6. The Coiled Coil Based Amyloid Design

Many naturally occurring proteins involved in amyloid diseases possess two defined, but markedly different conformations – a mostly partially helical, native conformation and the fibrillar amyloid form. Since the structural conversion from the native conformation to the β-sheet rich amyloid form has been found to be the most critical and sensitive event during the course of amyloid association, the approach was to implement features of two distinct folds into a *de novo* designed amyloid model. In order to mimic a native conformation which is rich in α-helix, we fused the design principles of the well known α-helical coiled coil folding motif with patterns present in amphiphilic β-sheets and amyloids. In principle, this idea is not new and similar approaches have been reported in the literature (see section 3.4.2). However, many of the published systems contain non-natural building blocks or linkers which represent very unnatural conditions. Furthermore, all reported coiled coil amyloid models need elevated temperatures to trigger the conversion into amyloids which precludes the direct observation of amyloid formation processes at native-like conditions. To overcome these drawbacks, the basic idea of this work was to expand the concept of a coiled coil based amyloid model by incorporating structural switches that can be used to control and direct conformational transitions and the consecutive formation into amyloids at physiological conditions. Therefore, functionalities that sensitively react to subtle changes in the environmental conditions such as altered pH or the presence of metal ions have been incorporated.

6.1 The α-Helical Coiled Coil Folding Motif

The α-helical coiled coil folding motif (see also section 3.4.2) is one of the most widespread structural motifs in nature. [124-126,190] Approximately 3-5% of amino acids in naturally occurring peptides and proteins are involved in the formation of coiled structures. α-Helical coiled coils typically consist of two to five right-handed α-helices which are wrapped around each other to form a left-handed superhelical twist. Figure 6.1 shows a molecular modeling structure of an antiparallel, 41 residue coiled coil peptide.

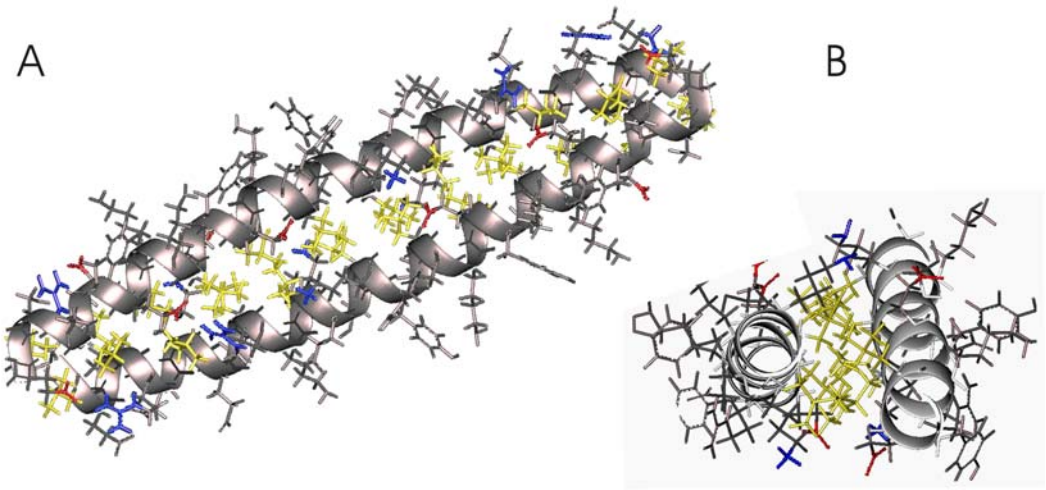

Figure 6.1. *Molecular modelling structure of an antiparallel, 41 residue coiled coil peptide. (A) View perpendicular to the helix axis. (B) View along the helix axis. Yellow: hydrophobic residues. Blue, red: positively and negatively charged residues, respectively.*

The primary structure of each helix is characterized by a periodicity of seven residues, the so-called 4-3 *heptad* repeat which is commonly denoted $(abcdefg)_n$. Figure 6.2 shows a helical wheel presentation which is generally used as a simplified description of the *heptad* repeat. Positions a and d are typically occupied by apolar residues (Leu, Ile, Val, Met) that form a special interaction surface at the interface of the helices by hydrophobic core packing ("knobs-into-holes").[124-126] In contrast, the positions e and g are frequently occupied by charged amino acids (most commonly Glu, Arg and Lys) that form inter-helical ionic interactions.[126] Polar residues are often found in the remaining *heptad* repeat positions b, c, and f, which are located solvent exposed at the opposite side of the motif.

