9. Applications in Gas Phase Spectroscopy

Besides the previously presented characterization of the *de novo* designed amyloid forming models by common biophysical and bioanalytical methods, selected peptides and their complexes were studied by rather complex mass spectrometry based gas phase IR spectroscopy experiments. Since these investigations are currently in a very early stage and not directly related to amyloid formation, the obtained results are presented separately in the following section.

9.1 Proteins in Mass Spectrometry and Gas Phase Studies

Mass spectrometric techniques applying soft and non-destructive ionization methods such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) evolved as one of the most widely used techniques for the basic characterization of biomolecules in the last twenty years. Especially in combination with sensitive, high resolution mass analyzers and sophisticated work-up procedures these methods provide detailed information on the sequence of proteins, even if they are embedded into complex biological matrices. Thus, mass spectrometry is the key method for the entire field of proteomics.[257]

Besides these routinely performed proteomics techniques, biophysical mass spectrometry approaches addressing structure and dynamics of biomacromolecules and their complexes in solution attracted increased attention in recent years.[258] Hydrogen deuterium exchange (HDX) reactions for example provide valuable information on the presence or absence of hydrogen bonding within the protein in solution, and are therefore widely used for the characterization of protein structure and dynamics.[259,260] Also chemical cross-linking reactions at solution conditions with a subsequent MS detection of the covalently linked products evolved a common method for the investigation of tertiary and quaternary contacts within proteins and protein aggregates.[258,261,262] Furthermore, the direct observation of intact non-covalent protein-protein, protein-peptide, protein-metal, and protein-DNA complexes by mass spectrometry yields valuable insights on the binding and dissociation properties present in solution.[263-265] Most of these approaches are very well established and detailed information can be obtained from several books[258] and review articles.[259-265]

More importantly, numerous investigations using electrospray ionization at very soft and native-like conditions in combination with ion mobility or gas phase spectroscopy experiments showed that structural features of proteins and protein complexes are at least partially retained in the gas phase.[266,267] These findings are currently subject of an intense debate and require a consideration of the unique properties of a solvent free environment. Rapid removal of the solvent, as occurring during the ESI process, doubtlessly affects structure and dynamics of the biomacromolecule, although not all of these changes will have a negative effect on the integrity of the higher order structure. In solution, much of the structural stability of proteins and their complexes is governed by the burial of hydrophobic side chains and consecutive shielding against polar water molecules which are consequently located at the polar protein exterior. At gas phase conditions, these hydrophobic interactions are expected to be dramatically reduced due to the removal of the solvent. Thus, it has been suggested that the vacuum conditions can be viewed as highly apolar medium, similar to the hydrophobic interiors of membranes. At these conditions the protein may turn “inside-out” exposing the hydrophobic residues on the outside, while burying polar residues at the interior.[268] On the other hand, the two other contributors to protein stability in solution, namely, hydrogen bonds and electrostatic interactions, are expected to become more stable in the absence of solvent molecules and may therefore rather stabilize a solution-like structure in the gas phase.[258] Thus, the scenario of an “inside-out” protein structure in the absence of solvent molecules seems rather unlikely. However, since ESI-generated macromolecular ions carry similar charges, electrostatic repulsions between these charges may also destabilize the solution-like protein structure and rather induce an extended conformation. The charge state of the proteins significantly influences the gas phase conformation and must consequently be considered for experiments addressing structural aspects of proteins in a solvent free environment.[267]

Nevertheless, recent investigations on proteins and protein complexes in the gas phase indicate that several structural features including a compact globular shape, secondary structure elements, hydrogen bonding patterns, and characteristic quaternary contacts can be retained in the solvent free environment of a mass spectrometer.[267] Thus it can be assumed that the rapid depletion of solvent during the ESI process affects hydrophobic interactions and residues at the exterior of the protein or protein complex, but electrostatic interactions and residues buried in the center of proteins do not seem to be disturbed significantly by the desolvation process.[267]

Recently, von Helden and co-workers reported the first IR spectroscopic data for gas phase Cytochrome C in a conformation sensitive wavelength range from 1400 to 1800 wavenumbers (Figure 9.1).[269] These data clearly reveal a narrow distribution of hydrogen bonded structures in the gas phase, similar to those observed at solution conditions. Furthermore, evidence for a coulombically driven unfolding was obtained for higher charge states of Cytochrome C, which is in accordance to previous ion mobility experiments.[270]

