Computational molecular biophysics of membrane reactions

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Habilitationsschrift

Submitted to the

Department of Physics of the Freie Universität Berlin, February 2020

Dedication

I dedicate my thesis to the girl with a curious mind.

Contents

1. Introduction

Proteins are nanoscale molecules that perform functions essential for biological life. Membranes surrounding cells, for example, contain receptor proteins that mediate communication between the cell and the external milieu, membrane transporters that transport ions and larger compounds across the membranes, and enzymes that catalyze chemical reactions. Likewise, soluble proteins found in interior of the cell include motor proteins that move other proteins around, enzymes that bind to and repair breaks in the DNA, and proteins that help control the cellular clock. Mutations in genes that encode proteins can cause disease, as is the case of cystic fibrosis, a disease that associates with mutation of a chloride channel called the cystic fibrosis transmembrane conductance regulator.[1] The essential functions they perform in the cell makes proteins essential drug targets for modern bio-medical applications. An important example here is the programmed-death ligand-1 (PD-L1), which is a valuable target for modern immunotherapy.[2-4] Predicting how a protein responds to a drug molecule, or using the protein as inspiration for bio-technological applications, require knowledge of how that protein works. As proteins are dynamic entities and protein dynamics are essential for function,[5-8] describing the mechanism of action of a protein requires knowledge about the protein motions in fluid environments. Theoretical biophysics provides valuable tools to characterize protein reaction mechanisms and protein motions at the atomic level of detail.

This Habilitation Thesis presents research on using theoretical biophysics approaches to decipher how proteins work. The focus of the research is on membrane proteins and reactions that occur at lipid membrane interfaces. The central question I address is the role of dynamic hydrogen (H) bonds in protein function and membrane interactions. The methods used include quantum mechanical (QM) computations of small molecules, combined quantum mechanics/molecular mechanics (QM/MM) of chemical reactions in protein environments, classical mechanical computations of large protein and membrane systems, and bridging numerical simulations to bioinformatics. In my research group we developed algorithms to identify H-bond networks in proteins and membrane environments, and to characterize the dynamics of these networks. To extend the applicability of numerical computations to bio-systems that bind drug-like compounds, we derive parameters for a potential energy function widely used in the field. The main research topics and specific questions addressed are summarized below together with a discussion of the computational approaches used.

2. Main scientific questions and model systems

2.1 H-bond networks and proton transfers in membrane proteins

Proton transfer is a reaction fundamental to bioenergetics. Proton pumps translocate protons across cell membranes, generating proton electrochemical gradients that can then be used, e.g., for secondary active transport across membranes or for the functioning of ion channels.[9-12] By contrast to the pumps, proton channels mediate the passive flow of protons down the electrochemical gradient.

The transport of protons across the ~30Å distance of the hydrophobic region of the lipid membrane occurs stepwise, and it involves protein groups and H-bonded water molecules. This stepwise transfer of protons –i.e., the change in the protonation state of specific protein groups- couples to changes in protein conformational dynamics, and in the dynamics of the internal water molecules.

2.1.1 Microbial rhodopsins are membrane proteins that function as ion transporters or as sensory proteins. These proteins bind covalently a retinal chromophore via a protonated Schiff base located approximately at the center of the hydrophobic region of the protein (Figure 1A). Absorption of light by the retinal triggers a reaction cycle that involves changes in the protonation state of specific protein groups, and structural rearrangements.

Microbial rhodopsins are excellent model systems to investigate the general physical-chemical principles of the coupling between protonation dynamics, protein and water dynamics. Indeed, decades of experimental and theoretical studies of bacteriorhodopsin and, over more recent years, of an increasing number of microbial rhodopsins, provide the fundament we can build upon to address questions pertaining to structure-function-relationship in membrane proteins (for reviews and perspectives see, e.g., refs.[13-17]). Importantly, three-dimensional structures of a number of microbial rhodopsins have been solved –e.g., for the bacteriorhodopsin proton pump,[18-22] for the halorhodopsin proton pump,[23] for a sodium pump,[24-25] for channelrhodopsins,[26-27] and for sensory rhodopsins,[28-31] providing a detailed view of the architecture of microbial rhodopsins of different function. For theoretical biophysics, reliable three-dimensional structures of microbial rhodopsins are invaluable in that they serve as starting points for computations.

Perhaps the best-studied microbial rhodopsin is bacteriorhodopsin. Extensive experimental and theoretical studies led to a detailed description of the reaction cycle of bacteriorhodopsin (see, e.g., refs. [13-15, 32-43]). Briefly, the photoisomerization of the all-trans retinal to 13-cis triggers a reaction cycle during which five proton transfer steps result in the net transport of one proton from the cytoplasmic to the extracellular side of the membrane (Figure 1A). The first proton-transfer step is from the retinal Schiff base to D85.[44] A proton is

then released from an extracellular proton release group that includes E194, D204, and H-bonded water.[45-48] The retinal Schiff base reprotonates from D96, which in turn accepts a proton from the bulk –potentially with participation of other carboxylate groups at the cytoplasmic side of the protein;[49-50] the last proton transfer step is from D85 to the extracellular proton release group, and might involve transient protonation of D212.[51-52]

Discrete water molecules appear to participate in all proton transfer steps of bacteriorhodopsin, and the role of water in bacteriorhodopsin proton transfer has been studied extensively (see, e.g., refs.[15, 37, 47, 53-54]). Water molecules that H bond to the proton donor and/or acceptor impact the relative orientation and proton affinity of these groups,[53] and the path and energetics of the proton-transfer reaction;[54-55] internal H-bonded waters help store a proton at the extracellular proton release group,[56-58] and transfer a proton from the cytoplasmic D96 to the retinal Schiff base.[59-60] This highlights the importance of accurate information regarding the location of internal water molecules in a cellular ionic pump, and of how the location and dynamics of these waters respond to changes in protonation during the reaction cycle of the pump. Bacteriorhodopsin remains an excellent model system to probe the coupling between protein conformational dynamics and water dynamics, and findings from such studies on bacteriorhodopsin can inform research on much more complex membrane transporters.

2.1.2 Water and H-bond networks of the AHA2 plasma membrane proton pump. That absence of reliable information about the location and dynamics of internal waters makes it difficult to understand how a proton pump works is illustrated by, e.g., *the AHA2 plasma membrane proton pump* from *Arabidopsin thaliana* (Figure 1B). This proton pump is a member of the P-type ATPases, which are proteins that hydrolase adenosine triphosphate (ATP) to power vectorial ion transfer across cell membranes; the reaction cycle of these proteins involves a phosphorylated intermediate, hence their being P-type.

Although AHA2 has a more complex fold than the simpler bacteriorhodopsin proton pump (Figure 1), there are important similarities in the three-dimensional arrangement of groups important for proton transfer within the transmembrane domain:[10] In AHA2 the primary proton donor group, D684, H bonds to N106, and R655 is though to function as a counterion for D684;[10, 61] at the extracellular side of the pump, carboxylate groups such as D92 and D95 could participate in proton release.[10, 61] As the crystal structure of AHA2[61] and the newer structural model improved with a flexible fitting procedure and structural refinement[62] lack coordinates for internal water molecules, the path that could be followed by a proton across the ~18Å distance from D684 to the D92/D95 pair is unclear.

In principle, water molecules could be added to the protein structure by using protein hydration software such as DOWSER++.[63] A potential artifact arising from such an approach is description of internal water-mediated H bonds in intermediate states of the pump. As we had noted before in work on bacteriorhodopsin, the location of a internal water molecule along the reaction path of a pump depends not only on the free energy for inserting water molecule at a particular site, but also on the free energy barrier associated with the relocation of the water to that site.[54] That is, internal water location and dynamics are part of the reaction coordinate of the pump, and setting a priori coordinates for internal waters may provide a biased description of the reaction coordinate of the pump.

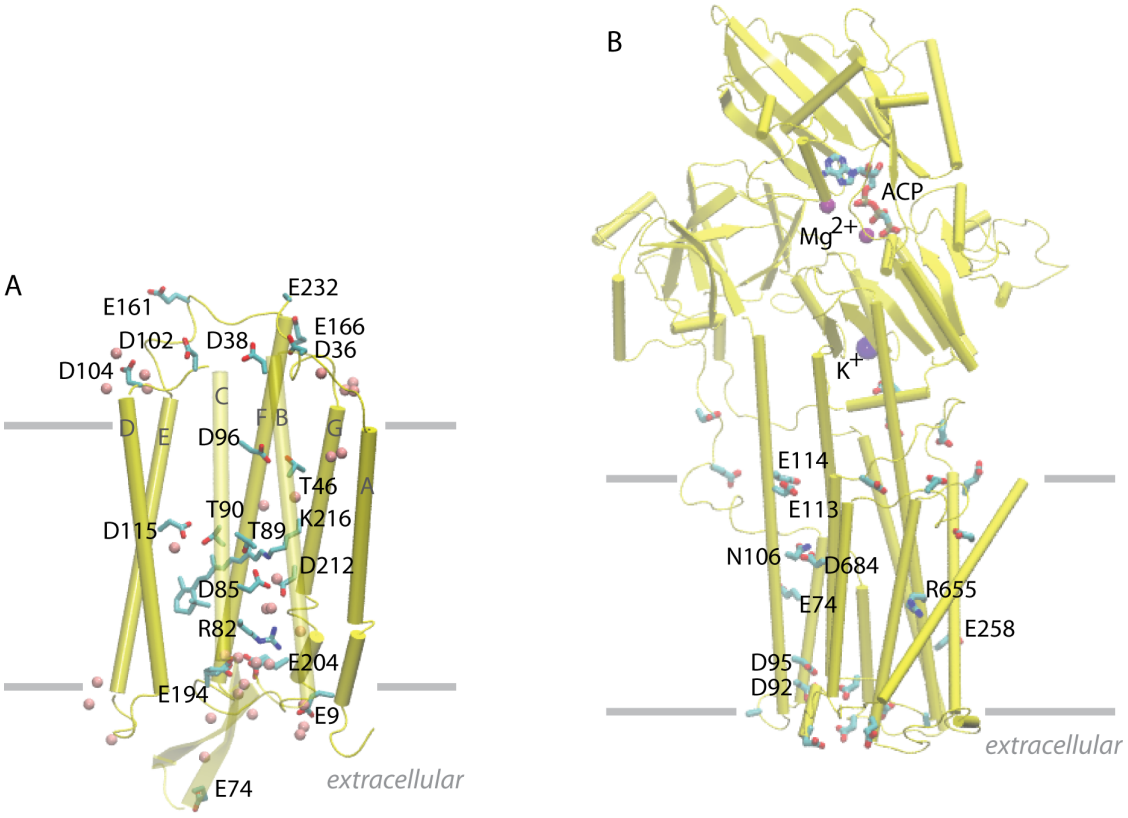

Figure 1. Architecture of proton pumps. The proteins are shown as cartoons and selected protein groups as bonds with carbon atoms colored cyan, nitrogen, blue, and oxygen, red. Water molecules are shown as small pink spheres. (A) The bacteriorhodopsin proton pump. The transmembrane (TM) helices of bacteriorhodopsin are labeled with letters A to G. For clarity, helices B and C are shown as transparent cartoons. The molecular graphics is based on the crystal structure PDB ID:5ZIM solved at 1.3Å resolution.[22] (B) The AHA2 plasma membrane proton pump. The central proton donor/acceptor group is located within the TM region of the protein. The carboxylate groups shown have at least one atom within 30Å from D684. Mg^{2+} and K^{+} ions are shown as van der Waals spheres colored purple and violet, respectively. ACP indicates phosphomethylphosphonic acid adenlyate ester.[64] The molecular graphics is based on chain A of the crystal structure PDB ID:5KSD solved at 3.5Å resolution.[62] The distance between the Cγ atoms of D95 and D684 is 17.5Å. Unless specified otherwise, molecular graphics were prepared using Visual Molecular Dynamics, VMD.[65]

One possible solution to the problem of finding water-mediated paths that could connect transiently proton donor and acceptor groups is to pursue atomistic molecular dynamics (MD) simulations of the protein in a hydrated lipid membrane environment, and monitor the simulations to observe whether water molecules visit the inter-helical region of the protein. Moreover, to derive a glimpse into how the protein and water dynamics could respond to changes in the protonation state, MD simulations of a transporter could be performed with different protonation states. Limitations to this approach include the timescale that can be simulated with current atomistic simulations, which might be insufficient to sample correctly the protein and water dynamics, and a dependence of the protein and water dynamics on protonation states chosen for the computations.

2.1.3 Retinal-protein coupling and role of water in Jumping Spider Rhodopsin-1

Jumping Spider Rhodopsin-1 (JSR-1**)** is a compelling example of a protein in which discrete internal waters are likely essential for function, and for which computations serve as valuable tool to understand how waters impact protein function. The crystal structure of JSR-1[66] indicates a water molecule that bridges a Glu and a Ser sidechains, both of which engage in additional H bonding, such that the Schiff base of the 9-*cis* retinal is part of a network of H bonds that includes the water molecule and 5 protein sidechains (Figure 2B). The H-bond network and hydrophobic packing make the binding pocket of the retinal appear as rather tight (Figure 2A). Close interactions between protein and retinal might be important to couple the geometry of the retinal to protein conformational changes –as required for the functioning of JSR-1 as a G Protein Coupled Receptor (GPCR).

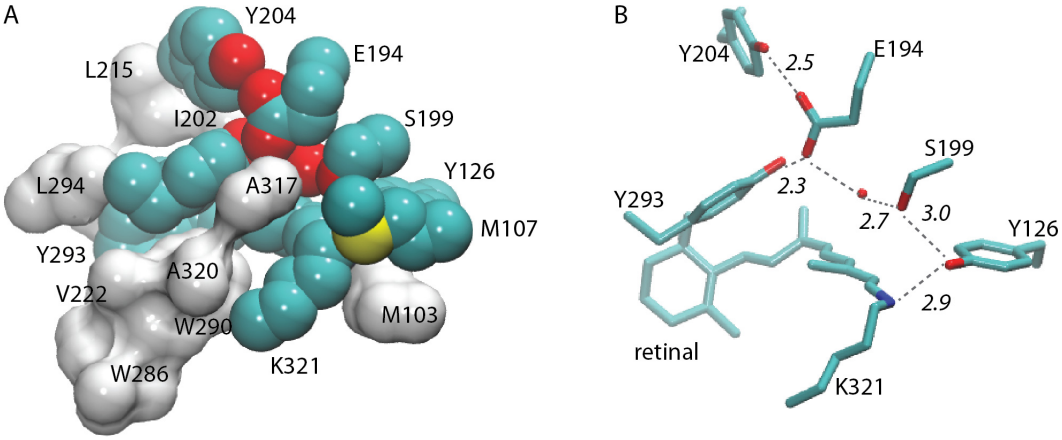

Figure 2. H bonding and hydrophobic packing at the retinal binding pocket of JSR-1. (A) Tight packing of 9-cis retinal in the crystal structure. The retinal molecule bound to K321 and protein sidechains of the H-bond network, together with the active-site water molecule, are depicted as van der Waals spheres, and hydrophobic siedchains within 5Å of the retinal chain are shown as a gray surface. (B) H-bond network of the retinal Schiff base. The dotted lines indicate H bonding, and numbers in italics indicate distances in Ångstrom. The images are based on PDB ID: 6I9K.[66]

H bonding between the protonated Schiff base and Y126 might contribute to a key feature of the reaction cycle of JSR-1:[66] the retinal Schiff base remains deprotonated during the reaction cycle, and retinal re-isomerizes from all-trans back to 11-cis by absorbing a second proton. This distinguishes JSR-1 from bovine rhodopsin, whose reaction cycle involves Schiff base deprotonation and hydrolysis.[66-67]

Computer simulations can help deciphering the role of water in the H-bond network proposed to stabilize a protonated retinal Schiff base. A first question that needs to be addressed is whether the water molecule and the H-bond network the water is part of are present in resting-state JSR-1 bound to 11-cis retinal, and in JSR-1 with isomerized all-trans retinal. To address this question, atomistic MD simulations can be used to model these two JSR-1 intermediate states, and to probe their dynamics at room temperature. Once models of JSR-1 with 11-cis vs. all-trans retinal have been derived, QM/MM computations can be used to compute reaction coordinates for proton transfer.

2.1.4 Water-mediated H-bonding in ion pumps: a molecular picture of functional interconversions in microbial rhodopsins. To accomplish orchestrated transfers of protons across the membrane, proton pumps rely on specific three-dimensional arrangements of titratable protein groups and discrete protein-bound waters. Understanding which specific structural motifs are essential for proton pumps vs. other ion pumps could serve as basis to alter the functionality of the pump. Prominent examples include the conversion of the bacteriorhodopsin proton pump into a chloride pump accomplished via the single amino acid mutation of D85 into Thr,[68] and into a sensory receptor via the mutation of 3 amino acid residues.[69]

More recently, *Krokinobacter eikastus* rhodopsin 2, KR2, could be converted into a proton pump via mutation of 4 amino acid residues as in the *Gloeobacter* rhodopsin (GR) proton pump;[70] the reverse mutation of GR into a sodium pump could not be achieved, being suggested that the two pumps have as common ancestor a proton pump, and that functional convergence could be achieved only when mutations reverse changes occurred during evolution.[70]

A difficulty with predicting mutations based on sequence analyses and crystal structures is that the response of a H-bond network to mutations can be difficult to predict. Computer simulations and thorough analyses of the dynamics of internal H-bond networks in wild-type vs. mutant proteins can be used to probe and short-list mutations for further study with experiments, and to provide a molecular interpretation of the mutations.

2.2 H-bond networks for proton transfer in photosystem II

Photosystem II is a membrane-embedded macro-molecular complex composed of multiple protein subunits, cofactor molecules, and special lipids (Figure 3), that uses the energy of light to oxidize water, catalyzing a chemical reaction fundamental for life: photosystem II splits two water molecules into molecular oxygen, protons, and electrons. As photosystem II could be coupled to a hydrogenase to generate molecular hydrogen (H_2) in an artificial environment,[71] and photosystem II is also a target for some of the herbicides,[72] understanding the reaction mechanism of photosystem II could potentially facilitate applications for biotechnology.

I focus on understanding how protons that are generated during water splitting at the oxygen evolving complex (OEC, Figure 3) transfer to the bulk lumen. This is a challenging aspect of the functioning of photosystem II, because proton transfer appears to involve long distances, and the reaction coordinate for proton transfer is largely unclear.

Analyses of crystal structures of photosystem II, spectroscopy, and theoretical studies have provided valuable information about putative pathways for proton transfer in photosystem II (see, e.g., refs.[73-76]); particularly important are thought to be groups such as D61 and E65 of subunit D1 and E312 of subunit D2, and H-bonded water molecules. Fourier Transform Infrared (FTIR) difference spectroscopy indicated that specific transitions between intermediate states of photosystem II associate with changes in the pKa or in the environment of three distinct carboxylate groups. [77-78] Recently, it was inferred that proton release might involve storage as 'a protonated Eigen cation'.[79]

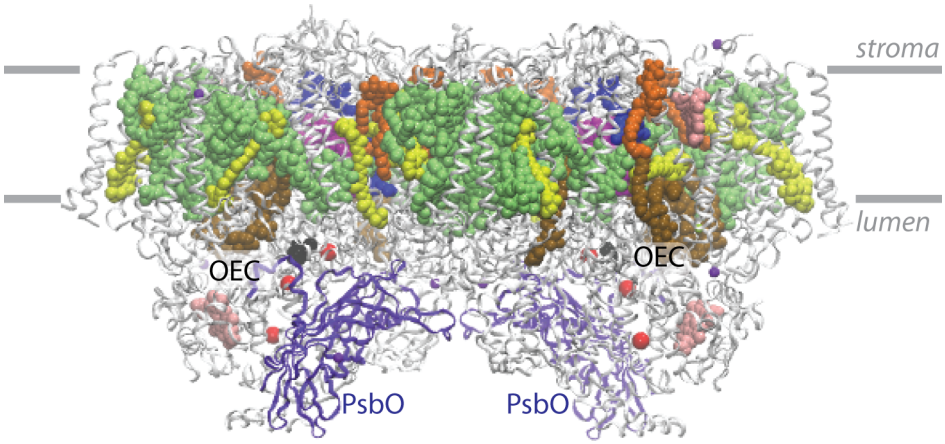

Figure 3. Architecture of the photosystem II dimer. The image is based on the X-ray free-electron laser structure PDB ID: 6JLJ. The protein subunits are shown as ribbons colored violet for the PsbO subunit, and white for all other subunits. The OEX is shown as van der Waals spheres colored black, sulfoquinovosyldiacylglycerol, dark orange, chlorophyll *a*, lime, beta carotene, dark yellow, heme, pink, plastoquinone 9, blue, pheophytin *a*, magenta, digalactosyl diacyl glycerol, ochre, Cl^- ions, red, Ca^{2+}, violet, and Mg^{2+}, yellow.

