Short Conceptual Overview

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The impact of water on the ambivalent behavior and paradoxical phenomenon of the amyloid-β fibril protein

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Abstract: The crucial role of water in amyloid-β(Aβ) fibril proteins is evaluated in several ways including the water's thermodynamic and kinetic solvation effects. As regards the water's character, its hindered-rotation barriers are also considered. The following protein molecules considered here are: the $A\beta_{40}$ (PDB ID: 2LMN), $A\beta_{42}$ (PDB ID: 5KK3 and 2NAO) and the double-layered $A\beta_{17-42}$ fibril. We discuss: (i) extracellular $A\beta_{40}$ and $A\beta_{42}$ fibril monomers exhibit an ambivalent propensity to transform into a helical form toward the N-term region and a β-strand-like form near the C-terminal; (ii) interfacial water molecules play a crucial role in protein-protein interactions, as molecular dynamics simulations have shown a significant impact on the protein-protein binding; (iii) it is shown that the spontaneous dimerization process of the $A\beta_{42}$ fibril protein in water occurs via a two-step nucleation-accommodation mechanism; (iv) MD simulations of the double-layered $A\beta_{17-42}$ fibril model show that the C↔C interface appears more energetically favorable than the N↔N interface due to large hydrophobic contacts; (v) the water's role in the HET-s prion and in the Aβ fibrillar aggregates; (vi) it was found that the monomer-oligomer equilibrium spontaneously dissociates into stable monomeric species when they are incubated up to 3 μM for a longer time (>1 week) in a physiological buffer.

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Introduction

Together with other key publications, we have previously shown the essential role of water with respect to structure, dynamics and activity of proteins (1–5), termed dynamics-structure-activity relationships (DSARs) (6), a key feature of water now extended to amyloid-β(Aβ) fibril proteins (7–11). It is well known, that the accumulation of the Aβ peptide in the brain is responsible for debilitating diseases, as in Alzheimer's and Parkinson's diseases (12). It may be mentioned that the toxic properties of Aβ also requires the cooperation of the intrinsically disordered tau protein (13, 14). A variety of amyloid diseases begin as benign amyloid-β monomer fibril proteins which then develop into toxic amyloid dimers/oligomers (15). Nevertheless, the important difference between proteins in general and that of the Aβ fibril ones should be kept in mind. Namely, both experimental and theoretical thermodynamic stabilities of a series of amyloid fibrils proteins revealed that this structural form is likely to be the most stable one, a stability that can be acquired even under physiological conditions. Perczel and his team have shown that the β-pleated sheet conformation of the peptide backbone is the thermodynamically most stable structure of all possible polypeptides both in vacuum, in aqueous environments and even in a crystalline state (16). According to this team, this also means that once the aggregation has started, there is no thermodynamic reason for it to stop (16). The other protein systems, however, are also metastable in monomer and oligomer forms (17).

Therefore, from a thermodynamics point of view the other secondary structures of the polypeptide chains can be considered as being of proteins in a metastable state of high Gibbs free energy. According to their

kinetics, however, the α-helix, hairpins, γ-turns, etc., conformations are existing in local minima isolated by kinetic energy barriers, i.e. they are kinetically trapped (17).

Concerning the toxicity of Aβ fibrils proteins, in spite of the earlier conception of the need of large fibril aggregates for this purpose, the newer experimental and theoretical results have proven the efficiency of oligomers as small as a dimer is. Amyloids appear to be structures that were not selected for molecular evolution. Recent studies have shown that the ability to form amyloid proteins is given not by some specific sequences, but it can be considered as a generic feature of the polypeptide chains.

The surfaces of the Aβ fibril proteins alter the structure and behavior of the surrounding layer of hydrating water molecules (5). Hydration water molecules typically have slower correlation times than those of the bulk. The effects of interactions with surfaces typically extend only into the water's one to two hydration shells. Polar groups favor direct interactions with water molecules, whereas non-polar groups enhance interactions among the protein molecules themselves. Water plays several roles on the surface of proteins, e.g. filling in gaps and cavities, bridging unsatisfied hydrogen bonds, mediating fibril-fibril interactions.

