnar® sera may have originate due to the size reduction and conjugation procedure used to make the conjugate. In addition, the sera also bound the cell wall polysaccharide-CWPS (sub-block 8) with high intensity indicating that a substantial amount of antibodies were raised against this structure. However, in the CPS3-GSL and CRM_{197}-CPS3 formulations the binding to CWPS was very minimal. The CWPS is a common contaminant in the capsular polysaccharide (CPS) preparation and might have been carried over in the final Prevnar® formulation. Since the commercial vaccine Prevnar® is composed of 13 serotypes that are individually conjugated, the final formulation might have contained a substantial amount of CWPS. Although the day 21 Prevnar® sera bound all the CPS-3 substructures with high intensity, the day 110 and 117 sera showed a substantial decrease in binding indicating hyporesponsiveness due to polysaccharide immunization.

Next, the binding of antisera to the CPS-3 polysaccharide structures (sub-block 3) was quantified by analyzing the mean fluorescence intensity (MFI) values. As the Prevnar® immunized sera bound strongly to the CWPS (sub-block 8); these values were also included in the analysis. The MFI values for Prevnar® sera (Figure 34, red bars) were exceptionally high compared to the other soluble formulations. There was a sharp decrease in the MFI

values by more than 50% in the sera collected one week post challenge (day 117) compared to the day 21 sera indicating polysaccharide induce hyporesponsiveness. The same was true for the CRM_{197}-CPS3 conjugate (green bars) prepared in-house which showed a high titer on day 21 but failed to respond on subsequent challenge by polysaccharide. The sera from the group immunized with only the CPS-3 (blue bars) failed to induce any IgG response and the values were comparable to the corresponding pre-immune values (white bars). However, the MFI values of the sera from the CPS-3 and GSL physical mixture (PM) group not only produced IgG titers above the CPS-3 group (blue bars) but also responded positively to polysaccharide challenge. Although the titers were very low, the trend definitely highlights the role of the T cell adjuvant GSL in enhancing the CPS-3 specific IgG titers and limiting the hyporesponsiveness to subsequent challenges with the polysaccharide. However, the post challenge antibody titer (MFI) for the Prevnar® group (sub-block 3) was very high in microarray analysis compared to that obtained by ELISA (Figure 34). This observation may

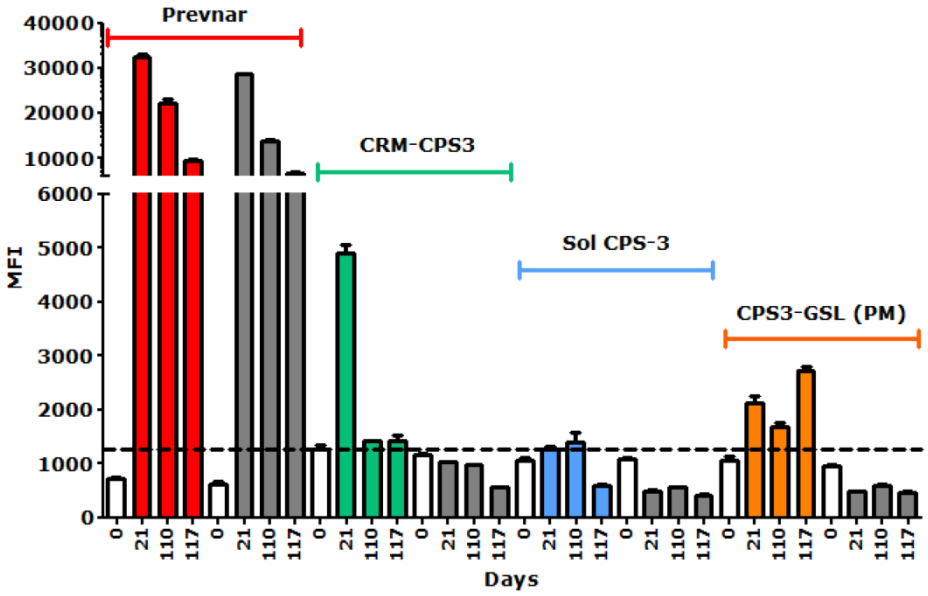

Figure 34. Glycan array quantification. Quantification of sera binding to natural CPS-3 and CWPS (sub-blocks 3 and 8) from the experimental groups immunized with various soluble formulations. Detection was done using an Alexafluor-647 conjugated mouse secondary antibody (1:400). Results are shown as mean fluorescence intensity (MFI) values ± SD.

be due to the presence of CWPS antibodies in the Prevnar® sera (grey bars) that bind the CPS-3 polysaccharide (sub-block 3), known to contain a considerable amount of CWPS contamination there by giving an enhanced MFI value. However, the CWPS titers for the CPS3-GSL physical mixtures were below the corresponding pre-immune values (day 0, white bar).

3.3 Conclusion

In this part of the thesis, as a surrogate to the commercial vaccine against T independent carbohydrate antigens, a fully synthetic and well defined vaccine formulation comprising the capsular polysaccharide virulence factors of *S.pneumoniae* was designed.

Vaccine development against polysaccharide antigens is often challenging due to poor immunogenicity, T cell independent immunological properties (polysaccharide vaccine) or variables like the nature of the carbohydrate antigen, the carrier protein, the conjugation chemistry and the type of adjuvant used as in case of glycoconjugate vaccines. In the studies reported in this thesis, two important formulation parameters of the commercial vaccine was changed in order to have better control of formulation parameters that would ultimately enhance the efficacy of the vaccine. In the first part, the adjuvant system alum used in the commercial vaccine was replaced by the glycolipid α-GalCer (KRN 7000). Adjuvant systems like alum forms an important bottle-neck in the success of glycoconjugate vaccine, the formulation processes of which are poorly defined with limitations such as poor adsorption of glycoconjugates due to interference of the attached glycans [32, 217]. On the other hand, numerous glycolipids and glycolipid based formulations are reported to have superior immunomodulatory functions as they specifically activate iNKT cell population in a CD 1d dependent manner. Recent reports suggest that co-delivering the carbohydrate antigen and glycolipid adjuvant [α-GalCer (KRN 7000)] to specific B cell would augment the immunogenicity of the carbohydrate antigen and the necessary ancillary help for B-cell to undergo isotype switching and affinity maturation would come from the recognized iNKT cell dependent manner [108, 218].

With this rational, when the polysaccharide (CPS-3) was injected in mice along with the glycolipid α-GalCer (KRN 7000) there was an increase in post booster (day 21) IgG titers. This indicated the adjuvant property of the glycolipid to elicit polysaccharide specific IgG responses. However the antibody titers were much lower than the commercial vaccine Prevnar 13®, a glycoconjugate vaccine administered in presence of alum as adjuvant. In order to increase the immunogenicity and antibody titers, attempts were made to co-deliver both the polysaccharide and the glycolipid adjuvant as a single entity. This was accomplished by developing a particulate delivery system made up of polylactic acid where both the antigen and the adjuvant were co-encapsulated. The PLA particulate system was qualitatively and quantitatively analyzed for both the polysaccharide and the glycolipid adjuvant confirming the co-encapsulation on the same polymeric particle. However, immunization of the particulate

system into mice elicited much lower IgG titers compared to the physical mixture formulations which were in turn lower than that observed for the commercial vaccine. Three important factors were assumed to be responsible for this reduced immunogenicity; the polysaccharide size, route of immunization and release property of the polymer particle.

Several conflicting reports discuss the influence of the polysaccharide size on the outcome of the immune response [163, 219, 220]. As the formulation details of the commercial vaccine are protected by intellectual property, the information on the size of the CPS-3 antigen used is not accessible. Hence, CRM_{197}-CPS3 glycoconjugates were prepared in-house using the same polysaccharide used in the physical mixture formulations and were immunized in mice with alum as adjuvant. Analysis of the antibody titers indicated that the physical mixture formulation and the CRM_{197}-CPS3 glycoconjugate prepared in-house elicited similar antibody titers indicating the adjuvant potential of GSL on par with that of alum. Although there was no differences in the antibody titers produced between the two adjuvant systems, the greatest advantage of the GSL adjuvant was the ease of administiring which was by just mixing with the polysaccharide and injecting. The CRM_{197}-CPS3 glycoconjugate on the other hand involved several chemical modification and characterization steps which might be not cost effective compared to the physical mixture formulations. However, the commercial vaccine Prevnar 13®, produced antibody titers several folds higer compared to both the physical mixture and CRM_{197}-CPS3 formulations. Limited acid hydrolysis is one of the preferred methods of polysaccharide size reduction in the manufacture of the commercial vaccine Prevnar 13® . Hence the use of such oligomeric structures in the commercial vaccine formulation might have resulted in a better immune response correlating with high titer antibodies. Although, the polymeric particulate and physical mixture formulations used the same polysaccharide, the decreased antibody titers seen in the particulate formulations compared to the physical mixture formulations might be due to factors like the route of immunization, uptake, presentation and the release property of the PLA polymer apart from the polysaccharide size. The uptake and presentation of the GSL in the polymeric particulate formulations is key important for iNKT cell activation to mediate the adjuvant effect. The uptake analysis and the splenocyte activation by the polymeric particulate formulations in an *ex vivo* splenocyte assay releasing the T cell cytokine IL-2 indicates the functionality of the adjuvant GSL formulated in a particulate form. The particulate formulation produced similar levels of cytokines IL-2,-4 and IFNγ even though it contained only half the amount of GSL compared to the soluble formulation. This further indicated that the reduced antibody titers produced against the particulate formulations could

be on account of the route of immunization which in this case was the sub cutaneous route. Several studies have demonstrated the shortcomings of sub-cutaneous (s.c) route of vaccine administration over the intra-muscular (i.m) route. One of the major disadvantages attributed to s.c route is the presence of deltoid tissue which might hinder the acquition of the vaccine by the antigen presenting cells. Intramuscular route on the other hand has been shown to be better for vaccine administration due to the increased circulation and better dispersion of the antigen [214, 215] . This is particularly true in case of polymeric particles which have been shown to undergo aggregation and lysis when administerd through the the sub-cutaneous route [221] . Hence, all the experimental groups were boosted with their corresponding antigens for the second time and were rested for a total of 110 days with the aim to facilitate the uptake of the particles resulting in shaping of immunological memory.

In order to evaluate the memory immune responses, all the experimental groups were challenged with 5 ug of the CPS-3 polysaccharide on day 110 and the sera were analyzed after one week at day 117. It was interesting to note that all the experimental groups demonstrated hyporesponsiveness on account of polysaccharide challenge except the group immunized with the physical mixture formulation comprising the polysaccharide and the glycolipid α-GalCer. The polysaccharide induced hyporesponsiveness is a common feature observed accross different age groups from neonates to adults mediated by either depletion or apoptosis of memory B cell populations [180, 222-226]. However, the increased antibody titers observed in the physical mixture immunized groups holds a promising future for the use of glycolipid adjuvants in polysaccharide vaccines. This however has to be validated for the other vaccine serotypes along with further experimental studies pertaining to the protection offered on account of bacterial challenge. The sera from the commercial vaccine and the physical mixture immunized groups when analysed by glycan array analysis showed a differential recognition of various CPS-3 sub-structures. Although both the Prevnar 13® and physical mixture immunized sera bound to the natural polysaccharide, the Prevnar 13® sera recognized diverse epitiopes ranging from mono saccharide, di-saccharide, tri-saccharide and tetra saccharide structures. The physical mixture immunized sera on the other hand only bound to the tri- and tetrasaccharide structures in addition to the natural polysaccharide. This highlights two important observations; either the different adjuvant systems, alum and GSL influence different immune outcomes or the nature of the polysaccharide antigen used plays an important role. The latter seems to responsible for the outcome as the CRM_{197}-CPS3 protein conjugate prepared in-house also recognized the same sub-structures recognized by the physical mixture immunized sera, however associated with hyporesponsiveness. Another

important observation between the Prevnar 13® sera and the physical mixture immunized sera is the abundance of antibodies against the cell-wall polysaccharide structures in the former. This might have originated on account of the conjugation reactions which further get amplified. Hence, immunizing the polysaccharide without conjugating to a protein but with a glycolipid adjuvant seems to produce very specific antibodies against the thereby minimizing unwanted immune responses against contaminating glycans.

Collectively the results presented here highlight the potential of using glycolipid adjuvants with T-independent antigens to increase the efficacy of polysaccharide vaccines with a reduced cost. The results obtained here have to be further validated using the other vaccine serotypes of *S. pneumoniae*. Further knowledge of the minimal epitope required to produce a robust and protective immune response can facilitate the chemical synthesis of the antigen resulting in a completely synthetic and controlled vaccine formulation. The particulate delivery system fabricated here were less robust in eliciting an immune response due to the various parameter constraints discussed above which have to be addressed methodically. However, the polymeric particulate system developed here hold great promise for the future vaccine delivery systems, some of which are mentioned here

i. The polymeric system fabricated here used a simple encapsulation process to introduce the polysaccharide and the glycolipid adjuvant as a single component, compared to complex chemistries involved in the glycoconjugate vaccines.
ii. The encapsulation of both the antigen and the adjuvant can be easily quantified and this allows better control of the formulation parameters.
iii. As the glycoconjugate vaccines are formulated by individually conjugating carbohydrate antigens to the carrier protein CRM_{197}, the general problem associated with carrier protein induced immunosuppression can be overcome by using polylactide delivery systems with glycolipid adjuvants
iv. The polymeric system can act as excellent systems in the future development of multicomponent vaccines comprising of many antigens but a single adjuvant type formulated on a common carrier platform.
v. A tremendous potential of polymeric carrier systems is the formulation of dry powder form of the vaccine that can be packed in inhalers with increased shelf life promoting self-administration.

4. *Toxoplasma gondii* Exosome Like Vesicles (ELVs) act as Carriers of Virulence Factors

This chapter describes the experiments performed to study the role of extracellular vesicles in the Host-Pathogen interaction of the protozoan parasites *Toxoplasma gondii*. The study highlights the capacity of the parasite *Toxoplasma gondii* to produce exosome like extracellular vesicles that might function as unconventional modes of communication between the parasite and the host. The study focuses on the isolation and characterization of extracellular vesicles from *Toxoplasma gondii* tachyzoites and characterizes the various protein and glycolipid cargo of the vesicles. The study further compares the extracellular vesicles produced by the virulent strain Type-I (RH) and the avirulent strain Type-II (PTG) and highlights their strain specific antigen expression and effector functions.

4.1 Introduction

4.1.1 Extracellular Vesicles

Intercellular communication is pivotal in multicellular organisms, both in healthy and diseased conditions. Most of the intercellular communication is mediated either by a direct cell-cell contact or by hormones and chemical messengers in a paracrine or endocrine manner [227]. In addition to the above mentioned modes of cellular communication, extra cellular vesicles in the form of exosomes, ectosomes, microvesicles and microparticles are fast gaining importance as modulators of both systemic and cellular responses in higher eukaryotes [228, 229]. Though initially thought as contaminants of cellular debris, such vesicular components have now been purified and characterized in great detail both from *in vivo* and *in vitro* sources with origins from various pathological conditions , biological fluids and an array of cells types ranging from macrophages, B cells, T cells, dendritic cells and endothelial cells [230-233].

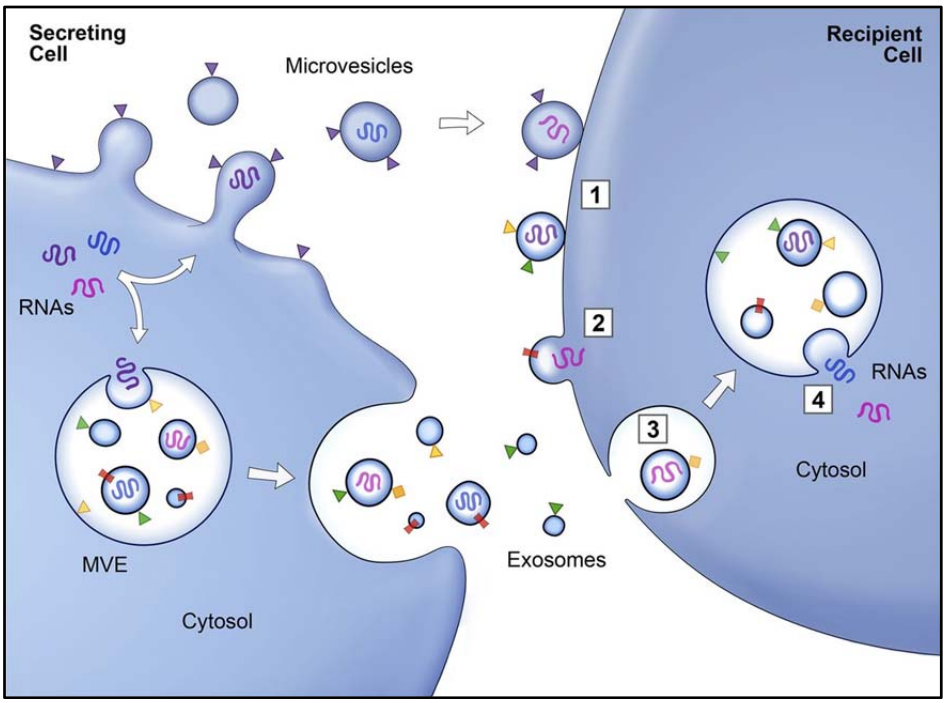

Figure 35. Schematic of intercellular communication mediated by exosomes and microvesicles. Formation of microvesicles and exosomes either by direct budding or fusion and release of multivesicular bodies contain membrane proteins (triangles and bars), RNA (curves), soluble proteins and lipids of the secreting cells. The released microveiscles and exosomes interact with the recipient cells either by 1) docking, 2) direct fusion or 3) endocytosis followed by 4) fusion to the endocytic compartment resulting in the release of cargo (proteins, RNA, lipids) leading to modulation of the recipient cell. Figure adopted from [234].

The extracellular vesicles have defined and characteristic functions ranging from reticulocyte maturation, immune modulation and also serve the potential as a delivery system [235, 236]. The biogenesis of extracellular vesicles is from the plasma membrane and hence is classified into either exosomes or microvesicles [237]. Exosomes are formed by the inward invagination of the plasma membrane forming endosomes which later fuse forming multivesicular endosomes with the plasma membrane to release their content as exosomes [238, 239]. On the other hand, microvesicles are thought to be produced by the outward blebbing of the plasma membrane [240]. In either case, the extracellular vesicles encompass proteins, lipids and nucleic acids (RNA, mRNA) molecules which serve as a cargo forming a signalasomes that can modulate the properties of the interacting cell [241, 242]. Although exosomes are microvesicles are characterized based on biochemical and biophysical properties of size, density, morphology, protein composition a clear distintion cannot be made between the two[243]. However, the role of exosomes and microvesicles in disease conditions is under intense investigation as both lower eukaryotes and prokaryotes are also known to secrete extracellular vesicles.

4.1.2 Extracellular Vesicles of Pathogens

In the recent past, there has been renewed interest and reports of unicellular and infectious pathogens like *Trypanosama cruzi, Lesihmania, Cryptococcous neoforms,* secreting extracellular vesicles. The secreted vesicles are involved in modulation of macrophage monocyte functions, carriers of virulence factors; innate and adaptive immunity and intercellular protein transport [244-247]. In the case of *Apicomplexcan* parasites, such extracellular vesicles of parasite origin have been isolated from the plasma of mice infected with *P. bergeie* 9 (ANKA) and were shown to be highly pro inflammatory [248]. However these vesicles in this case were defined as Microparticles. Quite recently, Reticulocyte derived extracellular vesicles of parasite origin in the form of exosomes have been reported from mice infected with *Plasmodium yoelii* and these exosomes have been used as sub unit vaccine component protecting the mice from lethal infections [249]. Exosomes from *Plasmodium falciparum* infected red blood cells have been shown to be associated with transfer of genetic information within a population [250]. In the case of *Toxoplasma gondii*, exosomes derived from dendritic cells exposed to *Toxoplasma gondii* lysates have been shown to be protective against infection in a mice model [251].

4.1.3 Toxoplasmosis

Toxoplasma gondii is one of the most important Protozoan pathogen with a seroprevalance of over 30%. It is an obligate intracellular parasite and is one of the most successful pathogens capable of infecting almost every warm blooded animal resulting in Toxoplasmosis [252]. The lifecycle of the parasite comprises of the asexual and sexual stages comprising different parasitic forms like tachyzoites, bradyzoites, tissue cysts and fecal oocystoocyst. The members of the cat family (*Felids*) form the definitive hosts of *Toxoplasma* for sexual reproduction forming fecal oocysts and other warm blooded animals serve as intermediary hosts for asexual reproduction forming tachyzoites, bradyzoite and oocysts. Primary infection of a non-immune host (humans/ animals) might start with the ingestion of food contaminated with fecal oocysts from the cat or direct ingestion of animal harbouring tissue cysts. Humans are at high risk either directly or indirectly as undiagnosed benign

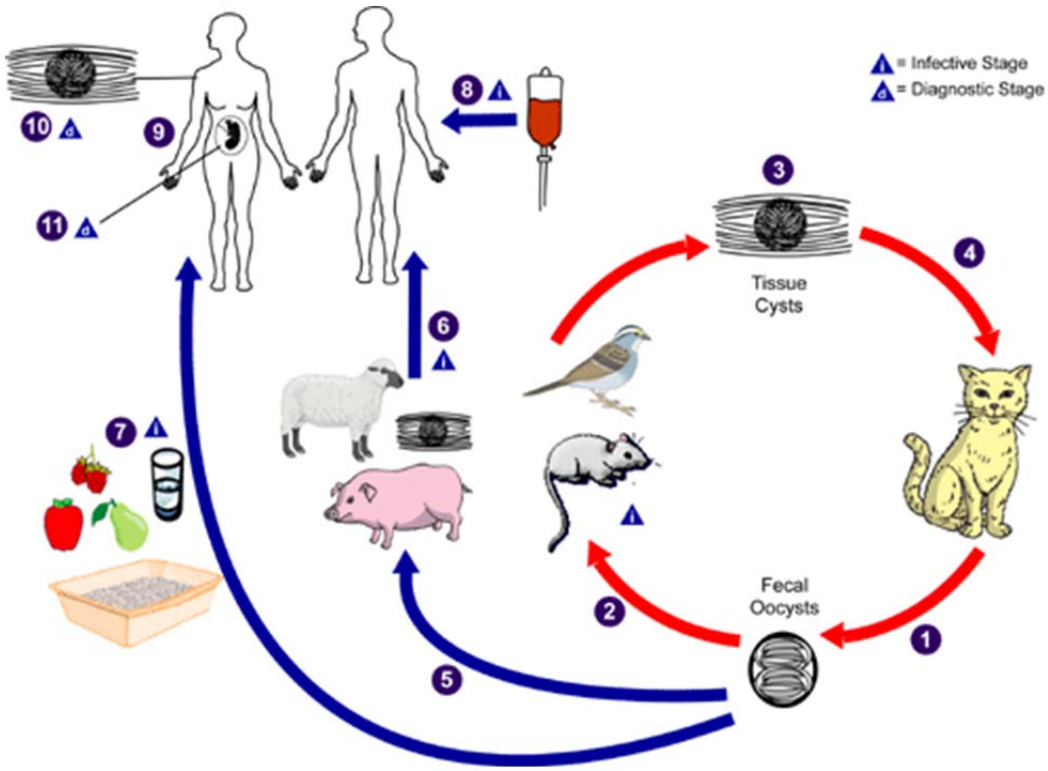

Figure 36. Life Cycle of *Toxoplasma gondii*. The life cycle of *T. gondii* traversing through tissue cyst and oocyst forms (1-4) in the definitive host (cats) represented by red arrows and their acquition by humans either by consumption of other infected animals (5-6) which act as intermediary hosts represented by blue arrows. Parasite acquisition by humans can also be through the consumption of contaminated fruits, vegetables and diary (7), by blood transfusion (8) or by direct materno-foetal transmission (11). Figure adopted from [253]

infections cause miscarriage and abortion in gestating mothers and loss of vision and encephalitis in new born babies, immune compromised individuals undergoing transplantation therapy and those suffering from HIV or cancer. The infection of farm animals with this pathogen also leads to the death and miscarriages resulting in huge economic burden for humans [254].

4.1.4 Infection Biology of *Toxoplasma gondii*

The primary infection in humans by *T. gondii* is asymptomatic followed by flu like symptoms. An important feature of toxoplasma infection is the efficiency with which it infects and disseminates in the various tissues of the host. Part of this feat is achieved by the remarkably ability of the parasite to hijack the host cell machinery and modulate the host responses to its favor . The primary route of infection in humans is the gastro intestinal tract by ingestion of contaminated or uncooked meat or by fecal contamination by cats where the parasite exists as a dormant tissue cyst or fecal oocyst. Upon ingestion, the parasite rapidly develops into the invasive form called tachyzoites which are capeable of infecting every nucleated and can also cross barriers like the placenta and brain. The tachyzoite form of the parasite gains entry through the gut epithelial cells, and primarily interacts with the cells of the Lamina propia and Peyers patch which are rich in tissue resident macrophages and dendritic cells [255]. This breach of the gut epithelia exposes the gut microflora which along with with the parasitic components synergistically interacts with the innate immune cells like resident macrophages and dendritic cells through their PRR initiating an inflammatory response [256, 257]. A, remarkable property of the parasite is to hijack the host cell machinery, especially the dendritic cells , which it used as a Trojan horse to traverse and disseminate to different parts of the body [258]. The disseminated parasites undergo rapid replication and establish itself in various tissues by which time the adaptive immunity kicks in with overwhelming production of IFN-γ and NO which now limits the infection [256, 259]. Under this pressure exerted by the host immune system, the parasite now converts into a more slow growing form called Bradyzoite; a slow growing form limits the adverse effect of host cell destruction and forms tissue cysts which when transferred to the definite host (*Felids*) forms the oocyst thereby restarting the infection cycle [260].

4.1.5 Host Resistance and Immunity to *Toxoplasma gondii* Infections

Apart from the first line innate defense mechanisms, adaptive immunity characterized by $CD4^+CD8^+$ T_H1 response and a T_H2 humoral immunity mediated by antibodies are critical for long lasting protection against *T. gondii* infections with $CD4^+$ T cells mediating a critical role [261-263]. The professional antigen presenting cells, mainly dendritic cells are involved in the phagocytosis of the parasite and presentation of the antigens to $CD4^+$ T cells thereby bridging the innate and adaptive immune responses [264]. Furthermore, it has been shown that uninfected DC can also present antigens and it is yet not clear how the antigens apart from dead parasites are acquired by the dendritic cell. One of the proposed mechanisms for this unconventional antigen uptake is by abortive infections, or by direct acquisition of parasite debris or soluble antigens. This presence of soluble antigens is further confirmed by the observation that parasite specific protein antigens can be detected in circulation as early as day 1 post infection and peaks up at day 5-7 to high levels [265, 266]. More recently, ROP family of proteins which are virulence factors were identified in uninfected host cells which suggests that toxoplasma have the ability to modulate distant uninfected host cells [267] through an un-conventional transport of parasite virulence factors. Several parasite virulence factors (proteins and glycolipids) have been identified which are thought to be important for the parasite survival, host cell modulation and evasion. Of these virulence factors, the family of surface proteins namely Sag-1, -2 and -3, the dense granule family namely Gra-2, -6, -7 and proteins belonging to the apical membrane region and microneme family namely MIC-1 and -2 are thought to play a very important role in the invasion and infection process . Pathogenicity associated with *T. gondii* infections is further complicated due to the three different clonal lineages of the parasite namely Type-I, II and III. The parasite strains differ considerably in their capacity to cause infection and the resulting pathogenesis with the Type-I strain being the most virulent with a LD 50 of 1 as compared to the Type-II&III with a LD 50 of 1000 [268, 269].

Hence, extracellular vesicles might function as important mediators of host-pathogen interaction and a understanding of such intercellular communications might give useful insights for designing reliable prevention and prophylactic strategies against Toxoplasmosis.

4.2 Results and Discussion

4.2.1 *Toxoplasma gondii* Tachyzoites Secrete Exosome Like Vesicles (ELVs) in a Time Dependent Manner

The role of extracellular vesicles like exosome and microvesicles in the infection biology of *Toxoplasm gondii,* a unicellular pathogen capable of infecting every nucleated cell and modulating its function has not been explored. *Toxoplasma gondii* tachyzoite, the unicellular form of the parasite causing active infections is ultrastructurally similar to a eukaryotic cell. Hence, I investigated whether the *Toxoplasma gondii* tachyzoites are qualified to secrete extracellular vesicles. Towards this aim, tachyzoites of the mouse virulent strain Type-I (RH) grown in Human Foreskin Fibroblasts (HFF) as the host cell system were incubated for different times (1, 3, 6, 18 h) to elucidate the kinetics of vesicle production. The spent medium from these incubation time-points was subjected to an ELV isolation protocol using sequential centrifugation steps, to first remove large cellular debris followed by a high speed centrifugation step to pellet the secretory vesicles. It was envisaged that the secreted vesicles were formed either by the inward/ outward blebbing of the cell membrane with the incorporation of membrane/ cellular proteins and hence the protein profile of the pellets obtained from the different time-point isolations were analyzed by SDS-PAGE. As shown in Figure 37A, an increase in protein content was observed at different time- points of vesicle isolation with the optimal time being 6 h. This time point was employed for all further isolations. There was no major qualitative difference observed between isolations at different time points but the quantitative representation of individual proteins varied, especially in the 35 KDa regions and below. It is also evident that some protein bands were selectively enriched in the ELV fractions as compared to the total tachyzoite lysate. This could be due to the complexity of the tachyzoite cell lysate that contain far more proteins than the pellet of secretory vesicles. To confirm the presence of vesicular components in the isolated pellet, electron microscopy analysis was performed. Exosomes and microvesicles are reported to have a size in the range of 100-200 nm in diameter with a cup-shaped morphology [270]. To conserve the morphology of the secreted vesicles, cryo-electron microscopy analyses were performed as a less invasive technique of sample processing. As seen in Figure 37B, (Left panel) vesicular structures in the size range of 100-200 nm matching that of exosomes and microvesicles were observed but did not exhibit a cup-shaped morphology. However, when the same set of vesicles was analyzed by TEM analysis using uranyl acetate staining, the

vesicles displayed a cup-shaped morphology with a size of 100 nm, a descriptive feature of exosomes (Figure 37B, Right panel). It is worth to note that the cup shaped morphology

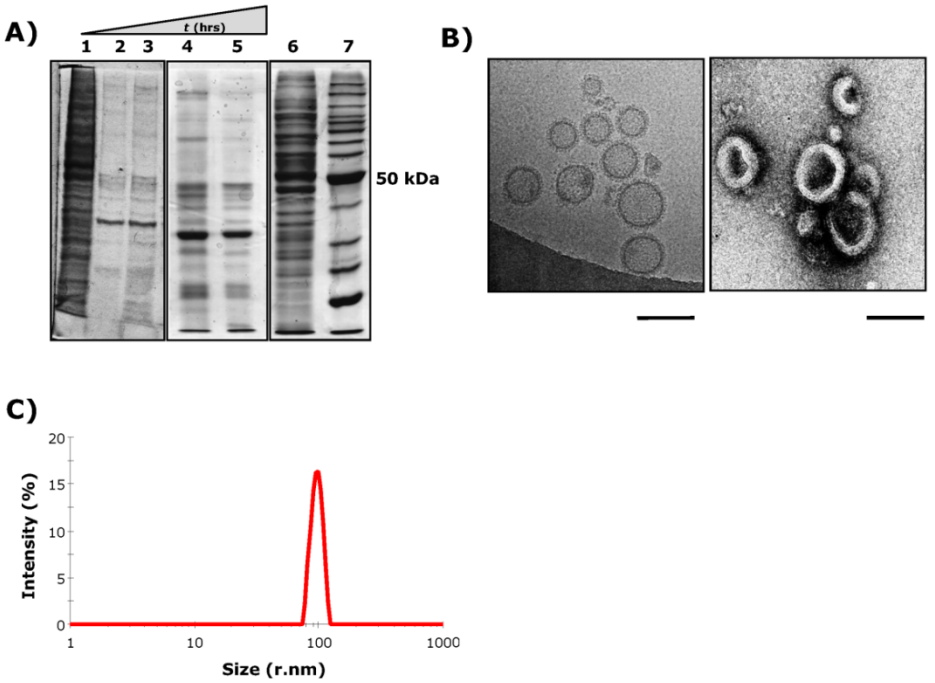

Figure 37. Characterization of ELVs isolated from the spent medium of *in vitro* grown *Toxoplasma gondii* RH strain tachyzoites. (**A**) Coomassie stained SDS-PAGE profiles depicting the time course of vesicle production in hours 1. *Toxoplasma* whole cell lysate, 2. Pellet of 1 h spent medium, 3. Pellet of 3 h spent medium, 4. Pellet of 6 h spent medium, 5. Pellet of 18 h spent medium, 6. *Toxoplasma* whole cell lysate, 7. Protein ladder ranging from 250-12 KDa M.Wt with highlighted 50 KDa band). (**B**) Electron micrographs of 6 h spent medium pellet as observed by Cryo-TEM analysis (left panel) and by TEM with negative staining using Uranyl acetate (right panel). Scale bars represent 100 nm. (**C**) Size distribution and polydispersity of 6 h spent medium pellet as measured by Dynamic Light Scattering.

described for identification of exosomes might be staining artifacts originating on account of harsh sample treatment as compared with the softer technique of cryo-imaging. To obtain information on the size and distribution of the isolated vesicles, Dynamic Light Scattering measurements were performed. As seen in (Figure 37C), the isolated vesicles were very homogenous and had an average size of 116 nm with a polydispersity index of 0.330. Based on the above experiments, the size and morphology of the isolated extracellular vesicles were in-between that of microvesicles and exosomes and hence are referred as Exosome Like Vesicles (ELVs).

4.2.2 *Toxoplasma gondii* ELVs Contain Parasitic Proteins and Glycolipids

The cell surface of *Toxoplasma gondii* tachyzoite is densely covered with the protein Sag-1/ Protein-30 (P-30) which belongs to the SAG (Surface antigen) protein family. Sag-1 protein is used by the parasite for host cell invasion and it is known to have lectin like activity. It is also known that this protein is rapidly shed from the parasite surface during the course of infections and the protein can be detected in circulation. The Sag protein is posttranslationally modified by the addition of a linker glycosylphosphatidylinositol (GPI), a glycolipid that anchors the protein to the cell membrane. Apart from the protein anchored form of GPI, the parasite membrane is also composed of the free form of the GPI anchor in large excess, the function of which is currently unknown. Hence it was predicted that the isolated ELVs that are membranous particles as inferred from the electron microscopic analysis may be composed of these protein (Sag-1) and glycolipid (GPI) moieties. The presence of Sag-1 in the ELVs was confirmed by western blot analysis or by binding the ELVs to aldehyde functionalized latex beads that covalently trapped all amine containing surface molecules of the ELVs, mostly proteins. The latex beads and the blots were probed using the Anti- Sag-1 antibody as the primary antibody followed by detection with a FITC conjugated secondary antibody for latex bead assay or HRP conjugated secondary antibody for western blots. As seen in Figure 38 A, the beads bound to the ELVs showed major shift when probed with the Anti-Sag-1 antibody as compared to the control beads shown as tinted histograms. The same result was observed in western blot analysis (Figure 38B) which showed the presence of Sag-1 at approximately 35 KDa, the reported molecular weight of Sag-1 protein. The comparison between the *Toxoplasma* whole cell lysate (lane 2) with the ELV fraction (lane 1) showed the over representation of the protein Sag-1 although the concentration of the ELVs loaded was 1/5 th of that of the cell lysate. Next, to analyze the presence of GPI, the ELVs and the total parasite pellet (approximately 10^8) were subjected to GPI isolation protocol [271] to separate the protein bound form from the free form of the GPI that formed a pellet. This protein free GPI pellet was subjected to dot blot analysis and probed with the *Toxoplasma* GPI specific monoclonal antibody T5 4E10 [272]. As seen in Figure 38C, both the ELV and whole parasite GPI fractions stained positive for the *Toxoplasma* GPI antibody as compared to the *Plasmodium* specific antibody that served as a control. Next, to demonstrate the specific localization of the protein (Sag-1) and the glycolipid (GPI) on the surface of the vesicles immune electron microscopy analysis was performed on the ELVs.

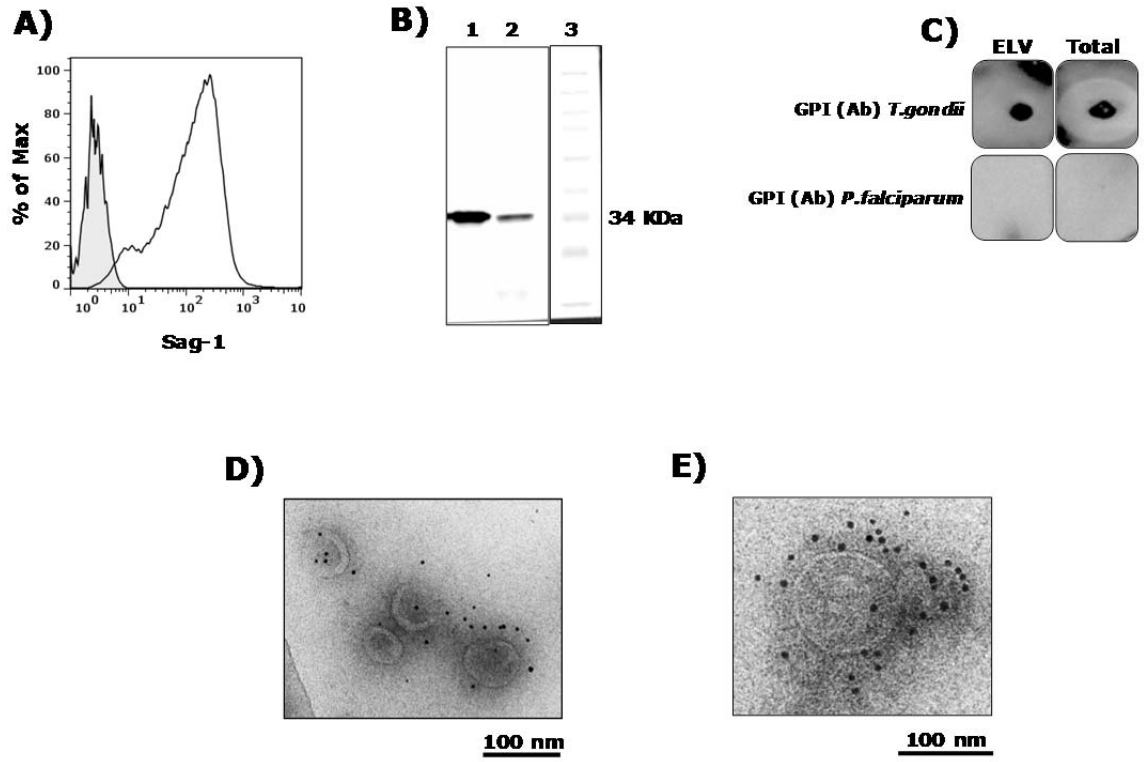

Figure 38. Characterization of the protein and glycolipid content of ELVs. (**A)** Histogram representation of SAG-1 expression analyzed by flow cytometry where ELVs were coated on latex beads and Sag-1 protein analyzed by monoclonal Sag-1 antibody (1:100) probed with a FITC conjugated secondary detection antibody (1:500) and the fluorescent beads detected by flow cytometry (clear histograms). Corresponding controls included the beads with primary and secondary antibodies but no ELVs (tinted histogram). **(B**) The presence of surface protein Sag-1 analyzed by western blot using the Sag-1 monoclonal antibody (1:1000) and probed with a HRP conjugated secondary antibody (1:5000) where 1.2 µg ELV fractions isolated at 6 h production time, 2. 10 µg whole cell parasite lysate, 3. Dual color protein ladder ranging from 250-12.5 KDa). (**C**) Dot blots of ELV isolated GPI content (ELV) and total GPI isolated from parasite (Total) probed with monoclonal antibody T5 4E10 (1:100) and Plasmodium specific antibody T3 (1:100). (**D & E**) Immuno-gold TEM micrographs showing the surface localization of Sag-1 protein and glycolipid GPI probed with Sag-1 and T5 4E10 primary antibodies (1:50) and detected by 5 nm gold conjugated secondary antibody (1:100) seen as black dots. Scale bar represents 100 nm.

Here, the ELVs were separately probed with the primary antibodies against Sag-1 and T5 4E10 against GPI followed by detection using a 5 nm gold conjugated secondary antibody. As seen in Figure 38D, there was specific localization of the Sag-1 and GPI (Figure 38E) on the ELVs, indicated by the black dots representing the gold conjugate. These experiments confirmed the specific localization of parasitic protein (Sag-1) and glycolipid (GPI) on the ELVs ruling out their presence as co-contaminants of the ELV isolation procedure. It can also be inferred that the Sag-1 protein is one of the major constituents of the ELVs and along with the GPI; the ELVs are to be of parasite membrane origin.

