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Review

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The Role of Molecular Simulations in the Development of Inhibitors of Amyloid β-Peptide Aggregation for the Treatment of Alzheimer's Disease

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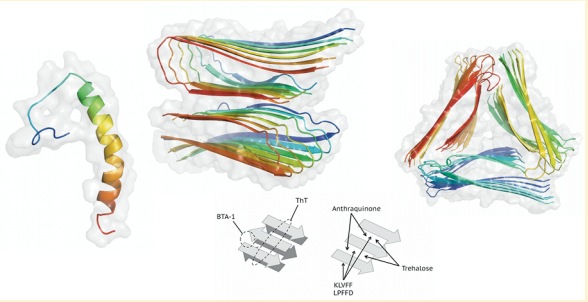
ABSTRACT: The pathogenic aggregation of the amyloid β-peptide (Aβ) is considered a hallmark of the progression of Alzheimer's disease, the leading cause of senile dementia in the elderly and one of the principal causes of death in the United States. In the absence of effective therapeutics, the incidence and economic burden associated with the disease are expected to rise dramatically in the coming decades. Targeting Aβ aggregation is an attractive therapeutic approach, though structural insights into the nature of Aβ aggregates from traditional experiments are elusive, making drug design difficult. Theoretical methods have been used for several years to augment experimental work and drive progress forward in Alzheimer's drug design. In this Review, we will describe how two common techniques, molecular docking and molecular dynamics simulations, are being applied in developing small molecules as effective therapeutics against monomeric, oligomeric, and fibrillated forms of Aβ. Recent successes and important limitations will be discussed, and we conclude by providing a perspective on the future of this field by citing recent examples of sophisticated approaches used to better characterize interactions of small molecules with Aβ and other amyloidogenic proteins.



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Alzheimer's disease is a debilitating neurodegenerative disorder afflicting millions of individuals worldwide. In the absence of effective drugs, the incidence of the disease is expected to rise rapidly over the coming years. In the United States alone, over 5.4 million people currently suffer from Alzheimer's disease, a figure that may triple by the year 2050.[1] The economic burden associated with Alzheimer's disease is staggering, with annual expenditures in the United States alone exceeding $183 billion.

The most widely accepted theory regarding the etiology of Alzheimer's disease is known as the "amyloid hypothesis," which features the amyloid β-peptide (Aβ) as the central pathological agent. Aβ is a short peptide, ranging in length from 38 to 43 residues, with its most common alloforms being 40 and 42 residues in length. The aggregation and deposition of Aβ in neural tissue is believed to be linked to the neuronal cell death and loss of cognitive function seen in patients with Alzheimer's disease.[2] Though the characteristic lesion of Alzheimer's disease is a fibrillated form of Aβ that gives rise to large plaques, the most toxic forms of Aβ are generally believed to be soluble oligomers.[3,4]

Current therapeutics largely target the breakdown of acetylcholine but are unable to halt the advancement of the disease.[5] Due to the central role of Aβ aggregation in the progression of Alzheimer's disease, inhibiting the aggregation cascade is an attractive approach for therapeutic development. Efforts have been made over many years to study different classes of small molecules and peptides that may interfere with the formation of higher-order Aβ species that may be neurotoxic.[6] A pioneering effort by Ghanta et al. in 1996 demonstrated that it was possible to use small peptides to interfere with Aβ aggregation.[7] These peptides had two sequence motifs, one designed to specifically bind Aβ based on the KVLFF sequence motif described by Tjernberg et al.[8] and another designed to interfere with β-strand formation. Subsequent work expanded upon these original findings.[9,10] In 2004, Gestewicki et al. detailed the design of nonpeptidyl bifunctional Aβ aggregation inhibitors that were capable of binding to both a protein chaperone and Aβ, thus inhibiting the self-association of Aβ.[11] The chemical moiety intended to target Aβ was designed to be similar to the dye known as Congo Red, which binds to Aβ fibrils. Though that study was successful in designing compounds that would bind Aβ, interfere with its aggregation, and reduce its neurotoxicity, no information was presented as to the residues through which the targeting molecules bound to Aβ, and the antiaggregation activity was attributed to the binding of chaperones rather than the small molecules themselves. Around the same time, Ono and co-workers produced a series of papers examining natural compounds that were potent inhibitors of Aβ aggregation.[12−17] In general, many of the compounds were

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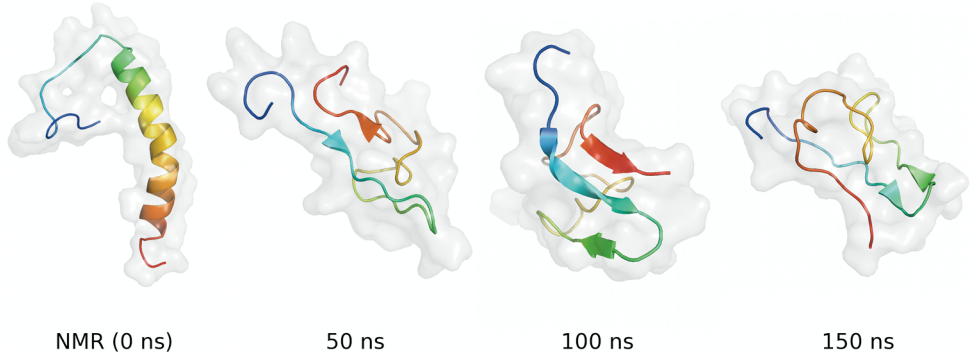

