2. Common Characteristics of Amyloids

The term amyloid with its current meaning was first introduced in 1854 by the german scientist Rudolf Virchow to describe a matter with an abnormal macroscopic appearance found in the brain and spinal cord of humans.[16] Utilizing the methodology of that time he used iodine to stain these deposits and found a starch-like behavior indicated by a pale blue color, which turned to violet upon treatment with sulphuric acid. Due to these observations he assumed in his first paper *"Ueber eine im Gehirn und Rueckenmark des Menschen aufgefundene Substanz mit der chemischen Reaction der Cellulose"* these *corpa amylacea* to be cellulose and, therefore, termed them amyloids. In 1859 Friedreich and Kekule simultaneously demonstrated the presence of protein in a mass of amyloid and furthermore stated the absence of carbohydrates based on the high nitrogen content. After that attention shifted to the field of proteins, first to classify amyloid as a single protein and later on as a complete protein class.[17,18] More recent investigations by Dobson and co-workers have shown that even proteins that are not relevant to amyloid diseases are able to form amyloid fibrils under certain conditions.[6] Thus, they hypothesized that *"the potential for amyloid deposition may be a common property of proteins, and not only a few proteins associated with disease."* In other words, almost every peptide and protein has the intrinsic potential to adopt an amyloid structure at appropriate conditions.[3,5,7] This history of a misleading terminology and interpretation intriguingly describes the whole dilemma of research in this field – the term amyloid describes a common property of proteins rather than a unique feature of a particular protein.[19] Nevertheless, amyloids of different protein origin show many similarities such as structure and formation pathway.

Per definition, all amyloids share three general features. (I) After staining with the dye Congo red they show a characteristic apple green birefringence under polarized light.[20] Although there are some practical difficulties, this was the first and still widely used indicator to characterize amyloid deposits from *in vitro* studies. (II) Amyloid deposits exhibit a non-amorphous, unbranched fibrous morphology as shown by early electron microscopy (EM) studies and (III) they consist of stacked β-pleated sheets forming the so-called cross-β structure.[21] This structural element was proven by X-ray diffraction studies of dried fibrils in the 60s of the last century. As a characteristic a strong meridional reflection at 4.7Å and a much weaker equatorial reflection at approximately 10Å was found.[22,23] This indicates a repeating pattern of β-sheets along the fibril axis with a

constant distance of 4.7Å and lamination with a spacing of 10Å perpendicular to this axis. Additionally, circular dichroism (CD) spectroscopy as well as Fourier transform infrared (FTIR) spectroscopy support the presence of high β–sheet content.

Beside the rather general features described above, modern bioanalytical, biophysical, and biochemical approaches provided a closer view on molecular architecture and folding pathways of amyloids. These common characteristics are described in the following section.

2.1 Current Structural Models – Cross-β or Nanotubes?

2.1.1 The Macroscopic Structure of Amyloid Fibrils

In recent years, structural studies by electron microscopy (EM) and atomic force microscopy (AFM) have revealed a much more detailed view on the macromolecular structure of amyloid fibrils. In general, amyloid fibers consist of up to six single strands, the so-called protofilaments, which are usually wound around each other and form a supercoiled structure.[23,24] Examples of macroscopic structural models containing 2,4, and 6 protofilaments are shown in Figure 2.1.[25] These structural models have been obtained by 3D reconstruction of cryo-transmission electron microscopy (cryo-TEM) data of bovine insuline.[25,26] Although the primary structure as well as the environmental conditions have been kept unchanged, different morphologies of the resulting fibrils were observed. Despite the fact of a varying number of protofilaments, the results suggest that size and shape of the individual filaments is similar. For the cross section a typical oval shape with diameters of 30-40Å has been determined. Beyond this compact or ribbon like arrangement of human insulin, similar 3D reconstruction approaches characterizing the macrostructure of SH3 fibrils suggested that some fibrils may exhibit a hollow core that is surrounded by several protofilaments.[27] Investigations on the islet amyloid polypeptide (IAPP) which is deposited in Type II diabetes furthermore showed that, depending on the sample preparation, single protofilaments, twisted ribbons, and single layered sheet like arrays can also be found.[28] Even tubular assemblies are possible as shown for the $A\beta_{(1\text{-}40)}$.[29] Additionally, more recent data provide evidence that the fibril morphology can be directed by concurrent multiple assembly and seeding pathways.[25,30]