Aβ peptides are cleaved from a transmembrane protein called the amyloid precursor protein (APP) by proteolytic cleavages of the β- and γ-secretases (18). There are two types of these fibril protein molecules: namely the $A\beta_{40}$ and $A\beta_{42}$ monomers. Although both species sample β-rich conformers in solution that may represent prefibrillar intermediates, the probability that $A\beta_{42}$ sample these prefibrillar states is roughly an order magnitude larger than that of $A\beta_{40}$ (19). Nevertheless, it is important to note that a fibrillar structure has been determined for the $A\beta_{42}$ protein by solid-state nuclear magnetic resonance (NMR) spectroscopy, as was investigated by three research groups (20–22). The third team also studied this molecule by cryo-electron microscopy (22). On the other hand, a hairpin-like structure of the full-length $A\beta_{42}$ monomer was determined by NMR (18) and by small angle neutron scattering (23) in the presence of organic co-solvents (e.g. hexafluoroisopropanol), as shown in Figure 1.

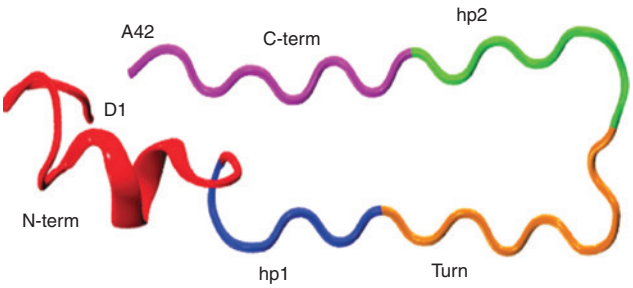

Figure 1: A typical conformation of the full-length $A\beta_{42}$. Reproduced from ref. (18).

The $A\beta_{42}$ peptide monomer

The amino acid residues of the $A\beta_{42}$ fibril peptide chain is given below (Scheme 1) (18), where the colors correlate to the hydropathy index (24): green = polar [(−3.2) – (−4.5)] and red = non-polar [(+4.5) – (+2.8)]. As can be seen, the $A\beta_{42}$ molecule's sequence includes a polar/non-polar proportion of 14/14, where the N-terminal half has 10 polar and the C-terminal has eight non-polar residues, i.e. this implies a polar dominance, which means therefore, the ambivalent behavior of the $A\beta_{42}$ fibril protein.

Definitely, if the polar N-terminal part dominates in a given conformer then the monomer will be strongly hydrated (≈5 Å is the first hydration layer), while non-polarity of the C-terminal part results in the oligomerization, all depending on the molecular conditions such as temperature, pH, ionic strength, etc.

The basic structural motif and major biophysical properties of Aβ are included in the fragment 18–35. In this case, the N-terminal part includes four polar and the C-terminal part has seven non-polar residues, which thus means a non-polar dominance (25). Nevertheless, to see the ambivalent character it is a mandatory requirement to deal with the full molecule. Perczel, Császár and their coworkers investigated the interactions between the non-covalent aromatic rings and ions including the most interesting ones in our case, namely: the interactions between the aromatic F, Y, W and the cationic R amino acid of the peptide chain (26, 27). In the case of the $A\beta_{42}$ fibril, the most likely interaction might be between the F4 and R5 amino acid residues, according to the CP⊥ perpendicular cationic position [see figure 1 of ref. (27)]. For the described type of stabilizations, the authors give interaction energy of 23–37 kcal mol^{-1}.

Bandyopadhyay and his coworkers (18) carried out molecular dynamics (MD) simulations of the $A\beta_{42}$ peptide monomer at room temperature. The different segments of the peptide chain were examined, namely the N-term and C-term, the central hydrophobic regions (hp1 and hp2)

$A\beta_{42}$ DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Scheme 1: The amino acid sequence of the $A\beta_{42}$ fibril peptide (ref. 18, p. 30145).

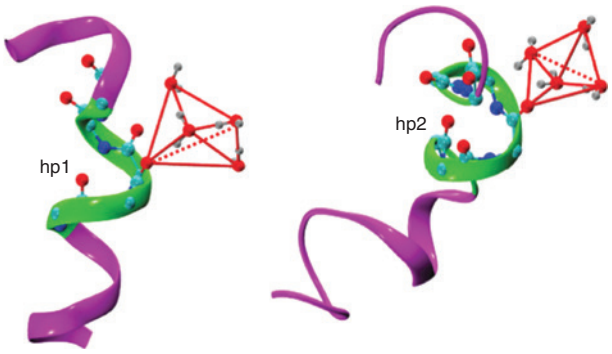

Figure 2: Representative snapshots of the Aβ peptide monomer (partial view) showing water molecules tetrahedrally bound to the backbone oxygen atoms of hp1 (left) and hp2 (right). Reproduced from ref. (18).

and the central turn spatial arrangements and binding energies of the surrounding water molecules were also studied. The Aβ monomer exhibits a propensity to either retain or transform into a helical form toward the N-term region and a β-strand-like form near the C-term segment. Besides, heterogeneous conformational flexibility of the Aβ monomers has been found to be correlated with the corresponding nonuniform entropy gains. Furthermore, it was found that irrespective of the Aβ peptide conformations and their nonuniform fluctuations, water molecules around the hydrophobic hp1 and hp2 segments are relatively weakly bound. This also means that the weakly bound water molecules around hp1 and hp2 are expected to be easily displaced during the hydrophobic collapse that leads to the Aβ fibril protein aggregation.