Figure 1. Structures of $A\beta_{40}$ along a 150 ns MD trajectory, indicating the large structural changes that are possible. The peptide is rendered as a cartoon to illustrate secondary structure, with an overlaid translucent surface to give an impression of the overall shape of the peptide. The peptide is colored as a rainbow gradient from N-terminus (blue) to C-terminus (red).

effective at inhibiting aggregation and protecting cultured cells at low micromolar concentrations, but no mechanistic evidence was presented to explain how these compounds were exerting their effects. Only several years later did structural insights emerge with respect to the residues on Aβ to which these compounds bind.[18]

Very recent work by Sinha and co-workers has described the identification of lysine and arginine residues as targets for anionic "molecular tweezers" that inhibit aggregation.[19,20] In contrast to this specific interaction, other compounds that show strong inhibitory interaction are poorly characterized by experimental methods.[19] Thus, despite some progress, mechanistic details have only slowly emerged using experimental techniques, and the specific chemical features of the small molecules responsible for antiaggregation activity remain largely uncharacterized.

Efficient development of new therapeutics for targeting Aβ aggregation requires detailed information about the mechanism of action of these compounds. Computational methods such as molecular docking and virtual screening are routinely used to evaluate compounds for their ability to bind to proteins with well-defined binding sites. Targeting Aβ aggregation presents a considerable challenge. That is, how can the ability of a compound to bind Aβ be assessed when the target peptide undergoes large and continual conformational changes? This type of information is difficult to gather using traditional experimental techniques and thus represents an area well suited to theoretical methods.[21] Information obtained from molecular dynamics (MD) simulations will likely accelerate the process of novel Alzheimer's drug development and has recently been successfully employed in designing Aβ aggregation inhibitors.[22] Here, we summarize and evaluate current progress in application of docking and MD studies and give perspective on the future of these techniques in the development of compounds that may inhibit Aβ aggregation and thus serve as potential therapeutic agents against Alzheimer's disease.

COMPUTATIONAL STRATEGIES FOR DRUG DESIGN

Docking. The ability to quickly screen libraries of compounds that may potentially act as drugs is extremely cost-effective and informative. Molecular docking derives its efficacy from the assumption that a small molecule (ligand) can associate with a predefined binding site of some macromolecule, and that the calculated energy of interaction is reflective of the affinity of the ligand for this binding site. Docking is thus composed of two main components, the generation of configurations of the ligand in the binding site and the energetic scoring of those configurations (poses) to determine their favorability. Docking requires high-quality structures of a receptor molecule, typically an enzyme or receptor protein to which the ligand will bind. The candidate drug molecule may be treated as flexible, while the receptor is predominantly rigid, though most modern algorithms allow for a subset of residues to be treated as flexible, as well. This treatment of the receptor structure thus requires that large structural changes do not occur upon ligand binding, which may not reflect reality. It is generally established that proteins exist as an ensemble of states to which small molecules can bind, and these binding events may have consequences for the ensemble of structures the receptor may sample, as reviewed elsewhere.[23−25] This rigidity of the binding site in docking studies is a limitation of current capabilities.

The requirements described above (high-quality receptor structure and minimal structural change upon ligand binding) account for the main reasons why docking studies are particularly challenging in the context of drug development for targeting Aβ aggregation. Very little is known about the structure(s) of soluble Aβ oligomers, which are generally regarded as the most toxic Aβ species.[3,4] Moreover, Aβ displays considerable structural heterogeneity in solution, making it difficult to choose a suitable structure or set of structures that could serve as a receptor for docking. The problem in the context of Aβ is even greater than in the case of well-folded proteins, since Aβ samples many structures along a very complex free energy surface.[26] Examples of configurations adopted by $A\beta_{40}$ in solution during the course of a single MD simulation are shown in Figure 1, illustrating the challenge of choosing configurations suitable for docking.

Molecular Dynamics Simulations. MD simulations provide atomic-resolution insights into molecular systems. By integrating Newton's laws of motion over time, the dynamics of the system can be probed in great detail. MD simulations have been applied to a vast number of biological problems and have provided valuable information in the study of protein dynamics, protein−ligand interactions, lipid membranes, and membrane proteins. Interested readers are directed to refs 27−29 for fundamental reviews on the topic of MD simulations of biomolecules. Given the difficulty in obtaining high-resolution structural information about Aβ and its aggregated states, MD simulations serve as an excellent tool for augmenting the current understanding of Aβ structure and dynamics.[30]

MD simulations require a starting configuration of all of the molecules to be studied and a corresponding topology that

describes the properties of each of the atoms. The dynamics are governed by potential energy functions that account for bonded interactions (bonds, angles, dihedrals, and planarity terms called "improper dihedrals") and nonbonded interactions (van der Waals interactions and electrostatics). Together, the atomic properties and potential energy functions are called a "force field." The choice of a force field for a given study is not a trivial exercise; rather, it constitutes a major choice that will have implications for the outcome and interpretation of the simulations. There are two classes of force fields, atomistic and coarse-grained, both of which have been applied to simulations of Aβ and related molecules. Atomistic force fields (both all-atom and united-atom) explicitly account for all atoms in the system. Popular force field families for biomolecules include AMBER,[31−36] CHARMM,[37−44] GROMOS,[45−49] and OPLS-AA.[50,51] Atomistic force fields are the most computationally demanding, but also provide the greatest level of detail about the system being studied. Coarse-grained force fields describe molecules in a more abstract way, such that functional groups are represented by so-called "coarse particles," thus reducing the number of degrees of freedom in the system in order to speed up calculations. Several coarse-grained force fields are widely used, including OPEP[52] and MARTINI,[53,54] with OPEP having been successfully applied to the study of a variety of Aβ systems.[55−58] Only recently has the MARTINI force field been adapted to be better suited to the study of amyloidogenic proteins,[59] though it has not yet been used in studies of Aβ dynamics.

