Published in final edited form as:

Org Biomol Chem. 2017 October 04; 15(38): 7993–8005. doi:10.1039/c7ob01290j.

Molecular Simulations of Peptide Amphiphiles

Anjela Manandhar^{a,b}, Myungshim Kang^a, Kaushik Chakraborty^a, Phu K. Tang^{a,b}, and Sharon M. Loverde^{a,b}

^aDepartment of Chemistry, College of Staten Island, City University of New York, Staten Island, NY 10314, USA

^bPh.D. Program in Biochemistry, The Graduate Center of the City University of New York, New York, NY 10016, USA

Abstract

This review describes recent progress in the area of molecular simulations of peptide assemblies, including peptide-amphiphiles, and drug-amphiphiles. The ability to predict the structure and stability of peptide self-assemblies from the molecular level up is vital to the field of nanobiotechnology. Computational methods such as molecular dynamics offer the opportunity to characterize intermolecular forces between peptide-amphiphiles that are critical to the self-assembly process. Furthermore, these computational methods provide the ability to computationally probe the structure of these supramolecular assemblies at the molecular level, which is a challenge experimentally. Herein, we briefly highlight progress in the areas of all-atomistic and coarse-grained simulation studies investigating the self-assembly process of short peptides and peptide amphiphiles. We also discuss recent all-atomistic and coarse-grained simulations of the self-assembly of a drug-amphiphile into elongated filaments. Next, we discuss how these computational methods can provide further insight on the pathway of cylindrical nanofiber formation and predict their biocompatibility by studying the interaction of these peptide-amphiphile nanostructures with model cell membranes.

Graphical abstract

Introduction

An increasing number of researchers are utilizing the dynamic and hierarchical self-assembly properties of proteins and peptides to design novel biofunctional nanostructures.¹ For example, *Zhang et al.*² was amongst the first few groups to show the self-assembly of a short designed peptide; they designed a sixteen-residue peptide with alternating positive and negative amino acids that self-assembles into nanofibers in aqueous solution. With natural amino acids, one can design numerous sequences of engineered polypeptides. Due to such high versatility, there have been multitudes of experimental studies with peptides of differing lengths/sequences that self-assemble into nanostructures such as nanofibers, bilayers and nanovesicles.¹ These nanostructures have potential use as drug carriers,³ nanostructures for brain damage repair,⁴ stabilized photosynthetic complexes,⁵ as well as food and cosmetic additives⁶. Computational simulations offer an emerging tool to characterize the structure and properties of peptide-based supramolecular assemblies.^{1,7,8} In this review, we focus on computational simulations of the self-assembly of amphiphilic peptides, including the self-assembly of peptide-amphiphiles into filamentous nanofibers.

With increasing developments in the field of nanobiotechnology, it has become easier to design, synthesize, and characterize a variety of peptide sequences and tune their directed self-assembly. For example, *Hartgerink et al.* was the first to design and characterize the self-assembly of a peptide-amphiphile, a peptide sequence with additional hydrophobic tail, into cylindrical fibers.^{9,10} This class of materials encompassing these peptide sequences with additional hydrophobic entities has been termed peptide-amphiphiles (PAs). Experimentally these PAs have been found to self-assemble into nanostructures ranging from spherical micelles to vesicles, bilayers, nanofibers, nanotubes, as well as nanoribbons.^{11–15} In particular, cylindrical nanofibers have gained recognition for their biomedical applications such as nerve regeneration,¹⁶ wound healing,¹⁷ cartilage regeneration,¹⁸ as well as drug delivery devices¹⁹. In the last decade, an increasing number of computational groups have provided major insights into the structure and self-assembly of PA nanofibers as shown in Table 1 and in discussed within the context of this review.^{8,20–33}

The thermodynamic stability of nanostructures formed due to the assembly of peptide-based amphiphiles is dependent on a delicate balance of intermolecular forces such as hydrophobic interactions, hydrogen bonding, and electrostatic interactions.^{34–36} Hydrogen bonding is one of the main driving forces behind supramolecular peptide-based self-assemblies into elongated filamentous assemblies. In particular, PA molecules are designed with peptide sequences that have high propensities to form hydrogen bonds along the filament axis. Thus, formed β -sheets assemble to form diverse 1-D, 2-D and 3-D nanostructures.^{37–39} It is suggested that the balance between hydrophobic collapse and hydrogen bonding determines the resulting shape of the self-assembly.^{8,39} Moreover, the local electrostatic environment (salt concentration) has been shown to modulate the strength of hydrogen bonding along the filament, as well as the mechanical properties of the 3D filament network.⁴⁰ Furthermore, the presence of aromatic rings in the peptide sequence, or else in the conjugated hydrophobic tail, can add directional π - π stacking interactions that may play a role in determining the stability and shape of the assembled filament. Indeed, it has been shown that aromatic compounds with a strong π - π stacking interactions may be critical to the initial

stages of the self-assembly process for these peptide-amphiphiles.³⁸ Compared to above-mentioned non-covalent forces, electrostatic interactions are the strongest with a strength of $\sim 400 \text{ kJ mol}^{-1}$. These interactions are crucial in PA self-assembly, in particular where charged residues are incorporated into the peptide sequence. For example, electrostatic interactions between PA monomers can also be tuned through varying the pH of the solvent through the protonation and deprotonation of carboxylate or ammonium groups of peptide side chains.³⁰ Recently, *Tantakitti et al.*⁴⁰ showed that the contour length and mechanical stability of the PA filament can be tuned by varying the salt concentration. Thus, a balance of hydrophobic, hydrogen-bonding, aromatic, and electrostatic interactions are critical to determine the self-assembly pathway as well as the structure and stability of the resulting assemblies.