4.2.3 Peritonial Fluid of *Toxoplasma gondii* Infected Mice Contains ELVs with Parasite Proteins

To corroborate the *in vitro* findings on the production of ELVs from tachyzoites it was decided to passage/ infect mice intraperitoneally with the tachyzoites and isolate the ELVs from the intraperitoneal fluid. Therefore, BALB/c mice were infected intraperitoneally with *Toxoplasma gondii* tachyzoites and ELVs were isolated from the peritoneal lavage. The ELV pellet obtained from the centrifugation step was analyzed by transmission electron microscopy following uranylacetate staining. As seen in Figure 39A, the pellet comprised of vesicular structures with exosome like morphology were coarse compared to ELVs obtained by growing parasites *in vitro.* This can be due to the low sample volume, complexity of the peritoneal fluid and handling. To obtain information on the size and distribution of the ELVs, Dynamic Light Scattering measurements were performed. As seen in Figure 39B the vesicles had an average size of 80 nm with a polydispersity index of 0.260. This lower size distribution of ELVs obtained from *in vivo* source in comparison to ELVs isolated from *in vitro* grown parasites can be due to the presence of small particulate vesicular structures that are clearly visible in the electron micrographs (Figure 39A). Analysis of the protein content by SDS-PAGE showed enrichment in the band at 35 KDa corresponding to Sag-1 protein (Figure 39C). However, the other protein bands that were present in the protein profile of ELVs obtained by *in vitro* culturing of tachyzoites were not visible or were very weak. This can again be due to the complexity and low volume of the peritoneal fluid resulting in a lower yield of ELVs. Here again, the presence of surface antigen (Sag-1) in the ELVs was confirmed by latex beads which covalently trapped all amine containing surface molecules of the ELVs, which was probed using the Anti-sag-1 antibody as the primary antibody followed by detection with a FITC conjugated secondary antibody (Figure 39D). These results indicate a significant similarity between the *Toxoplasma* ELVs isolated from *in vivo* infections and ELVs obtained from *in vitro* grown *Toxoplasma*. Hence, it can be inferred that *Toxoplasma gondii* tachyzoite secrete or shed exosome like membranous vesicles composed of parasitic proteins and glycolipids.

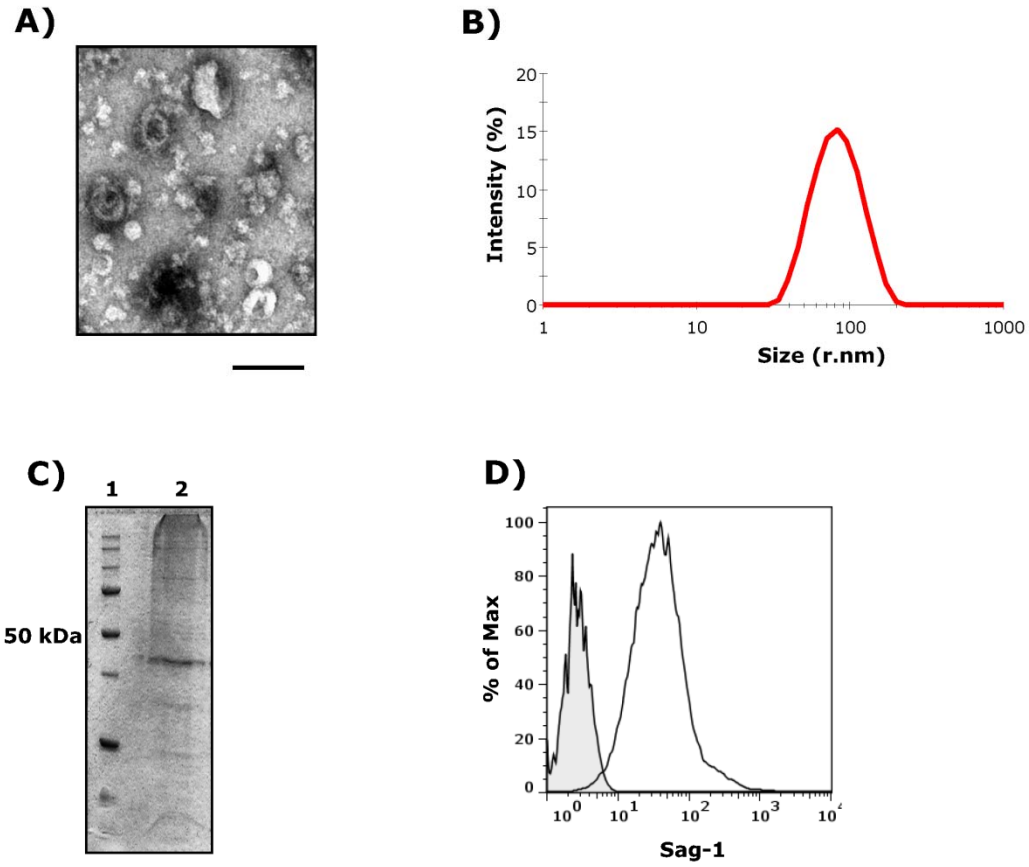

Figure 39. Characterization of ELVs from peritoneal fluid *Toxoplasma* infected mice (**A**) Electron micrographs of peritoneal fluid isolated ELVs as observed byTEM-with uranyl acetate negative staining. Scale bar represents 100 nm. (**B**) ELV size distribution and polydispersity as measured by Dynamic Light Scattering. (**C**) Coomassie stained SDS-PAGE profile of ELV fraction (1. Dual color protein ladder with 250-12.5 KDa, 2. Peritoneal fluid isolated ELV pellet). (**D)** Histogram representation of SAG-1 expression analyzed by flow cytometry where in *Toxoplasma* infected peritoneal fluid ELVs were coated on latex beads and Sag-1 protein analyzed by monoclonal Sag-1 antibody (1:100) probed with a FITC conjugated secondary detection antibody (1:500) and the flourecsnt beads were detected by flow cytometry (clear histograms). Corresponding controls included the beads with primary and secondary antibodies but no ELVs as shown by the (tinted histogram).

4.2.4 *Toxoplasma* ELVs from RH and PTG Strains are Composed of Different Parasitic Proteins

Toxoplasma gondii is a ubiquitous pathogen capable of infecting a wide variety of species and different cell types. Based on the pathogenicity, the parasites are divided into three different clonal lineages with Type-I (RH) being the hypervirulent strain with a LD 50 of 1 capable of killing a immunocompetent host whereas Type-II (PTG) &Type-III are

lowvirulent strains causing latent infections with a LD 50 of 1000. This difference in parasite pathogenicity of the parasite is due to their resistance of host responses mediated by parasite related virulence factors. However, it has been reported that there is no major difference in the total proteome between the RH and PTG strains [273]. Hence it was envisaged that the ELVs secreted by the parasite can act as carriers for the unconventional delivery of virulence factors. This would be important in devising novel prevention methods or for the design of new drugs. In order to identify such novel virulence factors, the ELVs were isolated from the spent medium of the RH and PTG parasite strains grown in HFF cells. To determine the role of the host cells and their effects on the quality of the ELVs produced, parallel experiments were performed where RH and PTG strains were grown in VERO cells and the ELVs were isolated and their protein profiles were compared. The isolated ELVs from the RH and PTG strains grown in two different host cell systems were first analyzed for their protein expression by SDS-PAGE. There was no major difference in the protein profile of ELVs isolated from the tachyzoites grown in HFF cells or VERO cells (Figure 40A and B). This demonstrates that *Toxoplasma gondii* produce ELVs and in a strain specific manner irrespective of the host/ cell type infected.

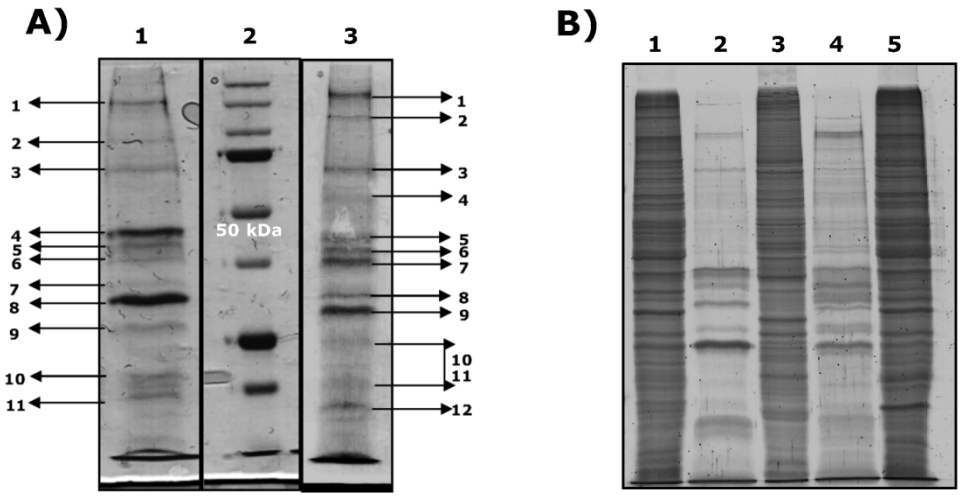

Figure 40. Proteomic profile of RH and PTG ELVs. (**A**) SDS–PAGE profile of ELVs isolated from RH and PTG strains grown in HFF cells. 1. RH-ELV (2 µg), 2. Dual color protein ladder with 250-12.5 KDa bands. 3. PTG-ELV (2 µg), (**B)** SDS–PAGE profile of ELVs isolated from RH and PTG strains grown in VERO cells as host. (1. Strain-RH whole cell lysate (10 µg), 2. RH-ELV (2 µg), 3. VERO whole cell lysate (10 µg), 4.PTG-ELV (2 µg), 5. Strain-PTG whole cell lysate (10 µg).)

When the protein profile of the ELVs isolated from RH and PTG strains grown in HFF cells were analyzed, there was a significant difference in the protein expression between the two strains. There was also a significant difference in the protein content of the bands that are

common to both strains (Fig 40A). It is worth to note that in the RH strain, the protein at 35 kDa range (band 8) was over-expressed when compared to that on the PTG strain (band 9). There was a visible difference in the expression of proteins in the 50-43 kDa range (bands 4, 5, 6 for RH and 5, 6, 7 for PTG) and a major difference in the low molecular weight region with the band at position 9 of RH sample missing in the PTG strain. The bands corresponding to 10 and 11 of the RH strain were also missing or underexpressed in the PTG strain. Equal concentrations of ELVs in terms of protein content were used in both cases.

Proteomic analysis of the bands revealed several surface antigen (SAG) and dense granule (GRA) family of proteins. The proteins SAG-1 and GRA-6 have been identified as immunodominant epitopes, antibodies against which are used for diagnostic purposes. The presence of GRA-7 protein also draws attention as this is the only protein of parasitic origin found to be present on the surface of an infected cell that is constitutively expressed in all forms of the parasite lifecycle.

4.2.5 *Toxoplasma* ELVs from RH and PTG Strains Differ in Parasitic Glycolipid (GPI) Composition.

Next, the differences in the glycolipid content of ELVs between the RH and PTG strains grown in HFF as host cell were analyzed. There are two forms of GPI anchor reported in the parasite that differ in their glycan architecture particularly in their side chains either terminating with a *N*-acetylgalactosamine (GalNac) (Figure 41A) residue or with a glucose residue terminally attached to the GalNac (Figure 41B) [274].

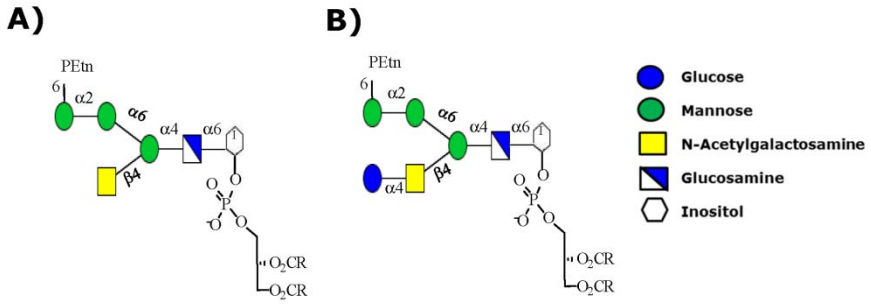

Figure 41. Schematic representation of the two glycan isoforms of *Toxoplasma gondii* GPI. (**A**) GPI core glycan ending with *N*-acetylgalactosamine and (**B**) core glycan ending with glucose attached to *N*-acetylgalactosamine.

The ELVs obtained from the two parasite strains RH and PTG were analyzed with the monoclonal antibodies T3 3F12 that binds specifically the GalNac structure and T5 4E10 that binds specifically to the glucose terminating structure. To evaluate the differences in GPI expression, the latex bead assay combined with flow cytometry approach was used as mentioned earlier. As seen in Figure 42(left panel), the glucose terminated structure (red histogram) is expressed higher in the RH strain when compared to the PTG strain Figure 42 (right panel), whereas the GalNac containing structure (green histogram) was expressed more in the PTG strain when compared to the RH strain. It is well known that many of the parasitic membrane proteins are GPI anchored. Hence, it can be reasoned that the differences in the staining pattern observed are due to differences in the amount of free and protein bound GPI expressed on the ELVs.

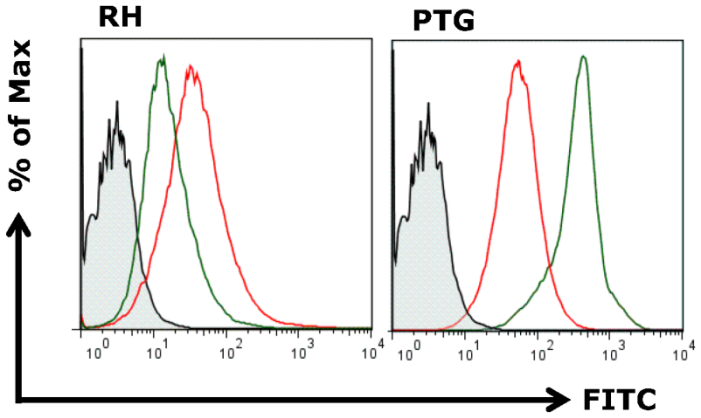

Figure 42. Glycolipid profile of RH and PTG ELVs. Histogram representation of GPI expression analyzed by flow cytometry ELVs from RH and PTG strains were coated on latex beads and the GPI isoforms analyzed by monoclonal antibodies T33F12 (Green histogram) and T54E10 (Red histogram) (1:100) probed with a FITC conjugated secondary detection antibody (1:500) and the flourecsnt beads were detected by flow cytometry. Corresponding controls included the beads with primary and secondary antibodies but no ELVs depicted as tinted histogram.

4.2.6 *Toxoplasma* ELVs Enhance Apoptosis of RAW Macrophages in the Presence of Exogenous IFN-γ

Although the proteins and glycolipids represented on the ELVs might potentially serve as a sub-unit vaccine component, the virulence and evasion mechanism between the two strains especially RH is partly attributed to the Rophtry associated injectable protein ROP18, kinase which along with the pseudo-kinase ROP5 and certain unidentified parasite components, target the IFN-γ inducible immunity related GTPase (IRG) thereby preventing destruction of the parasite by the host [275-278]. It is also known that *Toxoplasma gondii* can

deliver proteins to distant cells that it does not invade and modulate the behavior of these cells [267]. However, the mechanism of delivery of parasite factors and proteins is not completely understood. Such mechanisms might act as a decoy to direct the immune response away from the actual infected cell forming a mechanism of host resistance by the parasite. It has also been reported that one of the mechanisms of host resistance employed by *Toxoplasma gondii* is by immunosuppression which is mediated by the apoptosis of uninfected bystander macrophages inhibiting monocyte function and hence favoring parasite growth. It has been reported that macrophages in the lower chamber of a trans-well assay system undergo apoptosis when co-cultured with *Toxoplasma gondii* parasites in the upper chamber. This by-stander cell apoptosis has been shown to be mediated by IFN- γ and NO in addition with certain unidentified parasite molecules [279]. Hence, it was hypothesized that the ELVs, by virtue of their size, form perfect particulate systems that pack a rich cargo of proteins and glycolipids to form unconventional delivery systems of virulence factors. To confirm this hypothesis, a trans-well system was used with a 0.45 μm filter disc, where tachyzoites were incubated in the upper chamber for 6 h with the lower chamber containing culture medium. After the incubation time, the medium from the lower chamber was analyzed for the presence of ELVs that might have traversed from the upper chamber. As expected, parasite derived vesicular components were observed in the lower chamber of the trans-well system when analyzed by electron microscopy where the vesicles had a size of 80-100 nm with a surface morphology similar to that of ELVs (Figure 43A). The vesicles from the lower chamber had an average size 83 nm and a polydispersity index of 0.26 and their protein profile was similar to that of ELVs isolated under normal conditions (Figure 43B and C). With this observation, it was investigated whether the ELVs can induce apoptosis in the murine macrophages (RAW 264.7) and if there was a strain specific effect. Hence, ELVs isolated from the RH and PTG strains of the parasite were incubated with the murine macrophage cell-line RAW264.7 for 12 h followed by the addition of 50 IU/ml of IFN- γ for an additional 24 h. After the incubation time, the level of apoptosis was measured using fluorescently labelled Annexin-V which binds phososerine residues on the apoptotic cell. As seen in Figure 43D, there was a 10% increase in apoptosis in cells treated with ELVs from RH and PTG strains incubated with IFN- γ as compared to the cells which were treated with IFN- γ alone. The control cells that were incubated with ELVs alone showed apoptotic levels similar to the mock treated cells that were incubated with PBS.

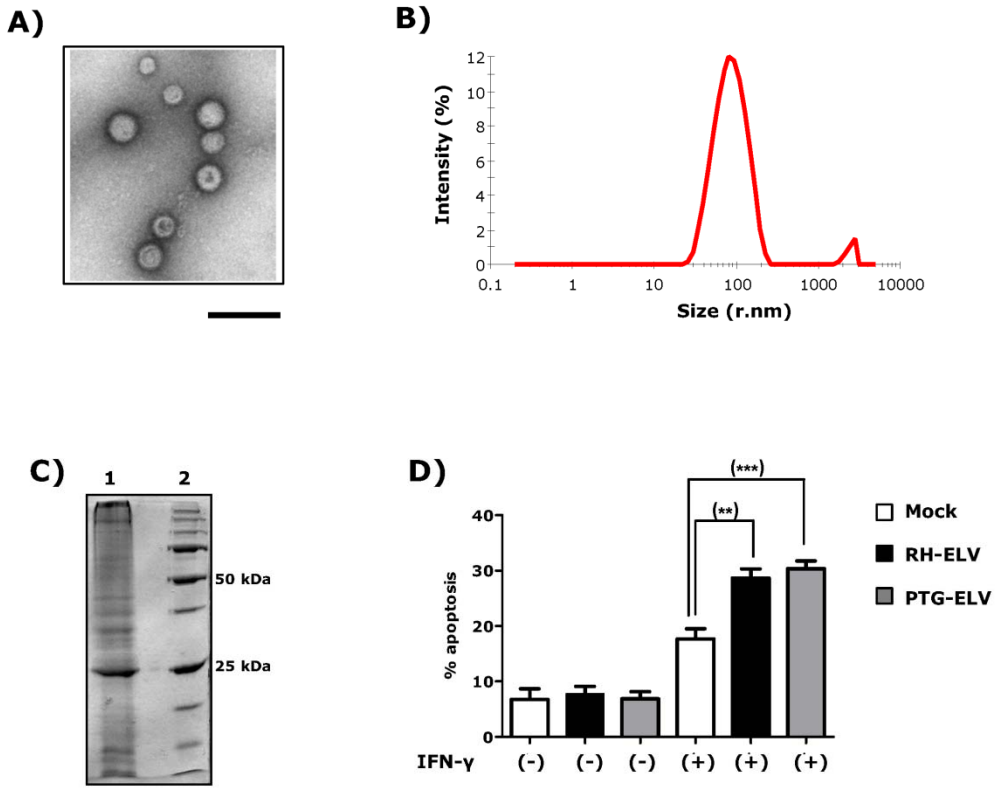

Figure 43. Apoptotic assay of RAW 264.7 macrophages with RH and PTG ELVs. (A) Electron micrograph of ELVs isolated from the lower chamber of the transwell assay system observed by TEM-with uranyl acetate negative staining. Scale bar represents 100 nm **(B)** Coomassie stained SDS-PAGE profile of ELV pellet from lower chamber (1. Dual color protein ladder with 250-12.5 KDa bands, 2.2 µg RH- ELV). (**C**) ELV size distribution and polydispersity measured by Dynamic Light Scattering. (**D**) Percentage apoptosis of RAW macrophages induced by 2 µg ELVs of RH and PTG strains in presence/ absence of 50 U/mL IFN-γ. (n=3 independent experiments)

These results imply that ELVs by themselves do not induce apoptosis under the experimental conditions but in the presence of an external inducer of apoptosis like IFN-γ they enhance the apoptotic process. These results also gain importance as the mechanism of immune response against *Toxoplasma gondii* is primarily mediated by a Th-1 response with IFN-γ being one of the most important host-resistance factors. However, both the RH and PTG ELVs induced a similar level of apoptosis and there was no significant strain specific difference between the two at the level of apoptosis. These results also indicate that the ELVs described in this thesis might have a greater role in the innate immune mechanisms involved during *Toxoplasma gondii* infections *in vivo*.

4.3 Conclusion

In the present study the isolation and characterization of extracellular vesicles of *Toxoplasma gondii* both from *in vivo* and *in vitro* sources are reported. The term Exosome Like Vesicels (ELV) was used as the morphology and size of these vesicles was in range between that of exosomes and microvesicles and a clear distintion could not be made [270]. However, this finding is important as both exosomes and microvesicles are known to be involved in unconventional transport of cargo in other cell types and also in other unicellular pathogens. Hence the ELVs might represent a novel mode of pathogenesis used by *T. gondii*. A large body of literature points to the fact that *T. gondii* can be induced to secret or release certain effector molecules into the surrounding medium [280]. This is also true in the *in vivo* context where *Toxoplasma* protein antigens are detected in circulation at high quantities as early as 6 Hrs post parasite infection/invasion [281]. Although the total time required for the successful invasion event of the host ranges between 1-2 mins, this high abundance of secretory proteins can be explained by clinical observation of live parasites in the peripheral blood of immunocompetent individuals with either acute or chronic infection [282]. It has been shown that tachyzoites of *Toxoplasma gondii* modify the invading host cell by secretion of membranous vesicles comprising of the SAG and GRA family of proteins which impair the normal endocytic machinery of the host cell, favouring parasite growth [283, 284]. Hence the ELVs described in this thesis might be involved in these processes.

The isolated ELVs contained several important parasitic proteins in addition to SAG and GRA family of proteins. However, the analysis of the ELVs between the RH and PTG strains showed no major differences in the protein profile. This was in agreement with the total proteome of the two strains reported [273]. The virulence between the two strains especially RH strain is attributed to the Rophtry associated injectable protein ROP18 kinase along with the pseudo-kinase ROP5 which together target the IFN-γ inducible imunity related GTPase (IRG) thereby preventing destruction of the parasite by the host [275-278]. We did not find any ROP family of proteins in the ELV fraction and also the apoptosis assay showed no significant difference between the RH and PTG strains. However, the increase in apoptosis as compared to the mock might have been due to other parasitic components. GPIs are known to induce the production of significant amounts of TNF-α and IL-12 with no distinction between the two strains and the isolated pure fractions of GPI are shown to have neither pro nor anti-apoptotic activity [285, 286] [287]. However, in case of ELVs, immuno electron microscopy analysis reveal that the GPIs are present in a particulate form along with various

parasitic proteins and this combination could have promoted apoptosis. Recent reports suggest that *T. gondii* can deliver proteins to distant host cells that it does not invade and can modulate the behavior of these cells [267]. Hence the ELVs isolated here might function as a decoy by directing the immune response away from the actual infected cell. Extracellular vesicles like exosomes and microparticles are involved in the horizontal transfer of RNA. *Toxoplasma gondii* when exposed to extracellular stress is shown to produce RNA granuels which improve the fitness and survival of the parasite [288]. Exosome mediated RNA transfer from HFF cells infected with *Toxoplasma gondii* has been recently reported [289]. The authors however could not detect any RNA of parasite origin. The ELVs characterized in this thesis contained mi-RNA like species but no m-RNA when subjected to RNA isolation protocols. This was in agreement with exosomal RNAs analysed from other cell types [290]. However, these were prilimary observations that needs further investigation and is not part of this thesis.

The observations reported here draws attention to the previously unnoticed role of Toxoplama ELVs. The role of ELVs in the context of *in vivo Toxoplasma* infection needs further investigation and the results might give useful insights for designing reliable prevention and propylactic strategies against Toxoplasmosis.

5. References

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Sample Info & Protocols
Name: RH1

Search Type Combined MS/MS - ProteinExtractor
Search Result RH1_2014-08-20 16:00:10
Search Location /BOPS/RH1/

Protein 1: Chain A, Crystal Structure Of A Parasite Protein
Accession: gi|22219177 **Score:** 758.55
Database: NCBInr **MW [kDa]:** 29.80
Seq. Coverage [%]: 48.40 % **pI:** 7.73
No. of Peptides: 15

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
176	3	815.8800	-55.64	2	33.6	41.9	0	38-52	K.TALTEPPTLAYSPNR.Q	
21	3	749.2950	-56.37	2	20.1	47.4	0	53-66	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
252	9	798.8860	-50.51	2	40.3	64.9	0	103-116	K.FPVTTQTFVVGCIK.G	Carbamidomethyl: 12
137	1	869.3720	-30.32	2	30.7	45.6	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7
95	1	877.8790	-10.05	2	26.9	43.3	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8; Deamidated: 5
39	4	508.7550	-44.82	2	21.8	64.9	0	133-142	R.ASSVVNNVAR.C	
15	3	509.2610	-17.30	2	20.6	62.8	0	133-142	R.ASSVVNNVAR.C	Deamidated: 6
44	3	509.2330	-72.25	2	22.4	48.6	0	133-142	R.ASSVVNNVAR.C	Deamidated: 7
96	3	677.7710	-70.83	2	27.4	76.9	0	143-155	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
157	2	782.8480	-52.51	2	32.5	39.8	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
131	3	790.8560	-38.65	2	30.2	29.7	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
78	1	585.3310	-50.63	1	25.2	23.9	0	198-202	K.DILPK.L	
104	4	774.3190	592.81	2	27.8	45.7	0	203-216	K.LTENPWQGNASSDK.G	
77	2	652.7850	-67.98	2	25.3	54.6	0	233-245	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6
75	2	511.2350	-67.05	2	26.0	52.7	0	262-262	K.LEFAGAAGSAK.S	

Protein 2: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484605 **Score:** 471.10
Database: NCBInr **MW [kDa]:** 34.90
Seq. Coverage [%]: 35.40 % **pI:** 8.07
No. of Peptides: 10

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
165	1	645.8010	-12.35	2	32.7	48.0	0	42-52	K.CDQDGSLLNLR.V	Carbamidomethyl: 1
39	1	534.2440	-52.45	2	22.8	57.9	0	53-62	R.VTGNSSVEFK.C	
56	1	534.7150	-91.68	2	24.3	26.5	0	53-62	R.VTGNSSVEFK.C	Deamidated: 4
45	1	473.2500	24.62	3	23.3	39.5	0	63-76	K.CGGAVPNLHPNPGK.T	Carbamidomethyl: 1
7	1	525.7390	-27.53	2	19.2	67.6	0	126-135	K.CSSLANTAAR.V	Carbamidomethyl: 1
1	1	476.5580	-37.11	3	17.1	30.5	1	136-149	R.VGAGQPGQEAEKEK.T	
222	1	793.3900	-42.82	2	37.9	32.0	0	150-163	K.TCNQTSVWGPPIK.G	Carbamidomethyl: 2
27	1	540.6750	-114.58	2	21.8	43.6	0	167-175	K.EYPTPDACK.N	Carbamidomethyl: 8
148	1	630.2830	-70.64	2	31.5	43.3	0	176-187	K.NGTLSLEVNPSK.K	Deamidated: 1
146	1	785.7180	1.57	3	31.3	68.2	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.I	Carbamidomethyl: 2

Protein 3: actin [Toxoplasma gondii ME49]
Accession: gi|237840731 **Score:** 443.08
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 38.30 % **pI:** 4.91
No. of Peptides: 14

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
137	2	571.8350	-26.65	2	29.9	35.8	0	30-40	R.AVFPSIVGKPK.N	
103	2	610.7770	-14.94	2	27.1	24.5	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5
65	2	618.7540	-47.81	2	24.3	26.3	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5, 8
24	2	636.2200	-69.26	2	21.2	31.7	0	52-62	K.DCYVGDEAQSK.R	Carbamidomethyl: 2
209	1	973.9500	-1.32	2	35.6	24.1	0	70-88	K.YPIEHGIVTNWDDMEK.I	
172	1	981.9100	-39.45	2	33.0	23.9	0	70-88	K.YPIEHGIVTNWDDMEK.I	Oxidation: 14
140	3	505.8980	-45.93	3	30.2	43.4	0	86-96	K.IWHHTFYNELR.V	

193	2	651.9830	-66.44	3	34.0	29.2	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
150	1	523.7000	-57.83	2	31.1	37.8	0	185-192	R.DLTEYMMK.I	Oxidation: 7
71	1	530.7140	-69.56	2	24.9	23.2	0	198-207	R.GYGFTTSAEK.E	
225	1	888.9170	-27.87	2	37.0	50.2	0	240-255	K.SYELPDGNIITVGNER.F	
70	1	479.7010	-62.83	2	24.7	24.8	0	278-288	R.TTFDSIMK.C	Oxidation: 7
115	4	597.2600	-79.94	2	28.2	24.0	0	317-327	K.ELETSLAPSTMK.I	Oxidation: 10
75	1	759.3140	-43.43	2	28.1	27.3	0	361-373	K.EEYDESGPSIVHR.K	

Protein 4: actin [Symbiodinium sp. clade C]
Accession: gi|86562730 **Score:** 357.04
Database: NCBInr **MW [kDa]:** 41.80
Seq. Coverage [%]: 13.00 % **pI:** 5.22
No. of Peptides: 4

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	488.7170	-22.06	2	22.2	74.5	0	20-29	K.AGFAGDDAPR.A	
17	2	599.7230	-69.62	2	20.5	52.7	0	52-62	K.DSYVGDEAQSK.R	
195	1	651.6790	496.29	3	33.9	21.7	0	97-114	R.PAPEEHPVLLTEAPLNPK.A	
93	1	566.7440	-40.76	2	26.6	32.7	0	198-207	R.GYSFTTTAER.E	

Protein 5: actin [Pyrocystis lunula]
Accession: gi|27450759 **Score:** 321.12
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 4.30 % **pI:** 5.83
No. of Peptides: 1

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
240	1	895.9210	-31.92	2	38.3	56.7	0	239-254	K.SYELPDGQVITIGNER.F	

Protein 6: SAG-3 [Toxoplasma gondii]
Accession: gi|300680036 **Score:** 288.09
Database: NCBInr **MW [kDa]:** 41.70

Seq. Coverage [%]: 12.70 % **pI:** 6.59
No. of Peptides: 5

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
179	3	586.2790	-72.51	2	33.0	41.1	0	55-64	K.ITYFGTLTQK.A	
79	2	470.5620	-47.37	3	25.4	35.0	0	76-88	R.ANEEVVGHVTLNK.E	
258	2	591.2790	-58.66	2	39.3	35.6	0	135-145	K.GFLTDYIPGAK.Q	
91	1	584.2690	-54.95	3	26.2	42.4	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
81	2	690.8160	-29.98	2	25.7	57.6	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 3

Protein 7: microneme protein MIC3 [Toxoplasma gondii ME49]
Accession: gi|237841071 **Score:** 268.58
Database: NCBInr **MW [kDa]:** 37.90
Seq. Coverage [%]: 27.00 % **pI:** 6.02
No. of Peptides: 7

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
80	1	932.3890	-52.57	2	25.1	51.2	0	71-86	K.QETQLCAISSEGKPCR.N	Carbamidomethyl: 6, 15
131	1	603.6130	-1.53	3	30.3	29.7	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 1
146	2	603.2630	-37.99	3	30.4	33.4	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14
58	4	610.5670	-56.81	3	24.4	35.9	0	157-173	K.NAECVENLDAGGGVHCK.C	Carbamidomethyl: 4, 16
173	2	915.3660	-23.66	2	32.6	37.4	0	176-192	K.DGFVGTGLTCSEDPCSK.R	Carbamidomethyl: 10, 15
100	1	831.5920	-71.07	3	26.5	23.1	0	249-269	R.CIDDASHENGYTCECPTGYSR.E	Carbamidomethyl: 1, 13, 15; Deamidated: 9
92	2	543.2140	-66.69	2	26.1	58.8	0	294-303	K.EFGISASSCK.C	Carbamidomethyl: 9

Protein 8: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640 **Score:** 261.19
Database: NCBInr **MW [kDa]:** 33.90
Seq. Coverage [%]: 19.70 % **pI:** 6.04
No. of Peptides: 6

Modification(s): Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
14	1	637.2820	-63.98	2	19.9	41.7	0	36-50	R.AAVAAAAANGAETGK.A	Deamidated: 9
23	1	636.7540	-73.43	2	20.6	87.7	0	36-50	R.AAVAAAAANGAETGK.A	
42	1	517.2620	-28.19	2	22.1	42.9	0	56-65	K.LASQMSLAAR.S	Oxidation: 5
53	1	627.7970	-77.55	2	22.9	43.0	0	114-124	K.APPYKPIPESR.F	
288	1	817.3980	-42.64	2	41.9	51.0	0	180-192	K.QPFTILYLEPEQR.T	
165	1	751.8670	-7.04	2	31.9	24.9	0	193-205	R.TFAEPEQPDIEVK.D	

Protein 9: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163 **Score:** 177.82
Database: NCBInr **MW [kDa]:** 82.10
Seq. Coverage [%]: 6.40 % **pI:** 5.21
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	551.7730	-64.82	2	22.1	51.3	1	240-250	K.AVAELKAGESK.A	
137	1	541.2890	54.54	3	29.6	42.9	0	540-553	R.IDDPEHPAGELCLR.G	Carbamidomethyl: 12
170	1	554.8010	14.56	2	32.0	38.5	0	554-563	R.GPTITPGYFR.N	
143	1	801.3460	-9.56	2	30.0	25.4	0	732-744	K.EIDALYSEMETER.A	Oxidation: 9

Protein 10: dense granule protein [Toxoplasma gondii]
Accession: gi|2062409 **Score:** 176.49
Database: NCBInr **MW [kDa]:** 25.30
Seq. Coverage [%]: 23.30 % **pI:** 4.80
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
71	1	493.7560	-22.09	2	24.4	23.8	0	72-80	K.ASVESQLPR.R	
123	1	713.9800	-32.08	3	28.4	56.0	1	81-97	R.REPLETEPDEQEEVHFR.K	
87	1	670.2630	-30.93	3	26.0	47.8	0	103-119	R.SDAEVTDDNIYEEHTDR.K	

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
39	1	671.3710	25.21	2	28.9	30.6	0	156-166	R.LVPELTEEQQR.G	

Protein 11: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 173.95
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 13.70 % **pI:** 7.76
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
255	3	702.8260	-65.59	2	38.9	51.8	0	136-148	K.EWVTGDSVLTGLK.I	
102	3	702.3150	-66.77	2	27.2	34.3	0	149-161	K.ISVPESQYPANAK.S	
112	2	548.9320	-1.09	3	28.0	25.3	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5; Oxidation: 8
151	2	543.5920	-16.48	3	30.9	36.6	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5
59	2	650.2650	-50.61	2	24.2	26.0	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1

Protein 12: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499 **Score:** 148.54
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 11.70 % **pI:** 5.66
No. of Peptides: 4

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
10	5	847.9980	-29.05	3	18.4	51.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 21
5	3	848.0140	-10.18	3	17.8	38.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 18
5	1	848.3400	-12.54	3	18.4	25.1	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 17, 21
13	3	795.9600	-36.35	3	19.1	26.4	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 20

Protein 13: surface antigen P22 [Toxoplasma gondii]

Accession: gi|13957751 **Score:** 137.13
Database: NCBInr **MW [kDa]:** 19.00
Seq. Coverage [%]: 21.50 % **pI:** 9.35
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
76	1	785.8590	592.83	2	35.2	24.7	0	61-75	K.LTISPSGEGDVFYGK.E	
66	2	706.4370	-15.26	2	33.8	49.8	1	82-95	K.KLTTVLPGAVLTAK.V	
5	3	529.6970	-111.46	2	19.2	62.6	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1

Protein 14: subtilisin-like protein [Toxoplasma gondii]
Accession: gi|15419013 **Score:** 136.55
Database: NCBInr **MW [kDa]:** 85.00
Seq. Coverage [%]: 6.50 % **pI:** 5.07
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
27	1	603.2110	-88.52	2	21.1	21.1	0	271-280	R.DNMEVNQAER.D	
24	1	547.2600	-46.53	2	20.8	44.7	0	615-625	R.TPPSAPSPSPR.T	
2	3	561.6180	2.99	3	18.4	25.2	0	706-721	R.SGSQPKPPQDNTTTPK.M	
122	1	862.3040	-68.05	2	28.4	45.5	0	746-760	K.EEEPPTDEDDFSSVK.G	

Protein 15: phosphofructokinase, putative [Toxoplasma gondii ME49]
Accession: gi|237834103 **Score:** 133.85
Database: NCBInr **MW [kDa]:** 131.30
Seq. Coverage [%]: 2.80 % **pI:** 5.82
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
178	1	647.3240	6.64	2	32.8	50.0	0	406-416	R.SVFEELPESTR.R	

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
94	1	676.8230	-22.54	2	26.0	24.9	1	744-754	R.TEREEAELFTK.E	
119	1	644.3100	-18.19	2	28.1	58.9	0	798-809	R.VVCVEPSGDLGR.F	Carbamidomethyl: 3

Protein 16: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826 **Score:** 133.11
Database: NCBInr **MW [kDa]:** 45.10
Seq. Coverage [%]: 10.00 % **pI:** 7.74
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
329	1	780.3650	-48.99	2	46.1	51.9	0	58-70	R.FLDPIDELPEPFK.A	
169	1	547.2190	-122.67	3	32.7	42.9	0	101-115	K.SPISPDVAVYVHAER.L	
210	1	705.2920	-68.21	2	35.7	38.3	1	389-400	K.LCDAFTDVAIRE.-	Carbamidomethyl: 2

Protein 17: dense granule protein 3 [Toxoplasma gondii ME49]
Accession: gi|237834147 **Score:** 120.94
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 8.60 % **pI:** 9.71
No. of Peptides: 3

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	608.3400	-17.76	2	31.4	39.9	1	114-123	K.KVEEELSLLR.R	
194	1	544.2780	-46.52	2	34.4	59.5	0	115-123	K.VEEELSLLR.R	
15	1	548.2680	-29.93	2	19.6	21.8	1	192-200	K.RQPFMSSVK.N	Oxidation: 5

Protein 18: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688 **Score:** 120.50
Database: NCBInr **MW [kDa]:** 144.50
Seq. Coverage [%]: 1.90 % **pI:** 5.80
No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
19	1	621.8260	9.86	2	20.1	59.6	0	46-58	K.VGSQPVAAGAEEK.V	
189	1	702.8200	-56.23	2	33.5	25.9	0	673-684	R.VQELEVPFNSSR.K	

Protein 19: heat shock protein 70 [Toxoplasma gondii]
Accession: gi|5738968 **Score:** 120.12
Database: NCBInr **MW [kDa]:** 68.50
Seq. Coverage [%]: 0.00 % **pI:** 5.03
No. of Peptides: 0

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

Protein 20: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049 **Score:** 118.79
Database: NCBInr **MW [kDa]:** 39.40
Seq. Coverage [%]: 11.30 % **pI:** 4.66
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
328	1	748.9970	54.79	2	45.0	51.4	0	133-146	R.LVDVSTLIPTAIVR.G	
122	1	503.7470	-72.67	2	28.4	25.2	0	188-196	R.SCIVTVTVK.K	Carbamidomethyl: 2
350	1	1072.5000	-25.51	2	48.4	25.3	0	313-332	R.QDEGLGVCIMQALLPASEGR.R	Carbamidomethyl: 8

Protein 21: hypothetical protein TGME49_003600 [Toxoplasma gondii ME49]
Accession: gi|237836689 **Score:** 87.16
Database: NCBInr **MW [kDa]:** 47.20
Seq. Coverage [%]: 4.80 % **pI:** 8.46
No. of Peptides: 2

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
50	1	559.7660	-1.04	2	22.6	22.3	0	194-204	K.TGSPVCAVGDR.D	Carbamidomethyl: 6
209	1	548.7660	-35.16	2	35.5	64.9	0	322-331	K.SGYSLSDLVR.S	

Protein 22: MORN repeat-containing protein [Toxoplasma gondii ME49]
Accession: gi|237838513 **Score:** 85.36
Database: NCBInr **MW [kDa]:** 49.20
Seq. Coverage [%]: 3.40 % **pI:** 5.15
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
4	1	745.8750	15.65	2	17.3	85.4	0	408-423	R.QTASTAGSAQPATGSR.A	

Protein 23: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635 **Score:** 68.23
Database: NCBInr **MW [kDa]:** 70.50
Seq. Coverage [%]: 1.90 % **pI:** 5.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
49	1	560.7540	-84.66	2	21.7	68.2	0	579-590	R.LSGTGSTGATIR.V	

Protein 24: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221480679 **Score:** 65.86
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 4.10 % **pI:** 6.59
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

234	1	797.8430	-67.22	2	37.4	49.6	0	115-129	K.GIDASAFTFTPPSQR.A	

Protein 25: smc family/structural maintenance of chromosome [Babesia bovis]
Accession: gi|156084430 **Score:** 54.17
Database: NCBInr **MW [kDa]:** 137.40
Seq. Coverage [%]: 0.70 % **pI:** 8.63
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
86	1	479.7500	947.99	2	25.8	54.2	1	955-962	R.VVSDRLLR.D	

Protein 26: Fructose-bisphosphate aldolase class-I family protein [Tetrahymena thermophila]
Accession: gi|118401200 **Score:** 50.04
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 3.90 % **pI:** 7.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
129	2	666.8350	-28.35	2	29.3	50.0	0	21-34	K.GILAADESTGTIGK.K	

Protein 27: microneme protein, putative [Toxoplasma gondii GT1]
Accession: gi|221486835 **Score:** 49.39
Database: NCBInr **MW [kDa]:** 36.30
Seq. Coverage [%]: 3.80 % **pI:** 5.01
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
21	1	724.7930	-4.96	2	20.4	49.4	0	249-261	R.YGGCASDNAGSYR.C	Carbamidomethyl: 4

Search Type Combined MS/MS - ProteinExtractor
Search Result RH1_2nd_2014-09-10 10:27:36
Search Location /BOPS/RH1/