This Review will begin with a discussion of studies utilizing docking to characterize interactions of antiaggregation compounds with Aβ. We follow with an analysis of studies that have utilized MD simulations as the primary technique. Though some studies utilize both docking and MD, studies that rely primarily on docking will be discussed first, with their MD results incorporated into the later discussion. We conclude by analyzing the current state of the field and provide an outlook for how investigators might apply docking and MD in more advantageous and sophisticated ways to improve our understanding of the molecular mechanisms of antiaggregation compounds.

MODELING Aβ AGGREGATION INHIBITORS

Molecular Docking. Efforts have been made to use molecular docking to characterize the interactions of small molecules with Aβ monomers. Teper et al. conducted a docking study of hydroxycholesterol derivatives, finding that different compounds could bind to large regions of the Aβ surface, encompassing nearly half the sequence.[60] Recently, Braymer et al. analyzed the binding of stilbene derivatives to Aβ monomers, finding that these compounds could bind to polar N-terminal residues that are believed to bind metal ions and contribute to neurotoxicity.[61]

In a similar study, Wang et al. docked a xanthone derivative to Aβ and found that it stabilized the α-helical conformation of the peptide during a short (20 ns) MD simulation.[62] Using a similar approach, Yang et al. docked a pentapeptide (LPFFD) to Aβ and analyzed the conformations adopted over the course of a short (30 ns) MD simulation, with the initial α-helical character of Aβ being retained as a result of LPFFD binding.[63] Liu et al. used MD prior to docking to generate a conformation that may be more representative of solution conditions.[64] The compound examined in that work bound to a large portion of the Aβ surface, but did not inhibit β-strand formation. The authors concluded that likely its inhibitory mechanism involved interfering with interpeptide hydrogen bonding. Viet et al.[65] also used a combined docking/MD approach to evaluate the binding energy of KVLFF and LPFFD peptides (the latter of which is known as a "β-sheet breaker") on monomeric Aβ_{40}, finding that LPFFD did not decrease total β-strand content, but it did prevent the formation of β-strands in aggregation-prone regions. Further, LPFFD inhibited the transition of α-helix to random coil structures. All of these studies represent first efforts in understanding the interactions of small molecules and peptides with monomeric Aβ.

Inhibiting Aβ aggregation at the level of the monomer is an attractive therapeutic approach, as it represents the earliest opportunity to interfere with the aggregation cascade, but the above-mentioned studies all suffer from several limitations. The first major challenge in these studies is the choice of starting structures for Aβ. The NMR structures that were used were obtained in nonaqueous solvents. It is not clear that the structure of Aβ in water bears any resemblance to any of the NMR structures, and it is widely accepted that Aβ populates many different conformations in water, as has been demonstrated experimentally[66−70] and through the use of simulations.[26,55,71−77] The next problem that has not been adequately addressed is sufficient MD sampling. The simulations that were conducted by Wang et al.[62] and Yang et al.[63] were very short (20 and 30 ns, respectively), and no replicates (starting from the same configuration and different velocities, or alternatively different starting configurations) were performed. Liu et al.[64] employed a simulation of 50 ns in length, which is still relatively short, and the structure obtained was very similar to the starting model. To more thoroughly describe the dynamics of Aβ in aqueous solvent, considerably longer simulations are generally required for exhaustive sampling[77] and additional simulations would improve statistical reliability and allow for a more thorough evaluation of convergence within and across simulations.

Additional docking studies have been performed on models of the Aβ fibril. Numerous structural studies have been performed on fibrils formed by both Aβ_{40}[78,79] and Aβ_{42}.[80] Results have shown that fibril architecture is dependent upon the conditions used to grow them, as well as the principal peptide (Aβ_{40} or Aβ_{42}). Thus, though more extensively characterized than the monomeric form of Aβ, fibrils represent a challenge for molecular docking, as well. To date, the only docking studies reported in the literature that have been conducted on Aβ fibrils have used Aβ_{40} models.[81−83]

Chen et al. employed docking of several β-sheet breaker peptides to design molecules that successfully inhibited Aβ aggregation.[81] Their study predicted two possible binding sites for the model peptides on the Aβ_{40} fibril with 2-fold symmetry,[78] one toward the polar N-terminal residues and the other at the interface of the two layers of the fibril. The focus of the study was on the β-sheet breaker peptide LPFFD and other compounds with greater calculated binding affinity. Recently, Viet et al.[65] employed a similar strategy by docking KVLFF and LPFFD peptides to the same fibril structure, as well as another model of the Aβ_{40} fibril that has 3-fold symmetry.[79] The results obtained in this docking study were different from those obtained by Chen et al. Specifically, Viet et al. found that LPFFD bound consistently in the bend region (D_{23}VGSNKGAI_{31}) of the 2-fold symmetric Aβ_{40} fibril, with only a few contacts occurring at the interface between the two layers. The different results may be attributed to the use of different docking algorithms. Chen et al. utilized AutoDock,[84] while Viet et al. used AutoDock Vina[85] due to its greater speed. AutoDock Vina has been found to produce

clusters of ligand poses with lower root-mean-square deviation (rmsd), indicating it produces very similar poses.[85]

