Computer Simulations of Alzheimer's Amyloid β-Protein Folding and Assembly

Brigita Urbanc*, Luis Cruz*, David B. Teplow[†] and H. Eugene Stanley*

Center for Polymer Studies, Department of Physics, Boston University, Boston, MA 02215, USA; [†]Department of Neurology, David Geffen School of Medicine, and Molecular Biology Institute and Brain Research Institute, University of California, Los Angeles, CA 90095, USA

Abstract: Pathological folding and aggregation of the amyloid β-protein (Aβ) are widely perceived as central to understanding Alzheimer's disease (AD) at the molecular level. Experimental approaches to study Aβ self-assembly are limited, because most relevant aggregates are quasi-stable and inhomogeneous. In contrast, simulations can provide significant insights into the problem, including specific sites in the molecule that would be attractive for drug targeting and details of the assembly pathways leading to the production of toxic assemblies. Here we review computer simulation approaches to understanding the structural biology of Aβ. We discuss the ways in which these simulations help guide experimental work, and in turn, how experimental results guide the development of theoretical and simulation approaches that may be of general utility in understanding pathologic protein folding and assembly.

Keywords: Alzheimer's disease, amyloid β-protein, computer simulations, coarse-grained protein model, folding, oligomer formation, fibril formation.

I. INTRODUCTION

Searching for the primary causative event leading to neuronal injury and loss in Alzheimer's disease (AD) has been at the heart of AD research for the past century. Alois Alzheimer first defined the clinicopathological syndrome that bears his name at a meeting in Munich in 1906. Discovery of electron microscopy in the 1960's allowed Michael Kidd and Robert Terry to describe the ultrastructure of two types of lesions in the cerebral cortex of Alzheimer patients, senile plaques and neurofibrillary tangles [1,2]. However, it was only in 1984, when Glenner and Wong isolated and sequenced amyloid β-protein (Aβ) from cerebrovascular deposits, that plaques were linked to accumulation of Aβ [3]. It has been postulated that pathological aggregates of Aβ are the cause of all forms of AD, whereas the appearance of neurofibrillary tangles and other neuropathological changes are a consequence of the pathological Aβ assembly [4]. If this postulate, also known as the amyloid hypothesis, is correct, then genetic alterations that produce AD should be related to Aβ aggregation. Studies of familial forms of AD indeed show that naturally-occurring mutations strongly influence Aβ metabolism and aggregation [5-7]. In addition, the amyloid hypothesis has received strong support by experiments using transgenic animal models of AD [8-11].

Aβ is a normally-secreted, soluble proteolytic product of the amyloid β-protein precursor (APP). APP is a transmembrane protein that undergoes sequential endoproteolytic cleavage to produce predominately two forms of Aβ, Aβ(1-40) and Aβ(1-42). Although Aβ(1-40) is the major species produced, the principal species deposited within the parenchyma of the AD brain is Aβ(1-42) [12,13]. Senile plaques, which are believed to represent the final product of Aβ aggregation, contain Aβ fibrils. Aβ fibrils have specific tinctorial properties, including birefringence when treated with the amyloidophilic dye Congo Red and visualized using cross-polarized light. Fibrils can be observed in the electron microscope, which reveals filaments ranging from 60 Å to 130 Å in width [14], and characterized by powder X-ray diffraction, which produces patterns consistent with a cross-β core structure [15].

In vitro studies have shown that Aβ fibril assembly involves the formation of a number of intermediates, including oligomers and protofibrils (for a recent review, see Ref. [16]). A precise determination of the precursor–product relationships among the many different types of Aβ assemblies that have been described is lacking. There may be more than one pathway of assembly. For example, in addition to senile plaques that are associated with the fibrillar form of Aβ, there exist diffuse plaques with amorphous Aβ aggregates and these are far more abundant in the AD brain (for a review, see Ref. [17]). According to a traditional view, after Aβ is generated from APP, it is secreted in a monomeric form into the extracellular space where it forms aggregates that eventually form senile plaques. On the other hand, there is evidence that some Aβ is generated intracellularly and that the intracellular Aβ could play a pathophysiologic role in AD and Downs syndrome (for a review, see Ref. [18]).

In vitro studies demonstrate that synthetic Aβ monomers aggregate into Aβ oligomers that eventually undergo structural changes and assemble into protofibrils and fibrils [19,20,21,22]. During the last decade, *in vitro* and *in vivo* evidence has implicated the soluble Aβ oligomers as the predominant neurotoxic species (for reviews, see

*Address correspondence to this author at the Boston University/ Physics Department, Center for Polymer Studies, 590 Commonwealth Avenue, Boston, MA 02215, USA; Tel: (617) 353-3411; Fax: (617) 353-3783; E-mail: brigita@bu.edu

Refs. [23,24,25]). Even at nanomolar concentrations, Aβ oligomers were shown to kill neurons in hippocampal slices [26] and inhibit long-term potentiation *in vivo*, which might lead to impairment of learning and memory in AD [27,28]. In addition, Aβ oligomers were found to disrupt the membrane and cause calcium dysregulation in cells in culture, which might lead to cell death [29]. Human Aβ oligomers injected into rats were shown to directly interfere with cognitive function without inducing permanent neurological deficits [30]. Recently, a new and highly stable oligomer species made of 12 Aβ(1-42) peptides, named Aβ(1-42) globulomers, that also exists in the AD brain, was prepared *in vitro* and shown to block long-term potentiation in rat hippocampal slices [31].

Detailed, quantitative analyses of the three-dimensional structure, energetics, and dynamics of oligomer formation are necessary steps toward a molecular understanding of Aβ assembly neurotoxicity. During the formation of fibrils, oligomers of different sizes co-exist with monomers and larger aggregates such as protofibrils and fibrils. The relative amounts of each oligomer type are small. Although important information can be obtained by using solid state NMR [32], quasielastic light scattering [33], and other methods, *in vitro* structure determination of oligomers is limited. Limitations also are caused by the metastability and short lifetimes of oligomers. Computer simulations, in contrast, are not subject to the same kinds of problems, allowing small oligomers to be studied at full atomic resolution.

During the past two decades, computational power has increased, allowing longer, all-atom molecular dynamics simulations, development of efficient new methods for sampling configurational space, and optimization of force-field parameterization to enable study of explicitly-solvated proteins on time scales of up to several hundreds of nanoseconds (for reviews, see Refs. [34,35]). However, to examine biologically-relevant dynamics and interactions, such as folding, protein-protein docking, rearrangement upon ligand binding, and protein assembly, time scales of microseconds and longer are needed. To achieve such long simulation times, simplified representations of proteins have been employed, a strategy proposed three decades ago [36]. In the past decade, a variety of coarse-grained protein models, which integrate a large number of degrees of freedom into a few, have been developed and used extensively (for reviews, see Refs. [37,38,39]).

In this review, we classify the computational approaches and present up-to-date simulation studies of Aβ folding and assembly. The simulation studies are organized into two major sections, the first one on Aβ monomer folding and the second one on Aβ assembly. Within each of these two sections, studies on smaller fragments are presented first, followed by larger fragments, and finally full-length Aβ(1-40) and Aβ(1-42). To ensure biological relevance of the computational studies, most were based on prior experimental approaches and findings. For this reason, we also present results of selected experimental studies.

II.   CLASSIFICATION OF COMPUTATIONAL APPROACHES

Computational methods applied to study amyloid aggregation *in silico* can be characterized by:

(a) the type of the method used (molecular dynamics, discrete molecular dynamics, Monte Carlo, Langevin dynamics, Brownian dynamics, activation-relaxation technique);

(b) the way the solvent is treated (implicitly or explicitly);

(c) the technique used to sample the configurational space (umbrella sampling, replica-exchange, weighted histogram analysis method); and

(d) the degree of complexity of amino acid description (one-bead, two-bead, four-bead, six-bead, united-atom models as described below).

Molecular dynamics (MD) and discrete molecular dynamics (DMD) are the most commonly used methods to study protein folding and aggregation. To emphasize that in MD the protein is usually modeled by all atoms, the term "all-atom MD" is sometimes used. In DMD a simplified description of the protein is mostly used: each amino acid can be represented by only one "atom" or "bead" (one-bead model), by one backbone and one side chain beads (two-bead model), by three backbone and one side chain beads (four-bead model), by four backbone and up to two side chain beads (six-bead model) or by all atoms except hydrogens (united-atom model). Even though MD and DMD are the most commonly used method, other methods, such as Monte Carlo, Langevin, and Brownian dynamics, typically associated with a simplified description of a protein, have been applied to study Aβ folding and aggregation. Monte Carlo techniques are mostly used to obtain global knowledge of the phase space, including the intermediate and denatured states of proteins [40]. Because folding is a slow process compared to microscopic motion of a protein chain, overdamped Langevin dynamics is applied to the study of proteins. The Langevin equations of motion include the viscosity of the solvent as well as a white Gaussian random force with zero mean derived from the fluctuation-dissipation theorem that models thermal fluctuations at a given temperature [41,42,43,44]. In Brownian dynamics, each particle moves in a force field given by the interparticle potential with individual diffusion coefficient, while thermal fluctuations are accounted for in a similar way as in the Langevin dynamics [45].

Simulations of complex systems such as biopolymers at a constant volume and temperature are subject to a multiple-minima problem, because systems simulated at low temperatures tend to get trapped in one of multiple local-minimum energy states (for a review, see Ref. [46]). To overcome this problem, several techniques have been proposed, such as umbrella sampling [47,48,49,50], replica-exchange [51], simulated tempering [52], and weighted histogram analysis methods [53]. A simulation in such a generalized ensemble realizes a random walk in the potential energy space, alleviating the multiple-minima problem. These techniques can be used in combination with MD, DMD, Monte Carlo, or Langevin dynamics.