Multiple computational studies have utilized both all-atomistic (AA) and coarse-grained (CG) molecular dynamics (MD) simulation techniques to explore both the self-assembly of short peptide sequences⁷ as well as PAs^{23,27} and the resulting stability of the assemblies. MD uses the classical Newtonian equations of motion to calculate the dynamic behaviour of the system. Indeed, MD simulations are important tools to characterize both the structure and function of biophysical self-assemblies.⁴¹ AA simulations to study short peptide and PA self-assembly from random to the resulting nanostructures have been a challenge because of the large size and long timescale requirement. For this reason, multiple computational laboratories use a CG approach to explore the development of fibril-type assemblies. A CG approach involves the representation of groups of atoms with a single CG interaction site or bead. Such simpler models with decreased degrees of freedom (compared to AA models) make the simulation of larger systems for longer timescales possible. In general, CG models are 2 to 3 times faster than AA simulations and provide access to time and length-scales which cannot be accessed using AA simulations.^{42,43} Within this review, we highlight several types of CG models used to characterize short peptide and PA self-assembly. For example, the MARTINI⁴⁴ force field is a widely used CG tool for simulations of lipids, proteins and carbohydrates, as well as short peptide sequences^{45–47} and PAs^{23,32}. Two other CG force fields recently used for PA simulations are the Shinoda-DeVane-Klein (SDK) force field^{43,48,49} as well as ePRIME,²⁴ an extension of PRIME (Protein Intermediate Resolution Model),⁵⁰ which was developed by *Fu et al.*^{24,27,28} to CG their PA monomer, Palmitoyl-VVVAEEEE. However, CG MD simulations of such large systems have their own challenges which include: *i*) the accuracy of the force field for PAs, *ii*) loss of detailed chemical resolution with CG modelling, and *iii*) ability to computationally characterize structure and properties of these assemblies to compare with experimental results, validate computational force fields, and establish a feedback loop between experiment and computation. In order to increase the accuracy of CG systems, hybrid models consisting mixture of both AA and CG systems, and CG to AA reverse mapping techniques can be used as well.⁵¹

In the first section of this review we highlight AA and CG simulations of short peptide sequences, followed by AA and CG simulations of PA self-assembly. Next, we discuss recent results by Kang *et al.*⁵² utilizing AA and CG MD simulations to probe the structure and self-assembly process of drug amphiphiles (DAs) into elongated filaments. Finally, we

briefly discuss the challenges and highlight the opportunities in utilizing MD simulations to probe the interactions of PAs with model cell membranes.

AA and CG Simulations of Short Peptide Sequences

In the field of nanobiotechnology, short peptides like di- and tri-peptides that self-assemble into elongated fibrillar nanostructures have growing interest in recent years due to their potential applications in biomedicine,⁵³ photonics,^{53,54} and nanotechnology⁵⁵. These short peptides can self-assemble to form a range of shapes from nanosheets to nanotubes to nanovesicles.⁵⁶ Figure 1A shows cartoon representations of vesicle, nanotube and rectangular box formed by a Lys-Phe-Gly (KFG) tripeptide as schematically drawn by *Moitra et al.*⁴⁷ Due to the wide variety of peptide building blocks, the morphologies of such nanostructures display a rich structural diversity even for these short phenylalanine-based peptide sequences. *Gazit et al.*⁵⁷ first recognized that the π - π stacking of phenylalanine rings could play an important role in the self-assembly process. Thereafter, a large number of studies revealed that, dependent on the experimental conditions, phenylalanine-based peptides can form nanostructures ranging from nanovesicles, nanowires, nanoribbons to hydrogels.^{56,58,59} Indeed, diphenylalanine (FF) is one of the most known and extensively studied peptide building blocks.

Despite the growing interest and the enormous efforts toward structural characterization, very little is actually known about the peptide self-assembly process itself. Due to the complex nature and the difficulties in obtaining high-resolution structure of the aggregates, it is challenging to characterize the aggregation process experimentally. In this context, computational methods, especially MD simulation can play important roles both to understand the underlying mechanism of self-assembly process and also for designing new peptide building blocks for various applications. Indeed, a substantial number of AA^{60,61} and CG^{45,62} MD simulations have already been reported. In an early work, *Flöck et al.*⁶³ used AA MD simulation to study the aggregation process of various amyloidogenic and non-amyloidogenic short peptides. More recently, Tamamis and co-workers⁶⁰ used replica exchange methods in an implicit solvent to investigate the self-assembly of FF and FFF (triphenylalanine) peptides. In agreement with experiments, they observed that the aggregated peptides often contain open and ring-like peptide networks. Later, Jeon and co-workers⁶⁴ performed AA MD simulations of both zwitterionic (charged) and capped (uncharged) FF peptides. Their study revealed that due to strong electrostatic interactions between the charged termini, zwitterionic peptides can form a more ordered, clustered and compact structure than the uncharged FF peptides. *German et al.*⁶¹ also studied self-assembly of F and FF at various temperature and concentrations. In agreement with experiments, they found that both F and FF aggregates form layered structure and nanotube morphologies respectively. Effects of solvent on the self-assembly of F and FF were also investigated by Rissanou and co-workers.⁶⁵ Compared to methanol, it is found that the self-assembly propensity of FF is higher in water, which is clearly evident from their results.

Villa et al.^{66,67} developed CG models to study the aggregation process of FF peptide. They explored the conformational properties of FF both in explicit and implicit solvent. Based on the MARTINI⁴⁴ force field, Frederix and co-workers⁶⁸ calculated the aggregation propensity

of 400 different peptide sequences. A snapshot of a hollow tube formed by FF dipeptide is shown in Figure 1B. Water molecules present inside the tube are shown in a blue color. On the other hand, *Guo et al.*⁶² used a similar CG model to investigate FF peptide self-assembling pathways at different peptide concentrations. Their study shows that the assembly pathway is highly concentration dependent. In another study,⁴⁶ they reported a wide variety of nanostructures formed by FF and FFF co-assemblies with different mass ratios. In an important work, Tuttle, Ulijn, and co-workers⁴⁵ demonstrated a screening protocol for the aggregation behaviour of peptide based on aggregation propensity and applied this protocol to 8,000 gene-coded tripeptides. In agreement with experiments, they also found that aggregation propensity is highest for F, WF, FF and WW. Very recently, *Moitra et al.*⁴⁷ reported extensive MD simulations of KFG tripeptide self-assembly. At low peptide concentrations, KFG self-assembles to form vesicle type structures and at high concentrations it forms nanotubes, which agrees well with experimental results. Aggregation of relatively longer peptides (up to hexapeptide sequences) are also reported from other simulation studies.^{69–72}

Although MD simulation has been successfully used to probe the morphologies of these short peptide assemblies, simulating the aggregation process of longer peptides, like the amyloid beta ($\alpha\beta$), Prion, and Tau peptides, etc., is still extremely challenging. In a recent review, Andrews and Shea⁷³ highlighted computational approaches to study peptide aggregation using both CG and AA simulation studies. *Hall et al.* has simulated the aggregation of several polyalanine⁵⁰ and polyglutamine⁷⁴ peptides with various chain lengths. Self-assembly of FA32 peptide was studied by Thota and co-workers.⁷⁵ Using the MARTINI⁴⁴ CG model Xu and co-workers⁷⁶ explored the aggregation process of monomers, oligomers and fibril seeds of the A β peptide. Self-assembly of peptides on a lipid bilayer was also studied by the Shea group using CG MD in implicit solvent, as shown in Figure 1C.⁷⁷ In this particular work, they studied a model peptide named as CPHPPHA, where H and P are hydrophobic and polar groups respectively and cationic and anionic side chains at the ends are C and A.