The hydrophobic core provides the major contribution to the structural stability of the α-helical coiled coil. The allocation of the two a and d positions of this interface in regard to the different β- and γ-branched hydrophobic residues controls the order of aggregate formation.[191-193] Introducing cavities and protuberances or specific, buried polar interactions within the hydrophobic core additionally provides an efficient way to direct the relative helix alignment.[194-197] In contrast, the inter-helical ionic pairing positions e and g mainly dictate the specificity of folding (parallel versus antiparallel) as well as promote

the preference for homo- or heterotypic α-helical coiled coil formation.[130,196-202] Amino acids in these positions provide less overall stability for this folding motif and have less effect on the direction of the oligomerization state than hydrophobic core residues. An additional recognition domain which is not related to the coiled coil oligomerization is formed by intramolecular Coulomb interactions between positions c/g and b/e, respectively, of the single helices. These interactions indirectly influence the stability of α-helical coiled coil folding by stabilizing or destabilizing the single helices.[203] Position f of the *heptad* repeat is not part of any of the three recognition domains. Therefore, it has not yet been defined if it contributes to helix stability.

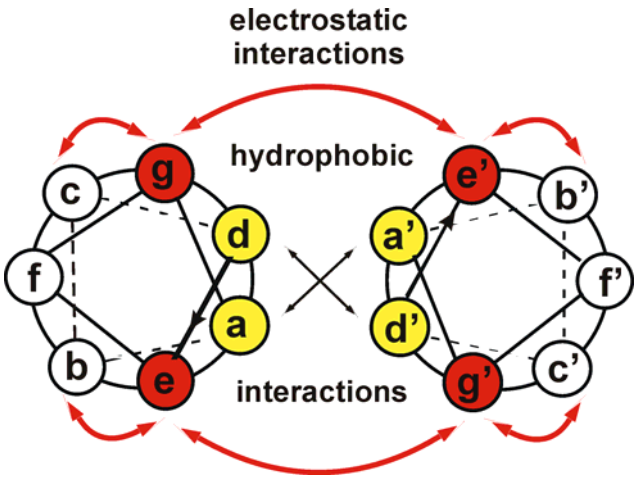

Figure 6.2. *Helical wheel diagram of a parallel coiled coil dimer. Yellow: residues forming the hydrophobic core. Red: residues involved in the formation of hydrophobic interactions.*

6.2 β-Sheets and Amyloids

β-Sheets, the subunits of amyloids, are not as uniform as α-helices and their basic design principles and folding characteristics are much more complicated. Despite the importance of β-sheets as one of the most important secondary structure elements in proteins, the principles underlying their formation and stability are not understood in detail.[204] In general, two major problems complicate detailed elucidation of the β-sheet secondary structure motif. The intrinsic tendency to aggregate usually results in solubility problems that make sample handling difficult. Furthermore, the absence of well de-

termined cross-strand amino acid preferences in protein β-sheets constrains the use of long-range interactions in the *de novo* design. Thus, the design of β-sheets that fold with high specificity is a challenging topic in modern peptide chemistry.

In general, β-sheets consist of at least two extended β-strands with five to ten residues which are arranged adjacent to each other forming an extensive inter-strand hydrogen bond network between backbone amide N-H and carbonyl C=O groups. Thereby, the neighboring strands can be aligned in a parallel or antiparallel fashion. Furthermore, the amino acid side chains point to the up- or down-side of the β-sheet in an alternating fashion. Figure 6.3 shows the chemical structure and a stick-and-balls model of an antiparallel β-sheet. In principle, the term “cross β-structure,” which is commonly used in the context of amyloids (see section 2.1.2), refers to the same arrangement of extended β-strands. Nevertheless, β-sheets do not necessarily aggregate into the highly ordered amyloid quaternary structure.

