TEACHING STATEMENT

Andriy Baumketner

I enjoy teaching and sharing my learning process to students and colleagues. During my two postdoctoral appointments I mentored a number of graduate students. I strongly believe that teaching is a very important part of a scientific career. Teaching is beneficial not only for students but also for professors. My teaching philosophy will be based on three principles:

- **Encouragement of independent and critical thinking**
  I will do my best to share the knowledge that I have with my students. But at the same time I think that students should be capable of self-direction and self-motivation in their learning. They should be capable of taking on small tasks at first and larger ones later without close supervision from me. If they are to become successful professionals, self-discipline and self-sufficiency are essential characteristics. To encourage my students to develop a greater sense of independence, I will give them as much freedom in their research as they can take.

- **Emphasis on examples and problem sets**
  I believe that the best way of learning is through practical application. In my classes, theoretical concepts will always be explained in examples. Homework assignments will be largely composed of problem sets rather than dry reading. This will allow students to demonstrate their own creativity and encourage them to do additional reading if solving the problem requires so.

- **Individual approach to students**
  Every student is a unique individual. Accordingly, every grad/undergrad program should be tailored to fit the needs of all students. In my classes, there will exist a core of knowledge that should be mastered by all students. For those who want to learn more I plan on organizing specialized meetings in the form of chemistry/physics clubs.

As a faculty member, I am capable of teaching courses across both undergraduate and graduate levels in statistical mechanics, quantum mechanics, classical dynamics or other theoretical disciplines. Specific to the graduate level, I would like to organize courses on advanced topics in modern computational science. These could include introduction to computer simulations of proteins, advanced sampling techniques for simulations of biomolecules or efficient energy optimization algorithms.

STATEMENT OF RESEARCH INTERESTS

Andriy Baumketner

My research interests are in theoretical approaches to problems in chemical and biological physics. Particular systems of interest are complex liquids, proteins and other biologically relevant molecules. Characterized by inherent disorder, heterogeneity of constituent components, structural complexity and the lack of symmetry these systems are extremely difficult to describe theoretically. Below I briefly summarize my past projects in which a variety of computational approaches as well as analytical tools were used to investigate various aspects of protein folding and the structure and dynamics of liquids.

Structure of Alzheimer's disease amyloid β peptides

The onset of Alzheimer's disease, a serious neurological disorder, is directly linked to the deposits of amyloid β (Aβ) peptides. Aβ peptides are produced through the cleavage of the β-amyloid precursor protein (APP) by α-, β- and γ-secretases. Of a large variety of known Aβ peptides, the naturally occurring and cytotoxic alloforms Aβ40/42 are 40 and 42 aa long. While the exact mechanism of the toxicity of amyloid deposits is unknown, it has been noted that small oligomers of Aβ peptides are more pathogenic than the larger fibrillar aggregates. A better understanding of the molecular structure of these oligomers may help to develop efficient therapeutic strategies for the disease. A first step along this path is to characterize the structure of monomers.

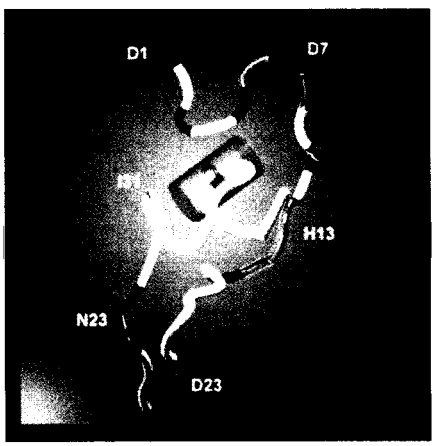

FIG. 1 A representative conformation of the Aβ42 peptide identified in our replica exchange simulations (1). The residues are color-coded according to their type: blue – positively charged, red – negatively charged, green – polar, white – nonpolar, cyan – histidines. There are 3 positively charged and 6 negatively charged residues in this peptide. The total charge state is -3. Characteristic feature of this conformation is that hydrophobic residues tend to form a core, buried inside a shell of polar and charged residues that serve as a shield against water molecules.

We applied the replica exchange molecular dynamics method to study conformational ensembles of Aβ42 peptide (1). The modeling was performed using an atomically accurate peptide representation and an accurate implicit solvent model. Molecular dynamics simulations produced a set of conformations which represent the thermally populated ensemble under physiological conditions. One of such representative conformations is shown in Fig. 1. Among other results, we find that monomeric Aβ42 in aqueous solutions can exist either as extended coils of gyration radius $R_g \sim$ 14-15 Å or collapsed coils of $R_g \sim 11$ Å. Several aspects of our theoretical analysis are directly corroborated by experiments. Specifically, I) distribution of the scattering cross section obtained theoretically agrees very well with the cross section obtained in ion-mobility experiments, II) the amount of secondary structure in monomeric states observed in our simulations agrees well with earlier circular dichroism measurements and NMR studies, III) the major structural motif, so-called central hydrophobic cluster (CHC), revealed by NMR studies of Aβ10-35 (which is the most closely sequence-related peptide to our model) was observed in the simulations.

Chaperone-assisted folding

While proteins in general are very fast and efficient folders, there exist a distinct class of proteins whose spontaneous *in vivo* folding is strongly inhibited. Molecular chaperonins, large multi-subunit protein complexes, are implicated in helping such proteins to fold. Chaperonins, such as the GroE complex of the bacteria E. Coli, act by providing a cavity in which newly translated or translocated proteins can be encapsulated. One of the theories that have recently been proposed to explain the mechanism of chaperonin-assisted folding, the Anfinsen cage model, posits that inhibited folding is due to protein aggregation. Rather than folding to their native states, proteins choose to self-assemble into large, biologically inactive aggregates. The chaperonin cavity serves the purpose of shielding proteins from the cellular environment and thus prevents aggregation. Within the framework of the Anfinsen cage model, we studied the implications of the encapsulation of proteins into cavities. We used a minimalist bead-and-spring model to simulate an

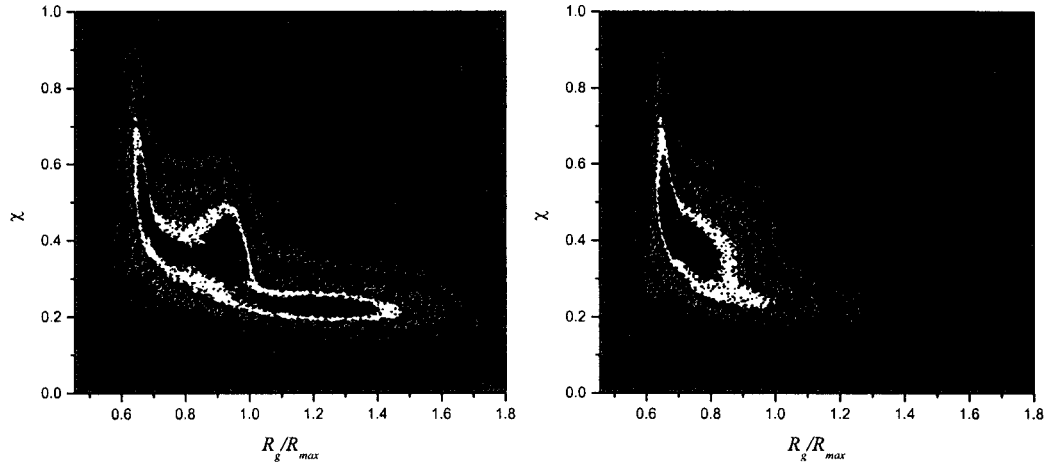

FIG. 2 Folding maps for and α-β protein studied (a) in the bulk and (b) inside chaperonin cavity. The maps are plotted as a function of the gyration radius R_g and a measure of nativeness χ. They reflect relative amount of time the protein spends during folding in each part of the map. Red and orange regions are visited most frequently. Panel (b) shows that the effect of the encapsulation is to eliminate unfolded conformations with relatively large gyration radius $R_g/R_{max} \sim 1.2$ from the folding pathways. This results in an accelerated folding.