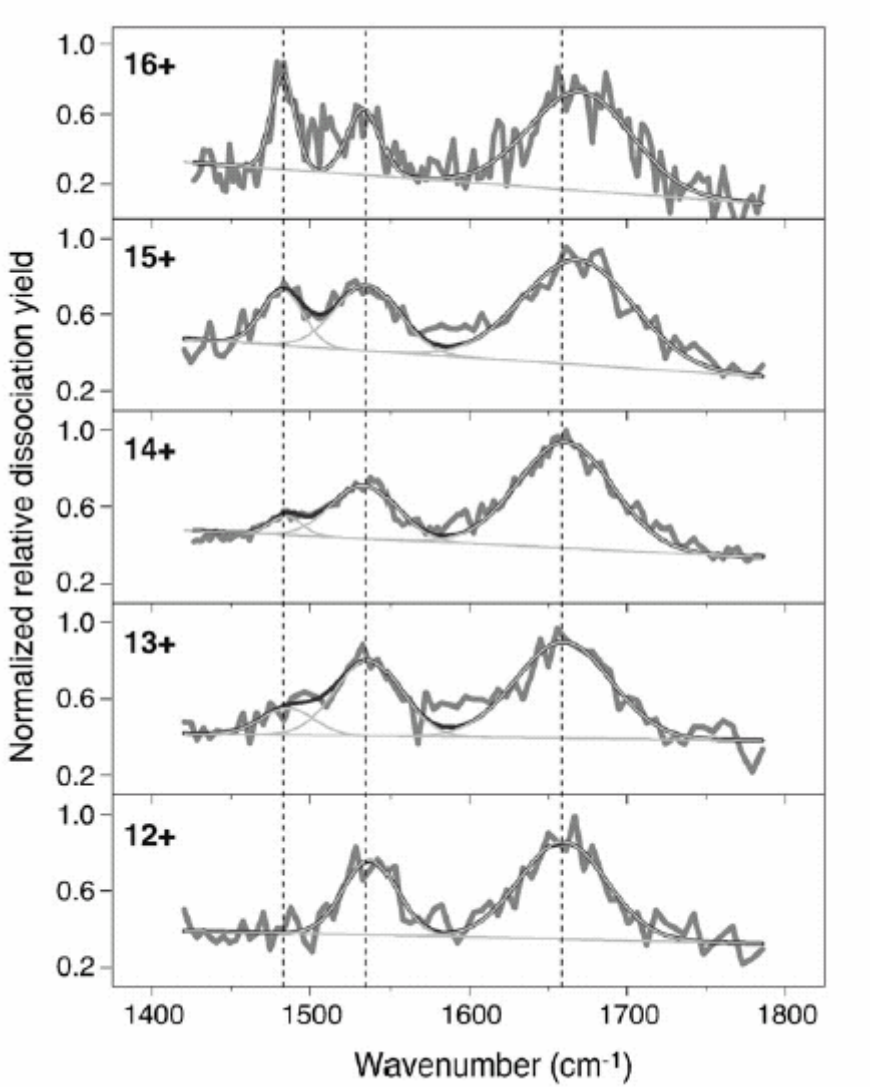

Figure 9.1. *Infrared photodissociation spectra for different charge states of bovine cytochrome c. The black curves represent the average of several independently recorded scans. All spectra are individually normalized. The band at 1483cm^{-1}(amide I, N-H bend) clearly grows in with increasing charge state, while the band near 1660cm^{-1} (amide I, C=O stretch) shows a blue shift as the charge increases. Thus, less hydrogen bonding can be assumed for an increasing charge state of Cytochrome C (according to J. Oomens et al.)*[269]

At lower charged states, the amide I (C=O stretch) band falls in the range were usually solvated α-helices are found in solution. In contrast, unfolding of Cytochrome C at higher charge states is accompanied by a blue shift of the amide I band and the appearance of a new, red shifted band around 1480 wavenumbers. While the origin of the band at 1480 wavenumbers is still unclear, the blue shifted amide I band can be interpreted as a result of a weaker hydrogen bonding network that occurs due to unfolding of a presumably α-helical conformation.