Protein 1: Chain A, Crystal Structure Of A Parasite Protein
Accession: gi|22219177 **Score:** 758.55
Database: NCBInr **MW [kDa]:** 29.80
Seq. Coverage [%]: 48.40 % **pI:** 7.73
No. of Peptides: 15

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
176	3	815.8800	-55.64	2	33.5	41.9	0	38-52	K.TALTEPPTLAYSPNR.Q	
21	3	749.2950	-56.37	2	20.1	47.4	0	53-66	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
252	9	798.8860	-50.51	2	40.3	64.9	0	103-116	K.FPVTTQTFVVGCIK.G	Carbamidomethyl: 12
137	1	869.3720	-30.32	2	30.7	45.6	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7
95	1	877.8790	-10.05	2	26.9	43.3	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8; Deamidated: 5
39	4	508.7550	-44.82	2	21.8	64.9	0	133-142	R.ASSVVNNVAR.C	
15	3	509.2610	-17.30	2	20.6	62.8	0	133-142	R.ASSVVNNVAR.C	Deamidated: 6
44	3	509.2330	-72.29	2	22.4	48.6	0	133-142	R.ASSVVNNVAR.C	Deamidated: 7
96	3	677.7710	-70.83	2	27.4	70.9	0	143-155	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
157	2	782.8480	-52.51	2	32.8	39.8	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
131	3	790.8560	-38.65	2	30.2	29.7	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
78	1	585.3310	-50.63	1	25.2	23.9	0	198-202	K.DILPK.L	
104	4	774.3190	592.81	2	27.8	45.7	0	203-216	K.LTENPWQGNASSDK.G	
77	2	652.7850	-67.98	2	25.3	54.0	0	233-245	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6
75	2	511.2350	-67.05	2	26.0	52.7	0	252-262	K.LEFAGAAGSAK.S	

Protein 2: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484605 **Score:** 471.10
Database: NCBInr **MW [kDa]:** 34.90
Seq. Coverage [%]: 35.40 % **pI:** 8.07

No. of Peptides: 10

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
165	1	645.8010	-12.38	2	32.7	48.0	0	42-52	K.CDQDGSLLNLR.V	Carbamidomethyl: 1
39	1	534.2440	-52.48	2	22.8	57.0	0	53-62	R.VTGNSSVEFK.C	
56	1	534.7150	-91.68	2	24.3	26.5	0	53-62	R.VTGNSSVEFK.C	Deamidated: 4
45	1	473.2500	24.62	3	23.3	39.5	0	63-76	K.CGGAVPNLHPNPGK.T	Carbamidomethyl: 1
7	1	525.7390	-27.53	2	19.2	67.6	0	126-139	K.CSSLANTAAR.V	Carbamidomethyl: 1
1	1	476.5580	-37.11	3	17.1	30.5	1	136-149	R.VGAGQPGQEAEKEK.T	
222	1	793.3900	-42.82	2	37.9	32.0	0	150-163	K.TCIVQTSVWGPPIK.G	Carbamidomethyl: 2
27	1	540.6750	-114.58	2	21.8	43.6	0	167-175	K.EYPTPDACK.N	Carbamidomethyl: 8
148	1	630.2830	-70.64	2	31.5	43.3	0	176-187	K.NGTLSLEVNPSK.K	Deamidated: 1
146	1	785.7180	1.57	3	31.3	68.2	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.T	Carbamidomethyl: 2

Protein 3: actin [Toxoplasma gondii ME49]
Accession: gi|237840731
Database: NCBInr
Seq. Coverage [%]: 38.30 %
Score: 443.08
MW [kDa]: 41.90
pI: 4.91
No. of Peptides: 14

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
137	2	571.8350	-26.65	2	29.9	35.8	0	30-40	R.AVFPSIVGKPK.N	
103	2	610.7770	-14.94	2	27.1	24.5	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5
65	2	618.7540	-47.81	2	24.3	26.3	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5, 8
24	2	636.2200	-69.28	2	21.2	31.7	0	52-62	K.DCYVGDEAQSK.R	Carbamidomethyl: 2
209	1	973.9500	-1.32	2	35.6	24.1	0	70-85	K.YPIEHGIVTNWDDMEK.I	
172	1	981.9100	-39.48	2	33.0	23.9	0	70-85	K.YPIEHGIVTNWDDMEK.I	Oxidation: 14
140	3	505.8980	-45.93	3	30.2	43.4	0	86-90	K.IWHHTFYNELR.V	
193	2	651.9830	-66.44	3	34.0	29.2	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
150	1	523.7000	-57.83	2	31.1	37.8	0	185-192	R.DLTEYMMK.I	Oxidation: 7
71	1	530.7140	-69.56	2	24.9	23.2	0	198-207	R.GYGFTTSAEK.E	
225	1	888.9170	-27.87	2	37.0	50.2	0	240-255	K.SYELPDGNIITVGNER.F	

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
70	1	479.7010	-62.83	2	24.7	24.5	0	279-289	R.TTFDSMK.C	Oxidation: 7
115	4	597.2600	-79.94	2	28.2	24.6	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10
75	1	759.3140	-43.43	2	25.1	27.3	0	361-373	K.EEYDESGPSIVHR.K	

Protein 4: actin [Symbiodinium sp. clade C]
Accession: gi|86562730
Database: NCBInr
Seq. Coverage [%]: 13.00 %
Score: 357.04
MW [kDa]: 41.80
pI: 5.22
No. of Peptides: 4

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	488.7170	-22.06	2	22.2	74.5	0	20-29	K.AGFAGDDAPR.A	
17	2	599.7230	-69.62	2	20.5	52.7	0	52-62	K.DSYVGDEAQSK.R	
195	1	651.6790	498.29	3	33.9	21.7	0	97-114	R.PAPEEHPVLLTEAPLNPK.A	
93	1	566.7440	-40.76	2	26.5	32.7	0	198-207	R.GYSFTTTAER.E	

Protein 5: actin [Pyrocystis lunula]
Accession: gi|2740759
Database: NCBInr
Seq. Coverage [%]: 4.30 %
Score: 321.12
MW [kDa]: 41.70
pI: 5.83
No. of Peptides: 1

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
240	1	895.9210	-31.92	2	38.3	56.7	0	239-254	K.SYELPDGQVITIGNER.F	

Protein 6: SAG-3 [Toxoplasma gondii]
Accession: gi|300680036
Database: NCBInr
Seq. Coverage [%]: 12.70 %
Score: 288.09
MW [kDa]: 41.70
pI: 6.59
No. of Peptides: 5

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
179	3	586.2790	-72.51	2	33.0	41.1	0	55-64	K.ITYFGTLTQK.A	
79	2	470.5620	-47.37	3	25.4	35.0	0	76-88	R.ANEEVVGHVTLNK.E	
258	2	591.2790	-58.66	2	39.3	35.6	0	135-145	K.GFLTDYIPGAK.Q	
91	1	584.2680	-54.95	3	26.2	42.4	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
81	2	690.8150	-29.98	2	28.7	57.6	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 3

Protein 7: microneme protein MIC3 [Toxoplasma gondii ME49]
Accession: gi|237841071
Database: NCBInr
Seq. Coverage [%]: 27.00 %
Score: 268.58
MW [kDa]: 37.90
pI: 6.02
No. of Peptides: 7

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
80	1	932.3890	-62.57	2	25.1	51.2	0	71-86	K.QETQLCAISSEGKPCR.N	Carbamidomethyl: 6, 15
131	1	603.6130	-1.53	3	30.3	29.7	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 1
146	2	603.2630	-37.99	3	30.4	33.4	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14
58	4	610.5670	-56.81	3	24.4	35.9	0	157-173	K.NAECVENLDAGGGVHCK.C	Carbamidomethyl: 4, 16
173	2	915.3660	-23.68	2	32.6	37.4	0	176-192	K.DGFVGTGLTCSEDPCSK.R	Carbamidomethyl: 10, 15
100	1	831.5920	-71.07	3	26.5	23.1	0	249-269	R.CIDDASHENGYTCECPTGYSR.E	Carbamidomethyl: 1, 13, 15; Deamidated: 9
92	2	543.2140	-66.69	2	26.1	58.0	0	294-303	K.EFGISASSCK.C	Carbamidomethyl: 9

Protein 8: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640
Database: NCBInr
Seq. Coverage [%]: 19.70 %
Score: 261.19
MW [kDa]: 33.90
pI: 6.04
No. of Peptides: 6

Modification(s): Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
14	1	637.2820	-63.98	2	19.9	41.7	0	36-50	R.AAVAAAAANGAETGK.A	Deamidated: 9

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
23	1	636.7840	-73.43	2	20.6	57.7	0	36-50	R.AAVAAAAANGAETGK.A	
42	1	517.2620	-28.19	2	22.1	42.9	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5
53	1	627.7970	-77.55	2	22.9	43.8	0	114-124	K.APPYKPIPESR.F	
288	1	817.3980	-42.64	2	41.9	51.0	0	180-192	K.QPFTILYLEPEQR.T	
165	1	751.8670	-7.04	2	31.9	24.9	0	193-205	R.TFAEPEQPDIEVK.D	

Protein 9: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163
Database: NCBInr
Seq. Coverage [%]: 6.40 %
Score: 177.82
MW [kDa]: 82.10
pI: 5.21
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	551.7730	-64.82	2	22.1	51.3	1	240-250	K.AVAELKAGESK.A	
137	1	541.2890	54.54	3	29.6	42.9	0	540-553	R.IDDPEHPAGELCLR.G	Carbamidomethyl: 12
170	1	554.8010	14.56	2	32.0	38.5	0	554-563	R.GPTITPGYFR.N	
143	1	801.3460	-9.50	2	30.0	25.4	0	732-744	K.EIDALYSEMETER.A	Oxidation: 9

Protein 10: dense granule protein [Toxoplasma gondii]
Accession: gi|2062409
Database: NCBInr
Seq. Coverage [%]: 23.30 %
Score: 176.49
MW [kDa]: 25.30
pI: 4.80
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
71	1	493.7560	-22.09	2	24.4	23.8	0	72-80	K.ASVESQLPR.R	
123	1	713.9800	-32.08	3	28.4	56.0	1	81-97	R.REPLETEPDEQEEVHFR.K	
87	1	670.2630	-30.93	3	26.0	47.8	0	103-119	R.SDAEVTDDNIYEEHTDR.K	
39	1	671.3710	25.21	2	28.9	30.6	0	156-166	R.LVPELTEEQQR.G	

Protein 11: SRS domain-containing protein [Toxoplasma gondii ME49]

Accession: gi|237837819
Database: NCBInr
Seq. Coverage [%]: 13.70 %
Score: 173.95
MW [kDa]: 39.10
pI: 7.76
No. of Peptides: 5
Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
255	3	702.8260	-65.59	2	38.9	51.8	0	136-148	K.EWVTGDSVLTGLK.I	
102	3	702.3150	-66.77	2	27.2	34.3	0	149-161	K.ISVPESQYPANAK.S	
112	2	548.9320	-1.09	3	28.0	25.3	0	173-186	K.TGNTCMLTHVEPR.D	Carbamidomethyl: 5; Oxidation: 6
151	2	543.5920	-16.48	3	30.9	36.6	0	173-186	K.TGNTCMLTHVEPR.D	Carbamidomethyl: 5
59	2	650.2650	-50.61	2	24.2	26.6	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1

Protein 12: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499
Database: NCBInr
Seq. Coverage [%]: 11.70 %
Score: 148.54
MW [kDa]: 23.90
pI: 5.66
No. of Peptides: 4
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
10	5	847.9980	-29.05	3	18.4	51.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 21
5	3	848.0140	-10.18	3	17.8	38.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 18
6	1	848.3400	-12.64	3	18.4	25.1	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 17, 21
13	3	795.9600	-36.35	3	19.1	26.4	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGN EGR.G	Deamidated: 20

Protein 13: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751
Database: NCBInr
Seq. Coverage [%]: 21.50 %
Score: 137.13
MW [kDa]: 19.00
pI: 9.35

No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
76	1	785.8590	592.83	2	35.2	24.7	0	61-75	K.LTISPSGEGDVFYGK.E	
66	2	706.4370	-15.26	2	33.8	49.8	1	82-95	R.KLTTVLPGAVLTAK.V	
5	3	529.6970	-111.40	2	19.2	62.6	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1

Protein 14: subtilisin-like protein [Toxoplasma gondii]
Accession: gi|15419013
Database: NCBInr
Seq. Coverage [%]: 6.50 %
Score: 136.55
MW [kDa]: 85.00
pI: 5.07
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
27	1	603.2110	-88.52	2	21.1	21.1	0	271-280	R.DNMEVNQAER.D	
24	1	547.2600	-46.53	2	20.8	44.7	0	615-625	R.TPPSAPSPSPR.T	
2	3	561.6180	2.99	3	18.4	25.2	0	706-721	R.SGSQPKPPQDNTTTPK.M	
122	1	862.3040	-68.05	2	28.4	45.5	0	746-760	R.EEEPPTDEDDFSSVK.G	

Protein 15: phosphofructokinase, putative [Toxoplasma gondii ME49]
Accession: gi|237834103
Database: NCBInr
Seq. Coverage [%]: 2.80 %
Score: 133.85
MW [kDa]: 131.30
pI: 5.82
No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
178	1	647.3240	6.64	2	32.5	50.0	0	406-416	R.SVFEELPESTR.R	
94	1	676.8230	-22.54	2	26.0	24.9	1	744-754	R.TEREEAELFTK.E	
119	1	644.3100	-18.19	2	28.1	58.9	0	798-809	R.VVCVEPSGDLGR.F	Carbamidomethyl: 3

Protein 16: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826
Database: NCBInr
Seq. Coverage [%]: 10.00 %
Score: 133.11
MW [kDa]: 45.10
pI: 7.74
No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
329	1	780.3650	-48.99	2	46.1	51.9	0	58-70	R.FLDPIDELPEPFK.A	
169	1	547.2190	-122.67	3	32.7	42.9	0	101-115	K.SPSPDVAVVVNAER.L	
210	1	705.2920	-68.21	2	35.7	38.3	1	389-400	K.LCDAFTDVAIRE.-	Carbamidomethyl: 2

Protein 17: dense granule protein 3 [Toxoplasma gondii ME49]
Accession: gi|237834147
Database: NCBInr
Seq. Coverage [%]: 8.60 %
Score: 120.94
MW [kDa]: 23.90
pI: 9.71
No. of Peptides: 3
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	608.3400	-17.76	2	31.4	39.9	1	114-123	K.VEEEELSLLR.R	
194	1	544.2780	-46.52	2	34.4	59.8	0	115-123	K.VEEELSLLR.R	
15	1	548.2680	-29.93	2	19.6	21.6	1	192-200	K.RQPFMSSVK.N	Oxidation: 5

Protein 18: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688
Database: NCBInr
Seq. Coverage [%]: 1.90 %
Score: 120.50
MW [kDa]: 144.50
pI: 5.80
No. of Peptides: 2
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
19	1	621.8260	9.86	2	20.1	59.6	0	46-58	K.VGSQPVAAGAEEK.V	

189	1	702.8200	-56.23	2	33.5	25.9	0	673-684	R.VQELEVPFNSSR.K	

Protein 19: heat shock protein 70 [Babesia gibsoni]
Accession: gi|156511059
Database: NCBInr
Seq. Coverage [%]: 6.40 %
Score: 120.12
MW [kDa]: 39.50
pI: 6.36
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
188	1	744.3300	-32.61	2	31.9	85.6	0	36-48	R.TTPSYVAFTDTER.L	
144	1	574.7890	10.87	2	28.7	34.5	0	61-70	R.NPENTIFDAK.R	

Protein 20: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049
Database: NCBInr
Seq. Coverage [%]: 11.30 %
Score: 118.79
MW [kDa]: 39.40
pI: 4.66
No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
328	1	748.9970	54.79	2	45.0	51.4	0	133-146	R.LVDVSTLIPTAIVR.G	
122	1	503.7470	-72.87	2	28.4	25.2	0	188-196	R.SCIVTVTVK.K	Carbamidomethyl: 2
350	1	1072.5000	-25.51	2	48.4	25.3	0	313-332	R.QDEGLGVCIMQALLPASEGR.R	Carbamidomethyl: 8

Protein 21: hypothetical protein TGME49_003600 [Toxoplasma gondii ME49]
Accession: gi|237836689
Database: NCBInr
Seq. Coverage [%]: 4.80 %
Score: 87.16
MW [kDa]: 47.20
pI: 8.46
No. of Peptides: 2
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

50	1	559.7660	-1.04	2	22.6	22.3	0	194-204	K.TGSPVCAVGDR.D	Carbamidomethyl: 6
209	1	548.7660	-35.16	2	35.8	64.9	0	322-331	K.SGYSLSDLVR.S	

Protein 22: MORN repeat-containing protein [Toxoplasma gondii ME49]
Accession: gi|237838513 **Score:** 85.36
Database: NCBInr **MW [kDa]:** 49.20
Seq. Coverage [%]: 3.40 % **pI:** 5.15
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
4	1	745.8750	15.65	2	17.3	85.4	0	408-423	R.QTASTAGSAQPATGSR.A	

Protein 23: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635 **Score:** 68.23
Database: NCBInr **MW [kDa]:** 70.50
Seq. Coverage [%]: 1.90 % **pI:** 5.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
49	1	560.7540	-84.66	2	21.7	68.2	0	579-590	R.LSGTGSTGATIR.V	

Protein 24: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221480679 **Score:** 65.86
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 4.10 % **pI:** 6.59
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
234	1	797.8430	-67.22	2	37.4	49.6	0	115-129	K.GIDASAFTFTPPSQR.A	

Protein 25: smc family/structural maintenance of chromosome [Babesia bovis]
Accession: gi|156084430 **Score:** 54.17
Database: NCBInr **MW [kDa]:** 137.40
Seq. Coverage [%]: 0.70 % **pI:** 8.63
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
86	1	479.7500	947.99	2	25.6	54.2	1	955-962	R.VVSDRLLR.D	

Protein 26: Fructose-bisphosphate aldolase class-I family protein [Tetrahymena thermophila]
Accession: gi|118401200 **Score:** 50.04
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 3.90 % **pI:** 7.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
129	2	666.8350	-28.35	2	29.3	50.0	0	21-34	K.GILAADESTGTIGK.K	

Protein 27: microneme protein, putative [Toxoplasma gondii GT1]
Accession: gi|221486835 **Score:** 49.39
Database: NCBInr **MW [kDa]:** 36.30
Seq. Coverage [%]: 3.80 % **pI:** 5.01
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
21	1	724.7930	-4.96	2	20.4	49.4	0	249-261	R.YQGCASDNAGSYR.C	Carbamidomethyl: 4

Search Type Combined MS/MS - ProteinExtractor

Search Result RH1_3rd_2014-09-10 10:35:52
Search Location /BOPS/RH1/

Protein 1: Chain A, Crystal Structure Of A Parasite Protein
Accession: gi|22219177 **Score:** 758.55
Database: NCBInr **MW [kDa]:** 29.80
Seq. Coverage [%]: 48.40 % **pI:** 7.73
No. of Peptides: 15

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
176	3	815.8800	-55.64	2	33.5	41.9	0	38-52	K.TALTEPPTLAYSPNR.Q	
21	3	749.2950	-56.37	2	20.1	47.4	0	53-66	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
252	9	798.8960	-50.51	2	40.3	64.9	0	103-116	K.FPVTTQTFVVGCIK.G	Carbamidomethyl: 12
137	1	869.3720	-30.32	2	30.7	45.6	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7
95	1	877.8790	-10.05	2	26.9	43.3	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8; Deamidated: 5
39	4	508.7550	-44.82	2	21.8	64.9	0	133-142	R.ASSVVNNVAR.C	
15	3	509.2610	-17.30	2	20.6	62.8	0	133-142	R.ASSVVNNVAR.C	Deamidated: 6
44	3	509.2330	-72.29	2	22.4	48.6	0	133-142	R.ASSVVNNVAR.C	Deamidated: 7
96	3	677.7710	-70.83	2	27.4	70.9	0	143-155	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
157	2	782.8480	-52.51	2	32.5	39.8	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
131	3	790.8560	-38.68	2	30.2	29.7	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
78	1	586.3310	-50.63	1	28.2	23.9	0	198-202	K.DILPK.L	
104	4	774.3190	592.81	2	27.8	48.7	0	203-216	K.LTENPWQGNASSDK.G	
77	2	652.7850	-67.98	2	25.3	54.0	0	233-248	K.SVIGCTGGSPEK.H	Carbamidomethyl: 8
75	2	511.2350	-67.05	2	26.0	52.7	0	252-262	K.LEFAGAAGSAK.S	

Protein 2: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484605 **Score:** 471.10
Database: NCBInr **MW [kDa]:** 34.90
Seq. Coverage [%]: 35.40 % **pI:** 8.07
No. of Peptides: 10

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
165	1	645.8010	-12.35	2	32.7	48.0	0	42-52	K.CDQDGSLLNLR.V	Carbamidomethyl: 1
39	1	534.2440	-52.46	2	22.8	57.0	0	53-62	R.VTGNSSVEFK.C	
86	1	534.7150	-91.68	2	24.3	26.5	0	53-62	R.VTGNSSVEFK.C	Deamidated: 4
45	1	473.2500	24.62	3	23.3	39.5	0	63-76	K.CGGAVPNLHPNPGK.T	Carbamidomethyl: 1
7	1	525.7390	-27.53	2	19.2	67.6	0	126-135	K.CSSLANTAAR.V	Carbamidomethyl: 1
1	1	476.5580	-37.11	3	17.1	30.5	1	136-149	R.VGAGGPGQEAEKEK.T	
222	1	793.3900	-42.82	2	37.9	32.8	0	150-163	K.TCVQTSVWGPPIK.G	Carbamidomethyl: 2
27	1	540.6750	-114.58	2	21.8	43.8	0	167-175	K.EYPTPDACK.N	Carbamidomethyl: 8
148	1	630.2830	-70.64	2	31.5	43.3	0	176-187	K.NGTLSLEVNPSK.K	Deamidated: 1
146	1	785.7180	1.57	3	31.3	68.2	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.T	Carbamidomethyl: 2

Protein 3: actin [Toxoplasma gondii ME49]
Accession: gi|237840731 **Score:** 443.08
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 38.30 % **pI:** 4.91
No. of Peptides: 14

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
137	2	571.8350	-26.66	2	29.9	35.8	0	30-40	R.AVFPSIVGKPK.N	
103	2	610.7770	-14.94	2	27.1	24.6	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5
65	2	618.7540	-47.81	2	24.3	26.3	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5, 8
24	2	636.2200	-69.26	2	21.2	31.7	0	52-62	K.DCYVGDEAQSK.R	Carbamidomethyl: 2
209	1	973.9500	-1.32	2	35.6	24.1	0	70-85	K.YPIEHGIVTNWDDMEK.I	
172	1	981.9100	-39.48	2	33.0	23.8	0	70-85	K.YPIEHGIVTNWDDMEK.I	Oxidation: 14
140	3	505.8980	-45.93	3	30.2	43.4	0	86-96	K.IWHHTFYNELR.V	
193	2	651.9830	-66.44	3	34.0	29.2	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
150	1	523.7000	-57.83	2	31.1	37.8	0	185-192	R.DLTEYMMK.I	Oxidation: 7
71	1	530.7140	-69.56	2	24.9	23.3	0	198-207	R.GYGFTTSAEK.E	
225	1	888.9170	-27.87	2	37.0	50.2	0	240-256	K.SYELPDGNIITVGNER.F	
70	1	479.7010	-62.83	2	24.7	24.5	0	278-285	R.TTFDSIMK.C	Oxidation: 7
115	4	597.2600	-79.94	2	28.2	24.0	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10

75	1	759.3140	-43.43	2	25.1	27.3	0	361-373	K.EEYDESGPSIVHR.K	

Protein 4: actin [Symbiodinium sp. clade C]
Accession: gi|86562730 **Score:** 357.04
Database: NCBInr **MW [kDa]:** 41.80
Seq. Coverage [%]: 13.00 % **pI:** 5.22
No. of Peptides: 4

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	488.7170	-22.06	2	22.2	74.5	0	20-29	K.AGFAGDDAPR.A	
17	2	599.7230	-69.62	2	20.5	52.7	0	52-62	K.DSYVGDEAQSK.R	
195	1	651.6790	498.29	3	33.9	21.7	0	97-114	R.PAPEEHPVLLTEAPLNPK.A	
93	1	566.7440	-40.76	2	26.8	32.7	0	198-207	K.GYSFTTTAER.E	

Protein 5: actin [Pyrocystis lunula]
Accession: gi|27450759 **Score:** 321.12
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 4.30 % **pI:** 5.83
No. of Peptides: 1

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
240	1	895.9210	-31.92	2	38.3	56.7	0	239-254	K.SYELPDGQVITIGNER.F	

Protein 6: SAG-3 [Toxoplasma gondii]
Accession: gi|300680036 **Score:** 288.09
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 12.70 % **pI:** 6.59
No. of Peptides: 5

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

179	3	686.2790	-72.51	2	33.0	41.1	0	55-64	K.ITYFGTLTQK.A	
79	2	470.5620	-47.37	3	25.4	35.0	0	76-88	R.ANEEVVGHVTLNK.E	
258	2	591.2790	-58.66	2	39.3	35.6	0	135-145	K.GFLTDYIPGAK.Q	
91	1	584.2680	-54.95	3	26.2	42.4	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
81	2	690.8150	-29.98	2	25.7	57.6	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 3

Protein 7: microneme protein MIC3 [Toxoplasma gondii ME49]
Accession: gi|237841071 **Score:** 268.58
Database: NCBInr **MW [kDa]:** 37.90
Seq. Coverage [%]: 27.00 % **pI:** 6.02
No. of Peptides: 7

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
80	1	932.3890	-52.57	2	25.1	51.2	0	71-86	K.QETQLCAISSEGKPCR.N	Carbamidomethyl: 6, 15
131	1	603.6130	-1.53	3	30.3	29.7	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 1
146	2	603.2630	-37.99	3	30.4	33.4	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14
58	4	610.5670	-56.81	3	24.4	35.9	0	157-173	K.NAECVENLDAGGGVHCK.C	Carbamidomethyl: 4, 16
173	2	915.3660	-23.66	2	32.6	37.4	0	176-192	K.DGFVGTGLTCSEDPCSK.R	Carbamidomethyl: 10, 15
100	1	831.5920	-71.07	3	26.5	23.1	0	249-269	R.CIDDASHENGYTCECPTGYSR.E	Carbamidomethyl: 1, 13, 15; Deamidated: 9
92	2	543.2140	-66.69	2	26.1	58.0	0	294-303	K.EFGISASSCK.C	Carbamidomethyl: 9

Protein 8: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640 **Score:** 261.19
Database: NCBInr **MW [kDa]:** 33.90
Seq. Coverage [%]: 19.70 % **pI:** 6.04
No. of Peptides: 6

Modification(s): Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
14	1	637.2820	-63.98	2	19.9	41.7	0	36-50	R.AAVAAAAAANGAETGK.A	Deamidated: 9
23	1	636.7840	-73.43	2	20.6	57.7	0	36-50	R.AAVAAAAAANGAETGK.A	
42	1	517.2620	-28.19	2	22.1	42.9	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5

53	1	627.7970	-77.55	2	22.9	43.0	0	114-124	K.APPYKPIPESR.F	
288	1	817.3980	-42.64	2	41.9	51.0	0	180-192	K.QPFTILYLEPEQR.T	
165	1	751.8670	-7.04	2	31.9	24.9	0	193-205	R.TFAEPEQPDIEVK.D	

Protein 9: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163 **Score:** 177.82
Database: NCBInr **MW [kDa]:** 82.10
Seq. Coverage [%]: 6.40 % **pI:** 5.21
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	551.7730	-64.82	2	22.1	51.3	1	240-250	K.AVAELKAGESK.A	
137	1	541.2890	54.54	3	29.6	42.9	0	540-553	R.IDDPEHPAGELCLR.G	Carbamidomethyl: 12
170	1	554.3010	14.56	2	32.0	38.5	0	554-563	R.GPTITPGYFR.N	
143	1	801.3460	-9.58	2	30.0	25.4	0	732-744	K.EIDALYSEMETER.A	Oxidation: 9

Protein 10: dense granule protein [Toxoplasma gondii]
Accession: gi|2062409 **Score:** 176.49
Database: NCBInr **MW [kDa]:** 25.30
Seq. Coverage [%]: 23.30 % **pI:** 4.80
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
71	1	493.7560	-22.09	2	24.4	23.8	0	72-80	K.ASVESQLPR.R	
123	1	713.9800	-32.08	3	28.4	56.0	1	81-97	R.REPLETEPDEQEEVHFR.K	
87	1	670.2630	-30.93	3	26.0	47.8	0	103-119	R.SDAEVTDGNIYEEHTDR.K	
39	1	671.3710	25.21	2	28.9	30.6	0	156-166	R.LVPELTEEQQR.G	

Protein 11: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 173.95
Database: NCBInr **MW [kDa]:** 39.10

Seq. Coverage [%]: 13.70 % **pI:** 7.76
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
255	3	702.8260	-65.59	2	38.9	51.8	0	136-148	K.EWVTGDSVLTGLK.I	
102	3	702.3150	-66.77	2	27.2	34.3	0	149-161	K.ISVPESQYPANAK.S	
112	2	548.9320	-1.09	3	28.0	25.3	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5; Oxidation: 6
151	2	543.5920	-16.48	3	30.9	36.6	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5
59	2	650.2650	-50.61	2	24.2	26.0	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1

Protein 12: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499 **Score:** 148.54
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 11.70 % **pI:** 5.66
No. of Peptides: 4

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
10	5	847.9980	-29.05	3	18.4	51.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 21
5	3	848.0140	-10.18	3	17.8	38.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 18
5	1	848.3400	-12.54	3	18.4	25.1	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 17, 21
13	3	795.9600	-36.38	3	19.1	26.4	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 20

Protein 13: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751 **Score:** 137.13
Database: NCBInr **MW [kDa]:** 19.00
Seq. Coverage [%]: 21.50 % **pI:** 9.35
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
76	1	785.8590	592.83	2	35.2	24.7	0	61-75	K.LTISPSGEGDVFYGK.E	
66	2	706.4370	-15.26	2	33.8	49.8	1	82-95	R.KLTTVLPGAVLTAK.V	
5	3	529.6970	-111.48	2	19.2	62.6	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1

Protein 14: subtilisin-like protein [Toxoplasma gondii]
Accession: gi|15419013 **Score:** 136.55
Database: NCBInr **MW [kDa]:** 85.00
Seq. Coverage [%]: 6.50 % **pI:** 5.07
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
27	1	603.2110	-88.52	2	21.1	21.1	0	271-280	R.DNMEVNQAER.D	
24	1	547.2600	-46.53	2	20.8	44.7	0	615-625	R.TPPSAPSPSPR.T	
2	3	561.6180	2.99	3	18.4	28.2	0	706-721	R.SGSQPKPPQDNTTTPK.M	
122	1	862.3040	-68.05	2	28.4	45.8	0	746-760	R.EEEPPTDEDDFSSVK.G	

Protein 15: phosphofructokinase, putative [Toxoplasma gondii ME49]
Accession: gi|237834103 **Score:** 133.85
Database: NCBInr **MW [kDa]:** 131.30
Seq. Coverage [%]: 2.80 % **pI:** 5.82
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
178	1	647.3240	6.64	2	32.6	50.0	0	406-416	R.SVFEELPESTR.R	
94	1	676.8230	-22.54	2	26.0	24.9	1	744-754	R.TEREEAELFTK.E	
119	1	644.3100	-18.19	2	28.1	58.9	0	798-809	K.VVCVEPSGDLGR.F	Carbamidomethyl: 3

Protein 16: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826 **Score:** 133.11

Database: NCBInr **MW [kDa]:** 45.10
Seq. Coverage [%]: 10.00 % **pI:** 7.74
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
329	1	780.3650	-48.99	2	46.1	51.9	0	58-70	R.FLDPIDELPEPFK.A	
169	1	547.2190	-122.67	3	32.7	42.9	0	101-115	K.SPISPDVAVYVHAER.L	
210	1	705.2920	-68.21	2	35.7	38.3	1	389-400	K.LCDAFTDVAIRE.-	Carbamidomethyl: 2

Protein 17: dense granule protein 3 [Toxoplasma gondii ME49]
Accession: gi|237834147 **Score:** 120.94
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 8.60 % **pI:** 9.71
No. of Peptides: 3

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	608.3400	-17.76	2	31.4	39.9	1	114-123	K.KVEEELSLLR.R	
194	1	544.2790	-46.52	2	34.4	59.5	0	115-123	K.VEEELSLLR.R	
15	1	548.2680	-29.93	2	19.6	21.6	1	192-200	K.RQPFMSSVK.N	Oxidation: 5

Protein 18: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688 **Score:** 120.50
Database: NCBInr **MW [kDa]:** 144.50
Seq. Coverage [%]: 1.90 % **pI:** 5.80
No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
19	1	621.8260	9.86	2	20.1	59.6	0	46-58	K.VGSQPVAAGAEEK.V	
189	1	702.8200	-56.23	2	33.5	25.9	0	673-684	R.VQELEVPFNSSR.K	

Protein 19: heat shock protein 70 [Babesia gibsoni]
Accession: gi|156511059 **Score:** 120.12
Database: NCBInr **MW [kDa]:** 39.50
Seq. Coverage [%]: 6.40 % **pI:** 6.36
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
188	1	744.3300	-32.61	2	31.9	85.6	0	36-48	R.TTPSYVAFTDTER.L	
144	1	574.7890	10.87	2	28.7	34.9	0	61-70	R.NPENTIFDAK.R	

Protein 20: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049 **Score:** 118.79
Database: NCBInr **MW [kDa]:** 39.40
Seq. Coverage [%]: 11.30 % **pI:** 4.66
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
328	1	748.9970	54.79	2	45.0	51.4	0	133-146	R.LVDVSTLIPTAIVR.G	
122	1	503.7470	-72.87	2	28.4	25.2	0	188-196	R.SCIVTVTVK.K	Carbamidomethyl: 2
350	1	1072.5000	-25.51	2	48.4	25.3	0	313-332	R.QDEGLGVCIMQALLPASEGR.R	Carbamidomethyl: 8

Protein 21: hypothetical protein TGME49_003600 [Toxoplasma gondii ME49]
Accession: gi|237836689 **Score:** 87.16
Database: NCBInr **MW [kDa]:** 47.20
Seq. Coverage [%]: 4.80 % **pI:** 8.46
No. of Peptides: 2

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
50	1	559.7660	-1.04	2	22.6	22.3	0	194-204	K.TGSPVCAVGDR.D	Carbamidomethyl: 6
209	1	548.7660	-35.16	2	35.5	64.9	0	322-331	K.SGYSLSDLVR.S	

Protein 22: MORN repeat-containing protein [Toxoplasma gondii ME49]
Accession: gi|237838513 **Score:** 85.36
Database: NCBInr **MW [kDa]:** 49.20
Seq. Coverage [%]: 3.40 % **pI:** 5.15
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
4	1	745.8760	15.66	2	17.3	85.4	0	408-423	R.QTASTAGSAQPATGSR.A	

Protein 23: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635 **Score:** 68.23
Database: NCBInr **MW [kDa]:** 70.50
Seq. Coverage [%]: 1.90 % **pI:** 5.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
49	1	560.7540	-84.66	2	21.7	68.2	0	579-590	R.LSGTGSTGATIR.V	

Protein 24: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221480679 **Score:** 65.86
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 4.10 % **pI:** 6.59
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
234	1	797.8430	-67.22	2	37.4	49.6	0	115-129	K.GIDASAFTFTPPSQR.A	

Protein 25: smc family/structural maintenance of chromosome [Babesia bovis]
Accession: gi|156084430 **Score:** 54.17

Database: NCBInr
Seq. Coverage [%]: 0.70 %
MW [kDa]: 137.40
pI: 8.63
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
86	1	479.7500	947.99	2	25.5	54.2	1	955-962	R.VVSDRLLR.D	

Protein 26: Fructose-bisphosphate aldolase class-I family protein [Tetrahymena thermophila]
Accession: gi|118401200
Database: NCBInr
Seq. Coverage [%]: 3.90 %
Score: 50.04
MW [kDa]: 38.60
pI: 7.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
129	2	666.8350	-26.35	2	29.3	50.0	0	21-34	K.GILAADESTGTIGK.K	

Protein 27: microneme protein, putative [Toxoplasma gondii GT1]
Accession: gi|221486835
Database: NCBInr
Seq. Coverage [%]: 3.80 %
Score: 49.39
MW [kDa]: 36.30
pI: 5.01
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
21	1	724.7930	-4.96	2	20.4	49.4	0	249-261	R.YQGCASDNAGSYR.C	Carbamidomethyl: 4

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23

Search Location /BOPS/RH1/Bops_No10_BC3_01_621.d/

Protein 1: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751
Database: NCBInr
Seq. Coverage [%]: 21.50 %
Score: 133.84
MW [kDa]: 19.00
pI: 9.35
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
76	1	785.8590	592.83	2	35.2	24.7	0	61-75	K.LTISPSGEGDVFYGK.E	
66	1	706.4370	-15.26	2	33.8	49.8	1	82-95	R.KLTTVLPGAVLTAK.V	
1	1	529.7270	-54.77	2	19.3	59.3	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No11_BC4_01_622.d/

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No12_BC5_01_623.d/

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No1_BB2_01_610.d/

Protein 1: phosphofructokinase, putative [Toxoplasma gondii ME49]
Accession: gi|237834103
Database: NCBInr
Seq. Coverage [%]: 2.80 %
Score: 133.85
MW [kDa]: 131.30
pI: 5.82
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
178	1	647.3240	6.64	2	32.6	50.0	0	406-416	R.SVFEELPESTR.R	
94	1	676.8230	-22.54	2	26.0	24.9	1	744-754	R.TEREEAELFTK.E	
119	1	644.3100	-18.19	2	28.1	58.9	0	798-809	R.VVCVEPSGDLGR.F	Carbamidomethyl: 3

Protein 2: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688
Database: NCBInr
Seq. Coverage [%]: 1.90 %
Score: 120.50
MW [kDa]: 144.50
pI: 5.80
No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
19	1	621.8260	9.86	2	20.1	59.6	0	46-58	K.VGSQPVAAGAEEK.V	
189	1	702.8200	-56.23	2	33.5	25.9	0	673-684	R.VQELEVPFNSSR.K	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No2_BB3_01_611.d/

Protein 1: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163
Database: NCBInr
Score: 177.82
MW [kDa]: 82.10

Seq. Coverage [%]: 6.40 %
pI: 5.21
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	551.7730	-64.82	2	22.1	51.3	1	240-250	K.AVAELKAGESK.A	
137	1	541.2890	54.54	3	29.6	42.9	0	540-553	R.IDDPEHPAGELCLR.G	Carbamidomethyl: 12
170	1	554.8010	14.56	2	32.0	38.5	0	554-563	R.GPTITPGYFR.N	
143	1	801.3460	-9.50	2	30.0	25.4	0	732-744	K.EIDALYSEMETER.A	Oxidation: 9

Protein 2: subtilisin-like protein [Toxoplasma gondii]
Accession: gi|15419013
Database: NCBInr
Seq. Coverage [%]: 6.50 %
Score: 136.55
MW [kDa]: 85.00
pI: 5.07
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
27	1	603.2110	-88.52	2	21.1	21.1	0	271-280	R.DNMEVNQAER.D	
24	1	547.2600	-46.53	2	20.8	44.7	0	615-625	R.TPPSAPSPSPR.T	
2	1	561.6180	2.99	3	18.4	25.2	0	706-721	R.SGSQPKPPQDNTTTPK.M	
122	1	862.3040	-68.05	2	28.4	45.5	0	746-760	R.EEEPPTDEDDFSSVK.G	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No3_BB4_01_612.d/

Protein 1: heat shock protein 70 [Babesia gibsoni]
Accession: gi|156511059
Database: NCBInr
Seq. Coverage [%]: 6.40 %
Score: 120.12
MW [kDa]: 39.50
pI: 6.36

No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
188	1	744.3300	-32.61	2	31.9	85.6	0	36-48	R.TTPSYVAFTDTER.L	
144	1	574.7890	10.87	2	28.7	34.5	0	61-70	R.NPENTIFDAK.R	

Protein 2: MORN repeat-containing protein [Toxoplasma gondii ME49]
Accession: gi|237838513 **Score:** 85.36
Database: NCBInr **MW [kDa]:** 49.20
Seq. Coverage [%]: 3.40 % **pI:** 5.15
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
4	1	745.8750	15.65	2	17.3	85.4	0	408-423	R.QTASTAGSAQPATGSR.A	

Protein 3: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635 **Score:** 68.23
Database: NCBInr **MW [kDa]:** 70.50
Seq. Coverage [%]: 1.90 % **pI:** 5.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
49	1	560.7540	-84.66	2	21.7	68.2	0	579-590	R.LSGTGSTGATIR.V	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No4_BB5_01_613.d/

Protein 1: actin [Toxoplasma gondii ME49]
Accession: gi|237840731 **Score:** 413.90
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 33.50 % **pI:** 4.91
No. of Peptides: 13
Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
137	1	571.8350	-26.65	2	29.9	35.8	0	30-40	R.AVFPSIVGKPK.N	
65	2	618.7540	-47.81	2	24.3	26.3	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5, 8
103	2	610.7770	-14.94	2	27.1	24.9	0	41-51	K.NPGIMVGMEEK.D	Oxidation: 5
24	1	636.2200	-69.26	2	21.2	31.7	0	52-62	K.DCYVGDEAQSK.R	Carbamidomethyl: 2
209	1	973.9500	-1.32	2	35.6	24.1	0	70-86	K.YPIEHGIVTNWDDMEK.I	
172	1	981.9100	-39.45	2	33.0	23.9	0	70-86	K.YPIEHGIVTNWDDMEK.I	Oxidation: 14
140	3	505.8980	-45.93	3	30.2	43.4	0	86-96	K.IWHHTFYNELR.V	
150	1	523.7000	-57.83	2	31.1	37.8	0	185-193	R.DLTEYMMK.I	Oxidation: 7
71	1	530.7140	-69.56	2	24.9	23.2	0	198-207	R.GYGFTTSAEK.E	
225	1	888.9170	-27.87	2	37.0	50.2	0	240-258	K.SYELPDGNIITVGNER.F	
70	1	479.7010	-62.83	2	24.7	24.5	0	278-286	R.TTFDSIMK.C	Oxidation: 7
115	3	597.2600	-79.94	2	28.2	24.9	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10
75	1	759.3140	-43.43	2	25.1	27.3	0	361-373	K.EEYDESGPSIVHR.K	