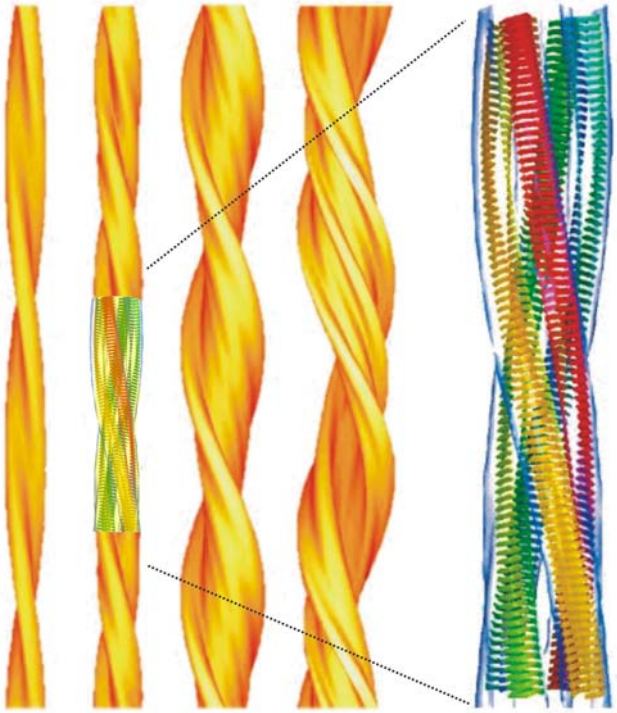

Figure 2.1. *(Yellow) 3D maps of 4 different insulin fibril morphologies. The fibrils are made up of different numbers of component strands (protofilaments; from left: 2, 4, 6, and probably 6). The protofilaments are twisted around each other in either compact or ribbon-like arrangements. (Coloured) model highlighting the β-strand arrangement within protofibrils (according to J.L. Jimenez et al.)*[25]

Due to the high variability of resulting morphologies, the question arises how protofilaments interact with each other. This question cannot be answered satisfactorily yet, but the macroscopic appearance of fibrils at least provides evidence. The twist of β-strands in β-sheets is left-handed and caused by the peptides chirality. The macroscopic twist of amyloid fibrils in which the hand has been determined are left-handed as well. Thus, an interaction of protofilaments can only occur if they exhibit the same twist. Based on the results of the insulin fibrils, Dobson and co-workers suggested two possibilities (Figure 2.2).[25] A protofilament twist that follows the overall twist of the fibril ensures a consistent interacting interface between the filaments (Figure 2.2a). Contrarily, if the twist of the β-strands is shorter than the overall twist of the fibril, the region of interactions progressively rotates around the protofibril. Consequently, interactions between the protofibril surfaces do not occur in this case (Figure 2.2b). The twist of the protofibril has to accompany the twist of the fibril to ensure a constant region of interactions. This structural model is consistent with the assumption that non-interacting, unordered resi-

dues are present on the outside of the β-core. These residues would not interfere with the inner interacting surface between the fibrils.[31] Thus, it is likely that protofilaments possess a twisted rectangular shape with two different surfaces, which are either exposed to the outer shell of the fibril or packed in an interacting core on the inside. However, to clarify the nature of interactions between the protofibrils in detail, it is essential to consider the internal molecular structure of amyloid fibrils.

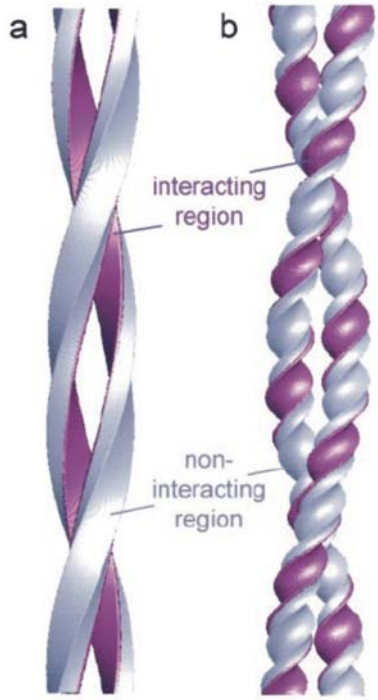

Figure 2.2. *Models for protofilament packing. (a) A twisted pair of rectangular protofilaments with the interacting surface highlighted in purple. (b) A supercoiled pair of protofilaments in which the regions involved in packing interactions rotate around each protofilament (according to J.L. Jimenez et al.)*[25]

2.1.2 The Internal Structure of Protofilaments

Although many attempts to elucidate the molecular arrangement of the amyloid core have been carried out in recent years there is no incontrovertible structural model.

The most widely accepted motif is the cross-β structure, which represents one of the common features of amyloids. Based on X-ray diffraction studies it has (in this context) first been described in the 1960s.[21,22] Recently, the Eisenberg group succeeded in characterizing amyloid like microcrystals derived from the amyloid forming segments

GNNQQNY and NNQQNY of the yeast prion Sup35 by X-ray microchristallography.[32-34] These outstanding high resolution data of a short peptide provide a very precise view on the cross-β architecture of the inner amyloid core (Figure 2.3). The GNNQQNY peptide chains are extended in conformation with a characteristic hydrogen bonding pattern of parallel β-sheets. The β-strands within one sheet are exactly in register and exhibit the typical strand-strand separation distance of 4.87Å (Figure 2.3 a). Along the a axis a second sheet of β-strands is aligned in an antiparallel manner with a slight vertical shift relative to the first sheet. The shift results from a perfect packing of the side chains within the interface of both sheets (Figure 2.3 b). Such a packing of sheet pairs along the c axis is called lamination. The lamination of more than two sheet pairs in the case of GNNQQNY clearly indicates that there are two distinctly different interfaces, which were termed dry and wet interface (Figure 2.3 c and d). The wet interface is completely surrounded by water molecules that separate the hydrogen bonded side chain residues from the neighboring sheet. Thus, the distance between sheets at this interface is large and is about 15Å. The occurrence of hydrogen bonded side chain residues is in agreement with previous studies that proposed a “polar zipper” stabilization of the cross-β structural motif by intermolecular hydrogen bonds of glutamine-rich and asparagine-rich sequences in addition to the backbone hydrogen bonds which are present in all amyloid fibrils.[35,36]