The method with the most detailed information is the all-atom MD with explicit solvent, where the solvent molecules are explicitly modeled. Many different force fields are available that differ in the way electrostatic interactions are im-

plemented and in the model for water (SPC, TIP3P, TIP4P, TIP5P). In MD of proteins in explicit solvent, the simulation time is limited to several hundred nanoseconds. Longer times are accessible using all-atom MD with implicit solvent [54], where the absence of solvent molecules drastically reduces the number of particles in the simulation. Recently, a replica-exchange MD approach [51] with implicit solvent has been applied to study dimer formation of amyloidogenic peptides [55]. Within the replica-exchange technique, simulations of a number of copies (replicas) of the system under study are conducted in parallel. Temperature is a dynamic variable and is individually assigned to each replica. Periodically, after a fixed number of simulation steps, two replicas are swapped with a probability that depends on the potential energy and the temperature difference. This technique allows for unrestrained walks for each replica up and down in temperature between two limiting temperatures. Consequently, the dynamical processes are accelerated due to crossing of the potential energy barriers that would not be possible in constant temperature simulations.

The protein aggregation process occurs over time scales larger than a microsecond, so all-atom MD with explicit solvent is not usable. The activation-relaxation technique (ART) was recently introduced using the implicit solvent incorporated into the interaction potential [56,57,58] (for a review, see Ref. [59]). ART consists of three general steps: (1) the first activation step during which a random deformation is applied to "kick" the initial conformation out of the local minimum; (2) the second activation step during which the conformation is pushed in the direction of the lowest negative curvature on the energy landscape, while the energy is minimized; and (3) the relaxation step during which the energy is minimized until the total force approaches zero. The advantage of this method is that the system moves through the conformational space rapidly without need to wait for a rare event to occur. However, because the entropy is not taken into account, temperature is not defined by this technique. In addition, the dynamics of the system cannot be directly related to the observed pathways of protein folding and assembly.

The idea of applying a fast and efficient discrete molecular dynamics method (DMD) [60] to study protein folding was proposed in 1996 [61]. Soon after, the method was applied to study protein folding and assembly [62,63,64,65,66]. The main simplification in this method is to replace continuous interparticle potentials by a square-well or a combination of square-well potentials. Consequently, particles move along straight lines with constant velocities until a pair of particles reaches a distance at which the interparticle potential is discontinuous. A collision event then takes place during which the velocities and directions of the particles are updated while preserving the total kinetic energy, momenta, and angular momenta. Because DMD is event-driven, it is faster than all-atom MD. Even though it is not a requisite, DMD is typically used without explicit solvent. Solvent effects are implemented through effective interparticle potentials. In addition, protein models are usually coarse-grained to further increase the efficiency. Among the coarse-grained models, the four-bead [67,68,69,70], six-bead [71], and united-atom models [72] are particularly important as they allow for an *ab initio* simulation approach [73].

III. Aβ FOLDING

The primary structure of Aβ(1-42) is defined by the amino acid sequence: DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVV**IA**. The last two amino acids I41 and A42 are missing in the alloform Aβ(1-40). At physiological conditions and neutral pH amino acids D, E, K, and R are charged, while H is typically considered neutral. Most hydrophobic amino acids are concentrated in the central hydrophobic core (L17-A21) and in the C-terminal part of the peptide (A30-A42), where with exception of G33, G37, and G38, all amino acids are hydrophobic. The first 15 N-terminal residues are predominantly hydrophilic.

In this section we describe first the experimental findings on folding of specific Aβ peptides, and review the relevant computer simulation work. The computer simulation studies encompass folding of a decapeptide, Aβ(21-30), folding of a longer fragment Aβ(10-35) and its Dutch mutant, and folding of full-length Aβ(1-40) and Aβ(1-42).

A. Folding Events of Aβ(21-30)

Lazo *et al.* used limited proteolysis with mass spectrometry to identify protease-resistant segments of Aβ(1-40) and Aβ(1-42) [74]. They showed that a ten-residue segment, A21-A30, was protease resistant in both alloforms. A similar protease resistance was observed with the Aβ(21-30) decapeptide alone. Solution–state NMR studies of the decapeptide revealed a turn formed by the peptide segment V24-K28. Lazo *et al.* postulated that this turn nucleates the folding of a full-length Aβ monomer, which represents the first step in Aβ assembly. They also proposed three factors contributing to the formation and stability of the turn: (1) the innate propensity of G25, S26, and N27 to exist in turns; (2) the salt bridges E22-K28 or D23-K28; and (3) hydrophobic interactions between the isopropyl side chain of V24 and the *n*-butyl portion of the K28 side chain. Comparing the protease-resistant regions of Aβ(1-40) and Aβ(1-42), they also found that the fragment V36-A42 was protease resistant in Aβ(1-42) only. This result is consistent with previous experimental studies suggesting a β-helix model of Aβ(34-42) fibrils with a turn at G37-G38 stabilized by hydrophobic interactions [75], leading to the observed loop or turn-like conformation in the G37-G38 region of Aβ(34-42) [76].

Borreguero *et al.* used DMD simulations and a united-atom protein model with implicit solvent to study folding events of Aβ(21-30) [72]. All heavy atoms (all atoms except hydrogens) were modeled and the amino acid-specific interactions between pairs of side chain atoms were derived using an experimental estimation of the gain/loss of the free energy on transferring a particular amino acid from an aqueous solution to a gas phase [77]. The results of this study showed that Aβ(21-30) folds into a loop-like conformation driven by the hydrophobic attraction between the side chains of V24 and K28. Comparing the simulation results to the *in vitro* study [74], Borreguero *et al.* found that transient D23-K28 and E22-K28 salt bridges stabilized the loop conformation, with the E22-K28 interaction being more favorable. They also showed that at high strengths of the electrostatic interaction, which occur in desolvated regions inside proteins and aggregates, Aβ(21-30) folding is destabilized by the D23-

K28 salt bridge interaction, in agreement with studies of molecular modeling of full-length Aβ [78].

Recently, Cruz *et al.* studied the dynamics of Aβ(21-30) and its E22Q ("Dutch") mutant using all-atom MD and explicit water, salted water, and reduced-density water [79]. The folding events due to hydrophobic interaction between V24 and K28 side chains were observed in the wild type peptide in water at physiological conditions, in agreement with Borreguero *et al.* [72]. In contrast, the Dutch mutant did not display any stable conformation events. In salted water, the salt bridges E22-K28 and D23-K28 played an important role in stabilizing the Aβ(21-30) folding events, while in reduced-density water stable α-helix conformations were observed. These results indicate that the folding of Aβ(21-30) decapeptide, and possibly of full-length Aβ, is strongly sensitive to small solvent changes. If folding of the fragment A21-A30 nucleates the folding of full-length Aβ, as suggested by Lazo *et al.* [74], then subtle changes in the solvent environment could disrupt the folded structure and consequently change the pathway of Aβ assembly.

Conformational space accessible to the Aβ(21-30) fragment was investigated by Baumketner *et al.* using replica exchange MD in explicit solvent [80]. Baumketner *et al.* found conformations belonging to the global free energy minimum in good agreement with reported NMR structures [74].

B. Folding and Solubility of Aβ(10-35)

NMR experiments on Aβ(10-35) in an aqueous solution revealed a collapsed structure with loops, strands, and turns without any significant amount of α-helical or β-strand content [81]. This is in contrast to the full-length Aβ monomer structures in a membrane-like environment that show two α-helical regions separated by a kink at V24-G29 [82,83].

Massi *et al.* performed all-atom MD simulations of Aβ(10-35) monomer in an aqueous environment at the nanosecond time scale [84]. The starting structures for the simulations were derived from distance geometry calculations employing NMR-derived constraints. Although their simulations indicated that the peptide is somewhat disordered in solution, the central hydrophobic core, L17-A21, and the turn region, V24-N27, were particularly stable. The results indicated that these two structural motifs are cooperatively stabilized through intramotif hydrogen bonds. Massi *et al.* expanded their study by performing all-atom MD of the Aβ(10-35) monomer in an aqueous environment in both the wild type and the Dutch mutant forms [85]. They generated and analyzed multiple nanosecond time scale MD trajectories using measures of the peptide's average structure, hydration, conformational fluctuations, and dynamics. The results showed that the Dutch mutant, as well as the wild type peptide, are stable in collapsed coil conformations consistent with the experimentally-derived structure of Zhang *et al.* [81]. However, the Dutch mutant is more open and flexible and the E22Q substitution leads to a change in the first solvation layer of water molecules close to the peptide's hydrophobic patch that results in increased solvation. Massi *et al.* argued that these results may explain the increased propensity for fibril deposition in the Dutch mutant.

An increased fibril elongation rate of the Dutch mutant of the Aβ relative to the wild type peptide was reported by several experimental groups [86,87]. Massi *et al.* disproved the hypothesis that the increased fibril elongation rate is due to a larger propensity for the formation of β-structure in the Dutch mutant monomer relative to the wild type peptide [88]. They applied all-atom MD simulations to study the wild type and the Dutch mutant forms of Aβ(10-35) in an aqueous solution at a four nanosecond time scale. The simulation results indicated that the propensity for formation of a β-structure is no greater in the E22Q mutant peptide than in the wild type peptide. Massi *et al.* proposed that the greater stability of the solvated wild type peptide relative to the Dutch mutant leads to a decreased fibril elongation rate in the former. They concluded that this stability difference is due to the differing charge state of the two peptides.