The study by Viet et al. also illustrated the importance of choosing a biologically relevant model for Aβ fibril docking studies. In addition to the model described above, they also analyzed the docking of KVLFF and LPFFD to the Aβ_{40} fibril with 3-fold symmetry, finding differences in the binding site depending on the model used. They also found that subtle differences in the binding modes of the short peptides emerged when the size of the receptor fibril was increased. For KLVFF, increasing the size of the 2-fold symmetric Aβ_{40} fibril (from 6 to 12 peptides) led to more consistent clustering in the bend region of the Aβ_{40} peptide, while for LPFFD the results were consistent between the smaller and larger fibrils. For the 3-fold symmetric Aβ_{40} fibril, use of the smaller model fibril (9 peptides) caused both KLVFF and LPFFD to bind at the interfaces between peptide layers, while increasing the fibril size (to 18 peptides) led to both peptides binding through the core of the fibril structure, along the long axis of the structure. Despite the differences in positioning, the authors were able to consistently demonstrate that LPFFD bound with greater affinity to the fibril models through hydrogen bonding and side chain contacts, but the observed differences underscore the importance of choosing a biologically relevant model for docking studies, as the structural characteristics of binding interactions may be quite different.

Work by Yang et al. on flavones concluded that these molecules bound within the fibril core, between the N- and C-terminal β-strands, associating with Phe19 residues (numbered as Phe11 in that study due to the missing 8 residues at the N-terminus of each peptide chain) via π-stacking and with polar residues via hydrogen bonding.[82] The authors found a good correlation between experimental binding affinities and the calculated binding free energies, leading them to speculate that the predicted binding sites were a good model to explain the experimental findings, though it is known that enthalpic factors alone do not necessarily produce accurate binding energies in docking studies.[86] In the final example of docking small molecules to Aβ fibrils, Keshet et al. docked a small library of structurally distinct molecules to 20 different Aβ_{40} fibril models. The authors concluded that Congo Red (a dye molecule), myricetin (a flavonoid), and melatonin (a neurotransmitter) shared common binding sites on the fibril. These sites were (i) the bend connecting the two β-strands in each layer (A_{21}EDVGSNKGAII$_{32}$) of the fibril (inside the turn and on its solvent-exposed surface) and (ii) the hydrophobic C-terminus, particularly residues Val39 and Val40.[83] The results from molecular docking thus indicate that many binding poses may be possible on any given structure and that binding affinity calculations should be interpreted with care.

Figure 2 summarizes the binding sites of various compounds on the Aβ_{40} fibril from the docking studies discussed.

Molecular Dynamics Simulations of Aβ Fragments. MD simulations provide a molecular-level view of a system as it evolves over time, making them particularly useful in studying Aβ and its interactions with proteins, membranes, and small molecules. Since Aβ adopts a variety of structures along the aggregation pathway,[87] MD simulations can be used to examine how these different structures may interact with small molecules that may serve as therapeutic antiaggregation compounds. To date, numerous MD simulation studies have been conducted on full-length Aβ in water[26,55,71−75,88] and in association with membranes[89−98] that have elucidated useful details regarding its structure and potential deleterious effects on membrane environments, which may contribute to cytotoxicity.[3,4]

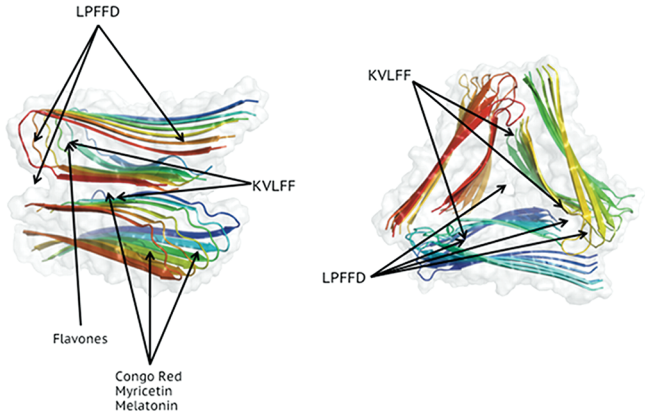

Figure 2. Binding sites of small molecules and peptides from docking to two different Aβ_{40} fibrils. Individual peptide layers are colored as a rainbow gradient along each chain.

Building on these studies, a number of investigators have used MD simulations to examine the interactions of Aβ with small molecules, potentially providing mechanistic information that can aid in drug design. Convertino et al. were among the first to use MD simulations in this respect, examining the interactions of 9,10-anthraquinone and anthracene with a fragment of Aβ encompassing the sequence H_{14}QKLVFF$_{20}$.[99] Using Aβ_{14-20} trimers as a model Aβ aggregate, they determined that 9,10-anthraquinone could compete for backbone hydrogen bonds, resulting in what they authors termed a "butter-knife" mechanism for separating the β-strands. Anthracene, lacking carbonyl moieties, did not exhibit this effect. The hydrogen bonding interactions of 9,10-anthraquinone with Aβ_{14-20} were augmented by $\pi^+\delta^-$ interactions between the aromatic rings of 9,10-anthraquinone and carbonyl oxygens of the peptide backbone. In the case of anthracene, only hydrophobic contacts were formed with the peptide, which were insufficient to destabilize the aggregate structure.