In contrast, the dry interface does not contain water molecules and exhibits a much shorter sheet-sheet separation of 8.5Å. Whereas the polar side chains at the wet interface are completely hydrated by water molecules, the polar side chains of the dry interface are tightly interdigitated with that of the opposing sheet. These residues do not form hydrogen bonds with each other and their close packing indicates instead the presence of van der Waals interactions. Furthermore, π-stacking between aromatic residues, as suggested by Gazit and co-workers, may appear at the dry interface.[37] Due to the fact that the interdigitated residues at the dry interface look like the teeth of a zipper the authors decided to call it a “steric zipper” interaction (Figure 2.3 d). Comprising the knowledge about the macroscopic appearance of amyloid fibrils with these structural data, one might conclude that two sheet layers connected by “steric zipper” interactions represent one protofibril and further lamination is driven by interactions of the wet interface. This would also be in agreement with X-ray diffraction data with a 4.7Å reflex describing the distance between strands of one sheet and a weaker 10Å reflex denoting the characteristic spacing between sheets at the dry interface. Unfortunately, the authors do not address this question.

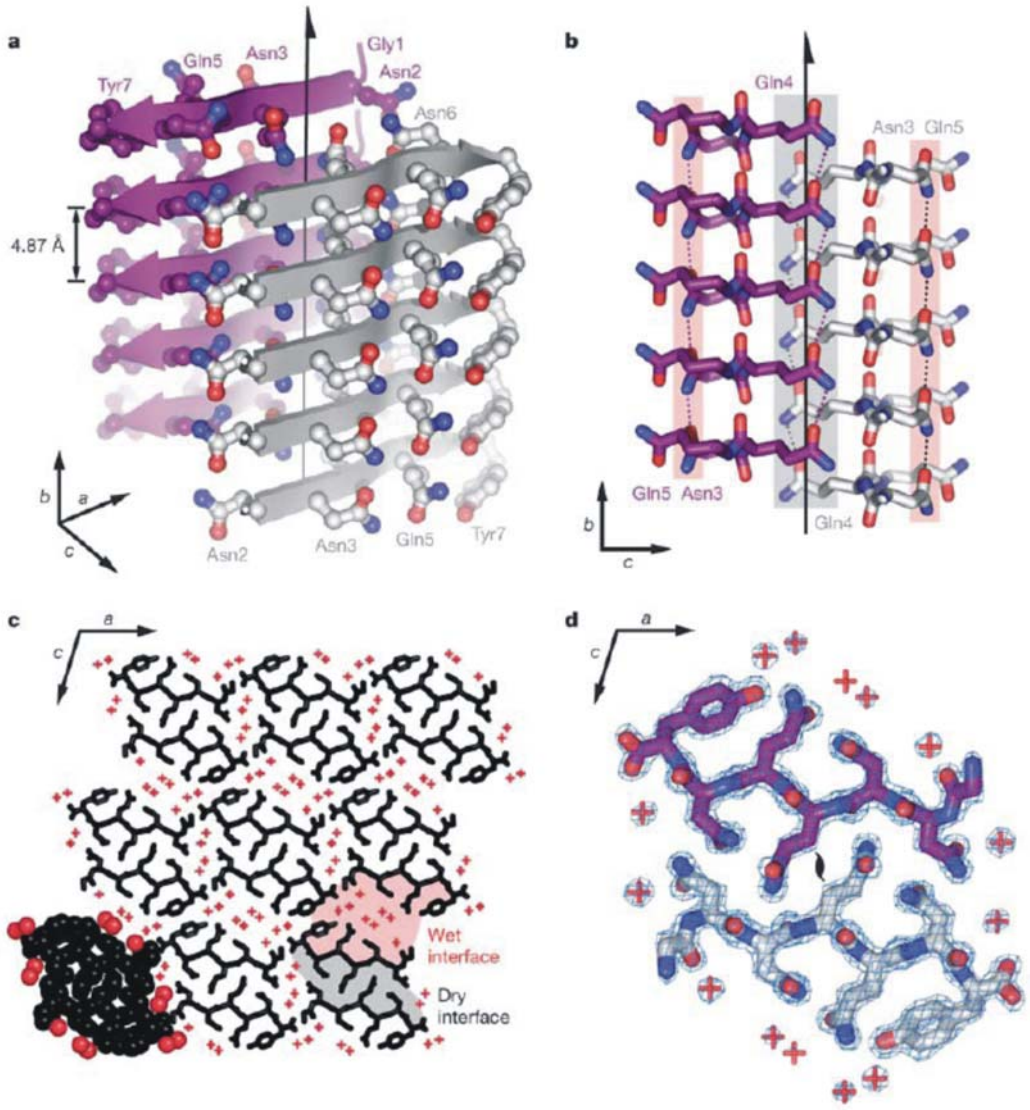

Figure 2.3. *Structure of GNNQQNY. (a) The pair of sheets structure, the backbone of each strand is depicted as an arrow with protruding side chains. (b) View along the sheets with the dry interface on the inside and separated residues on the outside of the fibril. The vertical shift of the sheets relative to each other indicates the tight packing within the dry interface. (c) View along the fibril axis representing dry and wet interface. Lamination occurs along the c axis. (d) A closer view along the fibril axis emphasising the "steric zipper" interaction (according to R. Nelson et al.)*[32]