Simulations of Peptide-Amphiphile Self-Assemblies

In a biologically functional PA, the peptide head is composed of three main domains: i) a peptide sequence able to form inter-peptide hydrogen bonds, ii) charged peptide residues for solubility, and, iii) a bio-functional epitope (Figure 2A).^{21,78,79} Experimental studies have shown that the hydrophobic tails of these PAs allow them to cross the cell membrane barrier, while the peptide epitope can be used to target various cells through a ligand-receptor complex.⁸⁰ In the past decade computational studies by Schatz, Stupp, Olvera de la Cruz and others have provided major insights into structure of PAs and the intermolecular forces governing their self-assembly into cylindrical nanofibers.^{8,12,21,25,81} This section reviews the AA and CG studies discussing the forces governing self-assembly process of cylindrical nanofiber.

Experimental study by Paramonov et al.³⁵ established β -sheets as the driving force for PA self-assembly into cylindrical nanofiber. The study reported the first four amino acids closest to the core participate in inter-peptide hydrogen bonds parallel to fiber axis and form β -

sheets. *Tsonchev et al.*²⁰ performed one of the earliest AA simulations of PAs. Within this study, a system of 4×4 clusters of optimized PA quartets was simulated. These early AA simulation results showed formation of β -sheets parallel to the fiber axis, as well as the tendency of β -sheet clusters to curve around the fiber axis resulting in a cylindrical micelle. In this early study, they concluded that for PAs (Palmitoyl-CCCCGGGS(P)RGD) the cylindrical nanostructure is more stable compared to bilayers, driven by β -sheet formation along the cylindrical axis of the fiber. The first CG simulation of PA assembly performed by *Velichko et al.*⁸ also supported the requirement of β -sheet to drive PA self-assembly into cylindrical nanofiber. The study used a simple united atom model, treating chemically similar segments as one monomeric unit. Using this model, the PA monomer was CG using three units—hydrophobic, peptide and epitope. Here, a Morse potential defined the hydrophobic interactions. To maintain the distance between monomer units and avoid chains from crossing, finite extendable nonlinear elastic potential (FENE) was used. The result of this study is summarized in Figure 3A. The resulting elongated shape of the PA aggregate self-assemblies depends on the temperature, which determines the balance of hydrophobic interaction energy and a directional hydrogen bonding energy. The results indicate that under conditions of pure hydrophobic interaction and below the critical micelle temperature (CMT), PAs organize into monodisperse finite-size micelles (Figure 3A i, ii). Whereas, a pure hydrogen bonding condition results in PAs organizing into one-dimensional β -sheets (Figure 3A vi). Also increase in the concentration of PAs increases the hydrophobic units resulting in stronger hydrophobic attraction between β -sheets and the formation of rolls of β -sheets (Figure 3A v). With further increase in hydrogen bonding, elongated β -sheet aggregates form resulting in several amorphous aggregates (Figure 3A vii). Above the CMT, frustrated micellar structures form (Figure 3A iii). These structures are geometrically frustrated due to steric interactions between the planar β -sheets. With further increase of the strength of the hydrogen bonding, the micellar corona becomes unstable, breaking spherical symmetry and reorganizing into a one-dimensional elongated cylindrical fiber (Figure 3A iv) with β -sheets aligned parallel to the fiber axis. Overall, in order for the formation of elongated cylindrical nanofibers, *Velichko et al.* suggests that β -sheet formation acts as the nucleation step followed by the cooperative effect of hydrogen bonding between peptide segments and the hydrophobic collapse of alkyl tails. These results are also supported by recent CG simulation studies by *Fu et al.*⁸² as shown in Figure 3B that suggests with increasing hydrophobicity, PAs first aggregate into a network of β -sheets and then assemble into cylindrical nanofibers followed by cylindrical micelles. The necessity of β -sheets for cylindrical nanofiber assembly was further explored by a comparative study of two cylindrical nanofibers composed with two PA monomers (Alanine rich and Valine rich).²² Although both PAs assembled into nanofibers, the fiber composed of Valine rich PAs had a higher β -sheet population, as well as a higher number of hydrogen bonds compared to the fiber composed of Alanine-rich PAs. Additionally, the study agreed with previous experimental studies reporting that the mechanical stiffness of peptide fibres depends on the population of β -sheets in the corona of the fiber.⁸³

Following, a more detailed understanding on the balance of intermolecular forces in maintaining the stable cylindrical nanofiber was provided by 40 ns simulation of pre-assembled nanofiber composed of 144 PAs (Palmitoyl-SLSLAAEIKVAV) by the Schatz

group (Figure 2B).²¹ After 20 ns simulation, the radius of fiber stabilized at ~44 Å (Figure 2C), which agreed well with the experimental values obtained via cryo-TEM.⁸³ In this study, the average population of β -sheets was only 14% and they were arranged parallel to the fiber axis. The study contradicted previous studies^{8,35,36} that suggested β -sheets as the driving force for cylindrical nanofiber formation. Instead, the study suggested that Van der Waals interactions between PA monomers as well as the electrostatic interactions between the PAs and the sodium ions of the solvent as the prominent interactions in the cylindrical nanofiber assembly. This work was further continued using a MARTINI⁴⁴ CG model. In order to mimic AA simulation results, secondary structures were assigned in the ratio of 25/15/60 for turn, β -sheet and coil PAs. This study showed the self-assembly of a nanofiber from a random organization of PAs in solution. As shown in Figure 2D, at 16 μ s PAs self-assemble such that hydrophobic tails (red) aggregate at the centre forming the core and peptide segments are exposed to water molecules. The results obtained from this CG simulation agreed well with their AA simulation study (Figure 2E). Another study by *Schatz et al.*³² investigated the difference in the potential mean force of PAs in the free unimer, and in the assembled micellar state. This study also reported that electrostatics and van der Waals interactions are the major intermolecular forces stabilizing cylindrical assemblies of PAs. They suggested that the self-assembly process is driven by enthalpy, and that this process takes place in a two-step mechanism. The first self-assembly step is the reorganization of PAs to form aggregates, followed by the second step driven by the formation of secondary structures.

