10. Summary

The aim of this work was to establish a simple and manageable coiled coil based model peptide system that can be applied to study the impact of a changed environment on the amyloid formation process. Therefore, a strategy involving the generation of an amyloid forming coiled coil peptide system and the consecutive incorporation of either pH or metal ion sensitive domains was followed.

A model peptide that exhibits a balanced distribution of structural features from α-helical coiled coils as well as amyloids was developed first. The starting point was a 26 residue peptide that strictly follows the characteristic *heptad* repeat pattern of a parallel α-helical coiled coil (VW01 and VW02). This optimized coiled coil peptide was modified vigorously at the solvent exposed b, c, f as well as the e and g positions to gain a sequence that is rather characterized by a β-sheet preferring alternating pattern of hydrophobic and hydrophilic amino acids. To maintain a certain α-helix propensity, residues at a and d positions, which govern the major thermodynamic driving force for the coiled coil formation, have been kept unchanged. The obtained peptide VW11, however, adopts a clear and invariable β-sheet conformation in a wide range of concentrations and pH values. Based on these findings, an evolutional, bottom-up design approach was followed to sequentially adjust the intrinsic structural propensities of both structural motifs, α-helix and β-sheet. A series of VW11 related peptides with successively increased α-helix propensity was designed, synthesized, and characterized (VW12 to VW18). This evolutional approach succeeded and revealed that three β-sheet preferring Val residues located at the solvent exposed b, c, and f positions within the *heptad* repeat are sufficient to make the ideally helically folded coiled coil peptide prone to amyloid formation. At pH 7.4 and concentrations between 100 and 300μM the optimized sequence VW18 forms amyloids within three days.

Subsequently, excessively charged domains as pH sensitive functionalities that, depending on the pH, destabilize the helical structure without affecting both coiled coil recognition motifs have been incorporated into the design of VW18. The validity of this approach was proven by a design based on the 26 residue coiled coil (VW03 and VW04). These data unquestionably revealed that an accumulation of similarly charged residues at adjacent c, f, and g or b, e, and f positions within the coiled coil *heptad* repeat provides a powerful tool for the pH directed destabilization of the helical structure.

Thus, an excessively charged domain formed by Lys residues has been incorporated into the sequence of VW18 gaining peptide VW19 which now forms amyloids in a highly pH and concentration dependent manner. At moderate concentrations, unfolding was observed at acidic as well as neutral pH. Higher concentrated samples clearly revealed a slow amyloid formation at acidic pH, at which the excessively charged domain represents the “on” state of the pH switch. Contrarily, the “off” state of the pH switch at neutral conditions yielded non-amyloidogenic α-helical fibrils at elevated concentrations. Taken together, VW19 is the first example of a peptide that, without changes in the primary structure, responds to environmental conditions by adopting three completely different conformations (Figure 10.1). These data impressively illustrate the enormous impact of an altered pH value on peptide structure and amyloid formation. Further studies on the VW19 related peptide RR01, which contains Glu instead of Lys residues at position f of the coiled coil *heptad* repeat, led to an extension of the presented design principles. RR01 exhibits a reverse pH dependence of folding and, thus, facilitates a controlled amyloid formation at neutral conditions (Figure 10.1).

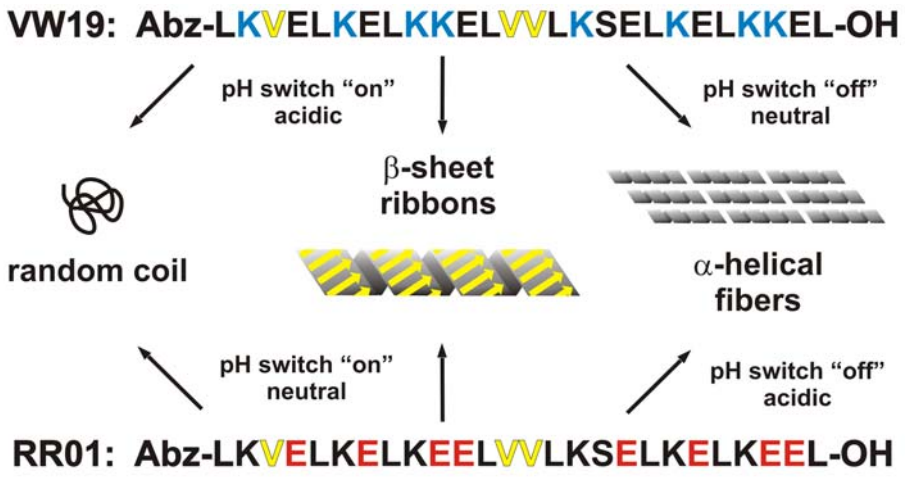

Figure 10.1. *Schematic description of the reverse folding behavior of peptides VW19 and RR01. Depending on peptide concentration and pH value both peptides adopt three different conformations (according to K. Pagel et al. and Wagner et al.)*[227,228]