Expanding on their previous work, Convertino et al. more recently conducted simulations to explore the binding of 9,10-anthraquinone, anthracene, peptides, and several peptide derivatives to the Aβ fragment encompassing V_{12}HHQKLVFF-AEDVGSNK$_{28}$.[100] Control simulations showed the peptide fragment to be largely unstructured in solution. Among the compounds tested, the most frequent interactions involved residues H_{13}HQKLVFF$_{20}$, with the Phe dyad being involved in the most intermolecular contacts. Many of the compounds assessed in that work contained aromatic moieties, and thus the association with Phe was expected. Overall, the authors found that a variety of compounds could induce somewhat generic effects with respect to Aβ_{12-28} structure. Despite the lack of a prevailing binding mode for any of the compounds examined, subtle changes in the peptide structure could be induced through allosteric effects. That is, contacts between polar residues in the Aβ_{12-28} sequence were perturbed upon small molecule binding, even if the molecule was not directly bound to the affected residues. These findings were attributed to entropic terms arising from the intrinsic disorder and flexibility of the Aβ_{12-28} sequence and thus illustrate an important contribution of MD simulations to understanding small molecule binding to Aβ.

Similar studies have been carried out by Liu et al., who have analyzed the binding of the sugar trehalose to the Aβ_{16-22} fragment and full-length Aβ_{40}.[101] The authors found that trehalose destabilized the β-sheet structure adopted by Aβ_{16-22}, concomitant

with a change in the hydration of the aggregates which gave rise to both direct and water-mediated hydrogen bonding of trehalose to Aβ_{16-22}. These hydrogen bonding interactions destabilized existing aggregates and inhibited association of any additional Aβ_{16-22} peptides with the aggregate, though a large molar excess of trehalose was required to produce this phenomenon. Viet et al. examined the ability of KVLFF and LPFFD peptides to inhibit Aβ_{16-22} aggregation at substoichiometric amounts, conducting simulations of two Aβ_{16-22} peptides with one molecule of each pentapeptide.[65] They concluded that LPFFD was more effective at inhibiting Aβ_{16-22} aggregation, as it increased the time required for the Aβ_{16-22} peptides to align, and it bound more strongly than did KVLFF, an effect the authors attributed to the greater hydrophobicity of LPFFD.

The Aβ_{16-22} fragment also served as a useful model for Wu et al., who studied the binding of the fluorescent dye thioflavin T (ThT) and its neutral analogue BTA-1 to a double-layer protofibril of Aβ_{16-22}.[102] Their results showed two principal binding modes for both ThT and BTA-1 on the model protofibril, in "grooves" on the protofibril surface and on the ends of the protofibril itself. The surface grooves arise from repetition in the structure along the protofibril axis; repeated appearance of the same sequence leads to generic binding pockets that may be present in all amyloidogenic sequences, and thus, Wu et al. proposed a rationale for why dye molecules bind to many different amyloid structures. The binding of ThT and BTA-1 was found to be principally due to hydrophobic interactions, which were augmented in BTA-1, which bears no net charge, relative to ThT, which is cationic. The differences in these structures led to slightly different binding modes, with ThT preferring the so-called "central groove" (flanked by Phe19 in two strands of neighboring peptides in the upper sheet layer), while BTA-1 preferred the so-called "side groove" near the ends of the β-sheets, flanked by Lys16/Val18 on one strand and Phe20/Glu22 in the neighboring strand. These results shed light on experimental observations that there may be multiple sites to which these molecules can bind, each with different affinity. Though Aβ_{16-22} assembles in an antiparallel β-sheet structure, and full-length Aβ fibrils are composed of parallel β-sheets, Wu et al. proposed that the binding of dye molecules is independent of strand orientation, and that the generic repetition of structure in amyloid fibrils is what allows such molecules to bind. Indeed, such a pose was also observed in the docking study by Keshet et al. discussed earlier in the context of Congo Red[83] and a recent MD study by Hochdörffer et al., who studied the interactions of a variety of compounds with Aβ_{42} protofibrils.[103]

The Aβ_{16-22} fragment has also served as a useful model for N-methylated peptides. These peptides have been characterized experimentally[104−107] and are shown to inhibit Aβ aggregation by competing for backbone hydrogen bonding. Simulations of such systems have been carried out recently by Chebaro and Derreumaux[108] and Soto et al.[109] Both of these studies indicate that N-methylated peptides manifest complex interactions with Aβ_{16-22} peptide fragments, binding to (i) the ends of peptide layers to inhibit elongation, (ii) the surface of peptide layers to prevent stacking, and (iii) between peptides (intercalation) that destabilize Aβ_{16-22} assembly. These complex binding modes may explain the ability of N-methylated peptides to inhibit aggregation and/or promote disassembly or lock Aβ aggregates in conformations that do not lead to higher-order neurotoxic assemblies.

Binding poses of small molecules and peptides for the Aβ fragments discussed here are shown in Figure 3.