Given the complexity of the system, an important question is how protein and water dynamics enable transient sampling of proton-transfer paths at room temperature, and how changes in the protonation state of carboxylate groups involved in proton transfer might couple to changes in protein and water dynamics.

Atomistic simulations are a valuable approach to study dynamic H-bond networks of photosystem II. Challenges with such simulations include the size of the system, which can make it difficult to sample the protein motions for long times, the presence of cofactor molecules and special lipids, which might lack accurate representations in atomistic force fields, and the large number of H bonds whose dynamics need to be analyzed.

2.3 The PsbO subunit of photosystem II as a putative proton antenna

PsbO is an extrinsic subunit of photosystem II also denoted as the 33kDa protein and as the manganese stabilizing protein[80-82] – a name that indicates an essential functional role of PsbO in the structural stability of the manganese cluster.[83] In the crystal structure of the photosystem II complex PsbO has a β-barrel core and long loops (Figure 4); this is compatible with NMR data on free PsbO indicating that, in solution, the protein has a folded core and flexible loops whose motions might become restricted upon binding to the complex.[84] Binding of PsbO to photosystem II occurs late in the assembly of the complex,[85] and it requires two conserved Arg groups (R151 and R161 in spinach photosystem II) whose mutation to Gly lowers not only the affinity for the binding of PsbO to photosystem II, but also impair chloride binding to the OEC and the oxygen evolution activity, which in these mutants is as low as 20% (R151G) and 40% (R161G) of wild-type photosystem II.[82]

In addition to contributing to the integrity of the manganese cluster, PsbO might directly participate in the functioning of photosystem II by contributing carboxylate groups such as PsbO-D224[75] to an extended H-bond network that could conduct protons from the interior of the complex to the bulk lumen. D224 is part of a cluster of carboxylate groups on the surface of PsbO hypothesized to function as a proton antenna.[86] Proton antennas have been discussed, e.g., for bacteriorhodopsin[49-50, 87] and for cytochrome *c* oxidase.[87-88] Proton antennas consist of clusters of carboxylate groups, possibly also with histidine groups, and their role is to increase the time that a proton spends on the surface of a protein.[87, 89]

The PsbO antenna cluster suggested for *T. elongatus* photosystem II includes 16 carboxylate groups, including D158 and D224[86] that are close to the two essential Arg groups (Figure 4). But the carboxylate groups of the antenna cluster are only about half of the PsbO Asp and Glu groups: in the case of photosystem II from *T. vulcanus*, for example,

the PsbO protein chain solved in a recent crystal structure contains no fewer than 35 Asp and Glu groups (PDB ID:6JLJ,[79] Figure 4), some of which are at the interface between PsbO, PsbU and CP43 (Figure 4).

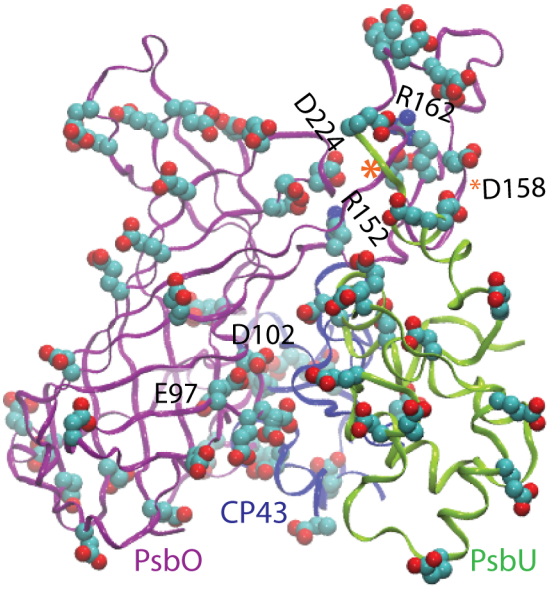

Figure 4. The carboxylate groups of PsbO and the charged interface between PsbO, PsbU, and CP43. The molecular graphics is based on PDB ID:6JLJ. For clarity, only a fragment of CP43, which is within 15Å of PsbO, is shown as blue ribbons, and only selected PsbO groups are labeled. Sidechains of the Asp and Glu groups of PsbO, PsbU, and of the CP43 fragment, are depicted as van der Waals spheres. The orange star indicates the location of PsbO-D158. E97 and D102 are within 2.6Å distance.

In the hypothesis that PsbO functions as a proton antenna, key questions include how conserved are the carboxylate groups of the putative proton antenna, and whether the pH at which the organism lives could determine the size and spatial distribution of the cluster. Given the significant spatial extent of the putative antenna cluster, it is unclear whether all these carboxylates would be directly involved in proton binding, or whether protons are more likely to bind at a particular site of the cluster. Transfer of protons between carboxylate groups of the antenna cluster could associate with changes in the local structure, dynamics, and water interactions of the protein; at the interface between PsbO, PsbU and CP43, changes in protonation state could even associate with altered protein dynamics. These aspects would need to be addressed via experiments and computations that together could provide a detailed description of the mechanism by which PsbO participates in proton transfer of photosystem II.

2.4 The bacterial *Sec* protein secretion path and conformational dynamics of the SecA protein motor

The *Sec* secretion path is a major protein biosynthesis path, and mutations in genes that encode for *Sec* proteins have been linked to human disease.[90]

In bacterial protein secretion, the cytosolic SecA protein motor (Figure 5A) couples the binding and hydrolysis of adenosine triphosphate (ATP) to the binding and stepwise translocation of secretory pre-proteins through the membrane embedded SecYEG protein

translocon (Figure 5B). SecA recognizes the signal sequence of the newly synthesized protein –these are N-terminal sequences of variable length and amino acid composition that have three regions, a positively-charged N terminus, an intermediate hydrophobic stretch, and a polar C-region.[91-92]

Extensive experimental and computational studies (see, e.g., refs.[93-96]) together with structural biology of individual components (e.g., refs.[97-102]) and of protein complexes[103-104] have provided invaluable insight into how *Sec* protein secretion works. The highly complex reaction coordinate of the bacterial Sec protein secretion, which involves protein conformational dynamics, interactions between protein, lipids, and water, and electronic structure changes during the catalytic cleavage of ATP, remains, however, incomplete. A fundamental open question is how electronic structure changes during ATP cleavage couple to conformational changes of SecA and protein secretion through the translocon.

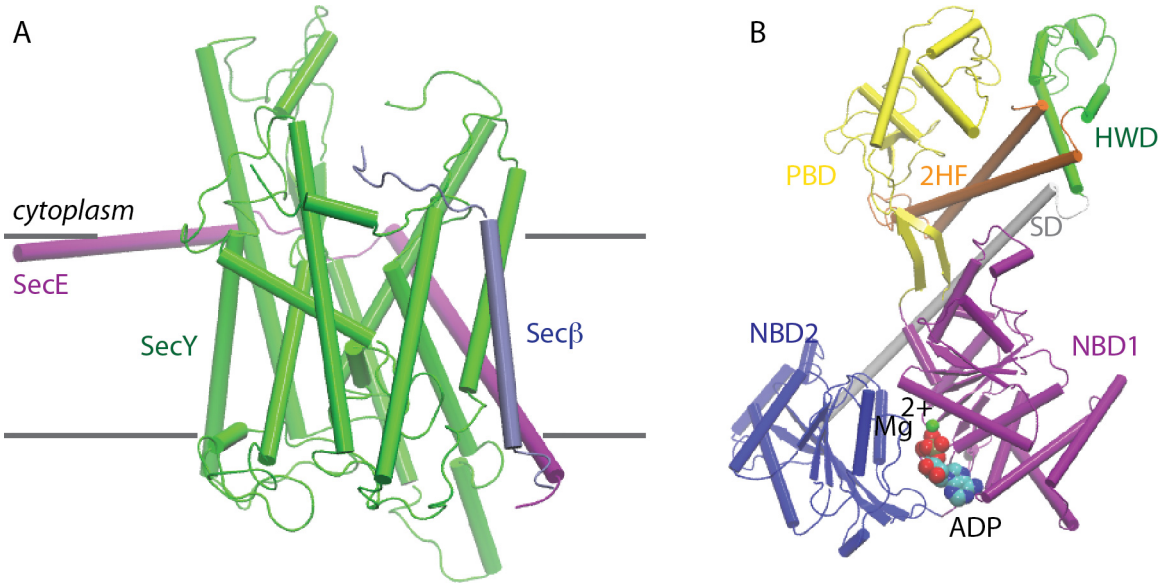

Figure 5. Architecture of the protein translocon and of the bacterial protein motor. (A) The SecYEβ translocon from the archaeon *Methanocaldococcus jannaschii*, PDB ID:1RHZ.[105] The three protein chains are shown as cartoons. (B) The functional domains of the SecA protein motor, based on the crystal structure PDB ID:1M74 of ADP-bound *B. subtilis* SecA.[106] The nucleotide and the Mg^{2+} ion are bound at the interface between nucleotide binding domains NBD1 and NBD2. The two ends of the protein binding domain (PBD) are linked to NBD1. The other three functional domains of SecA are the scaffold domain (SF), the helical wing domain (HWD), and the two-helix finger domain (2HF).

Understanding the reaction coordinate of conformation-coupled ATP hydrolysis of SecA would require description of the sequence of structural rearrangements and associated free energy profile along the path. Such a description could be derived, e.g., by using QM/MM computations. Once QM/MM reaction paths of the reaction coordinate have been computed, they could be dissected to find out why the release of ADP is rate limiting.[107]

Computations of the reaction coordinate of SecA are challenging due to the size and complex interactions of the protein. Yet another challenge is how to derive accurate structural models of ATP-bound SecA, which are required as starting point for the computations: Whereas the structure of ADP-bound SecA has been well characterized with X-ray crystallography (e.g., Figure 5B), the three-dimensional structure of ATP-bound SecA is somewhat poorly characterized. A crystal structure of ATP-bound SecA has been presented for *E. coli* SecA.[100] Difficulties with this structure include lack of coordinates for the active-site Mg^{2+} ion, and for a large part of the PBD. A structure of *E. coli* SecA with the PBD and bound to a signal peptide model, but without nucleotide, was solved with NMR.[99] To characterize the reaction coordinate of SecA, one possible solution is to start from a crystal structure of, e.g., ADP-bound *B. subtilis* SecA, study the protein conformational dynamics and the dynamics of nucleotide binding, and dock ATP to *B. subtilis* SecA to derive a structural model with which ATP hydrolysis can then be computed.

2.5 Protein binding at lipid membrane interfaces

Understanding how membrane proteins acquire their fold is a fundamental question in biophysics. An intriguing model system to study how proteins interact with membranes is Mistic (membrane-integrating sequence for translation of IM protein constructs', where 'IM' indicates integral proteins[108]). Mistic is a small protein, 110-amino acid residues long, that associates with the *E. coli* membrane[108] even though it contains numerous charged and polar groups (Figure 6). Mistic is thought to fold without the help of the *Sec* translocon machinery, and it is useful for the expression of other membrane proteins in *E. coli*.[108] In its native *B. subtilis* cells, the gene encoding Mistic, mstX, might be involved in biofilm production.[109-110]

The NMR structure of Mistic solubilized in the detergent lauryl dimethylamine oxide, LDAO[108] contains 4 relatively short helical segments – though it was noted that ends of the helices might partially unravel in detergent (Figure 6, PDB ID: 1YGM).[108] In this detergent, circular dichroism measurements indicated that ~60% of Mistic is α-helical;[111] helices 3 and 4 are though to form secondary structure first, with subsequent packing of helices 1 and 2.[111] In early atomistic simulations of Mistic in a LDAO micelle, helix 4 displaced relative to the other three helical segments.[112] A limitation of these simulations might have been the description of the non-bonded interactions between protein and detergent, as it is unclear whether deriving HF/6-31G* partial charges for LDAO was sufficient for reliable description of protein-detergent-water interactions.

The conformational dynamics of Mistic was found to depend on physical-chemical properties of the detergent micelle, with a more compact, but conformationally dynamic

protein, in a zwitterionic detergent, and structure heterogeneity in a nonionic detergent.[113] Likewise, dimerization of Mistic depends on the lipid membrane environment.[114]

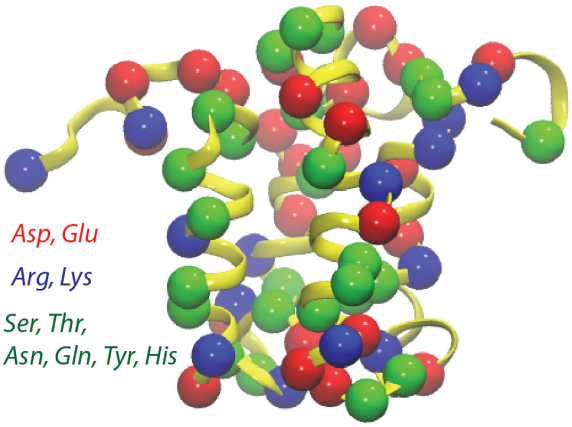

Figure 6. Architecture and charge distribution of Mistic. The protein is depicted as yellow ribbons. The colored spheres are van der Waals spheres for the Ca atoms of Asp and Glu (red), Arg and Lys (blue), and Ser; The, Asn, Glrn, Tyr, and His groups (green). Note that the protein is loaded with charged and polar groups, which raise the question of how it bonds at the lipid membrane interface. The image is based on a coordinate set from the NMR ensemble PDB ID:1YGM.

The Mistic protein chain solved with NMR contains no fewer than 25 Asp and Glu groups, i.e., ~22% of the protein chain is acidic; when considering the Asp/Glu and Arg/Lys sidechains in their standard protonation states, the net charge of this protein chain is -11e. In a sequence conservation analysis based on a small set of Mistic proteins from 4 *Bacillus* species, it was noted that the distribution of acidic amino acid residues tends to be conserved.[115]

How a protein as hydrophilic as Mistic associates with the membrane is difficult to envision, and its was hypothesized that binding to the membrane likely involves conformational changes of the protein.[115] Both the *E. coli* and the *B. subtilis* membranes contain negatively charged lipids,[116-118] which raises the question as to whether conformational changes of Mistic might be required to shield Asp/Glu sidechains from negatively-charged lipids of the membrane. The negatively-charged lipids of the bilayer could also serve as anchors for the protein, as they could H bond to Arg/Lys sidechains. The potential role of lipid-protein H bonds in the binding of Mistic at lipid membrane interfaces highlights the importance of atomistic computer simulations to derive a molecular picture of Mistic-membrane complexes.

2.6 Role of lipids in membrane protein function: the GlpG rhomboid protease

The lipid bilayer can impact significantly the reaction cycles of membrane-embedded proteins. A prominent example here is the visual rhodopsin G Protein Coupled Receptor (GPCR), for which experiments demonstrated that phosphatidylethanolamine (PE) lipids favor the transition from the intermediate state metarhodopsin I to metarhodopsin II.[119] H bonds between tyrosine groups and lipids were observed in an early crystal structure of bacteriorhodopsin;[120] squalene and phosphatidyl glycerophsophate methyl ester, two native

lipids of the purple membrane, appear required for a normal reaction cycle of bacteriorhodopsin.[121]

Intriguing observations about the role of lipids in membrane protein function were made for rhomboid proteases –these are proteases that are embedded in the membrane, where they cleave other transmembrane substrates and in doing so release molecules that participate in, e.g., cell signaling. The cleavage activity of rhomboid proteases from different organisms was tested against a model transmembrane substrate, using different lipid or detergent environments. One of the best studied rhomboid proteases, the GlpG protease from *Escherichia coli* (Figure 7), cleaved substrate in PE lipids and in detergent, but had poor activity in, e.g., PC, PG, or *E. coli* lipids;[122] YqgP, the rhomboid protease from *Bacillus subtilis*, cleaved substrate in lipids such as PG and PC, but showed poor activity in PE.[122] That is, catalytic activity by rhomboid proteases depends on lipids, and rhomboids from different organisms respond differently to a particular lipid.

That lipids impact enzymatic activity was also observed for the functional domain of signal peptide peptidase,[123] and for γ-secretase,[124] the protein complex whose cleavage of the amyloid precursor protein was implicated in Alzheimer's disease.[125]

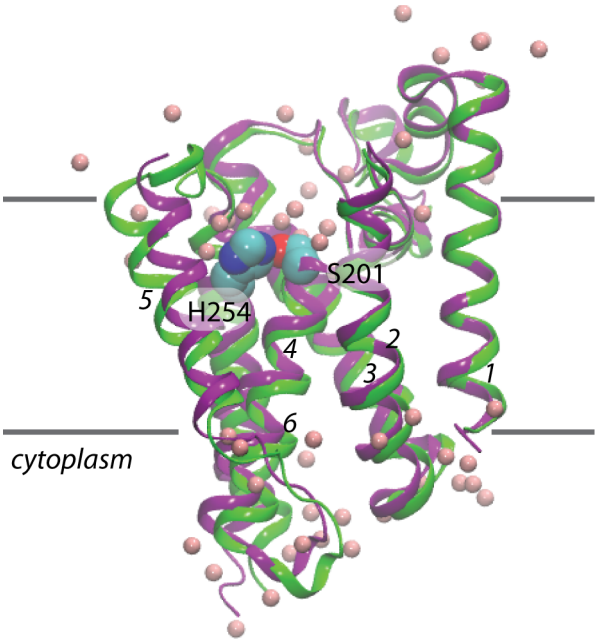

Figure 7. Architecture of the GlpG rhomboid protease. The molecular graphics is based on PDB ID:2IRV with chains A and B depicted as purple and green ribbons, respectively. The catalytic groups S201 and H254 are shown as van der Waals spheres in atom colors. Water molecules solved for chain B are shown as small pink spheres. Note the different orientation of helix 5 in the two protein structures. For simplicity, a lipid headgroup that is bound at the active site is not shown.

Our earlier atomistic simulations of GlpG embedded in hydrated lipid bilayers provided insights into how lipids might impact the functioning of the protease.[126] We found that lipid bilayers composed of 1-palmytoyl-2-oleoyl-*sn*-glycero-3 phosphatidylcoline (POPC) or 1-palmytoyl-2-oleoyl-*sn*-glycero-3 phosphatidylethanolamine (POPE) thin close to the protease to adjust to the size and shape of the protein, and that this thinning, measured as the difference between the bilayer thickness far away and close to the protease, is about 3.8-4.4Å.[126] Thinning of the membrane close to the protease is likely important for the

functioning of the protease, because it could influence the local structure and dynamics of the substrate as it encounters the protease.[126-127]

Substrate docking by GlpG is thought to involve lateral movement of transmembrane helix 5[128] and motions of loops 4[129] and 5[130-131] (Figure 7, based on PDB ID:2IRV[132]). As lateral movement of helix 5 would occur in the membrane, motions of the helix are likely to couple to changes in the local dynamics of the lipids, e.g., the local membrane thinning could change during the reaction cycle of GlpG. Knowledge of the reaction coordinate of substrate binding and cleavage by GlpG thus requires description of the lipid-protein interactions for different conformations of the protein.

2.7 Overview of main research program

I work on understanding mechanisms of reactions at lipid membrane interfaces. I am particularly interested in proteins whose functioning involves proton transfers, and proteins that couple to lipids. A unifying question concerns the role of dynamic H-bond networks in protein conformational dynamics and protein function. The bio-molecules used as model systems are representative to classes of proteins and reaction mechanisms.

The reaction coordinate of an enzyme that catalyzes a chemical reaction involves changes in electronic structure associated with bond breaking and forming, dynamics of the protein environment, interactions with the solvent –aqueous solution for a soluble protein, hydrated lipid membrane environment for a membrane protein. Describing such complex reaction coordinates requires advanced computational techniques to model reaction intermediates and probe their dynamics in relevant environments, compute energy profiles for the reaction, probe the response of the protein environment to changes in protonation or mutation. For proteins that bind cofactor molecules, computational studies of protein dynamics might first require tests for the reliability of existing force-field parameters and even computations to derive such parameters. Moreover, numerical simulations of large biomolecules in hydrated lipid environments would generate large amounts of data whose analyses require efficient algorithms.

In what follows I introduce methods used for computations, and summarize main results from our research.

3. Theoretical biophysics approach

3.1 Classical all-atom MD simulations of membrane proteins

Membrane proteins are embedded in lipid membranes that surround biological cells and compartments of these cells, and the communication with lipids is essential for how the protein works. That lipids impact various aspects of membrane protein function has been observed, e.g., for bovine rhodopsin,[119] rhomboid proteases,[122] γ-secretase,[124] and the multidrug transporter LmrP.[133] Classical mechanical MD simulations are a valuable approach to dissect mechanisms by which lipids can impact membrane protein function, because with this approach we can study the motions of the protein in a hydrated lipid environment, generate a MD trajectory that describes the time evolution of each atom of the simulation system, and then use that trajectory to calculate various parameters to characterize the system.