α-β sandwich protein confined to a spherical cavity. In our simulations, the cavity was found to strongly influence folding kinetics of the protein. In doing so, the lining of the cavity plays a critical role. Cavities with repulsive walls (2) were found to accelerate folding of proteins with unfrustrated sequences by a maximum amount of 2. The mechanism for this acceleration was seen to be through the elimination of local potential energy minima. Weakly hydrophobic cavities, on the other hand (3), were shown to affect folding much stronger. Accelerations of up to an order of magnitude were observed for frustrated sequences under certain conditions. Enhanced folding rates were due to the lowering and flattening of the folding transition state barrier. We demonstrated in our simulations that rather than being a passive encapsulating cage, a chaperonin cavity can play a more active role in folding.

Protein folding kinetics

An understanding of folding kinetics is essential for our understanding of protein folding in general. Theoretical approaches to folding kinetics are met with a number of difficulties. The primary challenge is related to the high dimensionality of the configuration space underlying folding. As modeling of multidimensional dynamical processes is rather problematic, the idea of developing simple low-dimensional models for protein folding kinetics has been actively pursued. The simplest approach in this direction lies in designating a single dynamical variable as the reaction coordinate of folding. It is required that the dynamics of this coordinate be much slower than of the rest of the degrees of freedom. If the folding reaction coordinate exists, and how to find it remain open questions. To gain more insights into protein folding kinetics we investigated the folding of an off-lattice minimalist model. We applied the Brownian, or diffusion-equation, formalism to describe the dynamics of a chosen folding reaction coordinate x. Within this formalism, folding time is associated with the mean first passage time τ_f of one-dimensional diffusion along x from the ensemble of unfolded states x_d to the native state x_f. τ_f can be evaluated using the Kramers formula from known free energy profile $F(x)$ and the generalized diffusion coefficient D. While $F(x)$ is rather straightforward to

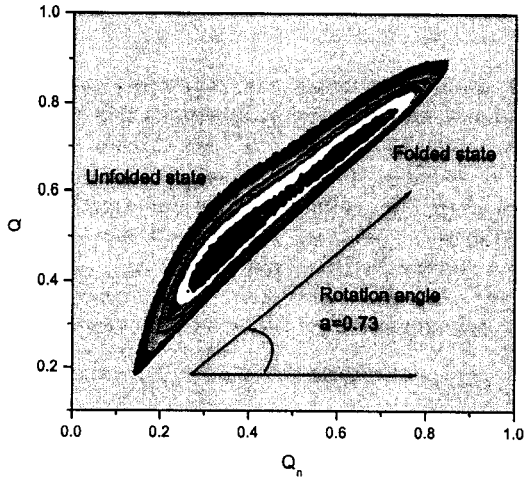

FIG. 3 Free energy map plotted as a function of total number of native contacts Q_n and the total number of contacts Q for a three-helix bundle protein. By directing the initial poor choice of the reaction coordinate $x = Q_n$, along the line connecting the minima corresponding to the unfolded and folded state ensembles, $x = Q_n cos(\alpha) + Q sin(\alpha)$, the agreement between theory and simulations can be improved 6-fold.

generate in computer simulations, a numerical evaluation of D is more challenging. We proposed a formula for D that involves time correlations between x and $F(x)$ and can be easily applied in computer simulations. Using this formula, we studied the folding kinetics of a β-hairpin off-lattice minimalist model. Theoretical prediction for τ_f was found to be only within a factor of 2 off the direct evaluation in simulations (4). When we extended our study to a three-helix bundle protein (5), we found that the initial choice of the reaction coordinate was not successful. We then demonstrated that by considering rotations in the phase space spanned by the trial reaction coordinate and a second dynamical variable relevant for folding, the agreement between simulation and theory can be improved 6-fold (5). A recipe for how to construct a better reaction coordinate is illustrated in Fig. 3.

Structure of liquids from molecular dynamics simulations

Computer simulations play an increasingly important role in theoretical studies of liquid state. Seemingly free from approximations, the method of molecular dynamics requires interparticle potential as its only input. Typically,

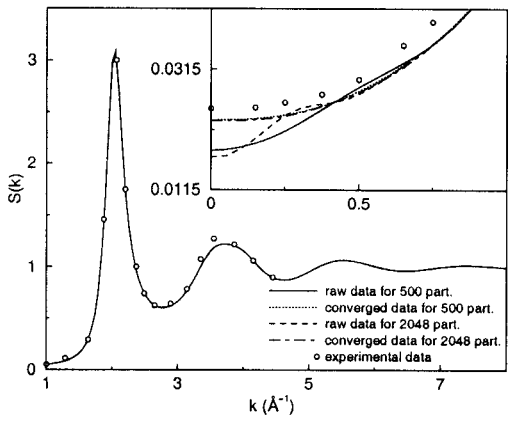

FIG. 4 Static structure factors of liquid sodium obtained in simulations of 500- and 2048-particle systems directly and with the help of the self-consistent procedure. Finite-size effects are clearly seen in the results of the direct simulations. They are effectively eliminated by the developed procedure even for the smaller simulation box. Open symbols correspond to experimental data.

the structural functions of a liquid, pair distribution function $g(r)$ and its Fourier transform static structure factor $S(k)$, extracted from simulation trajectories are directly compared to those obtained in X-ray and neutron-scattering

experiments. But is this comparison truly legitimate? The main reason for concern here is that the theoretical and experimental structural functions are obtained in two different ensembles. Computer models consider finite number of particles in a simulation box and thus correspond to the canonical (NVT) ensemble. Experiments, on the other hand, correspond to the grand canonical ensemble (μPT), since they are carried out under conditions where the particle exchange is not inhibited. The difference in the averages in these two ensembles is termed finite-size effects in computer simulations. Among other properties, finite-size effects are revealed in the structural functions of a liquid, especially when the simulations are carried out for a small number of particles N. For small N, the asymptotic behavior $g(r) \sim 1$ at large r observed in experiments differs significantly from the $g(r) \sim 1 - 1/N$ asymptotics, appropriate for a simulation. Consequently, the static structure factor obtained from simulated $g(r)$ through Fourier transformation is strongly distorted, especially in the $k \sim 0$ region. We developed a self-consistent procedure to correct for finite-size effects in computer simulations. The procedure builds on analytical theory and integral equation approaches (6; 7). Designed to improve the cost-efficiency of liquid structure calculations, our procedure allows for an accurate determination of $S(k)$ from simulations of small systems. As illustrated in Fig. 4, the procedure delivers satisfactory results for a simulation box comprising as few as 500 particles, even in the $k \to 0$ limit (7).