Since CG simulations provide the characterization of the self-assembly process at longer time-scales, it has been possible to observe the multi-step aggregation process of PA self-assembly (Figure 3B). Studies have reported spherical micelles as the intermediate structures which later evolves into cylindrical fibers^{23,25,84}. For instance, in study by Fu *et al.*²⁴ using ePRIME model, PAs first assembled into spherical micelle and later merged to form cylindrical nanofiber (Figure 4A). Similarly, Lee *et al.*²³ used the MARTINI⁴⁴ force field to perform a simulation of 140 CG PAs in explicit water self-assembling from random. The study reported that first, due to hydrophobic collapse, spherical micelles form followed by cylindrical nanofibers that evolve due to van der Waals interactions between these micelles. On the contrary, cluster analysis of the system consisting of a mixture of the same peptide sequence with three different secondary structural distributions reported that the spherical micelle as only a transient intermediate and a pillar-like non-cylindrical fiber as the metastable intermediate that evolves into cylindrical nanofibers.²⁵ This study also indicated that the peptide-head segments aggregate faster than the tail segments suggesting that hydrophobic collapse may not be the driving force for cylindrical nanofiber formation. Similarly, a study utilizing the ePRIME force field²⁴ to CG PAs reported a spherical micelle to rod shape nanostructure transformation.²⁷ Within this study, with increasing hydrophobicity strength as low, moderate and high, three clusters were observed—random clusters, cylindrical nanofibers and elongated micelles.

PA self-assemblies that display peptide sequences on their surface can act as stimuli responsive hydrogels; indeed, these peptide-based hydrogels can be engineered to respond to external stimuli such as pH, light, temperature, or the presence of small molecules.⁸⁵ For example, experimental studies by Hartgerink *et al.*⁸⁶ demonstrated that a PA molecule

consisting of a cell binding epitope and a matrix metalloprotease cleavable sequence is degraded by cell-mediated proteolysis. Furthermore, the study indicated that at a neutral pH, an increase in Ca^{2+} ions facilitates the growth of cylindrical micelles into long nanofibers. *Stupp et al.* have also demonstrated control of PA nanofiber self-assembly by the screening of charged peptide residues by metal ions or pH adjustments.³⁶ Furthermore, recent studies by *Stupp et al.* using a PA containing a photoresponsive 2-nitrobenzyl group reported a light triggered sol-to-gel transformation.⁸⁷ Simulations have also demonstrated that PA self-assemblies can be responsive to various stimuli such as temperature and pH. For example, *Tekin et al.*⁸¹ performed united atom MD simulations on PA fibers with different layers with varying number of PAs in each layer. They found that a 19-layered nanofiber with 12 PAs in each layer as the most stable structure. The parallel β -sheet formation was found to possess a stepwise mechanism where first a dimer was formed followed by a trimer. As a continuation to the study, the stable nanofiber was simulated in varying temperatures in a range of pH's and counterion conditions. The results showed that the disintegration temperature of nanofibers is dependent on the pH, as well as the counterions in the solution. As shown in Figure 4B, the disintegration temperature for the PA being simulated at pH 7 with Na^+ counterions is 358K.³⁰ Recent studies by *Chen et al.*⁸⁴ and *Cote et al.*²⁶ reported under pH levels with low electrostatic repulsion, hydrogen bond formation between peptide segments is facilitated, disturbing the spherical micelle and forming the cylindrical nanofiber. A recent study of PAs with positively charged head groups using X-ray scattering, transmission electron microscopy, and Monte Carlo simulations showed that spherical micelle morphologies transform to cylindrical nanofibers to crystalline bilayer membranes with increasing pH.³¹

Simulation of Drug Amphiphile Filaments

Self-assembling PAs have also been shown to possess great potential as efficient drug delivery systems.^{88–94} Drug amphiphiles (DAs) are a special class of PAs, where hydrophobic drugs are conjugated to a short peptide through a biodegradable linker.⁸⁸ DAs have been suggested as a potential self-delivery strategy for hydrophobic drugs since they can self-assemble into discrete, stable, well-defined supramolecular nanostructure with a high and quantitative drug loading.⁸⁸ Formation of supramolecular nanostructures can protect drugs from rapid clearance as well as premature degradation, which can lead to a longer circulation time in the bloodstream. Furthermore, the monodispersity of peptide-drug amphiphiles provides an advantage over polydisperse polymeric carriers utilized in drug delivery. Experimental studies have been done on both the tunability of the morphologies and the resulting drug loading content of peptide assemblies. For example, *Cheetham et al.*⁸⁸ reported the morphological shift in the self-assembly shape of supramolecular nanostructures of DAs as they varied the number of the conjugated drug per DA. They found that 'mCPT-buSS-Tau' with one drug per DA self-assembles into an elongated 'one-dimensional' nanofilament, while 'qCPT-buSS-Tau' with four drugs per DA forms a hollow nanotube with a central water channel.

Kang et al. reported on MD simulation studies of these peptide-drug amphiphiles with conjugated aromatic rings.⁵² In their study, they performed long-time AA simulations of DA self-assembly, as well as characterized the chirality of pre-assembled supramolecular