Classical mechanical MD simulations rely on a potential energy function (force field) to compute interactions between atoms of the system. All classical mechanical simulations discussed here were performed with the CHARMM potential energy function (Chemistry at Harvard Molecular Mechanics),[134] which is a sum of energy terms for bonded and non-bonded interactions,

$$V(\boldsymbol{r}) = \sum_{bonds} k_b(b - b_0)^2 + \sum_{angles} k_\theta(\theta - \theta_0)^2 + \sum_{dihedrals} k_\chi\big(1 + cos(n\chi - \delta)\big) + \sum_{impropers} k_\omega(\omega - \omega_0)^2 + \sum_{UB} k_{UB}(d - d_0)^2 + \sum_{i,j}\left(\epsilon_{ij}\left[\left(\frac{R_{ij}}{r_{ij}}\right)^{12} - \left(\frac{R_{ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{D r_{ij}}\right) + E_{CMAP} \quad (1)$$

where b_0, θ_0 and ω_0 are reference values for covalent bond lengths, valence angles, and improper angles, 1-3 Urey-Bradley (UB) interactions, k_b, k_θ, k_χ k_ω, and k_{UP} are force constants, χ - dihedral angles, δ – phase, n – multiplicity of the dihedral angle, r_{ij} is the distance between atoms i and j, ε_{ij} and R_{ij} are the Lennard Jones well depth and distance at minimum, q_i is the partial atomic charge of atom i, and D = 1 is the permittivity of vacuum. E_{CMAP} is an energy correction map term.[135]

The force constants, reference values, partial atomic charges, Lennard Jones distances and well depths, are collectively denoted as force field parameters. The classical mechanical computations reported here were performed with CHARMM force field parameters for proteins,[135-136] lipid[137-138] and nucleotide molecules,[139] and ions,[140] and with the TIP3P water model.[141]

To perform MD simulations of a membrane protein, the structure of the membrane protein –which is known from structural biology or derived with homology modeling, is first oriented in the membrane, and then embedded in a hydrated lipid membrane patch. Convenient platforms for orienting membrane proteins and embedding them in hydrated lipid membranes are the web-based tools Orientations of Proteins in Membranes (OPM)[142] and CHARMM-Graphical User Interface (CHARMM-GUI).[143-144] Ions are added for charge neutrality and/or to study the membrane system at a specific ion concentration in the bulk.

Protonation states of the titratable amino acid residues are chosen at the start of the MD simulation; during the course of a regular MD simulation with standard classical mechanics force fields, the protonation states remain unchanged. To choose protonation states, we rely on information from experiments –e.g., from Fourier Transform Infrared (FTIR) spectroscopy, and on careful inspection of hydrogen bonds in the crystal structure of the protein studied. We have also probed the likely protonation state and the response of the protein to changes in protonation by performing independent MD simulations with different protonation states of the protein.

The size of simulation systems consisting of the membrane protein in a hydrated lipid membrane patch depends on how many lipids are included in the membrane, and on the size and shape of the membrane protein –a membrane protein that has large solvent-exposed domains would also need a relatively large number of water molecules in the bulk. A typical size of the simulation systems discussed in this thesis is ~150.000-200.000 atoms.

The simulation system is then heated and equilibrated at room temperature, and the MD simulation prolonged as required to sample motions of interest to the question addressed. The MD simulation will generate a simulation trajectory that contains the coordinates of each atom of the system as a function of the simulation time. These coordinates are used to extract from the MD data to characterize the molecular system studied.

The lipid membrane composition of the lipid bilayer in which the membrane protein is embedded is set during the initial system setup. A very convenient platform for selecting a specific lipid membrane composition is that of CHARMM-Graphical User Interface (CHARMM-GUI).[143-145]

A typical lipid bilayer used in MD simulations of membrane proteins is 1-palmytoyol-2-oleoyl-sn-glycero-3-phosphatidylcholine, POPC. For bacterial proteins, bilayers composed of 1-palmytoyol-2-oleoyl-sn-glycero-3-phosphatidylethanolamine, POPE, are often used.

3.2. QM/MM computations of chemical reactions in protein environments

Chemical reactions such as proton transfer involve breaking and forming of covalent bonds; such reactions need to be described with quantum mechanical (QM) methods. For proton transfer reactions between donor and acceptor groups that are part of a protein chain, the protein environment needs to be accounted for, because motions and electrostatic interactions of the protein environment can influence significantly the energetics of proton transfer.[55, 146-147] Given the size of a membrane protein, its description with QM is not feasible. Instead, combined QM/MM approaches can be used, whereby only the reaction site is described with QM, the protein environment is described with MM, and the QM and MM regions interact via bonded and non-bonded. Thus, the total energy *E* of a QM/MM system has three terms,

$$E = E_{QM} + E_{MM} + E_{QM-MM} \quad (2)$$

where E_{QM}, the energy for the QM part of the system, is computed according to the QM treatment used, and E_{MM}, the energy for the MM part of the system, is computed according to the MM force field used. The QM/MM computations discussed here were performed with the approximate QM method Self Consistent Charge Density Functional Tight Binding, SCC-DFTB,[148] as implemented in CHARMM.[149] In this implementation E_{QM-MM}, which describes interactions between the QM and MM parts of the system, includes bonded interactions for atoms at the frontier between the QM and MM regions, and non-bonded interactions –van der Waals and Coulomb interactions; the latter interactions are computed between MM atomic partial charges as set in the force field, and atomic partial charges of the QM region derived with Mulliken analyses.[149] The empty valence at the QM frontier atom is treated, e.g., with a hydrogen link atom.[150-151]

3.3 Reaction path computations

Chemical reactions such as proton transfer or retinal isomerization can have significant energy barriers that would be difficult to overcome with regular MD simulations, particularly since bond breaking and forming would require a description with QM.

A relatively simple approach to derive reaction coordinates is coordinate driving, or adiabatic mapping. This method entails the *a priori* choice of a reaction coordinate, and sampling along this reaction coordinate by taking small increments; an energy optimization of the remaining degrees of freedom is performed for each value of the reaction coordinate. The resulting path is thus a minimum energy path. In the case of the isomerization of a retinal bond, for example, a simple choice of the reaction coordinate could be the dihedral angle whose value changes during isomerization. For proton transfer, a simple choice of the

reaction coordinate could be the relative distance between the proton and the donor heavy atom, vs. the distance between the proton and the acceptor heavy atom.

As we have noted in earlier work on bacteriorhodopsin, the a priori choice of a reaction coordinate can lead to discontinuities in the energy profile, and in the structural change along the reaction coordinate for groups that were not included in the definition of the reaction coordinate.[55] A solution to this problem is to compute minimum energy paths using the Conjugate Peak Refinement algorithm, CPR,[152] which starts from energy-minimized structures of the reactant and product states of the reaction to construct a path whose energy maxima are first-order saddle points.

3.4 Force field parameters for cofactor and drug-like molecules: assessing accuracy and deriving force-field parameters

Membrane proteins and membrane protein complexes can bind cofactor molecules, special lipids, or drug molecules. When such binding takes place, it is often of central interest to the understanding of protein function. Important examples here are the family of retinal proteins, the G Protein Coupled Receptors (GPCRs), and photosystem II. The retinal chromophore bound covalently to retinal proteins photo-isomerizes upon absorption of light, which triggers a reaction cycle that results in transport of ions or signal transduction.[153] The membrane embedded GPCRs bind a wide range of drug molecules, and thus are key targets for drug design.[154] In the case of photosystem II, several cofactor molecules are bound to protein subunits –including the manganese cluster where catalytic splitting of water molecules takes place.[155]

Numerical simulations of proteins that bind cofactor molecules can be challenging when these molecules are absent from the set of force-field parameters used for the protein and membrane atoms. Moreover, cofactor molecules might have complex electronic structure difficult to represent with a classical mechanical force field, and limitations in the force-field description of cofactor molecules can impact significantly protein conformational dynamics: Numerical simulations of squid rhodopsin performed with two different sets of retinal parameters indicated that the dynamics of internal water molecules depends on how retinal is described.[156]

When deriving force-field parameters for cofactor and other drug-like molecules, a key aspect is to ensure that these parameters are compatible with the force-field representation used for other components of the molecular system of interest –i.e., with how the protein, lipids, and water are represented in the force field.

The protocol for deriving CHARMM force-field parameters for drug-like molecules, CHARMM General Force Field (CGenFF), consists of an iterative procedure whereby

computations with QM and MM are performed to optimize the partial atomic charges of the compound of interest, bonded degrees of freedom and, where needed, Lennard-Jones parameters.[157] Optimization of the atomic partial charges involves geometry optimization of the compound with MP2, computations of water interaction energies and distances with Hartree-Fock (HF)/6-31G*, and adjustment of the MM partial atomic charges to fit the MM water interaction energies and distances to the QM target values; to ensure usefulness of the parameters for the bulk phase, in the case of neutral polar compounds water interaction energies are scaled, and distances are offset.[157] Parametrizing bonded interactions uses relaxed potential energy scans and vibrational frequencies for force constants.

3.5 Analyses of dynamic carboxylate-water interactions

Membrane-embedded proton transfer proteins often expose to the bulk clusters of carboxylate groups. Proton antennas have been discussed, e.g., for bacteriorhodopsin,[87] cytochrome c oxidase,[87] and the PsbO subunit of photosystem II.[86] A proton antenna has a size of ~10Å, and it consists of carboxylate groups that can bind a proton collectively and increase the dwell time for the proton.[87, 89]

When studying proton transfer at proton antennas, it is particularly important to understand how potential proton-binding sites interact with water molecules: H bonding water molecules bridging these sites can mediate proton transfers.[158] The need to address water interactions at carboxylate clusters brings about the challenge of how to accurately sample the conformational dynamics of the protein and the dynamics of the water molecules. Timescales relevant to protein dynamics range from fast fluctuations on the picosecond-nanosecond timescale to large structural rearrangements on the μs-ms timescale.[6] By contrast, timescales relevant to bulk water dynamics are significantly shorter, as the lifetime of water H bonding is on the order of picoseconds.[159]

Prolonged trajectories aimed at sampling protein conformational changes need not be sampled with the same frequency as needed to sample fast water motions, and doing so for a large simulation systems could lead to difficulties with storage of the trajectories. In computations presented here, trajectories where we sampled protein motions were saved each 10ps; to sample water motions we used equilibrated trajectories to select coordinate snapshots and then, starting from these coordinate snapshots, performed short (on the order of 100ps-1ns) trajectories with frequent writing of coordinates, such as 10fs.

3.6 Combining MD simulations with bioinformatics

Computer simulations are often performed on one or just a few members of a protein family. To find out whether specific H-bond motifs observed in such simulations are relevant for the entire protein family, MD simulations can be combined with bioinformatics approaches, in which sequences of proteins are aligned and subjected to subsequent data analyses. These analyses can aim to find out, e.g., the number of sequences in which a specific amino acid residue is conserved or replaced by an amino acid residue of similar H-bonding propensity. Moreover, the data set of aligned protein sequences can be analyzed to find out whether the net charge carried by the sequence is conserved: such an analysis could be performed by calculating the length of the amino acid sequences, and the number of positively-charged vs. negatively-charged groups present in that sequence.

As the reliability of the sequence analysis depends on the size of the dataset of sequences, and on the accuracy of the individual sequences included in the dataset, great care needs to be taken in generating the sequence dataset. For analyses of the net charge carried by an amino acid sequence, potential limitations include lack of precise information regarding the signal sequence vs. mature region of a protein, and lack of precise information about the protonation states of titratable amino acid sidechains in the cell environment of various organisms from which the protein was sequenced.

4. Results

The Results section is largely organized according to the topics discussed in Chapter 2, in which the main scientific topics and model systems are introduced. The publications are appended to the Thesis.

4.1 Dynamic H-bond networks in membrane proteins

To understand the role of dynamic H-bond networks in membrane protein function we used a broad range of computational approaches that we applied for membrane proteins that function as transporters or receptors. An important common observation from this body of work is that internal H-bond networks of membrane proteins typically have complex dynamics, with H bonds that break and reform, and that these complex dynamics, whose description would require prolonged atomistic simulations or a large data set of crystal structures, needs to be accounted for to understand how the protein responds to perturbations such as mutation or changes in protonation.

4.1.1 Coupling between inter-helical H bonding and water dynamics in proton pumps

Vectorial transport of ions by cellular ion pumps relies on structural elements that gate the accessibility of the pump to the two sides of the membrane, such that ions are translocated across the membrane.[160] Structural elements of the protein that gate ion access, denoted as gates, were suggested to sample two main conformations, open and closed.[160] A fundamental question is how the conformational dynamics of the protein, which would ensure coordinated opening and closing of the gates, couple to changes in the protonation state of the pump during its reaction cycle. To address this question I took advantage of the wealth of experimental data on mutants of the bacteriorhodopsin proton pump.

The kinetics of proton transfers in the reaction cycle of bacteriorhodopsin is altered by specific mutations that change H bonding. An example is Y57F, in which lack of the inter-helical H bond between helix-A Y57 and helix-G D212 (Figure 1A) associates with altered order of proton transfers: in Y57F, proton uptake from the cytoplasm occurs before proton release.[161]

Motivated by observations from site-directed mutagenesis work, in ref.[162] I performed simulations of wild-type and nine different mutants of bacteriorhodopsin, and focused on the effect that mutations have on water interactions at protein sites known to be important for proton transfers. The simulations indicated that mutations of H-bonding groups can alter drastically the dynamics and water interactions at proton-transfer sites.

One such site is D212 (Figure 1A), which might transiently bind a proton during the reaction cycle.[51-52] When D212 is mutated to Asn, local structural rearrangements of protein groups associate with enhanced hydration of inner cavities of bacteriorhodopsin.[162] Altered protein and water dynamics of D212N could explain why its photocycle is perturbed.[163] D85N, a mutant in which retinal deprotonation is delayed and the amount of deprotonated M retinal intermediate is reduced,[163] showed not only reorientation of R82 at the extracellular side, but also and enhanced hydration near the inter-helical H bond between T46 and the cytoplasmic proton donor D96 (Figure 1A).[162] That is, mutations can associate with changes in internal water dynamics and structural rearrangements that would be difficult to predict based on the crystal structure.

The H bond between D96 and T46 and that between D115 and T90 were both inter-helical H bonds between a protonated carboylate and a hydroxyl group. Yet, in the simulations these two H bonds had different dynamics and responded differently to perturbations induced by mutation, the H bond between D115 and T90 being overall more stable and less sensitive to mutations than that between D96 and T46.[162] The different behavior of these two carboylate-hydroxyl H bonds could originate from differences in nearby hydrophobic packing that can restrict the motions of the H-bonding groups.[162]

Taken together, these observations suggest that inter-helical H bonds at functionally important sites can play essential roles for the conformational dynamics and water interactions of bacteriorhodopsin.

In closely related work performed in my research group we probed how the AHA2 plasma membrane proton pump responds to changes in protonation (Figure 1B). The central proton donor/acceptor of AHA2, D684,[61] is located far away from the putative proton release groups D92 and D95, and absence in the crystal structure[61] of water molecules that could bridge the proton donor and acceptor groups make it difficult to predict the proton transfer path, and how the protein and water would respond to changes in the protonation state of the protein. In ref.[164] we reported classical mechanical atomistic simulations of AHA2 in five distinct protonation states of the protein. Water molecules visited the inter-helical region of the transmembrane domain of the protein.[164] Waters could be observed close to D684 for all protonation states considered, and a negatively-charged D95 appeared to associate with water molecules entering deeper into the protein at its extracellular side.[164] This could be interpreted to suggest that the number of internal waters of AHA2 could change during the reaction cycle of the pump.

When high-resolution crystal structures of AHA2 will become available, it will be important to probe again the protonation-coupled dynamics of the protein and compute proton-transfer paths.

Main publications discussed:

del Val C, Bondar ML and Bondar A-N. Coupling between water dynamics and inter-helical hydrogen bonds in a proton transporter. *Journal of Structural Biology* 186:95-111 (2014)

Guerra F and Bondar A-N. Dynamics of the plasma membrane proton pump. *Journal of Membrane Biology*, 248:443-453 (2015)

4.1.2 Intra-helical hydroxyl H bonding and inter-helical hydroxyl-carboxyl H bonds

Intra- and inter-helical H bonding of helical segments of membrane proteins impacts local helix structure and dynamics, and the overall protein dynamics. Simulations on bacteriorhodopsin[162] discussed in section 4.1.1 above, and inspection of crystal structures of other membrane transporters,[162] led me to think that inter-helical carboxylate-hydroxyl H bonds between Ser/Thr and Asp/Glu are motifs likely important for the functioning of the protein: Changes in the dynamics of the inter-helical H bond could couple to changes in the dynamics of intra-helical H bonding in which the hydroxyl group can engage, which in turn could alter local protein dynamics.[162] At a site where the carboxylate is a proton donor/acceptor group, we think that the H-bonded hydroxyl group could serve as an intermediate proton carrier.[17] In a subsequent study, I inspected additional membrane protein structures and noticed that inter-helical carboxylate-hydroxyl H bonds are also

present at functionally important sites of GPCRs.[165] Taken together, these observations suggested that analyses of inter-helical carboxylate-hydroxyl H bonds in membrane proteins could be used to identify sites potentially important for function.

Motivated in part by these observations from simulations and crystal structures of membrane proteins, in the Bridge graph-based algorithm for analyses of H-bond networks we implemented a search for inter-helical carboxylate-hydroxyl H bonds[166] (see section 4.1.4). In the future, this algorithm could be extended to analyze large data sets of high-resolution crystal structures to find out how common carboxylate-hydroxyl H-bond motifs–or other H-bond motifs- are in membrane proteins.

Main publications discussed:

Bondar A-N, Lemieux J. Reactions at membrane interfaces. *Chemical Reviews* 119:6162-6183 (2019)

Bondar A-N, Smith JC. Protonation-state coupled conformational dynamics in reaction mechanisms of channel and pump rhodopsins. *Photochemistry and Photobiology* 93, 1336-1344 (2017)

del Val C, Bondar ML and Bondar A-N. Coupling between water dynamics and inter-helical hydrogen bonds in a proton transporter. *Journal of Structural Biology* 186:95-111 (2014)

4.1.3 H-bonding groups in a protein family: channelrhodopsins as a test case

MD simulations are typically performed on one or a few members of a protein family. Bioinformatics sequence analyses, in which sequences of proteins are aligned and subjected to analyses, can inform on whether particular H bond motifs observed in simulations or the experimental structure of a protein are relevant for the entire protein family. In collaboration with Prof. Coral del Val from the University of Granada, I took such an approach to study the conservation of H-bonding groups in channelrhodopsins.[167] Our focus was on the conservation of charged and polar amino acid residues from transmembrane helical segments.

In the bacteriorhodopsin proton pump, the cytoplasmic proton donor D96 (Figure 1A) is followed by a stretch of 5 hydrophobic groups, D_{96}LALLV; within the set of sequences used for our analyses, the preferred sequence was HLSNLT, that is, though D96 is replaced by a His, the remaining of the sequence stretch is more polar in channelrhodopsins as compared to bacteriorhodopsin.[167]

The same picture of a more hydrophilic stretch was observed for helix D of channelrhodopsin, which can contain several Ser/Thr groups absent from the bacteriorhodopsin sequence.[167] In all but two of the channelrhodopsin sequences included in analyses, A114 that is adjacent to D115 of bacteriorhodopsin (Figure 1A) is replaced by Ser. Based on this observation and on previous work on the dynamics of model transmembrane peptides with Ser/Thr groups, we hypothesized that the Ser/Thr groups of

helix D are likely important for dynamics and thus for protein function.[167] A more hydrophilic environment at the helix D aspartic group of channelrhodopsins could be related to proton transfer events: in channelrhodopsin-2 from *Chlamidomonas reinhardtii*, D156 (corresponding to bacteriorhodopsin D115) is thought to be an internal proton donor for the retinal Schiff base.[168]

Main publications discussed:

del Val C, Royuela-Flor J, Milenkovic S, Bondar A-N. Channelrhodopsins: a bioinformatics perspective. *Biochimica et Biophysica Acta (Bioenergetics)* 1837:643-655 (2014)

4.1.4 Development of algorithms to identify networks of dynamic H bonds in proteins

Proteins can contain dynamic networks of H bonds that inter-connect different regions of the protein; as such networks of H bonds are likely important for the conformational dynamics and for the functioning of the protein, there is significant interest in identifying and characterizing the dynamics of such H bond networks.

With co-workers in my research group we dedicated significant efforts to design, implement and apply algorithms that enable efficient analyses of dynamic H-bond networks in proteins. These efforts of my research team are briefly summarized below.