Also the thermodynamic parameters of the $A\beta_{42}$ monomer fibril were calculated (18). Considering the calculated binding energies [(tagged potential energy (TPE) (U_{TPE})] of the hydration water according to the segments are as follows: As a reference, the average TPE of the pure bulk water is –19.7 kcal mol^{-1}. The minima in U_{TPE} for water molecules bound to hp1 and hp2 are around –20 kcal mol^{-1}, while the data of the N-term, C-term and turn regions are within –22 to –23 kcal mol^{-1}. The water molecules tetrahedrally bound to the backbone oxygen of hp1 and hp2 are shown in Figure 2.

The extracellular $A\beta_{40}$ and $A\beta_{42}$ fibril peptides

Petkova and coworkers (28) studied in detail the molecular structure of the $A\beta_{40}$ fibril protein using the solid NMR technique, and also using X-ray fiber diffraction, transmission electron microscopy and MD simulations. Although this molecule is missing the Ile and Ala amino acid (41–42) residues: the C-terminal part of the $A\beta_{42}$ fibril protein, does not make any essential difference in the structure and or in the conformation. [The hydropathy indices are: Ile = 4.5; Ala = 1.8 (23).]

A structural model is presented based on the above given toolkits and showing the following features. Approximately the first 10 residues of the $A\beta_{1-40}$ are structurally disordered, while the residues of 12–24 and 30–40 adopt β-strand conformations and form parallel β-sheets through intermolecular hydrogen bonds. Residues 25–29 contain a bend of the peptide backbone, which is then a double-layered β-sheet structure with a hydrophobic core and one hydrophobic face. The only charged side chains in the core are the D23 and K28 amino acid residues, which form a salt bridge that may stabilize the structure. In the low dielectric environment that may exist in the interior of an amyloid fibril, electrostatic repulsions between like charges might destabilize the parallel β-sheet structure by ≈100 kcal/mol, overwhelming the favorable hydrophobic energy.

In conclusion, the significant features of the $A\beta_{42}$ model are: (i) residues 1–8 are omitted because of the N-terminal structural disorder; (ii) the peptide conformation contains two β-strands, separated by a 180° bend formed by residues 25–29; (iii) the β-strands form two parallel β-sheets, which interact through side chain-side chain contacts; (iv) except for D23 and K28, the side chains in the core of the resulting double-layered structure are neutral and primarily hydrophobic; (v) side chains of D23 and K28 form a salt bridge across the bend; (vi) the side chains of A30, I32, L34, V36 and V40 form a hydrophobic face; and (vii) other charged and polar side chains are distributed on the opposite face, on the convex side of the bend, and in the N-terminal segment.

The role of interfacial water in the protein-protein binding

Interfacial water molecules are considered to play a crucial role in protein-protein interactions, as molecular dynamics simulations have shown this significant impact on the protein-protein binding thermodynamics. The emergence of water in the interfacial region that bridge two biomolecules through concurrent hydrogen bonds and exhibit extremely slow hydrogen-bond rearrangements (~1000 times slower than in bulk water) has been demonstrated. This extremely slow nature of

bridging water is due to the high number of hydrogen bonds involved, and also to the additional stabilization resulting from the strong electrostatic field between the binding surfaces of electrostatic complementarity (29, 30). Besides this protein-protein binding energy yield, however, the water's entropy gain should also be considered due to this protein dimerization.