Dynamical properties of binary liquids

Our present understanding of the dynamical properties of liquids is far from complete. Triggered by the inception of the inelastic neutron scattering technique (INS) more than 40 years ago, continuing research efforts in this direction have left many important questions unanswered. While simple liquids, composed of atoms of the same sort, have received considerable attention in the past, multicomponent liquids have hardly been studied. This is quite unfortunate given the omnipresence of complex liquids in nature. The recent discovery of the "fast sound" phenomenon in binary liquid mixtures further highlights the need for more research on the dynamics of complex liquids. Along this line, we developed a theoretical framework for the description of density fluctuations in binary mixtures.

Spontaneous fluctuations of atomic density represent the most basic dynamical process that can occur in liquids. Quantitatively, this process is characterized by incoherent and coherent intermediate scattering functions $F(k,t)$, depending on whether they describe self-particle or collective behavior, respectively. Fourier transform $S(k,\omega)$ of the coherent $F(k,t)$ is directly accessible in neutron scattering experiments. Termed dynamical structure factor, $S(k,\omega)$ measures the collective response of a liquid to the external perturbation due to incidental neutrons that transfer energy $\hbar\omega$ and momentum $\hbar\vec{k}$ to the system. We extended the viscoelastic approximation for the intermediate scattering functions, developed by Lovesey and colleagues, to binary mixtures. The approximation builds on the assumption that density fluctuations are decoupled from thermal fluctuations in the liquid. To construct an equation of motion for $F(k,t)$, correlations among density, particle flux and stress tensor operators only are considered. Conceptually, this approximation is wrong in the hydrodynamic limit $k \to 0$ where the energy-density coupling is essential. But at finite k, the viscoelastic approximation is expected to work well for systems that exhibit weak coupling between density and energy. Using molecular dynamics simulations, we found that this is the case for metal alloys, in particular alkali alloys. Partial intermediate scattering functions, computed using the viscoelastic approximation for a number of liquid alkali-metal alloys, including sodium-potassium and potassium-caesium alloys, were found to be in a good agreement with the results of molecular dynamics simulations (8–11).

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COMPUTER SIMULATION OF ALZHEIMER'S DISEASE AMYLOID β PEPTIDES.

Andriy Baumketner

Research proposal

Introduction

Proteins are among the most ubiquitous and important molecules in nature. The multitude of functions carried out by proteins in living organisms is truly colossal. Enzymatic activity, transport of oxygen, fatty acids and other agents, storage, control and structural properties, as well as communication and signaling functions, all require active involvement of proteins. To be biologically active, proteins need to reach specific three-dimensional conformations, or native states. Sequences of amino acid residues uniquely encode native states for each particular protein. Predicting the structure of the native state from a given sequence constitutes the elusive *protein folding* problem. Because of the extraordinary complexity of protein molecules, a mechanistic understanding of their folding in any appreciable detail poses a tremendous challenge in molecular biology. Over the past decades, main research efforts were concentrated on studying proteins while isolated from other molecular species. Called *in vitro*, such experiments examine purified solutions of proteins at low concentrations to avoid interactions among proteins or between proteins and other biomolecules that may be present in the cell. Considerable progress has been made in our understanding of the *in vitro* folding from both experimental and theoretical perspectives (1–4).

In contrast to the ideal environment of laboratory test tubes, proteins inside living cells (*in vivo* conditions), do not fold in the absence of other molecules. Immediately after having been synthesized, a nascent polypeptide chain finds itself in a crowded cellular interior where it faces a large ensemble of interaction partners. Intracellular interactions have profound effect on protein thermodynamics, especially the stability of the native state and, foremost, folding kinetics (5). Instead of folding directly to the native state, the next most likely scenario for a protein *in vivo* is aggregation. Driven by mutually attractive interactions, proteins can self-assemble into larger supra-molecular complexes commonly termed aggregates. While protein aggregates come in a large variety of sizes and shapes, their most prominent type is amyloid fibrils (6).

Amyloid fibrils, rod-like objects of mesoscopic dimensions (6-8 *nm* wide and up to 1 *mm* long) rich in β-sheet structure, are relevant to current research for a number of reasons. Firstly, they are a very promising material for nanotechnological applications (7). Pioneering reports of nanowire fabrications using amyloidogenic peptides as the casting matrix have recently appeared in the literature (6). Secondly, from a biological perspective, unique properties of amyloid structures have been utilized by some species, including bacteria and fungi, for specific and well-defined purposes (8). But most importantly, fibrils are interesting because of their implications in medicine.

The appearance of intra- and extra-cellular fibrils has long been suspected of causing disease (8; 9). Deposits of a number of proteins are believed to lead to certain neuropathological conditions, including Alzheimer's and Parkinson's diseases. Additionally, growing experimental evidence suggests that a larger class of severe illnesses, including those unrelated to the central nervous system, may be caused by amyloid deposition (8; 9). Multiple myeloma associated with the deposition of immunoglobulins, or systemic amyloidosis and familial Mediterranean fever that result from the deposition of the serum amyloid A (SAA) protein in various organs, including spleen, kidney or liver, are just two such diseases. Finally, recent experiments reveal that other proteins, which are not related to disease and which were thoroughly investigated in earlier *in vitro* studies, such as the SH3 domain family, can self-assemble into fibrils under appropriate conditions (10). This observation has laid the groundwork for an important conclusion in molecular biology stating that the ability to form amyloid is a generic property of all proteins and peptides, regardless of their sequence specificity (11; 12). As one of the common states in which proteins can exist, amyloid fibrils present an appealing subject for basic research. A variety of biochemical methods and techniques have been recently employed to investigate these systems at the molecular level (9; 13). Nevertheless, a plethora of important questions about amyloid formation remains unanswered. In particular, very little is known about the molecular structure of amyloid fibrils. While, using NMR and crystallographic means, microscopic three-dimensional structures have been solved for a few monomeric proteins embedded into fibrils, the detailed atomistic pictures of fibrils themselves are still missing. Further, it is poorly understood which protein characteristics govern propensity to form fibrillar aggregates,

their stability and possible distribution of sizes. Also, our present understanding of the mechanisms involved in fibril assembly is ambiguous. And, most importantly, the origins of amyloid pathogenicity remain unclear. Poor understanding of the molecular mechanics of amyloid and conditions leading to its occurrence have contributed to the fact that the recent progress in developing new and efficient therapies for amyloid-related diseases has been rather slow.

