11. Outlook

In the future, the presented model peptides can be used for a variety of investigations and applications. In comparison to naturally occurring systems, the advantages of these models are obvious - they follow clear and well investigated design principles, are easily synthetically accessible, exhibit a perceptibly elevated solubility and slowly convert into amyloids in a highly pH and metal ion controllable manner. Thus, further investigations using low as well as high resolution methods can help to elucidate the complex molecular events that occur during amyloid formation on a molecular level.

A systematic rearrangement of the β-sheet preferring Val residues within the sequence of VW18 for example, can be used to obtain valuable information on the validity of the so-called “amyloid stretch” hypothesis, where very short hydrophobic core segments (“amyloid stretches”) are discussed to be the essential amyloid inducing element. Also experiments on continuative peptides with a similar intrinsic amyloid formation propensity, but distinct stability of the coiled coil structural motif should provide a closer view on the impact of the competitive native conformation. Moreover, the presented model peptides can be easily equipped with spin labels or fluorescence tags which, in combination with sophisticated biophysical techniques, such as EPR spectroscopy and ultrafast time-resolved fluorescence spectroscopy, enable a systematic investigation of the amyloid formation kinetics in a millisecond to picosecond time scale that is usually not accessible by conventional spectroscopic approaches.

A detailed characterization of the presented model system by modern high resolution techniques such as NMR spectroscopy should furthermore help to understand both, the molecular arrangement within fibrils as well as events and intermediates that occur during the complex amyloid formation process. Thereby, the above mentioned advantages of the presented model peptides are beneficial for NMR experiments in general, regardless if at solid state conditions or in solution. Solid state NMR in combination with proline scanning analysis for example can be applied to determine the internal protofilament structure of the system. Such experiments are suitable to locate turn regions within the peptide strands and might help to identify specific side chain - side chain interactions that, beside hydrogen bonds, govern the extraordinarily high stability of amyloid fibrils. A structural characterization by solid state NMR can furthermore lead to a better understanding of the morphological distinctions of the resulting amyloid fibrils

that are driven by protofilament-protofilament interactions which are still very poorly understood. On the other hand, NMR spectroscopy in solution can help to obtain information on the dynamics of amyloid formation. Solution state NMR spectroscopy is very demanding in terms of solubility and sample consumption which, as a consequence, often hampers a detailed characterization of amyloid formation processes at solution conditions. As the model peptides presented here are easily synthetically accessible, isotope labels can be introduced selectively at sequence positions of special interest and importance. Additionally, the here presented peptide model exhibits a perceptibly elevated solubility and slowly converts into amyloids at native like aqueous conditions without the necessity of unusual stimuli. Consequently, it should be possible to directly monitor the molecular events which occur during the process of amyloid formation by solution state NMR. Such experiments are of current, paramount interest, since especially the early aggregates and intermediates, which are thought to be the real cause of many diseases associated with amyloids, are very poorly investigated so far.

Under the prerequisite of a well investigated internal protofilament structure, the presented model systems can furthermore be used for a variety of biology and materials science related applications. It is for example possible to use model peptide fibrils as rigid, stable, and, more importantly, switchable scaffolds for the presentation of several non-peptidic molecules such as oligosaccharides. The well defined amyloid ultra structure ensures a discrete alignment of these molecules on the outer surface of the fibril. Additionally, it should be possible to direct the formation of these fibrillar scaffolds by pH alteration or the addition of metal ions. In the context of biomedicine this switchable biopolymeric aggregation might provide a powerful tool for the controlled generation of functional, multivalent species at targets of special interest.

Additionally, a detailed structural knowledge can be used for the generation of a prospective model system to study interactions between natural and non-natural amino acids in the well defined environment of a cross-β quaternary structure. Our group has an enormous experience in the field of fluorinated amino acids, which have been implemented at hydrophobic and polar positions of coiled coil peptides and subsequently studied regarding their impact on the conformational stability. In contrast to the rather complex α-helical coiled coil folding motif, the cross-β quaternary structure displays only two defined interfaces and, thus, should provide a perfect environment to study the impact of non-natural amino acids on peptide-peptide and peptide-protein interactions.