Protein 2: actin [Symbiodinium sp. clade C]
Accession: gi|86562730 **Score:** 306.20
Database: NCBInr **MW [kDa]:** 41.80
Seq. Coverage [%]: 8.20 % **pI:** 5.22
No. of Peptides: 3
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
37	1	488.7170	-22.06	2	22.2	74.5	0	20-29	K.AGFAGDDAPR.A	
17	1	599.7230	-69.62	2	20.5	52.7	0	52-62	K.DSYVGDEAQSK.R	
93	1	566.7440	-40.76	2	26.5	32.7	0	198-207	R.GYSFTTTAER.E	

Protein 3: actin [Pyrocystis lunula]
Accession: gi|27450759 **Score:** 291.94
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 4.30 % **pI:** 5.83
No. of Peptides: 1
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
240	1	895.9210	-31.92	2	38.3	56.7	0	239-254	K.SYELPDGQVITIGNER.F	

Protein 4: SAG-3 [Toxoplasma gondii]
Accession: gi|300680036 **Score:** 195.97
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 12.70 % **pI:** 6.59
No. of Peptides: 5
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
174	1	586.2970	-41.81	2	33.1	35.5	0	55-64	K.ITVFGTLTQK.A	
79	1	470.5620	-47.37	3	25.4	35.0	0	76-88	R.ANEEVVGHVTLNK.E	
251	1	591.2990	-24.84	2	39.3	25.5	0	135-145	K.GFLTDYIPGAK.Q	
91	1	584.2680	-54.95	3	28.2	42.4	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
81	1	690.8150	-29.98	2	25.7	57.6	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 3

Protein 5: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826 **Score:** 133.11
Database: NCBInr **MW [kDa]:** 45.10
Seq. Coverage [%]: 10.00 % **pI:** 7.74
No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
329	1	780.3650	-48.99	2	46.1	51.9	0	58-70	R.FLDPIDELPEPFK.A	
169	1	547.2190	-122.67	3	32.7	42.9	0	101-115	K.SPISPDVAVYVHAER.L	
210	1	705.2920	-68.21	2	35.7	38.3	1	389-400	K.LCDAFTDVAIRE.-	Carbamidomethyl: 2

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No5_BB6_01_615.d/

Protein 1: hypothetical protein TGME49_032280 [Toxoplasma gondii ME49]
Accession: gi|237837601 **Score:** 219.47
Database: NCBInr **MW [kDa]:** 33.90
Seq. Coverage [%]: 19.70 % **pI:** 6.04
No. of Peptides: 5
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
23	1	636.7840	-73.43	2	20.6	57.7	0	36-50	R.AAVAAAAAANGAETGK.A	
42	1	517.2620	-28.19	2	22.1	42.9	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5
53	1	627.7970	-77.59	2	22.9	43.0	0	114-124	K.APPYKPIPESR.F	
288	1	817.3980	-42.64	2	41.9	51.6	0	180-192	K.QPFTILYLEPEQR.T	
165	1	751.8670	-7.04	2	31.9	24.9	0	193-205	R.TFAEPEQPDIEVK.D	

Protein 2: MIC3 protein [Toxoplasma gondii]
Accession: gi|172054126 **Score:** 191.00
Database: NCBInr **MW [kDa]:** 37.90
Seq. Coverage [%]: 22.60 % **pI:** 6.02
No. of Peptides: 5
Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
80	1	932.3890	-52.57	2	25.1	51.2	0	71-86	K.QETQLCAISSEGKPCR.N	Carbamidomethyl: 6, 15

71	1	610.5810	-33.88	3	24.4	21.4	0	157-173	K.NAECVENLDAGGGVHCK.C	Carbamidomethyl: 4, 16
173	1	916.3660	-23.66	2	32.6	37.4	0	176-192	K.DGFVSTGLTCSEDPCSK.R	Carbamidomethyl: 10, 15
100	1	831.5920	-71.07	3	26.5	23.1	0	249-269	R.CIDDASHENGYTCECPTGYSR.E	Carbamidomethyl: 1, 13, 15; Deamidated: 9
92	1	543.2140	-66.69	2	26.1	58.0	0	294-303	K.EFGISASSCK.C	Carbamidomethyl: 9

Protein 3: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488523 **Score:** 155.27
Database: NCBInr **MW [kDa]:** 39.20
Seq. Coverage [%]: 10.80 % **pI:** 8.76
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
255	1	702.8260	-65.59	2	38.9	51.8	0	136-148	K.EWVTGDSVLTGLK.I	
107	1	702.3070	-78.16	2	27.3	28.6	0	149-161	K.ISVPESQYPANAK.S	
116	1	548.9240	-15.66	3	27.9	22.7	0	173-186	K.TGNTCMLTHVEPR.D	Carbamidomethyl: 5; Oxidation: 6
151	1	543.5920	-16.48	3	30.9	36.6	0	173-186	K.TGNTCMLTHVEPR.D	Carbamidomethyl: 5

Protein 4: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049 **Score:** 118.79
Database: NCBInr **MW [kDa]:** 39.40
Seq. Coverage [%]: 11.30 % **pI:** 4.66
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
328	1	748.9970	54.79	2	45.0	51.4	0	133-146	R.LVDVSTLIPTAIVR.G	
122	1	503.7470	-72.87	2	28.4	25.2	0	188-196	R.SCIVTVTVK.K	Carbamidomethyl: 2
350	1	1072.5000	-25.51	2	48.4	25.3	0	313-332	R.QDEGLGVCIMQALLPASEGR.R	Carbamidomethyl: 8

Protein 5: microneme protein, putative [Toxoplasma gondii GT1]
Accession: gi|221486835 **Score:** 49.39
Database: NCBInr **MW [kDa]:** 36.30

Seq. Coverage [%]: 3.80 % **pI:** 5.01
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
21	1	724.7930	-4.96	2	20.4	49.4	0	249-261	R.YQGCASDNAGSYR.C	Carbamidomethyl: 4

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No6_BB7_01_616.d/

Protein 1: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 109.73
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 9.90 % **pI:** 7.76
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
250	1	702.8580	-20.06	2	38.9	49.4	0	136-148	K.EWVTGDSVLTGLK.I	
102	1	702.3150	-66.77	2	27.2	34.3	0	149-161	K.ISVPESQYPANAK.S	
59	1	650.2650	-50.61	2	24.2	26.6	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1

Protein 2: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221480679 **Score:** 65.86
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 4.10 % **pI:** 6.59
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
234	1	797.8430	-67.22	2	37.4	49.6	0	115-129	K.GIDASAFTFTPPSQR.A	

Protein 3: hypothetical protein TGME49_003600 [Toxoplasma gondii ME49]
Accession: gi|237836689 **Score:** 64.87
Database: NCBInr **MW [kDa]:** 47.20
Seq. Coverage [%]: 2.30 % **pI:** 8.46
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
209	1	548.7660	-35.16	2	35.5	64.9	0	322-331	K.SGYSLSDLVR.S	

Protein 4: surface antigen P22 [Toxoplasma gondii]
Accession: gi|269838870 **Score:** 62.61
Database: NCBInr **MW [kDa]:** 17.30
Seq. Coverage [%]: 6.50 % **pI:** 9.09
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
5	1	529.6970	-111.40	2	19.2	62.6	0	108-118	K.CVAEAGAPAGR.N	Carbamidomethyl: 1

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No7_BB8_01_617.d/

Protein 1: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484605 **Score:** 471.10
Database: NCBInr **MW [kDa]:** 34.90

Seq. Coverage [%]: 35.40 % **pI:** 8.07
No. of Peptides: 10

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
165	1	645.8010	-12.35	2	32.7	48.0	0	42-52	K.CDQDGSLLNLR.V	Carbamidomethyl: 1
39	1	534.2440	-52.45	2	22.8	57.0	0	53-62	R.VTGNSSVEFK.C	
56	1	534.7150	-91.68	2	24.3	26.5	0	53-62	R.VTGNSSVEFK.C	Deamidated: 4
45	1	473.2500	24.62	3	23.3	39.8	0	63-76	K.CGGAVPNLHPNPGK.T	Carbamidomethyl: 1
7	1	525.7390	-27.53	2	19.2	67.6	0	126-139	K.CSSLANTAAR.V	Carbamidomethyl: 1
1	1	476.5580	-37.11	3	17.1	30.5	1	136-149	R.VGAGQPGQEAEKEK.T	
222	1	793.3900	-42.82	2	37.9	32.0	0	150-163	K.TCIVQTSVWGPPIK.G	Carbamidomethyl: 2
27	1	540.6750	-114.58	2	21.8	43.0	0	167-179	K.EYPTPDACK.N	Carbamidomethyl: 8
148	1	630.2830	-70.64	2	31.5	43.3	0	176-187	K.NGTLSLEVNPSK.K	Deamidated: 1
146	1	785.7180	1.57	3	31.3	68.2	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.T	Carbamidomethyl: 2

Protein 2: surface antigen p30 [Toxoplasma gondii]
Accession: gi|50082488 **Score:** 444.33
Database: NCBInr **MW [kDa]:** 26.70
Seq. Coverage [%]: 40.50 % **pI:** 8.91
No. of Peptides: 10

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
176	1	815.8800	-55.64	2	33.5	41.9	0	8-22	K.TALTEPPTLAYSPNR.Q	
12	1	749.3070	-40.36	2	20.2	40.5	0	23-36	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
239	1	798.8970	-36.74	2	39.2	35.5	0	73-86	K.FPVTTQTFVVGCIK.G	Carbamidomethyl: 12
20	1	508.7440	-66.44	2	21.1	58.2	0	103-112	R.ASSVVNNVAR.C	
15	2	509.2610	-17.30	2	20.6	62.8	0	103-112	R.ASSVVNNVAR.C	Deamidated: 6
36	2	509.2510	-36.94	2	22.6	46.5	0	103-112	R.ASSVVNNVAR.C	Deamidated: 7
96	1	677.7710	-70.83	2	27.4	70.9	0	113-125	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
116	1	790.8410	-57.61	2	28.8	27.7	0	126-140	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
73	1	652.8310	2.49	2	25.8	37.6	0	203-215	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6

75	1	511.2350	-67.05	2	26.0	52.7	0	222-232	K.LEFAGAAGSAK.S	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No8_BC1_01_618.d/

Protein 1: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998 **Score:** 683.48
Database: NCBInr **MW [kDa]:** 26.60
Seq. Coverage [%]: 56.70 % **pI:** 7.78
No. of Peptides: 15

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
129	1	769.3890	-21.81	2	29.9	37.4	0	1-14	K.PPLVANQVVTCPDK.K	Carbamidomethyl: 11
112	1	555.9630	-14.87	3	28.2	33.6	1	1-15	K.PPLVANQVVTCPDKK.S	Carbamidomethyl: 11
177	2	815.8750	-61.76	2	34.3	29.8	0	36-50	K.TALTEPPTLAYSPNR.Q	
34	1	749.2580	-105.75	2	21.3	27.2	0	51-64	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
252	8	798.8860	-50.51	2	40.3	64.9	0	101-114	K.FPVTTQTFVVGCIK.G	Carbamidomethyl: 12
137	1	869.3720	-30.32	2	30.7	45.6	0	115-130	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7
95	1	877.8790	-10.05	2	26.9	43.3	0	115-130	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8; Deamidated: 5
39	3	508.7550	-44.82	2	21.8	64.9	0	131-140	R.ASSVVNNVAR.C	
44	1	509.2330	-72.29	2	22.4	49.2	0	131-140	R.ASSVVNNVAR.C	Deamidated: 6
96	2	677.7840	-51.65	2	27.0	43.4	0	141-153	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
157	2	782.8480	-52.51	2	32.8	39.8	0	154-168	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
131	2	790.8560	-38.65	2	30.2	29.7	0	154-168	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
78	1	585.3310	-50.63	1	25.2	23.9	0	196-200	K.DILPK.L	
104	3	774.3190	592.81	2	27.8	45.7	0	201-214	K.LTENPWQGNASSDK.G	
77	1	652.7850	-67.98	2	25.3	54.0	0	231-243	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:23
Search Location /BOPS/RH1/Bops_No9_BC2_01_620.d/

Protein 1: dense granule protein [Toxoplasma gondii]
Accession: gi|2062409 **Score:** 145.84
Database: NCBInr **MW [kDa]:** 25.30
Seq. Coverage [%]: 18.50 % **pI:** 4.80
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
71	1	493.7560	-22.09	2	24.4	23.8	0	72-80	K.ASVESQLPR.R	
123	1	713.9800	-32.08	3	28.4	56.0	1	81-97	R.REPLETEPDEQEEVHFR.K	
87	1	670.2630	-30.93	3	26.0	47.8	0	103-119	R.SDAEVTDDNIYEEHTDR.K	

Protein 2: dense granule protein 3 [Toxoplasma gondii ME49]
Accession: gi|237834147 **Score:** 120.94
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 8.60 % **pI:** 9.71
No. of Peptides: 3

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	608.3400	-17.76	2	31.4	39.9	1	114-123	K.KVEEELSLLR.R	
194	1	544.2780	-46.52	2	34.4	59.5	0	115-123	K.VEEELSLLR.R	
15	1	548.2680	-29.93	2	19.6	21.6	1	192-200	K.RQPFMSSVK.N	Oxidation: 5

Protein 3: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499 **Score:** 92.94
Database: NCBInr **MW [kDa]:** 23.90

Seq. Coverage [%]: 11.70 % **pI:** 5.66
No. of Peptides: 3

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
10	2	847.9980	-29.05	3	18.4	51.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 21
5	2	848.0140	-10.18	3	17.8	38.0	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 18
13	1	795.9600	-36.35	3	19.1	26.4	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGN EGR.G	Deamidated: 20

Sample Info & Protocols
Name: RH2

Search Type Combined MS/MS - ProteinExtractor
Search Result RH2_2014-08-20 16:19:08
Search Location /BOPS/RH2/

Protein 1: Chain A, Crystal Structure Of A Parasite Protein
Accession: gi|22219177 **Score:** 1801.72
Database: NCBInr **MW [kDa]:** 29.80
Seq. Coverage [%]: 80.60 % **pI:** 7.73
No. of Peptides: 32

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
286	1	623.2820	-67.80	3	26.9	58.6	1	1-17	K.SDPPLVANQVVTCPDKK.S	Carbamidomethyl: 13
315	1	623.5980	-87.01	3	28.3	20.9	1	1-17	K.SDPPLVANQVVTCPDKK.S	Carbamidomethyl: 13; Deamidated: 8
462	9	615.0630	96.72	3	36.0	68.7	0	18-34	K.STAAVILTPTENHFTLK.C	
488	2	615.3030	-46.35	3	37.2	49.2	0	18-34	K.STAAVILTPTENHFTLK.C	Deamidated: 12
411	1	743.3420	-73.05	3	33.4	24.5	1	18-37	K.STAAVILTPTENHFTLKCPK.T	Carbamidomethyl: 18
381	7	815.8920	-40.93	2	31.9	62.6	0	38-52	K.TALTEPPTLAYSPNR.Q	
82	3	749.2930	-59.04	2	16.0	68.0	0	53-66	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
275	2	400.2030	-117.00	2	26.3	29.8	0	96-102	K.LTVPIEK.F	
629	2	793.0860	-32.16	3	45.6	34.1	1	96-116	K.LTVPIEKFPVTTQTFVVGCIK.G	Carbamidomethyl: 19
226	2	586.2150	-87.66	3	23.7	49.2	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8
74	16	508.7520	-50.72	2	17.9	72.6	0	133-142	R.ASSVVNNVAR.C	
136	7	509.2140	-109.59	2	19.1	70.5	0	133-142	R.ASSVVNNVAR.C	Deamidated: 6
236	6	677.7820	-54.60	2	24.3	79.9	0	143-158	K.CSYGADSTLGPVK.L	Carbamidomethyl: 1
373	2	782.8400	-62.73	2	31.4	69.6	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
278	2	790.8560	-38.65	2	26.4	59.1	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
270	2	660.6440	-30.37	3	26.0	63.3	1	156-174	K.LSAEGPTTMTLVCGKDGVK.V	Carbamidomethyl: 13;

										Oxidation: 9
254	3	895.6970	-41.48	3	25.2	43.1	1	171-194	K.DGVKVPQDNNQYCSGTTLTGCNE K.S	Carbamidomethyl: 13, 21
264	1	896.0040	-64.91	3	25.7	49.8	1	171-194	K.DGVKVPQDNNQYCSGTTLTGCNE K.S	Carbamidomethyl: 13, 21; Deamidated: 9
196	2	762.6140	-64.99	3	22.1	30.2	0	175-194	K.VPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 9, 17
323	2	474.2360	-96.27	2	28.8	48.3	1	195-202	K.SFKDILPK.L	
230	2	516.2120	-59.26	3	24.0	61.8	0	203-216	K.LTENPWQGNASSDK.G	
247	2	774.3160	-46.83	2	24.8	91.8	0	203-216	K.LTENPWQGNASSDK.G	Deamidated: 9
388	3	744.3400	-55.89	3	32.2	41.6	1	203-223	K.LTENPWQGNASSDKGATLTIK.K	
295	3	787.0100	-88.84	3	27.5	50.9	2	203-224	K.LTENPWQGNASSDKGATLTIKK. E	
319	2	590.7650	-72.38	4	28.8	53.2	2	203-224	K.LTENPWQGNASSDKGATLTIKK. E	Deamidated: 9
97	2	416.1930	-181.50	2	16.9	41.3	1	217-224	K.GATLTIKK.E	
67	1	503.7510	-25.47	2	15.1	43.9	1	224-233	K.KEAFPAESK.S	
103	2	439.6550	-139.52	2	17.4	33.8	0	225-233	K.EAFPAESK.S	
211	2	652.7850	-67.98	2	22.9	94.3	0	233-245	K.SVIGCTGGSPEK.H	Carbamidomethyl: 6
187	1	614.6920	-30.44	5	28.0	56.8	2	233-262	K.SVIGCTGGSPEKHHCTVKLEFA GAAGSAK.S	Carbamidomethyl: 6, 16
206	4	446.6950	-74.23	4	22.6	54.5	1	246-262	K.HHCTVKLEFAGAAGSAK.S	Carbamidomethyl: 3
216	2	511.2110	-113.99	2	23.3	77.2	0	252-262	K.LEFAGAAGSAK.S	

Protein 2: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998 **Score:** 1617.63
Database: NCBInr **MW [kDa]:** 26.60
Seq. Coverage [%]: 5.90 % **pI:** 7.78
No. of Peptides: 1

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
250	2	555.9350	-65.23	3	25.1	84.6	1	1-15	K.PPLVANQVVTCPDKK.S	Carbamidomethyl: 11

Protein 3: surface antigen protein 1 [Toxoplasma gondii]
Accession: gi|56157026 **Score:** 1595.19

Database: NCBInr **MW [kDa]:** 33.00
Seq. Coverage [%]: 4.40 % **pI:** 9.75
No. of Peptides: 1

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
126	1	744.7630	-75.03	2	18.5	63.4	0	83-96	R.QICSAGTTSSCTSK.A	Carbamidomethyl: 3, 11; Deamidated: 1

Protein 4: actin [Toxoplasma gondii ME49]
Accession: gi|237840731 **Score:** 1148.04
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 49.70 % **pI:** 4.91
No. of Peptides: 21

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
54	2	464.6520	-163.06	2	13.9	85.6	0	20-29	K.AGVAGDDAPR.A	
367	2	571.8160	-59.88	2	29.6	65.7	0	30-40	R.AVFPSIVGKPK.N	
90	2	476.5080	-78.71	3	15.6	35.9	1	52-63	K.DCYVGDEAQSKR.G	Carbamidomethyl: 2
453	8	651.9560	-107.88	3	34.0	82.6	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
375	2	563.2210	-91.44	3	30.1	48.2	1	179-192	R.LDLAGRDLTEYMMK.I	Oxidation: 12, 13
209	2	530.6930	-109.13	2	21.7	41.0	0	198-207	R.GYGFTTSAEK.E	
352	2	519.9010	-65.59	3	28.9	49.2	1	198-211	R.GYGFTTSAEKEIVR.D	
47	2	532.6980	-80.28	2	13.2	69.6	0	230-239	K.AAEDSSDIEK.S	
512	1	941.3680	-86.11	3	36.8	32.2	1	230-255	K.AAEDSSDIEKSYELPDGNIITVG NER.F	
508	3	592.9430	-34.75	3	36.7	84.1	0	240-255	K.SYELPDGNIITVGNER.F	
596	2	599.6050	-63.81	3	41.1	46.5	1	256-270	R.FRCPEALFQPSFLGK.E	Carbamidomethyl: 3
605	2	747.3590	-23.41	2	41.5	76.2	0	256-270	R.CPEALFQPSFLGK.E	Carbamidomethyl: 1
521	1	554.2430	-73.19	4	37.3	20.7	1	258-277	R.CPEALFQPSFLGKEAAGVHR.T	Carbamidomethyl: 1
357	2	572.8850	-88.83	3	29.1	51.0	1	278-291	R.TTFDSIMKCDVDIR.K	Carbamidomethyl: 9; Oxidation: 7
449	4	792.3530	-46.40	3	33.7	56.7	1	292-313	R.KDLYGNVVLSGGTTMYEGIGER. L	Oxidation: 15

300	2	512.5910	-49.73	3	26.3	69.2	1	314-327	R.LTKELTSLAPSTMK.I	Oxidation: 13
254	2	597.2530	-91.66	2	24.0	66.7	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10
341	2	589.2690	-70.06	2	28.3	41.4	0	317-327	K.ELTSLAPSTMK.I	
287	2	478.9020	-66.67	3	25.7	46.2	1	317-329	K.ELTSLAPSTMKIK.V	Oxidation: 10
191	1	504.7530	-120.16	2	20.8	28.1	1	328-336	K.IKVVAPPER.K	
49	1	448.2210	-112.94	2	13.4	34.6	1	330-337	K.VVAPPERK.Y	

Protein 5: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751 **Score:** 822.07
Database: NCBInr **MW [kDa]:** 19.00
Seq. Coverage [%]: 59.10 % **pI:** 9.35
No. of Peptides: 14

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
319	1	585.2370	-62.47	3	27.7	22.6	0	44-60	K.TVDAPSSGSVVFQCGDK.L	Carbamidomethyl: 14
460	2	786.3580	-46.07	2	34.5	37.8	0	61-79	K.LTISPSGEGDVFYGK.E	
456	4	471.2400	-129.31	3	34.6	92.2	1	82-95	R.KLTTVLPGAVLTAK.V	
512	2	428.5840	-43.45	3	38.0	60.2	0	83-95	K.LTTVLPGAVLTAK.V	
497	1	645.6910	-54.35	3	36.9	29.6	1	83-101	K.LTTVLPGAVLTAKVQQPAK.G	
422	1	785.6250	-31.40	4	32.9	22.0	1	96-123	K.VQQPAKGPATYTLSYDGTPEKPQ VLCYK.C	Carbamidomethyl: 26
432	2	830.0520	-26.10	3	33.5	89.1	0	102-123	K.GPATYTLSYDGTPEKPQVLCYK. C	Carbamidomethyl: 20
45	7	529.7210	-66.10	2	15.1	61.6	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1
118	3	709.6410	-37.53	3	17.3	51.8	1	124-148	K.CVAEAGAPAGRNNDGSSAPTPK. D	Carbamidomethyl: 1
128	1	709.9700	-36.11	3	17.8	35.0	1	124-145	K.CVAEAGAPAGRNNDGSSAPTPK. D	Carbamidomethyl: 1; Deamidated: 13
47	3	544.7090	-68.60	2	12.8	76.3	0	135-148	R.NNDGSSAPTPK.D	Deamidated: 1
35	4	497.8510	-66.66	3	12.0	67.1	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13; Deamidated: 2
23	6	497.5170	-78.75	3	11.4	68.1	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13
311	1	576.8230	-39.91	2	27.4	37.8	1	149-159	K.LIRVPGADGR.V	

Protein 6: hypothetical protein TGME49_112630 [Toxoplasma gondii ME49]

Accession: gi|237930375
Database: NCBInr
Seq. Coverage [%]: 6.30 %
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Score: 801.71
MW [kDa]: 286.30
pI: 4.74
No. of Peptides: 16

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
133	1	482.7160	-91.63	2	17.0	28.1	0	333-342	K.IASMISGAAK.M	Oxidation: 4
166	1	501.7250	-78.48	2	19.2	67.0	0	702-710	R.ITGAENLER.C	
184	1	411.1980	-68.61	3	20.3	31.7	1	787-797	K.IDSNVGETLRK.C	
253	2	461.7000	-75.96	2	25.3	41.2	0	983-990	R.SAELAFER.N	
294	2	417.7130	-63.74	2	28.2	38.2	0	1191-1198	R.LGGELFAK.M	
267	1	565.7740	-51.19	2	26.3	40.3	0	1272-1281	R.LEGNVALVCR.R	Carbamidomethyl: 9
118	1	743.7520	-62.49	2	18.7	46.6	0	1452-1465	R.DCDALMGSNSSSAR.Q	Carbamidomethyl: 2; Oxidation: 6
330	2	738.8550	-45.04	2	30.8	77.8	0	1481-1493	K.AYNDALIQEQALK.K	
262	1	537.2260	-64.40	3	25.8	31.3	0	1563-1577	R.ALQGLSMADVNMTAR.T	Oxidation: 7, 12
255	1	525.2660	-36.04	2	25.4	41.9	0	1743-1751	K.LLQANEFSK.M	
421	1	701.8220	-86.15	2	37.5	57.1	0	1759-1771	K.GQADPEIVLYAVK.A	
296	1	413.6940	-78.61	2	28.4	30.7	0	1821-1826	R.YFLVER.T	
188	2	419.6900	-68.84	2	20.6	31.1	0	1965-1971	K.NTEIFSK.L	
247	2	434.7250	-94.71	2	24.6	66.6	0	1972-1980	K.LGGPALNVK.V	
357	2	613.3210	-65.49	2	32.7	65.4	0	2012-2024	K.LLGTNGAPAALVK.G	Deamidated: 5
135	1	599.7110	-89.63	2	17.0	71.0	0	2025-2035	K.GLSNYDGSEEK.T	

Protein 7: surface antigen [Toxoplasma gondii]
Accession: gi|13447088
Database: NCBInr
Seq. Coverage [%]: 24.20 %
Modification(s): Carbamidomethyl, Deamidated

Score: 795.18
MW [kDa]: 41.70
pI: 6.93
No. of Peptides: 14

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
358	2	462.8770	-105.34	3	29.2	78.2	1	53-64	R.SKITYFGTLTQK.A	

485	1	544.2760	-26.34	4	35.5	69.3	2	53-70	R.SKITYFGTLTQKAPNWYR.C	
413	2	586.2880	-67.16	2	32.0	50.4	0	55-64	K.ITYFGTLTQK.A	
537	2	653.6480	-46.84	3	38.1	59.7	1	55-70	K.ITYFGTLTQKAPNWYR.C	
207	2	403.6620	-96.20	2	21.6	38.7	0	65-70	K.APNWYR.C	
240	2	470.5510	-70.74	3	23.3	68.4	0	76-88	R.ANEEVVGHVTLNK.E	
35	1	426.4180	-66.48	4	12.0	48.4	2	119-132	R.VCHIDAKDKGDCER.N	Carbamidomethyl: 2, 12
471	2	476.2270	-64.21	3	34.9	60.6	1	133-145	R.NKGFLTDYIPGAK.Q	
559	2	591.2560	-97.56	2	39.2	64.3	0	135-145	K.GFLTDYIPGAK.Q	
244	2	584.2490	-87.47	3	23.8	69.3	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
220	2	690.7970	-56.04	2	22.4	66.9	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 4
220	2	690.7970	656.62	2	22.4	40.3	0	154-165	K.VENNGEQSVLYK.F	
695	1	700.3800	-39.42	2	54.2	25.8	0	166-177	K.FTVPWIFLPPAK.Q	
412	2	489.2310	-51.45	2	31.9	55.0	0	229-236	K.DANFIEIR.C	

Protein 8: actin I [Plasmodium falciparum]
Accession: gi|5911379
Database: NCBInr
Seq. Coverage [%]: 4.80 %
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Score: 733.50
MW [kDa]: 41.80
pI: 5.15
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
525	5	908.4060	489.93	2	37.5	52.1	0	2-19	M.GEEVVQALVVDNGSGNVK.A	Deamidated: 6
490	6	908.4170	503.15	2	35.8	70.4	0	2-19	M.GEEVVQALVVDNGSGNVK.A	Deamidated: 12
514	1	907.8560	427.39	2	36.9	20.9	0	2-19	M.GEEVVQALVVDNGSGNVK.A	

Protein 9: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640
Database: NCBInr
Seq. Coverage [%]: 54.80 %
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Score: 698.68
MW [kDa]: 33.90
pI: 6.04
No. of Peptides: 13

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

23	2	539.5180	-97.29	3	11.2	48.8	0	16-30	K.DSHAANADSQTPMQK.L	Oxidation: 13
184	2	455.7220	-54.81	4	20.5	61.0	1	36-55	R.AAVAAAANGAETGKAPHLK.L	Deamidated: 9
140	2	517.2580	-35.93	2	18.3	60.8	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5
314	2	481.0270	155.52	3	27.0	50.0	0	101-113	K.KPTEGQTVLLQVK.A	
169	2	418.8540	-108.74	3	19.7	37.3	0	114-124	K.APPYKPIPESR.F	
74	1	507.5510	-57.58	3	14.8	51.5	1	136-148	R.QNIETMKDTPEAK.A	Oxidation: 6
371	2	761.8180	-72.21	2	29.9	52.6	0	193-205	R.TFAEPEQPDIEVK.D	
97	2	548.8960	-61.41	3	16.2	74.4	1	222-237	K.AAETVAEKEGEQSAPK.N	
26	2	500.1710	-102.12	3	11.3	46.6	0	238-251	K.NESPESGHDATAQR.K	
198	2	414.5010	-111.66	3	21.1	27.4	0	257-267	R.YLGPESSVPHR.L	
678	1	762.4250	-28.75	2	47.1	55.8	0	268-281	R.LLATCGILPLPLDK.A	Carbamidomethyl: 5
107	2	406.6690	-141.21	2	16.8	28.8	0	284-290	K.ELIQEGK.R	
679	1	740.8600	-37.45	2	47.3	88.2	0	300-314	K.TIITEAGGFFGTPVA.-	

Protein 10: GPI-anchored surface protein [Toxoplasma gondii ME49]
Accession: gi|237837813
Database: NCBInr
Seq. Coverage [%]: 28.30 %
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Score: 469.89
MW [kDa]: 44.20
pI: 7.81
No. of Peptides: 10

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
426	4	788.3160	-49.89	2	32.6	67.0	0	132-145	K.GAQNDWLNGEDLSR.G	Deamidated: 8
407	6	787.8200	-54.99	2	31.7	52.7	0	132-145	K.GAQNDWLNGEDLSR.G	
326	1	459.2540	-60.95	2	27.6	20.3	1	146-153	R.GITFRVPK.N	
351	2	457.7090	-80.34	2	28.8	37.3	0	154-161	K.NNFPGLPR.I	
403	1	458.2230	-32.26	2	31.5	22.9	0	154-161	K.NNFPGLPR.I	Deamidated: 1
516	1	849.0560	-47.93	3	37.0	46.1	0	207-230	R.ITPATNHATIVCGPYGGISPLDY K.K	Carbamidomethyl: 12
75	1	586.2110	544.24	3	14.8	30.4	1	231-244	K.KEFCTGEPHTSCGR.Q	Carbamidomethyl: 4, 12
113	1	513.1830	-52.86	3	17.0	26.7	0	232-244	K.EFCTGEPHTSCGR.Q	Carbamidomethyl: 3, 11
129	1	1046.7170	-50.88	3	17.8	57.3	0	334-367	K.EAPNTNNSGGTPTGGGSDDDTTA PGTGSSGTDSR.G	
30	1	438.4120	-86.17	4	11.6	61.6	2	368-384	R.GDLGDGRDQDAKGGMSR.G	Oxidation: 15

Protein 11: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049
Database: NCBInr
Seq. Coverage [%]: 28.80 %
Modification(s): Carbamidomethyl

Score: 465.15
MW [kDa]: 39.40
pI: 4.66
No. of Peptides: 10

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
545	1	880.7030	-33.97	3	38.5	71.0	0	108-132	K.SQNLSPTELDTAFGVDAAGNCDT TR.L	Carbamidomethyl: 21
680	2	748.9320	-32.00	2	47.8	83.9	0	133-146	R.LVDVSTLIPTAIVR.G	
135	1	406.6160	-159.18	2	18.1	23.8	0	174-179	K.ACFVCR.E	Carbamidomethyl: 2, 5
77	1	463.2160	-73.04	2	15.0	23.7	0	227-234	R.SVVVHCPK.G	Carbamidomethyl: 6
670	1	567.9470	-53.19	3	45.3	60.3	1	235-249	K.GLPVLDIFKESDNVR.A	
360	1	604.0260	-76.97	4	29.3	42.2	1	258-280	K.AKLEDVVGSGSTIKPVNVDSQNR D	
342	1	718.3430	-50.97	4	28.4	49.5	2	258-284	K.AKLEDVVGSGSTIKPVNVDSQNR DVSR.S	
360	2	738.6960	-28.26	3	28.8	46.7	0	260-280	K.LEDVVGSGSTIKPVNVDSQNR.D	
330	1	668.5770	-29.31	4	27.8	21.2	1	260-284	K.LEDVVGSGSTIKPVNVDSQNRDV SR.S	
354	2	525.2490	-56.86	3	28.9	27.3	0	285-298	R.SYLITSTSSFSKPR.I	

Protein 12: SRS domain-containing surface antigen, putative [Toxoplasma gondii ME49]
Accession: gi|237839855
Database: NCBInr
Seq. Coverage [%]: 32.00 %
Modification(s): Carbamidomethyl

Score: 422.60
MW [kDa]: 34.90
pI: 8.07
No. of Peptides: 8

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
147	3	534.2230	-91.75	2	19.6	59.7	0	53-62	R.VTGNSSVEFK.C	
70	1	525.7130	-76.98	2	15.3	75.5	0	126-139	K.CSSLANTAAR.V	Carbamidomethyl: 1
38	1	585.7690	-37.74	2	12.8	50.2	0	136-147	R.VGAGQPGQEAEK.E	
40	2	476.5450	-64.38	3	12.9	81.0	1	136-149	R.VGAGQPGQEAEKEK.T	

434	2	619.9730	-45.53	3	33.8	24.1	1	150-160	K.TCIVQTSVWGPPIKGSK.E	Carbamidomethyl: 2
360	2	589.5260	-22.72	4	30.7	54.1	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.T	Carbamidomethyl: 2
391	2	629.0230	-67.73	4	32.4	52.9	1	235-267	R.TNAADDKKPAYTFSVTSLPTEEKT	
76	2	401.1420	-132.06	3	14.8	25.1	1	268-276	K.TLCYQCTKK.N	Carbamidomethyl: 3, 6

Protein 13: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221487802 **Score:** 372.61
Database: NCBInr **MW [kDa]:** 37.20
Seq. Coverage [%]: 26.00 % **pI:** 5.82
No. of Peptides: 6

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
282	1	586.7680	-74.15	2	26.6	83.2	0	81-92	K.GGISVEVDPATK.K	
631	2	947.9190	-46.10	2	45.9	85.3	0	122-139	R.ANLQGETPLAEFFGEGSK.A	
20	1	708.2780	-46.48	2	11.4	64.3	0	184-197	R.APTGDPSQNSDGNR.G	
540	2	778.7080	-37.73	3	40.1	63.6	0	266-287	K.LADVLPSATFQETSSAHVFSVK.E	
197	2	501.5520	-51.91	3	22.2	40.6	1	288-299	K.ELPKTEATYCYK.C	Carbamidomethyl: 10
51	2	511.8780	-42.66	3	14.2	35.6	1	300-314	K.CSPPVDSGDTADGKK.N	Carbamidomethyl: 1

Protein 14: SAG1-related sequence 3 [Toxoplasma gondii]
Accession: gi|2305258 **Score:** 363.35
Database: NCBInr **MW [kDa]:** 36.20
Seq. Coverage [%]: 21.20 % **pI:** 5.75
No. of Peptides: 8

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
414	2	831.8860	-47.71	2	40.2	53.1	0	91-105	K.CAIGTVTLPVDLMEK.Q	Carbamidomethyl: 1; Oxidation: 13
109	1	455.7340	-42.28	2	18.9	21.3	0	158-165	K.QNLPPVDK.E	
333	2	720.3300	-48.44	3	33.1	28.7	1	158-176	K.QNLPPVDKEFIVGCTQEGK.G	Carbamidomethyl: 14

215	2	634.2840	-29.95	2	25.2	67.5	0	166-179	K.EFIVGCTQEGK.G	Carbamidomethyl: 6
110	2	604.0100	-20.81	4	19.0	33.6	1	189-211	R.ASSKDSQTVTCAYGSASNADVHRV	Carbamidomethyl: 11; Deamidated: 18
120	4	603.7380	-63.88	4	19.5	65.3	1	189-211	R.ASSKDSQTVTCAYGSASNADVHRV	Carbamidomethyl: 11
318	2	560.6040	-46.20	3	32.1	52.5	0	278-292	K.AVLTIPHDNFPEAQK.K	
279	1	452.7110	-81.69	4	29.4	42.0	1	278-293	K.AVLTIPHDNFPEAQKK.V	

Protein 15: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221486446 **Score:** 345.80
Database: NCBInr **MW [kDa]:** 50.90
Seq. Coverage [%]: 13.80 % **pI:** 4.54
No. of Peptides: 6

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
16	2	483.8870	-49.27	3	11.5	80.5	2	55-67	K.VKVEDTEGDKSSR.G	
22	2	420.1580	-90.26	3	13.0	40.9	0	97-106	K.QHQMELEAEK.A	Oxidation: 4
86	2	487.2390	-34.55	3	20.6	48.3	1	146-157	K.ISAREEIEQQTR.K	
23	1	516.7240	-53.13	2	13.1	45.3	0	150-157	R.EEIEQQTR.K	
97	2	588.6090	-30.19	3	21.5	52.7	1	438-453	K.QTVEEASKQTVEEASK.Q	
50	1	846.8660	-39.88	2	17.2	62.6	0	470-485	K.QTASEVTKPTEEANTS.-	

Protein 16: microneme protein MIC3 [Toxoplasma gondii]
Accession: gi|343113243 **Score:** 337.20
Database: NCBInr **MW [kDa]:** 36.40
Seq. Coverage [%]: 18.30 % **pI:** 5.84
No. of Peptides: 4

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
338	6	603.5610	-87.67	3	28.2	94.1	0	80-95	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 6
82	2	550.2110	-53.47	3	14.1	22.5	1	226-239	R.TGCHAFRENCSPGR.C	Carbamidomethyl: 3, 10

415	2	765.3570	-21.93	3	32.1	47.8	1	261-281	R.EVTSKAEESCVEGVEVTLAEK.C	Carbamidomethyl: 10
45	4	583.7460	-27.78	2	13.0	82.8	0	312-323	K.GESGSEGSLSEK.M	

Protein 17: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 303.54
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 11.80 % **pI:** 7.76
No. of Peptides: 6

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
551	4	702.8400	-45.67	2	38.9	72.0	0	136-148	K.EWVTGDSVLTGLK.I	
204	5	702.3290	-46.84	2	24.7	66.3	0	149-161	K.LSVPESQYPANAK.S	
279	2	702.8110	-61.05	2	25.3	41.0	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 11
279	1	702.8110	-61.05	2	25.3	22.9	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 7
190	2	650.2570	-62.91	2	20.8	58.9	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
491	2	448.6870	-70.64	2	35.8	26.2	0	269-265	K.WFFGDPK.S	

Protein 18: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826 **Score:** 301.85
Database: NCBInr **MW [kDa]:** 45.10
Seq. Coverage [%]: 17.80 % **pI:** 7.74
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
685	1	780.3860	-22.08	2	49.1	74.2	0	58-70	R.FLDPIDELPEPFK.A	
421	2	440.5540	-62.83	3	32.4	69.4	1	89-100	K.LGIEGDKTVFLK.S	
419	2	547.2560	-55.06	3	32.3	33.0	0	101-115	K.SPISPDVAVYVHAER.L	
447	2	610.3190	-28.67	3	33.7	77.0	0	223-240	K.SVELTRPQAVLGAVGMGK.K	Oxidation: 16
414	2	484.9090	-49.56	3	32.0	48.2	1	269-281	R.ALQFGKDFQVTAK.K	

Protein 19: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499 **Score:** 299.63

Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 23.90 % **pI:** 5.66
No. of Peptides: 7

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
59	1	410.8420	-77.77	3	16.2	29.4	1	112-122	K.SEARGPSLEER.I	
162	1	534.2310	-71.18	3	25.5	28.5	1	116-129	R.GPSLEERIEEQGTR.R	
57	1	568.7620	-36.49	2	16.1	41.5	0	132-141	R.YSSVQEPQAK.V	
32	2	847.9820	-47.92	3	13.1	64.9	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 21
34	1	848.3060	-52.62	3	13.4	31.4	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 13, 21
48	2	795.9480	-51.42	3	13.8	49.5	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 23
36	2	795.9450	-55.19	3	13.8	61.1	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 20