Amyloid-β fibril protein dimerization

The spontaneous dimerization process of the full length $A\beta_{42}$ fibril protein in water was reported by Chong and Ham, as using fully atomistic, explicit-water molecular dynamics simulations (31). Based on the thermodynamic analysis, it is demonstrated that the dimerization in water occurs via a two-step nucleation-accommodation mechanism driven by water-induced force and by the protein internal force, respectively (Figure 3). The Aβ-dimer is particularly important as it is the smallest oligomeric toxic species. The $A\beta_{42}$ dimerization simulations were conducted at 300 K and 1 bar under neutral pH where each $A\beta_{42}$ monomer is in the –3 charged state due to its amino acid composition [3D, 3E/1R, 2K]. The two monomers were initially placed 45 Å apart from each other and they were solvated with 24 708 explicit water molecules and also six Na^+ counter ions were added to neutralize the system (31). Once the two approaching $A\beta_{42}$ monomers develop sufficient heavy atom contacts around potential nucleation sites, they do not fall apart and the dimerization proceeds to the next stage. The authors calculated the collision cross-sections along the dimerization trajectory. The computed collision cross-sections of both the initial $A\beta_{42}$ monomer structure (752 Å^2) and the final $A\beta_{42}$ dimer structure (1287 Å^2) are in good agreement with the experimental values (702 and 1256 Å^2), respectively). These simulations also demonstrated the possibility that the increased solvation free energy of a dimer at the end of the second step would play a role in inducing further oligomerization process in water (Figure 3).

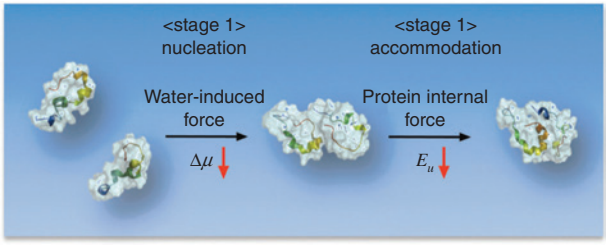

Figure 3: Schematic illustration of the two-step, nucleation-accommodation mechanism of Aβ42 dimerization in water.
The initial nucleation stage is driven by the water-induced force that originates from the decrease in the solvation free energy $\Delta\mu$. The subsequent structural accommodation stage is driven by the decrease in the protein internal energy E_u. Reproduced from ref. (31).

Modeling the $A\beta_{17-42}$ fibril architecture and oligomerization at different protonation sites

The $A\beta_{17-42}$ protofibril structure in solution using MD simulations was investigated (32). It is also important to study the 17–42 peptide chain's hydrophobic character, as the part of the ambivalent $A\beta_{42}$ fibril protein. Namely, this residue of the peptide chain is responsible for the molecular dimerization. The authors (32) generated double-layered models to gain an insight into how two β-sheets associate along the direction perpendicular to the fibril axis, as can be seen in Figure 4.

The analysis led to the following conclusions: interaction energy estimation indicated that both the C↔C and N↔N interfaces exhibit stable structures, but the C↔C interface appears more energetically favorable than the N↔N interface (not considering the N↔N hydration) due to large hydrophobic contacts. Aβ simulations also show that all oligomers with the U-bent strand-loop-strand structural motif have an inner hydrophobic cavity of 6–7 Å in diameter that allows small molecules such as ions and water molecules to be conducted through. The hydrated hydrophobic cavity is expected to facilitate an optimal intermolecular sheet-sheet packing. Intramolecular hydrated cavities run in the fibril axis direction.

The role of Water in the HET-s prion and in the Aβ fibrillar aggregates

The thermodynamic properties of the experimental fragments of the amyloid fibril of the HET-s prion protein (the infectious element of the filamentous fungus *Podospora anserina*) and of the Aβ proteins with using the three-dimensional molecular theory of solvation were studied by Kovalenko's team (33). This theory of solvation predicts that water molecules can be locked in the interior