Although the molecular details that lead to regular fibril spacing at the wet interface are currently unclear, recent solid-state nuclear magnetic resonance (NMR) investigations are addressing this issue more closely. The internal protofilament structure of many naturally occurring amyloids has been investigated by this method and showed similar

structural features like observed for GNNQQNY.[38-43] Goto and co-workers characterized protofilaments of a 22 amino acid fragment of β2-microglobulin and found closely packed, staggered β-strand-loop-β-strand segments, which are aligned in a parallel manner.[38] This study furthermore indicates that maximized intermolecular interactions at the dry interface are a result of a close "steric zipper" packing as well as an efficient π-π stacking of aromatic side chains. Moreover, investigations of a non-native 26 amino acid peptide showed analogical β-hairpin structures with a parallel alignment and "steric zipper" packing.[39] A proposed structural model for amyloid fibrils formed by residues 218-289 of the HET-s fungal prion protein revealed that one polypeptide chain can participate with two different strands in each of the two different sheets, as long as the peptide chain is long enough.[43] More recently, the same group stated that highly flexible regions of the 218-289 HET-s fragment are located on the outside of a protofilament.[31] This additional conformational space appears to be the requirement for this exceptional four-strand arrangement.

Solid state NMR also provided detailed information on the inner protofibrillar structure of $A\beta_{(1-40)}$.[40-42] A very accurate structural characterization of $A\beta_{(1-42)}$ with oxidized Met at position 35 ($^{35Mox}A\beta_{(1-42)}$) in combination with pair wise sequence mutagenesis, cryo-TEM and modeling approaches furthermore provides a clue on the protofibrillar arrangement within Aβ fibrils.[44] The obtained structure is shown in Figure 2.4. It consists of β-strand-loop-β-strand segments, which are aligned in a staggered manner as reported for β2-microglobulin. In other words, the β1-strand of the blue $^{35Mox}A\beta_{(1-42)}$ hairpin is adjacent to the β2-strand of the green $^{35Mox}A\beta_{(1-42)}$ hairpin (Figure 2.4 A and B). These findings of a staggered Aβ amyloid core structure with exclusively intermolecular *"steric zipper"* packing between the residues are distinct from previously reported models that proposed intramolecular interactions in Aβ protofibrils.[40-42] Beyond this, these experiments provide a slight evidence that lamination is indeed directly related to interactions between protofibrils at the wet interface. It is known from previous investigations that residue 4 of Aβ's poorly structured N-terminal part forms intermolecular cross-links to methionine 35 on the outside of the β2-strand (Figure 2.4 C).[45] Connecting this knowledge with cryo-TEM (Figure 2.4 E) and modeling data (Figure 2.4 D), which show paramount similarities, suggests an inter-protofilament binding between these residues at the wet interface. Furthermore, recent studies based on solid state NMR and computational approaches provided evidence that $A\beta_{(1-40)}$ lamination occurs via antiparallel aligned protofilaments.[41]