Protein 20: bradyzoite-specific surface protein, putative [Toxoplasma gondii ME49]
Accession: gi|237841177 **Score:** 284.11
Database: NCBInr **MW [kDa]:** 41.50
Seq. Coverage [%]: 16.80 % **pI:** 6.23
No. of Peptides: 5

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
251	1	626.2610	-87.98	2	27.7	72.5	0	93-104	K.GSLTATLECTAK.D	Carbamidomethyl: 9
196	1	401.5130	-136.74	3	24.2	37.8	1	169-179	K.AIQSQKWTLK.L	
227	1	725.8050	-33.86	2	26.2	62.6	0	190-205	K.TFDVGCTYTTTGK.A	Carbamidomethyl: 6
297	3	778.3850	-40.70	2	30.5	73.2	0	203-217	K.AAANPAVCQLTVNVK.A	Carbamidomethyl: 8
100	3	624.9410	-41.82	3	18.2	39.4	1	326-342	R.LGCVPNKAPQSDSQDTR.V	Carbamidomethyl: 3

Protein 21: myosin D, putative [Toxoplasma gondii ME49]
Accession: gi|237832241 **Score:** 239.13
Database: NCBInr **MW [kDa]:** 91.00

Seq. Coverage [%]: 7.90 %
pI: 9.39
No. of Peptides: 6

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
171	2	662.3210	-35.29	3	28.1	41.5	0	66-83	K.LQQVEPTADSQELTVQAK.E	
184	1	595.7550	-95.41	2	29.3	24.7	0	143-153	K.NAGPDTIALYR.D	
35	2	442.8700	-62.21	3	15.1	38.2	1	409-420	K.TTYAGSNKIESR.W	
214	1	443.7500	-76.69	2	33.2	26.5	0	526-533	K.LIETLLGK.G	
88	2	419.6990	-90.74	2	20.8	26.9	0	555-562	K.FVSSLASK.L	
25	2	431.1890	-83.19	2	13.5	49.1	0	614-621	R.ASTNDVVR.G	

Protein 22: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635
Database: NCBInr
Seq. Coverage [%]: 7.80 %
Score: 236.79
MW [kDa]: 70.50
pI: 5.55
No. of Peptides: 5

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
123	2	516.2540	-45.78	2	23.3	40.4	0	80-90	K.GGTLLVSGDGR.F	
43	1	445.1940	-91.88	2	18.9	51.1	0	520-528	K.SSPAEITGK.V	
47	1	419.1510	-124.23	2	16.4	28.1	0	551-557	K.YTDPVDK.Q	
59	2	560.7710	-54.35	2	18.0	71.5	0	579-590	R.LSGTGSTGATIR.V	
203	1	597.7710	-76.71	2	32.6	22.9	0	627-637	K.YIGTETPTVIT.-	

Protein 23: RecName: Full=Dense granule protein 5; Short=Protein GRA 5; AltName: Full=p21; Flags: Precursor
Accession: gi|2507039
Database: NCBInr
Seq. Coverage [%]: 35.00 %
Score: 221.83
MW [kDa]: 13.00
pI: 5.69
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
20	2	502.9610	-47.61	4	11.2	54.9	1	43-58	R.GREQQQVQQHEQNEDR.S	
14	2	599.2330	-61.71	3	10.9	47.8	0	45-58	R.EQQQVQQHEQNEDR.S	
204	1	607.7590	-45.01	4	21.7	47.8	1	45-63	R.EQQQVQQHEQNEDRSLFER.G	
270	1	779.6260	-62.65	3	25.2	71.3	1	100-120	R.AIQEESKESATAEEEEVAEEE.-	

Protein 24: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163
Database: NCBInr
Seq. Coverage [%]: 7.50 %
Score: 203.13
MW [kDa]: 82.10
pI: 5.21
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
40	1	554.2130	-82.25	2	15.6	35.8	0	172-181	R.SVVASEECAR.R	Carbamidomethyl: 8
150	1	480.2550	-39.63	2	26.3	50.1	0	183-191	R.LLDSIEAAK.S	
135	1	758.8450	-27.06	2	24.2	29.8	0	192-206	K.SSLAGEPEEAVNASR.V	
65	1	551.7570	-93.82	2	18.6	68.2	1	240-250	K.AVAELKAGESK.A	
41	1	547.2140	-123.39	2	15.7	20.6	0	677-687	R.AVTESMAAVAK.E	Oxidation: 6

Protein 25: actin [Pyrocystis lunula]
Accession: gi|27450759
Database: NCBInr
Seq. Coverage [%]: 7.20 %
Score: 187.99
MW [kDa]: 41.70
pI: 5.83
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
385	3	400.1990	-102.48	3	30.7	27.8	0	29-39	R.AVFPSIVGRPR.H	
538	2	895.9050	-49.78	2	38.1	59.8	0	239-254	K.SYELPDGQVITIGNER.F	

Protein 26: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688
Score: 184.05

Database: NCBInr
Seq. Coverage [%]: 5.60 %
MW [kDa]: 144.50
pI: 5.80
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
103	1	621.7870	-52.86	2	16.2	22.1	0	46-58	K.VGSQPVAAGAEEK.V	
77	1	501.2250	-42.35	3	14.5	33.8	0	82-97	R.LESTVGGSGTGHSQLGK.S	
316	2	803.8410	-65.88	2	27.8	82.1	0	338-352	K.TNEALLTGESEDISK.T	
415	1	702.8110	-69.04	2	32.8	25.4	0	673-684	R.VQELEVPFNSSR.K	
204	1	633.2750	-80.99	3	22.3	20.7	0	918-936	R.QGHTVAMTGDGVNDAPALK.A	Oxidation: 7

Protein 27: glyceraldehyde-3-phosphate dehydrogenase [Toxoplasma gondii ME49]
Accession: gi|237838193
Database: NCBInr
Seq. Coverage [%]: 0.00 %
Score: 179.64
MW [kDa]: 36.60
pI: 6.75
No. of Peptides: 0

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

Protein 28: microtubule-binding protein, putative [Toxoplasma gondii ME49]
Accession: gi|237836067
Database: NCBInr
Seq. Coverage [%]: 6.90 %
Score: 177.75
MW [kDa]: 92.50
pI: 9.15
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
168	2	848.8550	-37.30	2	27.8	30.1	0	187-200	R.LNESFDDQTEETLR.V	
193	1	648.2630	-46.00	2	23.9	27.3	0	285-295	R.MDVEVSDIDTR.L	Oxidation: 1
267	1	439.2030	-102.79	2	28.6	32.4	0	430-438	K.LFGGLGSAR.A	
158	2	526.2630	-23.26	3	19.2	64.3	1	734-749	R.APTCGATITSSGVAKR.F	Carbamidomethyl: 4

62	1	448.7170	-63.38	2	18.2	23.6	0	808-815	K.YTTTAIAR.V	

Protein 29: 14-3-3 protein homologue [Toxoplasma gondii]
Accession: gi|3123732
Database: NCBInr
Seq. Coverage [%]: 15.00 %
Score: 177.67
MW [kDa]: 30.70
pI: 4.55
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
164	2	458.5270	-66.80	3	20.4	70.3	1	143-154	R.YISEFSGEEGKK.Q	
46	2	683.7920	-34.09	2	13.3	57.7	0	155-166	K.QAADQAQESYQK.A	
251	2	574.9240	-62.76	3	25.0	49.7	0	167-182	K.ATETAEAELPSTHPIR.L	

Protein 30: hypothetical protein TGME49_093430 [Toxoplasma gondii ME49]
Accession: gi|237841695
Database: NCBInr
Seq. Coverage [%]: 13.40 %
Score: 159.38
MW [kDa]: 44.60
pI: 4.86
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
151	2	750.8300	-60.71	2	18.8	44.2	0	70-82	R.QQPVEANETTVER.G	
94	2	437.1790	-104.92	2	15.9	64.8	0	185-193	R.AAADIAADR.A	
523	2	708.3640	-1.26	3	38.6	20.3	1	226-244	R.QGGLLPDVPLDETVEPCRK.G	Carbamidomethyl: 17
340	2	688.8190	-36.11	2	28.3	30.1	0	398-411	R.HTPPGADQIELAGA.-	

Protein 31: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237844685
Database: NCBInr
Seq. Coverage [%]: 10.20 %
Score: 154.15
MW [kDa]: 40.90
pI: 6.17
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
184	2	401.6990	-140.08	2	23.4	36.0	0	87-94	K.ATALVLSK.Q	
81	1	644.2840	-39.02	2	16.8	51.8	0	101-113	K.LVCSGDGNAAAPR.N	Carbamidomethyl: 3
141	1	479.7060	-110.23	2	20.8	34.8	0	182-189	R.VNNAVQWK.K	
206	1	615.3040	-38.77	2	24.8	31.8	0	240-250	K.AVQVELTADQR.S	

Protein 32: SRS domain containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484162
Database: NCBInr
Seq. Coverage [%]: 13.20 %
Score: 154.10
MW [kDa]: 35.80
pI: 5.20
No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
280	1	507.7170	-97.24	2	25.3	37.7	0	108-116	K.WTVAPPESK.T	
406	2	494.9020	-82.87	3	31.6	58.6	0	117-129	K.THSLTLPAENFPR.V	
667	1	800.0660	-25.02	3	45.0	21.6	0	255-276	K.LVIPEGGFPAQEETIVLGCNVR.S	Carbamidomethyl: 19

Protein 33: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221485528
Database: NCBInr
Seq. Coverage [%]: 1.20 %
Score: 147.18
MW [kDa]: 226.10
pI: 5.11
No. of Peptides: 3
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
43	1	519.2140	-77.35	2	16.1	37.5	0	1201-1210	R.YQAGSGGELR.R	
49	1	455.1840	1000.74	2	15.3	52.5	0	1533-1540	R.VGSLMETR.V	Oxidation: 5
126	1	426.1770	-135.40	2	22.2	26.3	0	1545-1551	R.LYASELR.G	

Protein 34: hypothetical protein TGME49_109600 [Toxoplasma gondii ME49]

Accession: gi|237829839
Database: NCBInr
Seq. Coverage [%]: 2.90 %
Score: 141.54
MW [kDa]: 106.50
pI: 10.23
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
73	2	480.2150	-96.73	2	19.4	43.2	0	338-346	K.SQQLGGLEK.A	
61	3	509.7260	-70.27	2	18.3	69.8	0	561-569	K.TASVDEVAAR.L	
106	1	492.7410	-36.69	2	21.9	28.5	0	567-575	R.AAEPNVDIR.A	

Protein 35: ubiquitin, putative [Toxoplasma gondii ME49]
Accession: gi|237835791
Database: NCBInr
Seq. Coverage [%]: 12.00 %
Score: 126.22
MW [kDa]: 24.40
pI: 6.02
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
40	1	434.2020	-69.47	2	14.7	24.9	0	66-72	K.QLTTYNK.S	
84	1	658.2700	-63.40	3	18.7	32.0	1	198-216	K.KNAAVSSTPVNQDEAAQEN.-	
104	1	922.8640	-56.87	2	20.5	69.3	0	199-216	K.NAAVSSTPVNQDEAAQEN.-	

Protein 36: hypothetical protein TGME49_038150 [Toxoplasma gondii ME49]
Accession: gi|237834419
Database: NCBInr
Seq. Coverage [%]: 9.70 %
Score: 119.77
MW [kDa]: 37.00
pI: 5.43
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	534.7590	-19.91	2	25.0	43.3	0	44-53	K.DPVDDAAIPR.V	

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
92	2	438.6990	-68.59	2	19.6	50.7	0	121-128	K.STALDDVR.A	
113	1	859.8500	-45.82	2	21.3	25.8	1	334-349	R.NAAVKDEESVEQAEEA.-	

Protein 37: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221505321
Database: NCBInr
Seq. Coverage [%]: 3.40 %
Score: 119.63
MW [kDa]: 61.90
pI: 4.79
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
136	1	537.2760	-12.88	2	24.4	42.3	0	60-69	R.LQALDSVDGR.S	
55	2	583.2530	-42.58	2	17.6	59.2	0	347-356	R.VTFAENDNQK.V	

Protein 38: hypothetical protein NCLIV_021800 [Neospora caninum Liverpool]
Accession: gi|325116838
Database: NCBInr
Seq. Coverage [%]: 0.20 %
Score: 115.85
MW [kDa]: 522.10
pI: 5.40
No. of Peptides: 3
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
300	6	487.2190	-129.00	2	26.7	44.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2, 5
155	3	487.2080	859.00	2	26.8	26.5	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 5
300	2	487.2190	881.59	2	26.7	53.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2

Protein 39: MORN repeat-containing protein [Toxoplasma gondii ME49]
Accession: gi|237838513
Database: NCBInr
Seq. Coverage [%]: 10.30 %
Score: 111.65
MW [kDa]: 49.20
pI: 5.15
No. of Peptides: 3
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
48	2	510.2490	-32.79	3	16.5	30.6	1	393-407	R.SANAAPARTDTNAIR.Q	
28	2	745.8350	-37.98	2	12.8	58.0	0	408-423	R.QTASTAGSAQPATGSR.A	
91	1	789.3610	-23.23	2	20.9	23.0	0	424-440	R.ASLNGAQLSSQGSGSGR.T	Deamidated: 7

Protein 40: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221484778
Database: NCBInr
Seq. Coverage [%]: 21.50 %
Score: 107.24
MW [kDa]: 20.80
pI: 5.68
No. of Peptides: 2
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
398	2	898.4190	-42.24	3	31.6	50.3	0	44-69	R.ILVHSGDSVTIQCPGAIASNPQDVSK.Y	Carbamidomethyl: 13
164	1	870.8150	-56.06	2	19.7	56.9	0	70-84	K.YVCPGEEPNCTDATK.A	Carbamidomethyl: 3, 10

Protein 41: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221504434
Database: NCBInr
Seq. Coverage [%]: 7.00 %
Score: 103.36
MW [kDa]: 46.90
pI: 10.17
No. of Peptides: 2
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
67	2	502.1910	-141.34	2	14.4	60.7	0	199-208	R.VATVNAGTNR.L	Deamidated: 5
448	1	762.3590	-48.41	3	33.7	42.6	1	231-250	R.NRIPLTATQLSAESQEIQER.L	Deamidated: 9

Protein 42: hypothetical protein TGME49_093440 [Toxoplasma gondii ME49]
Accession: gi|237841697
Database: NCBInr
Seq. Coverage [%]: 5.20 %
Score: 102.72
MW [kDa]: 58.70
pI: 5.00
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
183	1	596.2670	-49.01	2	28.2	30.1	0	341-352	K.SEENSGSLIGAK.S	
486	2	618.6410	-36.18	3	36.3	57.1	0	434-449	K.LKPSDFADVQVITEWR.Q	

Protein 43: hypothetical protein [Paramecium tetraurelia strain d4-2]
Accession: gi|145498718 **Score:** 100.68
Database: NCBInr **MW [kDa]:** 52.30
Seq. Coverage [%]: 2.30 % **pI:** 8.76
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
324	8	595.7870	-93.34	2	32.3	51.4	0	129-138	K.INTNNIFIIK.S	Deamidated: 5
200	4	595.7930	743.08	2	32.3	49.3	0	129-138	K.INTNNIFIIK.S	

Protein 44: 60S ribosomal protein L7a, putative [Toxoplasma gondii ME49]
Accession: gi|237831983 **Score:** 95.74
Database: NCBInr **MW [kDa]:** 31.00
Seq. Coverage [%]: 5.40 % **pI:** 10.46
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
44	3	420.6730	-154.37	2	15.0	59.7	0	49-56	R.VGGNIQPR.R	
64	1	429.7050	-75.90	2	16.9	36.1	0	122-128	R.LLQEAER.Q	

Protein 45: facilitative glucose transporter, putative [Toxoplasma gondii ME49]
Accession: gi|237842935 **Score:** 94.70
Database: NCBInr **MW [kDa]:** 61.60
Seq. Coverage [%]: 4.20 % **pI:** 6.67
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
470	1	635.3130	-13.71	2	34.8	51.8	0	495-505	K.GLSIEESPYFK.G	
372	2	660.2830	-83.43	2	29.9	43.7	1	556-568	R.GVSDDLTKGTEVV.-	

Protein 46: myosin light chain 2 [Toxoplasma gondii]
Accession: gi|121281792 **Score:** 89.49
Database: NCBInr **MW [kDa]:** 40.50
Seq. Coverage [%]: 6.30 % **pI:** 5.29
No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
101	2	734.7740	-48.59	2	16.4	62.8	0	249-262	K.GGSMPQQDAGDYAR.S	Oxidation: 4
237	1	460.6940	-111.65	2	23.2	26.7	0	263-271	R.SLGFAPSNK.E	

Protein 47: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237833253 **Score:** 87.85
Database: NCBInr **MW [kDa]:** 40.00
Seq. Coverage [%]: 4.80 % **pI:** 5.60
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
173	2	429.2120	-120.68	2	22.5	54.6	0	199-206	K.VAVTLQAR.A	
37	1	566.2210	-91.98	2	12.2	33.3	0	321-330	K.ISDPNNQTSR.S	

Protein 48: fructose-1,6-bisphosphate aldolase [Toxoplasma gondii]
Accession: gi|25989716 **Score:** 76.31
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 4.70 % **pI:** 8.67
No. of Peptides: 2

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
119	1	533.7160	-81.61	2	17.3	47.8	0	175-183	R.YAAICQANR.L	Carbamidomethyl: 5
104	1	495.7200	-71.58	2	16.6	28.5	0	324-331	K.AGQVLMER.A	Oxidation: 6

Protein 49: hypothetical protein TTHERM_00357130 [Tetrahymena thermophila]
Accession: gi|118354497 **Score:** 75.95
Database: NCBInr **MW [kDa]:** 187.80
Seq. Coverage [%]: 0.60 % **pI:** 9.51
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
291	4	592.7730	-126.33	2	26.6	50.1	0	1240-1249	K.NVQINLLLQK.G	Deamidated: 3, 5
291	1	592.7730	704.16	2	26.6	25.8	0	1240-1249	K.NVQINLLLQK.G	Deamidated: 3

Protein 50: hypothetical protein [Paramecium tetraurelia strain d4-2]
Accession: gi|145541197 **Score:** 70.26
Database: NCBInr **MW [kDa]:** 45.30
Seq. Coverage [%]: 1.80 % **pI:** 9.44
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
327	1	430.7170	-10.76	2	29.0	50.4	0	97-103	K.QDLLDEK.C	

Protein 51: hypothetical protein TGME49_011630 [Toxoplasma gondii ME49]
Accession: gi|237843981 **Score:** 67.48
Database: NCBInr **MW [kDa]:** 23.60
Seq. Coverage [%]: 4.20 % **pI:** 9.06
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
90	2	486.2410	-79.48	2	19.4	67.5	0	193-201	R.AAQEALIQK.N	

Protein 52: Fructose-bisphosphate aldolase class-I family protein [Tetrahymena thermophila]
Accession: gi|11801200 **Score:** 63.42
Database: NCBInr **MW [kDa]:** 38.60
Seq. Coverage [%]: 3.90 % **pI:** 7.55
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
240	3	666.8190	-52.34	2	27.1	63.4	0	21-34	K.GILAADESTGTIGK.K	

Protein 53: ribosomal phosphoprotein P0 [Toxoplasma gondii]
Accession: gi|14579678 **Score:** 54.71
Database: NCBInr **MW [kDa]:** 34.10
Seq. Coverage [%]: 3.80 % **pI:** 5.34
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
148	2	422.1760	-176.63	3	19.6	54.7	1	102-113	R.IVAENKVPAPAR.Q	

Search Type Combined MS/MS - ProteinExtractor
Search Result RH2_2nd_2014-09-10 11:07:42
Search Location /BOPS/RH2/

Protein 1: Chain A, Crystal Structure Of A Parasite Protein
Accession: gi|22219177 **Score:** 1801.72

Database: NCBInr
Seq. Coverage [%]: 80.60 %
MW [kDa]: 29.80
pI: 7.73
No. of Peptides: 32
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
286	1	623.2820	-67.80	3	26.9	58.6	1	1-17	K.SDPPLVANQVVTCPDKK.S	Carbamidomethyl: 13
315	1	623.5980	-87.01	3	28.3	20.9	1	1-17	K.SDPPLVANQVVTCPDKK.S	Carbamidomethyl: 13; Deamidated: 8
462	9	615.0630	96.72	3	36.0	68.7	0	18-34	K.STAAVILTPTENHFTLK.C	
488	2	615.3030	-46.35	3	37.2	49.2	0	18-34	K.STAAVILTPTENHFTLK.C	Deamidated: 12
411	1	743.3420	-73.06	3	33.4	24.6	1	18-37	K.STAAVILTPTENHFTLKCPK.T	Carbamidomethyl: 18
381	7	815.8920	-40.93	2	31.9	62.6	0	38-52	K.TALTEPPTLAYSPNR.Q	
82	3	749.2930	-59.04	2	16.0	68.6	0	53-66	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
275	2	400.2030	-117.00	2	26.3	29.8	0	96-102	K.LTVPIEK.F	
629	2	793.0860	-32.16	3	45.6	34.1	1	96-116	K.LTVPIEKFPVTTQTFVVGCIK.G	Carbamidomethyl: 19
226	2	585.2150	-87.66	3	23.7	49.2	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8
74	16	508.7520	-80.72	2	17.9	72.6	0	133-142	R.ASSVVNNVAR.C	
136	7	509.2140	-109.59	2	19.1	70.5	0	133-142	R.ASSVVNNVAR.C	Deamidated: 6
236	6	677.7820	-54.60	2	24.3	79.9	0	143-155	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
373	2	782.8400	-62.73	2	31.4	69.0	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
278	2	790.8560	-38.65	2	26.4	59.1	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
270	2	660.6440	-30.37	3	26.0	63.3	1	156-174	K.LSAEGPTTMTLVCGKDGVK.V	Carbamidomethyl: 13; Oxidation: 9
254	3	895.6970	-41.48	3	25.2	43.1	1	171-194	K.DGVKVPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 13, 21
264	1	896.0040	-64.91	3	25.7	49.8	1	171-194	K.DGVKVPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 13, 21; Deamidated: 9
196	2	762.6140	-64.99	3	22.1	30.2	0	175-194	K.VPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 9, 17
323	2	474.2360	-96.27	2	28.8	48.3	1	195-202	K.SFKDILPK.L	
230	2	516.2120	-59.26	3	24.0	61.5	0	203-216	K.LTENPWQGNASSDK.G	
247	2	774.3160	-46.83	2	24.8	91.0	0	203-216	K.LTENPWQGNASSDK.G	Deamidated: 9
388	3	744.3400	-55.89	3	32.2	41.6	1	203-223	K.LTENPWQGNASSDKGATLTIK.K	
295	3	787.0100	-88.84	3	27.5	50.9	2	203-224	K.LTENPWQGNASSDKGATLTIKK.E	

319	2	590.7650	-72.38	4	28.5	53.2	2	203-224	K.LTENPWQGNASSDKGATLTIKK.E	Deamidated: 9
97	2	416.1930	-181.50	2	16.9	41.3	1	217-224	K.GATLTIKK.E	
67	1	503.7510	-25.47	2	15.1	43.9	1	224-232	K.KEAFPAESK.S	
103	2	439.6550	-139.52	2	17.4	33.8	0	225-232	K.EAFPAESK.S	
211	2	652.7850	-67.98	2	22.9	94.3	0	233-245	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6
187	1	614.6920	-30.44	5	28.0	56.8	2	233-262	K.SVIIGCTGGSPEKHHCTVKLEFAGAAGSAK.S	Carbamidomethyl: 6, 16
206	4	446.6950	-74.23	4	22.6	54.5	1	246-262	K.HHCTVKLEFAGAAGSAK.S	Carbamidomethyl: 3
216	2	511.2110	-113.99	2	23.3	77.2	0	252-262	K.LEFAGAAGSAK.S	

Protein 2: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998
Database: NCBInr
Seq. Coverage [%]: 5.90 %
Score: 1617.63
MW [kDa]: 26.60
pI: 7.78
No. of Peptides: 1
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
250	2	555.9360	-65.23	3	25.1	84.9	1	1-18	K.PPLVANQVVTCPDKK.S	Carbamidomethyl: 11

Protein 3: surface antigen protein 1 [Toxoplasma gondii]
Accession: gi|56157026
Database: NCBInr
Seq. Coverage [%]: 4.40 %
Score: 1595.19
MW [kDa]: 33.00
pI: 9.75
No. of Peptides: 1
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
126	1	744.7630	-75.03	2	18.5	63.4	0	83-96	R.QICSAGTTSSCTSK.A	Carbamidomethyl: 3, 11; Deamidated: 1

Protein 4: actin [Toxoplasma gondii ME49]
Accession: gi|237840731
Score: 1148.04

Database: NCBInr
Seq. Coverage [%]: 49.70 %
MW [kDa]: 41.90
pI: 4.91
No. of Peptides: 21
Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
54	2	464.6520	-163.06	2	13.9	85.6	0	20-29	K.AGVAGDDAPR.A	
367	2	571.8160	-59.88	2	29.6	65.7	0	30-40	R.AVFPSIVGKPK.N	
90	2	476.5080	-78.71	3	15.6	35.5	1	52-63	K.DCYVGDEAQSKR.G	Carbamidomethyl: 2
453	8	651.9560	-107.85	3	34.0	82.0	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
375	2	563.2210	-91.44	3	30.1	48.2	1	179-192	R.LDLAGRDLTEYMMK.I	Oxidation: 12, 13
209	2	530.6930	-109.13	2	21.7	41.0	0	198-207	R.GYGFTTSAEK.E	
352	2	519.9010	-65.59	3	28.9	49.2	1	198-211	R.GYGFTTSAEKEIVR.D	
47	2	532.6980	-80.25	2	13.2	69.6	0	230-239	K.AAEDSSDIEK.S	
512	1	941.3680	-86.11	3	36.8	32.2	1	230-255	K.AAEDSSDIEKSYELPDGNIITVGNER.F	
508	3	592.9430	-34.75	3	36.7	84.1	0	240-255	K.SYELPDGNIITVGNER.F	
596	2	599.6050	-63.81	3	41.1	46.5	1	256-270	R.FRCPEALFQPSFLGK.E	Carbamidomethyl: 3
605	2	747.3590	-23.41	2	41.5	75.2	0	258-270	R.CPEALFQPSFLGK.E	Carbamidomethyl: 1
521	1	554.2430	-73.19	4	37.3	20.7	1	258-277	R.CPEALFQPSFLGKEAAGVHR.T	Carbamidomethyl: 1
357	2	572.8850	-88.83	3	29.1	51.0	1	278-291	R.TTFDSIMKCDVDIR.K	Carbamidomethyl: 9; Oxidation: 7
449	4	792.3530	-46.40	3	33.7	56.7	1	292-313	R.KDLYGNVVLSGGTTMYEGIGER.	Oxidation: 15
300	2	512.5910	-49.73	3	26.3	69.2	1	314-327	R.LTKELTSLAPSTMK.I	Oxidation: 13
254	2	597.2530	-91.66	2	24.0	66.7	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10
341	2	589.2690	-70.06	2	28.3	41.4	0	317-327	K.ELTSLAPSTMK.I	
287	2	478.9020	-86.67	3	25.7	46.2	1	317-329	K.ELTSLAPSTMKIK.V	Oxidation: 10
191	1	504.7530	-120.16	2	20.8	28.1	1	328-336	K.IKVVAPPERK.K	
49	1	448.2210	-112.94	2	13.4	34.6	1	330-337	K.VVAPPERK.Y	

Protein 5: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751
Database: NCBInr
Seq. Coverage [%]: 59.10 %
Score: 822.07
MW [kDa]: 19.00
pI: 9.35
No. of Peptides: 14

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
319	1	586.2370	-62.47	3	27.7	22.6	0	44-60	K.TVDAPSSGSVVFQCGDK.L	Carbamidomethyl: 14
450	2	785.3580	-45.07	2	34.5	37.8	0	61-79	K.LTISPSGEGDVFYGK.E	
456	4	471.2400	-129.31	3	34.6	92.2	1	82-96	R.KLTTVLPGAVLTAK.V	
512	2	428.5840	-43.45	3	38.0	60.2	0	83-95	K.LTTVLPGAVLTAK.V	
497	1	645.6910	-54.35	3	36.9	29.0	1	83-101	K.LTTVLPGAVLTAKVQQPAK.G	
422	1	785.6250	-31.40	4	32.9	22.0	1	96-123	K.VQQPAKGPATYTLSYDGTPEKPQMLCYK.C	Carbamidomethyl: 26
432	2	830.0520	-26.10	3	33.5	89.1	0	102-123	K.GPATYTLSYDGTPEKPQVLCYK.C	Carbamidomethyl: 20
45	7	529.7210	-66.10	2	15.1	61.6	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1
118	3	709.6410	-37.53	3	17.3	51.8	1	124-145	K.CVAEAGAPAGRNNDGSSAPTPK.D	Carbamidomethyl: 1
128	1	709.9700	-36.11	3	17.8	35.0	1	124-145	K.CVAEAGAPAGRNNDGSSAPTPK.D	Carbamidomethyl: 1; Deamidated: 13
47	3	544.7090	-68.60	2	12.8	76.3	0	135-145	R.NNDGSSAPTPK.D	Deamidated: 1
35	4	497.8510	-66.66	3	12.0	67.1	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13; Deamidated: 2
23	6	497.5170	-78.75	3	11.4	68.1	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13
311	1	576.8230	-39.91	2	27.4	37.8	1	149-159	K.LIRVPGADGR.V	

Protein 6: hypothetical protein TGME49_112630 [Toxoplasma gondii ME49]
Accession: gi|237830375
Database: NCBInr
Seq. Coverage [%]: 6.30 %
Score: 801.71
MW [kDa]: 286.30
pI: 4.74
No. of Peptides: 16
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
133	1	482.7160	-91.63	2	17.0	28.1	0	333-342	K.IASMISGAAK.M	Oxidation: 4
166	1	501.7250	-78.48	2	19.2	67.0	0	702-710	R.ITGAENLER.C	
184	1	411.1980	-68.61	3	20.3	31.7	1	787-797	K.IDSNVGETLRK.C	
253	2	461.7000	-75.96	2	25.3	41.2	0	983-990	R.SAELAFER.N	

294	2	417.7130	-63.74	2	28.2	38.2	0	1191-1199	R.LGGELFAK.M	
267	1	565.7740	-61.19	2	26.3	40.3	0	1272-1281	R.LEGNVALVCR.R	Carbamidomethyl: 9
118	1	743.7520	-62.49	2	15.7	46.6	0	1452-1465	R.DCDALMGSNSSSAR.Q	Carbamidomethyl: 2; Oxidation: 8
330	2	738.8550	-45.04	2	30.8	77.8	0	1481-1493	K.AYNDALIQEQALK.K	
262	1	537.2260	-64.49	3	25.8	31.3	0	1563-1577	R.ALQGLSMADVNMTAR.T	Oxidation: 7, 12
255	1	525.2660	-36.04	2	25.4	41.9	0	1743-1751	K.LLQANEFSK.M	
421	1	701.8220	-86.15	2	37.5	57.1	0	1759-1771	K.GQADPEIVLYAVK.A	
296	1	413.6940	-78.61	2	28.4	30.7	0	1821-1826	R.YFLVER.T	
188	2	419.6900	-68.84	2	20.6	31.1	0	1965-1971	K.NTEIFSK.L	
247	2	434.7250	-94.71	2	24.6	66.6	0	1972-1980	K.LGGPALNVK.V	
357	2	613.3210	-65.49	2	32.7	65.4	0	2012-2024	K.LLGTNGAPAALVK.G	Deamidated: 5
135	1	599.7110	-89.63	2	17.0	71.0	0	2025-2035	K.GLSNYDGSEEK.T	

Protein 7: surface antigen [Toxoplasma gondii]
Accession: gi|13447088 **Score:** 795.18
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 24.20 % **pI:** 6.93
No. of Peptides: 14

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
358	2	462.8770	-105.34	3	29.2	78.2	1	53-64	R.SKITYFGTLTQK.A	
485	1	544.2760	-26.24	4	35.5	69.3	2	53-70	R.SKITYFGTLTQKAPNWYR.C	
413	2	586.2880	-57.16	2	32.0	50.4	0	55-64	K.ITYFGTLTQK.A	
537	2	653.6450	-46.84	3	38.1	59.7	1	55-70	K.ITYFGTLTQKAPNWYR.C	
207	2	403.6620	-96.20	2	21.6	38.7	0	65-70	K.APNWYR.C	
240	2	470.5510	-70.74	3	23.3	68.4	0	76-88	R.ANEEVVGHVTLNK.E	
35	1	426.4180	-66.48	4	12.0	48.4	2	119-132	R.VCHIDAKDKGDCER.N	Carbamidomethyl: 2, 12
471	2	475.2270	-64.21	3	34.9	60.6	1	133-145	R.NKGFLTDYIPGAK.Q	
559	2	591.2560	-97.56	2	39.2	64.3	0	135-145	K.GFLTDYIPGAK.Q	
244	2	584.2490	-87.47	3	23.5	69.3	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
220	2	690.7970	-66.04	2	22.4	66.9	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 4
220	2	690.7970	656.62	2	22.4	40.3	0	154-165	K.VENNGEQSVLYK.F	

695	1	708.3800	-39.42	2	54.2	25.8	0	166-177	K.FTVPWIFLPPAK.Q	
412	2	489.2310	-51.46	2	31.9	55.9	0	229-236	K.DANFIEIR.C	

Protein 8: actin I [Plasmodium falciparum]
Accession: gi|5911379 **Score:** 733.50
Database: NCBInr **MW [kDa]:** 41.80
Seq. Coverage [%]: 4.80 % **pI:** 5.15
No. of Peptides: 3

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
525	5	908.4050	489.93	2	37.5	52.1	0	2-19	M.GEEVVQALVVDNGSGNVK.A	Deamidated: 6
490	6	908.4170	503.15	2	35.8	70.4	0	2-19	M.GEEVVQALVVDNGSGNVK.A	Deamidated: 12
514	1	907.8560	427.39	2	36.9	20.9	0	2-19	M.GEEVVQALVVDNGSGNVK.A	

Protein 9: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640 **Score:** 698.68
Database: NCBInr **MW [kDa]:** 33.90
Seq. Coverage [%]: 54.80 % **pI:** 6.04
No. of Peptides: 13

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
23	2	539.5180	-97.29	3	11.2	48.8	0	16-30	K.DSHAAANDSGTPMQK.L	Oxidation: 13
184	2	455.7230	-54.81	4	20.5	61.9	1	36-55	R.AAVAAAAANGAETGKAPHLK.L	Deamidated: 9
140	2	517.2580	-35.93	2	18.3	60.8	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5
314	2	481.0270	155.52	3	27.0	50.0	0	101-113	K.PTEQGTVLLQVK.A	
169	2	418.8540	-108.74	3	19.7	37.3	0	114-124	K.APPYKPIPESR.F	
74	1	507.5510	-57.56	3	14.8	51.8	1	136-148	R.QNIETMKDTPEAK.A	Oxidation: 6
371	2	751.8180	-72.21	2	29.9	52.6	0	193-205	R.TFAEPEQPDIEVK.D	
97	2	548.8960	-81.41	3	16.2	74.4	1	222-237	K.AAETVAEKEGEQSAPK.N	
26	2	500.1710	-102.12	3	11.3	46.6	0	238-251	K.NESPESGHDATAQR.K	
198	2	414.5010	-111.66	3	21.1	27.4	0	257-267	R.YLGPESSVPHR.L	
678	1	762.4250	-28.75	2	47.1	55.8	0	268-281	R.LLATCGILPLPLDK.A	Carbamidomethyl: 5

107	2	408.6690	-141.21	2	16.8	28.8	0	284-290	K.ELIQEGK.R	
679	1	740.8600	-37.45	2	47.3	88.2	0	300-314	K.TIITEAGGFFGTPVA.-	

Protein 10: GPI-anchored surface protein [Toxoplasma gondii ME49]
Accession: gi|237837813 **Score:** 469.89
Database: NCBInr **MW [kDa]:** 44.20
Seq. Coverage [%]: 28.30 % **pI:** 7.81
No. of Peptides: 10

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
426	4	788.3160	-49.89	2	32.6	67.0	0	132-145	K.GAQNDWLNGEDLSR.G	Deamidated: 8
407	6	787.8200	-54.99	2	31.7	52.7	0	132-145	K.GAQNDWLNGEDLSR.G	
326	1	459.2540	-60.95	2	27.6	26.3	1	146-153	R.GITFRVPK.N	
351	2	457.7090	-80.34	2	28.8	37.3	0	154-161	K.NNFPGLPR.I	
403	1	458.2230	-32.26	2	31.5	22.9	0	154-161	K.NNFPGLPR.I	Deamidated: 1
516	1	849.0560	-47.93	3	37.0	46.1	0	207-230	R.ITPATNHATIVCGPYGGISPLDYK.K	Carbamidomethyl: 12
75	1	556.2110	544.24	3	14.8	30.4	1	231-244	K.KEFCTGEPHTSCGR.Q	Carbamidomethyl: 4, 12
113	1	513.1830	-52.86	3	17.0	26.7	0	232-244	K.EFCTGEPHTSCGR.Q	Carbamidomethyl: 3, 11
129	1	1046.7170	-50.88	3	17.9	57.3	0	334-367	K.EAPNTNNSGGTPTGGGSDDDTTAPGTGSSGTDSR.G	
30	1	438.4120	-86.17	4	11.6	61.6	2	368-384	R.GDLGDGRDQDAKGGMSR.G	Oxidation: 15

Protein 11: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049 **Score:** 465.15
Database: NCBInr **MW [kDa]:** 39.40
Seq. Coverage [%]: 28.80 % **pI:** 4.66
No. of Peptides: 10

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
545	1	880.7030	-33.97	3	38.6	71.6	0	108-132	K.SQNLSPTELDTAFGVDAAGNCDTTR.L	Carbamidomethyl: 21
680	2	748.9320	-32.09	2	47.8	83.9	0	133-146	R.LVDVSTLIPTAIVR.G	

135	1	406.6160	-159.18	2	18.1	23.8	0	174-179	K.ACFVCR.E	Carbamidomethyl: 2, 5
77	1	463.2160	-73.04	2	15.0	23.7	0	227-234	R.SVVVHCPK.G	Carbamidomethyl: 6
670	1	567.9470	-53.15	3	45.3	60.3	1	235-249	K.GLPVLDIFKESDNVR.A	
360	1	604.0260	-76.97	4	29.3	42.2	1	258-280	K.AKLEDVVGSGSTIKPVNVDSQNRD	
342	1	718.3430	-50.97	4	28.4	49.5	2	258-284	K.AKLEDVVGSGSTIKPVNVDSQNRDVSR.S	
360	2	738.6960	-28.26	3	28.8	46.7	0	260-280	K.LEDVVGSGSTIKPVNVDSQNR.D	
330	1	668.5770	-29.31	4	27.8	21.2	1	260-284	K.LEDVVGSGSTIKPVNVDSQNRDVSR.S	
354	2	525.2490	-56.86	3	28.9	27.3	0	285-298	R.SYLITSTSSFSKPR.I	

Protein 12: SRS domain-containing surface antigen, putative [Toxoplasma gondii ME49]
Accession: gi|237839855 **Score:** 422.60
Database: NCBInr **MW [kDa]:** 34.90
Seq. Coverage [%]: 32.00 % **pI:** 8.07
No. of Peptides: 8

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
147	3	534.2230	-91.75	2	19.6	59.7	0	53-62	R.VTGNSSVEFK.C	
70	1	525.7130	-76.98	2	15.3	75.8	0	126-135	K.CSSLANTAAR.V	Carbamidomethyl: 1
38	1	585.7690	-37.74	2	12.8	50.2	0	136-147	R.VGAGQPGQEAEK.E	
40	2	476.5450	-64.38	3	12.9	81.0	1	136-149	R.VGAGQPGQEAEKEK.T	
434	2	619.9730	-45.53	3	33.8	24.1	1	150-166	K.TCIVQTSVWGPPIKGSK.E	Carbamidomethyl: 2
360	2	589.5260	-22.72	4	30.7	54.1	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.T	Carbamidomethyl: 2
391	2	629.0230	-67.73	4	32.4	52.9	1	235-257	R.TNAADDKKPAYTFSVTSLPTEEKT	
76	2	401.1420	-132.06	3	14.8	25.1	1	258-266	K.TLCYQCTKK.N	Carbamidomethyl: 3, 6

Protein 13: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221487802 **Score:** 372.61
Database: NCBInr **MW [kDa]:** 37.20
Seq. Coverage [%]: 26.00 % **pI:** 5.82
No. of Peptides: 6

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
282	1	586.7680	-74.15	2	26.6	83.2	0	81-92	K.GGISVEVDPATK.K	
631	2	947.9190	-46.10	2	48.9	86.3	0	122-139	R.ANLQGETPLAEFFGEGSK.A	
20	1	708.2780	-46.48	2	11.4	64.3	0	184-197	R.APTGDPSQNSDGNR.G	
540	2	778.7080	-37.73	3	40.1	63.6	0	266-287	K.LADVLPSATFQETSSAHVFSVK. L	
197	2	501.5520	-51.91	3	22.2	40.6	1	288-299	K.ELPKTEATYCYK.C	Carbamidomethyl: 10
51	2	511.8780	-42.66	3	14.2	35.6	1	300-314	K.CSPPVDSGDTADGKK.N	Carbamidomethyl: 1

Protein 14: SAG1-related sequence 3 [Toxoplasma gondii]
Accession: gi|2305258
Database: NCBInr
Seq. Coverage [%]: 21.20 %
Score: 363.35
MW [kDa]: 36.20
pI: 5.75
No. of Peptides: 8