H-bond maps, shortest-distance H-bond paths, and water-mediated bridges. An early step towards this aim were the two-dimensional maps of H-bond donors and acceptors that we used to characterize H bonding of the SecA protein motor[169-170] (Figure 5B). With help of such maps we identified H bonds that inter-connect functional domains of SecA.[169]

To focus our analysis on inter-connections between the nucleotide-binding site and the PBD (Figure 5B), we implemented algorithms that relied on graphs of H bonds and searches of shortest-distance paths between two nodes (i.e., H-bonding amino acid residues) of the graph.[171] An H-bond path that we identified between NBD1 and the PBD includes no fewer than 17 protein groups, with most of the H bonds within the path being dynamic.[171]

The soluble PsbO subunit of photosystem II (Figure 4), for which a key issue is whether it functions as a proton antenna (section 2.3), the challenge was to identify and characterize the dynamics of carboxylate-water bridges on the surface of the protein. We developed an algorithm that searched for shortest-distance H-bond chains of water molecules between pairs of carboxylate groups.[172] Briefly, H-bonded water chains were identified by scanning the space between carboxylate pairs for water molecules that met the H-bonding criteria used. With this approach we found that a cluster of carboxylates that includes D224 (Figure 4) had persistent bridging via H-bonding waters.[172] More recently, we used a similar

approach to search for water-mediated bridges between phosphate groups at a lipid membrane interface.[173]

Water residence times. Clusters of surface carboxylate groups where a proton might bind could cage water molecules. In the inter-helical domain of a proton transporter protein, discrete water molecules bound to the protein could participate in H-bond networks relevant to proton transfers. Computations of water residence times allow us to characterize the dynamics of water molecules interacting with the protein, and to identify sites of a protein where water molecules spend prolonged times.

Water residence times can be estimated by first computing from simulations normalized time-correlation functions for water molecules within the first hydration shell (~4Å) of protein groups; the time-correlation function is fitted with a stretched exponential function – the Kohlrausch-Williams-Watts, KWW function, whose integral gives an average residence time.[174] Tests on simulations of the PsbO-PsbU complex (Figure 4) indicated relatively poor fits when using the KWW exponential,[175] which led us to implement an algorithm that computes water residence times with three additional exponential functions and selects the best fit, and thus the best estimation of the water residence times.[175]

Graph-based searches of protein-water wires. Our studies of the dynamics of a photosystem II monomer in a hydrated lipid membrane environment came with the challenge of how to evaluate efficiently the dynamics of H bond networks in such a large protein system (Figure 3), particularly since our interest was to identify dynamic water-protein H-bond networks that could transiently connect the vicinity of the reaction center to the bulk lumen. To tackle this issue we presented a graph-based algorithm that pre-computes chains of H-bonded water molecules, maps these H-bonded water chains onto protein side chains, and then searches for H-bonded water wires that inter-connect two protein sidechains.[176] By using this algorithm to analyze atomistic simulations of photosystem II we could identify two long-distance H-bond paths that connect the oxygen reaction center to the bulk lumen; both H-bond networks consist of a number of individual protein-water H-bond bridges, some of which can be rather dynamic.[176] The dynamics of the H-bond paths we identified, and of other pathsthat might be sampled, would need to be accounted for when considering long-distance proton transfer path: H-bond paths are likely to rearrange during proton transfers.

Bridge: algorithms for efficient analyses of dynamic H-bond networks. The graph-based search for water wires presented in ref.[176] served as basis for the development of the set of algorithms we denoted as Bridge.[166] We made Bridge available as a plugin for PyMol, a software that to my knowledge is widely used in the community.[177]

Bridge allows computations of all H-bonded paths starting from a particular protein group or between two protein groups, and of continuous H-bonded paths between two protein groups;[166] the percentage of time during which two protein groups connect via a continuous H-bonded path is evaluated by computing a joined occupancy of the individual segments of the path.[166] Given our interest in the functional role of inter-helical hydroxyl-carboxylate H bonds, Bridge allows the search of such motifs.[166]

Taken together, data analysis tools implemented in my research group allow efficient studies of the dynamics of H-bond networks in complex bio-molecular environments, and in particular of dynamic water-mediated H-bond networks for proton transfers.

Main publications discussed:

Guerra F, Siemers M, Mielack C, and Bondar A-N. Dynamics of long-distance hydrogen-bond networks in photosystem II. *Journal of Physical Chemistry B* 122:4625-4641 (2018)

Karathanou K, Bondar A-N. Using graphs of dynamic hydrogen bonds to dissect conformational coupling in a protein motor. *Journal of Chemical Information and Modeling* 15:1882-1896, doi: 10.1021/acs.jcim.8b00979 (2019)

Karathanou K, Bondar A-N. Dynamic water hydrogen-bond networks at the interface of a lipid membrane containing palmitoyl-oleoyl phosphatidylglycerol. *Journal of membrane Biology* (Topical Collection) 251:461-473 (2018)

Kemmler L, Ibrahim M, Dobbek H, Zouni A, Bondar A-N. Water hydrogen bonding and proton transfer at the interface between the PsbO and PsbU subunits of photosystem II. *Physical Chemistry Chemical Physics* 21:25449-25466 (2019)

Lorch S, Capponi S, Pieront F, Bondar A-N. Dynamic carboxylate/water networks on the surface of the PsbO subunit of Photosystem II. *Journal of Physical Chemistry B* 119, 12172-12181 (2015)

Milenkovic S, Bondar A-N. Motions of the SecA protein motor bound to signal peptide: Insights from molecular simulations. *Biochimica et Biophysica Acta (Biomembranes)* 1860, 416-427 (2018)

Milenkovic S, Bondar A-N. Mechanism of conformational coupling in SecA: Key role of hydrogen-bonding networks and water interactions. *Biochimica et Biophysica Acta (Biomembranes)* 1858, 374-385 (2016)

Siemers M, Lazaratos M, Karathanou K, Brown K, Bondar A-N. Bridge: A graph-based algorithm to analyze dynamic H-bond networks in membrane proteins. *Journal of Chemical Theory and Computation* 15:6781-6798, doi 10.1021/acs.jctc.9b00697 (2019)

4.1.5 Using computations to probe the architecture of ion pumps

Recent experimental studies of functional interconversions between microbial rhodopsins that function as ion pumps used as model proton and sodium pumps GR and KR2, respectively.[70] The sequence identity between these two proteins is 26%.[70] Important differences are observed near the retinal Schiff base. GR, as bacteriorhodopsin, contains the counterions D121 and D253 on helices C and G, respectively (Figure 8A, corresponding to bacteriorhodopsin D85 and D212, Figure 1B); in KR2, D85 is replaced by N112, but another carboxylate on helix C, D116 (Figure 8E), which replaces T89 of bacteriorhodopsin,[178] is thought to function as primary proton acceptor for the retinal Schiff base.[178]

Knowledge about the functional role of specific protein groups is essential in studies of functional interconversions, which in turn provide invaluable information about sequence-structure-function relationship. In experiments, a sextuple GR mutant that mimics groups of KR2, I83S/H87L/Y88L/D121N/T125D/E132Q was insufficient to convert GR into a sodium pump[70] (GR-6 in Figure 8B) Could we use MD simulations to understand why mutant GR-6 is not a sodium pump though it mimics interactions specific to KR2, and to predict which additional mutations might be required to achieve functional interconversion?

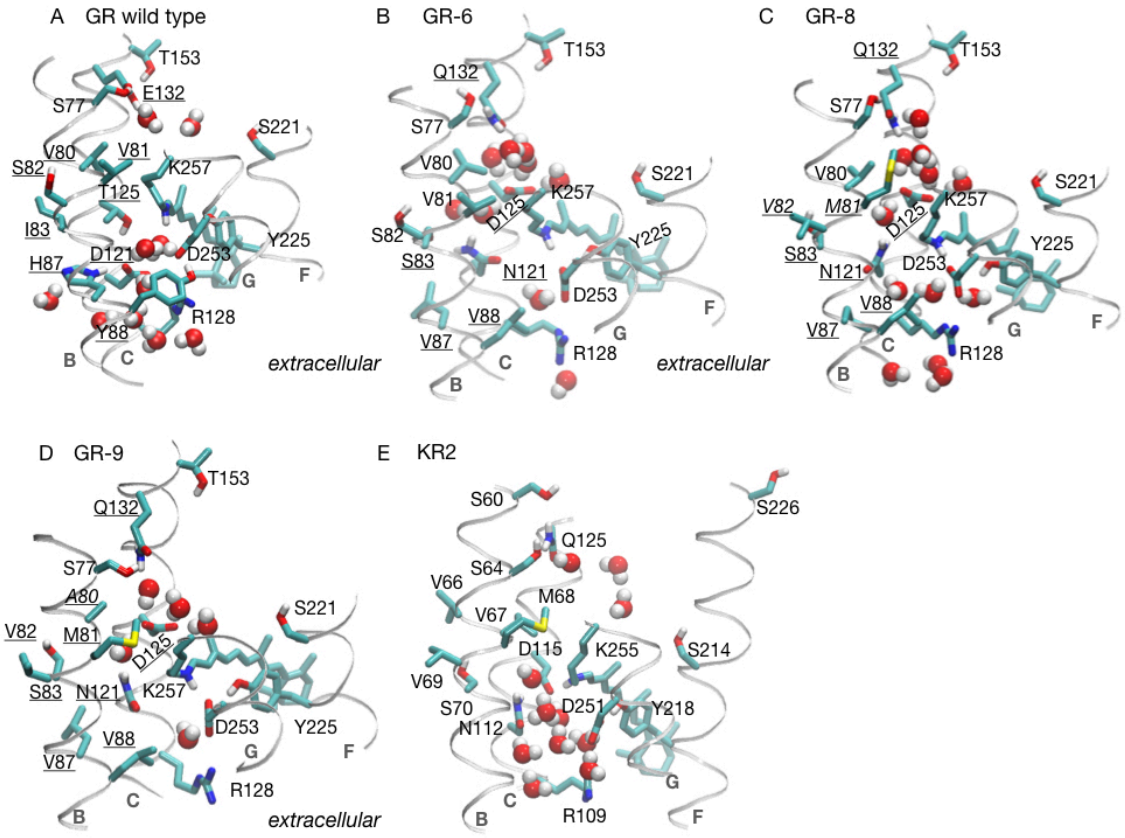

Figure 8. Towards a molecular description of mechanisms of functional interconversions in microbial pump rhodopsins. The molecular graphics are based on coordinate snapshots from MD simulations of GR and KR2 in hydrated lipid membranes. (A) Wild-type GR proton pump. Amino acid residues that are mutated in GR-6, GR-8 or GR-9 are underlined. (B-D) The mutant proteins GR-6, GR-8 and GR-9. (E) Wild type KR2 sodium pump. The GR and KR2 work relies for the starting protein coordinates on crystal structures PBD ID:6NWD[179] and PDB ID:6RF5,[180] respectively.

To this end, I initiated, in collaboration with Prof. Keiichi Inoue from the University of Tokyo, Japan, a series of atomistic MD simulations in which I probe the dynamics of GR, GR-6 and KR2, and of two additional mutations we selected based on inspections of crystal structures and knowledge of structural motifs. In GR-8, we added to GR-6 the mutations V81M and S82V (Figure 8C), and in GR-9 we further mutated V89 into Ala (Figure 8D). The preliminary simulations illustrated in Figure 8 suggest that the mutations alter the internal

water dynamics at the cytoplasmic side of the retinal Schiff base, where more waters appear to visit in mutant vs. wild-type GR. We also observe that in the mutants the retinal Schiff base tends to prefer to interact with D253 – though the strength of interactions between the Schiff base and a carboxylate could overestimated by the force field.[162] In the future, the equilibrated trajectories of GR and KR2 could be used to compute the free energy for the binding of a sodium ion at different locations inside the protein.

4.1.6 Control of the protonation state of the retinal Schiff base in JSR-1

An intriguing aspect of the reaction cycle of JSR-1 is that the retinal Schiff base stays protonated.[67] Based on the crystal structure of JSR-1 with the inverse agonist 9-cis retinal, it was proposed that the local H-bond network in which the retinal Schiff base connects to Y126 (Figure 2B) likely stabilizes the protonated state of the retinal Schiff base.[66] But the extent to which the H-bond network of the 9-cis retinal is representative for the 11-cis and all-trans retinals of the reaction cycle of JSR-1 remains unclear. To address this issue we pursue reaction path computations of isolated JSR-1, and atomistic simulations of JSR-1 embedded in hydrated lipid membrane environment. This research is a collaboration with Dr. Elena Lesca and Prof. Gebhard Schertler from the Paul Scherrer Institute/ETH Zürich.

My strategy is to first use coordinate driving and CPR[152] to compute MM minimum energy paths for the isomerization of the retinal from 9-cis to all-trans, and from all-trans to 11-cis, in isolated JSR-1. These reaction path computations generate structures of JSR-1 with 11-cis vs. all-trans retinal that I then refine by performing MD simulations of JSR-1 in hydrated lipid membrane environments. The MM minimum energy paths can also be decomposed according to equation (1) to dissect contributions to the total energy profile of the isomerization reactions, and thus identify which interactions help control the isomeric state of the retinal in JSR-1. Based on these analyses, I select amino acid residues to mutate and compute new minimum energy paths for the mutants.

Figure 9 illustrates a CPR minimum energy path computation for the isomerization of the retinal from 9-cis to all-trans in wild-type JSR-1 (see section 3.3). For efficiency, I performed the CPR computations with MM using for the retinal force-field parameters as described in ref.[181] based on refs.[36, 38].

The reaction path computation indicates that the retinal Schiff base changes its H-bond partner from Y126 in the 9-cis reactant state (Figure 7A) to A317 in the all-trans product state (Figure 9C). The total energy profile (Figure 9D) indicates a significant energy barrier of 30.2 kcal/mol, and a reaction energy of 4.1 kcal/mol. The finding here of a significant energetic penalty against isomerization of the retinal is not surprising, since the protein

crystal structure was solved with 9-cis retinal, there is tight packing of retinal protein groups at the binding pocket (Figure 2A), and most of the energy barrier is given by the intrinsic torsional energetics of the retinal (see red profile in Figure 9D). As the retinal isomerizes around the C_9=C_{10} bond, the H bond between the Schiff base and Y126 breaks (Figures 9E, 9F); the interaction energy between the retinal and A317 is unfavorable first, as there is steric repulsion between the C_{13} methyl group of the retinal and the methyl group of A317 (Figure 9E). As the retinal completes the isomerization and the Schiff base H bonds to the backbone carbonyl of A317 (Figure 9F), the interaction energy between the retinal and A317 becomes stabilizing (Figure 9E).

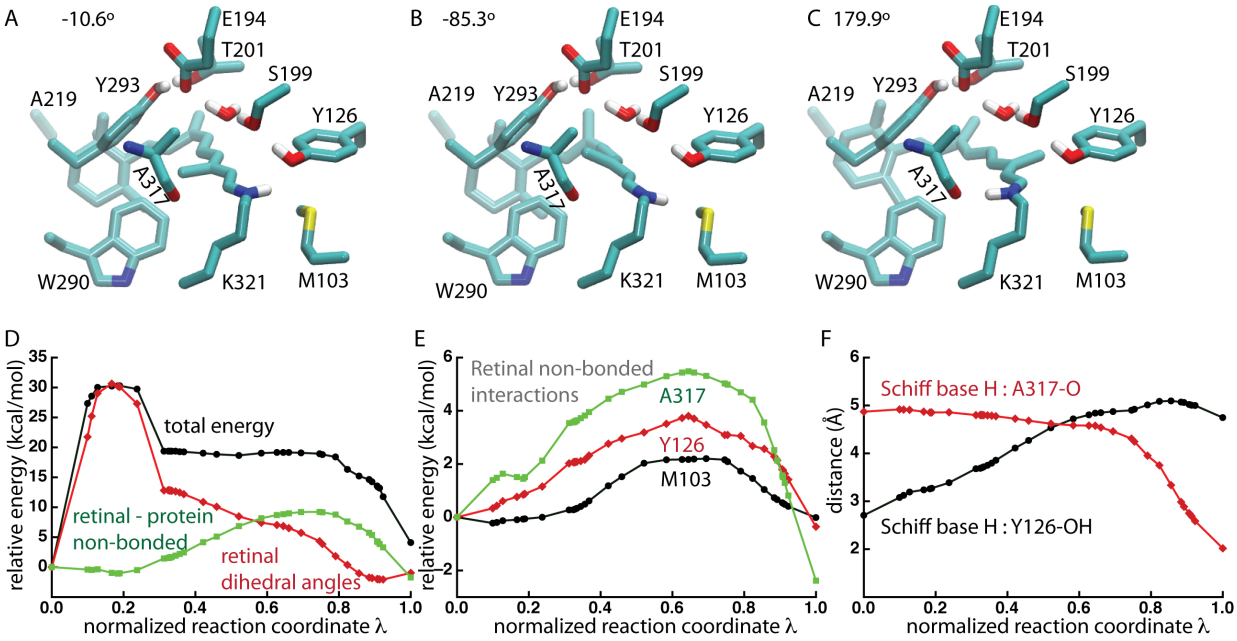

Figure 9. Reaction path for the clockwise isomerization of 9-cis retinal JSR-1 to all-trans. (A-C) Molecular graphics depicting the retinal and its immediate environment in the starting 9-*cis* reactant state of the path (panel A), an intermediate state with the C_9 = C_{10} bond twisted at -85.3° (panel B), and in the all-*trans* product state. Carbon atoms are colored cyan, H – white, oxygen – red, and nitrogen – blue. Only selected H atoms are depicted. (D) Energy profile of the path and decomposition of the energy profile. The reaction coordinate is normalized to the length of the path. (E) Non-bonded interaction energy of the retinal and K321 with selected protein groups from the retinal-binding pocket. Energy profiles for M103, Y126 and A317 are colored black, red and green, respectively. (F) Distance between the Schiff base H atom and H-bond partners in the binding pocket. The distance between the Schiff base H atom and Y126-OH (black) increases as the retinal isomerizes and the Schiff base of all-trans retinal H bonds with the backbone carbonyl of A317 (red).

The all-trans retinal obtained as a product state of the 9-cis isomerization reaction (Figure 9C) was then used as a reactant state to compute a path for the isomerization from all-trans to 11-cis retinal. For the clockwise isomerization, the energy barrier is 31.4kcal/mol.

To further probe the impact that nearby protein groups have on the energetics of retinal isomerization, I modeled the M103A and Y126A mutants and performed computations for

the isomerization of the retinal from 9-cis to all-trans. The clockwise isomerization of the retinal from 9-cis to all-trans costs ~29kcal/mol in the mutants, which is close to the energy barrier computed for the wild type (Figure 9D), and consistent with the energy decomposition indicating that the total energy barrier is largely given by the intrinsic retinal torsion (Figure 9D).

The CPR computations provide clues about structural and energetic determinants of the retinal isomeric state. Minimization-based approaches are, however, limited in their description of structural changes of the protein and of the dynamics of internal waters. To begin to understand how the protein and internal waters respond to changes in the isomeric state of the retinal, and thus to derive better models of JSR-1 with 11-cis and all-trans retinal, I used structures derived from reaction path computations to initiate atomistic MD simulations of wild-type JSR-1 in hydrated POPC lipid bilayers with 11-cis and all-trans retinals. I further performed simulations of wild-type 9-cis wild-type JSR-1 that serve as reference, and of the Y126A and M103A 9-cis JSR-1 mutants to probe dynamics at the active site with perturbed Schiff base interactions.

Preliminary MD simulations illustrated in Figure 10 indicate that waters visit the inter-helical region of JSR-1 (Figure 10A). At room temperature, the H bond network at the active site of 9-cis JSR-1 remains close that in the crystal structure (Figures 2B, 10A), suggesting that the force-field description used is reasonable.

The H-bond network at the active site of 11-cis wild-type JSR-1 is similar to 9-cis in that the Schiff base H interacts with Y126, though E194 H bonds directly to S199 and several waters visit the vicinity of E194 (Figure 10C). In all-trans JSR-1 the Schiff base H bonds to the backbone carbonyl of A320 (Figure 10D). H bonding between the Schiff base of all-trans retinal and a backbone carbonyl could explain why the Schiff base stays protonated in the reaction cycle of JSR-1, as a carbonyl group is an unlikely proton acceptor.

In the absence of the Y126 sidechain (Y126A mutant), S199 remains H bonded to E194 via the water molecule (Figure 10E), which suggests that the Y126 sidechain is not essential to maintain the water bridging of E194. In M107A, a chain of two water molecules connects the retinal Schiff base to the backbone carbonyl of M103 (Figure 10F). Taken together, the simulations on mutant JSR-1 suggest that in wild-type JSR-1 Y126 and M103 help optimize the H-bond network of the retinal Schiff base.

The simulations reported here for JSR-1 provide a qualitative understanding of the mechanism by which JSR-1 avoids deprotonation of the retinal Schiff base, in that, depending on the isomerization state of the retinal Schiff base, the Schiff base interacts with Y126 (Figures 2B, 10B, 10C), or with a backbone carbonyl group (Figure 10 D). In the

future, this mechanism will, however, need to be evaluated with QM/MM computations of proton transfers.