In a subsequent study, Massi and Straub performed all-atom MD simulations to analyze the structure, energetics, and dynamics of water in the first solvation shell of the Aβ(10-35) monomer and the Dutch mutant peptide [89]. They analyzed the hydrogen bond network, the water residence times for each residue in the peptide, the diffusion constant, experimentally-determined amide proton exchange, and the transition probabilities for water to move from one residue to another or into the bulk. The results indicated that in both peptides the water molecules at the peptide-solvent interface are organized in an ordered structure different from that of the bulk water. In addition, they showed that the peptide structure inhibits water diffusion perpendicular to the peptide surface by a factor of 3 to 5 relative to diffusion parallel to the peptide surface, which is comparable to the diffusion of the bulk water. Water in the first solvation shell showed dynamical relaxation on fast (~1-2 ps) and slow (~10-40 ps) time scales, indicating intricate water dynamics that may influence the peptide dynamics at different levels. The interaction between the peptide and the solvent was found to be stronger in the wild type than in the Dutch mutant peptide, suggesting that the wild type is characterized by a more stable folded structure than the Dutch mutant.

Straub *et al.* introduced the MaxFlux algorithm for the computation of likely pathways of global macromolecular conformational transitions [90]. The algorithm is capable of describing intermediate structural states between two known (initial and final) structural states, thus rendering a pathway. The algorithm assumes an overdamped diffusive dynamics for the biomolecule. As an application of the MaxFlux method, Straub *et al.* explored conformational transitions between α-helical, collapsed coil, and β-sheet conformations of the Aβ(10-35) monomer generating possible intermediate structures, i.e., pathways, between the two conformations. The results showed that there are significant energy barriers between the collapsed coil and α-helical conformations and between the collapsed coil and β-sheet conformations. Each transition pathway involves an early formation of the turn in the region V24-N27, which has well-preserved structure in both α-helical and collapsed coil conformations. This result suggested that by destabilizing this turn, the kinetics of α-helix and collapsed coil formation could be altered.

Recently, Han and Wu studied conformations of Aβ(10-35) peptide using all-atom MD with explicit water sol-

vent [91]. The results of this study revealed that the collapsed coil structure determined by experiments is stable at pH 5.6 for hundreds of nanoseconds, but it exchanges with a strand-loop-strand conformation on the milisecond time scale. The strand-loop-strand conformation is characterized by a loop at residues D23-K28 which allows the central hydrophobic core (L17-A21) to be in hydrophobic contact with the residues A30-M35. Because the strand-loop-strand conformation resembles an intramolecular arrangement of the Aβ(10-35) peptide in a fibril, Han and Wu suggested that this conformation represents an important intermediate on the pathway from monomer folding to fibril formation. They also performed simulations at pH 2.0, to mimic the mutations E22Q and D23N, and showed that at a temperature of 400 K the strand-loop-strand conformation is considerably populated, while the collapsed coil structure is disrupted. These results are in agreement with recent finding by Lazo *et al.* on folded Aβ(21-30) monomer conformation with a loop centered at G25-S26 [74] and give support to the hypothesis that the strand-loop-strand conformation plays a role of an intermediate structure on the pathway to fibril formation.

C. Folding of Full-Length Aβ

Experimental studies of Aβ(1-40) and Aβ(1-42) monomers in water–organic solvent mixtures showed that the monomer structure of both consisted of two α-helical regions connected through a flexible turn- or bend-like kink [82,83]. The temperature-dependence of the Aβ(1-40) monomer and dimer structures in water was experimentally studied by Gursky and Aleshkov [92]. They found a folded Aβ(1-40) monomer structure with no substantial amounts of α-helix or β-strand at low temperatures. As the temperature was increased to physiological, a substantial β-sheet content was detected. This structural transition was not accompanied by oligomer formation, thus it was attributed to Aβ(1-40) monomers and dimers. These studies showed that full-length Aβ monomer structure is very sensitive to external conditions, such as temperature, pH, and solvent.

Typically, all-atom MD studies are limited to ~100 ns time scales, which is not sufficient to span the entire folding process even in the case of a relatively short protein, such as full-length Aβ. Thus, numerous studies have taken simplified approaches by applying coarse-grained protein models. Using a two-bead amino acid model with one backbone and one amino acid-specific side chain bead and Brownian dynamics, Mukherjee and Bagchi showed that Aβ(1-40) folds into a conformation characterized by a β-bend at residues V24-K28 [45]. These simulations also showed that Aβ(1-40) folding dynamics consists of several stages: a two-stage fast hydrophobic collapse and a slow decay stage during which the protein formed more favorable pair contacts to replace the less favorable ones [45]. Despite the lack of a unique native state, the multistage dynamics of Aβ(1-40) folding is in accord with a predicted protein folding dynamics in which a slow, rate-determining step is related to a search through the conformational space to find a transition state from which the protein folds rapidly to the lowest-energy state [93].

A DMD study by Urbanc *et al.* using a four-bead amino acid model with hydrogen bonds and amino acid-specific interactions not only addressed the formation of Aβ(1-40)

and Aβ(1-42) oligomers, but also studied monomer folding prior to oligomer formation [94]. Results of this study showed that a folded Aβ(1-42) monomer, but not Aβ(1-40) monomer, possesses a turn at G37-G38 stabilized by a hydrophobic interaction between V36 and V39 [94]. This turn structure is in agreement with the solution ^{1}H NMR study of the C-terminal fragment Aβ(34-42) by Weinreb *et al.* who suggested that this C-terminal hydrophobic cluster nucleates amyloid formation in AD [95]. Moreover, Urbanc *et al.* showed that this turn is present in oligomers of both Aβ(1-40) and Aβ(1-42), implicating a possible important role of this turn in oligomer formation.

One of the most extensive all-atom MD studies of helix-to-coil conformational change in Aβ(1-40) monomer was reported by Xu *et al.*, who studied Aβ(1-40) folding in both aqueous and membrane-like environments [96]. In an aqueous solution, Aβ(1-40) trajectories showed an α-helix→β-sheet and a β-sheet→random coil conformational change. The residues V24-G37 represented the core of the β-sheet conformation that would presumably be important for fibril formation, while G25, G29, G33, and G37 were shown to be essential for formation of the β-sheet structure. Xu *et al.* showed that in a biomembrane environment, Aβ(1-40) prefers an α-helix conformation, however the protein has a tendency to exit the membrane environment. In another all-atom MD study of Aβ(1-42) folding in an aqueous solvent at various temperatures and pH conditions, Flöck *et al.* showed that at least one of the two α-helices is not stable, but rather rapidly converts to a random and β-strand rich conformation [97]. Both studies are in qualitative agreement with the experimental findings.

Recently, an Aβ(1-42) monomer structure was studied by Baumketner *et al.* using a combination of ion-mobility mass spectrometry and replica exchange MD simulations with implicit water solvent [98]. They showed that Aβ(1-42) did not adopt a unique fold, but rather a mixture of rapidly interconverting conformations that were classified into three distinct families. The secondary structure analysis revealed that these conformations were dominated by the loops and turns, but also showed some helical structure in the C-terminal hydrophobic tail. Baumketner *et al.* proposed that an increase in α-helical structure as observed in Aβ intermediates by Kirkitadze *et al.* [99] results from association of unstructured monomers into oligomers in such a way that the hydrophobic tails of the peptide become shielded from the solvent. This shielding would create an apolar microenvironment promoting α-helical structure to grow from pre-existing seeds. This hypothesis is amenable to both *in silico* and *in vitro* testing.

IV. Aβ AGGREGATION

Understanding pathways of oligomer and fibril formation of full-length Aβ as well as discerning the structures of folded monomers, oligomers, and fibrils of full-length Aβ is the aim of current computer simulation studies. However, due to computer limitations, many studies focused on Aβ fragments, of which Aβ(16-22) was the most frequently studied.

Solid-state NMR measurements on Aβ(1-40) [100,101], Aβ(10-35) [102,103], Aβ(16-22) [104,105,106], and Aβ(34-

42) [76] showed that the first two peptides formed parallel β-sheets whereas the latter two peptides adopted an antiparallel β-sheet organization. Gordon *et al.* showed that amphiphilicity was critical in determining the structural organization of β-sheets in the fibril [106]. Thus, all amyloid fibrils do not share a common supramolecular structure.

A. Assembly of Aβ(16-22)

Studies of Aβ(16-22) assembly are numerous because of the simplicity of the fragment (KLVFFAE), and more importantly, because this fragment comprises the central hydrophobic core (LVFFA) thought to be important in fibril formation of full-length Aβ. In addition, amino acid substitutions at the E22 position are linked to four familial forms of AD, referred to as Flemish (A21G), Dutch (E22Q), Arctic (E22G), and Italian (E22K). Experimental studies on Aβ(16-22) showed that Aβ(16-22) peptides assemble into an anti-parallel, in-register, fibrillar structure [104,105,106].

A stability study of Aβ(16-22) octamers packed in different parallel and antiparallel arrangements was conducted by Ma and Nussinov using all-atom MD in explicit solvent and physiological conditions [107]. Antiparallel β-sheet/parallel layer arrangement was found to be the most stable, in agreement with the solid state NMR findings [104]. The same study addressed the stability of Aβ(16-35) and Aβ(10-35). Neither of these two fragments was found to form stable extended β-strands because of the presence of residues G25, S26, N27 with high turn propensities, which create a bend stabilized by a D23-K28 salt bridge. The aim of this study was to understand the supramolecular organization of fibrils, but did not provide any insight into the oligomerization process and possible pathways of assembly.

Klimov and Thirumalai studied formation of an Aβ(16-22) trimer using all-atom MD in explicit water and a bias to facilitate interactions between peptides [108]. They found that Aβ(16-22) assembled through multiple pathways, each accompanied by the formation of α-helical intermediates, in agreement with experimental observations [20,21,99]. This tripeptide system, which forms a stable antiparallel β-sheet in water, was shown to be destabilized in urea solution due to hydrogen bond formation between urea and the peptide backbone, which gave rise to stable β-strand monomers [109].