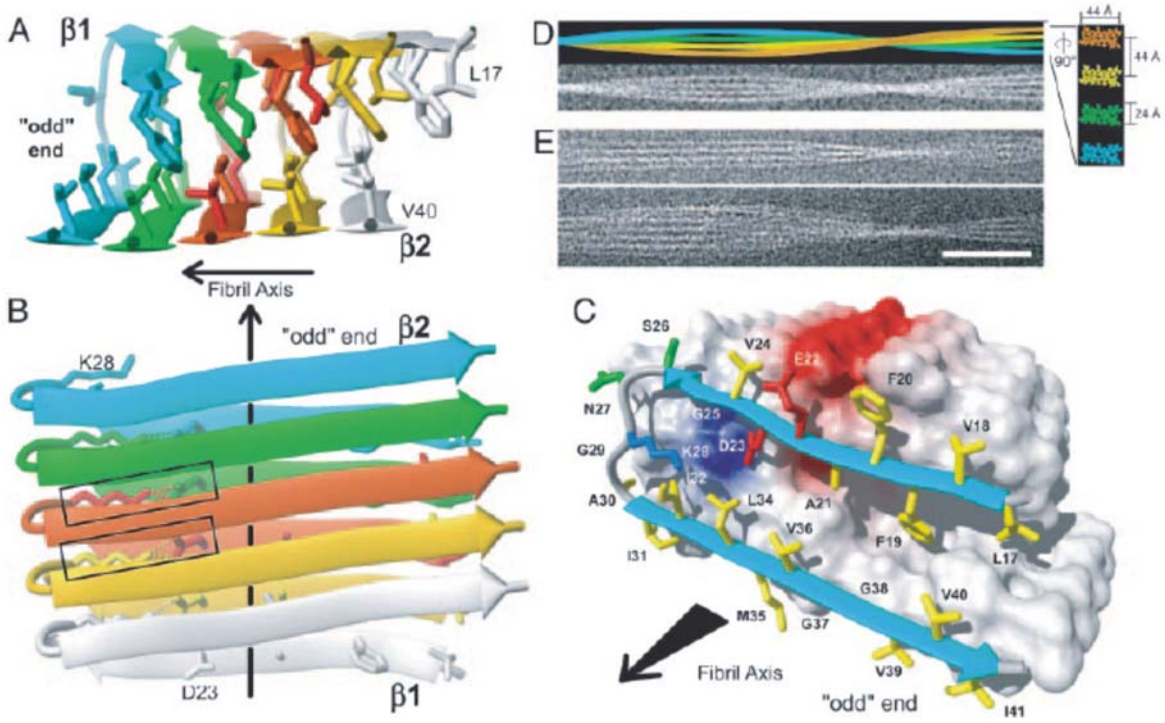

Figure 2.4. *The 3D structure of $^{35Mox}Aβ_{(1-42)}$. (A and B) Ribbon diagrams of the hair-pin structure of residues 17-42 aligned as a staggered "steric zipper" at the inner core of the protofilament. (C) Van der Waals contact surface polarity and ribbon diagram of one $Aβ_{(17-42)}$ hairpin. (D) (Upper) Simulation of an $^{35Mox}Aβ_{(1-42)}$ fibril that consists of four individually coloured protofilaments. (Lower) The same modelled fibril shown as a grey-scale image blurred to a resolution of 2nm. (E) Two examples of cryo-TEM micrographs of $^{35Mox}Aβ_{(1-42)}$ fibrils (scale bar 50nm) (according to T. Luehrs et al.)*[44]

Beside the widely accepted cross-β motif several groups have proposed that amyloid fibrils adopt a structure similar to that found in β-helical proteins or nanotubes.[36,46-50] This alternative model arose from the interpretation of X-ray diffraction and EM data and was first published in 1997.[49] The reasoning is based on the absence of the weak but characteristic X-ray diffraction reflex at 10Å in case of poly-L-glutamine peptide $D_2Q_{15}K_2$, model peptide KLKLKLELELELG, the exon-1 peptide of huntingtin and some Aβ fragments.[32] Furthermore, proline scanning analysis suggested this motif to be present in fibrils of full-length $Aβ_{(1-40)}$.[50] The structural idea is that one or more β-strands wrap around a hollow or water-filled core in a cylindrical or helical manner.[47] These helical nanotubes represent protofibrils, which bunch to form fibers with an already discussed macroscopic appearance. A computer rendered visualization based on the structure of poly-L-glutamine peptide $D_2Q_{15}K_2$ proposed by Perutz and co-workers is shown in Figure 2.5.[47,51] The central pore features a diameter of ~ 15Å, which would be

large enough to pass small ions. A recent investigation showed that this structural motif could serve as an explanation why Aβ and similar amyloid forming peptides are able to solubilize cell membranes.[51] Moreover, it provides evidence why early aggregates of non disease related amyloids show toxicity.[9] Thus, it appears likely that it is the common structural behavior of amyloids which is crucial for their toxicity. Unfortunately and due to the length scale of more than 5-6Å, solid-state NMR is inconclusive and not able to differentiate between a parallel in-register β-helix and an in-register cross-β structure. Nevertheless, NMR of Aβ fibrils clearly indicates the presence of two distinct β-sheet regions, while β-helices contain at least three β-strands per turn.

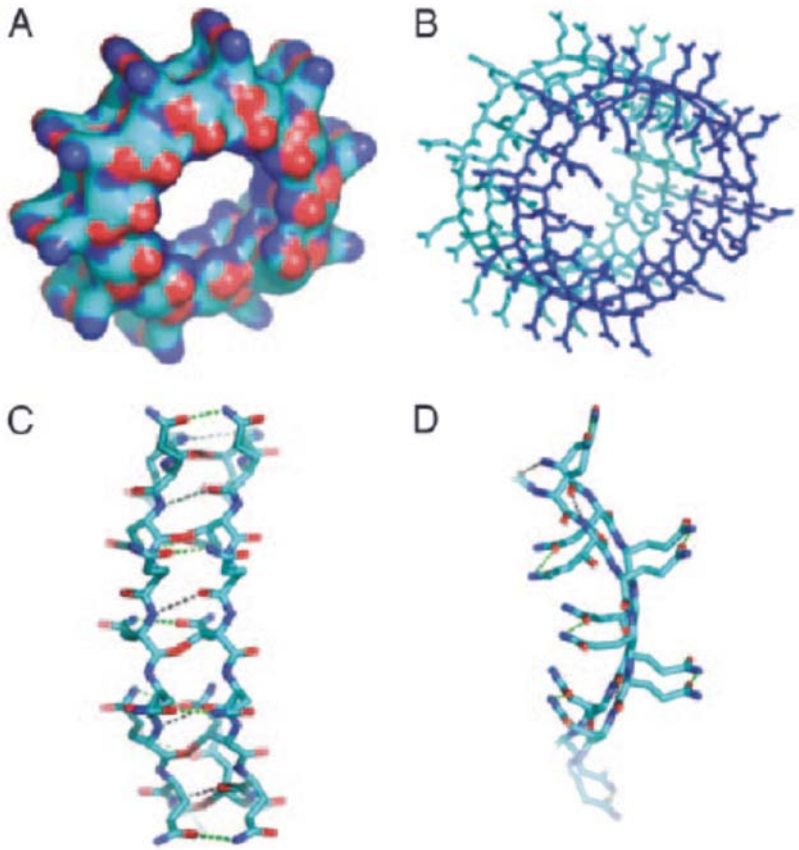

Figure 2.5. *(A) Space-filling model of one subunit of 42 Q residues with two turns of β-strands. (B) Stick model of two subunits of poly-Q, each subunit consisting of 42 Q residues. One subunit is depicted in dark blue. (C) Stick model of a small segment showing inter-β-strand hydrogen bonds between the backbone NH and C=O groups. (D) Stick model of a small segment hydrogen bonds between Q side chains (according to S.J. Singer et al.)*[51]

However, both structural models describe structure related features of amyloids and are therefore valuable. So far, none of these models has been proved or disproved. Thus, further investigations comparing structural properties of amyloid forming peptides and naturally occurring β-helices are needed to clarify this controversially discussed topic.