filaments as shown in Figure 5. In their pre-assembled filaments, the hydrophobic pentacyclic drug camptothecin (CPT) remains buried in the core of the filament, while the hydrophilic peptide groups wrap around the core, forming the outer shell, as shown in Figure 5A and B. The CPTs in the core stack into short helical strands with a preferential handedness. The formation of β -sheets of the selected peptide is also distinctive, indicating intermolecular hydrogen bondings. They also performed advanced free energy calculations (multiple walker metadynamics calculations) of aromatic ring stacking to probe the directional interaction of the planar CPT as shown in Figure 5C.⁵² The AA simulations performed probe the delicate balance of underlying forces driving filamentous self-assembly of 'mCPT-buSS-Tau'. The findings highlight the anchoring role of π - π stacking of planar CPTs in the early stage of the aggregation process, the potential rearrangement of hydrogen bonding network in the later stages of the self-assembly process, and the helical packing of CPTs in the core of the nanofilaments. In the multiple walkers metadynamics calculation, the calculated free energy as a function of distance and dihedral angle between 2 freely rotating CPTs has two minima, suggesting displaced parallel, and sandwiched arrangements in their stacking (Figure 5). This suggests that when strongly directional aromatic interactions are driving the hydrophobic self-assembly process in the early stages of the self-assembly, fibrils can elongate even when hydrogen-bonding interactions are suppressed. In a subsequent simulation study, CG models are built based on AA simulation results, providing access to the μ s-timescale molecular view on the assembly and disassembly processes.⁹⁵ These CG models successfully reproduce the growth of the molecular clusters and their elongation trends, compared with previously reported AA simulations. Furthermore the structure and the helicity of the 'mCPT-buSS-Tau' filaments are conserved in these CG simulations as shown in Figure 6. Figure 6 shows their mapping of AA representation of 'mCPT-buSS-Tau' to CG beads and the conserved structure of the CG 'mCPT-buSS-Tau' filaments after a 1 μ s simulation in Figure 6C and D. The disassembly process of the finite-length filaments of length 0.7 μ m at elevated temperatures are also characterized in this study. At timescales more than 1 μ s, the filament is shown to disassociate, starting from the endcap. These results have implications for the design of the stability of future drug delivery vehicles that self-assemble from DAs.

Interactions with Membranes

In 1988, a cationic amphiphilic peptide, namely, TAT from the human immunodeficiency virus 1 (HIV-1) was found to translocate the membrane.⁹⁶ In general, two different classes of peptide sequences can be categorized when interacting with the membranes: Antimicrobial peptides (AMPs) and cell-penetrating peptides (CPPs). AMPs disrupt and form pores within the membrane via three mechanisms: 'carpet', 'barrel-stave', and 'toroidal-pore'.⁹⁷ The sequences and/or lengths of the peptides determine whether they are AMP class, which is invasive to the membrane, or the CPP class, which spare and translocate phospholipid bilayers. For example, the hexapeptide, VQIVYK, derived from the Tau peptide sequence, has been shown by electron spin resonance spectroscopy, that it is prone to form β -sheets, one of the signatures of AMPs, when they make contact with the membrane.⁹⁸ CPPs interact with the membrane via a passive mechanism. On the other hand, an active mechanism is defined as endocytosis ('cell-eating'), pinocytosis ('cell-drinking'),

peptides designed to interact with and organize surface receptors to promote active-mechanisms of interaction such as endocytosis (Figure 7C).¹⁰ For example, the Cui laboratory has explored the pathway of DA cellular internalization.⁹⁹ With confocal microscopy, the lab has explored the pathway for free doxorubicin (DOX), as well as for DOX conjugated to the TAT peptide. As previously discussed, the TAT peptide sequence has been shown to translocate the membrane and accumulate in the cell nucleus.¹⁰⁰ Furthermore, MD simulations have shown that the peptide acts via a cooperative mechanism when it interacts with the cell surface,¹⁰¹ forming a pore in the membrane. Experiments have suggested that conjugation of the TAT sequence to DOX changes the mechanism of cellular internalization from a passive mechanism to an active or endocytosis-mediated mechanism.⁹⁹ Several questions are raised by this result, such as: *i*) what is the change in the binding free energy of TAT with the membrane when attaching a hydrophobic drug? Additionally, *ii*) why does the original mechanism of cooperative pore-formation for TAT translocation become inefficient? If the interaction between the PAs and the membrane is not strong enough, the energy required to dissociate the supramolecular filaments (as schematically sketched in Figure 7A and 7B will be too large, and the DAs will remain in stable filamentous self-assemblies. If elongated PA filaments are the stable structures, an active endocytosis-mediated mechanism may dominate.

Conclusion

Exploiting the natural phenomenon of protein self-assembly, researchers have developed various nanostructures from peptides synthesized in the lab. In this vastly emerging field, computational simulation has become an effective tool to study the processes of peptide self-assembly and thus aid experimental groups in the rational design of peptide structures. As discussed above, simulation studies have shown two-step mechanism for cylindrical nanofiber formation. However, the pathway for merging of nuclei, and assembly of micelles into filamentous structures is still unclear. The pathway may be dependent on various factors such as peptide sequence, length of alkyl tail, and pH of the solvent. Using computational simulations we can study the effect of each factor and its role on the self-assembly pathway of PAs. Moreover, computational simulations can be used to explore response of biological systems such as cell membranes to these PA nanostructures. It is crucial to understand the interaction of PA nanostructures with the cell membrane to explore their biocompatibility and determine their future prospects in bionanomedicine. Using MD simulation, we can predict and characterize the process of PA nanofiber interaction with model cell membranes, its possible insertion and its reorganization in the lipid bilayer. However, we cannot get the complete picture of the self-assembly process of PAs and their interaction with other systems using AA simulations. The CG methods discussed within this review have potential to characterize these dynamic pathways.

Acknowledgments

S.M.L. acknowledges support from the NIH (R15EB020343-01A1). Additionally, this research was supported, in part, by the NSF through XSEDE resources under grant number TG-CHE130099 and a grant of computer time from the City University of New York High Performance Computing Center under NSF Grants CNS-0855217, CNS-0958379 and ACI-1126113. S.M.L. also acknowledges start-up funding received from College of Staten Island and City University of New York. A. M. and M. K. also acknowledge partial support thanks to the Rosemary O'Halloran scholarship awarded to female chemists in the Chemistry department at College of Staten Island.

Biographies

Anjela Manandhar

Anjela Manandhar received her B.S. in Chemistry with Biochemistry concentration from McNeese State University in 2013. She is currently a PhD candidate at the Graduate Center of the City University of New York, under the supervision of Professor Sharon M. Loverde. She is studying the effect of nucleotide state on the protofilament conformation of tubulin octamers using all-atomistic (AA) and coarse-grained (CG) simulation methods.

Myungshim Kang

Myungshim Kang received her Ph.D. in Physical Chemistry from Kansas State University in 2009. Next, she completed a postdoctoral position with Chia-en Chang at University of California, Riverside. She is currently a postdoctoral fellow in the Loverde laboratory and an adjunct assistant professor at College of Staten Island. She is working on AA and CG simulations of drug amphiphiles.