However, the presence of an α-helical or a helix-like conformation in the gas phase has not yet been proven sufficiently. Thus, the advantage of the unique folding properties of the well investigated α-helical coiled coil folding motif was employed to provide further experimental evidence. Due to the absence of the well defined, thermodynamically stabilizing intermolecular interactions, coiled coil peptides are supposed to adopt an unordered random coil conformation in the monomeric state, while the oligomeric state is almost entirely α-helical in solution. Recent studies showed that coiled coil complexes can be transferred as intact oligomeric complexes into the gas-phase.[202,271] Thus, one might question whether the helical conformation present in solution is, at least partially, conserved during transfer, storage and detection of coiled coil oligomers in the gas phase environment of a mass spectrometer. If so, perceptible differences in the gas phase IR spectra of monomers and oligomers should occur. To answer this question, gas phase IR spectra of the previously presented peptides VW02Abz, VW03, and their oligomers have been measured.

9.2 Instrument Setup and Work Principles

For the presented experiments a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer installed at the free electron laser facility at FOM-Institute for Plasma Physics Rijnhuizen was used. In this section, only a short description of the instrument and the underlying work principles is given. Further details regarding the instrumental setup[269,272,273] and the free electron laser[274] can be obtained from the literature.

Figure 9.2 shows a schematic description of the used apparatus.[273,275] In principle, the instrument consists of two units. (I) A laboratory-constructed FT-ICR mass spectrometer equipped with a 4.7Tesla superconducting magnet, and (II) a free electron laser (FELIX) that provides widely tunable, pulsed IR radiation.

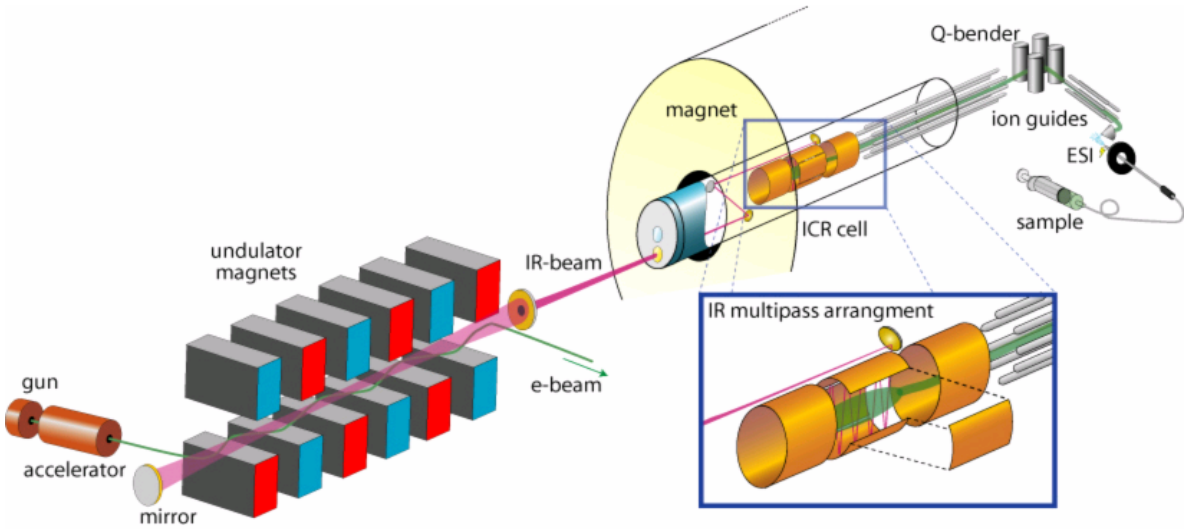

Figure 9.2. *Schematic description of the FT-ICR mass spectrometer for IRMPD experiments. Left: free electron laser for infrared experiments (FELIX) providing pulsed wavelength tunable IR radiation. Right: ESI-FT-ICR mass spectrometer and ion transfer optics. Frame: ICR cell showing a schematic description of the IR multipass arrangement (according to N.C. Polfer et al.)*[273]

A conventional Z-spray ESI interface is used to continuously produce multiply charged ions from aqueous analyte solutions. These so-called parent ions are accumulated in a hexapole ion guide and transferred into the FT-ICR cell via a quadropole deflector (Q-bender) and a linear ion transfer optic. In the ICR cell the ions are trapped and then dissociated by infrared multiple photon dissociation (IRMPD). Therefore, pulsed infrared radiation provided by FELIX is introduced into the cell via several mirrors and a nitrogen flushed transfer tube. In the cell, these IR pulses follow a multipass arrangement in order to maximize the overlap with the ion cloud. Subsequently, formed fragments and remaining, non-dissociated parent ions are detected in the FT-ICR mass spectrometer.