I propose to address some of the questions raised above using molecular dynamics simulations. Amyloid β peptides (Aβ), implicated in Alzheimer's disease, will be considered as a model amyloidogenic system in my study. As the protein aggregation problem is multifaceted and presents a rich variety of behaviors occurring on different length and time scales, it is desired that theoretical modeling be carried out at different levels of complexity. Along these lines, I propose to employ three types of protein models that differ in their degree of structural detail. A common element in these studies is that their results will be interpreted in terms of free energy landscapes. Energy landscape theory has proved very successful at explaining various aspects of protein folding (I). At the first stage of this project, I will begin from a fully atomistic protein description. A variety of Aβ peptides will be simulated using an all-atom force field. With the computational resources available today, systematic investigations of monomers only will be feasible. The purpose of these simulations, which will provide detailed structural characterization for a number of Aβ alloforms, is both specific and general. The specific aspect is to gain much needed insights into conformational statistics of Aβ peptides, especially their neurotoxic 40 and 42-aa long versions. The general part is related to the use of the atomistic simulations as a benchmark for the subsequent stages of the project.

At the second stage of the modeling, I plan to employ reduced, or minimalist, off-lattice protein models. Within these models, every amino acid residue is treated as a single interacting bead. This results in a significant reduction of the degrees of freedom that need to be taken into account. The beads are separated from one another by virtual bonds of fixed length. A variety of potentials are applied to the beads in an attempt to mimic the physical interactions present in real proteins. I propose that the parameters of these interactions be fitted so that major conformational characteristics of the reduced model match those of the fully-atomic protein representation. This can be done most conveniently by using free energy maps as functions of designated order parameters. Since I intend to simulate the self-assembly of these peptides, which occurs through water-exposed hydrophobic contacts, I propose that the chosen order parameters include, among other quantities, the degree of solvent exposure of hydrophobic residues. Accordingly, I will attempt to fine-tune the parameters of the reduced model so that the resulting free energy closely matches the free energy obtained in the atomistic simulations. Because of the reduced number of degrees of freedom, minimalist models allow for larger systems to be studied compared to fully-atomic approaches. I anticipate that equilibrium simulations of Aβ40-42 oligomers up to decamers will be feasible when special sampling techniques are employed. The main questions of interest in these simulations will be: (I) What types of oligomers are predominantly populated under given conditions? (II) Are the earliest kinetic intermediates termed *paranuclei* that were experimentally shown to consist of about 5-6 monomers, seen in the simulations? (III) What is the nature of the conformational changes occurring to monomers upon association?

At the third and final stage of the project the protein model will be further simplified in an attempt to capture the statistical nature of the proteins comprising a fibril. Amyloids are composed of thousands of monomeric proteins and as such should possess unique properties characteristic for large ensembles of particles. These statistical properties will be irrelevant for studies that consider only a few monomers (proteins/peptides), but will be clearly manifest in phenomena that require participation of many particles, such as phase transitions. To properly describe these properties a theoretical model needs to consider a sufficiently large number of particles. At present, this can be done only by eliminating all the internal degrees of freedom which are responsible, among other things, for the protein flexibility and conformational entropy. I propose that the proteins comprising a fibril be considered as rigid bodies, characterized by the translational degrees of freedom only. This is a rather crude approximation, but one that was successfully employed in the past to model other biological processes involving proteins. In my model, the proteins will be represented by spherocylinders that interact via asymmetric attractive force. The force will depend on both distance and mutual orientation of the molecules and will be parametrized using the results of the simulations of the more accurate off-lattice model discussed above. An immediate question to ask at this theoretical level is whether it is possible to devise an effective inter-protein potential such that the proteins are forced to self-assemble into geometrical objects that structurally resemble amyloid fibrils. If so, I will investigate the details of the phase diagram of this model. In particular, it will be interesting to map out equilibrium phases that comprise various oligomeric states in which the model protein can exist. From the kinetic perspective, the simulations will delineate aggregation pathways including possible on- and off-pathway intermediates to fibrillation. As an ultimate test for the quality of the proposed model I will try to reproduce the mechanical properties of fibrils.

In what follows I provide more detailed information about the present state of the art research on each of the three protein models discussed here. I will also shortly discuss my previous research projects that involved the use of these models. Finally, I state the specific aims and goals of the present proposal.

Multiple faces of Aβ peptides: monomers, oligomers, fibrils

The amyloid hypothesis (14), a conceptual framework proposed recently to explain the molecular basis of Alzheimer's disease (AD), directly links deposits of Aβ peptides with the onset of the disorder. As a result of normal cell metabolism (14), Aβ peptides are produced from the β-amyloid precursor protein (APP), a trans-membrane protein with unclear biological function, through cleavage by α-,β- and γ-secretases. Depending on the sequence details

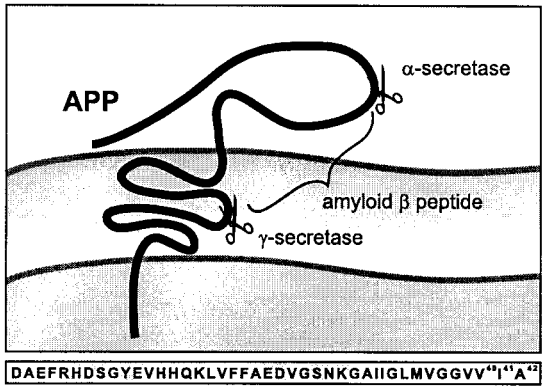

FIG. 1 Various alloforms of amyloid β peptides result from the cleavage of the membrane APP protein by α-,β- and γ-secretases. The length of the peptides can be from 40 to 43 amino acids, depending on the cleavage point in the intra-membrane part of APP. Highlighted in the figure are two regions of contiguous hydrophobic residues in Aβ peptides: the central hydrophobic cluster $LVFFA$, which is essential for the peptides' ability to fibrillate (15; 16), and the C-terminal hydrophobic patch.

of their intra-membrane C-terminal region, the naturally occurring β-amyloid peptides can be 40 to 43 amino acid residues long. Of these, Aβ42 is considered the most cytotoxic (17). Figure 1 explains schematically how Aβ are produced.

A majority of all known alloforms of Aβ peptides are capable of forming amyloid fibrils (16). Due to their pathogenic nature (18–20), these fibrils have recently attracted much research attention. Numerous studies have sought (6; 16) to characterize their structure at the microscopic level using a variety of experimental techniques. Although an atomically accurate model for amyloid fibrils has not yet emerged, considerable progress has been made in our understanding of these systems. Amyloid fibrils are thought to have a specific hierarchical structure. The present views on the molecular organization of amyloids are explained schematically in Fig. 2. According to the results of X-ray diffraction experiments (22), individual peptides comprising a fibril are rich in cross β structure, as evidenced by two specific maxima in the diffraction patterns at 4.7 Å and 10-11 Å spacings. Driven by inter-monomer hydrogen bonding, Aβ peptides are believed to self-assemble into β sheets by aligning their β strands in the direction perpendicular to the fiber axis. Both parallel (23; 24) and anti-parallel (25–27) β-sheet alignments were reported, depending on the length of the peptide and its amphiphilicity. As a general rule, it has been observed that short fragments of Aβ peptides adopt antiparallel orientations with the longer ones, parallel. Additionally, recent experiments have demonstrated that the degree of amphiphilicity is important for the β-sheet alignment. By making the C-terminal of Aβ16-22 peptide more hydrophobic, and as a result the entire molecule more amphiphilic, the preferred antiparallel sheet orientation was reversed to the parallel one (28). The influence of the amphiphilicity, inferred from these experiments, is consistent with the earlier findings concerning longer Aβ peptides. Thermodynamic stability of β sheets in various arrangements was studied recently using molecular dynamics simulations (29).