2.2 The Process of Amyloid Formation – One Way?

2.2.1 Proposed Pathways Leading to Amyloids

The formation of amyloids is a two-step reaction that consists of a slow, kinetically driven nucleation step followed by a molecular assembly step. Expectedly, seeding with preformed fibrils results in a perceptibly reduced lag phase for the nucleation step.[19,52,53] Thus, the early aggregation step yielding the nucleus appears to be crucial for the amyloid formation process in general. On the other hand, recent studies showed that some proteins may form amyloids with a non-nucleation dependent pathway. As a result, kinetically trapped aggregates with a fairly poor degree of ordering compared to classical fibrils are obtained.[54] Interestingly, the protein β2-microglobulin forms amyloids following both the nucleation dependent as well as the nucleation independent pathway, respectively. The applied environmental conditions were shown to control the kinetics and the resulting aggregate morphology for both pathways was substantially different. However, the general validity of the non-nucleation dependent pathway has neither been proven nor refuted. Though, it is still widely accepted that the nucleation dependent growth represents a common characteristic of amyloids.

The simple observation of the amyloid formation kinetics does not provide information on the underlying structural processes. Therefore, the question arises whether the kinetically driven early aggregation step that yields the nucleus is related to previous or concomitant structural changes.[55] It has been proposed that the amyloid formation process involves a series of folding and unfolding events. (Figure 2.6).[10,11,56] This theory of partially unfolded and partially folded intermediates is widely accepted. The basis is the observation that all amyloids, independent from their origin, share the cross-β structural motif. From this it follows that conformational changes transferring the molecule from the native conformation to the amyloid fold are obligatory. In detail, the native structure of the involved protein has to be considered. In case of highly ordered globu-

lar proteins, most of the residues are tightly packed in the inner core, which limits interactions with its surrounding. The crucial nucleation step, however, is mediated by specific intermolecular interactions such as hydrogen bonds, hydrophobic interactions, and salt bridges. Thus, it is necessary that the molecule at least partially unfolds to enable intermolecular interactions (Figure 2.6 i). On the other hand, amyloid forming peptides and proteins without defined native structure must exhibit a different transformation step. Due to the absence of ordering it appears likely that rather a partially folded precursor provides here the ability for the necessary intermolecular interactions (Figure 2.6 ii). Thus, combining these two pathways suggests that the transformation into a partially ordered intermediate is common to all fibril forming processes.[10,11] This intermediate, which contains a certain fraction of β-structure is stabilized by an ordered self assembly (Figure 2.6 iii). Alternatively, Tjernberg et al. suggested the formation of large amorphous aggregates (Figure 2.6 iv).[57] The amorphous aggregates may facilitate nucleation by providing a high local concentration of kinetically trapped amyloidogenic intermediates (Figure 2.6 v). Moreover, Dobson and co-workers showed that very different intermediate structures are able to form very similar aggregates.[58] However, the emergence of the nucleus is followed by the formation of prefibrillar aggregates and fibrils by amyloid extension (Figure 2.6 vi and vii).

In contradiction to the previously reported mechanism, recent investigations showed that the formation of early amyloid aggregates is not necessarily accompanied by structural changes.[59-61] Chiti et al. succeeded characterizing early aggregates of the 101 residue acylphosphatase from *Sulfolobus solfataricus* (Sso AcP), which showed native-like enzymatic activity and exhibit a structure that is more related to the native fold than to that observed for the protofibrillar amyloid precursors.[60] Therefore, they concluded that it is very unlikely that the conversion into protofibrils occurs via disaggregation and unfolding. The process could rather be described as an aggregation of native-like oligomers, which subsequently promote the conversion of the protein into amyloid assemblies. This suggests that at least some proteins may not require drastic destabilizing conditions to convert into amyloid protofibrils and fibrils. Thus, the generality of the partial folding and unfolding mechanism has been put into perspective again.