At constant laser power, the observed dissociation efficiency depends on the wavelength of the applied IR radiation. Figure 9.3 exemplarily shows mass spectra of fluorene after an irradiation at different FELIX wavelengths.[272] When FELIX is tuned to 1550cm^{-1}, resonance with an allowed infrared mode occurs and photodissociation is observed. In contrast, an off resonant irradiation at 1500cm^{-1} does not lead to fragmentation and, thus, yields a spectrum which is essentially the same as that obtained without FELIX radiation. The final gas phase IR spectrum is consequently generated by obtaining a series of mass spectra collected as a function of laser wavelength and then plotting fragmentation yield versus wavelength.

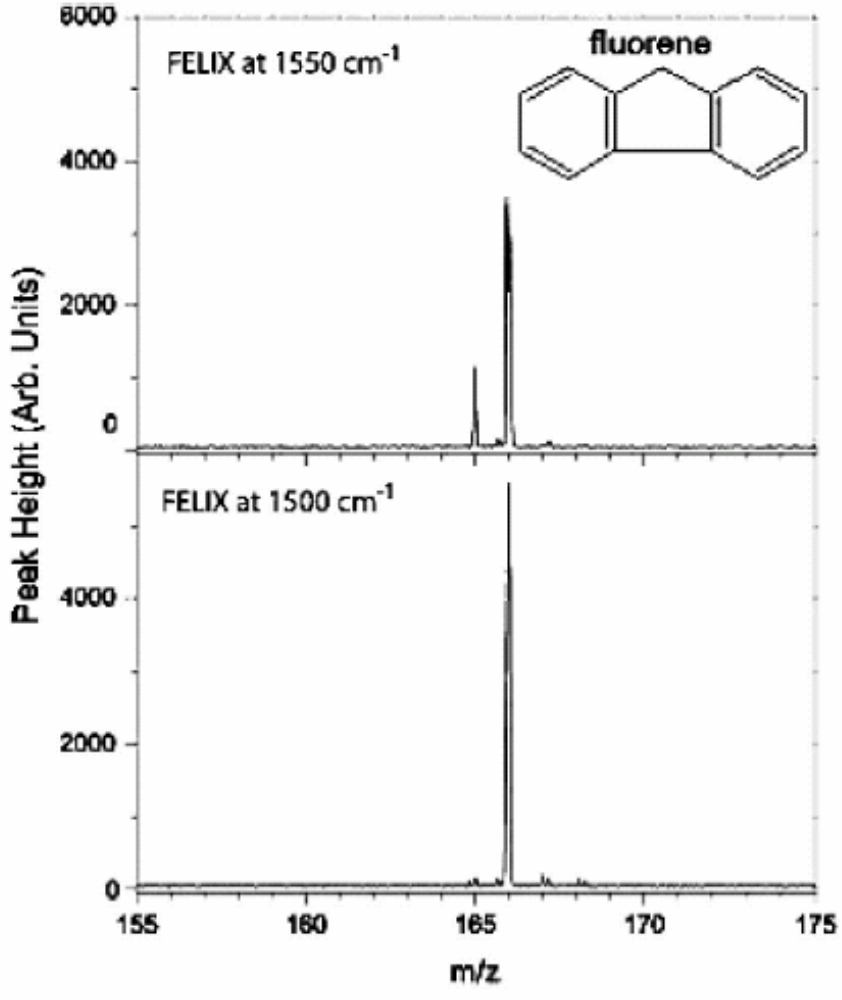

Figure 9.3. *FT-ICR mass spectra of fluorene when FELIX was tuned on resonance (top) and off resonance (bottom) with an allowed infrared mode (according to J.J. Valle et al.)*[272]

9.3 Gas Phase Characterization of Coiled Coil Peptides

Two *de novo* designed coiled coil peptides, VW02Abz and VW03, were used for the presented gas phase studies. Figure 9.4 shows the helical wheel diagram and the sequence of both peptides. Efficient electrospray ionization in the positive ion mode is usually achieved at slightly acidic conditions. Thus, VW02 was selected since it forms an ideally folded α-helical coiled coil in a wide pH range from 4.0 to 8.0 (see section 7.1.2). Previous experiments utilizing a commercially available ESI-ToF instrument furthermore revealed that dimeric aggregates of VW02 obtained from acidic solutions can be easily transferred as intact complex into the gas phase using ESI (data not shown). Thus, VW02Abz is an ideal system for a characterization of helical species in a

solvent free environment. In contrast, VW03 adopts a random coil conformation at pH 4.0 and 5.0 (see section 8.1.1) and does, consequently, not form dimers at acidic conditions. An α-helical coiled coil conformation is exclusively observed at neutral and slightly basic pH. Therefore, VW03 was used to serve as negative control, i.e., as standard for a fully unfolded conformation at acidic pH.