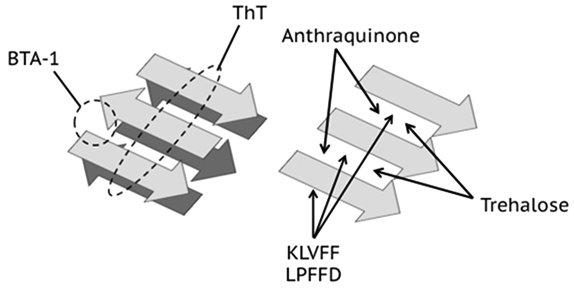

Figure 3. Representative binding sites of small molecules and peptides in antiparallel double-layer β-sheet protofibrils (left) modeled by Wu et al.[102] and model parallel β-strands (right) similar to those considered by Convertino et al.[99] The parallel β-sheet structure is also used to illustrate the approximate binding sites of the indicated molecules to antiparallel β-sheet models considered by Viet et al.[65] and Liu et al.[101]

Molecular Dynamics Simulations of Full-Length Aβ. Liu et al. also conducted simulations of the polyphenol (−)-epigallocatechin-3-gallate (EGCG) binding to Aβ_{42}, revealing 12 residues to which EGCG principally bound, with molecular mechanics−Poisson−Boltzmann surface area (MM-PBSA) analysis revealing that hydrophobic interactions accounted for the driving force for EGCG association with Aβ.[110] Polar interactions such as hydrogen bonding played only a minor role in this process. In those simulations, a high concentration of EGCG (10:1 EGCG:Aβ) was capable of preventing the emergence of any β-strand content in the peptide, a behavior that presumably inhibits aggregation. This behavior was similar to that of trehalose described above, in that exclusion of water from the surface of Aβ and the resulting interactions between EGCG and Aβ were responsible for the inhibition of structural change. Further, the affinity of EGCG for many residues in the Aβ sequence could explain its strong inhibitory effect toward aggregation and also the difficulty in assigning specific interactions to which this phenomenon can be attributed, as described by Sinha et al.[19]

Other studies have been conducted on larger Aβ aggregates, such as protofibrils and fibrils, on which more extensive structural analysis has been conducted experimentally via NMR spectroscopy. Though these models often lack several N-terminal residues that are assumed to be disordered in solution, these simulations will be discussed here in the context of full-length Aβ peptides, as they are far more complete than the model peptides described in the previous section.

A series of implicit solvent simulations has been conducted by Raman et al. and Takeda et al. describing the interactions of naproxen and ibuprofen with Aβ fibrils.[111−113] These studies employed an enhanced sampling technique known as replica exchange molecular dynamics (REMD), in which multiple simulations are conducted at different temperatures, with configurations periodically switched between temperatures to overcome energy barriers and improve sampling. In addition, an implicit solvent model was used to speed up calculations. In the first study on ibuprofen,[111] Raman et al. determined that ibuprofen bound in clusters to the concave edge of the Aβ_{10-40} fibril with a far greater probability than at the convex edge; see Figure 1 in ref 111. It is at the concave edge that new peptides would attach in the unidirectional growth model of the Aβ fibril. Ibuprofen formed clusters within the groove of the concave edge, precluding the attachment of additional Aβ peptides, a hypothesis confirmed by Chang et al.[114] The authors further observed that interactions between ibuprofen and the peptide side chains

were principally responsible for the ibuprofen−Aβ interaction, with few contacts formed involving Aβ backbone groups. Takeda et al. performed very similar simulations with naproxen,[112] finding that, like ibuprofen, naproxen bound to the concave edge of the $Aβ_{10–40}$ fibril in order to preclude the attachment of other Aβ peptides. Additionally, naproxen altered the conformational ensemble of monomeric Aβ to promote the emergence of β-strand structures, an effect that was not observed in the case of ibuprofen. The observation regarding the structural changes imparted by naproxen binding explained the experimental quandary that naproxen has a stronger affinity for Aβ than ibuprofen, but it is a less effective antiaggregation compound. These MD simulations revealed that ibuprofen bound only weakly to polar N-terminal residues of the $Aβ_{40}$ monomer but bound strongly to the $Aβ_{10–40}$ fibril to inhibit peptide attachment,[111] but naproxen promoted aggregation on the monomer level and inhibited aggregation on the fibril level[112] by binding strongly to both forms of the peptide. Takeda et al. further expanded upon these ideas with a detailed analysis of the structural features of naproxen and ibuprofen that contribute to their binding to the Aβ fibril.[113] They found that the naphthalene ring system of naproxen was particularly important for fibril binding, as it was important for ligand−ligand interactions that stabilized the clusters formed at the concave edge of the Aβ fibril.

We conclude the discussion on MD studies of small molecule-Aβ binding by describing our own efforts in this field. We conducted simulations of a flavonoid, morin, binding to a model of the $Aβ_{42}$ protofibril,[115] finding that morin bound to the ends of the protofibril and the C-terminal hydrophobic β-strands. These observations were consistent with the other studies described here, particularly the findings of Keshet et al. with respect to the dual binding modes of the flavonoid, myricetin, considered in that study.[83] Thus, despite the fact that we considered $Aβ_{42}$ and Keshet et al. used $Aβ_{40}$ as their model, there are underlying structural features that are common to both and allow for binding of flavonoids. We note that there is one important difference between our MD simulations and the docking study of Keshet et al. Morin molecules that were steered into the protofibril interior were not stable in that location, as was the case for myricetin in the docking study by Keshet et al. Instead, these morin molecules moved to the periphery of the protofibril structure, causing destabilization of backbone hydrogen bonding while still interacting with the Asp23-Lys28 salt bridge region of the $Aβ_{42}$ structure. Possible explanations for the observed differences are (i) subtle differences between $Aβ_{40}$ and $Aβ_{42}$ with respect to their ability to bind flavonoids, (ii) small differences in the chemical structure of morin and myricetin that give rise to this phenomenon, or (iii) MD simulations provide a better model of the dynamic interactions that occur when flavonoids bind to Aβ and induce structural change. Further studies using consistent methodology and techniques would be required to determine the exact reasons for these differences. We have also more recently examined the binding of morin to monomers and dimers of full-length $Aβ_{40}$ and $Aβ_{42}$.[116] Our results indicated that morin can alter the tertiary contacts within Aβ monomers and is capable of interfering with the formation of hydrophobic contacts, thus inhibiting the collapse of the monomeric structure into a compact conformation. We further concluded that preformed Aβ dimers were largely resistant to morin treatment, and thus aggregation is very difficult to reverse. However, morin was capable of modulating quaternary structure of dimers that were formed in its presence, and that these effects were dependent upon binding location. Binding of morin at the dimerization interface led to the greatest reduction in interpeptide contacts, while surface binding of morin did not manifest significant quaternary effects.