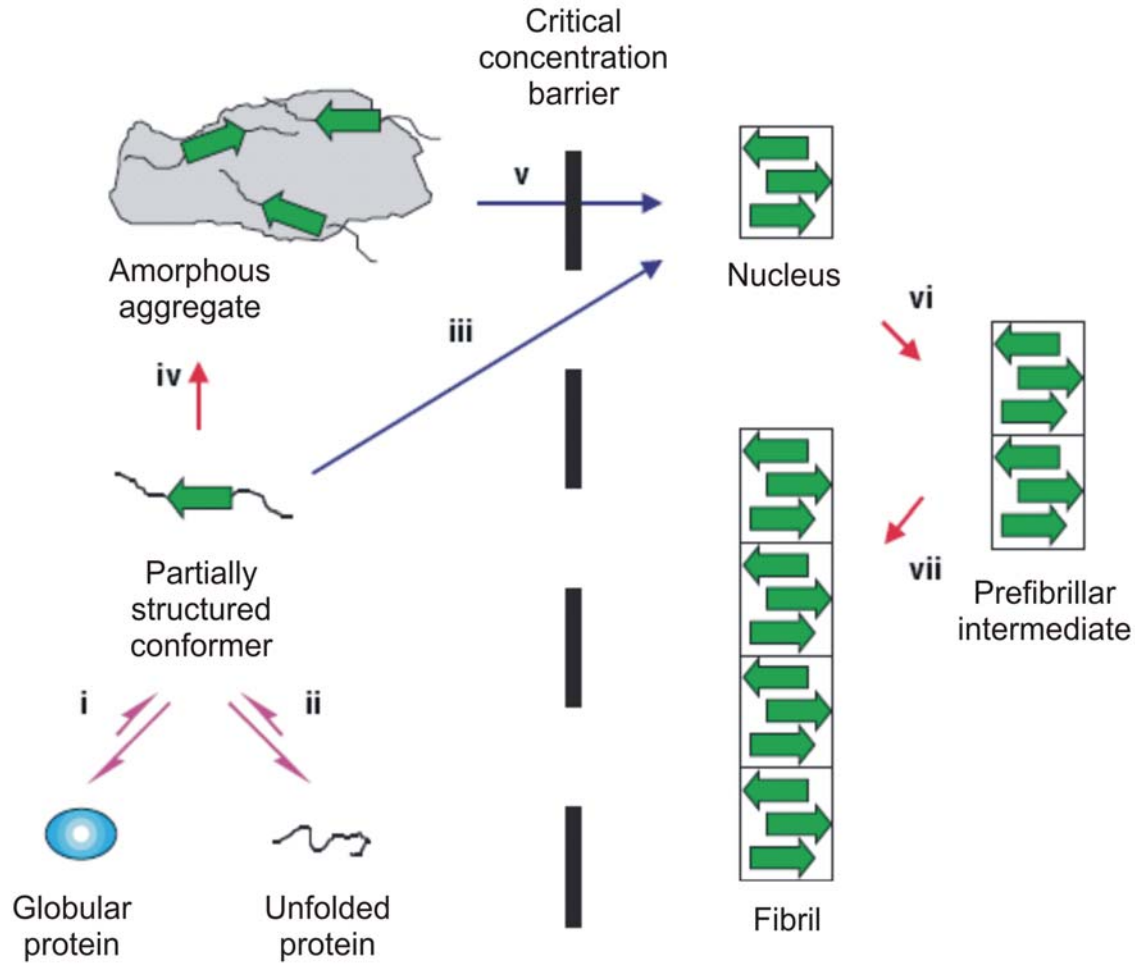

Figure 2.6. *Proposed mechanism for the formation of amyloids by partial folding and unfolding. (i and ii) Partial unfolding of folded proteins or partial folding of unfolded proteins, respectively, results in the formation of partially structured intermediates. These intermediates either self assemble to form the nucleus (iii) or alternatively associate forming amorphous aggregates followed by a realignment (iv and v). After development of the nucleus prefibrillar intermediates and fibrils are consecutively formed (vi and vii) (according to J.-C. Rochet et al.)*[10]

2.2.2 Environmental Changes Triggering a Conformational Transition

Although alternative aggregation pathways without the necessity of structural conversions are described, the partial folding and unfolding mechanism inescapably shifts the attention to the conformational transition causing aggregation and fibril formation. It is commonly accepted that mutations in the primary structure, as observed for early-onset familial Alzheimer's disease, yield structural changes and rapid progression of amyloidosis.[1,12] More importantly, a changed environment to which natural proteins are exposed at translocation or stress conditions can induce misfolding. In other words, changing the environment of a peptide or protein can alter its conformation and therefore has to be considered as a potential trigger or inhibitor for amyloid formation.[13]

In principle, various factors represent criteria of a changed environment. Alteration of the pH value is one of the most evident changes and its impact on conformation and amyloid association has been shown in many studies.[62-65] Furthermore, transition metal ions such as copper, zinc, manganese, iron, and aluminum are known to bind potential complexation sites in peptides and proteins, namely, side chain residues of cysteine, histidines, and tyrosine. Depending on the nature of the metal, this coordination requires a defined geometry at the surrounding metal center and therefore to some extent dictates the structure.[66-68] Moreover, the redox chemical properties of transition metals are the principal chemical origin of radicals and reactive oxygen species that are implicated in the pathogenesis of many neurodegenerative diseases.[69-71] Also changes of the ionic strength, which are allegorized by dialysis processes in nature, can substantially alter the conformation and yield or prevent amyloids as a result. Studies on β2-microglobulin clearly indicate that an increase of salt perceptibly accelerates the amyloid formation rate.[72] Furthermore, the absence or presence of membrane surroundings shows an impact on structural changes.[73] Even more obvious is the effect of solvents.[74] For instance it is undoubtedly clear that the presence of hexafluoroisopropanol or trifluoroethanol changes the protein structure to a preferably helical conformation. Although organic solvents represent rather harsh conditions which are far from nature they clearly indicate their potential to trigger or inhibit protein aggregation. More recently it has been shown that even in the absence of metal ions oxidative stress mediated by aldehydes can trigger the misfolding of Alzheimer's Aβ.[75] Owing to this huge variety of factors and examples, the impact of environmental conditions on the resulting

secondary structure of amyloid forming model peptides is described more precisely in the following sections.