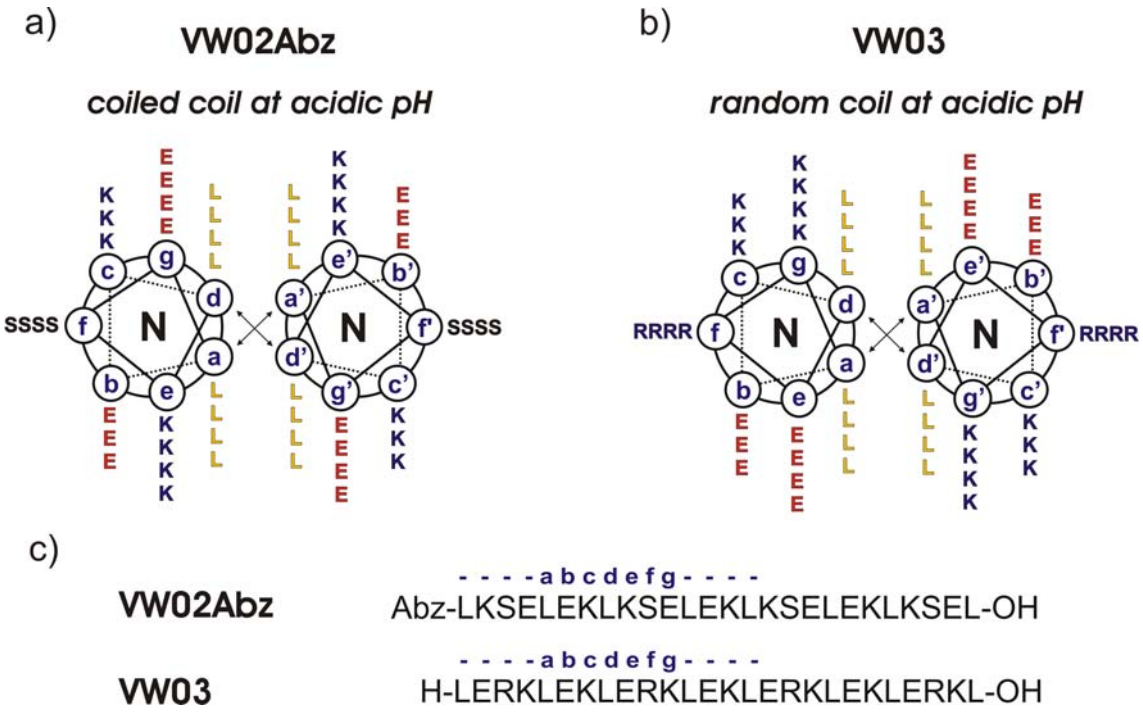

Figure 9.4. *(a and b) Helical wheel diagram and (c) Sequence of peptides VW02Abz and VW03.*

Gas phase IR spectra of monomeric VW02Abz, dimeric VW02Abz and monomeric VW03 were recorded. Therefore, ammoniumformate buffered solutions with pH 4.5 and moderate peptide concentrations of approx. 50µM were applied. Additionally, 40% MeOH were added in order to achieve a stable and reproducible electrospray. Figure 9.5 shows the obtained spectra. In all spectra, two resolved bands analogous to those occurring for lower charged ions of Cytochrome C (Figure 9.1) have been observed. These bands indicate a conformational distribution which is similar to those found at solution conditions. Although the spectra appear similar at a first glance, there are distinct differences in the position of the band maxima. Compared to the monomeric form of VW02Abz (Figure 9.5 black), the dimer (Figure 9.5 red) exhibits a slight red-shift of the Amide I band and a perceptible blue shift of the Amide II band. Interestingly, the Amide I as well as Amide II band maxima of monomeric VW02 and monomers of the

negative control *VW03* appear at similar wavenumbers, which indicates that both species may share common structural characteristics.