Using the ART and an approximate free energy model, Santini *et al.* showed that an in-register antiparallel β-sheet structure was the most stable structure for the Aβ(16-22) dimer, despite the existence of several hydrogen bond patterns in both parallel and antiparallel orientations that were thermodynamically possible [110,111]. The existence of alternative β-sheet organizations is important because it helps explain the dependence of β-sheet registry on pH [105] and amino acid composition [112]. Santini *et al.* also found multiple pathways, but α-helical intermediates were not found to be obligatory on the pathway to dimer formation, in contrast to previous findings by Klimov *et al.* [108].

Dimer formation of Aβ(16-22) and its Arctic (E22G) mutant was studied using all-atom MD with implicit solvent [113]. The results of this study showed that hydrophobic interactions oriented the peptides to minimize the solvent

accessible surface area leading to dimer structures that were kinetically trapped in energetically unfavorable conformations. Once hydrophobic contacts were present, hydrogen bonds formed rapidly in a zipper-like way. Aβ(16-22) dimers also showed preference for an antiparallel configuration, probably due to the electric dipole moment of this fragment.

An assembly of six Aβ(16-22) peptides was studied by Favrin *et al.* using an unbiased Monte Carlo method with simulated tempering [114]. They used a sequence-based atomic protein model with hydrogen bonding and effective hydrophobic attraction with no explicit water molecules. Favrin *et al.* found different supramolecular structures of Aβ(16-22) aggregates with a high β-strand content. Even though an antiparallel arrangement of β-strands was preferred, parallel arrangements were found as well. The preference for the antiparallel arrangement persisted even in the absence of the Coulombic interaction between two charged amino acids, which disagrees with the finding of Klimov *et al.* that Coulombic interactions are the main determinant for the antiparallel orientation in the Aβ(16-22) assembly [108].

Gnanakaran *et al.* applied replica exchange MD simulations at 38 different temperatures in explicit water solvent to study dimer formation of Aβ(16-22) [115]. They found that a predominant monomer conformation is polyproline-II-like, while there exist six stable dimer conformations, not necessarily limited to only parallel or antiparallel strands. In addition Gnanakaran *et al.* found that water molecules are directly involved in stabilizing certain dimer conformation that can not be predicted by implicit solvent models.

B. Aβ(10-35) Dimer Stability

Using several methods, Tarus *et al.* examined the initial steps of Aβ aggregation by studying the stability of two Aβ(10-35) dimers in aqueous solution [116]. They generated a pool of possible dimer configurations using a protocol based on shape complementarity. They evaluated these structures using estimates of the desolvation and electrostatic interaction energies to identify putative stable dimer structures. Two globular dimers were identified, the φ-dimer that minimizes the desolvation energy of the residues and the ε-dimer that minimizes the interpeptide electrostatic energy. The potential of the mean force associated with the dimerization of the peptides in aqueous solution was computed for both the hydrophobic- and the electrostatic-driven forces using umbrella sampling and all-atom MD simulation at constant temperature and pressure in explicit solvent. This calculation led Tarus *et al.* to obtain the free energy profiles for each dimer that suggested that the φ-dimer has a favorable structure originating from the desolvation of the hydrophobic residues at the interface. Furthermore, they generated MD trajectories originating from the two dimer structures and found that the φ-dimer is stabilized primarily through hydrophobic interactions while the ε-dimer is not stable. These results suggested that the preservation of the structure of the central hydrophobic core (L17-A21) plays an important role in the stabilization of the φ-dimer structure.

C. Understanding Aβ Fibril Assembly

Petkova *et al.* derived a structural model of Aβ(1-40) based on experimental constraints from solid state

NMR [78]. The model is consistent with the cross-β structural motif established by X-ray diffraction [15]. According to this model, the first 10 residues of Aβ(1-40) are structurally disordered, while segments 12-24 and 30-40 adopt a β-strand structure. Residues 25-29 contain a backbone bend which brings the two β-strand segments together through side chain–side chain interactions. The supramolecular fibril structure is formed through intermolecular hydrogen bonds along the fibril axis. Petkova *et al.* suggested that the salt bridge D23-K28 plays a key role in fibril stabilization. The importance of this salt bridge interaction was recently demonstrated by Sciarretta *et al.* who compared fibrillogenesis of a wild type Aβ(1-40) with Aβ(1-40) containing a lactam bridge between the side chains of D23 and K28 (Aβ(1-40)-Lactam(D23/K28)) [117]. Sciarretta *et al.* found, in contrast to the wild type, that fibrillogenesis of Aβ(1-40)-Lactam(D23/K28) occurred at a 1000-fold greater rate than observed in Aβ(1-40) and without a lag phase, possibly bypassing an unfavorable folding step in fibrillogenesis. Aβ(1-40)-Lactam(D23/K28) also showed a stronger tendency for self-association and formed oligomers at micromolar concentrations, at which the wild type Aβ(1-40) showed no detectable oligomers.

Intriguingly, in their subsequent work, Petkova *et al.* showed that different Aβ(1-40) fibrillar morphologies have different underlying molecular structures that can be controlled by subtle variations in fibril growth conditions [118]. Moreover, they found that different Aβ(1-40) fibrillar morphologies are associated with different toxicities in neuronal cell cultures.

Recently Lührs *et al.* reported a three-dimensional structure of [M35(O)]Aβ(1-42) fibrils based on *in vitro* hydrogen-bonding constraints from quenched hydrogen/deuterium-exchange NMR, side chain packing constraints from pairwise mutagenesis studies, and parallel, in-register arrangement determined by previous solid-state NMR studies [119]. In [M35(O)]Aβ(1-42) M35 is oxidized, which was shown by several groups to strongly affect Aβ(1-42) oligomer and fibril formation [120,121,122,123,124]. Results of Lührs *et al.* showed that the structure of [M35(O)]Aβ(1-42) fibrils consisted of disordered residues 1-17, while residues 18-42 formed a β-strand–turn–β-strand pattern. Denoting the β-strand of residues 18-26 by β_1 and the β-strand of residues 31-42 by β_2, they found that the odd-numbered residues of β_1 of the *n*th molecule formed contacts with the even-numbered residues of β_2 of the *n-1*th molecule. Between β_1 and β_2 strands within each molecule they found the following important side chain–side chain contacts: D23-K28 (the salt bridge interaction), F19-G38, and A21-V36. Together, these results suggest that [M35(O)]Aβ(1-42) fibrils are stabilized by intermolecular domain swapping-type of side chain interactions.

Using a kinetic theory approach, Massi and Straub derived kinetic equations governing Aβ deposition and fibril elongation [125]. These equations are applicable to full-length, fragment, and mutant forms of Aβ. Their approach is based on the "dock-and-lock" model of Aβ fibril assembly in which a monomer first "docks" with the fibril end in a reversible manner and then undergoes a conformational reorganization that locks it into place [20,126,127,128,129].

Their kinetic equations consider several possible scenarios for peptide deposition, including fast deposition from solution through an activation/nucleation event and deposition of peptide from solution onto existing fibrils followed by reorganization of the peptide/fibril deposit. The approach of Massi and Straub unifies several views of Aβ peptide deposition and elongation. They found that the proposed mechanism is consistent with experimental data on the rate of fibril elongation for wild type Aβ alloforms [130], Aβ fragments [131,132,81], and mutant Aβ peptide fragments [129].

A fibrillogenic fragment, Aβ(12-28), was studied by Simona *et al.* using all-atom MD with explicit solvent [133]. Simona *et al.* studied Aβ(12-28) monomer transition from an α-helix to a β-hairpin-like conformation. During this simulation the five consecutive hydrophobic side chains of the central hydrophobic core, LVFFA, were exposed to the solvent, which made the β-hairpin-like conformation prone to aggregation. The results show that while hydrophobic contacts are important to bring together individual molecules in a β-hairpin-like conformation, hydrogen bonding and Coulombic interactions are necessary to stabilize the nascent fibrillar aggregates.

Buchete *et al.* applied all-atom MD with explicit solvent and multiple force-fields to probe structural stability of several models of Aβ(9-40) fibril structure [134]. These structural models were based on solid state NMR data of Aβ(1-40) by Petkova *et al.* [78]. Buchete *et al.* considered four topologies that were all found to be stable in accord with experimentally-observed structural polymorphism at the molecular level [118]. The inward-pointing salt bridges D23-K28 were found to stabilize the protofilament structure and were hydrated by interior water molecules.

D. Full-Length Aβ Oligomer Formation

Understanding the assembly of full-length Aβ, especially that of Aβ(1-40) versus Aβ(1-42), is critical for gaining insight into oligomerization differences that may be relevant to the particularly strong linkage of the longer Aβ alloform with disease [135].

Bitan *et al.* used the method photo-induced cross-linking of unmodified proteins (PICUP) to study oligomerization of full-length Aβ [136,137]. PICUP enabled identification and quantification of short-lived metastable assemblies with no *pre facto* structural modification of the native peptide. Bitan *et al.* determined the oligomer size distributions of Aβ(1-40) and Aβ(1-42) [135]. Whereas the Aβ(1-40) size distribution comprise primarily monomers through tetramers, the Aβ(1-42) size distribution was multimodal, displaying peaks in the region of pentamer/hexamer, dodecamer, and octadecamer. The quasi-pentad/hexad periodicity suggested that a basic oligomer building block existed. The term "paranucleus" was used to refer to this pentamer/hexamer unit [135]. They established that I41 of Aβ(1-42) is essential for formation of paranuclei, while A42 is necessary for assembly of paranuclei into higher-order oligomers. Subsequent studies determined primary structure elements controlling early Aβ oligomer formation by systematically evaluating 34 physiologically-relevant alloforms [138]. Further studies of Bitan *et al.* showed that oxidation of M35 blocked Aβ(1-42) paranuclei

formation, producing oligomer distributions indistinguishable from those of Aβ(1-40) [122].