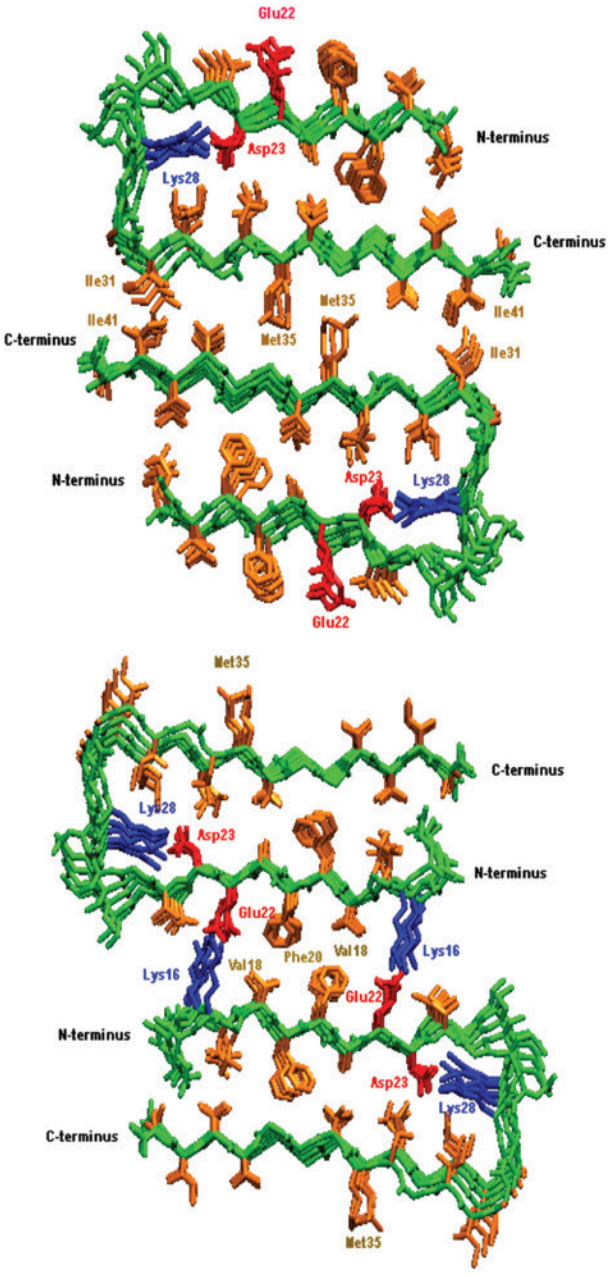

Figure 4: Double-layered structural models of Aβ oligomers. Two β-sheets were associated by Ile31/Ile41 and Ile41/Ile31 (upper) or by Glu21/Lys16 and Lys16/Glu22 (lower) along the direction perpendicular to fibril axis. Color identifications are: backbone (green); negatively charged residues (red); positively charged residues (blue); hydrophobic residues (orange). Reproduced from ref. (32).

cavities along the fibril axis for both the HET-s and Aβ proteins. The results suggest that the interior hydration plays an important role in the structural stability of fibrils. It has also been found that both the HET-s and Aβ pentamers have loose intermolecular packing with voids.

Monomer-oligomer equilibrium

The monomer-oligomer transition is not only the first step of aggregation, but is also the key event of the transformation of a benign protein to a neurotoxic molecule. To study this equilibrium Maiti and his team used, a fluorescent rhodamine-labeled Aβ peptide to characterize the monomeric state instead of the co-solvent technique. The monomer-oligomer transition has also been carried out with the fluorescent method at physiological concentrations in buffers mimicking very dilute cerebrospinal fluid (CSF: <1 μM). Considering this transition from a thermodynamic viewpoint: an aggregation-capable molecule should have a defined equilibrium between the monomer and dimer (oligomer), i.e. where it is monomeric below a certain concentration. Namely, oligomers spontaneously dissociate into monomeric species when they are incubated at a low concentration for a long time (>1 week) in a physiological buffer solution (34). Regarding this conception, it is not surprising that at equilibrium the Aβ fibril peptide molecule appears mainly monomeric up to 3 μM, above which it forms oligomers. This stable monomeric species is similar to what exists *in vivo*. The 3 μM concentration is much higher, however, than the estimated concentrations in the CSF of either normal or diseased brains. Concerning the size of the rhodamine-labeled Aβ monomer, a hydrodynamic radius of 0.9 ± 0.1 nm was detected (34).

Membrane affinity

The Maiti group (35) also detected that the Aβ monomer ($A\beta_{40}$ or $A\beta_{42}$) has a much lower membrane affinity than the small oligomers. The question arises then, why have monomers such a lower membrane affinity compared to the oligomers? Fluorescently labeled Aβ monomers show negligible binding to cell membranes of a neuronal cell line at physiological concentrations (250 nM), while oligomers (even 2–10 mers) at the same concentrations show strong binding within 30 min of incubation. It is likely that membrane binding precedes subsequent slower toxic events induced by Aβ.

These results, i.e. the monomer's low membrane binding (a) provide an explanation for the non-toxic nature of Aβ monomers and (b) offer a quick assay for monitoring the benign-to-toxic transformation of Aβ. At this point it is reasonable to mention that Suman Nag (in Maiti's laboratory) in his unpublished work suggests that the inter-terminal distances of the Aβ fibril are drastically

different between the monomers and oligomers, where such conformational change may then be the key to the increased affinity (see also: the paradox paragraph).