2.2.3 The Molecular Recycling Hypothesis

Due to their high degree of ordering, amyloid fibrils have been thought to represent the perfect example of a static assembly formed by an irreversible process.[5,76] It means that once the fibrillar structure has been formed, amyloid structures are indestructible at physiological conditions and no or only a minor exchange with the environment takes place. Although this has never been proven, it has been generally accepted. A recent and very convincing study by Dobson and co-workers suggested that this is not the case (Figure 2.7).[77] These groundbreaking investigations are a milestone in the field of amyloid research and therefore need to be described here.

Dobson and co-workers studied the dynamical behavior of amyloid fibrils formed by the α-subunit of bovine phosphatidylinositol-3'-kinase (PI(3)K-SH3) by H/D exchange NMR and mass spectrometry as well as electron microscopy experiments. Prior to dissolution with dimethylsulfoxide (DMSO) preliminarily grown fibrils were exposed to a deuterated buffer solution for a different amount of time. Only a minor exchange at the side chain amide groups would be expected for a static assembly of molecules. Interestingly, a perceptible degree of exchange has been observed in a time range of days, even at backbone amide groups. To clarify whether this exchange is a top-down process, the fibrils were subjected to repetitive pipetting and sonication. The obtained rate constants clearly indicate that the time for recycling half of the molecules within an ensemble of fibrils with an average length of 100nm is between 2 and 20 days. In a more general context, amyloid fibrils thus represent a dynamic equilibrium that constantly exchanges between fibrils and their soluble precursors in a timescale which is relevant for biology (Figure 2.7). As this is the first observation for an exchange process, it is so far unclear whether amyloid recycling is a common characteristic of all amyloids or just an exceptional feature of the particular protein investigated here.

Additionally, numerous studies from the Wetzel group led to the hypothesis that, for some amyloid forming proteins such as $A\beta_{(1\text{-}40)}$, the endpoint of fibril formation is a dynamic equilibrium between monomers and fibrils, which exhibits a characteristic equilibrium constant with an associated free energy of formation.[78,79] The residual monomer in solution, also denoted as "critical concentration" (c_r), can be quantified by HPLC and

provides information on the thermodynamics of the fibril elongation reaction and fibril stability. These findings are impressively consistent with the amyloid recycling hypothesis.

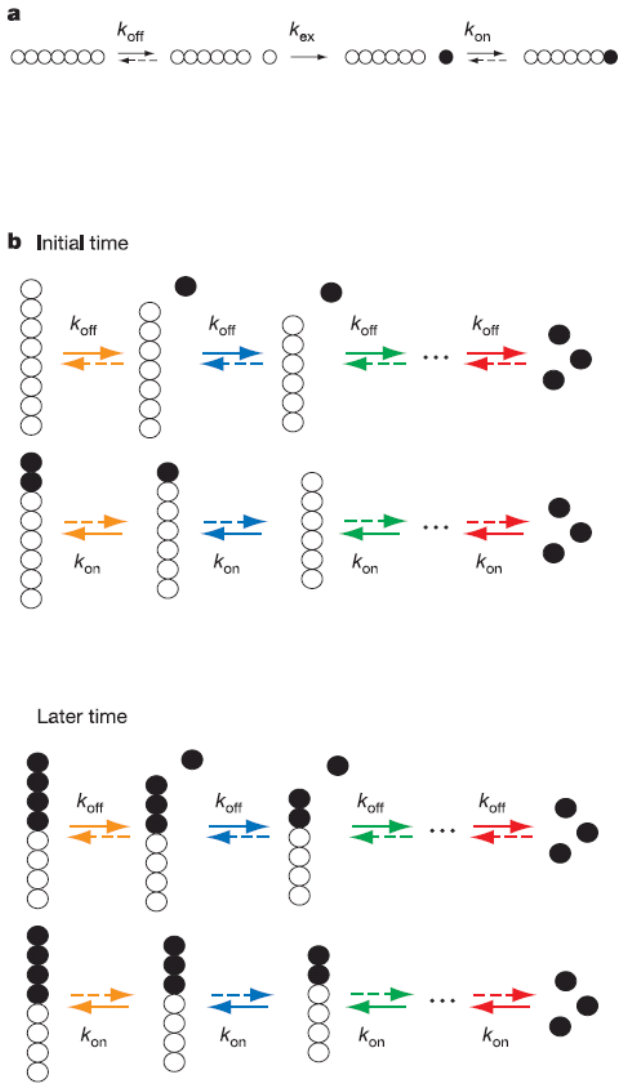

Figure 2.7. *The molecular recycling model. (a) Schematic representation of the recycling process involving a single amyloid fibril. A protonated protein molecule (open circle) dissociates from the fibril. Once in solution, hydrogen exchange takes place rapidly and the molecule is subsequently reincorporated in a fully deuterated state (filled circle). (b) Schematic representation of the recycling mechanism for a distribution of amyloid fibrils at different times of exchange. At a given time point the dissociation of a molecule from a fibril (above) is counteracted by the re-association of that molecule in another fibril (below) (according to N. Carulla et al.)*[77]