Kaushik Chakraborty

Kaushik Chakraborty received his M.S. from Jadavpur University Kolkatta in 2009. He next completed his PhD in Physical Chemistry at IIT Kharagpur in 2015. He is currently a postdoctoral fellow in the Loverde laboratory. He is working on large-scale simulations of the nucleosome core particle as well as diblock copolymer assemblies in soft materials.

Phu Khanh Tang

Phu Khanh Tang received his B.S in Biochemistry in 2015 from CUNY College of Staten Island. He is currently a PhD candidate at the Graduate Center of the City University of New York, under the supervision of Professor Sharon M. Loverde. He is currently studying the interactions of hydrophobic drugs with model cell membranes using molecular dynamics (MD) simulation techniques.

Sharon Loverde

Sharon Loverde is an assistant professor at CUNY College of Staten Island. She is a faculty member of the CUNY Graduate Programs in Physics, Chemistry, and Biochemistry. Her computational laboratory focuses on large-scale AA and CG simulation studies of macromolecular assemblies in soft materials and biophysical chemistry.

References

1. Uljén RV, Smith AM. Chem. Soc. Rev. 2008; 37:664–675. [PubMed: 18362975]
2. Zhang S. Nat. Biotechnol. 2003; 21:1171–1178. [PubMed: 14520402]
3. Liang L, Yang J, Li Q, Huo M, Jiang F, Xu X, Zhang X. J. Biomater. Nanobiotechnol. 2011; 2:622–625.
4. Ellis-Behnke RG, Liang Y-X, You S-W, Tay DKC, Zhang S, So K-F, Schneider GE. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:5054–9. [PubMed: 16549776]
5. Das R, Kiley PJ, Segal M, Norville J, Yu AA, Wang L, Trammell SA, Reddick LE, Kumar R, Stellacci F, Lebedev N, Schnur J, Bruce BD, Zhang S, Baldo M. Nano Lett. 2004; 4:1079–1083.
6. Zhao X, Pan F, Lu JR. Prog. Nat. Sci. 2008; 18:653–660.
7. Wu C, Shea J-E. Curr. Opin. Struct. Biol. 2011; 21:209–220. [PubMed: 21371882]
8. Velichko YS, Stupp SI, De La Cruz MO. J. Phys. Chem. B. 2008; 112:2326–2334. [PubMed: 18251531]
9. Hartgerink JD. Science. 2001; 294:1684–1688. [PubMed: 11721046]
10. Aida T, Meijer EW, Stupp SI. Science. 2012; 335:813–817. [PubMed: 22344437]
11. Kokkoli E, Mardilovich A, Wedekind A, Rexeisen EL, Garg A, Craig JA. Soft Matter. 2006; 2:1015–1024.
12. Cui H, Webber MJ, Stupp SI. Biopolymers. 2010; 94:1–18. [PubMed: 20091874]
13. Hartgerink JD, Beniash E, Stupp SI. Proc. Natl. Acad. Sci. U. S. A. 2002; 99:5133–8. [PubMed: 11929981]

14. Dehsorkhi A, Castelletto V, Hamley IW. *J. Pept. Sci.* 2014; 20:453–67. [PubMed: 24729276]
15. Miravet JF, Escuder B, Segarra-Maset MD, Tena-Solsona M, Hamley IW, Dehsorkhi A, Castelletto V. *Soft Matter*. 2013; 9:3558–3564.
16. Tysseling-mattiace VM, Sahni V, Niece KL, Birch D, Czeisler C, Fehlings MG, Stupp SI, Kessler JA. *Spinal Cord*. 2008; 28:3814–3823.
17. Hosseinkhani H, Da Hong P, Yu DS. *Chem. Rev.* 2013; 113:4837–4861. [PubMed: 23547530]
18. Shah RN, Shah NA, Del Rosario Lim MM, Hsieh C, Nuber G, Stupp SI. *Proc. Natl. Acad. Sci. U. S. A.* 2010; 107:3293–8. [PubMed: 20133666]
19. Rezler EM, Khan DR, Lauer-Fields J, Cudic M, Baronas-Lowell D, Fields GB. *J. Am. Chem. Soc.* 2007; 129:4961–4972. [PubMed: 17397150]
20. Tsonchev S, Troisi A, Schatz GC, Ratner MA. *Nano Lett.* 2004; 4:427–431.
21. Lee O-S, Stupp SI, Schatz GC. *J. Am. Chem. Soc.* 2011; 133:3677–3683. [PubMed: 21341770]
22. Lee OS, Liu Y, Schatz GC. *J. Nanoparticle Res.* 2012; 14:936.
23. Lee O-S, Cho V, Schatz GC. *Nano Lett.* 2012; 12:4907–4913. [PubMed: 22924639]
24. Fu IW, Markegard CB, Chu BK, Nguyen HD. *Adv. Healthc. Mater.* 2013; 2:1388–1400. [PubMed: 23554376]
25. Yu T, Schatz GC. *J. Phys. Chem. B.* 2013; 117:14059–14064. [PubMed: 24143994]
26. Cote Y, Fu IW, Dobson ET, Goldberger JE, Nguyen HD, Shen JK. *J. Phys. Chem. C.* 2014; 118:16272–16278.
27. Fu IW, Markegard CB, Nguyen HD. *Langmuir*. 2015; 31:315–324. [PubMed: 25488898]
28. Fu IW, Nguyen HD. *Biomacromolecules*. 2015; 16:2209–2219. [PubMed: 26068113]
29. Tekin ED. *RSC Adv.* 2015; 5:66582–66590.
30. Ozkan AD, Tekinay AB, Guler MO, Tekin ED. *RSC Adv.* 2016; 6:104201–104214.
31. Gao C, Li H, Li Y, Kewalramani S, Palmer LC, Dravid VP, Stupp SI, Olvera de la Cruz M, Bedzyk MJ. *J. Phys. Chem. B.* 2017; 121:1623–1628. [PubMed: 28145713]
32. Yu T, Schatz GC. *J. Phys. Chem. B.* 2013; 117:9004–9013. [PubMed: 23822638]
33. Fu IW, Markegard CB, Chu BK, Nguyen HD. *Langmuir*. 2014; 30:7745–7754. [PubMed: 24915982]
34. Toksöz S, Guler MO. *Nano Today*. 2009; 4:458–469.
35. Paramonov SE, Jun HW, Hartgerink JD. *J. Am. Chem. Soc.* 2006; 128:7291–7298. [PubMed: 16734483]
36. Stendahl JC, Rao MS, Guler MO, Stupp SI. *Adv. Funct. Mater.* 2006; 16:499–508.
37. Cui H, Webber MJ, Stupp SI. *Biopolymers*. 2010; 94:1–18. [PubMed: 20091874]
38. Wang J, Liu K, Xing R, Yan X. *Chem. Soc. Rev.* 2016; 225:177–193.
39. Han S, Cao S, Wang Y, Wang J, Xia D, Xu H, Zhao X, Lu JR. *Chem. - A Eur. J.* 2011; 17:13095–13102.
40. Tantakitti F, Boekhoven J, Wang X, Kazantsev RV, Yu T, Li J, Zhuang E, Zandi R, Ortony JH, Newcomb CJ, Palmer LC, Shekhawat GS, de la Cruz MO, Schatz GC, Stupp SI. *Nat. Mater.* 2016; 15:469–476. [PubMed: 26779883]
41. Karplus M, McCammon JA. *Nat. Struct. Biol.* 2002; 9:646–652. [PubMed: 12198485]
42. Ingólfsson HI, Lopez CA, Uusitalo JJ, de Jong DH, Gopal SM, Periole X, Marrink SJ. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* 2014; 4:225–248. [PubMed: 25309628]
43. Klein ML, Shinoda W. *Science*. 2008; 321:798–800. [PubMed: 18687954]
44. Marrink SJ, Risselada HJ, Yefimov S, Tieleman DP, De Vries AH. *J. Phys. Chem. B.* 2007; 111:7812–7824. [PubMed: 17569554]
45. FrederixPim WJM, Scott GG, Abul-Haija YM, Kalafatovic D, Pappas CG, Javid N, Hunt NT, Ulijn RV, Tuttle T. *Nat Chem.* 2015; 7:30–37. [PubMed: 25515887]
46. Guo C, Arnon ZA, Qi R, Zhang Q, Adler-Abramovich L, Gazit E, Wei G. *ACS Nano*. 2016; 10:8316–8324. [PubMed: 27548765]
47. Moitra P, Subramanian Y, Bhattacharya S. *J. Phys. Chem. B.* 2017; 121:815–824. [PubMed: 28045258]