As a final note, it is important to consider the representation of solvent in MD simulations. Previous theoretical[117,118] and experimental[119] studies have suggested that hydration of amyloid aggregates has an important role in structural stability. An explicit representation of water typically increases the number of atoms in a system by several orders of magnitude. Implicit solvent models utilize a potential function to mimic the effects of solvation. The latter approach was used in studies by Convertino et al.,[99,100] Raman et al.,[111] Takeda et al.,[112,113] and Chang et al.[114] Explicit solvent representation was used in the studies by Liu et al.,[101,110] Wu et al.,[102] Viet et al.,[65] and in our own work.[115] Our previous work[120] demonstrated the importance of water to the stability of the Asp23-Lys28 salt bridge, and ultimately the entire $Aβ_{42}$ protofibril structure. Our subsequent MD simulations[115] indicated that morin could disrupt this hydrogen bonding network and other important hydrophobic packing interactions that stabilize the bend region (A_{21}EDVGSNKGAIIGL$_{34}$) of the protofibril. When morin interacted with these residues, excess water molecules penetrated the protofibril core, competed for native hydrogen bonding, and ultimately destabilized peptide−peptide interactions. Such phenomena are not observed in implicit solvent simulations. Though less computationally demanding, it remains to be seen what interactions may be missing when using an implicit solvent representation. In addition, Liu et al. found an important role in hydration/dehydration in the binding of trehalose and EGCG to Aβ.[101,110] These results suggest that solvent representation is an important consideration in these simulations and that perhaps water-mediated interactions need to be explicitly considered to gain rigorous insight into interactions of some small molecules with Aβ.

CURRENT LIMITATIONS

Docking. The main limitation in using docking to identify binding modes of antiaggregation compounds against Aβ is the rigidity of the receptor structure. If an antiaggregation compound alters the structure of Aβ, such changes cannot be reflected during docking, and thus a pose is identified largely based on rigid interactions. Even if a large number of putative receptor structures are assigned to Aβ due to its inherent structural polymorphism, binding poses will only be identified and scored under the assumption that this receptor structure is unchanged upon binding, which may not necessarily be true.

Another challenge associated with docking is the scoring and refinement of docked poses. This particular hurdle is not unique to the study of Aβ; rather it affects all docking studies, even those involving well-characterized receptors.[121−123] The scoring of docked poses relies on computing energetic terms derived from the interactions between the ligand and receptor, including van der Waals and Coulombic interactions, as well as shape complementarity with the binding site and molecular mechanics terms related to the strain of the ligand while occupying the docked pose. Most common scoring functions make approximations for desolvation and entropic terms, which are among the principal deficiencies in scoring methodology.[121] In the context of Aβ-ligand interactions, these terms may be very important. Entropic effects were implicated in small molecule binding to the $Aβ_{12–28}$ fragment studied by Convertino et al.,[100] and rotational entropy terms may be particularly important for highly flexible ligands such as β-sheet breaker peptides that have been used in other studies.[65,81] In addition, desolvation terms may play a very important role in determining binding affinity of

small molecules and peptides for Aβ. Since Aβ lacks a well-defined binding site and the peptide has variable solvent exposure depending upon its conformation and aggregation state, ligands may bind to solvent-excluded or solvent-exposed regions on the peptide. In the absence of accurate metrics for desolvation effects, scoring these poses will remain challenging.

Molecular Dynamics Simulations. Though MD simulations provide the greatest level of detail in considering Aβ−ligand interactions, particularly with respect to the persistence of interactions over time and the response of the Aβ structure to the presence of the ligand, considerable computational resources are required to produce this information. Even with parallelized and highly optimized MD codes, generating a trajectory of sufficient length (hundreds of nanoseconds) may require weeks or even months of real time and a large number of processors on traditional CPU hardware. It is important to note that recent advancements in customized CPU[124,125] and GPU[126] hardware may propel progress in this field by allowing for far more efficient data collection that allows for extensive trajectories. These advances allow for more detailed analysis of protein conformational sampling and protein-small molecule interactions.

Two common techniques for reducing the time required for MD simulations include implicit solvent representation and coarse-grained force fields for describing solute molecules. Implicit solvent representation is an attractive approach, as water molecules may account for 75−90% of the atoms in the simulation system. Though the solvent is often viewed as a passive entity in most simulations, several studies have shown that water-mediated interactions may play a significant role in Aβ aggregate stability[120] and the interactions of Aβ with small molecules.[101,110] Several other studies summarized here employed implicit solvent representation,[99,100,111−114] though it is unclear what, if any, effects can be attributed to the absence of explicit water molecules. The other technique used to reduce the degrees of freedom in the system is the application of coarse-grained force fields, which have been utilized in several studies of Aβ dynamics.[55−58] Though these force fields effectively model Aβ interactions, their extension to small molecules of arbitrary size and structure is not straightforward. In addition, representing groups of atoms as coarse particles reduces the level of detail provided by the simulation, though reverse transformation algorithms exist.[127] To our knowledge, no simulation studies exist that have examined Aβ-small molecule interactions using coarse-grained force fields, likely due to the difficulty in analyzing the results and generating ligand parameters.