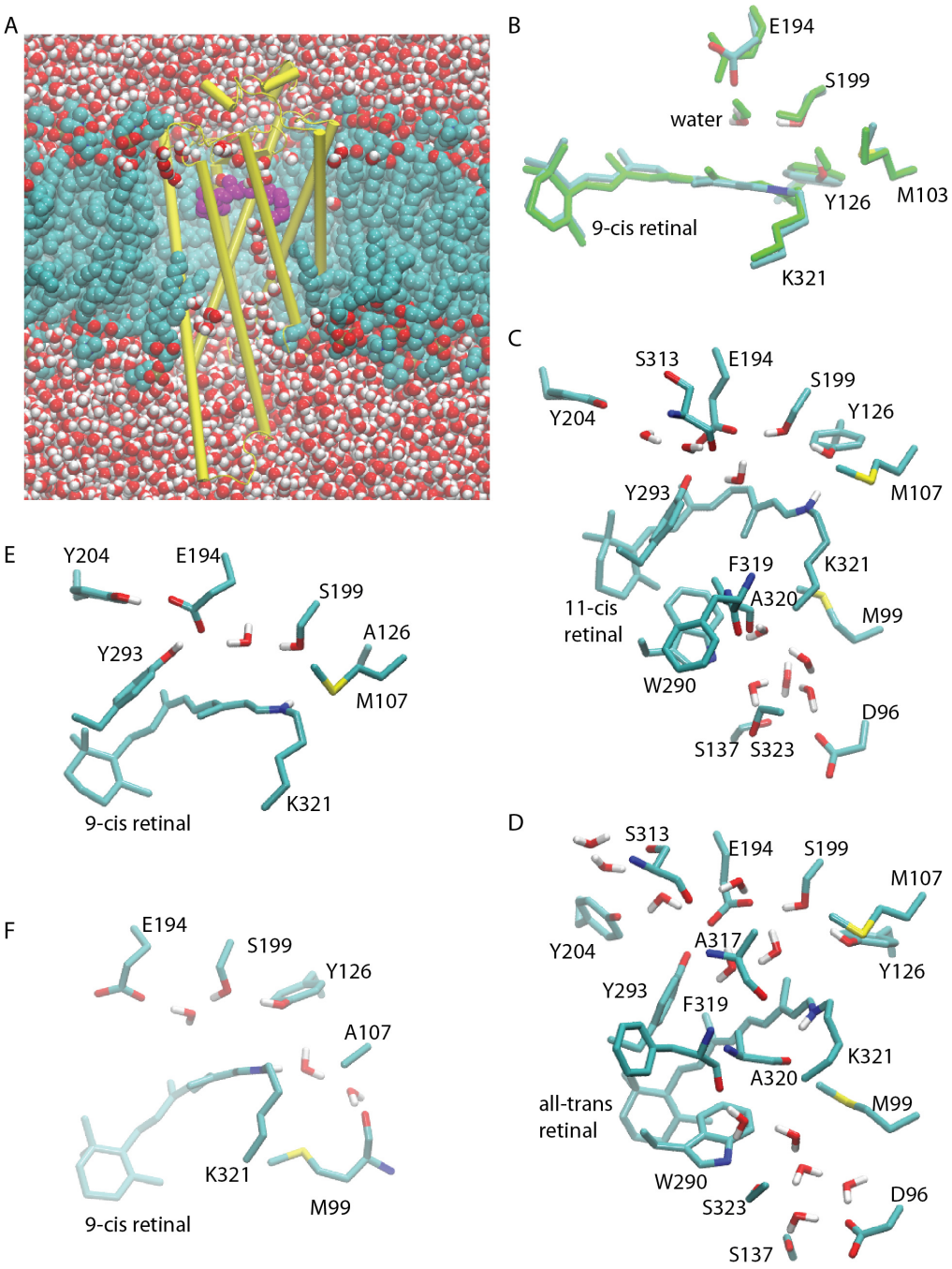

Figure 10. Preliminary simulations of JSR-1 in hydrated POPC lipid membrane environments. (A) Cut-away view of all-trans JSR-1 in a hydrated POPC lipid membrane environment. Heavy atoms of the retinal and of the K321 sidechain are shown as magenta van der Waals spheres. (B) Overlap of a coordinate snapshot from MD simulations of wild-type 9-cis JSR-1 in a hydrated lipid membrane environment (atom colors) and the starting coordinates of JSR-1 optimized with MM. The overlap was done for a short stretch of helix G that includes K321. The water-mediated H bond between E194 and S199 is preserved. (B) Coordinate snapshot from MD simulations of 11-cis retinal JSR-1. The Schiff base H bonds to Y126, and S199 H bonds directly to E194. (C) Coordinate snapshot from MD simulations of all-trans retinal JSR-1. The Schiff base H bonds to the backbone carbonyl of A317. (E) Coordinate snapshot from MD simulations of 9-cis retinal Y126A JSR-1. (F) Coordinate snapshot from MD simulations of 9-cis retinal M103A JSR-1.

4.1.7 Related research on retinal proteins

I collaborate with Prof. Leonid S. Brown (University of Guelph, Canada) on understanding sequence-structure-function relationships in microbial rhodopsins, particularly H bonding and proton transfer. As part of this collaboration I contributed structural models to aid interpretation of experiments.[182-183]

In my laboratory, we used QM/MM proton-transfer computations to dissect the role of water and protein electrostatic interactions for channelrhodopsin.[184]

I contributed with QM/MM computations to the paper reporting structural dynamics of bacteriorhodopsin based on time-resolved serial femtosecond crystallography.[21]

In collaboration with Prof. Jeremy C. Smith (Oak Ridge National Laboratory) and Prof. Marcus Elstner (Karlsruhe Institute of Technology) we completed work initiated during my Heidelberg years on the atomistic picture of retinal untwisting in bacteriorhodopsin.[185]

4.1.8 Perspectives on retinal proteins research and development of data analysis tools for H-bond networks

The graph-based algorithm we developed for analyses of dynamic H-bond networks, Bridge,[166] allows efficient analyses of large sets of structures. In the future, I envision that we will work on expanding the tool to mine for H-bonding motifs in protein data bases.

For the particular case of proton transfer in microbial rhodopsins, I anticipate that in the future we will use Bridge to identify putative proton-transfer paths in MM atomistic simulations, and then use a QM/MM description to compute the energetics for proton transfers along these paths.

The research I initiated together with external collaborators, on sequence-structure-function relationships in microbial rhodopsins and control of retinal dynamics in JSR-1, will contribute to a mechanistic description of how specific structural motifs shape the energetics of reactions in microbial rhodopsins. What is needed, and I envision working on in the future, is an accurate force-field description for twisted retinals and their water and protein interactions.

4.2 H-bond networks for long-distance proton transfers in photosystem II

Protons that are generated during water splitting reactions at the oxygen reaction center of photosystem II would need to be transferred to the bulk lumen across relatively long distances (Figure 3). A fundamental open question is that of the paths for proton transfer:

which protein groups are directly involved in proton-transfer reactions, and how do water and protein dynamics respond to changes in protonation as protons are transferred.

As a first step towards understanding how proton transfers might occur in photosystem II, I performed, in collaboration with Prof. Holger Dau from the Freie Universität Berlin, a detailed analysis of H-bond networks in the high-resolution crystal structure of photosystem II that had recently become available at the time.[186] Based on my analyses of H bonding, we identified clusters of local H bonds involving protein groups and water molecules, and presented a scheme illustrating the remarkable connectivity between these local clusters of H bonds that could be important for proton transfer and/or conformational coupling.[186] Based on the short distance between the carboxylate groups of PsbO-E97 and D102 in the crystal structure, we suggested that one of these two carboxylate groups could be protonated.[186]

In my research group, we established atomistic MD simulations of a monomer of photosystem II in a hydrated lipid membrane environment.[176] To analyze water wires in photosystem II, we used a graph-based approach[176] as described in section 4.1.3. Our analyses indicated for the H-bonded networks a complex dynamics that is directly relevant to mechanistic interpretations of proton transfer. An important observation from these analyses was that intermediate segments –or water wires- of a long-distance H-bond path can have rather different dynamics – the shorter the water wire, the less dynamical it tends to be; how long a water wire persists, that is its endurance time, depends likewise on the length of the wire, and also on the location of the wire and whether waters are available nearby and can exchange with waters of the wire.[176] These features of long-distance H-bond networks observed for photosystem II are likely relevant to other bio-systems.

Main publications discussed:

Bondar A-N and Dau H. Extended protein/water H-bond networks in photosynthetic water oxidation. *Biochimica et Biophysica Acta* (Bioenergetics) 1817: 1177-1190 (2012)

Guerra F, Siemers M, Mielack C, and Bondar A-N. Dynamics of long-distance hydrogen-bond networks in photosystem II. *Journal of Physical Chemistry B* 122:4625-4641 (2018)

Selected for the ACS Editor's Choice. Listed among the five most-read articles of 2018 for the Journal of Physical Chemistry B

4.3 PsbO as a model system to understand proton antennas in bio-molecules

Proton antennas are of general interest to proton transfer in bio-systems (see section 2.3). What makes PsbO a particularly suitable model system for computational analyses of a proton antenna system is the small size of the protein, the large number of carboxylate groups on its surface (Figure 4), and the fact that it is a soluble protein that can be studied in aqueous solution.

Dynamic carboxylate-water bridges on the surface of PsbO. In a first study of PsbO we performed in my group,[172] our aim was to understand how carboxylate clusters on the surface of PsbO interact with each other, particularly whether there are carboxylate clusters in which carboxylates interact closely with each other via short chains of H-bonded waters, as such clusters would be more likely to bind transiently a proton. With this aim in mind, we performed extensive atomistic MD simulations to probe the motions of PsbO in aqueous solution, and augmented the prolonged simulation probing protein motions with short simulations with frequent writing of coordinates to probe the fast motions of water molecules. Using the data-analysis algorithm we implemented for the water-mediated bridges between PsbO carboxylate groups (section 4.1.4), we indeed could identify a carboxylate cluster that included carboxylates bridged via short distance water bridging.[172] This clusters included D224, an amino acid residue along a long-distance protein-water H-bond path that in the crystal structure of photosystem II connects the reaction center to the bulk lumen.[172]

The protocol we implemented for simulations and data analyses of PsbO (see section 4.1.4) is generally applicable to analyses of carboxylate-water bridges on protein surfaces.

Protonation-dependent dynamics of a surface carboxylate pair of PsbO. A key question about proton antennas is where protons might bind transiently. Within SFB 1078 we addressed this question by combining crystallography experiments performed by experimentalist colleagues (Dr. Martin Bommer, Profs. Athina Zouni, Holger Dobbek, Holger Dau) with MD simulations I performed. The model system we used was a truncated version of PsbO without its long loops.

Taken together, the experiments and computations suggested that D102 (Figure 4) is likely protonated.[187] This observation is consistent with the discussion above of H-bond networks in the crystal structure of the photosystem II complex,[188] and provides an important starting point towards understanding of the potential role of PsbO in transient proton storage during the reaction cycle of photosystem II.

Overall, on the timescale of simulations on truncated PsbO local interactions at the D102 site were preserved somewhat better with D102 protonated than negatively charged; protonated D102 could, however, also interact with E97 via water.[187] A limitation of the computations is that the force-field parameters used might be insufficiently accurate in their description of short-distance H bonds between water-exposed carboxylates.

Carboxylate-water bridges at the interface between PsbO and PsbU, and energetics of proton transfer. Our studies of isolated PsbO in aqueous solution have identified the complex dynamics of carboxylate-water bridges.[172] As the putative proton antenna functionality of PsbO is of interest in the context of photosystem II, the important question

that arises is how the carboxylate cluster at the putative proton-binding site PsbO-D102 interacts with charged groups of PsbU across the PsbO-PsbU interface (Figure 4). To address this question, in my research group we studied with classical MD simulations the motions of the isolated PsbO-PsbU complex, characterized the dynamics of water and carboxylate-water bridges, and then used QM to calculate the energetics of proton transfer in relevant clusters of protein groups and water molecules.[175] The computational work was augmented by analyses of water binding sites in crystal structures of photosystem II – these analyses were performed by our collaborators within SFB 1078 Dr. Mohamed Ibrahim, Profs. Holger Dobbek and Athina Zouni.

We found that most waters visit the protein only for short times, <25ps.[175] At some sites, however, water molecules are caged by interactions with protein groups, or waters can visit for as long as ~320-540ps.[175] At the interface between PsbO and PsbU, several amino acid residues, including PsbO-E97 (Figure 4) have relatively long water residence times ranging from ~43ps to ~134ns, which is indicative of interactions between protein groups and water being somewhat stronger than at other sites more exposed to the bulk. Nevertheless, the lifetimes of the carboxylate-water bridges, including at the interface between PsbO and PsbU, is quite short, mostly in the sub-picosecond range.[175] Proton transfer at the cluster, from PsbO D102 to E97 or to D99, tended to have relatively unfavorable energetics, which could be interpreted to suggest that a proton bound at the D102 site could be stored.[175]

Conservation of charged groups of PsbO, and a protocol for sequence analyses of PsbO. Amino acid residues that are essential for function tend to be conserved in protein families. To what extend are the carboxylates of PsbO conserved in sequences from different organisms? And, are the specific carboxylate groups thought to be a proton antenna conserved, or is there rather a conservation of general features, such as the net charge carried by sequences of PsbO?

With these questions in mind we –my collaborator Prof. Coral del Val from the University of Granada and I–embarked in an extensive study whereby we combined bioinformatics sequence analyses with MD simulations of wild type and mutant PsbO proteins.

Our aim with the bioinformatics analyses was twofold: identify carboxylate groups that are highly conserved, and estimate the net charge of sequences of PsbO. We used datasets with 25 sequences for cyanobacteria PsbO, and 53 sequences for plant PsbO.[189] To estimate the net charge of sequences of PsbO, we calculated the difference between the number of Asp/ Glu groups vs. the number of Arg/ Lys groups, and paid special attention to the length of the mature region of the protein for which we estimated the charge.[189] Overall, within the datasets of sequences we included in the analyses, most of the plant PsbO sequences have a net estimated charge of about -6*e*; by contrast, a wider

range of net estimated charges was observed for PsbO sequences from cyanobacteria, which also tended to be more negatively charged than plant PsbO.[189] At the positions in the sequence corresponding to the carboxylates proposed to be part of the proton antenna,[86] most plant and bacterial PsbO sequences have at least 10 carboxylate groups.[189]

I studied with independent MD simulations the D158A and D224A PsbO mutants, which I chose because these two carboxylate groups are close to the two functionally important Arg groups (see section 2.3 and Figure 4). The simulations suggested that these two carboxylate groups could assist binding of PsbO to photosystem II by contributing to dynamic H bonding of the arginine sidechains.

Taken together, the bioinformatics sequence analyses suggest that a net negative charge is a common feature of PsbO sequences, and that carboxylate groups on the surface of PsbO could be important for protein-protein interactions. A limitation to the simple estimation of the net charge by counting charged groups is that the protonation state of titratable groups depends on the pH of the environment in which PsbO is found.

Main publications discussed:

Bommer M, Bondar A-N, Zouni A, Dobbeck H, and Dau H. Crystallographic and computational analysis of the barrel part of the PsbO protein of photosystem II: carboxylate-water clusters as putative proton transfer relays and structural switches. *Biochemistry* 55, 4626-4635 (2016)

del Val C and Bondar A-N. Charged groups at binding interfaces of the PsbO subunit of photosystem II: a combined bioinformatics and simulation study. *Biochimica et Biophysica Acta (Bioenergetics)* 1858, 432-441 (2017)

Kemmler L, Ibrahim M, Dobbek H, Zouni A, Bondar A-N. Water hydrogen bonding and proton transfer at the interface between the PsbO and PsbU subunits of photosystem II. *Physical Chemistry Chemical Physics* 21:25449-25466 (2019)

Lorch S, Capponi S, Pieront F, and Bondar A-N. Dynamic carboxylate/water networks on the surface of the PsbO subunit of photosystem II. *Journal of Physical Chemistry B* 119, 12172-12181 (2015)

4.3.1 Related research on PsbO

Within SFB 1078 my research group contributed recently with analyses of the electrostatic potential on the surface of truncated PsbO;[190] I contributed a molecular picture of the distribution of charged protein groups in the crystal structure of a photosystem II monomer.[191]

4.3.2 Perspectives on photosystem II and PsbO research

The research discussed above establishes the fundament for future work on computations for proton-transfer paths in photosystem II. To characterize the mechanisms of proton transfer, H-bond networks that we identified would need to be subjected to QM/MM computations to derive energy barriers for proton transfer. A key question that would need to be addressed with such computations is the response of the protein and water dynamics

to changes in protonation. In a long-distance H-bond path as we identified for photosystem II, where a path can consist of intermediate protein-water wires,[176] transfer of a proton within one such intermediate segment could cause rearrangement of the remaining path, and indeed other paths could be sampled.

4.4. Role of lipids in membrane reactions

Experiments have documented the important role that lipids can have in membrane protein reactions (section 2.6). As model system to dissect mechanisms by which lipids impact membrane protein reactions I use the GlpG rhomboid protease (Figure 7). This choice is motivated by the fact that an understanding of how GlpG works can inform on more general principles of lipid-protein coupling. Indeed, the drastic impact that the lipid membrane composition has on the catalytic activity of GlpG and of other could arise, a priori, from an impact of lipids on any segment of the reaction coordinate of GlpG: *i)* the fluidity of the membrane and the hydrophobic mismatch between GlpG and the membrane and between the transmembrane substrate and the membrane could govern the dynamics of substrate docking to the enzyme active site, and thus the dynamics of the formation of the enzyme-substrate complex; *ii)* the hydrophobic thickness of the lipid membrane surrounding the enzyme the lipid membrane could influence the tilt and partial unwinding of the transmembrane substrate;[126-127] *iii)* the energetics of the chemical reaction could be shaped by electrostatic interactions of the protein environment, but also of the lipid headgroups; *iv)* once the transmembrane substrate is cleaved, the dynamics of substrate release from the enzyme active site could depend on interactions with lipids –e.g., H bonding at the lipid membrane interface could delay substrate release.

Atomistic MD simulations are a valuable tool that allows us to address such questions, because we can study the motions of GlpG in membranes of specific lipid composition and dissect interactions between lipids and protein groups.

I used extensive MD simulations to characterize the motions of GlpG in membranes with different thickness and membranes with different lipid headgroups. As the timescale of an atomistic simulation of a membrane protein is unlikely to suffice to sample the full conformational dynamics of the protein, I studied the dynamics of GlpG with independent simulations initiated from three protein crystal structures[132, 192] that were suggested to represent GlpG in different conformations, and simulations of two mutant GlpG proteins. The total sampling time of the 15 independent simulations reported was ~2.9μs.

I found that the thickness of the lipid membrane surrounding GlpG depends not only on the composition of the lipid membrane, but also on the conformation of GlpG. This

observation would imply that the thickness of the membrane close to GlpG might change during the reaction cycle of GlpG. The largest membrane thinning observed, regardless of the protein conformation and lipid membrane composition, was ~4Å, i.e., about one helical turn.

GlpG is found in the membrane of *E. coli*, which is a rather thin membrane – the thickness of the cytoplasmic *E. coli* membrane is 37.5 ± 0.5Å.[193] In simulations of GlpG in POPE and in POPE:POPG membranes, which are often used as model membrane for bacterial membranes, the thickness of the membrane far away from GlpG, estimated as the distance between the peaks of the phosphate groups distributions for the two lipid leaflets, was 42.2 - 42.6Å for POPE, and 40 - 41Å for POPE:POPG. In the future, the distinct possibility needs to be considered that simulations of GlpG in model lipid membranes overestimate the thinning of the membrane close to GlpG.

Depending on the composition of the lipid membrane and on the conformation of GlpG, during simulations a lipid molecule could visit transiently the active site of GlpG. Binding of a lipid at the active site could prevent binding of a transmembrane substrate, and could help explain the observation from experiments[122] that specific lipid membrane compositions associate with poor substrate cleavage activity.

Main publication discussed:

Bondar A-N. Mechanisms by which lipids influence conformational dynamics of the GlpG intramembrane rhomboid protease'. *Journal of Physical Chemistry B* 123:4159-4172 (2019)

4.4.1 Related research on rhomboid proteases

I contributed with a protein homology model to the interpretation of experimental data on the human rhomboid RHBDL4.[194] I wrote a review with focus on H bonding in GlpG,[195] and co-wrote a review article that included a discussion of GlpG.[165]

4.4.2 Perspectives on GlpG research

A fundamental open question for GlpG remains the molecular movie of the sequence of structural changes along the reaction coordinate of GlpG. I am particularly interested in understanding whether GlpG, in the absence of a substrate, samples conformations compatible with docking of a transmembrane substrate at the active site. To address this question I now collaborate with Prof. Joanne Lemieux from the University of Alberta on combining simulations and experiments of wild type and mutant rhomboid protease from *Haemophilus influenzae*. On longer term, I envision pursing QM/MM computations of substrate cleavage by GlpG.