Recently, Bernstein *et al.* applied mass spectroscopy and ion mobility spectroscopy to the study of Aβ(1-42) and [P19]Aβ(1-42), a substitution that blocks Aβ fibril formation [139]. They observed solution-like structures of Aβ(1-42) that consist of monomers, dimers, tetramers, hexamers, and an aggregate of two hexamers, which correspond to the first step in protofibril formation. These results are consistent with the PICUP findings of Bitan *et al.* [135]. Further experiments by Bernstein *et al.* have extended these findings by demonstrating that monitoring formation of "oligo-paranuclear" assemblies is possible using a novel chemical form of Aβ(1-42) and ion mobility spectroscopy detection (Bernstein, unpublished data).

Aggregation propensity of Aβ(1-40) and its fragment Aβ(1-28) was studied by Valerio *et al.* using all-atom MD in an explicit water environment [140]. They used nonlinear signal analysis [141] to study aggregation and folding propensities by considering the distribution of hydrophobic and charged residues. Valerio *et al.* concluded that in the most "aggregation-prone" environment Aβ(1-40) has a higher conformational mobility than does Aβ(1-28) due to the most mobile part of the molecule, the hydrophobic tail, which is not present in Aβ(1-28).

The first DMD study of Aβ(1-40) aggregation was done by Peng *et al.* who used a two-bead peptide model with Go interactions based on the Aβ(1-40) structure in a membrane-like environment [142]. Peng *et al.* showed that molecules assemble into fibril-like aggregates with parallel, in-register organization.

Folding and dimer formation of Aβ(1-40) and Aβ(1-42) was studied by Urbanc *et al.* using a combination of DMD and all-atom MD simulations [143]. The explicit solvent/implicit solvent MD method was applied to estimate the free energy of different dimer conformations of both Aβ(1-40) and Aβ(1-42). DMD simulations using a four-bead protein model with hydrogen bond interactions [70] predicted a folded Aβ(1-42) monomer with a β-hairpin structure and a turn at residues G25-S26-N27. Two such β-hairpin monomers assembled into planar β-sheet dimer conformations, which then were studied for stability using all-atom MD. Urbanc *et al.* showed that all planar β-sheet dimers had a higher free energy than the corresponding monomeric states and that there was no significant free energy difference between Aβ(1-40) and Aβ(1-42) dimers. These results suggest that dimer conformations other than planar β-sheets are responsible for the experimentally-observed differences in oligomerization between Aβ(1-40) and of Aβ(1-42) [135]. At the molecular level, this result implies that interactions other than hydrogen bond interactions are needed to study Aβ oligomer formation in DMD.

Oligomer formation of Aβ(1-40) and Aβ(1-42) was studied by Urbanc *et al.* using DMD and the four-bead protein model with hydrogen bonding and amino acid-specific interactions [94]. Initially, the separated Aβ peptides folded into collapsed coil structures and then assembled into oligomers of different sizes [94]. After these simulations reached a steady state and the oligomer size distribution did not significantly change in time, the respective Aβ(1-40) and Aβ(1-42) size distributions differed significantly. Aβ(1-42) formed significantly more pentamers than Aβ(1-40) and Aβ(1-40) formed significantly more dimers than did Aβ(1-42). These results showed that the effective hydrophobic attraction of I41 has a direct impact on formation of Aβ(1-42) oligomers. Urbanc *et al.* also pointed out structural differences between Aβ(1-40) and Aβ(1-42) oligomers that are amenable to experimental study.

V. DISCUSSION

In a recent review, Snow *et al.* pointed out that in recent years important advances in validation of computer simulation methodology have been made possible through quantitative comparisons with experiments for small, rapidly-folding proteins [144]. These advances also have influenced computational studies of amyloidogenic proteins, in particular Aβ. In this review, we have discussed the unique insights into Aβ folding and assembly into oligomers and fibrils that can be obtained through computer simulation methods. Along with the simulation results, we also have reviewed experimental findings to emphasize the importance of bidirectional *in vitro⟷in silico* interactions for obtaining biologically-relevant information on Aβ structure and dynamics. In contrast to understanding fibril structure, for which a combination of NMR methods and pairwise mutagenesis recently yielded a [M35(O)]Aβ(1-42) fibrillar structure with atomic-level details [119], experiments seeking to elucidate the structure of early oligomers are significantly more challenging. Computational studies of full-length Aβ fibrils using all-atom MD currently are limited primarily to the investigation of the stability of pre-constructed Aβ fibril models [145]. In contrast, the elucidation of early folding events and oligomerization is more computationally accessible and can potentially yield new information amenable to *in vitro* testing.

A hierarchy of computational approaches extending from the most coarse-grained models with simplified dynamics, through intermediate resolution models such as four-bead and united-atom, to all-atom models with MD in explicit solvent is needed to understand the structure and dynamics of Aβ at the molecular level. Different levels of simplicity in the model and dynamics can address different questions. All-atom MD with explicit solvent is the most realistic approach and the only one that can address questions related to solvation of proteins, but it cannot be applied to study processes that occur in a time regime exceeding ~100–200 ns. The most coarse-grained approaches, e.g., one-, two-, or four-bead DMD models with implicit solvent, are efficient enough to study oligomer or fibril formation starting from initially-separated peptides. However, the degree of detail strongly depends on the protein model and implemented amino acid-specific interactions.

There are differences among the coarse-grained protein models in which an amino acid is represented by one, two, four, six, or all heavy atom beads. Although one- and two-bead models are too coarse-grained to model realistically the protein backbone, they allow the study of general properties of protein folding in more complex environments. An example of such an application is a recent study of molecular crowding effects on native state stability and protein folding

dynamics [146]. The models with four or more beads per amino acid, i.e., intermediate resolution models, have a well-described backbone geometry. If, in addition, the backbone hydrogen bond is implemented, these intermediate resolution models are capable of accounting for α-helix and β-hairpin monomer conformations by simply varying the temperature, and most importantly, without any knowledge of the native protein state [70]. Using a four-bead model of polyalanine, Nguyen and Hall studied mechanisms of fibril formation [147,148,149]. They derived a phase diagram in dependence on temperature and molar concentration that shows regions of amorphous aggregates, fibrillar aggregates, as well as regions with no aggregation [147,148,149]. Ding *et al.* recently used a coarse-grained model with four backbone beads and up to several side chain beads to fold a small Trp-cage protein to an NMR-resolution native structure, demonstrating that all-atom protein description is not necessary to successfully simulate folding into a native state [71]. All these properties of intermediate resolution models, and the above validation studies, illustrate that these models can form a solid foundation upon which *ab initio* modeling of specific proteins can be conducted.

Experimental findings motivate the development of computational approaches. Can computer simulation findings direct *in vitro* research? Results of DMD simulations of Aβ(1-40) and Aβ(1-42) oligomer formation suggest the answer is "yes" [94]. These results are consistent with existing experimental data, which defined the roles of individual amino acids in oligomerization of both allo-forms [122,135,138]. More importantly, they yield new structural predictions. Even though the main difference in conformational dynamics between the two alloforms was driven by the hydrophobic residues I41 and A42, the simulations showed that the N-termini of Aβ(1-40) and Aβ(1-42) oligomers were arrayed in a structurally distinct manner. The 3D structure of oligomers is tightly related to their neurotoxic properties, thus the N-terminal structural differences between Aβ alloforms revealed through simulation provide a theoretical basis for direct experimental testing of structure–neurotoxicity correlations *in vitro* and *in vivo*.

ACKNOWLEDGMENTS

ABBREVIATIONS

AD   = Alzheimer's disease

Aβ   = amyloid β-protein

APP   = amyloid β-protein precursor

MD   = molecular dynamics

DMD = discrete molecular dynamics

ART   = activation-relaxation technique

REFERENCES

[1]   Kidd M. Alzheimer's disease—an electron microscopical study. Brain 87: 307-320 (1964).

[2]   Terry RD, Gonatas NK and Weiss M. Ultrastructural studies in Alzheimer's presenile dementia. Am. J. Pathol. 44: 269-297 (1964).

[3]   Glenner GG and Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. 122: 1131-1135 (1984).

[4]   Hardy J and Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. Science 297: 353-356 (2002).

[5]   Kamino K, Orr HT, Payami H, Wijsman EM, Alonso E, Pulst SM et al. Linkage and mutational analysis of familial Alzheimer disease kindreds for the APP gene region. Am. J. Hum. Genet. 51: 998-1014 (1992).

[6]   Suzuki N, Cheung TT, Cai XD, Odaka A, Otvos LJr, Eckman C et al. An increased percentage of long amyloid β-protein secreted by familial amyloid β-protein precursor (β-APP(717)) mutants. Science 264: 1336-1340 (1994).

[7]   Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N et al. Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat. Med. 2: 864-870 (1996).

[8]   Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G et al. Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. Science 293: 1487-1491 (2001).

[9]   Gotz J, Chen F, van Dorpe J and Nitsch RM. Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Aβ 42 fibrils. Science 293: 1491-1495 (2001).

[10]   Oddo S, Billings L, Kesslak JP, Cribbs DH and LaFerla FM. Aβ immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. Neuron 43: 321-332 (2004).

[11]   Lesné S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M and Ashe KH. A specific amyloid β-protein assembly in the brain impairs memory. Nature Lett. 440: 352-357 (2006).

[12]   Seubert P, Vigo-Pelfrey C, Esch F, Lee M, Dovey H, Davis D, Sinha S et al. Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids. Nature 359: 325-327 (1992).

[13]   Gravina SA, Ho LB, Eckman CB, Long KE, Otvos LJr, Younkin LH et al. Amyloid β-protein (Aβ) in Alzheimer's disease brain — Biochemical and immunocytochemical analysis with antibodies specific for forms ending at Aβ-40 or Aβ-42(43). J. Biol. Chem. 270: 7013-7016 (1995).

[14]   Cohen AS and Calkins E. Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature 183: 1202-1203 (1959).

[15]   Kirschner DA, Abraham C and Selkoe DJ. X-ray diffraction from intraneuronal paired helical filaments and extraneuronal amyloid fibers in Alzheimer disease indicates cross-β conformation. Proc. Natl. Acad. Sci. USA 83: 503-507 (1986).