The observed band shifts of the oligomeric form of VW02Abz may indeed result from a conservation of the helical conformation in the gas phase. Within helices, backbone carbonyl oxygen atoms and the amide protons are involved in the formation of hydrogen bonds. Compared to free C=O, such H-bonded oxygen atoms would be bound weaker to the carbonyl C and consequently yield a red shift of the vibration mode (Amide I). In contrast, the N-H bend vibration of hydrogen bonded amide protons would be blue shifted due to a more rigid alignment within the hydrogen bond. Thus, it can be hypothesized that the observed band shifts result from the hydrogen bond driven helical or helix-like organization of the peptide backbone.

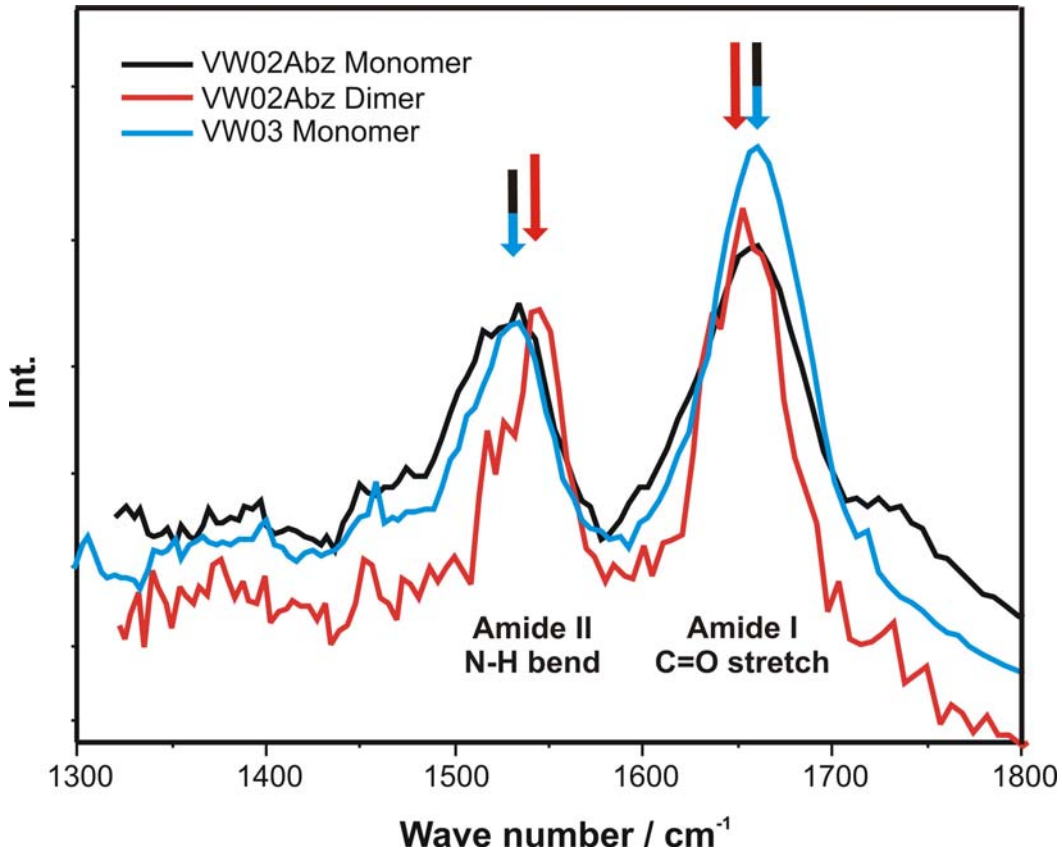

Figure 9.5. *Infrared photodissociation spectra of (black, M^{4+}) VW02Abz monomers, (red, D^{5+}) VW02Abz dimers, and (blue, M^{4+}) VW03 monomers (c ~ 50µM, 10mM ammonium formate buffer, pH 4.5, 40%MeOH)*

However, a general conclusion on basis of the current, very early results would be merely speculative. The observed band shifts could also result from repulsive electrostatic interactions between similarly charged groups on the outside of the coiled coil complex. Thus, further measurements on several lower charged states of monomers as well as dimers are necessary to support the observed effects. Also measurements on other artificial and naturally occurring coiled coil peptides are needed to prove a general validity. Additionally, computational approaches in the absence of solvent may provide further information on the structural organization of coiled coil complexes in the gas phase. These experiments are currently in progression.

From a more general point of view, embracing the forthcoming experiments with the previous investigations would enable us to set standards for the investigation of protein structures in the gas phase. In the future, such experiments may help to investigate structural features of early aggregates and intermediates which occur during the evolution of protein misfolding diseases such as Alzheimer's disease and Parkinson's disease.