4.4.3 Protein binding at lipid membrane interfaces

The work I pursue on understanding how lipids shape the reaction coordinate of GlpG is closely related to research initiated recently on the binding of Mistic (Figure 6) to lipid membranes. This research, which is in collaboration with the laboratory of Prof. Bernd Reif from the Technical University München, aims to derive a molecular picture of Mistic bound at the interface of lipid bilayers and lipid nanodiscs. How does a protein as polar as Mistic bind at lipid membrane interfaces? How do the protein groups interact with lipids? Do the conformational dynamics of the protein depend on lipid interactions?

To prepare starting coordinates for Mistic bound at lipid membranes I used OPM to find the orientation of the protein relative to the membrane, and CHARMM-GUI to generate simulation systems consisting of the protein and a hydrated lipid bilayer or lipid nanodisc (Figure 11). The preliminary computations suggest that interactions at the N terminus of Mistic largely determine binding of the protein at membrane interfaces. Two hydrophobic Phe groups of the N terminus help anchor Mistic into the membrane core (Figure 11).

The binding pose of Mistic seems to be different in POPE:POPG vs. POPC:POPG membranes (Figure 11). Although this observation is compatible with the different capabilities of POPE and POPG to H bond to protein groups, to conclude on how Mistic binds at membranes of different lipid composition, the current simulations would need to be prolonged and augmented by simulations performed from different starting coordinates.

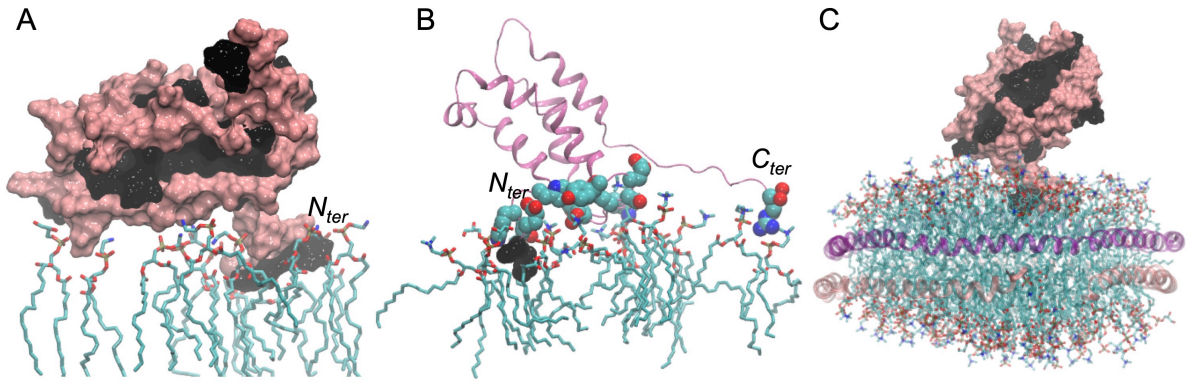

Figure 11. Binding of Mistic at interfaces of lipid bilayers and lipid nanodiscs. Panels A and B display as bonds lipids within 3.5Å of the protein. Panels A and C show the protein as a surface with hydrophobic groups colored black, and all other protein groups colored pink. (A) Mistic at the interface of a POPE:POPG lipid bilayer. Note that the protein anchors to the membrane via the N terminus. (B) Mistic at the interface of a POPC:POPG lipid bilayer. The two Phe groups at the N terminus of Mistic are shown as van der Waals spheres colored black; other protein sidechains within 3.5Å of lipids are shown as van der Waals spheres in atom colors. (C) Mistic bound to a nanodisc with POPC:POPG lipids. Protein chains of the nanodisc are depicted as ribbons.

4.5 Conformational dynamics and the reaction coordinate of the SecA protein motor

The SecA protein motor (Figure 5B) is a key component of the bacterial protein secretion pathway (section 2.4). Its complex reaction coordinate, which involves large-scale conformational changes, protein-protein interactions, and ATP hydrolysis, remains unclear.

Describing the reaction coordinate of an enzyme requires the three-dimensional coordinates of the reactant, product, and transition state(s) of the reaction, as these structures inform on the structural changes along the reaction pathway. Moreover, the free energy difference between the reactant and the product state, and the free energy barrier, are required to evaluate the validity of putative reaction mechanisms and to identify interactions that determine rate-limiting steps of the chemical reaction.

In this context, studies of the reaction coordinate of SecA face the challenge that the dynamics of nucleotide binding at the active site of SecA is unclear. There is insufficient information from structural biology about ATP-bound SecA, as the structural model of ATP-bound *E. coli* SecA[100] lacks coordinates for the active-site Mg^{2+} ion and for a large segment of the PBD (see section 2.4). And, the molecular origin of the release of ADP being rate-limiting[107] remains unclear.

In collaboration with Prof. Yuko Okamoto and his post-doctoral colleague Dr. Hirokazu Mishima (University of Nagoya) we attempted to use umbrella-sampling computations to derive a reaction coordinate for the release of ADP from SecA. As model system we used a simpler construct, based on the crystal structure of the motor domain (NBD1 and NBD2, Figure 5B) of *E. coli* SecA.[196] The simpler construct allows us to study nucleotide dynamics at the nucleotide binding-site of SecA without having to account for the conformational dynamics of the other domains of the protein (Figure 5B).

A reliable distance-based reaction coordinate to sample ADP release could not be easily identified, which might be a clue that more degrees of freedom could contribute to the reaction. To probe the dynamics of nucleotide binding at the active site, we relied instead on an extensive set of MD simulations that I performed for the SecA motor domain with different starting locations for the nucleotide and Mg^{2+}.

When both ADP and Mg^{2+} were bound at the active site, the location of the nucleotide was stable, which could be explained by the nucleotide being part of an extensive network of H bonds with protein groups. By contrast, when we probed the motions of the protein without Mg2+ at the active site, during the course of the simulations, interactions between ADP and protein groups were perturbed. In two of the simulations performed starting with the Mg^{2+} ion bound at the active site and the nucleotide in the bulk, the nucleotide (ADP or ATP) could bind spontaneously at the interface between NBD1 and NBD2, where it interacted bind to charged and polar protein groups.

Taken together, the ensemble of the simulations I performed suggest that charged and polar groups at the interface between NBD1 and NBD2 stabilize the nucleotide when bound at the active site, but could also stabilize transiently a nucleotide at a different site, and thus contribute to ADP release being rate limiting. The Mg^{2+} ion at the active site might need to be released prior to the release of ADP.

Manuscript discussed:

Bondar A-N, Mishima H, Okamoto Y. Molecular movie of nucleotide binding to a motor protein. Submitted to *Biochim Biophys Acta General Subjects*, Special Issue.

4.5.1 Bioinformatics analyses of SecA identify organism-specific sequence features

To a large extent, experimental research on SecA uses as model systems SecA from *B. subtilis* and *E. coli*. To the best of my knowledge, three-dimensional structures have been solved for SecA from a handful of organisms – *B. subtilis*,[98, 106] *E. coli*,[99-100] *Thermus thermophilus*,[197] *Thermotoga maritima*,[198-199] and *Mycobacterium tuberculosis*.[200] SecA from different organisms have the same key functional domains as *B. subtilis* SecA (Figure 5B), but some SecA sequences have additional stretches of amino acid residues, or insertions – *E. coli* SecA, for example, has an insertion denoted as VAR in NBD2,[100] and *T. maritima* SecA has an insertion in NBD1. [198]

As the PBD of SecA (Figure 5B) binds the pre-protein that is to be secreted, and the SecA-SecY complex is more stable when negatively-charged lipids are present,[201] the important question arises as to whether diverse bacterial species, which might be need to secrete different proteins and have characteristic lipid composition of their their inner membranes, have organism-specific preferences for the sequence of SecA. Description of the organism-specific SecA sequences would inform on the general physical-chemical principles of protein secretion in bacteria, and could help select of a wider set of model SecA proteins for experimental studies.

With this aim in mind, I worked together with Prof. Coral del Val (University of Granada, Spain) on bioinformatics analyses of SecA sequences. The hand-curated dataset we used included 425 sequences. We found that SecA sequences can be as short as 787 amino-acid residues, and as long as 1160 amino-acid sequences. For comparison, the *E. coli* SecA sequence has 901 amino acid residues. Most of the SecA sequences carry an estimated net negative charge, which for about half of the sequences is about -39.5*e* to -25.5*e*, but it can be as large as -107*e* in the case of SecA from the halophile *Salinibacter ruber*; some outliers, such as SecA from the symbiont *Buchnera aphidicola*, had an estimated positive charge. Sequences of the PBD can be highly different among SecA from different organisms; by contrast, NBD1 is highly conserved.

The remarkable diversity we identified for the length, net estimated charge, and PBD sequences within the large dataset we used highlights the importance of extending experimental and theoretical studies on SecA to proteins from a more diverse set of organisms, particularly to understand how SecA proteins that include large numbers of charged groups interact with the membrane-bound translocon of those organisms.

Manuscript discussed:

Del Val C, Bondar A-N. Sequence analyses identify remarkable diversity of the bacterial SecA protein motor. Submitted to *Biochimica Biophysica Acta Biomembranes* Special Issue, under revision

4.5.2 Related research on SecA

In my research group we pursued over the years work aiming to characterize the reaction coordinate of SecA. To facilitate analyses of the complex H-bond networks of SecA, our research on SecA included the development of specialized data analysis tools (see section 4.1.4). We identified dynamic H bonds that inter-connect functional domains of SecA, and thus could contribute to long-distance conformational coupling.[169] We presented a model of *B. subtilis* SecA bound to ADP and to a signal peptide model, and showed that presence of the signal peptide alters motions of SecA, including of NBD2.[170] We further constructed models of *B. subtilis* bound to ATP and Mg^{2+}, finding that changes in nucleotide-protein interactions at the nucleotide binding interface associate with altered dynamics at the PBD, and identified H-bond paths that could contribute to such long-distance conformational coupling.[171] Analyses of a set of crystal structures of SecA led us to conclude that, at least in the absence of interaction partners, the conformational dynamics of SecA could be described in terms of the intra- vs. inter-domain contacts between protein groups of a functional domain: whereas the number of unique internal, intra-domain H bonding and hydrophobic contacts, showed little variation among the different crystal structures we analyzed (although intra-domain contacts of the PBD had somewhat larger variation), the number of inter-domain contacts of PBD groups varied.[202]

4.5.3 Related research on the SecY protein translocon

Within the collaboration with Prof. Peter Pohl (University of Linz) I contributed with a homology model of the *E. coli* SecY translocon and with estimations of dipole moments that were used to interpret data on voltage sensing by SecY.[203]

4.5.4 Perspectives on *Sec* proteins research

The discussion above documents our contributions to the understanding of mechanisms of long-distance conformational coupling in SecA, particularly the role of dynamic H bonds and dynamics at the nucleotide-binding site. In collaboration with Prof. Anastassios Economou (KU Leuven) we started to explore the conformational dynamics of SecA dimers; we are particularly interested in finding out how the dynamics of the PBD depends on the oligomeric state of SecA.

In the future, I plan to build upon this foundation to pursue QM/MM computations of the reaction coordinate of SecA. The recent bioinformatics analyses we performed, which identified a remarkable diversity of the sequences of SecA, highlights the need to expand studies of SecA to proteins from other organisms. A direction of research I envision working on is binding of SecA from different organisms to membranes whose lipid composition is relevant to the organism in which SecA works, as the charge of the SecA sequences could be related to the physiological environment in the cell. This research I plan on SecA binding to lipid membranes is thus closely related to the current work on Mistic (section 4.4.3).

4.6 Perspective: towards potential energy functions for bio-medical applications

MD simulations are our days often used to characterize the conformational dynamics of bio-molecules and to verify reaction mechanisms. As MD simulations rely on the potential energy function that describes how atoms of the system interact with each other, and this potential energy function includes force-field parameters (section 3.1), how reliable the MD simulations are –and, thus, how reliable predictions made from MD-will necessarily depend on the accuracy of the force-field parameters.

My interest in how force-field parameters impact results of MD simulations started with earlier work on retinal and retinal proteins. In that work we had showed, for example, that torsional barriers of the retinal polyene chain depend on constraints used to twist retinal bonds and on the model compound used,[204] and that dynamics of internal waters of squid rhodopsin depend on how retinal is treated.[156]

In my research group, we have dedicated significant efforts to derive accurate force-field parameters for cofactors of photosystem II,[205-206] and for an azobenzene-based photo-switchable lipid molecule that can be used to control lateral pressure in membranes.

During the research stay in my laboratory of Dr. Samo Lešnik from the National Institute of Chemistry Slovenia, Ljubljana, we initiated parametrization of fentanyl, which is an opioid drug of interest for the treatment of pain. In the future, I envision that we will use these

parameters to study binding of fentanyl to membranes of inflamed tissue characterized by low pH, i.e., protonation-dependent binding of fentanyl to opioid receptor GPCRs.

5. Summary

I presented here research that uses computational biophysics approaches to address the dissect mechanisms of reactions at membrane interfaces. Reactions at membrane interfaces are considered in a broad sense to include the chemical reaction of proton binding at a protein surface, and interactions between proteins, lipids and water molecules. We focus on exemplary proteins whose reaction mechanisms have implications for our general understanding of how proteins work, particularly proteins whose functioning involves changes in protonation, lipid interactions, and long-distance coupling between bond breaking and forming and protein conformational dynamics.

Long-distance proton transfer in protein environments is thought to occur via H-bonded chains, or wires, of protein titratable groups and water molecules. As such wires are dynamic, computational biophysics approaches are particularly valuable in that we can they allow us to evaluate protein and water motions in fluid lipid bilayers at room temperature, and to identify transient events during which proton donor and acceptor groups connect to each other via H-bonded wires. Moreover, we can probe the response of the protein and waters to changes in protonation, or to mutations that impact protein function. To be able to analyze dynamic H-bonded networks in complex environments such as channelrhodopsin, photosystem II, and lipid membrane interfaces, we implemented efficient data analysis algorithms, including a graph-based algorithm, Bridge, that we made openly available to the community[166] (section 4.1.4).

The knowledge we acquired on conformational dynamics, H-bond dynamics and reaction mechanisms of retinal proteins informs our current research on sequence-structure-function relationship in microbial rhodopsins. We demonstrated that mutations that alter H bonding can change protein and water dynamics of the bacteriorhdopsin proton pump[162] (section 4.1.1). From MD simulations, bioinformatics analyses, and inspection of crystal structures of membrane proteins, we identified an inter-helical hydroxyl-carboxylate motif we think important for proton transporters[17, 162, 165] (sections 4.1.2, 4.1.3) As a natural development of research on sequence-structure-function relationships of retinal proteins, we now probe with simulations which mutations might need to needed to convert a mutant proton pump into a sodium pump (Figure 8, section 4.1.5), and dissect interactions that control retinal dynamics and retinal protonation in a visual rhodopsin (Figures 9-10, section 4.1.6).

Photosystem II (Figure 3) and its soluble subunit PsbO (Figure 4) are model systems we use to decipher mechanisms of long-distance proton transfers via protein-water H-bonded wires, and for the potential function of carboxylate clusters as proton antennas. The data analyses protocols we presented for simulations of carboxylate-water clusters on the surface of PsbO,[172, 175] and for the conservation of charge in sequences of PsbO,[189] could be applied to study other proteins with carboxylate clusters on their surface. Our observations on the dynamics of protein-water wires inside photosystem II[176] and at the surface of PsbO[172, 175] (sections 4.2, 4.3) suggest that long-distance proton transfers in complex protein environments involves transient H bonds whose dynamics depends on the length of the wires, and on interactions with the environment.

As membrane proteins function in lipid bilayers, it is of paramount importance to understand how the lipid membrane composition influences the structure, dynamics, and reactions of membrane proteins. The enzyme I used as a model system, the intramembrane protease GlpG (Figure 7), is a particularly useful model system because it is relatively small, its catalytic activity has been tested in different lipid membrane environments, and because deciphering intramembrane proteolysis catalyzed by GlpG could inform on how the much more complex γ-secretase cleaves the amyloid precursor protein. Simulations suggest that one potential mechanism by which lipids could shape the reaction coordinate of GlpG is by transient binding the active site where the substrate needs to dock[207] (section 4.4).

A highly complex reaction that occurs at the membrane interface is SecA-mediated protein secretion. We have found that conformational dynamics of SecA and long-distance conformational coupling of SecA involve dynamic H bonds, derived structural models that describe molecular interactions along the reaction coordinate of SecA, probed the dynamics of nucleotide binding, and identified features that characterize sequences of SecA from a large dataset (see section 4.5). Our analyses of SecA sequences indicate that SecA proteins tend to be highly negatively charged, which is intriguing given that negatively-charged lipids appear important for the stability of the SecA-SecY complex.[201] Work we do on the binding of the smaller protein Mistic to lipid membranes (section 4.4.4) might inform on how a charged protein can interact with membranes.

6. Perspectives

Computational biophysics provides valuable tools to explore the workings of biomolecules. I envision that our experience with proton-transfer systems will serve as foundation for research I plan on protonation reactions of biomolecules of direct relevance to human disease, particularly cancer, since the extracellular pH of cancer cells can be acidic.[208]

Another important challenge of studies on proteins of direct interest to medical applications is that these proteins might interact with drug molecules that are poorly represented in current force fields. Yet, understanding how the drug molecule binds to the protein target could be essential for the process of drug design. A possible solution to this issue, the derivation of accurate force-field parameters, can be time consuming, and additive force fields can be limited in their description of, e.g., pH-dependent binding of a drug molecule to a protein. In the future, developments in polarizable force fields and more efficient implementations of quantum mechanical computations could facilitate efficient and accurate screening of protein-drug interactions.

Advances in computational power and methodologies might enable accurate studies of entire protein interaction networks in physiological environments, e.g., of protein interaction networks involved in GPCR cell signaling. Such large-scale computations will bring about the challenge of data analysis for the simulation trajectories, but also the challenge of working together with experimentalist colleagues to incorporate into simulations data from, e.g., biochemistry. One methodological development I anticipate working on is extending the graph-based algorithms we developed in my research group[166] to catalogue H-bond networks of exemplary proteins –e.g., GPCR proteins and their G protein counterparts- from computations of the wild-type and mutant proteins, combine the catalogues of H-bond networks from protein simulations with machine learning approaches that rely on both the simulations and experimental structural biology data, and then develop algorithms that can predict how the proteins will respond to interactions within their signaling interaction network.

7. Publications explicitly listed in the various sections of this thesis

Names of members of my research group are underlined. Corresponding authorship is indicated by '*', and co-corresponding authorship by '#'. A description of my contribution is indicated for each publication.

7.1 Publications for main discussion of research

1. del Val C, Bondar A-N#. Sequence analyses identify remarkable diversity of the bacterial SecA protein motor. Submitted to *BBA - Biomembranes*

   Research co-design, data co-interpretation, manuscript co-writing

2. Bondar A-N#, Mishima H, Okamoto Y. Molecular movie of nucleotide binding to a motor protein. Submitted to *BBA – General subjects*

   Research design, performed MD simulations, prepared figures, wrote manuscript with input from collaborator Yuko Okamoto

3. Siemers M, Lazaratos M, Karathanou K, Brown K, Bondar A-N. Bridge: A graph-based algorithm to analyze dynamic H-bond networks in membrane proteins. *Journal of Chemical Theory and Computation* 15:6781-6798, doi 10.1021/acs.jctc.9b00697 (2019)

   Research design and supervision, provided MD simulation trajectories, manuscript co-writing together with co-authors

4. Bondar A-N#, Lemieux J. Reactions at membrane interfaces. *Chemical Reviews* 119:6162-6183 (2019)

   Co-wrote review article

5. Bondar A-N*. Mechanisms by which lipids influence conformational dynamics of the GlpG intramembrane rhomboid protease'. *Journal of Physical Chemistry B* 123:4159-4172 (2019)

   Designed and performed research, wrote manuscript

6. Kemmler L, Ibrahim M, Dobbek H, Zouni A, Bondar A-N. Water hydrogen bonding and proton transfer at the interface between the PsbO and PsbU subunits of photosystem II. *Physical Chemistry Chemical Physics* 21:25449-25466 (2019)

   Research design and supervision, manuscript co-writing together with co-authors

7. Karathanou K, Bondar A-N*. Using graphs of dynamic hydrogen bonds to dissect conformational coupling in a protein motor. *Journal of Chemical Information and Modeling* 15:1882-1896, doi: 10.1021/acs.jcim.8b00979 (2019)

   Research design and supervision, preliminary MD simulations, manuscript co-writing

8. Guerra F, Siemers M, Mielack C, Bondar A-N*. Dynamics of long-distance hydrogen-bond networks in photosystem II. *Journal of Physical Chemistry B* 122:4625-4641 (2018)

   Research design and supervision, manuscript co-writing

9. Karathanou K, Bondar A-N*. Dynamic water hydrogen-bond networks at the interface of a lipid membrane containing palmitoyl-oleoyl phosphatidylglycerol. *Journal of membrane Biology* (Topical Collection) 251:461-473 (2018)

   Research design and supervision, manuscript co-writing

10. Milenkovic S, Bondar A-N*. Motions of the SecA protein motor bound to signal peptide: Insights from molecular simulations. *Biochimica et Biophysica Acta (Biomembranes)* 1860, 416-427 (2018)

    Research design and supervision, manuscript co-writing

11. Bondar A-N#, Smith JC. Protonation-state coupled conformational dynamics in reaction mechanisms of channel and pump rhodopsins. *Photochemistry and Photobiology* 93, 1336-1344 (2017).