[16]   Lazo ND, Maji SK, Fradinger EA, Bitan G and Teplow DB. The amyloid β-protein. In 'Amyloid Proteins-The Beta Sheet Conformation and Disease' (Ed Sipe JC), Wiley-VCH, Weinheim, p. 385-492 (2005).

[17]   Morgan, Colombres CM, Nuñez MT and Inestrosa NC. Structure and function of amyloid in Alzheimer's disease. Progress in Neurobiology 74: 323-349 (2004).

[18]   Tsang BP, Kitazawa M and LaFerla FM. Amyloid β-peptide: the inside story. Current Alzheimer Research 1: 231-239 (2004).

[19]   Pike CJ, Walencewicz AJ, Glabe CG and Cotman CW. *In vitro* aging of β-amyloid protein causes peptide aggregation and neurotoxicity. Brain Res. 563: 311-314 (1991).

[20]   Walsh DM, Lomakin A, Benedek GB, Condron MM and Teplow DB. Amyloid β-protein fibrillogenesis: detection of a protofibrillar intermediate. J. Biol. Chem. 272: 22364-22372 (1997).

[21]   Walsh DM, Hartley DM, Kusumoto Y, Fezoui Y, Condron MM, Lomakin A et al. Amyloid β-protein fibrillogenesis - Structure and biological activity of protofibrillar intermediates. J. Biol. Chem. 274: 25945-25952 (1999).

[22] Chromy BA, Nowak RJ, Lambert MP, Viola KL, Chang L, Velasco PT *et al.* Self-assembly of $A\beta_{1-42}$ into globular neurotoxins. Biochemistry 42: 12749-12760 (2003).

[23] Klein WL. ADDLs & protofibrils - the missing links? Neurobiol. Aging 23: 231-233 (2002).

[24] Klein WL, Stine WBJr and Teplow DB. Small assemblies of unmodified amyloid β-protein are the proximate neurotoxin in Alzheimer's disease. Neurobiol. Aging 25: 569-580 (2004).

[25] Glabe CG. Amyloid accumulation and pathogensis of Alzheimer's disease: significance of monomeric, oligomeric and fibrillar Aβ. Subcell Biochem. 38: 167-177 (2005).

[26] Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M *et al.* Diffusible, nonfibrillar ligands derived from Aβ(1–42) are potent central nervous system neurotoxins. Proc. Natl. Acad. Sci. USA 95: 6448-6453 (1998).

[27] Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS *et al.* Naturally secreted oligomers of amyloid β-protein potently inhibit hippocampal long-term potentiation *in vivo*. Nature 416: 535-539 (2002).

[28] Gong Y, Chang L, Viola KL, Lacor PN, Lambert MP, Finch CE *et al.* Alzheimer's disease-affected brain: presence of oligomeric Aβ ligands (ADDLs) suggests a molecular basis for reversible memory loss. Proc. Natl. Acad. Sci. USA 100: 10417-10422 (2003).

[29] Demuro A, Mina E, Kayed R, Milton SC, Parker I and Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. J. Biol. Chem. 280: 17294-17300 (2005).

[30] Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ *et al.* Natural oligomers of the amyloid-β protein specifically disrupt cognitive function. Nat. Neurosci. 8: 79-84 (2005).

[31] Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Jansen B *et al.* Globular amyloid β-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. J. Neurochem. 95: 834-847 (2005).

[32] Chimon S and Ishii Y. Capturing intermediate structures of Alzheimer's β-amyloid, Aβ(1-40), by solid-state NMR spectroscopy. J. Am. Chem. Soc. 127: 13472-13473 (2005).

[33] Lomakin A and Teplow DB. Quasielastic scattering study of amyloid β-protein fibril formation. Prot. Pept. Lett. 13: 247-254 (2006).

[34] Karplus M. Molecular dynamics of biological macromolecules: a brief history and perspective. Biopolymers 68: 350-358 (2003).

[35] Schleif R. Modeling and studying protein with molecular dynamics. Methods Enzymol. 383: 28-47 (2004).

[36] Levitt M. A simplified representation of protein conformations for rapid simulation of protein folding. J. Mol. Biol. 104: 59-107 (1976).

[37] Head-Gordon T and Brown S. Minimalist models for protein folding and design. Curr. Opin. Struct. Biol. 13: 160-167 (2003).

[38] Tozzini V. Coarse-grained models for proteins. Curr. Opin. Struct. Biol. 15: 144-150 (2005).

[39] Ding F and Dokholyan NV. Simple but predictive protein models. Trends Biotech. 23: 450-455 (2005).

[40] Hansmann UHE and Okamoto Y. New Monte Carlo algorithms for protein folding. Curr. Opin. Struct. Biol. 9: 177-183 (1999).

[41] Takada S, Luthey-Schulten Z and Wolynes PG. Folding dynamics with nonadditive forces: A simulation study of a designed helical protein and a random heteropolymer. J. Chem. Phys. 110: 11616-11629 (1999).

[42] Brown S, Fawzi NJ and Head-Gordon T. Coarse-grained sequences for protein folding and design. Proc. Natl. Acad. Sci. USA 100: 10712-10717 (2003).

[43] Takagi F, Koga N and Takada S. How protein thermodynamics and folding mechanisms are altered by the chaperonin cage: Molecular simulations. Proc. Natl. Acad. Sci. USA 100: 11367-11372 (2003).

[44] Fawzi NL, Chubukov V, Clark LA, Brown S and Head-Gordon T. Influence of denatured and intermediate states of folding and protein aggregation. Prot. Sci. 14: 993-1003 (2005).

[45] Mukherjee A and Bagchi B. Contact pair dynamics during folding of two small proteins: Chicken villin head piece and the Alzheimer protein β-amyloid. J. Chem. Phys. 120: 1602-1612 (2004).

[46] Okamoto Y. Generalized-ensemble algorithms: enhanced sampling techniques for Monte Carlo and molecular dynamics simulations. J. Mol. Graph. Mod. 22: 425-439 (2004).

[47] Mezei M. Adaptive umbrella sampling—self-consistent determination of the non-Boltzmann bias. J. Comput. Phys. 68: 237-248 (1987).

[48] Hansmann UHE, Okamoto Y and Eisenmenger F. Molecular dynamics, Langevin and hybrid Monte Carlo simulations in a multicanonocal ensemble. Chem. Phys. Lett. 259: 321-330 (1996).

[49] Nakajima N, Nakamura H and Kidera A. Multicanonical ensemble generated by molecular dynamics simulation for enhanced conformational sampling of peptides. J. Phys. Chem. 101: 817-824 (1997).

[50] Bartels C and Karplus M. Probability distributions for complex systems: adaptive umbrella sampling of the potential energy. J. Phys. Chem. B 102: 865-880 (1998).

[51] Sugita Y and Okamoto Y. Replica-exchange molecular dynamics for protein folding. J. Chem. Phys. 314: 141-151 (1999).

[52] Marinari E and Parisi G. Simulated tempering: a new Monte Carlo scheme. Europhys. Lett. 19: 451-458 (1992).

[53] Kumar S, Bouzida D, Swendsen RH, Kollman PA and Rosenberg JM. The weighted histogram analysis method for free-energy calculations on biomolecules. J. Comput. Chem. 13: 1011-1021 (1992).

[54] Schaefer M, Bartels C and Karplus M. Solution Conformations and Thermodynamics of Structured Peptides: Molecular Dynamics Simulation with an Implicit Solvation Model. J. Mol. Biol. 284: 835-848 (1998).

[55] Baumketner A and Shea JE. Free Energy Landscape for Amyloidogenic Tetrapeptides Dimerization. Biophys. J. 89: 1493-1503 (2005).

[56] Barkema GT and Mousseau N. Event-based relaxation of continuous disordered systems. Phys. Rev. Lett. 77: 4358-4361 (1996).

[57] Malek R and Mousseau N. Dynamics of Lenard-Jones clusters: A characterization of the activation-relaxation technique. Phys. Rev. E 62: 7723-7728 (2000).

[58] Wei GH, Mousseau N and Derreumaux P. Exploring the energy landscape of proteins: a characterization of the activation-relaxation technique. J. Chem. Phys. 117: 11379-11387 (2002).

[59] Mousseau N and Derremaux P. Exploring the Early Steps of Amyloid Peptide Aggregation by Computers. Acc. Chem. Res. 38: 885-891 (2005).

[60] Rapaport DC. The art of molecular dynamics simulation. Cambridge University Press (1997).

[61] Zhou Y, Hall CK and Karplus M. First-Order Disorder-to-Order Transition in an Isolated Homopolymer Model. Phys. Rev. Lett. 77: 2822-2825 (1996).

[62] Zhou Y and Karplus M. Folding thermodynamics of a three-helix-bundle protein. Proc. Natl. Acad. Sci. USA 94: 14429-14432 (1997).

[63] Zhou Y, Karplus M, Wichert JM and Hall CK. Equilibrium thermodynamics of homopolymers and clusters: molecular dynamics and Monte-Carlo simulations of system with square-well interactions. J. Chem. Phys. 107: 10691-10708 (1997).

[64] Dokholyan NV, Buldyrev SV, Stanley HE and Shakhnovich EI. Discrete molecular dynamics studies of folding of a protein-like model. Folding and Design 3: 577-587 (1998).

[65] Zhou Y and Karplus M. Folding of a Model Three-helix Bundle Protein: A Thermodynamic and Kinetic Analysis. J. Mol. Biol. 293: 917-951 (1999).

[66] Dokholyan NV, Buldyrev SV, Stanley HE and Shakhnovich EI. Identifying the protein folding nucleus using molecular dynamics. J. Mol. Biol. 296: 1183-1188 (2000).