The dominant view on the internal structure of amyloid fibrils envisions that at a late stage in aggregation, pre-assembled β-sheets coalesce, presumably through water-exposed hydrophobic surfaces, to form larger supra-molecular complexes called protofilaments. The main structural elements of fibrils, protofilaments, can reach the full length of the mature fibrils. But measuring about 25-30 Å across, they are considerably thinner in diameter, compared with 60-80 Å of fibrils. While the exact number is unknown, and may as well vary with the peptide sequence, geometrical considerations suggest that there could be five or six protofilaments wound up around a common axis to make up a fibril (16).

Alternative explanations of amyloid structure include the polar zipper idea (30) and β-helix model (22). It should be noted, however, that none of the existing models has been rigorously proven. A number of important issues remain unclear for each of these models. For example, for the scheme involving protofilaments, questions that need clarification are: What is the molecular structure of monomers? How many β-sheets comprise a protofilament? How many protofilaments are contained in a fibril?

The recent groundbreaking discovery that it may not be Aβ fibrils that inflict most damage to the cell but their low

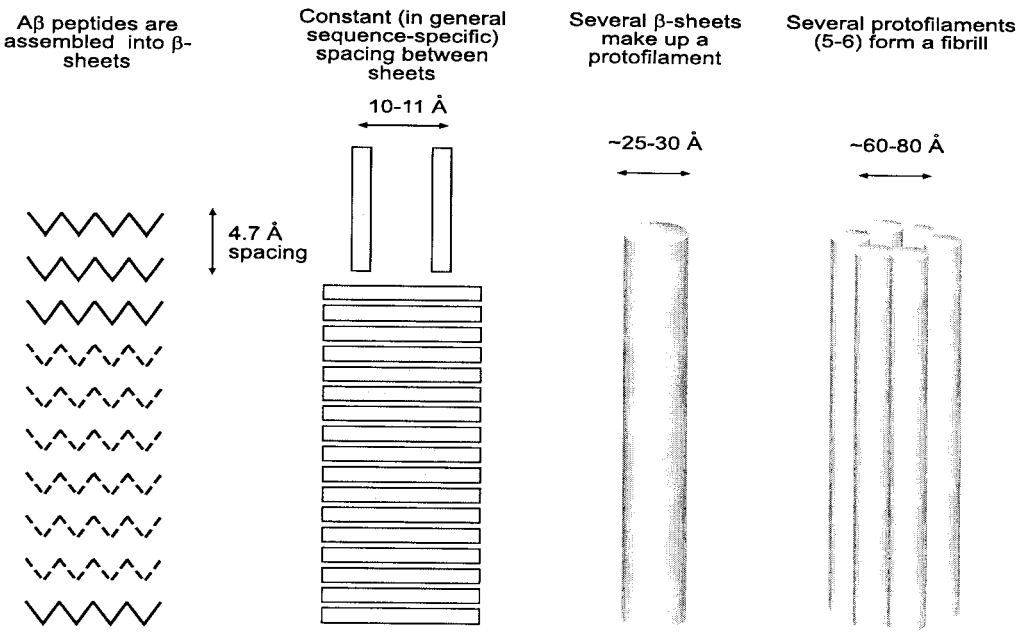

FIG. 2 Hierarchical structure of amyloid fibrils. $A\beta$ peptides assemble into β sheets. Several β sheets then form a protofilament which is the main structural element of fibrils. Five to six protofilaments make up a fibril. The microscopic structure of protofilaments is not known. Molecular conformations of monomeric peptides incorporated into protofilaments are available for a few peptides (6; 21)

molecular weight oligomers has prompted a radical shift in structural studies on amyloid formation. Soluble oligomeric intermediates that precede formation of fibrils and may coexist with them in dynamic equilibrium (14; 31; 32) have become a new focus of the research. These oligomers were observed both *in vivo* (33; 34) and *in vitro* (35; 36), and are believed to be formed at early stages of the aggregation process. In a number of recent papers (33; 37–40), these oligomers were seen to be more cytotoxic than insoluble amyloid fibrils. The exact structure of these mobile oligomers, the pathways underlying their formation, or the mechanism by which they elicit apoptosis remain unknown. Structural studies on these intermediates are expected not only to provide a better understanding of the main principles of protein aggregation, which is an interesting current problem in structural biology, but also to aid in the development of possible therapeutic strategies for Alzheimer's disease. The starting point for such studies is the characterization of the structure of non-aggregated, monomeric peptides.

Experimental efforts to solve the structure of monomeric $A\beta$ peptides are hampered by poor solubility of these molecules in water (13). Four solution NMR studies in water have been reported to date for various $A\beta$ peptides. Two of them were concerned with the shorter 12-28 (41) and 10-35 (42; 43) fragments which do not occur naturally. The other two (44; 45) considered the regular length peptides, 40 and 42 amino acids long, in which residue Met35 was oxidized ($A\beta 40^{ox}$,$A\beta 42^{ox}$). These NMR investigations produced valuable insights into structural organization of amyloid β peptides. Among other things, the content of the secondary structure was shown to be negligible in all alloforms studied. More interestingly, it was found that the peptides possess localized regions of high structural stability while the rest of the sequence is completely flexible. Due to the lack of long-range constraints, however, a thorough structural analysis of $A\beta$ peptides was not carried out. To date, molecular conformations in aqueous solutions have been solved for a single $A\beta$ peptide, $A\beta 10\text{-}35$ (42; 43).

In summary, investigations on $A\beta$ peptides remain a very dynamic research area in which a variety of open questions exist that concern all levels of our understanding of these systems: from monomers to oligomers to fibrils. Due to their size, phenomena in which they can be involved, and their physicochemical properties, $A\beta$ peptides have proven a formidable challenge to both experimental and theoretical approaches. Among many important questions that need to be answered about $A\beta$ peptides, solving their molecular structure in monomeric form and when they are incorporated into small oligomers seems to be the most urgent one.

Atomistic simulations of $A\beta$ peptides

I propose here to use fully atomic molecular dynamics (MD) simulations to characterize the structure of $A\beta$ monomers. Computer simulations are better suited for studies of monomeric peptides than most experimental tech-

niques. With the exception of single-molecule experiments, which were reported for proteins (46) but not for peptides, most experimental approaches produce ensemble-averaged pictures of the studied system. In contrast, computational methods are able to analyze a system at the single-conformation level. Why is this important for the proposed study? The reason is that the Aβ peptides studied here are capable of forming amyloid structures. Characteristic pathways leading to amyloid formation may depend on the existence of specific, low statistical-weight conformations that do not contribute greatly to the ensemble average and thus are undetected in experiments such as solution NMR. These conformations, however, may serve as transition states on the aggregation pathway and thus determine the mechanism of self-assembly.