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
414	2	831.8860	-47.71	2	40.2	53.1	0	91-105	K.CAIGTVTLPVDLMEK.Q	Carbamidomethyl: 1; Oxidation: 13
109	1	455.7340	-42.28	2	18.9	21.3	0	158-165	K.QNLPPVDK.E	
333	2	720.3300	-48.44	3	33.1	28.7	1	158-176	K.QNLPPVDKEFIVGCTQEGK.G	Carbamidomethyl: 14
215	2	634.2840	-29.95	2	28.2	67.5	0	166-176	K.EFIVGCTQEGK.G	Carbamidomethyl: 6
110	2	604.0100	-20.81	4	19.0	33.6	1	189-211	R.ASSKDSQTVTCAYGSASNADVHR V	Carbamidomethyl: 11; Deamidated: 18
120	4	603.7380	-63.88	4	19.6	65.3	1	189-211	R.ASSKDSQTVTCAYGSASNADVHR V	Carbamidomethyl: 11
318	2	560.6040	-46.20	3	32.1	52.5	0	278-292	K.AVLTIPHDNFPEAQK.K	
279	1	452.7110	-81.69	4	29.4	42.0	1	278-293	K.AVLTIPHDNFPEAQKK.V	

Protein 15: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221486446
Database: NCBInr
Seq. Coverage [%]: 13.80 %
Score: 345.80
MW [kDa]: 50.90
pI: 4.54

No. of Peptides: 6

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
16	2	483.8870	-49.27	3	11.5	80.5	2	55-67	K.VKVEDTEGDKSSR.G	
22	2	420.1580	-90.26	3	13.0	40.9	0	97-106	K.QHQMELEAEK.A	Oxidation: 4
86	2	487.2380	-34.55	3	20.6	48.3	1	146-157	K.ISAREEIEQQTR.K	
23	1	516.7240	-53.13	2	13.1	45.3	0	150-157	R.EEIEQQTR.K	
97	2	588.6090	-30.19	3	21.5	52.7	1	438-453	K.QTVEEASKQTVEEASK.Q	
50	1	846.8660	-39.88	2	17.2	62.6	0	470-485	K.QTASEVTKPTEEEANTS.-	

Protein 16: microneme protein MIC3 [Toxoplasma gondii]
Accession: gi|343113243
Database: NCBInr
Seq. Coverage [%]: 18.30 %
Score: 337.20
MW [kDa]: 36.40
pI: 5.84
No. of Peptides: 4

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
338	6	603.5610	-87.67	3	28.2	94.1	0	80-95	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 6
82	2	550.2110	-53.47	3	14.1	22.5	1	226-239	R.TGCHAFRENCSPGR.C	Carbamidomethyl: 3, 10
415	2	765.3570	-21.93	3	32.1	47.8	1	261-281	R.EVTSKAEESCVEGVEVTLAEK.C	Carbamidomethyl: 10
45	4	583.7460	-27.78	2	13.0	82.5	0	312-323	K.GESGSEGSLSEK.M	

Protein 17: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819
Database: NCBInr
Seq. Coverage [%]: 11.80 %
Score: 303.54
MW [kDa]: 39.10
pI: 7.76
No. of Peptides: 6

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

551	4	702.8400	-45.67	2	38.9	72.0	0	136-148	K.EWVTGDSVLTGLK.I	
204	5	702.3290	-46.84	2	24.7	66.3	0	149-161	K.ISVPESQYPANAK.S	
279	2	702.8110	-61.05	2	25.3	41.0	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 11
279	1	702.8110	-61.05	2	25.3	22.9	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 7
190	2	650.2570	-62.91	2	20.8	58.9	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
491	2	448.6870	-70.64	2	35.8	26.2	0	269-265	K.WFFGDPK.S	

Protein 18: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826
Database: NCBInr
Seq. Coverage [%]: 17.80 %
Score: 301.85
MW [kDa]: 45.10
pI: 7.74
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
685	1	780.3860	-22.08	2	49.1	74.2	0	58-70	R.FLDPIDELPEPFK.A	
421	2	440.5540	-82.83	3	32.4	69.4	1	89-100	K.LGIEGDKTVFLK.S	
419	2	547.2560	-55.06	3	32.3	33.0	0	101-115	K.SPISPDVAVYVHAER.L	
447	2	610.3190	-28.67	3	33.7	77.0	0	223-240	K.SVELTRPQAVLGAVGMGK.K	Oxidation: 16
414	2	484.9090	-49.58	3	32.0	48.2	1	269-281	R.ALQFGKDFQVTAK.K	

Protein 19: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499
Database: NCBInr
Seq. Coverage [%]: 23.90 %
Score: 299.63
MW [kDa]: 23.90
pI: 5.66
No. of Peptides: 7

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
59	1	410.8420	-77.77	3	16.2	29.4	1	112-122	K.SEARGPSLEER.I	
162	1	534.2310	-71.18	3	28.8	28.8	1	116-129	R.GPSLEERIEEQGTR.R	
57	1	568.7620	-36.49	2	16.1	41.5	0	132-141	R.YSSVQEPQAK.V	
32	2	847.9820	-47.92	3	13.1	64.9	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 21

34	1	848.3060	-52.62	3	13.4	31.4	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGG NEGR.G	Deamidated: 13, 21
48	2	795.9480	-51.42	3	13.8	49.5	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGN EGR.G	Deamidated: 23
36	2	795.9450	-55.19	3	13.8	61.1	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGN EGR.G	Deamidated: 20

Protein 20: bradyzoite-specific surface protein, putative [Toxoplasma gondii ME49]
Accession: gi|237841177
Database: NCBInr
Seq. Coverage [%]: 16.80 %
Score: 284.11
MW [kDa]: 41.50
pI: 6.23
No. of Peptides: 5

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
251	1	626.2610	-87.98	2	27.7	72.5	0	93-104	K.GSLTATLECTAK.D	Carbamidomethyl: 9
196	1	401.5130	-136.74	3	24.2	37.6	1	169-179	K.AIQSQKWTLK.L	
227	1	725.8050	-33.86	2	26.2	62.0	0	190-202	K.TFDVGCTYTTTGK.A	Carbamidomethyl: 6
297	3	778.3850	-40.70	2	30.5	73.2	0	203-217	K.AAANPAVCQLTVNVK.A	Carbamidomethyl: 8
100	3	624.9410	-41.82	3	18.2	39.4	1	326-342	R.LGCVPNKAPQSDSQDTR.V	Carbamidomethyl: 3

Protein 21: myosin D, putative [Toxoplasma gondii ME49]
Accession: gi|237832241
Database: NCBInr
Seq. Coverage [%]: 7.90 %
Score: 239.13
MW [kDa]: 91.00
pI: 9.39
No. of Peptides: 6

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
171	2	662.3210	-35.29	3	28.1	41.5	0	66-83	K.LQQVEPTADSQELTVQAK.E	
184	1	595.7550	-95.41	2	29.3	24.7	0	143-153	K.NAGPDTIALYR.D	
35	2	442.8700	-52.21	3	15.1	38.2	1	409-420	K.TTYAGSNKIESR.W	
214	1	443.7500	-76.69	2	33.2	25.5	0	526-533	K.LIETLLGK.G	
88	2	419.6990	-90.74	2	20.8	26.9	0	555-562	K.FVSSLASK.L	

25	2	431.1890	-83.19	2	13.8	49.1	0	614-621	R.ASTNDVVR.G	

Protein 22: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635 **Score:** 236.79
Database: NCBInr **MW [kDa]:** 70.50
Seq. Coverage [%]: 7.80 % **pI:** 5.55
No. of Peptides: 5

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
123	2	516.2540	-45.78	2	23.3	40.4	0	80-90	K.GGTLLVSGDGR.F	
43	1	445.1940	-91.88	2	15.9	51.1	0	520-528	K.SSPAEITGK.V	
47	1	419.1510	-124.23	2	16.4	25.1	0	551-557	K.YTDPVDK.Q	
59	2	560.7710	-54.35	2	18.0	71.5	0	579-590	K.LSGTGSTGATIR.V	
203	1	597.7710	-75.71	2	32.6	22.9	0	627-637	K.YIGTETPTVIT.-	

Protein 23: RecName: Full=Dense granule protein 5; Short=Protein GRA 5; AltName: Full=p21; Flags: Precursor
Accession: gi|2507039 **Score:** 221.83
Database: NCBInr **MW [kDa]:** 13.00
Seq. Coverage [%]: 35.00 % **pI:** 5.69
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
20	2	502.9610	-47.61	4	11.2	54.9	1	43-58	R.GREQQQVQQHEQNEDR.S	
14	2	599.2330	-61.71	3	10.9	47.8	0	45-58	R.EQQQVQQHEQNEDR.S	
204	1	607.7590	-45.01	4	21.7	47.8	1	45-63	R.EQQQVQQHEQNEDRSLFER.G	
270	1	779.6260	-62.65	3	25.2	71.3	1	100-120	R.AIQEESKESATAEEEEVAEEE.-	

Protein 24: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163 **Score:** 203.13
Database: NCBInr **MW [kDa]:** 82.10
Seq. Coverage [%]: 7.50 % **pI:** 5.21

No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
40	1	554.2130	-82.28	2	15.6	35.8	0	172-181	R.SVVASEECAR.R	Carbamidomethyl: 8
150	1	480.2550	-39.63	2	26.3	50.1	0	183-191	R.LLDSIEAAK.S	
135	1	758.8450	-27.06	2	24.2	29.0	0	192-206	K.SSLAGEPEEAVNASR.V	
65	1	551.7570	-93.82	2	18.6	68.2	1	240-250	K.AVAELKAGESK.A	
41	1	547.2140	-123.39	2	15.7	20.9	0	677-687	R.AVTESMAAVAK.E	Oxidation: 6

Protein 25: actin [Pyrocystis lunula]
Accession: gi|27450759 **Score:** 187.99
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 7.20 % **pI:** 5.83
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
385	3	400.1990	-102.48	3	30.7	27.8	0	29-39	R.AVFPSIVGRPR.H	
538	2	895.9050	-49.78	2	38.1	59.8	0	239-254	K.SYELPDGQVITIGNER.F	

Protein 26: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688 **Score:** 184.05
Database: NCBInr **MW [kDa]:** 144.50
Seq. Coverage [%]: 5.60 % **pI:** 5.80
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
103	1	621.7870	-82.86	2	16.2	22.1	0	46-58	K.VGSQPVAAGAEEK.V	
77	1	501.2250	-42.35	3	14.5	33.8	0	82-97	R.ESTVGGSGTGHSQLGK.S	
316	2	803.8410	-65.88	2	27.8	82.1	0	338-352	K.TNEALLTGESEDISK.T	
415	1	702.8110	-69.04	2	32.8	25.4	0	673-684	R.VQELEVPFNSSR.K	

204	1	633.2750	-50.95	3	22.3	20.7	0	918-936	R.QGHTVAMTGDGVNDAPALK.A	Oxidation: 7

Protein 27: glyceraldehyde 3-phosphate dehydrogenase, related [Neospora caninum Liverpool]
Accession: gi|325115564 **Score:** 179.64
Database: NCBInr **MW [kDa]:** 36.50
Seq. Coverage [%]: 17.40 % **pI:** 6.83
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
345	1	736.6810	-15.86	3	34.2	58.4	0	142-162	K.SSDVIVSNASCTTNCLAPLAK.I	Carbamidomethyl: 11, 15
264	2	678.8600	-26.19	2	28.4	51.1	0	203-217	R.SAGVNIIPASTGAAK.A	
260	1	504.2410	-65.50	2	28.2	30.8	0	257-265	K.YEDIVAAVK.E	
285	2	774.8220	-64.23	2	29.6	21.9	0	327-340	R.LVELAHYMSVQDGA.-	Oxidation: 8

Protein 28: microtubule-binding protein, putative [Toxoplasma gondii ME49]
Accession: gi|237836067 **Score:** 177.75
Database: NCBInr **MW [kDa]:** 92.50
Seq. Coverage [%]: 6.90 % **pI:** 9.15
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
168	2	848.8550	-37.39	2	27.8	30.1	0	187-200	R.LNESFDDQTEETLR.V	
193	1	648.2630	-46.00	2	23.9	27.3	0	285-295	R.MDVEVSDIDTR.L	Oxidation: 1
267	1	439.2030	-102.79	2	28.6	32.4	0	430-438	K.LFGGLGSAR.A	
158	2	526.2630	-23.26	3	19.2	64.3	1	734-749	R.APTCGATITSSGVAKR.F	Carbamidomethyl: 4
62	1	448.7170	-63.38	2	18.2	23.6	0	808-815	K.YTTTAIAR.V	

Protein 29: 14-3-3 protein homologue [Toxoplasma gondii]
Accession: gi|3123732 **Score:** 177.67
Database: NCBInr **MW [kDa]:** 30.70
Seq. Coverage [%]: 15.00 % **pI:** 4.55
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
164	2	458.5270	-66.80	3	20.4	70.3	1	143-154	R.YISEFSGEEGKK.Q	
46	2	683.7920	-34.09	2	13.3	57.7	0	155-166	K.QAADQAQESYQK.A	
251	2	574.9240	-62.76	3	25.0	49.7	0	167-182	K.ATETAEAELPSTHPIR.L	

Protein 30: hypothetical protein TGME49_093430 [Toxoplasma gondii ME49]
Accession: gi|237841695 **Score:** 159.38
Database: NCBInr **MW [kDa]:** 44.60
Seq. Coverage [%]: 13.40 % **pI:** 4.86
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
151	2	750.8300	-50.71	2	18.8	44.2	0	70-82	R.QQPVEANETTVER.G	
94	2	437.1790	-104.92	2	15.9	64.8	0	185-193	R.AAADIAADR.A	
523	2	708.3640	-1.26	3	38.6	20.3	1	226-244	R.QGGLLPDVPLDETVEPCRK.G	Carbamidomethyl: 17
340	2	688.8190	-36.11	2	28.3	30.1	0	398-411	R.HTPPGADQIELAGA.-	

Protein 31: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237844685 **Score:** 154.15
Database: NCBInr **MW [kDa]:** 40.90
Seq. Coverage [%]: 10.20 % **pI:** 6.17
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
184	2	401.6990	-140.08	2	23.4	36.0	0	87-94	K.ATALVLSK.Q	
81	1	644.2840	-39.02	2	16.8	51.8	0	101-113	K.LVCSGDGNAAAPR.N	Carbamidomethyl: 3
141	1	479.7060	-110.23	2	20.8	34.8	0	152-159	R.VNNAVQWK.K	
206	1	615.3040	-38.77	2	24.8	31.8	0	240-250	K.AVQVELTADQR.S	

Protein 32: SRS domain containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484162 **Score:** 154.10
Database: NCBInr **MW [kDa]:** 35.80
Seq. Coverage [%]: 13.20 % **pI:** 5.20
No. of Peptides: 3
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
280	1	507.7170	-97.24	2	28.3	37.7	0	108-116	K.WTVAPPESK.T	
406	2	494.9020	-52.87	3	31.6	58.6	0	117-129	K.THSLTLPAENFPR.V	
667	1	800.0660	-25.02	3	45.0	21.6	0	265-276	K.LVIPEGGFPAQEETIVLGCNVR.S	Carbamidomethyl: 19

Protein 33: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221485528 **Score:** 147.18
Database: NCBInr **MW [kDa]:** 226.10
Seq. Coverage [%]: 1.20 % **pI:** 5.11
No. of Peptides: 3
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
43	1	519.2140	-77.35	2	16.1	37.5	0	1201-1210	R.YQAGSGGELR.R	
49	1	455.1840	1000.74	2	15.3	52.8	0	1533-1540	R.VGSLMETR.V	Oxidation: 5
126	1	426.1770	-135.46	2	22.2	26.3	0	1545-1551	R.LYASELR.G	

Protein 34: hypothetical protein TGME49_109600 [Toxoplasma gondii ME49]
Accession: gi|237829839 **Score:** 141.54
Database: NCBInr **MW [kDa]:** 106.50
Seq. Coverage [%]: 2.90 % **pI:** 10.23
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

73	2	480.2150	-96.73	2	19.4	43.3	0	339-349	K.SQQLGGLEK.A	
61	3	509.7260	-70.27	2	18.3	69.8	0	551-560	K.TASVDEVAAR.L	
106	1	492.7410	-36.69	2	21.9	28.5	0	567-575	R.AAEPNVDIR.A	

Protein 35: ubiquitin, putative [Toxoplasma gondii ME49]
Accession: gi|237835791 **Score:** 126.22
Database: NCBInr **MW [kDa]:** 24.40
Seq. Coverage [%]: 12.00 % **pI:** 6.02
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
40	1	434.2020	-69.47	2	14.7	24.9	0	66-72	K.QLTTYNK.S	
84	1	658.2700	-63.40	3	18.7	32.6	1	198-216	K.KNAAVSSTPVNQDEAAQEN.-	
104	1	922.8640	-56.87	2	20.5	69.3	0	199-216	K.NAAVSSTPVNQDEAAQEN.-	

Protein 36: hypothetical protein TGME49_038150 [Toxoplasma gondii ME49]
Accession: gi|237834419 **Score:** 119.77
Database: NCBInr **MW [kDa]:** 37.00
Seq. Coverage [%]: 9.70 % **pI:** 5.43
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	534.7590	-19.91	2	25.0	43.3	0	44-53	K.DPVDDAAIPR.V	
92	2	438.6990	-58.59	2	19.6	50.7	0	121-128	K.STALDDVR.A	
113	1	859.8500	-45.82	2	21.3	25.8	1	334-349	R.NAAVKDEESVEQAEEA.-	

Protein 37: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221505321 **Score:** 119.63
Database: NCBInr **MW [kDa]:** 61.90
Seq. Coverage [%]: 3.40 % **pI:** 4.79
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
136	1	537.2760	-12.88	2	24.4	42.3	0	60-69	R.LQALDSVDGR.S	
55	2	583.2530	-42.88	2	17.6	59.2	0	347-356	R.VTFAENDNQK.V	

Protein 38: hypothetical protein NCLIV_021800 [Neospora caninum Liverpool]
Accession: gi|325116838 **Score:** 115.85
Database: NCBInr **MW [kDa]:** 522.10
Seq. Coverage [%]: 0.20 % **pI:** 5.40
No. of Peptides: 3
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
300	5	487.2190	-129.00	2	26.7	44.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2, 5
155	3	487.2080	869.00	2	26.8	26.5	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 5
300	2	487.2190	881.59	2	26.7	53.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2

Protein 39: MORN repeat-containing protein [Toxoplasma gondii ME49]
Accession: gi|237838513 **Score:** 111.65
Database: NCBInr **MW [kDa]:** 49.20
Seq. Coverage [%]: 10.30 % **pI:** 5.15
No. of Peptides: 3
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
48	2	510.2490	-32.79	3	16.5	30.6	1	393-407	R.SANAAPARTDTNAIR.Q	
28	2	745.8350	-37.98	2	12.8	58.0	0	408-423	R.QTASTAGSAQPATGSR.A	
91	1	789.3610	-23.23	2	20.9	23.0	0	424-440	K.ASLNGAQLSSQGSGSGR.T	Deamidated: 7

Protein 40: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221484778 **Score:** 107.24
Database: NCBInr **MW [kDa]:** 20.80

Seq. Coverage [%]: 21.50 % **pI:** 5.68
No. of Peptides: 2
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
398	2	898.4190	-42.24	3	31.6	50.3	0	44-69	R.ILVHSGDSVTIQCPGAIASNPQDVSK.Y	Carbamidomethyl: 13
164	1	870.8150	-56.06	2	19.7	56.9	0	70-84	K.YVCPGEEPNCTDATK.A	Carbamidomethyl: 3, 10

Protein 41: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221504434 **Score:** 103.36
Database: NCBInr **MW [kDa]:** 46.90
Seq. Coverage [%]: 7.00 % **pI:** 10.17
No. of Peptides: 2
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
67	2	502.1910	-141.34	2	14.4	60.7	0	199-208	R.VATVNAGTNR.L	Deamidated: 5
448	1	762.3590	-48.41	3	33.7	42.6	1	231-250	R.NRIPLTATQLSAESQEIQER.L	Deamidated: 9

Protein 42: hypothetical protein TGME49_093440 [Toxoplasma gondii ME49]
Accession: gi|237841697 **Score:** 102.72
Database: NCBInr **MW [kDa]:** 58.70
Seq. Coverage [%]: 5.20 % **pI:** 5.00
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
183	1	596.2670	-49.01	2	20.2	30.1	0	341-352	K.SEENSGSLIGAK.S	
486	2	618.6410	-36.18	3	36.3	57.1	0	434-449	K.LKPSDPADVQVITEWR.Q	

Protein 43: hypothetical protein [Paramecium tetraurelia strain d4-2]
Accession: gi|145498718 **Score:** 100.68

Database: NCBInr
Seq. Coverage [%]: 2.30 %
MW [kDa]: 52.30
pI: 8.76
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
324	8	595.7870	-93.34	2	32.3	51.4	0	129-138	K.INTNNIFIIK.S	Deamidated: 5
200	4	595.7930	743.08	2	32.3	49.3	0	129-138	K.INTNNIFIIK.S	

Protein 44: 60S ribosomal protein L7a, putative [Toxoplasma gondii ME49]
Accession: gi|237831983
Database: NCBInr
Seq. Coverage [%]: 5.40 %
Score: 95.74
MW [kDa]: 31.00
pI: 10.46
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
44	3	420.6730	-154.37	2	15.0	59.7	0	49-56	R.VGGNIQPR.R	
64	1	429.7050	-75.96	2	16.9	36.1	0	122-128	R.LLQEAER.Q	

Protein 45: facilitative glucose transporter, putative [Toxoplasma gondii ME49]
Accession: gi|237842935
Database: NCBInr
Seq. Coverage [%]: 4.20 %
Score: 94.70
MW [kDa]: 61.60
pI: 6.67
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
470	1	635.3130	-13.71	2	34.8	51.0	0	495-505	K.GLSIEESPYFK.G	
372	2	660.2830	-83.43	2	29.9	43.7	1	556-568	R.GVSDDLTKGTEVV.-	

Protein 46: myosin light chain 2 [Toxoplasma gondii]
Accession: gi|121281792
Score: 89.49

Database: NCBInr
Seq. Coverage [%]: 6.30 %
MW [kDa]: 40.50
pI: 5.29
No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
101	2	734.7740	-48.59	2	16.4	62.8	0	249-262	K.GGSMPQQDAGDYAR.S	Oxidation: 4
237	1	460.6940	-111.65	2	23.2	26.7	0	263-271	R.SLGFAPSNK.E	

Protein 47: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237833253
Database: NCBInr
Seq. Coverage [%]: 4.80 %
Score: 87.85
MW [kDa]: 40.00
pI: 5.60
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
173	2	429.2120	-120.68	2	22.5	54.6	0	199-206	K.VAVTLQAR.A	
37	1	566.2210	-91.98	2	12.2	33.3	0	321-330	K.ISDPNNQTSR.S	

Protein 48: fructose-1,6-bisphosphate aldolase [Toxoplasma gondii]
Accession: gi|25989716
Database: NCBInr
Seq. Coverage [%]: 4.70 %
Score: 76.31
MW [kDa]: 39.10
pI: 8.67
No. of Peptides: 2

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
119	1	533.7150	-8.61	2	17.3	47.8	0	175-183	R.YAAICQANR.L	Carbamidomethyl: 5
104	1	495.7200	-71.58	2	16.6	28.5	0	324-331	K.AQQVLMER.A	Oxidation: 6

Protein 49: hypothetical protein TTHERM_00357130 [Tetrahymena thermophila]
Accession: gi|118354497
Score: 75.95

Database: NCBInr
Seq. Coverage [%]: 0.60 %
MW [kDa]: 187.80
pI: 9.51
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
291	4	592.7730	-126.33	2	26.6	50.1	0	1240-1249	K.NVQINLLLQK.G	Deamidated: 3, 5
291	1	592.7730	704.16	2	26.6	25.8	0	1240-1249	K.NVQINLLLQK.G	Deamidated: 3

Protein 50: hypothetical protein [Paramecium tetraurelia strain d4-2]
Accession: gi|145541197
Database: NCBInr
Seq. Coverage [%]: 1.80 %
Score: 70.26
MW [kDa]: 45.30
pI: 9.44
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
327	1	430.7170	-10.76	2	29.0	50.4	0	97-103	K.QDLLDEK.C	

Protein 51: hypothetical protein TGME49_011630 [Toxoplasma gondii ME49]
Accession: gi|237843981
Database: NCBInr
Seq. Coverage [%]: 4.20 %
Score: 67.48
MW [kDa]: 23.60
pI: 9.06
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
90	2	486.2410	-79.48	2	19.4	67.5	0	193-201	R.AAQEALIQK.N	

Protein 52: ribosomal phosphoprotein P0 [Toxoplasma gondii]
Accession: gi|14579678
Database: NCBInr
Seq. Coverage [%]: 3.80 %
Score: 54.71
MW [kDa]: 34.10
pI: 5.34

No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
148	2	422.1760	-176.63	3	19.6	54.7	1	102-113	R.IVAENKVPAPAR.Q	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH1_GA2_01_2144.d/

Protein 1: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221487562
Database: NCBInr
Seq. Coverage [%]: 6.10 %
Score: 801.71
MW [kDa]: 293.50
pI: 4.78
No. of Peptides: 16

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
133	1	482.7160	-91.63	2	17.0	28.1	0	400-409	K.IASMISGAAK.M	Oxidation: 4
166	1	501.7250	-78.45	2	19.2	67.0	0	769-777	R.ITGAENLER.C	
184	1	411.1980	-68.61	3	20.3	31.7	1	854-864	K.IDSNVGETLRK.C	
263	2	467.7000	-75.96	2	25.3	41.2	0	1050-1057	R.SAELAFER.N	
294	2	417.7130	-63.74	2	28.2	38.2	0	1258-1265	R.LGGELFAK.M	
267	1	565.7740	-51.19	2	26.3	40.3	0	1339-1348	R.LEGNVALVCR.R	Carbamidomethyl: 9
118	1	743.7520	-62.49	2	15.7	46.6	0	1519-1532	R.DCDALMGSNSSSAR.Q	Carbamidomethyl: 2; Oxidation: 6
330	2	738.8550	-45.04	2	30.8	77.8	0	1548-1560	K.AYNDALIQEQALK.K	
262	1	537.2260	-64.40	3	25.8	31.3	0	1630-1644	R.ALQGLSMADVNMTAR.T	Oxidation: 7, 12
265	1	526.2660	-36.04	2	25.4	41.9	0	1810-1818	K.LLQANEFSK.M	
421	1	701.8220	-86.15	2	37.5	57.1	0	1826-1838	K.GQADPEVLYAVK.A	
296	1	413.6940	-78.61	2	28.4	30.7	0	1888-1893	R.YFLVER.T	

188	2	419.6900	-68.84	2	20.6	31.1	0	2032-2038	K.NTEIFSK.L	
247	2	434.7250	-84.71	2	24.6	66.6	0	2039-2047	K.LGGPALNVK.V	
357	2	613.3210	-65.48	2	32.7	65.4	0	2079-2091	K.LLGTNGAPAALVK.G	Deamidated: 5
135	1	599.7110	-89.63	2	17.0	71.0	0	2092-2102	K.GLSNYDGSEEK.T	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH2_GA3_01_2145.d/

Protein 1: P-type Ca(2+)-ATPase, putative [Toxoplasma gondii ME49]
Accession: gi|237842543 **Score:** 184.05
Database: NCBInr **MW [kDa]:** 144.60
Seq. Coverage [%]: 5.60 % **pI:** 5.77
No. of Peptides: 5
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
103	1	621.7870	-52.86	2	16.2	22.1	0	46-58	K.VGSQPVAAGAEEK.V	
77	1	501.2260	-42.35	3	14.6	33.8	0	82-87	R.ESTVGGSGTGHSQLGK.S	
316	1	803.8410	-65.88	2	27.8	82.1	0	338-352	K.TNEALLTGESEDISK.T	
415	1	702.8110	-69.04	2	32.8	25.4	0	673-684	R.VQELEVPFNSSR.K	
204	1	633.2750	-60.95	3	22.3	20.7	0	918-936	R.QGHTVAMTGDGVNDAPALK.A	Oxidation: 7

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH3_GA4_01_2146.d/

Protein 1: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221486446 **Score:** 330.22

Database: NCBInr **MW [kDa]:** 50.90
Seq. Coverage [%]: 13.80 % **pI:** 4.54
No. of Peptides: 6
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
16	2	483.8870	-49.27	3	11.5	80.5	2	55-67	K.VKVEDTEGDKSSR.G	
22	2	420.1580	-90.26	3	13.0	40.9	0	97-106	K.QHQMELEAEK.A	Oxidation: 4
86	2	487.2380	-34.55	3	20.6	48.3	1	146-157	K.ISAREEIEQQTR.K	
23	1	516.7240	-53.13	2	13.1	45.3	0	150-157	R.EEIEQQTR.K	
97	2	588.6090	-30.19	3	21.5	52.7	1	438-453	K.QTVEEASKQTVEEASK.Q	
50	1	846.8660	-39.88	2	17.2	62.6	0	470-485	K.QTASEVTKPTEEANTS.-	

Protein 2: myosin D, putative [Toxoplasma gondii ME49]
Accession: gi|237832241 **Score:** 239.13
Database: NCBInr **MW [kDa]:** 91.00
Seq. Coverage [%]: 7.90 % **pI:** 9.39
No. of Peptides: 6
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
171	2	662.3210	-35.29	3	28.1	41.5	0	66-83	K.LQQVEPTADSQELTVQAK.E	
184	1	595.7550	-95.41	2	29.3	24.7	0	143-153	K.NAGPDTIALYR.D	
35	2	442.8700	-52.21	3	15.1	38.2	1	409-420	K.TTYAGSNKIESR.W	
214	1	443.7500	-76.69	2	33.2	25.5	0	526-533	K.LIETLLGK.G	
88	2	419.6990	-90.74	2	20.8	26.9	0	555-562	K.FVSSLASK.L	
25	2	431.1890	-83.19	2	13.5	49.1	0	614-621	R.ASTNDVVR.G	

Protein 3: long-chain fatty acid CoA ligase, putative [Toxoplasma gondii ME49]
Accession: gi|237835163 **Score:** 203.13
Database: NCBInr **MW [kDa]:** 82.10
Seq. Coverage [%]: 7.50 % **pI:** 5.21
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
40	1	554.2130	-82.29	2	15.6	35.8	0	172-181	K.SVVASEECAR.R	Carbamidomethyl: 8
160	1	480.2650	-39.63	2	26.3	50.1	0	183-191	R.LLDSIEAAK.S	
135	1	788.8450	-27.06	2	24.2	29.0	0	192-206	K.SSLAGEPEEAVNASR.V	
65	1	551.7570	-93.82	2	18.6	68.2	1	240-250	K.AVAELKAGESK.A	
41	1	547.2140	-123.35	2	15.7	20.0	0	677-687	R.AVTESMAAVAK.E	Oxidation: 6

Protein 4: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221505321 **Score:** 119.63
Database: NCBInr **MW [kDa]:** 61.90
Seq. Coverage [%]: 3.40 % **pI:** 4.79
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
136	1	537.2760	-12.88	2	24.4	42.3	0	60-69	R.LQALDSVDGR.S	
55	2	583.2530	-42.58	2	17.6	59.2	0	347-356	R.VTFAENDNQK.V	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH4_GA5_01_2147.d/

Protein 1: phosphoglucomutase/parafusin related protein 1 [Toxoplasma gondii]
Accession: gi|15419635 **Score:** 236.79
Database: NCBInr **MW [kDa]:** 70.50
Seq. Coverage [%]: 8.90 % **pI:** 5.55
No. of Peptides: 6

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
123	2	516.2540	-45.78	2	23.3	40.4	0	80-90	K.GGTLLVSGDGR.F	
58	1	408.6760	-124.09	2	18.0	25.8	0	455-461	R.ELLQEGK.S	
43	1	445.1940	-91.88	2	15.9	51.1	0	520-528	K.SSPAEITGK.V	
47	1	419.1510	-124.23	2	16.4	25.1	0	551-557	K.YTDPVDK.Q	
59	2	560.7710	-54.35	2	18.0	71.5	0	579-590	R.LSGTGSTGATIR.V	
203	1	597.7710	-75.71	2	32.6	22.9	0	627-637	K.YIGTETPTVIT.-	

Protein 2: MORN repeat-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484177 **Score:** 111.65
Database: NCBInr **MW [kDa]:** 49.30
Seq. Coverage [%]: 10.30 % **pI:** 5.15
No. of Peptides: 3
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
48	2	510.2490	-32.79	3	16.5	30.6	1	393-407	R.SANAAPARTDTNAIR.Q	
28	2	745.8350	-37.98	2	12.8	58.0	0	408-423	R.QTASTAGSAQPATGSR.A	
91	1	789.3610	-23.23	2	20.9	23.9	0	424-440	R.ASLNGAQLSSQGSGSGR.T	Deamidated: 7

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH5_GA6_01_2148.d/

Protein 1: actin [Toxoplasma gondii ME49]
Accession: gi|237840731 **Score:** 1148.04
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 49.70 % **pI:** 4.91
No. of Peptides: 21
Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
54	2	464.6520	-163.06	2	13.9	85.6	0	20-29	K.AGVAGDDAPR.A	
367	2	571.8160	-59.88	2	29.6	65.7	0	30-40	R.AVFPSIVGKPK.N	
90	2	476.5080	-78.71	3	15.6	35.5	1	52-63	K.DCYVGDEAQSKR.G	Carbamidomethyl: 2
453	3	651.9560	-107.85	3	34.0	82.9	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
375	2	563.2210	-91.44	3	30.1	48.2	1	179-192	R.LDLAGRDLTEYMMK.I	Oxidation: 12, 13
209	2	530.6930	-109.13	2	21.7	41.0	0	198-207	R.GYGFTTSAEK.E	
352	2	519.9010	-65.59	3	28.9	49.2	1	198-211	R.GYGFTTSAEKEIVR.D	
47	2	532.6980	-80.25	2	13.2	69.6	0	230-239	K.AAEDSSDIEK.S	
612	1	941.3680	-86.11	3	36.8	32.2	1	230-255	K.AAEDSSDIEKSYELPDGNIITVG NER.F	
608	3	592.9430	-34.75	3	36.7	84.1	0	240-255	K.SYELPDGNIITVGNER.F	
596	2	599.6050	-63.81	3	41.1	46.5	1	256-270	R.FRCPEALFQPSFLGK.E	Carbamidomethyl: 3
605	2	747.3590	-23.41	2	41.8	75.2	0	268-270	R.CPEALFQPSFLGK.E	Carbamidomethyl: 1
521	1	554.2430	-73.19	4	37.3	20.7	1	268-277	R.CPEALFQPSFLGKEAAGVHR.T	Carbamidomethyl: 1
357	2	572.8850	-88.83	3	29.1	51.0	1	278-291	R.TTFDSIMKCDVDIR.K	Carbamidomethyl: 9; Oxidation: 7
449	2	792.3530	-46.40	3	33.7	56.7	1	292-313	R.KDLYGNVVLSGGTTMYEGIGER.	Oxidation: 15
300	2	512.5910	-49.73	3	26.3	69.2	1	314-327	R.LTKELTSLAPSTMK.I	Oxidation: 13
254	2	597.2530	-91.66	2	24.0	66.7	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10
341	2	589.2690	-70.06	2	28.3	41.4	0	317-327	K.ELTSLAPSTMK.I	
287	2	478.9020	-66.67	3	25.7	46.2	1	317-329	K.ELTSLAPSTMKIK.V	Oxidation: 10
191	1	504.7530	-120.16	2	20.8	28.1	1	328-338	K.IKVVAPPER.K	
49	1	448.2210	-112.94	2	13.4	34.6	1	330-337	K.VVAPPERK.Y	

Protein 2: surface antigen [Toxoplasma gondii]
Accession: gi|13447088 **Score:** 795.18
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 24.20 % **pI:** 6.93
No. of Peptides: 14

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
358	2	462.8770	-105.34	3	29.2	76.2	1	53-64	R.SKITYFGTLTQK.A	

485	1	544.2760	-26.34	4	35.5	69.3	2	53-70	R.SKITYFGTLTQKAPNWYR.C	
413	2	586.2880	-57.16	2	32.0	50.4	0	55-64	K.ITYFGTLTQK.A	
537	2	653.6480	-46.84	3	38.1	59.7	1	55-70	K.ITYFGTLTQKAPNWYR.C	
207	2	403.6620	-96.20	2	21.6	38.7	0	65-70	K.APNWYR.C	
240	2	470.5510	-70.74	3	23.3	68.4	0	76-88	R.ANEEVVGHVTLNK.E	
35	1	426.4180	-66.48	4	12.0	48.4	2	119-132	R.VCHIDAKDKGDCER.N	Carbamidomethyl: 2, 12
471	2	476.2270	-64.21	3	34.9	60.6	1	133-145	R.NKGFLTDYIPGAK.Q	
559	2	591.2560	-97.56	2	39.2	64.3	0	135-146	K.GFLTDYIPGAK.Q	
244	2	584.2490	-87.47	3	23.5	69.3	1	151-165	K.IEKVENNGEQSVLYK.F	Deamidated: 7
220	2	690.7970	-56.04	2	22.4	66.9	0	154-165	K.VENNGEQSVLYK.F	Deamidated: 4
220	2	690.7970	656.62	2	22.4	40.3	0	154-165	K.VENNGEQSVLYK.F	
695	1	708.3800	-39.42	2	54.2	25.8	0	166-177	K.FTVPWIFLPPAK.Q	
412	2	489.2310	-51.45	2	31.9	55.0	0	229-236	K.DANFIEIR.C	

Protein 3: actin I [Plasmodium falciparum]
Accession: gi|5911379 **Score:** 733.50
Database: NCBInr **MW [kDa]:** 41.80
Seq. Coverage [%]: 4.80 % **pI:** 5.15
No. of Peptides: 3

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
525	5	908.4060	489.93	2	37.5	52.1	0	2-19	M.GEEVVQALVVDNGSGNVK.A	Deamidated: 6
490	6	908.4170	503.15	2	35.8	70.4	0	2-19	M.GEEVVQALVVDNGSGNVK.A	Deamidated: 12
514	1	907.8560	427.39	2	36.9	20.9	0	2-19	M.GEEVVQALVVDNGSGNVK.A	

Protein 4: hypothetical protein TGME49_032280 [Toxoplasma gondii ME49]
Accession: gi|237837601 **Score:** 698.68
Database: NCBInr **MW [kDa]:** 33.90
Seq. Coverage [%]: 54.80 % **pI:** 6.04
No. of Peptides: 13

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

23	2	539.5180	-97.29	3	11.2	48.8	0	16-30	K.DSHAANADSQTPMQK.L	Oxidation: 13
184	2	455.7220	-54.81	4	20.5	61.0	1	36-55	R.AAVAAAANGAETGKAPHLK.L	Deamidated: 9
140	2	517.2580	-35.93	2	18.3	60.8	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5
314	2	481.0270	155.52	3	27.0	50.0	0	101-113	K.KPTEGQTVLLQVK.A	
169	2	418.8540	-108.74	3	19.7	37.3	0	114-124	K.APPYKPIPESR.F	
74	1	507.5510	-57.56	3	14.8	51.5	1	136-148	R.QNIETMKDTPEAK.A	Oxidation: 6
371	2	751.8180	-72.21	2	29.9	52.6	0	193-205	R.TFAEPEQPDIEVK.D	
97	2	548.8960	-61.41	3	16.2	74.4	1	222-237	K.AAETVAEKEGEQSAPK.N	
26	2	500.1710	-102.12	3	11.3	46.6	0	238-251	K.NESPESGHDATAQR.K	
198	2	414.5010	-111.66	3	21.1	27.4	0	257-267	R.YLGPESSVPHR.L	
678	1	762.4250	-28.75	2	47.1	55.8	0	268-281	R.LLATCGILPLPLDK.A	Carbamidomethyl: 5
107	1	406.6690	-141.21	2	16.8	28.8	0	284-290	K.ELIQEGK.R	
679	1	740.8600	-37.45	2	47.3	88.2	0	300-314	K.TIITEAGGFFGTPVA.-	

Protein 5: GPI-anchored surface protein [Toxoplasma gondii]
Accession: gi|1718389 **Score:** 469.89
Database: NCBInr **MW [kDa]:** 44.20
Seq. Coverage [%]: 28.30 % **pI:** 7.81
No. of Peptides: 10

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
426	4	788.3160	-49.89	2	32.6	67.0	0	132-145	K.GAQNDWLNGEDLSR.G	Deamidated: 8
407	6	787.8200	-54.99	2	31.7	52.7	0	132-145	K.GAQNDWLNGEDLSR.G	
326	1	459.2540	-60.95	2	27.6	20.3	1	146-153	R.GITFRVPK.N	
351	2	457.7090	-80.34	2	28.8	37.3	0	154-161	K.NNFPGLPR.I	
403	1	458.2230	-32.26	2	31.5	22.9	0	154-161	K.NNFPGLPR.I	Deamidated: 1
516	1	849.0560	-47.93	3	37.0	46.1	0	207-230	R.ITPATNHATIVCGPYGGISPLDY K.K	Carbamidomethyl: 12
75	1	586.2110	544.24	3	14.8	30.4	1	231-244	K.KEFCTGEPHTSCGR.Q	Carbamidomethyl: 4, 12
113	1	513.1830	-52.86	3	17.0	26.7	0	232-244	K.EFCTGEPHTSCGR.Q	Carbamidomethyl: 3, 11
129	1	1046.7170	-50.88	3	17.8	57.3	0	334-367	K.EAPNTNNSGGTPTGGGSDDDTTA PGTGSSGTDSR.G	
30	1	438.4120	-86.17	4	11.6	61.6	2	368-384	R.GDLGDGRDQDAKGGMSR.G	Oxidation: 15