Binding sites of cell plasma membrane

Verdier and Penke (36) studied the binding of Aβ peptides to the cell plasma membranes in AD, with regard to the toxicity on neurons. They discuss the membrane proteins that can mediate the interaction between the β-amyloid peptides and the cell plasma membranes. Aβ peptides can bind a variety of biomolecules such as lipids, proteoglycans and also proteins. According to the suggestion of Verdier and Penke, the binding of β-amyloid peptides to plasma membranes could be a promising possibility for intervention in the events leading to the development of AD.

Paradox phenomena

In the following it is be demonstrated that without the effect of water the paradoxical behavior is incomprehensible. As the paradox facts are referred to (see Expert opinion) a variety of amyloid diseases begin as a benign amyloid-β monomer fibril protein and then develop into toxic amyloid oligomers of fibrils molecules, which is already known for a dimer (34). As the monomer is also present in healthy individuals, it is unlikely therefore to be toxic. Also considering the monomer-oligomer Aβ fibril protein question in the membrane affinity, it can be recognized that this paradox is also related to the innocuous-toxic effects (35, 36).

It is likely that the monomer vs oligomer amyloid-β fibrils' structures differ not just in size, but also in conformation, and the conformational aspects may be the key to understand the toxicity and increased membrane-affinity for the oligomers, as several articles have been published to discuss these aspects. Therefore, here we give some excellent examples. Härd and coworkers (37) suggested that forcing the monomer fibril into a hairpin-like structure increases its toxicity. Smith and coworkers (38) proposed that different oligomeric species of similar size can have dissimilar toxicity, pointing toward the role played by folding. They have also proposed a specific pentameric oligomer model in which the monomers have a conformation which is different than that in the fibrils (39). Suman Nag's (Maiti's laboratory member) witty and important conformational observation is that the Aβ fibril protein shows a drastically different inter-terminal distance between the monomers and the oligomers (Nag, unpublished), where such conformational change may be the key to the above discussed effects.

As we suggest, the Aβ monomer is non-toxic because it has low affinity to the cell membranes, while a dimer of higher affinity can already perforate the membrane by making channels. The smallest aggregated species that we can distinguish (2–10 mers) are able to attack the cell membrane. Although, these above discussed conformational concepts are important determining factors, the essential role of water should not be ignored because otherwise the discussed questions remain unanswered.

To answer these significant paradox questions which are really important and exciting, our following proposed explanation can be considered as an effort to solve them. We assume that the monomer Aβ fibril protein should have a much stronger hydration shell, than the dimer/oligomer ones and in this manner the monomer molecule is much better protected. The oligomer's weaker hydrate shell is leaking through their channels. This hypothesis is also supported by the calculated thermodynamic solvation free energy data: μ^{solv}: –72.2 (monomer) kcal/mol; –44.5 (monomer of a tetramer) kcal/mol; $-(72.2+44.5)=-116.7$. The stated values are average data (40) (p. 726: table 1); (33) (p. 4543: table 2, f0–f2, f4 column 4).

Expert opinion and outlook

It is critical to understand the interaction of a protein with its hydrate shell and also the hindered-rotation barriers of water molecules. Based on this phenomenon a sophisticated wide-line NMR procedure was developed by the Tompa's team with several purposes (41). Among others, it delineates the bounded water's movements, reveals the monomeric and oligomeric protein states and also identifies the amyloid molecules in pathological processes. Although, the $A\beta_{42}$ fibril and the other disordered/ordered proteins are thermodynamically different, as only the amyloid protein is stable, this versatile NMR-method is also applicable for amyloids (41).

Gáspári and Perczel (42) published an interesting and important review of a highly advanced NMR theory/technique, in a book chapter. They concentrate on an NMR spectroscopy characteristics of the internal protein mobility, also including the disordered proteins like, e.g.: the tau-protein. As the authors emphasize, that the most

important goal is at present to get a realistic picture of protein motion at different time scales (42).

It is a well-known fact that as long as the amyloid-β fibril monomer is a benign molecule, its dimers/oligomers yield already the toxic Alzheimer's disease and several other diseases. However, Maiti's laboratory has shown that the thermodynamically stable Aβ monomers will be formed from oligomers when they are incubated at low concentration (<3 μM) for a long time (>1 week) in a physiological buffer solution (34), where this stable monomeric fibril protein may be similar to the *in vivo* form. It could be suggested therefore that a catalytic acceleration of this procedure may stop the oligomerization problem.

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