Within the proposed research plan, I intend to apply computer simulations to study Aβ40 and Aβ42 alloforms of the amyloid β peptide. These peptides have never been studied theoretically, and, most importantly, it is these peptides which are directly involved in the Alzheimer disease. Amyloid β peptides previously studied by full-atom simulations are Aβ10-35 (47–49), Aβ16-22 (50; 51) and Aβ12-28 (52; 53). These studies provided valuable insights into the structure of monomeric Aβ peptides (47–49; 52; 53), as well as possible mechanisms of oligomerization (50; 51). A common drawback of these simulations is that they were run over times that are significantly shorter than the

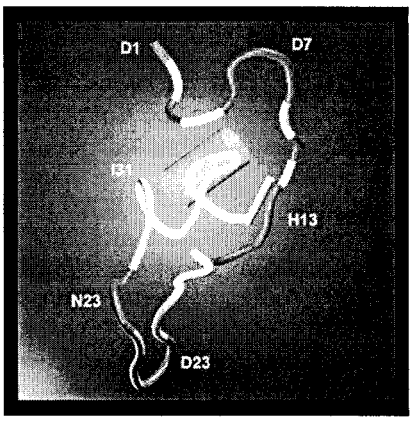

FIG. 3 A representative conformation of Aβ42 identified in our recent work (54). Characteristic feature of this conformation is that hydrophobic residues tend to form a core, buried inside a shell of polar and charged residues that serve as a shield against water molecules.

anticipated relaxation time for the studied peptides. Most likely, the simulations were unable to display the global equilibrium picture of the conformational landscape. Rather, they produced a "local" view, limited to the free energy minima nearest to the initial structures of the simulations.

Poor equilibration is a well-known problem in biomolecular simulations. As a rule, biologically relevant molecules such as proteins possess very complicated potential energy surfaces. Low energy minima on these surfaces serve as kinetic traps that considerably slow down protein dynamics, especially at low temperatures. A variety of computational techniques have been proposed to speed up equilibration and improve conformational sampling (55). I propose here to use the replica exchange method (56) for that purpose. This method considers a number of identical copies of the original system called *replicas* that are simulated in parallel, each at a different temperature. Periodically, the temperatures of adjacent replicas are swapped. This allows each replica to randomly travel up and down in temperature. As a result, replicas that traverse to higher temperatures experience accelerated equilibration, as at these temperatures the relaxation times are shorter. The replica exchange technique allows for both low free energy states to be mapped as well as the equilibrium thermodynamic functions to be extracted at varying temperature.

In our most recent work, we applied the replica exchange method to study conformational ensembles of the Aβ42 peptide (54). The modeling was performed using an atomically accurate peptide representation and an accurate implicit solvent model. Molecular dynamics simulations produced a set of conformations which represent the thermally populated ensemble under physiological conditions. One of these representative conformations is shown in Fig. 3 Among other results, we find in our simulations that monomeric Aβ42 in aqueous solutions can exist either as extended coils of gyration radius R_g ~14-15 Å or collapsed coils of R_g ~11 Å. Several aspects of our theoretical analysis are directly corroborated by experiments. Specifically, I) the distribution of the scattering cross section obtained theoretically agrees very well with the cross section obtained in ion-mobility experiments, II) the amount of secondary structure in monomeric states observed in our simulations agrees well with earlier circular dichroism measurements (35; 36; 57; 58) and NMR studies (45; 59), III) the major structural motif, the so-called central hydrophobic cluster (CHC), revealed by NMR studies of Aβ10-35 (which is the most closely sequence-related peptide to our model) (42; 43) is observed in the simulations. Despite these important findings, the work on Aβ42 needs

to be extended. Of foremost importance is to test the reproducibility of the obtained results. Our simulations were continued for about 10 ns which may not be long enough for a peptide of the given size. Also, the effects of the employed implicit solvent approximation need to be elucidated. For that purpose, simulations employing explicit water molecules will have to be initiated. Free energy surfaces as a function of various reaction coordinates will be produced as the output of these simulations. The temperature dependence of these surfaces will be studied in order to see how strongly the structure of Aβ peptides is affected by external perturbations such as heat. In particular, the transition state regions that correspond with free energy barriers will be analyzed in detail. It is hoped that these regions can shed some light onto possible oligomerization pathways of the peptides. I plan to carry out the same research program for Aβ40 and possibly Aβ10-35. This will allow for the effects of sequence specificity on the conformational ensembles of Aβ peptides to be analyzed.

Minimalist off-lattice model for Aβ peptides

In the previous sections I briefly described what can be learned about amyloidogenic peptides using full-atom simulations. I mentioned that only monomeric states of peptides can be studied using full-atom models because of the high computational requirements associated with them. Most often, however, it is not (or not only) the monomeric peptides but the pathways of their oligomerization that are of interest. This is certainly the case for Aβ peptides whose soluble, mobile oligomers were shown to be more toxic than both monomers and fibrils (17). In order to be able to address the oligomerization problem by means of simulations a radical reduction in the number of degrees of freedom of the studied model needs to be achieved. I propose here to employ minimalist off-lattice models which use a simplified representation of the polypeptide chain to study how Aβ peptides oligomerize.

Minimalist models have been widely used in biomolecular modeling. Their prominent applications include translocation of biopolymers through nanopores (60), DNA transport through membrane channels (61), or modeling of bilayers with reduced lipid representation (62). A few pioneering simulations for the aggregation problem have also been reported (63–65). But the most extensive use of the low-resolution modeling has been seen in the protein folding area (66). Important aspects of folding thermodynamics as well as kinetics have recently been elucidated with the help of simple minimalist models.

Modeling of the aggregation phenomenon using minimalist protein representations consists of two parts. The first part is to work out the structural details of the model. This includes the issue of how accurately the peptide backbone and side chains are represented. In an effort to achieve the highest level of simplification possible, I will follow the pioneering work of Thirumalai *et. al.* (67) and consider a model in which each amino acid residue is represented by a single interacting particle. A typical model of this type is shown in Fig. 4. The model consists of three types of

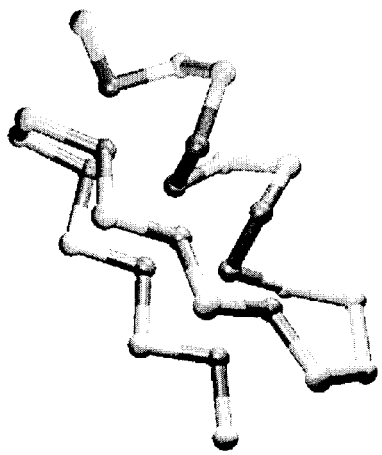

FIG. 4 A minimal model for an α-β sandwich protein that employs a single bead per residue convention. The model was used by us to study chaperone-assisted folding (68; 69).

residues, hydrophobic, hydrophilic and neutral, separated by virtual bonds of fixed length. We employed this model in our earlier work (68; 69) to study folding in the presence of molecular chaperones.