Protein 6: SRS domain containing protein [Toxoplasma gondii ME49]
Accession: gi|237841049 **Score:** 465.15
Database: NCBInr **MW [kDa]:** 39.40
Seq. Coverage [%]: 28.80 % **pI:** 4.66
No. of Peptides: 10

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
545	1	880.7030	-33.97	3	38.5	71.0	0	108-132	K.SQNLSPTELDTAFGVDAAGNCDT TR.L	Carbamidomethyl: 21
680	2	748.9320	-32.00	2	47.8	83.9	0	133-146	R.LVDVSTLIPTAIVR.G	
135	1	406.6160	-159.18	2	18.1	23.8	0	174-179	K.ACFVCR.E	Carbamidomethyl: 2, 5
77	1	463.2160	-73.04	2	15.0	23.7	0	227-234	R.SVVVHCPK.G	Carbamidomethyl: 6
670	1	567.9470	-53.15	3	45.3	60.3	1	235-249	K.GLPVLDIFKESDNVR.A	
360	1	604.0260	-76.97	4	29.3	42.2	1	258-280	K.AKLEDVVGSGSTIKPVNVDSQNR D	
342	1	718.3430	-50.97	4	28.4	49.5	2	258-284	K.AKLEDVVGSGSTIKPVNVDSQNR DVSR.S	
360	2	738.6960	-28.26	3	28.8	46.7	0	260-280	K.LEDVVGSGSTIKPVNVDSQNR.D	
330	1	668.5770	-29.31	4	27.8	21.2	1	260-284	K.LEDVVGSGSTIKPVNVDSQNRDV SR.S	
354	2	525.2490	-56.86	3	28.9	27.3	0	285-298	R.SYLITSTSSFSKPR.I	

Protein 7: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221481826 **Score:** 301.85
Database: NCBInr **MW [kDa]:** 45.10
Seq. Coverage [%]: 17.80 % **pI:** 7.74
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
685	1	780.3860	-22.08	2	49.1	74.2	0	58-70	R.FLDPIDELPEPFK.A	
421	2	440.5540	-82.83	3	32.4	69.4	1	89-100	K.LGIEGDKTVFLK.S	
419	2	547.2560	-55.06	3	32.3	33.0	0	101-115	K.SPISPDVAVYVHAER.L	
447	2	610.3190	-28.67	3	33.7	77.0	0	223-240	K.SVELTRPQAVLGAVGMGK.K	Oxidation: 16

414	2	484.9090	-49.56	3	32.0	48.2	1	269-281	R.ALQFGKDFQVTAK.K	

Protein 8: microneme protein, putative [Toxoplasma gondii VEG]
Accession: gi|221504324 **Score:** 296.02
Database: NCBInr **MW [kDa]:** 37.80
Seq. Coverage [%]: 13.60 % **pI:** 5.91
No. of Peptides: 3

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
338	2	603.5610	-87.67	3	28.2	94.1	0	89-104	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 6
415	1	765.3570	-21.93	3	32.1	47.8	1	270-290	R.EVTSKAEESCVEGVEVTLAEK.C	Carbamidomethyl: 10
45	2	583.7460	-27.78	2	13.0	82.5	0	321-332	K.GESGSEGSLSEK.M	

Protein 9: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 288.15
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 11.80 % **pI:** 7.76
No. of Peptides: 5

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
551	2	702.8400	-45.67	2	38.9	72.0	0	136-148	K.EWVTGDSVLTGLK.I	
279	2	702.8110	-61.08	2	28.3	41.0	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 11
279	1	702.8110	-61.05	2	28.3	22.9	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 7
190	2	650.2570	-62.91	2	20.8	58.9	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
491	2	448.6870	-70.64	2	35.8	26.2	0	269-265	K.WFFGDPK.S	

Protein 10: actin [Pyrocystis lunula]
Accession: gi|27450759 **Score:** 187.99
Database: NCBInr **MW [kDa]:** 41.70
Seq. Coverage [%]: 7.20 % **pI:** 5.83
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
385	3	400.1990	-102.48	3	30.7	27.8	0	29-39	R.AVFPSIVGRPR.H	
538	2	895.9080	-49.78	2	38.1	59.8	0	239-264	K.SYELPDGQVITIGNER.F	

Protein 11: hypothetical protein TGME49_093430 [Toxoplasma gondii ME49]
Accession: gi|237841695 **Score:** 155.59
Database: NCBInr **MW [kDa]:** 44.60
Seq. Coverage [%]: 8.80 % **pI:** 4.86
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
151	2	750.8300	-50.71	2	18.8	44.2	0	70-82	R.QQPVEANETTVER.G	
94	2	437.1790	-104.92	2	15.9	64.8	0	185-193	R.AAADIAADR.A	
340	1	688.8190	-36.11	2	28.3	30.1	0	398-411	R.HTPPGADQIELAGA.-	

Protein 12: SRS domain containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221484162 **Score:** 154.10
Database: NCBInr **MW [kDa]:** 35.80
Seq. Coverage [%]: 13.20 % **pI:** 5.20
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
280	1	507.7170	-97.24	2	25.3	37.7	0	108-116	K.WTVAPPESK.T	
406	2	494.9020	-52.87	3	31.6	58.6	0	117-129	K.THSLTLPAENFPR.V	
667	1	800.0660	-25.02	3	45.0	21.6	0	255-276	K.LVIPEGGFPAQEETIVLGCNVR.S	Carbamidomethyl: 19

Protein 13: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221504434 **Score:** 103.36

Database: NCBInr **MW [kDa]:** 46.90
Seq. Coverage [%]: 7.00 % **pI:** 10.17
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
67	1	502.1910	-141.34	2	14.4	60.7	0	199-208	R.VATVNAGTNR.L	Deamidated: 5
448	1	762.3590	-48.41	3	33.7	42.6	1	231-250	R.NRIPLTATQLSAESQEIQER.L	Deamidated: 9

Protein 14: facilitative glucose transporter, putative [Toxoplasma gondii ME49]
Accession: gi|237842935 **Score:** 94.70
Database: NCBInr **MW [kDa]:** 61.60
Seq. Coverage [%]: 4.20 % **pI:** 6.67
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
470	1	635.3130	-13.71	2	34.8	51.0	0	495-505	K.GLSIEESPYFK.G	
372	2	660.2830	-83.43	2	29.9	43.7	1	556-568	R.GVSDDLTKGTEVV.-	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH6_GA7_01_2149.d/

Protein 1: SAG1-related sequence 3 [Toxoplasma gondii]
Accession: gi|2305258 **Score:** 363.35
Database: NCBInr **MW [kDa]:** 36.20
Seq. Coverage [%]: 21.20 % **pI:** 5.75
No. of Peptides: 8

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
414	2	831.8860	-47.71	2	40.2	53.1	0	91-106	K.CAIGTVTLPVDLMEK.Q	Carbamidomethyl: 1; Oxidation: 13
109	1	465.7340	-42.28	2	18.9	21.3	0	158-165	K.QNLPPVDK.E	
333	2	720.3300	-48.44	3	33.1	28.7	1	158-176	K.QNLPPVDKEFIVGCTQEGK.G	Carbamidomethyl: 14
215	2	634.2840	-29.95	2	25.2	67.5	0	166-176	K.EFIVGCTQEGK.G	Carbamidomethyl: 6
110	2	604.0100	-20.81	4	19.0	33.0	1	189-211	R.ASSKDSQTVTCAYGSASNADVHRV	Carbamidomethyl: 11; Deamidated: 18
120	4	603.7380	-63.88	4	19.5	65.3	1	189-211	R.ASSKDSQTVTCAYGSASNADVHRV	Carbamidomethyl: 11
318	2	560.6040	-46.20	3	32.1	52.5	0	278-292	K.AVLTIPHDNFPEAQK.K	
279	1	482.7110	-81.69	4	29.4	42.9	1	278-293	K.AVLTIPHDNFPEAQKK.V	

Protein 2: bradyzoite-specific surface protein, putative [Toxoplasma gondii ME49]
Accession: gi|237841177 **Score:** 284.11
Database: NCBInr **MW [kDa]:** 41.50
Seq. Coverage [%]: 16.80 % **pI:** 6.23
No. of Peptides: 5

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
251	1	626.2610	-87.98	2	27.7	72.5	0	93-104	K.GSLTATLECTAK.D	Carbamidomethyl: 9
196	1	401.5130	-136.74	3	24.2	37.0	1	169-178	K.AIQSQKWTLK.L	
227	1	725.8090	-33.86	2	26.2	62.0	0	190-202	K.TFDVGCTYTTTGK.A	Carbamidomethyl: 6
297	2	778.3850	-40.70	2	30.5	73.2	0	203-217	K.AAANPAVCQLTVNVK.A	Carbamidomethyl: 8
100	1	624.9410	-41.82	3	18.2	39.4	1	326-342	R.LGCVPNKAPQSDSQDTR.V	Carbamidomethyl: 3

Protein 3: glyceraldehyde 3-phosphate dehydrogenase, related [Neospora caninum Liverpool]
Accession: gi|325115564 **Score:** 179.64
Database: NCBInr **MW [kDa]:** 36.50
Seq. Coverage [%]: 17.40 % **pI:** 6.83
No. of Peptides: 4

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
345	1	736.6810	-15.86	3	34.2	58.4	0	142-162	K.SSDVIVSNASCTTNCLAPLAK.I	Carbamidomethyl: 11, 15
264	2	678.8600	-26.10	2	28.4	51.1	0	203-217	R.SAGVNIIPASTGAAK.A	
260	1	504.2410	-65.50	2	28.2	30.8	0	257-265	K.YEDIVAAVK.E	
285	2	774.8220	-64.23	2	29.6	21.9	0	327-340	R.LVELAHYMSVQDGA.-	Oxidation: 8

Protein 4: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237844685 **Score:** 154.15
Database: NCBInr **MW [kDa]:** 40.90
Seq. Coverage [%]: 10.20 % **pI:** 6.17
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
184	2	401.6990	-140.08	2	23.4	36.0	0	87-94	K.ATALVLSK.Q	
81	1	644.2840	-39.02	2	16.8	51.8	0	101-113	K.LVCSGDGNAAAPR.N	Carbamidomethyl: 3
141	1	479.7960	-110.23	2	20.8	34.5	0	182-189	K.VNNAVQWK.K	
206	1	615.3040	-38.77	2	24.8	31.8	0	240-250	K.AVQVELTADQR.S	

Protein 5: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 120.99
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 3.50 % **pI:** 7.76
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
204	2	702.3290	-46.84	2	24.7	66.3	0	149-161	K.ISVPESQYPANAK.S	

Protein 6: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998 **Score:** 97.18
Database: NCBInr **MW [kDa]:** 26.60

Seq. Coverage [%]: 7.10 % **pI:** 7.78
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
94	2	508.7500	-54.65	2	17.8	69.4	0	131-140	R.ASSVVNNVAR.C	
89	1	439.6670	-112.23	2	17.5	27.8	0	223-230	K.EAFPAESK.S	

Protein 7: microneme protein MIC3 [Toxoplasma gondii]
Accession: gi|343113243 **Score:** 91.84
Database: NCBInr **MW [kDa]:** 36.40
Seq. Coverage [%]: 8.10 % **pI:** 5.84
No. of Peptides: 2

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
261	2	603.5800	-56.20	3	28.3	37.8	0	80-95	R.QLHTDNGYFIGASCPK.S	Carbamidomethyl: 14; Deamidated: 6
39	2	583.7520	-17.50	2	13.0	54.1	0	312-323	K.GESGSEGSLSEK.M	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH7_GA8_01_2151.d/

Protein 1: Chain A, Crystal Structure Of A Parasite Protein
Accession: gi|22219177 **Score:** 1685.20
Database: NCBInr **MW [kDa]:** 29.80
Seq. Coverage [%]: 80.60 % **pI:** 7.73
No. of Peptides: 30

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
286	1	623.2820	-67.80	3	26.9	58.6	1	1-17	-.SDPPLVANQVVTCPDKK.S	Carbamidomethyl: 13
315	1	623.5980	-87.01	3	28.3	20.9	1	1-17	-.SDPPLVANQVVTCPDKK.S	Carbamidomethyl: 13; Deamidated: 8
462	7	615.0630	96.72	3	36.0	68.7	0	18-34	K.STAAVILTPTENHFTLK.C	
488	2	615.3030	-46.35	3	37.2	49.2	0	18-34	K.STAAVILTPTENHFTLK.C	Deamidated: 12
411	1	743.3420	-73.05	3	33.4	24.5	1	18-37	K.STAAVILTPTENHFTLKCPK.T	Carbamidomethyl: 18
381	4	815.8920	-40.93	2	31.9	62.6	0	38-52	K.TALTEPPTLAYSPNR.Q	
82	2	749.2930	-59.04	2	16.0	68.0	0	53-66	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
275	1	400.2030	-117.00	2	26.3	29.8	0	96-102	K.LTVPIEK.F	
629	2	793.0860	-32.16	3	45.6	34.1	1	96-116	K.LTVPIEKFPVTTQTFVVGCIK.G	Carbamidomethyl: 19
226	2	585.2150	-87.66	3	23.7	49.2	0	117-132	K.GDDAQSCMVTVTVQAR.A	Carbamidomethyl: 7; Oxidation: 8
136	4	509.2140	-109.59	2	19.1	70.5	0	133-142	R.ASSVVNNVAR.C	Deamidated: 6
236	5	677.7820	-54.60	2	24.3	79.9	0	143-155	R.CSYGADSTLGPVK.L	Carbamidomethyl: 1
373	2	782.8400	-62.73	2	31.4	69.0	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
278	2	790.8560	-38.65	2	26.4	59.1	0	156-170	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
270	2	660.6440	-30.37	3	26.0	63.3	1	156-174	K.LSAEGPTTMTLVCGKDGVK.V	Carbamidomethyl: 13; Oxidation: 9
254	3	895.6970	-41.48	3	25.2	43.1	1	171-194	K.DGVKVPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 13, 21
264	1	896.0040	-64.91	3	25.7	49.8	1	171-194	K.DGVKVPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 13, 21; Deamidated: 9
196	2	762.6140	-64.99	3	22.1	30.2	0	175-194	K.VPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 9, 17
323	2	474.2360	-96.27	2	28.8	48.3	1	195-202	K.SFKDILPK.L	
230	2	516.2120	-59.26	3	24.0	61.5	0	203-216	K.LTENPWQGNASSDK.G	
247	2	774.3160	-46.83	2	24.8	91.0	0	203-216	K.LTENPWQGNASSDK.G	Deamidated: 9
388	3	744.3400	-55.89	3	32.2	41.6	1	203-223	K.LTENPWQGNASSDKGATLTIK.K	
295	3	787.0100	-88.84	3	27.5	50.9	2	203-224	K.LTENPWQGNASSDKGATLTIKK.E	
319	2	590.7650	-72.38	4	28.5	53.2	2	203-224	K.LTENPWQGNASSDKGATLTIKK.E	Deamidated: 9
97	2	416.1930	-181.50	2	16.9	41.3	1	217-224	K.GATLTIKK.E	
67	1	503.7510	-25.47	2	15.1	43.9	1	224-232	K.KEAFPAESK.S	
103	1	439.6550	-139.52	2	17.4	33.8	0	225-232	K.EAFPAESK.S	
211	2	652.7850	-67.98	2	22.9	94.3	0	233-245	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
206	2	446.6950	-74.23	4	22.6	54.5	1	246-262	K.HHCTVKLEFAGAAGSAK.S	Carbamidomethyl: 3
216	2	611.2110	-113.99	2	23.3	77.2	0	262-262	K.LEFAGAAGSAK.S	

Protein 2: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998 **Score:** 1557.93
Database: NCBInr **MW [kDa]:** 26.60
Seq. Coverage [%]: 5.90 % **pI:** 7.78
No. of Peptides: 1

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
250	2	555.9350	-65.23	3	25.1	84.0	1	1-15	-.PPLVANQVVTCPDKK.S	Carbamidomethyl: 11

Protein 3: surface antigen protein 1 [Toxoplasma gondii]
Accession: gi|56157026 **Score:** 1535.49
Database: NCBInr **MW [kDa]:** 33.00
Seq. Coverage [%]: 4.40 % **pI:** 9.75
No. of Peptides: 1

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
126	1	744.7630	-75.03	2	18.5	63.4	0	83-96	R.QICSAGTTSSCTSK.A	Carbamidomethyl: 3, 11; Deamidated: 1

Protein 4: SRS domain-containing surface antigen, putative [Toxoplasma gondii ME49]
Accession: gi|237839855 **Score:** 393.74
Database: NCBInr **MW [kDa]:** 34.90
Seq. Coverage [%]: 26.80 % **pI:** 8.07
No. of Peptides: 7

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

147	3	534.2230	-91.75	2	19.6	69.7	0	53-62	R.VTGNSSVEFK.C	
70	1	525.7130	-76.98	2	18.3	75.6	0	126-135	K.CSSLANTAAR.V	Carbamidomethyl: 1
38	1	585.7690	-37.74	2	12.8	50.2	0	136-147	R.VGAGQPSQEAEK.E	
40	2	476.5450	-64.38	3	12.9	81.0	1	136-149	R.VGAGQPSQEAEKEK.T	
360	1	589.5260	-22.72	4	30.7	54.1	0	213-234	K.ECTTSSTLAEHLSGASLVQHAR.T	Carbamidomethyl: 2
391	2	629.0230	-67.73	4	32.4	52.9	1	235-257	R.TNAADDKKPAYTFSVTSLPTEEKT	
59	1	401.1620	-82.21	3	14.7	20.4	1	258-266	K.TLCYQCTKK.N	Carbamidomethyl: 3, 6

Protein 5: SRS domain-containing protein, putative [Toxoplasma gondii GT1]
Accession: gi|221487802 **Score:** 372.61
Database: NCBInr **MW [kDa]:** 37.20
Seq. Coverage [%]: 26.00 % **pI:** 5.82
No. of Peptides: 6

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
282	1	586.7680	-74.15	2	26.6	83.2	0	81-92	K.GGISVEVDPATK.K	
631	2	947.9190	-46.10	2	45.9	85.3	0	122-139	R.ANLQGETPLAEFFGEGSK.A	
20	1	708.2780	-46.48	2	11.4	64.3	0	184-197	R.APTGDPSQNSDGNR.G	
640	2	778.7080	-37.73	3	49.1	63.6	0	266-287	K.LADVLPSATFQETSSAHVFSVK.E	
197	2	501.5520	-51.91	3	22.2	40.6	1	288-299	K.ELPKTEATYCYK.C	Carbamidomethyl: 10
51	2	511.8780	-42.66	3	14.2	35.6	1	300-314	K.CSPPVDSGDTADGKK.N	Carbamidomethyl: 1

Protein 6: 14-3-3 protein homologue [Toxoplasma gondii]
Accession: gi|3123732 **Score:** 177.67
Database: NCBInr **MW [kDa]:** 30.70
Seq. Coverage [%]: 15.00 % **pI:** 4.55
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

164	2	468.5270	-66.80	3	20.4	70.3	1	143-154	R.YISEFSGEEGKK.Q	
46	2	683.7920	-34.09	2	13.3	57.7	0	155-166	K.QAADQAQESYQK.A	
251	2	574.9240	-62.76	3	25.0	49.7	0	167-182	K.ATETAEAELPSTHPIR.L	

Protein 7: hypothetical protein [Paramecium tetraurelia strain d4-2]
Accession: gi|145541197 **Score:** 70.26
Database: NCBInr **MW [kDa]:** 45.30
Seq. Coverage [%]: 1.80 % **pI:** 9.44
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
327	1	430.7170	-10.76	2	29.0	50.4	0	97-103	K.QDLLDEK.C	

Protein 8: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499 **Score:** 56.22
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 11.30 % **pI:** 5.66
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
48	1	795.9480	-51.42	3	13.8	49.5	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 23

Protein 9: ribosomal phosphoprotein P0 [Toxoplasma gondii]
Accession: gi|14579678 **Score:** 54.71
Database: NCBInr **MW [kDa]:** 34.10
Seq. Coverage [%]: 3.80 % **pI:** 5.34
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

148	2	422.1760	-176.63	3	19.6	54.7	1	102-113	R.IVAENKVPAPAR.Q	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH8_GB1_01_2152.d/

Protein 1: major surface antigen P30 precursor [Toxoplasma gondii]
Accession: gi|10725 **Score:** 421.38
Database: NCBInr **MW [kDa]:** 33.00
Seq. Coverage [%]: 12.50 % **pI:** 9.62
No. of Peptides: 2

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
74	4	508.7520	-50.72	2	17.9	72.6	0	163-172	R.ASSVVNNVAR.C	
187	1	614.6920	-30.44	5	28.0	56.8	2	263-292	K.SVIIGCTGGSPEKHHCTVKLEFAGAAGSAK.S	Carbamidomethyl: 6, 16

Protein 2: dense granule antigen GRA6 [Toxoplasma gondii]
Accession: gi|89572499 **Score:** 273.88
Database: NCBInr **MW [kDa]:** 23.90
Seq. Coverage [%]: 23.90 % **pI:** 5.66
No. of Peptides: 6

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
59	1	410.8420	-77.77	3	16.2	29.4	1	112-122	K.SEARGPSLEER.I	
162	1	534.2310	-71.18	3	25.9	28.5	1	116-129	R.GPSLEERIEEQGTR.R	
57	1	568.7620	-36.49	2	16.1	41.5	0	132-141	R.YSSVQEPQAK.V	
32	1	847.9820	-47.92	3	13.1	64.9	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 21

34	1	848.3060	-52.62	3	13.4	31.4	1	177-203	R.RSPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 13, 21
36	1	795.9450	-55.19	3	13.8	61.1	0	178-203	R.SPQEPSGDGGGNDAGNNAGNGGNEGR.G	Deamidated: 20

Protein 3: ubiquitin, putative [Toxoplasma gondii ME49]
Accession: gi|237835791 **Score:** 126.22
Database: NCBInr **MW [kDa]:** 24.40
Seq. Coverage [%]: 12.00 % **pI:** 6.02
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
40	1	434.2020	-69.47	2	14.7	24.9	0	66-72	K.QLTTYNK.S	
84	1	658.2700	-63.40	3	18.7	32.0	1	198-216	K.KNAAVSSTPVNQDEAAQEN.-	
104	1	922.8640	-56.87	2	20.5	69.3	0	199-216	K.NAAVSSTPVNQDEAAQEN.-	

Protein 4: hypothetical protein TGME49_038150 [Toxoplasma gondii ME49]
Accession: gi|237834419 **Score:** 119.77
Database: NCBInr **MW [kDa]:** 37.00
Seq. Coverage [%]: 9.70 % **pI:** 5.43
No. of Peptides: 3

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
157	1	534.7590	-19.91	2	25.0	43.3	0	44-53	K.DPVDDAAIPR.V	
92	2	438.6990	-58.59	2	19.6	50.7	0	121-128	K.STALDDVR.A	
113	1	859.8500	-45.82	2	21.3	25.8	1	334-349	R.NAAVKDEESVEQAEEA.-	

Protein 5: 60S ribosomal protein L7a, putative [Toxoplasma gondii ME49]
Accession: gi|237831983 **Score:** 95.74
Database: NCBInr **MW [kDa]:** 31.00
Seq. Coverage [%]: 5.40 % **pI:** 10.46
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
44	1	420.6730	-154.37	2	15.0	69.7	0	49-56	R.VGGNIQPR.R	
64	1	429.7050	-75.90	2	16.9	36.1	0	122-128	R.LLQEAER.Q	

Protein 6: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221485528 **Score:** 78.76
Database: NCBInr **MW [kDa]:** 226.10
Seq. Coverage [%]: 0.70 % **pI:** 5.11
No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
49	1	455.1840	1000.74	2	15.3	52.5	0	1533-1540	R.VGSLMETR.V	Oxidation: 5
126	1	426.1770	-135.49	2	22.2	26.3	0	1545-1551	R.LYASELR.G	

Protein 7: hypothetical protein TGME49_011630 [Toxoplasma gondii ME49]
Accession: gi|237843981 **Score:** 67.48
Database: NCBInr **MW [kDa]:** 23.60
Seq. Coverage [%]: 4.20 % **pI:** 9.06
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
90	2	486.2410	-79.48	2	19.4	67.5	0	193-201	R.AAQEALIQK.N	

Protein 8: surface antigen P22 [Toxoplasma gondii]
Accession: gi|269938870 **Score:** 61.60
Database: NCBInr **MW [kDa]:** 17.30
Seq. Coverage [%]: 6.50 % **pI:** 9.09
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
45	1	529.7210	-66.10	2	15.1	61.6	0	108-118	K.CVAEAGAPAGR.N	Carbamidomethyl: 1

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:38
Search Location /BOPS/RH2/RH9_GB2_01_2153.d/

Protein 1: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751 **Score:** 815.11
Database: NCBInr **MW [kDa]:** 19.00
Seq. Coverage [%]: 59.10 % **pI:** 9.35
No. of Peptides: 13

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
319	1	585.2370	-62.47	3	27.7	22.6	0	44-60	K.TVDAPSSGSVVFQCGDK.L	Carbamidomethyl: 14
450	2	785.3580	-45.07	2	34.5	37.8	0	61-79	K.LTISPSGEGDVFYGK.E	
456	4	471.2400	-129.31	3	34.6	92.2	1	82-96	R.KLTTVLPGAVLTAK.V	
512	2	428.5840	-43.45	3	38.0	60.2	0	83-96	K.LTTVLPGAVLTAK.V	
497	1	645.6910	-54.35	3	36.9	29.8	1	83-101	K.LTTVLPGAVLTAKVQQPAK.G	
422	1	785.6250	-31.46	4	32.9	22.6	1	96-123	K.VQQPAKGPATYTLSVDGTPEKPQVLCYK.C	Carbamidomethyl: 26
432	2	830.0520	-26.10	3	33.5	89.1	0	102-123	K.GPATYTLSVDGTPEKPQVLCYK.C	Carbamidomethyl: 20
128	1	709.9700	-36.11	3	17.8	35.0	1	124-145	K.CVAEAGAPAGRNNDGSSAPTPK.D	Carbamidomethyl: 1; Deamidated: 13
118	3	709.6410	-37.53	3	17.3	51.8	1	124-145	K.CVAEAGAPAGRNNDGSSAPTPK.D	Carbamidomethyl: 1
47	3	544.7090	-68.60	2	12.8	76.3	0	135-148	R.NNDGSSAPTPK.D	Deamidated: 1
35	4	497.8510	-66.66	3	12.0	67.1	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13; Deamidated: 2
23	6	497.5170	-78.75	3	11.4	68.1	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13

311	1	576.8230	-39.91	2	27.4	37.8	1	149-159	K.LIVRVPGADGR.V	

Protein 2: RecName: Full=Dense granule protein 5; Short=Protein GRA 5; AltName: Full=p21; Flags: Precursor
Accession: gi|2507039 **Score:** 221.83
Database: NCBInr **MW [kDa]:** 13.00
Seq. Coverage [%]: 35.00 % **pI:** 5.69
No. of Peptides: 4

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
20	2	502.9610	-47.61	4	11.2	54.9	1	43-58	R.GREQQQVQQHEQNEDR.S	
14	2	599.2330	-61.71	3	10.9	47.8	0	45-58	R.EQQQVQQHEQNEDR.S	
204	1	607.7590	-45.01	4	21.7	47.8	1	45-63	R.EQQQVQQHEQNEDRSLFER.G	
270	1	779.6260	-62.65	3	25.2	71.3	1	100-120	R.AIQEESKESATAEEEEVAEEE.-	

Protein 3: SAG1 [Toxoplasma gondii]
Accession: gi|197281733 **Score:** 186.01
Database: NCBInr **MW [kDa]:** 17.90
Seq. Coverage [%]: 0.00 % **pI:** 7.47
No. of Peptides: 0

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

Protein 4: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221484778 **Score:** 107.24
Database: NCBInr **MW [kDa]:** 20.80
Seq. Coverage [%]: 21.50 % **pI:** 5.68
No. of Peptides: 2

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
398	2	898.4190	-42.24	3	31.6	50.3	0	44-69	R.ILVHSGDSVTIQCPGAIASNPQD	Carbamidomethyl: 13

									VSK.Y	
164	1	870.8160	-56.06	2	19.7	56.9	0	70-84	K.YVCPGEEPNCTDATK.A	Carbamidomethyl: 3, 10

Protein 5: hypothetical protein NCLIV_021800 [Neospora caninum Liverpool]
Accession: gi|325116838 **Score:** 98.55
Database: NCBInr **MW [kDa]:** 522.10
Seq. Coverage [%]: 0.20 % **pI:** 5.40
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
300	1	487.2190	-129.00	2	26.7	44.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2, 5
300	1	487.2190	881.59	2	26.7	53.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2

Protein 6: hypothetical protein TGME49_093440 [Toxoplasma gondii ME49]
Accession: gi|237841697 **Score:** 57.15
Database: NCBInr **MW [kDa]:** 58.70
Seq. Coverage [%]: 3.00 % **pI:** 5.00
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
486	2	618.6410	-36.18	3	36.3	57.1	0	434-449	K.LKPSDPADVQVITEWR.Q	

Sample Info & Protocols
Name: PTG

Search Type Combined MS/MS - ProteinExtractor
Search Result PTG2_2014-08-20 15:51:32
Search Location /BOPS/PTG2/

Protein 1: surface antigen 1 [Toxoplasma gondii]
Accession: gi|12523765 **Score:** 1322.33
Database: NCBInr **MW [kDa]:** 34.70
Seq. Coverage [%]: 48.20 % **pI:** 9.29
No. of Peptides: 22

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
385	3	615.0020	-2.46	3	36.0	76.9	0	65-81	K.STAAVILTPTENHFTLK.C	
336	1	557.7730	-46.70	4	33.3	74.0	1	65-84	K.STAAVILTPTENHFTLKCPK.T	Carbamidomethyl: 18
310	2	544.2640	-40.46	3	32.0	50.2	0	85-99	K.TALTEPPTLAYSPNR.Q	
36	2	749.3170	-27.01	2	16.1	63.1	0	100-113	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
60	5	508.7140	-125.41	2	17.8	54.2	0	180-189	R.ASSVVNNVAR.C	
84	4	509.2460	-46.76	2	19.3	81.3	0	180-189	R.ASSVVNNVAR.C	Deamidated: 6
169	2	677.7940	-36.90	2	24.2	64.2	0	190-202	R.CSYGANSTLGPVK.L	Carbamidomethyl: 1; Deamidated: 6
156	4	677.3090	-26.58	2	23.8	85.6	0	190-202	R.CSYGANSTLGPVK.L	Carbamidomethyl: 1
303	2	782.8550	-43.57	2	31.5	76.8	0	203-217	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
206	2	790.8600	-33.59	2	26.3	57.2	0	203-217	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
197	2	660.6490	-22.80	3	25.9	29.2	1	203-221	K.LSAEGPTTMTLVCGKDGVK.V	Carbamidomethyl: 13; Oxidation: 9
184	1	895.6980	-40.37	3	25.3	61.4	1	218-241	K.DGVKVPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 13, 21
133	2	762.6300	-44.01	3	22.2	39.8	0	222-241	K.VPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 9, 17
252	2	474.2690	-26.69	2	28.9	44.1	1	242-249	K.SFKDILPK.L	

318	6	773.0150	-32.36	3	32.3	54.6	0	250-271	K.LSENPWQGNASSDNGATLTINK.E	
330	3	773.3500	-23.30	3	32.9	71.5	0	250-271	K.LSENPWQGNASSDNGATLTINK.E	Deamidated: 14
330	3	773.3500	-23.30	3	32.9	48.5	0	250-271	K.LSENPWQGNASSDNGATLTINK.E	Deamidated: 9
374	1	1059.4900	-18.13	3	35.5	80.5	1	250-279	K.LSENPWQGNASSDNGATLTINKEAFPAESK.S	
387	1	1059.8180	-18.13	3	36.1	56.2	1	250-279	K.LSENPWQGNASSDNGATLTINKEAFPAESK.S	Deamidated: 14
372	1	795.1200	-12.24	4	35.4	34.5	1	250-279	K.LSENPWQGNASSDNGATLTINKEAFPAESK.S	Deamidated: 21
54	2	439.6620	-123.60	2	17.4	39.0	0	272-279	K.EAFPAESK.S	
148	2	652.8070	-34.28	2	22.9	67.1	0	280-292	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6

Protein 2: surface antigen protein 1 [Toxoplasma gondii]
Accession: gi|56157026 **Score:** 1045.73
Database: NCBInr **MW [kDa]:** 33.00
Seq. Coverage [%]: 4.40 % **pI:** 9.75
No. of Peptides: 1

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
74	1	744.7800	-52.20	2	18.4	49.3	0	83-96	R.QICSAGTTSSCTSK.A	Carbamidomethyl: 3, 11; Deamidated: 1

Protein 3: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998 **Score:** 1029.26
Database: NCBInr **MW [kDa]:** 26.60
Seq. Coverage [%]: 5.90 % **pI:** 7.78
No. of Peptides: 1

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
183	2	555.9710	-0.48	3	25.2	55.2	1	1-15	.PPLVANQVVTCPDKK.S	Carbamidomethyl: 11

Protein 4: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 667.49
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 24.70 % **pI:** 7.76
No. of Peptides: 13

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
320	4	702.8600	-17.21	2	38.9	90.6	0	136-149	K.EWVTGDSVLTGLK.I	
189	4	702.8100	-62.47	2	25.4	35.0	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 11
176	3	702.8280	-36.86	2	25.4	27.2	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 7
166	5	468.5520	-53.41	3	24.7	76.1	0	149-161	K.ISVPESQYPANAK.S	
205	4	548.9320	-1.09	3	28.4	44.2	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5; Oxidation: 8
17	2	585.7770	-33.67	2	12.5	25.4	1	187-196	R.DPAVERQEAR.C	
117	7	650.2740	-36.77	2	20.8	62.6	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
142	1	650.7570	-50.58	2	22.2	24.7	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1; Deamidated: 6
299	2	448.6890	-66.18	2	35.9	22.4	0	269-2665	K.WFFGDPK.S	
152	3	430.6910	-105.78	2	23.0	54.5	0	266-273	K.SPLGAMLR.I	Oxidation: 6
280	5	422.7030	-85.41	2	30.6	65.8	0	266-273	K.SPLGAMLR.I	
29	2	645.7590	-43.49	2	13.8	79.6	0	330-345	R.GGGQGGGGGLAGSDSR.Q	Deamidated: 4
22	8	645.2550	-62.11	2	12.7	80.5	0	330-345	R.GGGQGGGGGLAGSDSR.Q	

Protein 5: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957753 **Score:** 463.39
Database: NCBInr **MW [kDa]:** 19.10
Seq. Coverage [%]: 36.90 % **pI:** 9.33
No. of Peptides: 9

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
192	1	884.3860	-32.26	2	28.0	34.6	0	44-60	K.TVEAPSSGSVVFQCGDK.L	Carbamidomethyl: 14

244	1	785.3740	-24.70	2	34.2	23.5	0	61-79	K.LTISPSGEGDVFYGK.E	
266	1	556.3420	-28.68	2	36.7	49.0	0	83-93	K.LTTVLPGAVLK.A	
47	7	529.7410	-28.34	2	15.1	67.5	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1
47	2	728.6560	-25.79	3	17.4	44.9	1	124-146	K.CVAEAGAPAGRNNDGGSSAPTPKD	Carbamidomethyl: 1
51	1	728.9960	-9.32	3	17.8	48.5	1	124-149	K.CVAEAGAPAGRNNDGGSSAPTPKDDK	Carbamidomethyl: 1; Deamidated: 12
19	2	573.2280	-50.76	2	12.6	73.5	0	135-146	R.NNDGGSSAPTPK.D	Deamidated: 1
11	3	516.5330	-58.73	3	11.6	64.7	1	135-149	R.NNDGGSSAPTPKDCK.L	Carbamidomethyl: 14
15	2	516.8720	-37.42	3	12.2	42.1	1	135-149	R.NNDGGSSAPTPKDCK.L	Carbamidomethyl: 14; Deamidated: 1

Protein 6: surface antigen [Toxoplasma gondii ME49]
Accession: gi|237845243 **Score:** 449.64
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 20.00 % **pI:** 8.67
No. of Peptides: 11

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
273	2	586.2920	-50.34	2	32.0	35.0	0	55-64	K.ITYFGTLTQK.A	
131	2	403.6860	-36.75	2	21.6	40.5	0	65-70	K.APNWYR.C	
100	2	475.2460	-46.93	3	20.4	33.0	1	76-88	R.AKEEVVGHVTLNK.E	
28	2	440.9210	-39.96	4	13.8	28.8	1	119-132	R.VCHIDAKDQDDCER.N	Carbamidomethyl: 2, 12
355	2	591.2830	-51.90	2	39.3	59.2	0	135-149	R.GFLTDYPGAK.Q	
165	2	588.9550	-28.85	3	23.6	51.3	1	151-165	K.IEKVEQNGEQSVLYK.F	Deamidated: 7
165	2	588.9550	-28.85	3	23.6	39.0	1	151-165	K.IEKVEQNGEQSVLYK.F	Deamidated: 10
133	3	697.8150	-40.90	2	22.3	73.9	0	154-165	K.VEQNGEQSVLYK.F	Deamidated: 4
144	5	697.7980	640.24	2	22.3	34.9	0	154-165	K.VEQNGEQSVLYK.F	
274	5	488.7320	-66.81	2	30.2	70.4	0	229-236	K.NANFIEIR.C	
312	1	489.2150	-84.16	2	33.4	28.6	0	229-236	K.NANFIEIR.C	Deamidated: 1

Protein 7: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237830827 **Score:** 387.23
Database: NCBInr **MW [kDa]:** 34.20
Seq. Coverage [%]: 23.10 % **pI:** 5.55

No. of Peptides: 7

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
210	2	586.7720	-67.33	2	26.7	35.3	0	52-63	K.GGISVEVDPATK.K	
162	2	434.2040	-86.94	3	23.7	27.3	1	52-64	K.GGISVEVDPATKK.V	
304	2	638.3020	-25.32	3	31.5	65.4	0	93-110	R.VNLQGETPLAEHFGEGSK.A	
270	1	582.0330	-31.43	4	29.7	98.4	1	93-114	R.VNLQGETPLAEHFGEGSKANVK.K	
13	1	708.2830	-39.43	2	11.3	80.9	0	155-168	R.APTGDPSQNSDGNR.G	
134	1	501.5460	-63.87	3	22.2	46.1	1	269-270	K.ELPKTEATYCYK.C	Carbamidomethyl: 10
62	2	517.1830	-117.17	3	17.8	33.9	1	271-285	K.CSPLVDSGDTADGKK.N	Carbamidomethyl: 1

Protein 8: 14-3-3 protein homologue [Toxoplasma gondii]
Accession: gi|3123732
Database: NCBInr
Seq. Coverage [%]: 33.10 %
Score: 366.08
MW [kDa]: 30.70
pI: 4.55
No. of Peptides: 8

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
100	1	639.5920	-39.09	3	20.2	50.1	1	18-33	K.LAEQAERYDEMAEAMK.N	Oxidation: 11, 15
286	1	454.2440	-48.45	2	30.6	28.8	0	55-62	R.NLLSVAYK.N	
49	2	452.2000	-134.71	2	17.0	51.8	0	74-81	R.IISSVEQK.E	
115	1	454.2370	-82.67	3	21.2	24.6	1	74-85	R.IISSVEQKELSK.Q	
159	1	485.2160	-35.11	3	23.6	36.5	1	124-135	K.TSDSESKVFYYK.M	
102	1	458.5320	-55.89	3	20.5	42.0	1	143-154	R.YISEFSGEEGKK.Q	
19	2	683.7970	-26.78	2	13.2	89.5	0	155-166	K.QAADQAQESYQK.A	
180	1	574.9590	-1.89	3	25.0	42.7	0	167-182	K.ATETAEAELPSTHPIR.L	

Protein 9: actin [Toxoplasma gondii ME49]
Accession: gi|237840731
Database: NCBInr
Seq. Coverage [%]: 20.50 %
Score: 349.56
MW [kDa]: 41.90
pI: 4.91

No. of Peptides: 7

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
34	4	464.6880	-85.60	2	14.0	69.8	0	20-29	K.AGVAGDDAPR.A	
41	1	476.6250	-43.03	3	15.7	28.1	1	52-63	K.DCYVGDEAQSKR.G	Carbamidomethyl: 2
287	4	652.0080	-28.10	3	34.2	68.3	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
24	3	532.7100	-57.72	2	13.2	50.4	0	230-239	K.AAEDSSDIEK.S	
307	2	888.9120	-33.49	2	36.7	52.3	0	240-255	K.SYELPDGNIITVGNER.F	
221	1	589.2890	-36.13	2	28.3	20.7	0	317-327	K.ELTSLAPSTMK.I	
159	3	597.2780	-49.80	2	24.1	59.9	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10

Protein 10: lactate dehydrogenase [Toxoplasma gondii ME49]
Accession: gi|237837615
Database: NCBInr
Seq. Coverage [%]: 23.40 %
Score: 330.91
MW [kDa]: 35.50
pI: 6.03
No. of Peptides: 8

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
86	2	419.8610	-42.69	3	18.7	20.1	0	91-101	K.VPGKPDSEWSR.N	
52	2	401.1880	-96.92	2	16.1	25.8	0	114-120	R.EIGQNIK.K	
230	4	589.2790	-59.76	2	27.7	75.9	0	168-178	R.YVADALSVSPR.D	
283	1	695.3160	-44.52	3	33.3	20.6	0	179-197	R.DVQATVIGTHGDCMVPLVR.Y	Carbamidomethyl: 13; Oxidation: 14
248	1	648.8260	-29.83	2	30.0	29.5	0	198-208	R.YITVNGYPIQK.F	Deamidated: 5
271	1	648.3010	-80.74	2	30.0	33.0	0	198-208	R.YITVNGYPIQK.F	
59	3	408.6850	-115.81	2	17.0	44.5	0	229-236	K.VSGGEIVR.F	
211	1	546.2220	-94.65	2	27.5	46.1	0	313-322	K.SVDDVMALNK.A	