Co-wrote manuscript

12. del Val C, Bondar A-N#. Charged groups at binding interfaces of the PsbO subunit of photosystem II: a combined bioinformatics and simulation study. *Biochimica et Biophysica Acta (Bioenergetics)* 1858, 432-441 (2017)

Research co-design, performed MD simulations and analyses, manuscript co-writing

13. Milenkovic S, Bondar A-N*. Mechanism of conformational coupling in SecA: Key role of hydrogen-bonding networks and water interactions. *Biochimica et Biophysica Acta (Biomembranes)* 1858, 374-385 (2016)

Research design and supervision, manuscript co-writing

14. Bondar A-N*. Biophysical mechanism of rhomboid proteolysis: setting a foundation for therapeutics. *Seminars in Cell and Developmental Biology* 60, 46-51 (2016)

Wrote paper

15. Bommer M, Bondar A-N#, Zouni A, Dobbeck H, Dau H. Crystallographic and computational analysis of the barrel part of the PsbO protein of photosystem II: carboxylate-water clusters as putative proton transfer relays and structural switches. *Biochemistry* 55, 4626-4635 (2016)

Contributed MD simulations, data interpretation

16. Lorch S, Capponi S, Pieront F, Bondar A-N*. Dynamic carboxylate/water networks on the surface of the PsbO subunit of Photosystem II. *Journal of Physical Chemistry B* 119, 12172-12181 (2015)

Research design and supervision, manuscript co-writing

17. Guerra F, Bondar A-N*. Dynamics of the plasma membrane proton pump. *Journal of Membrane Biology*, 248:443-453 (2015)

Research design and supervision, manuscript co-writing

18. del Val C, Bondar ML, Bondar A-N*. Coupling between water dynamics and inter-helical hydrogen bonds in a proton transporter. *Journal of Structural Biology* 186:95-111 (2014)

Study design, performed and analyzed MD simulations, co-wrote manuscript

19. del Val C, Royuela-Flor J, Milenkovic S, Bondar A-N#. Channelrhodopsins: a bioinformatics perspective. *Biochimica et Biophysica Acta (Bioenergetics)* 1837:643-655 (2014)

Research design, homology modeling, co-analyzed data, manuscript co-writing

20. Bondar A-N# and Dau H. Extended protein/water H-bond networks in photosynthetic water oxidation. *Biochimica et Biophysica Acta* (Bioenergetics) 1817: 1177-1190 (2012)

Structure analyses, co-wrote manuscript

7.2. Publications mentioned in discussion of related research

1. Klaja O, Frank J, Trauner D, Bondar A-N*. Potential energy function for a photo-switchable lipid molecule. Submitted to the *Journal of Computational Chemistry*

   Research design and supervision, manuscript co-writing

2. Gerland L, Friedrich D, Hopf L, Donovan EJ, Wallmann A, Erdmann N, Diehl A, Bommer M, Buzar K, Ibrahim M, Schmieder P, Dobbek H, Zouni A, Bondar A-N, Dau H, Oschkinat H. pH-dependent protonation of surface carboxylates in PsbO enables local buffering and triggers structural changes. *ChemBioChem*, doi: 10.1002/cbic.201900739 (2020)

   Contributions to results discussion and writing

3. Knyazev, DG, Kuttner R, Bondar A-N, Zimmerman M, Siligan C, Pohl P. Voltage sensing in bacterial protein translocation. *Biomolecules* 10, 78. doi: 10.3390/biom10010078 (2020)

   Contributed homology model of *E. coli* SecY, dipole moment estimations

4. Pashkowsky S, Recinto SJ, Bondar A-N, Munter LM. Membrane cholesterol as regulator of human rhomboid protease RHBDL4. *Journal of Biological Chemistry*, doi: 10.1074/jbc.RA118.002640 (2018)

   Contributed homology model of RFHBL4 for data interpretation

5. Adam S, Knapp-Mohammady M, Yi J, Bondar A-N*. Revised CHARMM force-field parameters for iron-containing cofactors of photosystem II. *Journal of Computational Chemistry* 39, 7-20 (2018)

   Research design and supervision, manuscript co-writing

6. Adam S, Bondar A-N*. Mechanism by which water and protein electrostatic interactions control proton transfer at the active site of channelrhodopsin. *PLoS One*. 13(8), e0201298 (2018)

   Research design and supervision, manuscript co-writing

7. Karathanou K, Bondar A-N*. Dynamic hydrogen-bond networks in bacterial protein secretion. *FEMS Microbiology Letters* 365, fny 124 (2018)

   Invited minireview

   Co-wrote manuscript

8. Harris A, Saita M, Resler T, Hughes-Visentin A, Maia R, Sellnau F, Bondar A-N, Heberle J, Brown LS. Molecular details of the unique mechanism of chloride transport by a cyanobacterial rhodopsin. *Physical Chemistry Chemical Physics* 20, 3184-3199 (2018)

   Contributed homology model used for data interpretation

9. Nango A, Royant A, Kubo M, Nakane T, Wickstrand C, Kimura T, Tanaka T, Tono K, Song C, Tanaka R, Arima T, Yamashita A, Kobayashi J, Hosaka T, Mizohata E, Nogly P, Sugahara M, Nam D, Nomura T, Shimamura T, Im D, Fujiwara T, Yamanaka Y, Jeon B, Nishizawa T, Oda K, Fukuda M, Andersson R, Båth P, Dods R, Davidsson J, Matsuoka S, Kawatake S, Murata M, Nureki O, Owada S, Kameshima T, Hatsui T, Joti Y, Schertler G, Yabashi M, Bondar A-N, Standfuss J, Neutze R, Iwata S. A three-dimensional movie of structural changes in bacteriorhodopsin. *Science* 354, 1552-1557 (2016)

   Contributed QM/MM computations

10. Wolter T, Elstner M, Fischer S, Smith JC, and Bondar A-N#. Mechanism by which untwisting of retinal leads to productive bacteriorhodopsin photocycle states. *Journal of Physical Chemistry B*, *Special Issue on Photoinduced Proton Transfer in Chemistry and Biology Symposium*, 119:2229-2240 (2015)

    Research co-design, QM/MM computations for water translocation, manuscript co-writing

11. Karge O, Bondar A-N, Dau H. Photosystem II: Cationic screening of charged surface groups (carboxylates) affects electron transfer steps in water oxidation and quinone reduction. *Biochimica Biophysica Acta (Bioenergetics)* 1837:1625-1634 (2014)

    Contributed analysis of the distribution of charged groups in a photosystem II structure

12. Harris A, Ljumovic M, Bondar A-N, Shibata Y, Ito S, Inoue K, Kandori H, Brown LS. A new group of eubacterial light-driven retinal-binding proton pumps with an unusual cytoplasmic proton donor. *Biochimica et Biophysica Acta (Bioenergetics)* 1847, 1518-1529 (2015)

    Contributed structural model used for data interpretation

13. Guerra F, Adam S, Bondar A-N*. Revised force-field parameters for chlorophyll-*a*, pheophytin -*a* and plastoquinone-9. *Journal of Molecular Graphics and Modelling* 58, 30-39 (2015)

    Research design and supervision, manuscript co-writing

8. Acknowledgement of research funding and computing resources

I am grateful for the funding and computing resources that supported and made possible research presented in this Thesis. For research funding I thank the German Research Foundation (DFG) for funding within SPP 1926 Next Generation Optogenetics: Tool development and application (as co-PI), for the funding and renewal of Project C4 (as PI) within the Collaborative Research Center 'Protonation Dynamics in Protein Function', SFB 1078. I thank the European Commission for the Marie Curie International Reintegration Grant FP7-PEOPLE-2010-RG-276920 'Mechanisms of transport across cellular membranes', and for the project within the recently approved Innovative Training Network 'PROTON – Proton transport and proton-coupled transport'. I thank the Freie Universität Berlin for the funding within the Excellence Initiative of the German Research Foundation. I thank the Center for Research Strategy of the Freie Universität Berlin for funding the scholarship of a visiting post-doctoral scholar, the Collaborative Research Center SFB 1078 for doctoral scholarships that supported students in my laboratory and for funding a storage system, and the Collaborative Research Center SFB 1078, the Center for Research Strategy of the FU, and the Center for International Cooperation of the FU for travel funding and for funding that supported visiting collaborators. I thank the NanoScale Focus Area of the FU Berlin, and the Center for Supramolecular Interactions of the Institute for Chemistry and Biochemistry of the FU, for research funding. For computing resources I thank the North-German Supercomputing Alliance (HLRN), and the Freie Universität for ZEDAT and Physics Department computing.

9. Acknowledgements

Much of the research I presented here is the result of common efforts with colleagues - co-workers in my laboratory and collaborators. I consider myself privileged for having worked with my colleagues, whose names are mentioned throughout the text and in the publications enclosed with this thesis. I am deeply grateful to all my colleagues.

I would like to thank Prof. Joachim Heberle for his support of my research and career throughout these years.

Our research would not have been possible without the entire infrastructure of the FU Berlin, and I am grateful for the administration and computing assistance I, and my co-workers, have received over the years, particularly from Mrs. Sieglinde Endrias, Mrs. Sylvia Luther and Mr. Jens Dregger from the FU Berlin, and Dr. Christian Tuma from the HLRN. I am grateful to the late Mrs. Tanja Binder for her assistance with the Marie Curie grant.

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Appendix

Appendix 1: Publication List

PRIMARY RESEARCH PUBLICATIONS

Corresponding authorship is marked by '*', and shared corresponding authorship by '#'.
Berlin lab members are underlined.

53. Klaja O, Frank J, Trauner D, Bondar A-N*. Potential energy function for a photo-switchable lipid molecule. Submitted to the *Journal of Computational Chemistry*
52. del Val V, Bondar A-N#. Sequence analyses identify remarkable diversity of the bacterial SecA protein motor. Submitted to *BBA - Biomembranes*
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11. Bondar A-N, del Val C, and White SH. Rhomboid protease dynamics and lipid interactions. *Structure* 17: 395-405 (2009)

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12. Bondar A-N*. Biophysical mechanism of rhomboid proteolysis: setting a foundation for therapeutics. *Seminars in Cell and Developmental Biology* 60, 46-51 (2016)
11. Bondar A-N# and Dau H. Extended protein/water H-bond networks in photosynthetic water oxidation. *Biochimica et Biophysica Acta* (Bioenergetics) 1817: 1177-1190 (2012)
9. Bondar A-N# and White SH. Hydrogen bond dynamics in membrane protein function. *Biochimica et Biophysica Acta* (Biomembranes) 1818: 942-950 (2012)
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6. Bondar A-N. Bacteriorhodopsin proton pumping mechanism: insights from computer simulations. *Revue Roumaine de Chimie* (Special Issue) 54: 433-439 (2009)
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5. Lindow N, Baum D, Bondar A-N, and Hege H-C. Dynamic channels in biomolecular systems: Path analysis and visualization. *IEEE Symposium on Biological Data Visualization* 2012, October 14-15 Seattle, Washington, USA, 978-1-4673-4730-3, 99-106 (2012)
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5. Bondar A-N. Excelenta in educatia universitara: rolul cercetarii stiintifice (Excellence in University education: role of undergraduate research). *Revista de politica stiintei si scientometrie* – serie noua 1:67-68 (2012)
4. Bondar A-N, Woolf T and Tobias DJ. Festschrift in the honor of Stephen H. White's 70th Birthday. *Journal of Membrane Biology* 239: 1-3 (2011)
3. Bondar A-N and Frangopol PT. Young Roumanian Scientists. *Revue Roumaine de Chimie* 54: 409 (2009)
2. Bondar A-N. Homage to Petre T. Frangopol at his 75th Anniversary. *Revista Romana de Chimie* 59: 1173-1174 (2008)
1. Co-authored with P. T. Frangopol a piece on 'Quo vadis cercetarea stiintifica din Romania' (Quo vadis scientific research in Romania), in 'Pentru excelenta in Romania' (For excellence in Romania), eds. Petre T. Frangopol, Nicolae Victor Zamfir, Tibor Braun, Casa Cartii de Stiinta, Cluj-Napoca 2008, 50-64, also included as book chapter in 'Mediocritate si excelenta. O radiografie a stiintei si invatamantului din Romania' by Petre T. Frangopol, Casa Cartii de Stiinta, Cluj Napoca, 2008

CONFERENCE ABSTRACTS

Berlin lab members are underlined.

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20. Guerra F and Bondar A-N. Dynamic hydrogen-bonding networks in photosystem II. Biophysical Journal Supplement 112:507a (2017)
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17. Capponi C, M Heyden M, Bondar A-N, AJ Freites, Tobias DJ, and White SH. Dynamics of water inside the SecY translocon complex. *Biophysical Journal Supplement* 104: 410a (2013)
16. Heyden M, Jardon-Valadez HE, Bondar A-N, and Tobias DJ. GPCR activation on the microsecond timescale in MD simulations. *Biophysical Journal Supplement* 104: 115a (2013)

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14. Bondar A-N, Tobias DJ and White SH. Long-distance lipid:protein coupling in the protein translocon. *Biophysical Journal Supplement* 102:714a (2012)

13. Heyden M, Jardon-Valadez HE, Bondar A-N, and Tobias DJ. Microsecond dynamics of the G-protein coupled receptor squid rhodopsin in atomistic detail. *Biophysical Journal Supplement* 102: 622a (2012)

12. Radu I, Nack M, del Val C, Bondar A-N, Abbruzzetti S, Schulz B, Schlesinger R, Viappiani, C, and Heberle J. Kinetics of proton release and uptake by channerhodopsin-2. *Biophysical Journal Supplement* 102: 575a (2012)

11. Bondar A-N, Tobias DJ, and White SH. Opening the protein translocon. *Biophysical Journal Supplement* 100: 363a (2011)

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8. Bondar A-N, Tobias DJ, and White SH. How binding of the signal peptide unlocks the translocon. *Biophysical Journal Supplement* 98: 435a (2010)

7. Bondar A-N, Tobias DJ, and White SH. How prl mutations or binding of the signal peptide unlocks the translocon. *Protein Society* 19, Supplement 1: 232 (2010)

6. Bondar A-N and White SH. A relay mechanism in rhomboid intramembrane protease. *Biophysical Journal Supplement* 96: 592a (2009)

5. Jardon E, Bondar A-N, and Tobias DJ. Dynamics of the internal water molecules in squid rhodopsin. *Biophysical Journal Supplement* 96: 680a (2009)

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2. Bondar A-N and White SH. Lipids, water, and protein interactions of intramembrane rhomboid protease. *Biophysical Journal Supplement* 94: 42-Plat (2008)

1. Bondar A-N, Elstner M, Fischer S, Suhai S, and Smith JC. Quantum Mechanical/Molecular Mechanical investigation of bacteriorhodopsin proton pumping. *Chimia* 59: 406 (2005)

Appendix 2: Invited and Contributed Talks

CONFERENCE SESSION CHAIR OR PANEL MEMBER

8. Co-chair, platform session Membrane Proteins I, 63rd Biophysical Society Meeting, March 2-6, 2019, Baltimore, USA
7. Member of the Membrane Biophysics Subgroup Juror panel for the Student Research Achievement Award Poster Competition at the 63rd Biophysical Society Meeting, March 2-6, 2019, Baltimore, USA
6. Member of the Juror panel for selecting the Best Contributed Talk at the International workshop on lipid membranes, University of Helsinki, Finland, August 19-22, 2018
5. Invited chairperson, International Symposium on Physics of Life, on the occasion of Prof. Tsutomu Kouyama's retirement. University of Nagoya, Nagoya, Japan, March 25-25, 2017
4. Invited chairperson of the session on Intramembrane Proteases of the Gordon Research Conference on Protons and Membrane Reactions, February 23-28, 2014, Ventura, California, USA
3. Co-chair, platform session AU: Membrane Protein Function, Biophysical Society 56th Annual Meeting, San Diego, California, February 25-29, 2012
2. Co-chair, platform session AP: Membrane Pumps & Transporters. 54th Biophysical Society Meeting, San Francisco, California, USA, March 20-24, 2010
1. Co-chair of the platform session AR: Membrane protein function. 53rd Biophysical Society Meeting, Boston, USA, February 28- March 4, 2009

INVITED TALKS AT SCIENTIFIC MEETINGS

32. 'Proton binding at membrane interfaces'. CECAM workshop Frontiers in Multiscale Modeling of Photoreceptor Proteins. Tel Aviv, Israel, July 2-5, 2019
31. 'Protonation-coupled protein and water dynamics in retinal proteins'. 18th International Conference on Retinal Proteins, Hockley Valley Resort, Ontario, Canada, September 24-29, 2018
30. 'Reactions at lipid membrane interfaces'. International workshop on lipid membranes, University of Helsinki, Finland, August 19-22, 2018
29. 'Proton transfer at membrane and protein interfaces'. Telluride workshop on Proton Transfers in Biology. Telluride, Colorado, USA, July 8-13, 2018
28. 'Hydrogen bonding and membrane reactions'. Plenary talk. Prague Membrane Discussions, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, June 21, 2018
27. 'Mechanism by which lipids shape reaction mechanisms of rhomboid proteases'. Bridge over troubled waters – proteases in disease and aging. Universitätsmedizin Mainz, Mainz, Germany, August 24-26, 2017
26. 'Hydrogen bonding and membrane reactions'. Telluride workshop on Protein Electrostatics, Telluride, USA, June 26-30, 2017
25. 'Hydrogen bonding and proton transfer at molecular interfaces'. International Symposium on Physics of Life, on the occasion of Prof. Tsutomu Kouyama's retirement. University of Nagoya, Nagoya, Japan, March 25-25, 2017

24. ‘Hydrogen bonding at charged biomolecular interfaces’. Meeting ‘Proton transfer in complex environments’, on the occasion of Prof. Mechem Gutman’s 80th Birthday. University of Tel Aviv, Tel Aviv, Israel, October 25, 2016

23. ‘Hydrogen-bond dynamics in retinal protein function’. Satellite meeting of the 17th International Conference on Retinal Proteins, Potsdam, Germany, October 2, 2016

22. ‘Dynamic protein/water hydrogen-bond networks in proton transfers’. Telluride workshop on proton transfers in biology. Telluride, Colorado, USA, July 12-16, 2016

21. ‘Lipid-protein coupling in protein function’. Meeting of the DFG Research Group FG1279 Optogenetics, March 29-31, Chorin, Germany

20. ‘Ion antennas and transport across cell membranes’. Kick-off Symposium of the Frontier Research Institute for Materials Science, Nagoya Institute of Technology, Nagoya, Japan, March 1-2, 2016

19. ‘Proton transfer and water dynamics in retinal proteins’. Pacifichem 2015, Symposium on Chemistry and Applications of Retinal Proteins. Honolulu, Hawaii, USA, December 17-20, 2015

18. ‘Dynamics of the protein secretion machinery’. CECAM Workshop ‘Understanding function of proteins in membrane by atomistic and multiscale simulations’ Lugano, Switzerland, November 10-12, 2015

17. ‘Mechanisms of conformational coupling in membrane proteins’. DFG-Rungespräch Photoreceptors & Meeting of DFG-Forschergruppe 1279. Abtei Frauenwörth, Freuenchiemsee, Germany, October 8-12, 2015

16. ‘The protein secretion machinery: a view from all-atom simulations’. Annual meeting of the Swedish Chemical Society, Theoretical Chemistry Section 2015 Baltic Lights, Kalmar, Sweden, August 25-27, 2015

15. ‘The protein secretion machinery: a perspective from theoretical biophysics’. DK Summer school 2015, St. Wolfgang, Austria, July 5-8, 2015

14. ‘Hydrogen bonding and lipid interactions in GlpG protease function’. 18. Deutsche Physikerinnentagung, Dresden, Germany, October 16-19, 2014

13. ‘Hydrogen bond dynamics and directional proton transfers in microbial rhodopsins’. 16th International Conference on Retinal Proteins, Nagahama, Japan, October 5-10, 2014