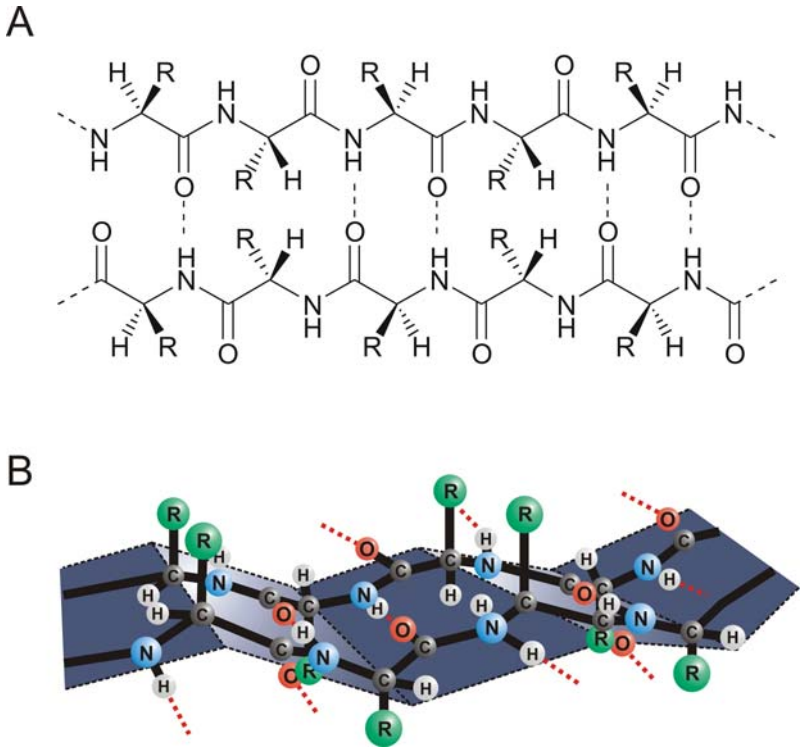

Figure 6.3. *(A) Chemical structure and (B) stick-and-balls model of an antiparallel β-sheet. The extended β-strands are aligned adjacent to each other and form a highly ordered inter-strand hydrogen bond network between backbone amide N-H and carbonyl C=O groups.*

However, various β-sheet rich folding motifs have been utilized as a basis for *de novo* design approaches. Due to their simplicity and frequent occurrence in natural proteins, β-hairpins are one of the best studied β-sheet rich, protein-like folding motifs. As the name implies, β-hairpins consist of two β-strands which are linked by a short loop or turn with two to five residues. In contrast to aggregating β-sheets, β-hairpins are able to adopt their well defined conformation in the monomeric state, as shown by Serrano and co-workers.[205] Based on this work, many other non-aggregating multi-stranded β-sheet peptides with up to eight β-strands have been designed, synthesized, and characterized by high resolution methods such as NMR spectroscopy.[206-209] As a result, valuable information for the *de novo* design of such multi-stranded β-sheets have been achieved and can be obtained from the literature.[210-212] Although the non-aggregating nature of these peptides facilitates a detailed investigation of the β-sheet secondary structure motif and protein folding in general, these systems are usually unsuitable models for the investigation of peptide aggregation and fibril formation.

The knowledge of design principles for the *de novo* generation of amyloids is suboptimal compared to those for β-hairpins, especially if the implementation of defined long range interactions is intended. As summarized in section 3.4.1, amyloids can be designed by implementation of a simple, alternating pattern of polar and nonpolar amino acids.[111-115,118-122] This results in a successive up-down-up-down alignment of polar and nonpolar residues which is characteristic for an extended β-sheet structure.[116] Since the resulting strands possess a hydrophobic and a hydrophilic face, nonpolar residues are buried in a dry interface (see section 2.1.2) and consecutively form large amyloid assemblies. On the other hand, Koide and co-workers showed that the formation of a hydrophobic face in β-sheets does not necessarily yield aggregation.[213,214] Nevertheless, the use of alternating hydrophobic-hydrophilic patterns for the *de novo* design of amphiphilic, amyloid forming β-sheets is a widely accepted strategy and has been used for many applications.

Another important feature with the potential to be used for the *de novo* design of amyloids are side-chain hydrogen bonds, which occur in fibrils of glutamine and asparagine rich sequences (see section 2.1.2).[32-36,215,216] To date, this unique property of glutamine and asparagine has not been applied for *de novo* design approaches, since the general validity of these systems would be highly restricted to the field of polyglutamine diseases such as Huntington's disease.