[67] Smith AV and Hall CK. α-helix formation: discontinuous molecular dynamics on an intermediate-resolution protein model. Proteins 44: 344-360 (2001).

[68] Smith AV and Hall CK. Assembly of a Tetrameric α-Helical Bundle: Computer Simulations on an Intermediate-Resolution Protein Model. Proteins 44: 376-391 (2001).

[69] Smith AV and Hall CK. Protein refolding versus aggregation: Computer simulations on an intermediate-resolution protein model. J. Mol. Biol. 312: 187-202 (2001).

[70] Ding F, Borreguero JM, Buldyrev SV, Stanley HE and Dokholyan NV. A Mechanism for the α-helix to β-hairpin transition. Proteins: Structure, Function, and Genetics 53: 220-228 (2003).

[71] Ding F, Buldyrev SV and Dokholyan NV. Folding Trp-cage to NMR resolution native structure using a coarse-grained protein model. Biophys. J. 88: 147-155 (2005).

[72] Borreguero JM, Urbanc B, Lazo ND, Buldyrev SV, Teplow DB and Stanley HE. Discrete molecular dynamics study of the amyloid β-protein decapeptide Aβ(21-30). Proc. Natl. Acad. Sci. USA 102: 6015-6020 (2005).

[73] Urbanc B, Borreguero JM, Cruz L and Stanley HE. Amyloid β-Protein Aggregation: *Ab Initio* Discrete Molecular Dynamics Approaches. Methods in Enzymology 412: 314-338 (2006).

[74] Lazo ND, Grant MA, Condron MC, Rigby AC and Teplow DB. On nucleation of amyloid β-protein monomer folding. Prot. Sci. 14: 1581-1596 (2005).

[75] Lazo ND and Downing DT. Fibril formation by amyloid-β proteins may involve β-helical protofibrils. J. Pept. Res. 53: 633-640 (1999).

[76] Lansbury PTJr, Costa PR, Griffiths JM, Simon EJ, Auger M, Halverson KJ *et al.* Structural model for the β-amyloid fibril based on interstrand alignment of an antiparallel-sheet comprising a C-terminal peptide. Nat. Struct. Biol. 2: 990-998 (1995).

[77] Wesson L and Eisenberg D. Atomic solvation parameters applied to molecular dynamics of proteins in solution. Protein Science 1: 227-235 (1992).

[78] Petkova AT, Ishii Y, Balbach JJ, Antzutkin ON, Leapman RD, Delaglio F *et al.* A structural model for Alzheimer's β-amyloid fibrils based on experimental constraints from solid state NMR. Proc. Natl. Acad. Sci. USA 99: 16742-16747 (2002).

[79] Cruz L, Urbanc B, Borreguero JM, Lazo ND, Teplow DB and Stanley HE. Solvent and mutation effects on the nucleation of amyloid β-protein folding. Proc. Natl. Acad. Sci. 102: 18258-18263 (2005).

[80] Baumketner A, Bernstein SL, Wyttenbach T, Lazo ND, Teplow DB, Bowers MT *et al.* Structure of the 21-30 fragment of amyloid β-protein. Prot. Sci. 15: 1239-1247 (2006).

[81] Zhang S, Iwata K, Lachenmann MJ, Peng JW, Li S, Stimson ER *et al.* The Alzheimer's peptide Aβ adopts a collapsed coil structure in water. J. Struct. Biol. 130: 130-141 (2000).

[82] Coles M, Bicknell W, Watson AA, Fairlie DP and Craik DJ. Solution structure of amyloid-β peptide (1–40) in a water-micelle environment. Is the membrane spanning domain where we think it is? Biochemistry 37: 11064-11077 (1998).

[83] Crescenzi O, Tomaselli S, Guerrini R, Salvatori S, D'Ursi AM, Temussi PA and Picone D. Solution structure of the Alzheimer amyloid β-peptide (1–42) in an apolar microenvironment. Similarity with a virus fusion domain. Eur. J. Biochem. 269: 5642-5648 (2002).

[84] Massi F, Peng JW, Lee JP and Straub JE. Simulation study of the structure and dynamics of the Alzheimer's amyloid peptide congener in solution. Biophys. J. 80: 31-44 (2001).

[85] Massi F and Straub JE. Probing the origins of increased activity of the E22Q "Dutch" mutant Alzheimer's β-amyloid peptide. Biophys. J. 81: 697-709 (2001).

[86] Wisniewski T, Ghiso J and Frangione B. Peptides homologous to the amyloid protein of Alzheimer's disease containing a glutamine for glutamic acid substitution have accelerated amyloid fibril formation. Biochem. Biophys. Res. Commun. 179: 1247-1254 (1991).

[87] Teplow DB, Lomakin A, Benedek GB, Kirschner DA and Walsh DM. Effects of β-protein mutations on amyloid fibril nucleation and elongation. In 'Alzheimer's Disease: Biology, Diagnosis and Therapeutics' (Eds: Iqbal K, Winblad B, Nishimura T, Takeda M and Wisniewski HM) John Wiley & Sons Ltd., Chichester, England, p. 311-319 (1997).

[88] Massi F, Klimov D, Thirumalai D and Straub JE. Charge states rather than propensity for β-structure determine enhanced fibrillogenesis in wild-type Alzheimer's β-amyloid peptide compared to E22Q Dutch mutant. Prot. Sci. 11: 1639-1647 (2002).

[89] Massi F and Straub JE. Structural and dynamical analysis of the hydration of the Alzheimer's β-amyloid peptide. J. Comput. Chem. 24: 143-153 (2002).

[90] Straub JE, Guevara J, Huo S and Lee JP. Long time dynamic simulations: exploring the folding pathways of an Alzheimer's amyloid Aβ-peptide. Acc. Chem. Res. 35: 473-481 (2002).

[91] Han W and Wu YD. A strand-loop-strand structure is possible intermediate in fibril elongation: long time simulations of amyloid β-peptide (10-35). J. Am. Chem. Soc. 127: 15408-15416 (2005).

[92] Gursky O and Aleshkov S. Temperature-dependent β-sheet formation in β-amyloid Aβ$_{1-40}$ peptide in water: uncoupling β-structure folding from aggregation. Biochim. Biophys. Acta 1476: 93-102 (2000).

[93] Sali A, Shakhnovich E and Karplus M. How do proteins fold? Nature 369: 248-251 (1994).

[94] Urbanc B, Cruz L, Yun S, Buldyrev SV, Bitan G, Teplow DB and Stanley HE. *In silico* study of amyloid β-protein (Aβ) folding and oligomerization. Proc. Natl. Acad. Sci. USA 101: 17345-17350 (2004).

[95] Weinreb PH, Jarrett JT and Lansbury PTJr. Peptide models of a hydrophobic cluster at the C-terminus of the β-amyloid protein. J. Am. Chem. Soc. 116: 10835-10836 (1994).

[96] Xu Y, Shen J, Luo X, Zhu W, Chen K, Ma J *et al.* Conformational transition of amyloid β-peptide. Proc. Natl. Acad. Sci. USA 102: 5403-5407 (2005).

[97] Flöck D, Colacino S, Colombo G and Di Nola A. Misfolding of the Amyloid β-protein: A Molecular Dynamics Study. Proteins 62: 183-192 (2006).

[98] Baumketner A, Bernstein SL, Wyttenbach T, Bitan G, Teplow DB, Bowers MT and Shea JE. Amyloid β-protein monomer structure: a computational and experimental study. Prot. Sci. 15: 420-428 (2006).

[99] Kirkitadze MD, Condron MM and Teplow DB. Identification and characterization of key kinetic intermediates in amyloid β-protein fibrillogenesis. J. Mol. Biol. 312: 1103-1119 (2001).

[100] Antzutkin ON, Balbach JJ, Leapman RD, Rizzo NW, Reed J and Tycko R. Multiple quantum solid-state NMR indicates a parallel, not antiparallel, organization of β-sheets in Alzheimer's β-amyloid fibrils. Proc. Natl. Acad. Sci. USA 97: 13045-13050 (2000).

[101] Balbach JJ, Petkova AT, Oyler NA, Antzutkin ON, Gordon DJ, Meredith SC *et al.* Supramolecular structure in full-length Alzheimer's β-amyloid fibrils: Evidence for a parallel β-sheet organization from solid-state nuclear magnetic resonance. Biophys. J. 83: 1205-1216 (2002).

[102] Benzinger TL, Gregory DM, Burkoth TS, Miller-Auer H, Lynn DG, Botto RE *et al.* Propagating structure of Alzheimer's β-amyloid (10-35) is parallel β-sheet with residues in exact register. Proc. Natl. Acad. Sci. USA 95: 13407-13412 (1998).

[103] Benzinger TL, Gregory DM, Burkoth TS, Miller-Auer H, Lynn DG, Botto RE *et al.* Two-dimensional structure of β-amyloid(10-35) fibrils. Biochemistry 39: 3491-3499 (2000).

[104] Balbach JJ, Ishii Y, Antzutkin ON, Leapman RD, Rizzo NW, Dyda F *et al.* Amyloid fibril formation by Aβ(16-22), a seven-residue fragment of the Alzheimer's β-amyloid peptide, and structural characterization by solid state NMR. Biochemistry 39: 13748-13759 (2000).

[105] Petkova AT, Buntkowsky G, Dyda F, Leapman RD, Yau WM and Tycko R. Solid state NMR reveals a pH-dependent antiparallel β-sheet registry in fibrils formed by a β-amyloid peptide. J. Mol. Biol. 335: 247-260 (2004).

[106] Gordon DJ, Balbach JJ, Tycko R and Meredith SC. Increasing the amphiphilicity of an amyloidogenic peptide changes the β-sheet structure in the fibrils from antiparallel to parallel. Biophys. J. 86: 428-434 (2004).