The second component necessary for minimalist modeling is an appropriate interaction scheme. The beads that make up a minimal protein are assumed to interact with each other via a number of interactions. The interactions are designed so as to capture implicitly the effect of the protein and solvent degrees of freedom that were neglected

in the modeling. Two conceptually different approaches to deriving these effective interactions are available: (I) first principles approach and (II) statistical, or "knowledge-based" approach. The first principles approach attempts to mimic direct interactions among peptide residues, irrespective of the associated amino acid sequence. The most important physical interactions such as dispersion forces, hydrophobic effect, Coulomb interaction or hydrogen bonding have all been adapted, in a variety of forms, for simulations of minimal protein models. The second category of inter-particle interactions is derived on the basis of statistical mechanics. Correlation patterns among groups of amino acid residues are detected and analyzed using the existing experimental data base of protein structures. These statistical correlations are then converted into effective potentials assuming Boltzmann statistics. Perhaps the best known example of a statistical potential is the Miyazawa-Jernigan energy matrix (70), derived for all 20 naturally occurring amino acids. In the minimal protein model that I propose here, elements of both first-principle and statistical approaches will be utilized. The physical interactions that will be implemented in the model are hydrophobic forces, electrostatic force and hydrogen bond forces.

As with protein folding, the hydrophobic effect plays a central role in protein aggregation (71). Aggregates of various proteins were seen to disintegrate at low temperatures, where hydrophobic forces are significantly weakened (72). In the context of the Aβ peptides, the importance of the hydrophobic residues was demonstrated by Selkoe and colleagues (73). In order to correctly reproduce the tendency of non-polar residues located on different peptides to compound, hydrophobic forces need to be taken into account explicitly through direct inter-residue interaction. Despite being extremely successful in folding simulations, implicit modeling of hydrophobic forces, as implemented in Go-type potentials for example, will not be adequate for an aggregation project. Accordingly, I propose to use Lennard-Jones (LJ) potentials to model mutual attraction among hydrophobic residues. The strength of this potential will be scaled according to one of the hydrophobicity rankings available for amino acids (74). The second interaction type, the electrostatic potential, is believed to play a dual role in protein aggregation. On the one hand, the Coulomb force was seen to have a cumulative effect on a protein's propensity for aggregation. Strong negative correlations were observed between the total charge carried by a protein and the rate at which this protein can self-assemble (75). On the other hand, the specific location of charges along the sequence was suggested to fine-tune the final structure of fibrillar β-sheets through formation of salt bridges (76). Screened versions of electrostatic forces will be implemented in the proposed model in order to take into account the presence of the solvated counterions. Finally, the third interaction type, hydrogen bonding, is responsible for holding β sheets together. If the possibility of forming β sheets is to be realized in a minimalist model, then the hydrogen bonding potential has to be included. The form of this potential is not trivial for full-atom modeling, but for reduced models the situation is even more complicated as in these models explicit hydrogen bond donors and acceptors are missing. To mimic hydrogen bonding, I will attempt to construct a potential that will depend on mutual orientation of three-residue fragments, linked by two consecutive virtual bonds, and located far apart from each other along the amino acid sequence. The potential will be designed to energetically favor β sheet conformations.

The purpose of the statistical potentials proposed for this project is to compensate for the lack of structural detail in the model architecture through the use of potentials of mean force. In particular, local conformational preferences of four-residue segments will be accounted for using the distribution of dihedral angles obtained in the fully atomic simulations. These distributions will be subsequently converted into dihedral angle potentials and used for simulations of the minimalist model. The main idea behind the proposed interaction scheme is to balance long-range and short-range interactions. The long-range potentials, which are derived from physical principles, will shape the global structure of the model and govern inter-peptide interactions. The purpose of the short-range forces is to ensure that local conformational statistics are properly captured. These forces will depend on bond angles and dihedrals and will be most important for monomeric states. The relative strength of the local and non-local interactions will be adjusted so that the main structural ensembles observed for Aβ peptides in atomistic simulations are satisfactorily reproduced in the simulations of the minimal model. Free energy surfaces, defined as a function of two order parameters, will be used to fine-tune the minimal model.

In many aspects, the minimal protein model that I propose here is similar to models introduced earlier, such as UNRES developed by Scheraga and colleagues (77). A unique feature of my model is that it will be optimized to reproduce statistical properties of particular molecules, Aβ peptides. The idea is to develop a model that is appropriate for simulations of Aβ monomers and then apply it to simulations of the oligomerization process. To the best of my knowledge, none of the previous minimalist models were custom-tailored for a particular problem.

With the present power of computational resources, I anticipate that simulations of up to decamers of Aβ40-42 will be feasible when using the proposed minimalist model. The Langevin dynamics method that implicitly takes into account the solvent friction will be used to perform the simulations. Problems of immediate interest that I will seek to address in the simulations concern: (I) folding yields as function of external conditions, (II) the nature of oligomeric intermediate states that can be populated during aggregation, (III) propensity for aggregation. A particularly intriguing question is that of the structure of *paranuclei*, the earliest kinetic intermediates to fibril formation. These paranuclei consist of about 5-6 monomers. It will also be interesting to test if the subtle differences

observed in the oligomerization pathways of Aβ40 and Aβ42 by Bitan *et al.* (35) can be reproduced in the simulations. The nature and extent of conformational changes that happen to monomers upon incorporation into oligomers is another important issue that will be investigated.

Coarse-grained model for amyloids

In the previous sections I reviewed two computational approaches that can be applied today to study the protein aggregation problem. One of these approaches relied on an accurate, fully-atomic representation of proteins while the other one employed simplified protein representation in which every amino acid residue is modeled as an interacting bead. Questions that can be addressed using these approaches concern conformational statistics of proteins in their monomeric states and oligomerization pathways of small clusters of proteins. In *in vivo* fibrillation or *in vitro* experiments on protein aggregation, the numbers of participating proteins or peptides are fairly large, much larger than those that can be simulated using the two approaches I mentioned above. It is well known that large ensembles of interacting particles (protein molecules in the case of aggregation) possess distinctive properties, which are not shared by systems of finite sizes. These special, statistical properties are most manifestly revealed in the modeling of phenomena that involve changes of physical phases in which the modeled system can exist. For example, when modeling peptide aggregation, the equilibrium distribution of oligomeric states that are populated under given conditions is strongly influenced by the total number of molecules that are considered. According to the laws of thermodynamics, dependence on the number of particles N will disappear only in the limit when N tends to infinity. Consequently, to properly capture the statistical nature of protein aggregation in computer simulations, a large number of simulated particles need to be considered. To put this number into a context, I note that phase equilibria of neutral Lennard-Jones particles are typically investigated using hundreds of interacting particles. In order to reach comparable numbers in simulations of protein aggregation, the protein model needs to be further simplified. In this section I describe a coarse-grained model for simulations of aggregation that uses a single interacting particle to represent proteins.