6.3 Coiled Coil versus Amyloids

The interactions that force a peptide to fold into a β-sheet rich amyloid are in general not that different to those found for α-helical coiled coils. A comparison of the major contributors to the stability of coiled coils and β-sheets is shown in Table 6.1.

Table 6.1. *Comparison of the major features that contribute to the stability of α-helical coiled coils and β-sheets.*

α-helical coiled coil	β-sheet
Amino acid intrinsic secondary structure propensities	
Side chain-side chain hydrophobic interactions	
Interstrand electrostatic interactions	
Intramolecular hydrogen bonds	Intermolecular hydrogen bonds

The intrinsic secondary structure propensities of amino acids are doubtlessly an important factor for both folding motifs. Values for these propensities - known as the Chou-Fasman parameters - have been determined in the 1970s by calculation of the total amino acid distribution in secondary structure elements of naturally occurring proteins.[217-220] However, the suitability of these values for *de novo* design is restricted due to the partial incomparability of the structural data of large proteins and those obtained for small peptides. The second factor that contributes to the stability of both structural elements is the hydrophobic effect. The impact of hydrophobic interactions for the stability of coiled coil has been extensively described in section 6.1. Additionally, numerous studies on β-hairpin peptides, multi-stranded β-sheets, as well as amyloids showed that the burying of hydrophobic residues within the β-strands is one of the major thermodynamic driving forces for the secondary structure formation.[112-115,120-122,210] As a third factor, electrostatic interactions and salt bridges contribute significantly to β-sheet stability. As in case of α-helical coiled coil peptides, these electrostatic interactions are only minor contributors to overall stability, even though they assist the secondary structure formation and direct the specificity of folding.[210] The last and perhaps most important contributor to β-sheet stability are hydrogen bonds. Unlike α-helical structures, where hydrogen bonds are formed intramolecularly within one helix, hydrogen bonds in β-sheets are formed between two different peptide strands. In conclusion, the important

factors for α-helical coiled coil folding as well as β-sheet formation appear to be a balanced mixture of intrinsic amino acid propensities, the hydrophobic effect and weak electrostatic interactions. The only difference is the fashion of hydrogen bond formation. Thus, the general interaction patterns of α-helices and β-sheets are more similar to each other than expected and it should, therefore, be possible to combine features of both folding motifs within one peptide sequence.

Consequently, we selected positions within the α-helical coiled coil *heptad* repeat which are suitable for the incorporation of modifications. Figure 6.4 shows a helical wheel diagram of a parallel coiled coil with the selected positions highlighted in blue. As known from the design principles of coiled coil peptides, residues at positions b,c, and f do not significantly contribute to the coiled coil oligomerization and stability. Thus, these positions are suitable for the incorporation of several modifications without affecting both helix forming domains.

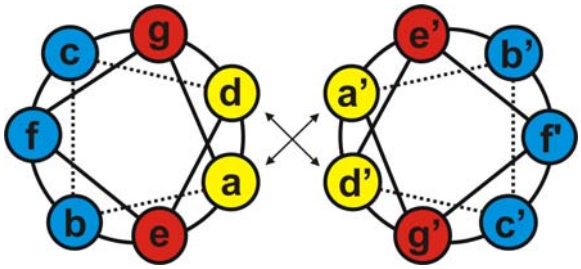

Figure 6.4. *Helical wheel diagram of a parallel coiled coil peptide. Yellow, red: positions forming the both coiled coil recognition motifs. Blue: positions suitable for the incorporation of modification.*

Within this work, two classes of modifications have been incorporated at the b, c, and f positions of the *heptad* repeat. (I) Valine residues have been inserted in order to make the system prone to β-sheet and amyloid formation. Valine is a typical β-sheet preferring amino acid and solvent exposition of these hydrophobic residues in case of a helical arrangement would be highly unfavorable. Thus, this modification results in a competition between both secondary structures – α-helix and β-sheet. In order to control the resulting structure sufficiently (II) trigger functions which sensitively react to environmental conditions such as altered pH or the presence of transition metal ions were introduced. Applying these structural switches enables a selective control of the intrinsic amyloid formation tendency induced by feature I.