The difficulty in generating high-quality topologies for small molecules is not a problem limited to coarse-grained force fields. The topologies must be derived in a manner consistent with the parent force field, most of which only consist of parameters for biomolecules such as proteins, nucleic acids, and lipids. For studies involving peptides as the antiaggregation agent, the task of topology generation is simple, since protein parameters are easily applied. For other molecules, even common organic functional groups are often difficult to parametrize in a manner compatible with the parent force field. These efforts have been aided by the development of generalized parameter sets compatible with the AMBER[128] and CHARMM[129] force fields. Several Web servers also exist for generating parameters compatible with the GROMOS family of force fields,[130−132] though we encourage all investigators to validate all parameters thoroughly, as some results obtained from such services are not reliable.[133] Parametrization of molecules for use with other force fields often requires careful quantum mechanical optimizations and charge calculations followed by empirical fitting of parameters to reproduce known behavior. These efforts can be very time-consuming, hindering the speed at which studies are conducted and completed.

CONCLUSION AND FUTURE PERSPECTIVES

Over the past few years, a growing number of theoretical studies have been conducted to analyze the interactions of Aβ with antiaggregation molecules. These early efforts have shown several strengths and limitations of current techniques. To solve the considerable challenge of using computational methods to design and study compounds that inhibit Aβ aggregation, it appears that combining docking and MD provides the most efficient and informative means of assessment of candidate molecules (Figure 4). The limitations of docking (rigidity and sampling) are complemented by MD, while the limitations of MD (computational expense) are complemented by docking. Long-time MD simulations of Aβ can be utilized to produce a heterogeneous ensemble of Aβ structures, a fact that is true for all levels of Aβ aggregation, from monomers and dimers to higher-order structures such as oligomers and fibrils. Docking can be conducted using multiple structurally distinct targets from these ensembles, and MD and other techniques can be applied again to analyze the stability of the docking poses and the persistence of various interactions that appear consequential in the docking scoring algorithm.

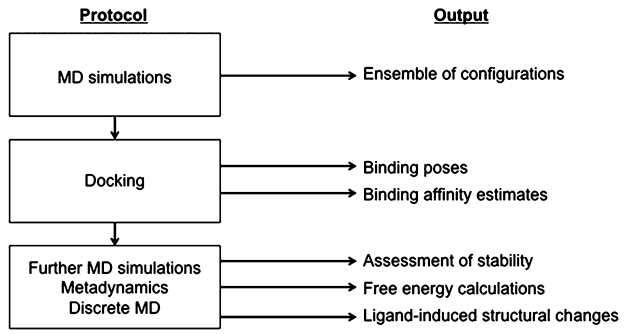

Figure 4. Schematic illustrating the advantages of MD simulations and docking and how they can be advantageously combined.

Such a combined approach was recently used to great effect by Kranjc et al. in the context of PrP.[134] In addition to canonical MD and docking procedures, the authors also employed metadynamics simulations, which allowed for a detailed analysis of many factors involved in ligand binding and stability as well as a reliable quantitation of the free energy associated with these interactions. Such procedures allowed the authors to overcome inherent challenges such as structural changes in the receptor and the possibility of multiple ligand binding sites to the PrP surface. A different hierarchical approach involving coarse grained REMD, followed by docking and atomistic MD simulations was recently used by Chebaro et al. to explore the interactions of $A\beta_{17-42}$ trimers.[135] The CG-REMD simulations allowed for efficient sampling of $A\beta_{17-42}$ trimer conformations, while the atomistic simulations provided detailed insights into the interactions of several molecules with the $A\beta_{17-42}$ trimers. These studies are models for methods that may be useful in the assessment of Aβ interactions with small molecules.

A final simulation technique that may be useful in evaluating Aβ-small molecule interactions is a technique known as discrete

molecular dynamics (DMD), which is computationally more efficient than traditional MD through the use of discretized potential energy functions. Recently, Proctor et al. utilized DMD to complement traditional static docking in identifying native binding poses in challenging protein targets.[136] They concluded that entropic contributions, particularly coupled ligand−protein dynamics, were particularly significant in determining native binding poses and residency time within the binding site. Such dynamic and entropic factors are absent in traditional docking. Despite the fact that the DMD method of Proctor et al. had difficulty in establishing native binding poses for flexible proteins such as kinases, this rapid sampling method presents an additional technique that can be used to identify Aβ-small molecule interactions and explore structural changes more efficiently than through the use of traditional MD. DMD simulations have frequently been combined with coarse grained models of Aβ to achieve greatly enhanced sampling,[137−139] and thus, the extension of these techniques to Aβ-small molecule interactions remains a viable avenue for future work.

From a review of the current literature, it is clear that, despite significant progress, a comprehensive study of Aβ−small molecule interactions is lacking. Traditional MD simulations must include trajectories of sufficient length that are evaluated for convergence, and multiple simulations are strongly recommended for statistical reliability. These criteria have not been met in some of the studies reviewed here and represent areas of improvement for all investigators to consider. Future investigations on this topic should carefully make use of the features of docking, traditional MD, and enhanced sampling techniques such as metadynamics, DMD, and REMD. Such a combined approach, employing a diverse set of Aβ structures as targets for docking and MD analysis, will likely shed light on interactions that can be exploited for the development of effective antiaggregation compounds.

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Notes

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