[107] Ma B and Nussinov R. Stabilities and conformations of Alzheimer's β-amyloid peptide oligomers (Aβ16-22, Aβ16-35 and Aβ10-35): Sequence effect. Proc. Natl. Acad. Sci. USA 99: 14126-14131 (2002).

[108] Klimov DK and Thirumalai D, Dissecting the assembly of Aβ16-22 amyloid peptides into antiparallel β-sheets. Structure 11: 295-307 (2003).

[109] Klimov DK, Straub JE and Thirumalai D. Aqueous urea solution destabilizes Aβ16-22 oligomers. Proc. Natl. Acad. Sci. USA 101: 14760-14765 (2004).

[110] Santini S, Mousseau N and Derreumaux P. In Silico Assembly of Alzheimer's Aβ16-22 Peptide into β-Sheets. J. Am. Chem. Soc. 126: 11509-11516 (2004).

[111] Santini S, Wei G, Mousseau N and Derreumaux P. Pathway Complexity of Alzheimer's β-Amyloid Aβ16-22 Peptide Assembly. Structure 12: 1245-1255 (2004).

[112] Tjernberg LO, Tjernberg A, Bark N, Shi Y, Ruzsicska BP, Bu Z *et al.* Assembling amyloid fibrils from designed structures containing a significant amyloid β-peptide fragment. Biochem. J. 366: 343-351 (2002).

[113] Hwang W, Zhang S, Kamm RD and Karplus M. Kinetic control of dimer structure formation in amyloid fibrillogenesis. Proc. Natl. Acad. Sci. USA 101: 12916-12921 (2004).

[114] Favrin G, Irback A and Mohanty S. Oligomerization of amyloid Aβ$_{16-22}$ peptides using hydrogen bonds and hydrophobicity forces. Biophys. J. 87: 3657-3664 (2004).

[115] Gnanakaran S, Nussinov R and García AE. Atomic-level description of amyloid β-dimer formation. J. Am. Chem. Soc. 128: 2158-2159 (2006).

[116] Tarus B, Straub JE and Thirumalai D. Probing the initial stage of aggregation of the Aβ(10–35)-protein: Assessing the propensity for peptide dimerization. J. Mol. Biol. 345: 1141-1156 (2005).

[117] Sciarretta KL, Gordon DJ, Petkova AT, Tycko R and Meredith SC. Aβ40-Lactam(D23/K28) models a conformation highly favorable for nucleation of amyloid. Biochemistry 44: 6003-6014 (2005).

[118] Petkova AT, Leapman RD, Guo Z, Yau WM, Mattson MP and Tycko R. Self-Propagating, Molecular-Level Polymorphism in Alzheimer's β-Amyloid Fibrils. Science 307: 262-265 (2005).

[119] Lührs TL, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Döbeli H *et al.* 3D structure of Alzheimer's amyloid-β(1-42) fibrils. Proc. Natl. Acad. Sci. USA 102: 17342-17347 (2005).

[120] Palmblad M, Westlind-Danielsson A and Bergquist J. Oxidation of methionine 35 attenuates formation of amyloid β-peptide 1-40 oligomers. J Biol Chem. 277: 19506-19510 (2002).

[121] Hou L, Kang I, Marchant RE and Zagorski MG. Methionine 35 oxidation reduces fibril assembly of the amyloid Aβ-(1-42) peptide of Alzheimer's disease. J. Biol. Chem. 277: 40173-40176 (2002).

[122] Bitan G, Tarus B, Vollers SS, Lashuel HA, Condron MM, Straub JE *et al.* A molecular switch in amyloid assembly: Met^{35} and amyloid β-protein oligomerization. J. Am. Chem. Soc. 125: 15359–15365 (2003).

[123] Hou L, Shao H, Zhang Y, Li H, Menon NK, Neuhaus EB *et al.* Solution NMR studies of the Aβ(1-40) and Aβ(1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. J Am Chem Soc. 126: 1992-2005 (2004).

[124] Butterfield DA and Boyd-Kimball D. The critical role of methionine 35 in Alzheimer's amyloid β-peptide (1-42)-induced oxidative stress and neurotoxicity. Biochim Biophys Acta. 1703: 149-156 (2005).

[125] Massi F and Straub JE. Energy landscape theory for Alzheimer's amyloid β-peptide fibril elongation. Proteins 42: 217-229 (2001).

[126] Lomakin A, Chung DS, Benedek GB, Kirschner DA and Teplow DB. On the nucleation and growth of amyloid β-protein fibrils: Detection of nuclei and quantitation of rate constants. Proc. Natl. Acad. Sci. USA 93: 1125-1129 (1996).

[127] Lomakin A, Teplow DB, Kirschner DA and Benedek GB. Kinetic theory of fibrillogenesis of amyloid β-protein. Proc. Natl. Acad. Sci. USA 94: 7942-7947 (1997).

[128] Kusumoto Y, Lomakin A, Teplow DB and Benedek GB. Temperature dependence of amyloid β-protein fibrillization. Proc. Natl. Acad. Sci. USA 95: 12277-12282 (1998).

[129] Esler WP, Stimson ER, Jennings JM, Vinters HV, Ghilardi JR, Lee JP *et al.* Alzheimer's Disease Amyloid Propagation by a Template-Dependent Dock-Lock Mechanism. Biochemistry 39: 6288-6295 (2000).

[130] Jarrett JT and Lansbury PTJr. Seeding "one-dimensional crystallization" of amyloid: A pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73: 1055-1058 (1993).

[131] Barrow CJ, Yasuda A, Kenny PTM and Zagorski MG. Solution conformations and aggregational properties of synthetic amyloid β-peptides of Alzheimer's disease — Analysis of circular-dichroism spectra. J. Mol. Biol. 225: 1075-1093 (1992).

[132] Zhang SS, Casey N and Lee JP. Residual Structure in the Alzheimer's Disease Peptide – Probing the Origin of a Central Hydrophobic Cluster. Folding and Design 3: 413-422 (1998).

[133] Simona F, Tiana G, Broglia RA and Colombo G. Modeling the α-helix to β-hairpin transition mechanism and the formation of oligomeric aggregates of the fibrillogenic peptide Aβ(12-28): insights from all-atom molecular dynamics simulations. J. Mol. Graph. Mod. 23: 263-273 (2004).

[134] Buchete NV, Tycko R and Hummer G. Molecular Dynamics Simulations of Alzheimer's β-Amyloid Protofilaments. J. Mol. Biol. 353: 804-821 (2005).

[135] Bitan G, Kirkitadze MD, Lomakin A, Vollers SS, Benedek GB and Teplow DB. Amyloid β-protein (Aβ) assembly: Aβ-40 and Aβ-42 oligomerize through distinct pathways. Proc. Natl. Acad. Sci. USA 100: 330-335 (2003).

[136] Bitan G, Lomakin A and Teplow DB. Amyloid β-protein oligomerization: Prenucleation interactions revealed by photo-induced cross-linking of unmodified proteins. J. Biol. Chem. 276: 35176-35184 (2001).

[137] Bitan G and Teplow DB. Rapid photochemical cross-linking - A new tool for studies of metastable, amyloidogenic protein assemblies. Acc. Chem. Res. 37: 357-364 (2004).

[138] Bitan G, Vollers SS and Teplow DB. Elucidation of primary structure elements controlling early amyloid β-protein oligomerization. J. Biol. Chem. 278: 34882-34889 (2003).

[139] Bernstein SL, Wyttenbach T, Baumketner A, Shea JE, Bitan G, Teplow DB *et al.* Amyloid β-Protein: Monomer Structure and Early Aggregation States of Aβ42 and Its Pro^{19} Alloform. J. Am. Chem. Soc. 127: 2075-2084 (2005).

[140] Valerio M, Colosimo A, Conti F, Giuliani A, Grottesi A, Manetti C *et al.* Early Events in Protein Aggregation: Molecular Flexibility and Hydrophobibic/Charge Interaction in Amyloid Peptides as Studied by Molecular Dynamics Simulations. Proteins 58: 110-118 (2005).

[141] Zbilut JP, Colosimo A, Conti F, Colafranceschi M, Manetti C, Valerio M *et al.* Protein Aggregation/Folding: The Role of Deterministic Singularities of Sequence Hydrophobicity as Determined by Nonlinear Signal Analysis of Acylphosphatase and Aβ(1-40). Biophys. J. 85: 3544-3557 (2003).

[142] Peng S, Ding F, Urbanc B, Buldyrev SV, Cruz L, Stanley HE *et al.* Discrete molecular dynamics simulations of peptide aggregation. Phys. Rev. E 69: 041908 (2004).

[143] Urbanc B, Cruz L, Ding F, Sammond D, Khare S, Buldyrev SV *et al.* Molecular Dynamics Simulation of Amyloid-β Dimer Formation. Biophys. J. 87: 2310-2321 (2004).

[144] Snow CD, Sorin EJ, Rhee YM, and Pande VS. How well can simulation predict protein folding kinetic and thermodynamics? Ann. Rev. Biophys. Biomol. Struct. 34: 43-69 (2005).

[145] Nelson R and Eisenberg D. Recent atomic models of amyloid fibril structure. Curr. Opin. Struct. Biol. 16: 260-265 (2006).

[146] Cheung MS, Klimov D and Thirumalai D. Molecular crowding enhances native state stability and refolding rates of globular proteins. Proc. Natl. Acad. Sci. USA 102: 4753-4758 (2005).

[147] Nguyen HD and Hall CK. Molecular dynamics simulations of spontaneous fibril formation by random-coil peptides. Proc. Natl. Acad. Sci. USA 101: 16180-16185 (2004).

[148] Nguyen HD and Hall CK. Phase diagrams describing Fibrillization by Polyalanine Peptides. Biophys. J. 87: 4122-4134 (2004).

[149] Nguyen HD and Hall CK. Kinetics of Fibril Formation by Polyalanine Peptides. J. Biol. Chem. 280: 9074-9082 (2005).