48. Shinoda W, DeVane R, Klein ML. *Mol. Simul.* 2007; 33:27–36.
49. Devane R, Shinoda W, Moore PB, Klein ML. *J. Chem. Theory Comput.* 2009; 5:2115–2124. [PubMed: 20161179]
50. Nguyen HD, Hall CK. *Proc. Natl. Acad. Sci. U. S. A.* 2004; 101:16180–16185. [PubMed: 15534217]
51. Nielsen SO, Buló RE, Moore PB, Ensing B. *Phys. Chem. Chem. Phys.* 2010; 12:12401–14. [PubMed: 20734007]
52. Kang M, Zhang P, Cui H, Loverde SM. *Macromolecules.* 2016; 49:994–1001.
53. Zhao X, Pan F, Xu H, Yaseen M, Shan H, Hauser CAE, Zhang S, Lu JR. *Chem. Soc. Rev.* 2010; 39:3480–3498. [PubMed: 20498896]
54. Zelzer M, Uljin RV. *Chem. Soc. Rev.* 2010; 39:3351–3357. [PubMed: 20676412]
55. Miyake H, Tsukube H. *Chem. Soc. Rev.* 2012; 41:6977–6991. [PubMed: 22850749]
56. Gazit E. *Chem. Soc. Rev.* 2007; 36:1263–1269. [PubMed: 17619686]
57. Reches M, Gazit E. *Science.* 2003; 300:625–627. [PubMed: 12714741]
58. Sedman VL, Adler-Abramovich L, Allen S, Gazit E, Tendler SJB. *J. Am. Chem. Soc.* 2006; 128:6903–6908. [PubMed: 16719470]
59. Yan X, Zhu P, Li J. *Chem. Soc. Rev.* 2010; 39:1877–1890. [PubMed: 20502791]
60. Tamamis P, Adler-Abramovich L, Reches M, Marshall K, Sikorski P, Serpell L, Gazit E, Archontis G. *Biophys. J.* 2009; 96:5020–5029. [PubMed: 19527662]
61. German HW, Uyaver S, Hansmann, Ulrich HE. *J. Phys. Chem. A.* 2015; 119:1609–1615. [PubMed: 25347763]
62. Guo C, Luo Y, Zhou R, Wei G. *ACS Nano.* 2012; 6:3907–3918. [PubMed: 22468743]
63. Flöck D, Rossetti G, Daidone I, Amadei A, Di Nola A. *Proteins Struct. Funct. Bioinforma.* 2006; 65:914–921.
64. Jeon J, Mills CE, Shell MS. *J. Phys. Chem. B.* 2013; 117:3935–3943. [PubMed: 23521630]
65. Rissanou AN, Georgilis E, Kasotakis E, Mitraki A, Harmandaris V. *J. Phys. Chem. B.* 2013; 117:3962–3975. [PubMed: 23510047]
66. Villa A, van der Vegt NFA, Peter C. *Phys. Chem. Chem. Phys.* 2009; 11:2068–2076. [PubMed: 19280017]
67. Villa A, Peter C, van der Vegt NFA. *Phys. Chem. Chem. Phys.* 2009; 11:2077–2086. [PubMed: 19280018]
68. Frederix PWJM, Uljin RV, Hunt NT, Tuttle T. *J. Phys. Chem. Lett.* 2011; 2:2380–2384. [PubMed: 23795243]
69. Liang L, Wang L-W, Shen J-W. *RSC Adv.* 2016; 6:100072–100078.
70. Slynghorg M, Fojan P. *Phys. Chem. Chem. Phys.* 2015; 17:30023–30036. [PubMed: 26499975]
71. Lakshmanan A, Cheong DW, Accardo A, Di Fabrizio E, Riekel C, Hauser CAE. *Proc. Natl. Acad. Sci.* 2013; 110:519–524. [PubMed: 23267112]
72. Hauser CAE, Deng R, Mishra A, Loo Y, Khoe U, Zhuang F, Cheong DW, Accardo A, Sullivan MB, Riekel C, Ying JY, Hauser UA. *Proc. Natl. Acad. Sci.* 2011; 108:1361–1366. [PubMed: 21205900]
73. Morriss-Andrews A, Shea J-E. *J. Phys. Chem. Lett.* 2014; 5:1899–1908. [PubMed: 26273871]
74. Marchut AJ, Hall CK. *Proteins Struct. Funct. Bioinforma.* 2007; 66:96–109.
75. Thota N, Luo Z, Hu Z, Jiang J. *J. Phys. Chem. B.* 2013; 117:9690–9698. [PubMed: 23927837]
76. Xu L, Chen Y, Wang X. *J. Phys. Chem. B.* 2014; 118:9238–9246. [PubMed: 25050788]
77. Morriss-Andrews A, Brown FLH, Shea J-E. *J. Phys. Chem. B.* 2014; 118:8420–8432. [PubMed: 24791936]
78. Cui H, Webber MJ, Stupp SI. *Biopolymers.* 2010; 94:1–18. [PubMed: 20091874]
79. Niece KL, Czeisler C, Sahni V, Tysseling-Mattiace V, Pashuck ET, Kessler JA, Stupp SI. *Biomaterials.* 2008; 29:4501–4509. [PubMed: 18774605]
80. Accardo A, Tesaro D, Mangiapia G, Pedone C, Morelli G. *Biopolymers - Peptide Science Section.* 2007; 88:115–121.