Furthermore, metal ion dependent conformational switches have been developed and incorporated into the amyloid forming model system. Studies on the folding behavior of the VW11 related peptide VW15 yielded a first indication for the feasibility of metal ion induced structural transitions in general, although unnatural solution conditions involving TFE were required for these experiments. All following peptides have been optimized to respond in the presence of transition metal ions at native-like aqueous condi-

tions. Therefore, the so-called *i,i+2* and *i,i+4* strategy has been implemented into the basic amyloid forming model. Following this approach, two metal binding His residues have been incorporated either at *i* and *i+2* position to disturb the helical structure or at *i* and *i+4* position to stabilize the helical conformation upon the addition of metal ions. The validity of the *i,i+2* and *i,i+4* concept was proven on the basis of the non-amyloidogenic coiled coil VW17 gaining peptide VW23 and VW24. Both sequences followed the expected folding behavior, exhibiting a Cu^{2+} induced unfolding (VW23, *i,i+2*) or stabilization of the helix (VW24, *i,i+4*). An incorporation of the *i,i+2* and *i,i+4* strategy into the amyloid forming coiled coil model finally yielded two peptides with well defined, metal ion sensitive amyloid formation properties (VW29, *i,i+2* and VW30, *i,i+4*; Figure 10.2). In the absence of metal ions both model peptides form amyloids within days. Independently of the His positions, coordination of Cu^{2+} inhibits the formation of amyloids by either metal directed unfolding (VW29, *i,i+2*) or helix stabilization (VW30, *i,i+4*). In contrast, Zn^{2+} was shown to accelerate the amyloid formation if two metal binding sites are placed one residue apart from each other (VW29, *i,i+2*) and thus, point to the opposite side of the helical cylinder. Zn^{2+} binding to peptide VW30 (*i,i+4)*, where both metal binding residues are adjacent towards each other at the helical cylinder, shows a similar amyloid inhibiting and helix-inducing behavior as observed for Cu^{2+}. Thus, it can be concluded that metal binding accomplishes inhibition or acceleration of the amyloid formation process by stabilization or destabilization of a competitive conformation. More importantly, the different Cu^{2+} and Zn^{2+} dependent amyloid formation properties of peptide VW30 show striking similarities to those observed for the naturally occurring Alzheimer's Aβ, although peptide VW29 is completely artificial and does not possess any sequence homologies. Thus, these findings impressively show that the intrinsic binding characteristic of the particular metal ion has a greater impact on the amyloid formation process than accepted so far.

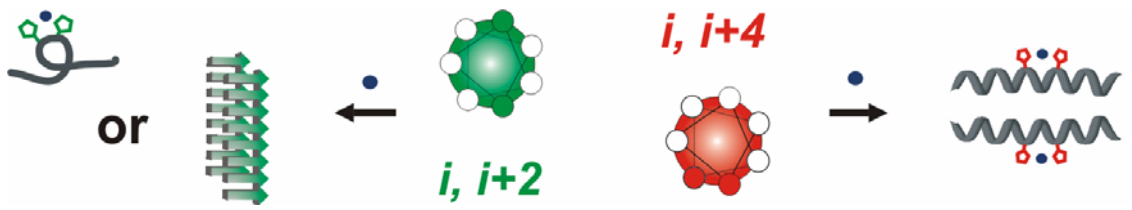

Figure 10.2. *Schematic description of the metal ion dependent folding behaviour of peptides VW29 (i,i+29) and VW30 (i,i+4). Without metal ion addition, both peptides form amyloids within days (according to K. Pagel et al.)*[247]

In conclusion, the presented results clearly emphasize and highlight the tremendous impact of a changed environment on the amyloid formation process. A pH directed destabilization of the competitive α-helical coiled coil structure, which serves as mimicry for the native and functional conformation, was shown to enable a controlled initiation of amyloid formation. On the other hand, pH and metal directed stabilization of the competitive helical isoform inhibited the amyloid formation. These observations are in good agreement with various data from natural systems and, thus, highlight the enormous potential of *de novo* designed models for the investigation of amyloid formation processes. Furthermore, metal ion binding studies revealed essential differences of the amyloid formation properties in presence of Cu^{2+} and Zn^{2+}. Similar effects have been observed for Alzheimer's Aβ and emphasize the outstanding impact of the metal ion's specific binding properties on peptide and protein folding.

In addition to investigations at solution conditions, two non-amyloidogenic coiled coil peptides and their oligomeric complexes (VW02 and VW03) have been studied by gas phase infrared multiple photon dissociation. Although these experiments are in a very early stage, the obtained data provide evidence that α-helical secondary structure elements of peptides and proteins may remain intact in the solvent free environment of a mass spectrometer. However, further experiments in combination with computational approaches are needed to validate these assumptions.