A number of recently proposed (78–82). models addressing protein aggregation on the basis of statistical thermodynamics suffer from two main drawbacks: (I) an inadequate lattice description of configuration space (78) and (II)

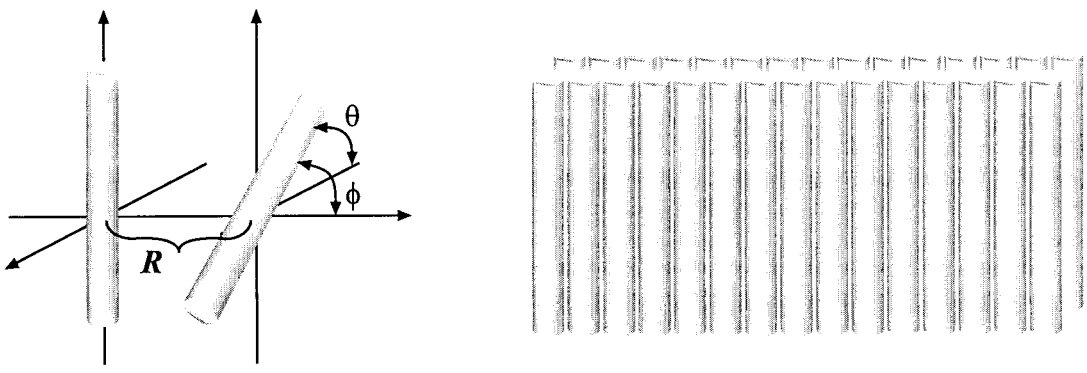

FIG. 5 A low-resolution, coarse-grained model proposed here for simulations of protein aggregation. Each protein is modeled as a spherocylinders. Proteins are assumed to interact among each other via an effective potential that depends on three parameters: the distance between centers of mass R, and two rotation angles ϕ and Θ. The parameters will be obtained through fitting to the interaction function of the more accurate, off-lattice model discussed in the previous section. It is anticipated that proteins will self-assemble into β-sheet structures in the aggregated phase.

the use of methods, perturbation theory (81) or density functional theory (79), that disallow directionally-dependent potentials to be employed. The model that I propose here will circumvent these difficulties. As the schematic in Fig. 5 shows, proteins will be represented as spherocylinders rather than spheres as in the previous models. This will allow the effects of the actual geometry of the protein conformations to be included. Spherocylindrical models have been extensively studied in the past to explain phase equilibria of colloidal dispersions. In particular, their ability to induce phase transitions that depend on orientational ordering, such as the smectic-nematic transition in liquid crystals, has thoroughly been investigated by a number of researchers, including Bolhuis *et al.* (83). Similar low-resolution models have recently been developed for other phenomena that involve proteins, such as membranes and protein membrane interactions (84).

In the proposed model, the interaction between a pair of proteins will depend on three parameters: the distance between the centers of mass of the cylinders R, and two rotation angles ϕ and Θ. The distance dependence will

have a form of an attractive interaction, built around either Lennard-Jones or Yukawa expressions. By design, the interparticle attraction will drive self-assembly of the proteins. The gain of potential energy due to the aggregation will be compensated for by the concurrent loss of translational and rotational entropies. The main characteristics of the aggregated phase will be governed by a subtle interplay of these two thermodynamic forces. The role of the angular dependence in the proposed interaction scheme is to enforce the formation of aggregated objects that resemble amyloid fibrils by shape. A prerequisite for such objects to appear is to choose the right symmetry of the interaction. For example, interaction types that are cylindrically symmetric will lead to the creation of layered structures with order present along one direction only (83). In order to induce planarity in the self-assembled aggregates, the cylindrical symmetry of the potential must be broken. This will be achieved by the introduction of potential terms that depend on angles ϕ and Θ. The exact form of these terms is not known. The angular potentials will provide energetic bias to planar orientations of the proteins and will be parametrized using the more accurate simulations of the off-lattice model I discussed in the previous section. Specifically, the most frequently populated monomeric states observed for the minimalist model will be chosen to serve as conformations that initiate aggregation. Potential energy surfaces for pairs of such conformations will be extracted as a function of ϕ and Θ. Analytical expressions that best fit the observed dependences will be derived and embedded into the interaction scheme of the coarse-grained model. This procedure may need to be repeated if more than one conformational state is required. Also, provisions can be made for conformational switching, by considering a mixture of particles of different geometries.

Monte Carlo simulations will be used to study phase equilibria of the proposed model. A question of immediate interest will concern the structural characterization of all possible phases in which the model protein can exist. In particular, what oligomers are present for the given set of conditions, and what is their size distribution? It will also be desirable to investigate if there are any metastable phases populated that may serve as kinetic intermediates to fibril formation. For the equilibrium phases, the roles of various physical interactions that contribute to the stability of coexisting phases will be investigated.

Conclusions

In this proposal I briefly explained how amyloid β (Aβ) peptides can be studied using computational means. Aβ peptides are directly linked to the onset of Alzheimer's disease, a serious neurological disorder. While it is not known what exactly causes the disease, a growing body of evidence points to large structured aggregates known as amyloid fibrils, and small, water-soluble oligomers as the main pathogenic species. A better understanding of the molecular structure of Aβ peptides, as well the mechanisms of their oligomerization, may help to develop efficient therapeutic strategies for the disease. Explaining general principles underlying fibril formation will also contribute to progress in technology. Recently, amyloid fibrils have emerged as a promising bio-material in certain technological areas, nanofabrication in particular.

I propose here three models for Aβ peptides that differ in their structural detail. The models are specifically tailored to target three different states in which Aβ peptides can be found: monomers, small oligomers and fibrils. Free energy landscape theory will be used to interpret the results of the simulations for all three states.

Full-atom simulations will be employed to explain the conformational statistics of a number of monomeric Aβ peptides. These will include cytotoxic alloforms Aβ40 and Aβ42. Using molecular dynamics simulations, free energy surfaces for these peptides will be obtained as a function of two reaction coordinates. One of these coordinates will measure the extent of solvent exposure of hydrophobic residues.

An off-lattice minimalist model for Aβ peptides that builds on the more accurate full-atom simulations will be developed. The model will use one interacting bead to represent each amino acid residue. The beads will interact via a variety of long-range and local potentials. The local interactions will be parametrized using dihedral angle distributions obtained in the fully atomic simulations. The balance between local and non-local interactions will be adjusted so that correct free energy surfaces are produced. The model will be used to simulate oligomerization of Aβ40 and Aβ42 peptides. Using the Langevin dynamics method, oligomers up to decamers will be studied.

Formation of fibrils will be viewed as a thermodynamic phase transition. In order to simulate a number of particles appropriately large for computer studies of phase transitions, a coarse-grained protein model will be constructed. The model will approximate interacting proteins as spherocylindrical particles. Potential energy functions for these particles will depend on both the distance between them as well their mutual orientations and will be extracted from the more detailed simulations of the minimalist model. Monte Carlo simulations will be carried out to study possible phases in which the model can exist. It is predicted that the aggregates formed by the particles will resemble the structure of fibrils.

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