12. ‘Hydrogen bonding and lipid interactions in membrane protein function’. 564. WE-Heraeus Seminar – Physical approaches to membrane protein function. Bad Honnef, Germany, May 25-28, 2014

11. ‘Water and hydrogen bond dynamics in proton transfer systems’. Les Houches – TSRC Workshop on protein dynamics, Les Houches, France, May 19-23, 2014

10. ‘Dynamics of substrate binding to GlpG rhomboid proteases’. Gordon Research Conference on regulated proteolysis of cell surface proteins. Ventura, California, USA, March 30-April 4, 2014

9. ‘Waters in proton transfer systems’. RESOLV IFF Meeting, Zürich, Switzerland, October 3-4, 2013

8. ‘Coupling between hydrogen bonding and water dynamics in proton transporters’. Optogenetics Forschergruppe Meeting, October 10-11, 2013, Heidelberg, Germany

7. ‘Proton-coupled water and protein dynamics in complex proton-transfer systems’. Workshop on Membrane Protein Folding and Functioning, August 5-9 2013 Telluride, Colorado, USA

6. ‘Lipid and hydrogen bonding in membrane protein function’. Workshop ‘From Computational Biophysics to Systems Biology (CBSB13)’ May 19-21, 2013, Norman, Oklahoma, USA

5. ‘Inter-helical hydrogen bonds and water dynamics in microbial rhodopsins’. 15th International Conference on Retinal Proteins, September 30-October 5, 2012, Ascona, Switzerland

4. ‘Long-distance conformational coupling in the GlpG intramembrane protease’. 7th Fabisch Symposium for cancer research and molecular cell biology – Regulated intramembrane proteolysis in cancer development and neurodegenerative diseases. Potsdam, Germany, October 5-7, 2011

3. ‘Role of hydrogen bonding in helix-gating membrane proteins’. International Biophysics Symposium at the Nagoya University, March 12-14, 2010, Nagoya, Japan

2. ‘Dynamics and lipid interactions of helix-gating membrane proteins’. Research Symposium on the occasion of Jeremy Smith’s 50th Birthday. IWR, University of Heidelberg, Germany, December 9, 2009

1. ‘Role of water molecules in bacteriorhodopsin proton transfer reactions’. Israel Science Foundation Workshop ‘Diffusion, solvation and transport of protons in complex and biological systems’. Eilat, Israel, January 13-17, 2008

INVITED SEMINARS

17. Proton binding at membrane interfaces. University of Bucharest, Department of Physics. Bucharest-Magurele, Romania, November 28, 2019

16. Proton binding at membrane interfaces. Forschungszentrum Jülich, Computational Biomedicine, Jülich, Germany, November 5, 2019

15. Proton binding at membrane interfaces. University of Tokyo, Institute of Solid State Physics, Kashiwa, Chiba, Japan, October 8, 2019. Part of the research visit supported by the JSPS FoS Program

14. Reactions at membrane interfaces. Paul Scherrer Institute, Department of Biology and Chemistry, Villingen, Switzerland, November 29, 2018

13. Reactions at membrane interfaces: Bacterial protein secretion and intramembrane proteolysis. Nagoya University, School of Science, Department of Physics. August 31, 2018, Nagoya, Japan, within the JSPS-BRIDGE fellowship

12. Dynamic hydrogen-bond networks for proton transfers in bio-systems. Nagoya University, School of Science, Department of Physics. August 30, 2018, Nagoya, Japan, within the JSPS-BRIDGE fellowship

11. Hydrogen bonding and membrane reactions. Weizmann Institute, Department of Structural Biology, Rehovot, Israel, April 29, 2018

10. Hydrogen bonding and membrane reactions. Hebrew University of Jerusalem, Fritz Haber Center for Molecular Dynamics Research. Jerusalem, Israel, April 25, 2018

9. ‘Hydrogen bonding and lipid interactions in membrane protein function’. Department of Biology, Technical University of Kaiserslautern, February 26, 2018

8. ‘Hydrogen bonding and lipid interactions in membrane protein function’. Department of Chemistry, Brandeis University, USA. February 16, 2017

7. ‘The Sec protein secretion machinery: understanding reaction coordinates with computer simulations. Nara Institute of Science and Technology, Nara, Japan, February 26, 2016.

6. ‘Water and hydrogen bonding in proton-transfer reactions’. Nagoya Institute of Technology, department of Frontier Materials, Nagoya, Japan, February 24, 2016

5. ‘Water and hydrogen bonding in proton-transfer reactions’. Nagoya University, Graduate School of Science, J-Lab, Nagoya, Japan, February 23, 2016

4. ‘Hydrogen bond dynamics in membrane protein function’. Tel-Aviv University / Freie Universität Berlin Membrane Workshop, Freie Universität Berlin, Berlin, Germany, September 15-16, 2014

3. ‘Membrane protein function and lipid interactions’. Johannes Keppler Universität Linz, Department of Physics, Linz, Austria, June 26, 2014

2. ‘Hydrogen-bond dynamics and lipid interactions in membrane protein function’. Humboldt-Universität zu Berlin, Institut für Biologie, Berlin, Germany, June 26, 2012

1. ‘Role of hydrogen bonding in the opening of the SecYEG protein translocon’. University of California, Davis, Department of Chemistry, the Stuchebrukhov research group. Davis, USA, April 10, 2009

CONTRIBUTED TALKS AT SCIENTIFIC MEETINGS

19. ‘Lipids, water and protons’. TSRC Telluride Workshop on Protein Electrostatics. Telluride, Colorado, USA, June 24-28, 2019

18. ‘Hydrogen bonding and proton transfer at complex biomolecular interfaces’. CECAM meeting ‘Tackling complexity of the nano-bio interface – computational and experimental approaches’. Bremen, Germany, June 12-16, 2017

17. ‘Mechanism by which lipids shape reaction coordinates of rhomboid proteases’, Gordon Research Conference on Membrane Protein Folding, Easton, MA, USA, June 4-9, 2017

16. ‘Mechanism of rhomboid intramembrane proteolysis’. DPG (German Physical Society) Meeting, Dresden, Germany March 19-20, 2017

15. ‘Proton-coupled water and hydrogen-bond dynamics in channelrhodopsin’. Platform presentation at the 2014 Biophysical Society Meeting, San Francisco, USA, February 14-19, 2014

14. ‘Lipid-coupled docking of transmembrane substrate by the GlpG rhomboid protease from Escherichia coli. Platform presentation at the 2012 Biophysical Society Meeting, San Diego, USA, February 25-29, 2012

13. ‘Lipid-mediated helix gating in the GlpG rhomboid protease from Escherichia coli’. Platform presentation at the 2011 Biophysical Society Meeting. Baltimore, Maryland, USA, March 5-9, 2011

12. ‘Lipid membrane composition has a dramatic effect on the dynamics of the GlpG rhomboid protease from *Escherichia coli*’. Platform presentation at the 2010 Biophysical Society Meeting, San Francisco, USA, February 20-24, 2010

11. ‘Pathways for opening the SecYEG protein translocon: role of hydrogen bonding and signal peptide interactions’. Gordon Research Conference on Protein Transport across Cell Membranes, Galveston, Texas, USA, March 7-12, 2010

10. ‘How prl mutations or binding of the signal peptide unlock the translocon’. Young Investigator Talk at the 24th Annual Symposium of the Protein Society, August 1-5, 2010, San Diego, California

9. ‘Opening the translocon: the role of hydrogen-bonding’. Gordon Research Conference on Protons and Membrane Reactions, Ventura, California, USA, February 22-27, 2009

8. ‘Opening the translocon: the role of hydrogen-bonding’. Platform presentation at the 2009 Biophysical Society Meeting, Boston, USA, February 28-March 4, 2009
7. ‘Lipids, water, and protein interactions of intramembrane rhomboid protease’. Platform presentation at the 2008 Biophysical Society Meeting, Long Beach, California, USA, February 2-6, 2008
6. ‘Quantum Mechanical/Molecular Mechanical investigation of bacteriorhodopsin proton pumping’. 11th International Conference on the Applications of Density Functional Theory in Chemistry and Physics. Geneva, Switzerland, September 11-15, 2005
5. ‘Mechanism of the retinal deprotonation step in the bacteriorhodopsin photocycle’. Workshop on Modeling interactions in Biomolecules II. Prague, Czech Republic, September 5-9, 2005
4. ‘Proton Transfer Pathways in Bacteriorhodopsin’. CECAM Meeting on Multiscale Modelling of Chemical Reactions. Lyon, France, September 3-5, 2003
3. ‘Theoretical Analysis of Proton Transfer Pathways in Bacteriorhodopsin’. CECAM (Centre Europeén de Calcul Atomique et Moléculaire) discussion meeting ‘Ion channels: from biology to physics’. Lyon, France, July 15-17, 2002
2. ‘Computer simulations of proton transfer in bacteriorhodopsin’. 2nd Fall Workshop. Complex Processes: modelling, simulation and optimisation. Bêdlewo, Poland, October 18-20, 2002.
1. ‘Quantum Mechanics/Molecular Mechanics study on the proton transport mechanism in Bacteriorhodopsin’. Human Frontier Science Program workshop on Bacteriorhodopsin, Heidelberg, Germany, August 26-28, 2001

Appendix 3: Brief Curriculum Vitae

Ana-Nicoleta Bondar

DEGREES

2004 **Doctor of Natural Sciences**, magna cum laudae, Ruprecht-Karls Universität Heidelberg, Germany, The Joint Faculty of Natural Sciences and Mathematics. Examination subject: biology. Doctoral research was in computational biophysics

1999 **Advanced studies**, 'Alexandru Ioan Cuza' University of Iasi, Romania, Faculty of Physics

1997 **Diploma in physics**, 'Alexandru Ioan Cuza' University of Iasi, Romania, Faculty of Physics

ACADEMIC EMPLOYMENT

01.12.2019- **Guest Professor**, Department of Physics, Freie Universität Berlin, Germany

2014-2019 **Professor** W2 fixed term, Department of Physics, Freie Universität Berlin, Germany

2010-2014 **Junior Professor W1**, Department of Physics, Freie Universität Berlin

2006-2010 **Assistant Project Scientist**, University of California, Irvine, Department of Physiology and Biophysics. Appointed as Assistant Project Scientist Step I in 2006, merit increase to Step II in 2008, and accelerated merit to Step IV in 2010

2004-2006 **Post-doctoral researcher** (scientific employee BAT IIA), University of Heidelberg, Interdisciplinary Center for Scientific Computing

2000-2004 **Doctoral researcher** (scientific employee BAT IIA/2) at the University of Heidelberg, Interdisciplinary Center for Scientific Computing; **doctoral student** at the the German Cancer Research Center Heidelberg Molecular Biophysics Department

2000 **Student Assistant**, University of Heidelberg, Interdisciplinary Center for Scientific Computing

NON-ACADEMIC EMPLOYMENT

1998-1999 **Referent**, National University Research Council, University of Bucharest, Romania

GRANTS

2019-2022 European Commission/ Marie Skłodowska Curie Actions, Innovative Training Network 'PROTON – Proton transport and proton-coupled transport' (PI)

2017-2020 German Research Foundation Collaborative Research Center 'Protonation Dynamics in Protein Function', SFB 1078, Project C4 'Coupling between

protein, water, and protonation dynamics in channelrhodopsins and photosystem II' (PI)

2016-2019 German Research Foundation Priority Program SPP 1926 Next Generation Optogenetics: Tool development and application. Grant on 'Photoswitchable lipids for optical control of mechanosensitive ion channels' (co-PI)

2012-2016 German Research Foundation Collaborative Research Center 'Protonation Dynamics in Protein Function', SFB 1078, Project C4 'Coupling between protein, water, and protonation dynamics in channelrhodopsins and photosystem II' (PI)

2011-2015 European Commission, Marie Curie International Reintegration Grant, FP7-PEOPLE-2010-RG-276920 'Mechanisms of transport across cellular membranes' (Fellow)

AWARDS AND HONORS

2018 Diploma of Excellence from the Embassy of Romania in Berlin. Berlin, October 30

2018 JSPS-BRIDGE Fellowship from the Japan Society for the Promotion of Science (16 days) Nagoya University, School of Science, Department of Physics. Nagoya, Japan

2018 Invited to attend the 14th Japanese-German Frontiers of Science Symposium of the Alexander von Humboldt Foundation and the Japanese Science for the Promotion of Science. Kyoto, Japan, September 6-9

2018 Certificate of Reviewing in recognition of the review made for BBA Bioenergetics

2012 Granada Excellence Network of Innovation Laboratories Strengthening through Short-Visits Fellowship (GENIL-SSV 2012) for the project 'Bioinformatics analyses of the SecA motor protein', November 6-18, 2012, University of Granada, Spain

2010 European Commission Marie Curie International Reintegration Grant

2006 Short-term Fellowship from the Japan Society for the Promotion of Science (20 days) Nagoya University, Nagoya, School of Science, Department of Physics

1992-1999 Merit stipend from the Al. I. Cuza University of Iasi, Faculty of Physics, for the 1st, 2nd and 3rd semesters of Advanced Studies. Merit stipend for the 8th semester of the undergraduate Diploma studies; study stipend for the 1st, 2nd, 6th, and 7th semesters

RESEARCH VISITS ABROAD

Tokyo University, Institute for Solid State Physics, Tokyo, Japan, planned for 27.09.2019-10.10.2019 Supported by the Follow-up Program of the JSPS Frontiers of Science (FoS) Symposium

Nagoya University, School of Science, Department of Physics. Nagoya, Japan. Supported by JSPS-BRIDGE fellowship. 26.08.2018 - 15.09.2018 (06.09 to 09.09: symposium in Kyoto)

University of Granada, Spain, Department of Computer Science and Artificial Intelligence. 06.11.2012 -18.11.2012. Supported by GENIL-SSV Fellowship

Nagoya University, School of Science, Department of Physics. Nagoya, Japan. Supported by JSPS short-term fellowship for 20 days, 2006

PROFESSIONAL SERVICE AND ACADEMIC MANAGEMENT

BOARD MEMBER

2018 - Member of the International Advisory Committee of the ICRP (International Conference on Retinal Proteins)

EDITORIAL SERVICE

2019 Initiated 'Young Investigator Featured', a new section of the Journal of Membrane Biology

2019 Co-Guest Editor of the Biochimica Biophysica Acta General subjects, Special Issue on Advances in Computational Molecular Biophysics. In progress

2019 Guest editor of the Chemical Reviews thematic issue 'Biomembrane Structure, Dynamics and Reactions'

2018 Co-Guest Editor of Journal of Membrane Biology Special Issue 'Lipid membranes & reactions at lipid interfaces'

2018- Section Editor – Biophysics Section, Journal of Membrane Biology

2016-2018 Associate Editor - Biophysics Section, Journal of Membrane Biology

ORGANIZING COMMITTEE MEMBER

2019 Co-applicant on the proposal for a Biophysical Society Conference entitled 'Protein Reactions: From Basic Science to Biomedical Applications', to be held in Ventura, California, August 20-24, 2021. Favorable review, we need to address sustainability for future meetings.

2018 CECAM (Centre Europeen de Calcul Atomique et Moleculaire) workshop 'Frontiers in computational biophysics: understanding conformational dynamics of complex lipid mixtures relevant to biology', January 10-12, Lugano, Switzerland

2013 CECAM workshop 'Coupling between protein, water, and lipid dynamics in complex biological systems: Theory and Experiments'. September 24-27, Lausanne, Switzerland

2010 Symposium 'Frontiers in Membrane and Membrane Protein Biophysics: Experiments and theory', University of California, Irvine, August 19-20

2008 CECAM workshop 'Membrane Protein Assembly: Theory and Experiments', Lausanne, Switzerland, September 3-6

PUBLIC OUTREACH

NEWSLETTER ARTICLE, NEWSPAPER INTERVIEW

2018 Report from the BRIDGE Fellowship 'Protein dynamics and enzyme reactions'. Neues von JSPS Club (Newsletter of the JSPS German Alumni Association, the Deutsche Gesellschaft der JSPS Stipendiaten e.V.) NvC Nr. 3/ 2018, Ausgabe 72, 6-8.

2016 'Uns verbindet die Physik', interview in the Free University Supplement to the Berlin Tagesspiegel, September 24, 2016, page B8.

TEACHING

PROFESSIONAL DEVELOPMENT

1-day seminar attended at the Further Education Center of the Freie Universität Berlin

2019 Assignment, guidance, and support of student tutors for natural sciences teaching (Einsatz, Anleitung, und Begleitung studentischer Tutor*inen für die naturwissenschaftliche Lehre)

2-day seminars attended at the Berlin Center for University Teaching (Berliner Zentrum für Hochschullehre, BZHL):

2019 Organization of oral examinations (Mündliche Prüfungen kompetenzorientiert gestalten)

2018 Activate the class with digital media (Aktivieren des Hörsaals mit digitalen Medien)

2018 Teaching International Students

2018 Competent advising of students (Studierende kompetent beraten)

German evening language courses attended at the Goethe Institute Berlin:

2019 Level C2_2

2019 Level C2_1

2018 Level C1

2019 Private German classes with Mr. Burkart Encke, Berlin

DIRECT SUPERVISION OF BACHELOR, DIPLOMA AND MASTER THESES

By month of completion of the thesis:

08/2019 Lukas Kemmler (Master), Water motions at negatively-charged protein interfaces

05/2017 Alexandra Krause (Bachelor), Study of water dynamics around ATP

10/2015 Malte Siemers (Bachelor), Water structures in channelrhodopsin

04/2015 Christian Spakowski (Master), Investigation of the effects of retinal isomerization and its conformational dynamics in channelrhodopsin-2 addressed by theoretical biophysical methods

08/2013 Christoph Mielack (Master), Proton-coupled water and hydrogen bond dynamics in channelrhodopsin

05/2013 Sebastian Lorch (Diploma), Proton-coupled water and hydrogen-bond dynamics of the SERCA calcium pump

04/2013 Suliman Adam (Master), The parametrization of thiamine and riboflavin for the application in ECF transporters

04/2013 Florian Pieront (Master), Analysis of hydrogen-bonding networks and surface water dynamics in photosystem II's PsbO subunit my molecular dynamics simulation

12/2012 Federico Guerra (Master), Proton-coupled dynamics in wild-type and mutant plasma membrane proton pumps

08/2012 Michael Paris (Bachelor), Towards accurate force field description of phosphorylated aspartate systems

On-going Master projects, by month of project start:

05/2019 Thomas Giannos, Numerical simulations of morphine binding to opioid receptors

12/2018 Eva Bartalan, Numerical simulations of the kappa- and delta-opioid receptors

DOCTORAL THESES, COMPLETED

By month of public thesis defense:

03/2018 Federico Guerra, 'Dynamic hydrogen-bonded networks of photosystem II'

05/2018 Suliman Adam, 'Towards accurate computations of cofactor-containing biosystems'

11/2017 Stefan Milenkovic, 'Hydrogen bonding and conformational coupling of the SecA protein motor'

DOCTORAL THESES, ON-GOING

By month of starting doctoral research:

02/2018 Michalis Lazaratos, Water networks in retinal proteins

04/2017 Krzystof Buzar, Computer simulations of photosystem II

12/2016 Oskar Klaja, Photo-switchable lipids

02/2016 Konstantina Karathanou, Development of data analysis tools for H-bond systems

COURSES, SEMINARS AND TUTORIALS TAUGHT

Role	Level	Topic	Hours	Period
Lecturer	Master	Quantum and statistical mechanics computations of molecular structure	2 + 1/ week	WS 2018
		bio(Nanotransporters): Theoretical biophysics and bioinformatics methods. Lecture and exercise class.	2	SS 2018
		Modern Methods in Physics: Electronic Structure Methods	2	SS 2011, SS 2012, WS 2014, WS 2018
		Advanced Quantum Mechanics	4	WS 2016, WS 2017
		Advanced Theoretical Biophysics	2	WS 2011, WS 2012, WS 2015, WS 2019
		Photobiophysics and Photosynthesis, shared 50%	2 + 1	WS 2010
	Bachelor	Analytical Mechanics (Theory 2) Taught in English/ German	4	SS 2013, SS 2014, 2015
		Experimental Physics III, shared 50%	4	WS 2012
Research-oriented seminars				
Lecturer	Master	Journal Club on Molecular Biophysics – Theory and Experiment, shared 50%	2	SS 2014, WS 2014, SS 2015, WS 2015
		Literature Seminar on Statistical Mechanics for Computer Simulations	2	SS 2016, WS 2016
	Doctoral, Master, Bachelor	Theoretical Molecular Biophysics, regular research seminars of the lab	2	Regular lab meetings
Practical computer classes				
Tutor	Master	Advanced Theoretical Biophysics – 50%	2	WS 2011
		Modern Methods in Theoretical Physics: Electronic Structure Methods	2	SS2011
	Bachelor	Computational Physics	2	WS 2010