Protein 11: hypothetical protein TGME49_002390 [Toxoplasma gondii ME49]
Accession: gi|237836453
Database: NCBInr
Seq. Coverage [%]: 10.70 %
Score: 293.62
MW [kDa]: 61.80
pI: 4.80

No. of Peptides: 7

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
46	2	441.6930	-59.82	2	18.9	26.2	0	52-59	R.LTSFSDGR.L	
99	2	537.2770	-11.02	2	24.4	41.5	0	60-69	R.LQALDSVDGR.S	
151	2	628.2820	-26.69	3	32.7	57.8	0	70-86	R.SAPPEFTDTVTGQYDVR.T	
158	1	571.7800	-17.57	2	33.9	35.2	0	323-332	K.TFFSAEELAK.I	
44	2	576.7540	-45.44	2	18.6	81.8	0	347-356	R.VTFAENDTQK.V	
49	1	577.2620	-35.02	2	19.3	26.9	0	347-356	R.VTFAENDTQK.V	Deamidated: 6
53	2	412.7120	-85.90	2	19.6	24.1	0	401-408	R.EIVAPAPK.G	

Protein 12: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957751
Database: NCBInr
Seq. Coverage [%]: 15.10 %
Score: 238.14
MW [kDa]: 19.00
pI: 9.35
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
196	2	471.2790	-46.56	3	34.7	62.1	1	82-95	R.KLTTVLPGAVLTAK.V	
213	2	642.3970	-5.13	2	38.1	35.6	0	83-95	K.LTTVLPGAVLTAK.V	
24	2	497.5300	-52.62	3	11.5	44.3	1	135-148	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13

Protein 13: bradyzoite-specific surface protein, putative [Toxoplasma gondii ME49]
Accession: gi|237841177
Database: NCBInr
Seq. Coverage [%]: 17.80 %
Score: 232.47
MW [kDa]: 41.50
pI: 6.23
No. of Peptides: 5

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
215	1	626.2990	-27.31	2	27.8	45.1	0	93-104	K.GSLTATLECTAK.D	Carbamidomethyl: 9

204	1	726.8020	-37.99	2	26.3	24.1	0	190-202	K.TFDVGCTYTTTGK.A	Carbamidomethyl: 6
278	2	778.3830	-43.27	2	30.4	65.6	0	203-217	K.AAANPAVCQLTVNVK.A	Carbamidomethyl: 8
77	2	624.9420	-40.22	3	18.2	44.3	1	326-342	R.LGCVPNKAPQSDSQDTR.V	Carbamidomethyl: 3
270	1	732.3680	-33.24	2	29.9	36.5	0	353-366	R.TSAPTTCSVLVTVK.A	Carbamidomethyl: 7

Protein 14: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237829715
Database: NCBInr
Seq. Coverage [%]: 16.10 %
Score: 211.33
MW [kDa]: 38.00
pI: 5.76
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
310	1	720.3510	-19.29	3	33.3	22.1	1	173-191	K.QNLPPVDKEFIVGCTQEGK.G	Carbamidomethyl: 14
96	1	804.6390	-75.39	3	19.5	72.6	1	204-226	R.ASSKDSQTVTCAYGSASNADVHR.V	Carbamidomethyl: 11
274	4	560.6280	-3.39	3	32.1	49.9	0	293-307	K.AVLTIPHDNFPEAQK.K	
262	2	452.7260	-48.56	4	29.5	51.1	1	293-308	K.AVLTIPHDNFPEAQKK.V	

Protein 15: ubiquitin, putative [Toxoplasma gondii ME49]
Accession: gi|237835791
Database: NCBInr
Seq. Coverage [%]: 21.30 %
Score: 186.11
MW [kDa]: 24.40
pI: 6.02
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
130	1	448.5470	-89.12	3	23.1	21.4	0	40-51	K.IIDMRPGLVASR.I	Oxidation: 4
24	1	434.1940	-87.90	2	14.6	29.2	0	66-72	K.QLTTYNK.S	
225	2	537.2840	17.93	2	31.0	25.3	0	182-189	K.LFYFNNQK.A	
59	1	658.2740	-57.32	3	18.6	35.6	1	198-216	K.KNAAVSSTPVNQDEAAQEN.-	
86	1	922.8880	-30.86	2	20.5	74.7	0	199-216	K.NAAVSSTPVNQDEAAQEN.-	

Protein 16: conserved hypothetical protein [Toxoplasma gondii GT1]

Accession: gi|221482255
Database: NCBInr
Seq. Coverage [%]: 6.90 %
Score: 184.94
MW [kDa]: 63.20
pI: 5.30
No. of Peptides: 4

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
27	1	435.2150	-57.42	2	14.2	30.3	0	10-18	R.TPATPSPAK.V	
29	1	610.2620	-46.57	2	14.6	53.4	0	36-46	K.NAGPVTQTEMR.N	Oxidation: 10
93	2	602.3560	-44.18	2	23.2	71.3	0	174-186	R.VVELVGGGSASTK.H	
103	1	422.7390	-21.25	2	25.1	30.0	0	274-280	R.LSNELLR.V	

Protein 17: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640
Database: NCBInr
Seq. Coverage [%]: 15.60 %
Score: 168.98
MW [kDa]: 33.90
pI: 6.04
No. of Peptides: 4

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
7	4	539.5450	-47.25	3	11.0	45.9	0	16-30	K.DSHAANADSQTPMQK.L	Oxidation: 13
67	2	517.2530	-45.59	2	18.3	50.5	0	56-65	K.LAGQMSLAAR.S	Oxidation: 5
102	3	418.8590	-96.89	3	19.8	39.5	0	114-124	K.APPYKPIPESR.F	
32	2	507.5650	-29.98	3	14.8	33.2	1	136-148	R.QNIETMKDTPEAK.A	Oxidation: 6

Protein 18: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688
Database: NCBInr
Seq. Coverage [%]: 2.30 %
Score: 127.51
MW [kDa]: 144.50
pI: 5.80
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification

45	2	601.2290	-34.37	3	14.4	84.7	0	82-97	R.ESTVGGSGTGHSQLGK.S	
149	1	803.8820	-14.87	2	27.9	42.8	0	338-352	K.TNEALLTGESEDISK.T	

Protein 19: glyceraldehyde-3-phosphate dehydrogenase, putative [Toxoplasma gondii VEG]
Accession: gi|221505688
Database: NCBInr
Seq. Coverage [%]: 9.90 %
Score: 127.51
MW [kDa]: 40.40
pI: 7.16
No. of Peptides: 3

Modification(s): Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
243	4	678.8580	-29.04	2	28.4	63.7	0	236-250	R.SAGVNIIPASTGAAK.A	
181	1	421.7190	1085.48	2	25.7	21.5	0	255-262	K.IIPSLNGK.L	
265	1	774.8110	-78.43	2	29.7	24.6	0	360-373	R.LVELAHYMSVQDGA.-	Oxidation: 8

Protein 20: surface protein, putative [Toxoplasma gondii VEG]
Accession: gi|221509030
Database: NCBInr
Seq. Coverage [%]: 8.10 %
Score: 115.59
MW [kDa]: 44.20
pI: 7.81
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
269	3	787.8260	-47.38	2	31.7	49.8	0	132-145	K.GAQNDWLNGEDLSR.G	
52	1	513.1930	-33.38	3	17.1	23.8	0	232-244	K.EFCTGEPHTSCGR.Q	Carbamidomethyl: 3, 11
181	2	417.2110	-44.17	2	24.8	42.8	0	247-255	K.YADVLPR.Y	

Protein 21: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221501829
Database: NCBInr
Seq. Coverage [%]: 5.40 %
Score: 99.27
MW [kDa]: 40.90
pI: 5.61
No. of Peptides: 2

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
153	1	401.7130	-105.23	2	23.6	25.0	0	87-94	K.ATALVLSK.Q	
49	4	644.2970	-18.85	2	16.7	74.2	0	101-113	K.LVCSGDGNAAAPR.N	Carbamidomethyl: 3

Protein 22: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237833253
Database: NCBInr
Seq. Coverage [%]: 4.80 %
Score: 74.94
MW [kDa]: 40.00
pI: 5.60
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
147	1	429.2370	-82.44	2	22.6	26.1	0	199-206	K.VAVTLQAR.A	
17	2	566.2550	-31.93	2	12.2	48.8	0	321-330	K.ISDPNNQTSR.S	

Protein 23: DNA-directed RNA polymerase, omega subunit family protein [Tetrahymena thermophila]
Accession: gi|118362960
Database: NCBInr
Seq. Coverage [%]: 0.20 %
Score: 74.60
MW [kDa]: 513.50
pI: 4.78
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
256	1	430.7190	-6.11	2	29.0	55.4	0	2354-2360	K.QNLIDEK.E	Deamidated: 2

Protein 24: 40S ribosomal protein S3a, putative [Toxoplasma gondii ME49]
Accession: gi|237837685
Database: NCBInr
Seq. Coverage [%]: 7.30 %
Score: 67.07
MW [kDa]: 29.40
pI: 9.63
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
121	2	659.3130	-34.70	3	21.5	67.1	2	241-259	R.KVQAESGEAQNTLTAETKA.-	

Protein 25: C2 domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237836043
Database: NCBInr
Seq. Coverage [%]: 2.60 %
Score: 49.74
MW [kDa]: 40.80
pI: 9.08
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
172	1	543.2930	-48.42	2	24.3	49.7	0	142-151	K.VVVLTAEQAR.A	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG1_GB3_01_2154.d/

Protein 1: surface antigen P22 [Toxoplasma gondii]
Accession: gi|269838870
Database: NCBInr
Seq. Coverage [%]: 22.90 %
Score: 199.45
MW [kDa]: 17.30
pI: 9.09
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
196	2	471.2790	-46.56	3	34.7	52.1	1	66-79	R.KLTTVLPGAVLTAK.V	
213	2	642.3970	-5.13	2	38.1	35.6	0	67-79	K.LTTVLPGAVLTAK.V	
47	2	529.7410	-28.34	2	15.1	67.5	0	108-118	K.CVAEAGAPAGR.N	Carbamidomethyl: 1
24	2	497.5300	-52.62	3	11.5	44.3	1	119-132	R.NNDGSSAPTPKDCK.L	Carbamidomethyl: 13

Protein 2: P-type ATPase, putative [Toxoplasma gondii VEG]
Accession: gi|221502688 **Score:** 127.51
Database: NCBInr **MW [kDa]:** 144.50
Seq. Coverage [%]: 2.30 % **pI:** 5.80
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
45	2	501.2290	-34.37	3	14.4	84.7	0	82-97	R.ESTVGGSGTGHSQLGK.S	
149	1	803.8820	-14.87	2	27.9	42.8	0	338-352	K.TNEALLTGESEDISK.T	

Protein 3: dense granule protein 5 precursor [Toxoplasma gondii ME49]
Accession: gi|237839947 **Score:** 69.25
Database: NCBInr **MW [kDa]:** 12.80
Seq. Coverage [%]: 17.50 % **pI:** 5.33
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
122	1	779.6490	-33.15	3	28.1	69.3	1	100-120	R.AIQEESKESATAEEEEVAEEE.-	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG2_GB4_01_2155.d/

Protein 1: hypothetical protein TGME49_002390 [Toxoplasma gondii ME49]
Accession: gi|237836453 **Score:** 293.62
Database: NCBInr **MW [kDa]:** 61.80
Seq. Coverage [%]: 10.70 % **pI:** 4.80
No. of Peptides: 7
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
46	2	441.6930	-59.82	2	18.9	26.2	0	52-59	R.LTSFSDGR.L	
99	2	537.2770	-11.02	2	24.4	41.5	0	60-69	R.LQALDSVDGR.S	
151	2	628.2820	-26.69	3	32.7	57.8	0	70-86	R.SAPPEFTDTVTGQYDVR.T	
158	1	571.7600	-17.57	2	33.9	35.2	0	323-332	K.TFFSAEELAK.I	
44	2	576.7540	-45.44	2	18.6	81.8	0	347-356	R.VTFAENDTQK.V	
49	1	577.2620	-35.02	2	19.3	26.9	0	347-356	R.VTFAENDTQK.V	Deamidated: 6
53	2	412.7120	-85.90	2	19.6	24.1	0	401-408	R.EIVAPAPK.G	

Protein 2: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221482255 **Score:** 184.94
Database: NCBInr **MW [kDa]:** 63.20
Seq. Coverage [%]: 6.90 % **pI:** 5.30
No. of Peptides: 4
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
27	1	435.2150	-57.42	2	14.2	30.3	0	10-18	R.TPATPSPAK.V	
29	1	610.2620	-46.57	2	14.6	53.4	0	36-46	K.NAGPVTQTEMR.N	Oxidation: 10
93	2	602.3060	-44.18	2	23.2	71.3	0	174-186	R.VVELVGGGSASTK.H	
103	1	422.7390	-21.25	2	25.1	30.8	0	274-280	R.LSNELLR.V	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG3_GB5_01_2157.d/

Search Type Combined MS/MS - ProteinExtractor

Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG4_GB6_01_2158.d/

Protein 1: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 608.08
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 22.00 % **pI:** 7.76
No. of Peptides: 11
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
348	2	702.8400	-45.67	2	38.8	83.7	0	136-148	K.EWVTGDSVLTGLK.I	
189	2	702.8100	-62.47	2	25.4	35.0	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 11
178	3	468.5500	-57.67	3	24.7	65.7	0	149-161	K.ISVPESQYPANAK.S	
205	2	548.9320	-1.09	3	26.4	44.2	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5; Oxidation: 8
117	3	650.2740	-36.77	2	20.8	62.6	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
142	1	650.7570	-50.58	2	22.2	24.7	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1; Deamidated: 6
326	1	448.6790	-68.47	2	35.9	20.8	0	269-265	K.WFFGDPK.S	
280	2	422.7030	-85.41	2	30.6	65.8	0	266-273	K.SPLGAMLR.I	
162	1	430.6910	-105.78	2	23.0	54.5	0	266-273	K.SPLGAMLR.I	Oxidation: 6
22	3	645.2550	-62.11	2	12.7	80.5	0	330-345	R.GGGQGGGGGLAGSDSR.Q	
29	1	645.7590	-43.49	2	13.9	79.6	0	330-345	R.GGGQGGGGGLAGSDSR.Q	Deamidated: 4

Protein 2: surface antigen [Toxoplasma gondii ME49]
Accession: gi|237845243 **Score:** 352.55
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 17.40 % **pI:** 8.67
No. of Peptides: 9
Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
131	1	403.6860	-36.75	2	21.6	40.5	0	65-70	K.APNWYR.C	

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
111	1	476.2510	-36.41	3	20.3	20.8	1	76-88	R.AKEEVVGHVTLNK.E	
32	1	440.9120	-60.38	4	13.9	21.6	1	119-132	R.VCHIDAKDQDDCER.N	Carbamidomethyl: 2, 12
355	2	591.2830	-51.90	2	39.3	59.2	0	135-148	R.GFLTDYIPGAK.Q	
165	2	588.9550	-28.85	3	23.6	51.3	1	151-165	K.IEKVEQNGEQSVLYK.F	Deamidated: 7
165	2	588.9550	-28.85	3	23.6	39.0	1	151-165	K.IEKVEQNGEQSVLYK.F	Deamidated: 10
144	2	697.7980	-65.26	2	22.3	60.3	0	154-165	K.VEQNGEQSVLYK.F	Deamidated: 4
312	1	489.2150	-84.16	2	33.4	28.6	0	229-236	K.NANFIEIR.C	Deamidated: 1
274	3	488.7320	-65.81	2	30.2	70.4	0	229-236	K.NANFIEIR.C	

Protein 3: actin [Toxoplasma gondii ME49]
Accession: gi|237840731 **Score:** 268.14
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 17.30 % **pI:** 4.91
No. of Peptides: 5
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
34	2	464.6880	-85.60	2	14.0	69.8	0	20-29	K.AGVAGDDAPR.A	
315	2	652.0160	-15.83	3	34.1	65.8	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
28	2	532.7140	-50.21	2	13.2	45.6	0	230-239	K.AAEDSSDIEK.S	
335	1	888.9200	-24.49	2	36.7	44.2	0	240-255	K.SYELPDGNIITVGNER.F	
169	1	597.2710	-61.52	2	24.0	42.6	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10

Protein 4: Chain A, T. Gondii Bradyzoite-Specific Ldh (Ldh1) In Complex With Nad And Oxq
Accession: gi|310689950 **Score:** 216.14
Database: NCBInr **MW [kDa]:** 35.50
Seq. Coverage [%]: 14.60 % **pI:** 6.06
No. of Peptides: 5

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
86	1	419.8610	-42.69	3	18.7	20.1	0	89-99	K.VPGKPDSEWSR.N	
52	1	401.1880	-96.92	2	16.1	25.8	0	112-118	R.EIGQNIK.K	
230	2	589.2790	-59.76	2	27.7	75.9	0	166-176	R.YVADALSVSPR.D	

271	1	648.3010	-80.74	2	30.0	33.0	0	196-206	R.YITVNGYPIQK.F	
59	1	408.6850	-115.81	2	17.0	44.5	0	227-234	K.VSGGEIVR.F	

Protein 5: SAG1-related sequence 3 [Toxoplasma gondii]
Accession: gi|2305258 **Score:** 200.94
Database: NCBInr **MW [kDa]:** 36.20
Seq. Coverage [%]: 16.80 % **pI:** 5.75
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
310	1	720.3510	-19.29	3	33.3	22.1	1	158-176	K.QNLPPVDKEFIVGCTQEGK.G	Carbamidomethyl: 14
96	1	804.6390	-76.39	3	19.5	72.6	1	189-211	R.ASSKDSQTVTCAYGSASNADVHR V	Carbamidomethyl: 11
298	2	560.6170	-23.01	3	32.1	39.5	0	278-292	K.AVLTIPHDNFPEAQK.K	
262	1	452.7260	-48.56	4	29.5	51.1	1	278-293	K.AVLTIPHDNFPEAQKK.V	

Protein 6: bradyzoite-specific surface protein, putative [Toxoplasma gondii ME49]
Accession: gi|237841177 **Score:** 187.35
Database: NCBInr **MW [kDa]:** 41.50
Seq. Coverage [%]: 14.80 % **pI:** 6.23
No. of Peptides: 4

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
204	1	725.8020	-37.99	2	26.3	24.1	0	190-202	K.TFDVGCTYTTTGK.A	Carbamidomethyl: 6
278	1	778.3830	-43.27	2	30.4	65.6	0	203-217	K.AAANPAVCQLTVNVK.A	Carbamidomethyl: 8
77	1	624.9420	-40.22	3	18.2	44.3	1	326-342	R.LGCVPNKAPQSDSQDTR.V	Carbamidomethyl: 3
270	1	732.3680	-33.24	2	29.9	36.5	0	353-366	R.TSAPTTCSVLVTVK.A	Carbamidomethyl: 7

Protein 7: glyceraldehyde 3-phosphate dehydrogenase, related [Neospora caninum Liverpool]
Accession: gi|325115564 **Score:** 88.23
Database: NCBInr **MW [kDa]:** 36.50
Seq. Coverage [%]: 8.50 % **pI:** 6.83

No. of Peptides: 2

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
243	2	678.8580	-29.04	2	28.4	63.7	0	203-217	R.SAGVNIIPASTGAAK.A	
265	1	774.8110	-78.43	2	29.7	24.6	0	327-340	R.LVELAHYMSVQDGA.-	Oxidation: 8

Protein 8: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237833253 **Score:** 74.94
Database: NCBInr **MW [kDa]:** 40.00
Seq. Coverage [%]: 4.80 % **pI:** 5.60
No. of Peptides: 2

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
147	1	429.2370	-62.44	2	22.5	26.1	0	199-206	K.VAVTLQAR.A	
17	1	566.2550	-31.93	2	12.2	48.8	0	321-330	K.ISDPNNQTSR.S	

Protein 9: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221501829 **Score:** 61.04
Database: NCBInr **MW [kDa]:** 40.90
Seq. Coverage [%]: 3.30 % **pI:** 5.61
No. of Peptides: 1

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
57	2	644.3040	-7.98	2	16.7	61.0	0	101-113	K.LVCSGDGNAAAPR.N	Carbamidomethyl: 3

Protein 10: C2 domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237836043 **Score:** 49.74
Database: NCBInr **MW [kDa]:** 40.80
Seq. Coverage [%]: 2.60 % **pI:** 9.08
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
172	1	543.2930	-48.42	2	24.3	49.7	0	142-151	K.VVVLTAEQAR.A	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG5_GB7_01_2159.d/

Protein 1: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 558.89
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 24.70 % **pI:** 7.76
No. of Peptides: 11

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
320	2	702.8600	-17.21	2	38.9	90.6	0	136-148	K.EWVTGDSVLTGLK.I	
176	1	702.8280	-36.86	2	25.4	27.2	0	149-161	K.ISVPESQYPANAK.S	Deamidated: 7
166	2	468.5520	-53.41	3	24.7	76.1	0	149-161	K.ISVPESQYPANAK.S	
189	2	548.9330	0.73	3	26.3	32.6	0	173-186	K.TGNTCMLTIHVEPR.D	Carbamidomethyl: 5; Oxidation: 8
17	1	585.7770	-33.67	2	12.6	25.4	1	187-196	R.DPAVERQEAR.C	
105	2	650.2830	-22.93	2	20.7	47.9	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
299	1	448.6890	-66.18	2	35.9	22.4	0	259-265	K.WFFGDPK.S	
142	1	430.7020	-80.24	2	22.8	30.6	0	266-273	K.SPLGAMLR.I	Oxidation: 6
259	2	422.7090	-71.22	2	30.8	48.8	0	266-273	K.SPLGAMLR.I	
26	1	645.7550	-49.69	2	13.5	61.8	0	330-345	R.GGGQGGGGGLAGSDSR.Q	Deamidated: 4
18	3	645.2470	-74.51	2	12.7	69.8	0	330-345	R.GGGQGGGGGLAGSDSR.Q	

Protein 2: actin [Toxoplasma gondii ME49]

Accession: gi|237840731 **Score:** 336.78
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 20.50 % **pI:** 4.91
No. of Peptides: 7

Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
29	2	464.6780	-107.12	2	14.1	57.1	0	20-29	K.AGVAGDDAPR.A	
41	1	476.5250	-43.03	3	15.7	28.1	1	52-63	K.DCYVGDEAQSKR.G	Carbamidomethyl: 2
287	2	682.0080	-28.10	3	34.2	68.3	0	97-114	R.VAPEEHPVLLTEAPLNPK.A	
24	1	532.7100	-57.72	2	13.2	50.4	0	230-239	K.AAEDSSDIEK.S	
307	1	888.9120	-33.49	2	36.7	52.3	0	240-256	K.SYELPDGNIITVGNER.F	
221	1	589.2890	-36.13	2	28.3	20.7	0	317-327	K.ELTSLAPSTMK.I	
159	2	597.2780	-49.80	2	24.1	59.9	0	317-327	K.ELTSLAPSTMK.I	Oxidation: 10

Protein 3: surface antigen [Toxoplasma gondii ME49]
Accession: gi|237845243 **Score:** 281.59
Database: NCBInr **MW [kDa]:** 41.90
Seq. Coverage [%]: 16.40 % **pI:** 8.67
No. of Peptides: 6

Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
273	2	586.2920	-50.34	2	32.0	35.0	0	55-64	K.ITYFGTLTGK.A	
122	1	403.6600	-101.16	2	21.7	29.9	0	65-70	K.APNWYR.C	
100	1	475.2460	-46.93	3	20.4	33.0	1	76-88	R.AKEEVVGHVTLNK.E	
28	1	440.9210	-39.96	4	13.8	28.8	1	119-132	R.VCHIDAKDQDDCER.N	Carbamidomethyl: 2, 12
133	1	697.8150	-40.90	2	22.3	73.9	0	154-165	K.VEQNGEQSVLYK.F	Deamidated: 4
282	2	488.7450	-39.21	2	30.2	51.9	0	229-236	K.NANFIEIR.C	

Protein 4: Chain A, T. Gondii Bradyzoite-Specific Ldh (Ldh1) In Complex With Nad And Oxq
Accession: gi|310889950 **Score:** 260.09
Database: NCBInr **MW [kDa]:** 35.50
Seq. Coverage [%]: 20.10 % **pI:** 6.06

No. of Peptides: 6

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
44	1	401.1610	-164.21	2	16.2	24.4	0	112-118	R.EIGQNIK.K	
214	2	589.2780	-61.46	2	27.9	69.4	0	166-176	R.YVADALSVSPR.D	
283	1	695.3160	-44.52	3	33.3	20.6	0	177-195	R.DVQATVIGTHGDCMVPLVR.Y	Carbamidomethyl: 13; Oxidation: 14
248	1	648.8260	-29.83	2	30.0	29.5	0	196-206	R.YITVNGYPIQK.F	Deamidated: 5
51	2	408.6980	-84.09	2	17.1	34.1	0	227-234	K.VSGGEIVR.F	
211	1	546.2220	-94.65	2	27.5	46.1	0	311-320	K.SVDDVMALNK.A	

Protein 5: conserved hypothetical protein [Toxoplasma gondii GT1]
Accession: gi|221488640 **Score:** 119.34
Database: NCBInr **MW [kDa]:** 33.90
Seq. Coverage [%]: 12.10 % **pI:** 6.04
No. of Peptides: 3

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
9	2	539.5370	-62.07	3	11.1	20.3	0	16-30	K.DSHAAANADSQTPMQK.L	Oxidation: 13
67	1	517.2530	-45.59	2	18.3	50.5	0	56-65	K.LAQQMSLAAR.S	Oxidation: 5
32	1	507.5650	-29.98	3	14.8	33.2	1	136-146	R.QNIETMKDTPEAK.A	Oxidation: 6

Protein 6: bradyzoite-specific surface protein, putative [Toxoplasma gondii ME49]
Accession: gi|237841177 **Score:** 112.72
Database: NCBInr **MW [kDa]:** 41.50
Seq. Coverage [%]: 6.80 % **pI:** 6.23
No. of Peptides: 2

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
215	1	626.2990	-27.31	2	27.8	45.1	0	93-104	K.GSLTATLECTAK.D	Carbamidomethyl: 9

257	1	778.3800	-47.12	2	30.5	48.8	0	203-217	K.AAANPAVCQLTVNVK.A	Carbamidomethyl: 8

Protein 7: SAG1-related sequence 3 [Toxoplasma gondii]
Accession: gi|2305258 **Score:** 110.06
Database: NCBInr **MW [kDa]:** 36.20
Seq. Coverage [%]: 11.30 % **pI:** 5.75
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
85	2	603.7300	-77.13	4	19.6	31.5	1	189-211	R.ASSKDSQTVTCAYGSASNADVHR V	Carbamidomethyl: 11
274	2	560.6280	-3.39	3	32.1	49.9	0	278-292	K.AVLTIPHDNFPEAQK.K	
241	1	452.7260	-48.56	4	29.4	28.7	1	278-293	K.AVLTIPHDNFPEAQKK.V	

Protein 8: conserved hypothetical protein [Toxoplasma gondii VEG]
Accession: gi|221501829 **Score:** 99.27
Database: NCBInr **MW [kDa]:** 40.90
Seq. Coverage [%]: 5.40 % **pI:** 5.61
No. of Peptides: 2

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
153	1	401.7130	-105.23	2	23.6	25.6	0	87-94	K.ATALVLSK.Q	
49	2	644.2970	-18.85	2	16.7	74.2	0	101-115	K.LVCSGDGNAAAPR.N	Carbamidomethyl: 3

Protein 9: surface protein, putative [Toxoplasma gondii VEG]
Accession: gi|221509030 **Score:** 94.93
Database: NCBInr **MW [kDa]:** 44.20
Seq. Coverage [%]: 8.10 % **pI:** 7.81
No. of Peptides: 3

Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
269	1	787.8260	-47.38	2	31.7	49.8	0	132-145	K.GAQNDWLNGEDLSR.G	
52	1	513.1930	-33.38	3	17.1	23.8	0	232-244	K.EFCTGEPHTSCGR.Q	Carbamidomethyl: 3, 11
170	1	417.1610	-164.01	2	24.9	21.3	0	247-253	K.YADVLPR.Y	

Protein 10: glyceraldehyde-3-phosphate dehydrogenase, putative [Toxoplasma gondii VEG]
Accession: gi|221505688 **Score:** 91.24
Database: NCBInr **MW [kDa]:** 40.40
Seq. Coverage [%]: 6.20 % **pI:** 7.16
No. of Peptides: 2

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
224	2	678.8610	-24.62	2	28.4	52.0	0	236-250	R.SAGVNIIPASTGAAK.A	
181	1	421.7180	1085.48	2	25.7	21.5	0	255-262	K.IIPSLNGK.L	

Protein 11: hypothetical protein TTHERM_00037160 [Tetrahymena thermophila]
Accession: gi|118346523 **Score:** 49.36
Database: NCBInr **MW [kDa]:** 10.70
Seq. Coverage [%]: 8.00 % **pI:** 10.35
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
60	1	401.6900	-131.19	2	17.9	49.4	2	21-27	K.KAVKNNK.K	Deamidated: 6

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG6_GB8_01_2160.d/

Protein 1: major surface antigen p30 [Toxoplasma gondii ME49]
Accession: gi|237837815 **Score:** 1322.33
Database: NCBInr **MW [kDa]:** 32.90
Seq. Coverage [%]: 50.80 % **pI:** 9.29
No. of Peptides: 22

Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
385	3	615.0020	-2.46	3	36.0	76.9	0	48-64	K.STAAVILTPTENHFTLK.C	
336	1	587.7730	-46.76	4	33.3	74.6	1	48-67	K.STAAVILTPTENHFTLKCPK.T	Carbamidomethyl: 18
310	2	544.2640	-40.46	3	32.0	50.2	0	68-82	K.TALTEPPTLAYSPNR.Q	
36	2	749.3170	-27.01	2	16.1	63.1	0	83-96	R.QICPAGTTSSCTSK.A	Carbamidomethyl: 3, 11
60	5	508.7140	-125.41	2	17.8	54.2	0	163-172	R.ASSVVNNVAR.C	
84	4	509.2460	-46.76	2	19.3	81.3	0	163-172	R.ASSVVNNVAR.C	Deamidated: 6
169	2	677.7940	-36.90	2	24.2	64.2	0	173-185	R.CSYGANSTLGPVK.L	Carbamidomethyl: 1; Deamidated: 6
156	4	677.3090	-26.58	2	23.5	85.6	0	173-185	R.CSYGANSTLGPVK.L	Carbamidomethyl: 1
303	2	782.8550	-43.57	2	31.5	76.8	0	186-200	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13
206	2	790.8600	-33.59	2	26.3	57.2	0	186-200	K.LSAEGPTTMTLVCGK.D	Carbamidomethyl: 13; Oxidation: 9
197	2	660.6490	-22.80	3	25.9	29.2	1	186-204	K.LSAEGPTTMTLVCGKDGVK.V	Carbamidomethyl: 13; Oxidation: 9
184	1	895.6980	-40.37	3	25.3	61.4	1	201-224	K.DGVKVPQDNNQYCSGTTLTGCNE K.S	Carbamidomethyl: 13, 21
133	2	762.6300	-44.01	3	22.2	39.5	0	205-224	K.VPQDNNQYCSGTTLTGCNEK.S	Carbamidomethyl: 9, 17
252	2	474.2690	-26.69	2	28.9	44.1	1	225-232	K.SFKDILPK.L	
318	5	773.0150	-32.36	3	32.3	54.6	0	233-254	K.LSENPWQGNASSDNGATLTINK. E	
330	3	773.3500	-23.30	3	32.9	71.9	0	233-254	K.LSENPWQGNASSDNGATLTINK. E	Deamidated: 14
330	3	773.3500	-23.30	3	32.9	48.5	0	233-254	K.LSENPWQGNASSDNGATLTINK. E	Deamidated: 9
374	1	1059.4900	-18.13	3	35.5	80.5	1	233-262	K.LSENPWQGNASSDNGATLTINKE AFPAESK.S	
387	1	1059.8180	-18.13	3	36.1	56.2	1	233-262	K.LSENPWQGNASSDNGATLTINKE AFPAESK.S	Deamidated: 14
372	1	795.1200	-12.24	4	35.4	34.9	1	233-262	K.LSENPWQGNASSDNGATLTINKE	Deamidated: 21

									AFPAESK.S	
54	2	439.6620	-123.69	2	17.4	39.0	0	255-262	K.EAFPAESK.S	
148	2	652.8070	-34.28	2	22.9	67.1	0	263-275	K.SVIIGCTGGSPEK.H	Carbamidomethyl: 6

Protein 2: surface antigen protein 1 [Toxoplasma gondii]
Accession: gi|56157026 **Score:** 1045.73
Database: NCBInr **MW [kDa]:** 33.00
Seq. Coverage [%]: 4.40 % **pI:** 9.75
No. of Peptides: 1
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
74	1	744.7800	-52.26	2	18.4	49.3	0	83-96	R.QICSAGTTSSCTSK.A	Carbamidomethyl: 3, 11; Deamidated: 1

Protein 3: Chain F, Structure Of The Immunodominant Epitope Displayed By The Surface Antigen 1 (Sag1) Of Toxoplasma Gondii Complexed To A Monoclonal Antibody
Accession: gi|85543998 **Score:** 1029.26
Database: NCBInr **MW [kDa]:** 26.60
Seq. Coverage [%]: 5.90 % **pI:** 7.78
No. of Peptides: 1
Modification(s): Carbamidomethyl, Oxidation, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
183	2	555.9710	-0.48	3	25.2	55.2	1	1-15	-.PPLVANQVVTCPDKK.S	Carbamidomethyl: 11

Protein 4: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237830827 **Score:** 387.23
Database: NCBInr **MW [kDa]:** 34.20
Seq. Coverage [%]: 23.10 % **pI:** 5.55
No. of Peptides: 7
Modification(s): Carbamidomethyl

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
210	2	586.7720	-67.33	2	26.7	35.3	0	52-63	K.GGISVEVDPATK.K	
162	2	434.2040	-86.94	3	23.7	27.3	1	52-64	K.GGISVEVDPATKK.V	
304	2	638.3020	-25.32	3	31.6	65.4	0	93-110	R.VNLQGETPLAEHFGEGSK.A	
270	1	582.0330	-31.43	4	29.7	98.4	1	93-114	R.VNLQGETPLAEHFGEGSKANVK.T	
13	1	708.2830	-39.43	2	11.3	80.9	0	155-168	R.APTGDPSQNSDGNR.G	
134	1	501.5460	-63.87	3	22.2	46.1	1	259-270	K.ELPKTEATYCYK.C	Carbamidomethyl: 10
62	2	517.1830	-117.17	3	17.8	33.9	1	271-285	K.CSPLVDSGDTADGKK.N	Carbamidomethyl: 1

Protein 5: 14-3-3 protein homologue [Toxoplasma gondii]
Accession: gi|3123732 **Score:** 366.08
Database: NCBInr **MW [kDa]:** 30.70
Seq. Coverage [%]: 33.10 % **pI:** 4.55
No. of Peptides: 8
Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
100	1	639.5920	-39.09	3	20.2	50.1	1	18-33	K.LAEQAERYDEMAEAMK.N	Oxidation: 11, 15
286	1	454.2440	-48.45	2	30.6	28.8	0	55-62	R.NLLSVAYK.N	
49	2	452.2000	-134.71	2	17.0	51.8	0	74-81	R.IISSVEQK.E	
115	1	454.2370	-52.67	3	21.2	24.6	1	74-85	R.IISSVEQKELSK.Q	
159	1	485.2160	-35.11	3	23.6	36.5	1	124-135	K.TSDSESKVFYYK.M	
102	1	458.5320	-55.89	3	20.5	42.0	1	143-154	R.YISEFSGEEGKK.Q	
19	2	683.7970	-26.78	2	13.2	89.5	0	155-166	K.QAADQAQESYQK.A	
180	1	574.9590	-1.89	3	26.0	42.7	0	167-182	K.ATETAEAELPSTHPIR.L	

Protein 6: 40S ribosomal protein S3a, putative [Toxoplasma gondii ME49]
Accession: gi|237837685 **Score:** 67.07
Database: NCBInr **MW [kDa]:** 29.40
Seq. Coverage [%]: 7.30 % **pI:** 9.63
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
121	2	659.3130	-34.70	3	21.8	67.1	2	241-259	R.KVQAESGEAQNTLTAETKA.-	

Protein 7: hypothetical protein [Plasmodium chabaudi chabaudi]
Accession: gi|70925394 **Score:** 55.37
Database: NCBInr **MW [kDa]:** 13.60
Seq. Coverage [%]: 6.00 % **pI:** 6.73
No. of Peptides: 1
Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
256	1	430.7190	-48.35	2	29.0	55.4	1	30-36	R.KNIINEK.S	Deamidated: 2, 5

Protein 8: hypothetical protein [Paramecium tetraurelia strain d4-2]
Accession: gi|145545301 **Score:** 48.87
Database: NCBInr **MW [kDa]:** 92.80
Seq. Coverage [%]: 0.90 % **pI:** 6.10
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
23	1	436.7100	-39.50	2	14.8	48.9	0	174-180	K.TPEELQR.I	

Search Type Combined MS/MS - ProteinExtractor
Search Result Amazon_Bops_NCBI_2014-08-20 15:22:07
Search Location /BOPS/PTG2/PTG7_GC7_01_2161.d/

Protein 1: surface antigen P22 [Toxoplasma gondii]
Accession: gi|13957753 **Score:** 461.48
Database: NCBInr **MW [kDa]:** 19.10

Seq. Coverage [%]: 42.80 % **pI:** 9.33
No. of Peptides: 10
Modification(s): Carbamidomethyl, Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
192	1	884.3860	-32.28	2	28.0	34.6	0	44-60	K.TVEAPSSGSVVFQCGDK.L	Carbamidomethyl: 14
244	1	785.3740	-24.70	2	34.2	23.5	0	61-75	K.LTISPSGEGDVFYGK.E	
256	1	556.3420	-28.58	2	36.7	49.0	0	83-93	K.LTTVLPGAVLK.A	
26	5	529.7270	-54.77	2	14.9	65.6	0	124-134	K.CVAEAGAPAGR.N	Carbamidomethyl: 1
47	2	728.6560	-25.79	3	17.4	44.9	1	124-148	K.CVAEAGAPAGRNNDGGSSAPTPK.D	Carbamidomethyl: 1
51	1	728.9960	-9.32	3	17.8	48.5	1	124-148	K.CVAEAGAPAGRNNDGGSSAPTPK.D	Carbamidomethyl: 1; Deamidated: 12
19	2	573.2280	-50.76	2	12.6	73.5	0	135-146	R.NNDGGSSAPTPK.D	Deamidated: 1
11	3	516.5330	-58.73	3	11.6	64.7	1	135-149	R.NNDGGSSAPTPKDCK.L	Carbamidomethyl: 14
15	2	516.8720	-37.42	3	12.2	42.1	1	135-149	R.NNDGGSSAPTPKDCK.L	Carbamidomethyl: 14; Deamidated: 1
181	1	576.8320	-24.31	2	27.4	15.2	1	150-160	K.LIVRVPGADGR.V	

Protein 2: SRS domain-containing protein [Toxoplasma gondii ME49]
Accession: gi|237837819 **Score:** 204.18
Database: NCBInr **MW [kDa]:** 39.10
Seq. Coverage [%]: 9.40 % **pI:** 7.76
No. of Peptides: 4
Modification(s): Carbamidomethyl, Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
89	2	650.2850	-19.86	2	20.8	57.8	0	197-207	R.CSYTENSTLPK.I	Carbamidomethyl: 1
126	1	430.7100	-61.67	2	22.9	44.3	0	266-273	K.SPLGAMLR.I	Oxidation: 6
222	1	422.6980	-97.24	2	30.7	22.2	0	266-273	K.SPLGAMLR.I	
20	2	645.2800	-23.37	2	12.8	79.9	0	330-345	R.GGGQGGGGGLAGSDSR.Q	

Protein 3: ubiquitin, putative [Toxoplasma gondii ME49]
Accession: gi|237835791 **Score:** 186.11
Database: NCBInr **MW [kDa]:** 24.40

Seq. Coverage [%]: 21.30 %
pI: 6.02
No. of Peptides: 5

Modification(s): Oxidation

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
130	1	448.5470	-89.12	3	23.1	21.4	0	40-51	K.IIDMRPGLVASR.I	Oxidation: 4
24	1	434.1940	-87.90	2	14.6	29.2	0	66-72	K.QLTTYNK.S	
225	2	537.2840	17.93	2	31.0	25.3	0	182-189	K.LFYFNNQK.A	
59	1	668.2740	-87.32	3	18.6	38.6	1	198-216	K.KNAAVSSTPVNQDEAAQEN.-	
86	1	922.8880	-30.86	2	20.5	74.7	0	199-216	K.NAAVSSTPVNQDEAAQEN.-	

Protein 4: hypothetical protein NCLIV_021800 [Neospora caninum Liverpool]
Accession: gi|325116838
Database: NCBInr
Seq. Coverage [%]: 0.20 %
Score: 75.65
MW [kDa]: 522.10
pI: 5.40
No. of Peptides: 1

Modification(s): Deamidated

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
168	1	487.2280	-110.53	2	26.7	48.8	0	1088-1096	K.IQLSQLAAK.W	Deamidated: 2, 5

Protein 5: major surface antigen p30 [Toxoplasma gondii]
Accession: gi|4324684
Database: NCBInr
Seq. Coverage [%]: 8.50 %
Score: 50.08
MW [kDa]: 26.60
pI: 6.38
No. of Peptides: 1

Cmpd.	No. of Cmpds.	m/z meas.	Δ m/z [ppm]	z	Rt [min]	Score	P	Range	Sequence	Modification
234	1	773.0150	-32.36	3	32.5	50.1	0	173-194	K.LSENPWGGNASSDNGATLTINK.E	