81. Tekin ED. RSC Adv. 2015; 5:66582–66590.
82. Fu IW, Markegard CB, Chu BK, Nguyen HD. Langmuir. 2014; 30:7745–7754. [PubMed: 24915982]
83. Pashuck ET, Cui H, Stupp SI. J. Am. Chem. Soc. 2010; 132:6041–6046. [PubMed: 20377229]
84. Chen Y, Gan HX, Tong YW. Macromolecules. 2015; 48:2647–2653.
85. Mart RJ, Osborne RD, Stevens MM, Ulijn RV, Somayaji R, Uludag H, Brenner RE, Möller M, Mutter M. Soft Matter. 2006; 2:822.
86. Jun H-W, Yuwono V, Paramonov SE, Hartgerink JD. Adv. Mater. 2005; 17:2612–2617.
87. Muraoka T, Koh C-Y, Cui H, Stupp SI. Angew. Chemie Int. Ed. 2009; 48:5946–5949.
88. Cheetham AG, Zhang P, Lin Y, Lock LL, Cui H. J. Am. Chem. Soc. 2013; 135:2907–2910. [PubMed: 23379791]
89. MacKay JA, Chen M, McDaniel JR, Liu W, Simnick AJ, Chilkoti A. Nat. Mater. 2009; 8:993–9. [PubMed: 19898461]
90. Shen Y, Jin E, Zhang B, Murphy CJ, Sui M, Zhao J, Wang J, Tang J, Fan M, Van Kirk E, Murdoch WJ. J. Am. Chem. Soc. 2010; 132:4259–4265. [PubMed: 20218672]
91. Li X-Q, Wen H-Y, Dong H-Q, Xue W-M, Pauletti GM, Cai X-J, Xia W-J, Shi D, Li Y-Y. Chem. Commun. (Camb). 2011; 47:8647–9. [PubMed: 21725532]
92. Gao Y, Kuang Y, Guo ZF, Guo Z, Krauss IJ, Xu B. J. Am. Chem. Soc. 2009; 131:13576–13577. [PubMed: 19731909]
93. Liu L, Xu K, Wang H, Tan PKJ, Fan W, Venkatraman SS, Li L, Yang Y-Y. Nat. Nanotechnol. 2009; 4:457–463. [PubMed: 19581900]
94. Xu X, Li Y, Li H, Liu R, Sheng M, He B, Gu Z. Small. 2014; 10:1133–1140. [PubMed: 24155260]
95. Kang M, Cui H, Loverde SM. Soft Matter. In Review.
96. Frankel A, Pabo C. Cell. 1988; 55:1189–1193. [PubMed: 2849510]
97. Brogden KA. Nat. Rev. Microbiol. 2005; 3:238–250. [PubMed: 15703760]
98. Georgieva ER, Xiao SF, Borbat PP, Freed JH, Eliezer D. Biophys. J. 2014; 107:1441–1452. [PubMed: 25229151]
99. Zhang P, Cheetham AG, Lock LL, Cui H. Bioconjug. Chem. 2013; 24:604–613. [PubMed: 23514455]
100. Brooks H, Lebleu B, Vives E. Adv. Drug Deliv. Rev. 2005; 57:559–577. [PubMed: 15722164]
101. Herce HD, Garcia AE. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:20805–20810. [PubMed: 18093956]

Figure 1.

A. A cartoon representation of the cross section of a vesicle, a nanotube and a rectangular box formed by the Lys-Phe-Gly (KFG) tripeptide. Structure of the peptide is also shown on the top. Hydrophilic and hydrophobic residues are shown in red and black beads and water and ions are in blue and green. Adapted with permission from ref ⁴⁷. Copyright (2017) American Chemical Society. **B.** Aggregation of diphenylalanine from a CG MARTINI simulation. Adapted with permission from ref 68. Copyright (2011) American Chemical Society. This figure is licensed under the ACS AuthorChoice license. **C.** Aggregation of a peptide on a lipid bilayer. Adapted with permission from ref 77. Copyright (2014) American Chemical Society.

Figure 2.

MD simulations of PAs by Lee et al.^{21,23} **A.** Monomeric unit of PA (Palmitoyl-SLSLAAAEIKVAVPA) showing hydrophilic alkyl tail in black, β-sheet forming sequence in green, spacer in magenta and epitope head in blue. **B.** Snapshot showing self-assembled PA after 40ns of AA simulation. Here, hydrophobic core is represented in blue, α-helices in yellow, turns in cyan, and coils in grey. **C.** Radius of fiber during simulation. The radius stabilizes to ~44Å after 20ns simulation. Adapted with permission from ref 21. Copyright (2011) American Chemical Society. **D.** Snapshot of CG PA fiber after 16 μs. Here three PA monomers—PA1, PA2, and PA3 with different assigned secondary structures are shown in

blue, green, and yellow respectively, and tails are shown in red color. **E.** Peptide secondary structure obtained from all atom simulation. Here, turn is represented in green, β -sheet in yellow, helix in purple, random coil in grey, and tail in red. Adapted with permission from ref 23. Copyright (2017) American Chemical Society.

Figure 3.

A. Various PA aggregate formation due to combined effect of hydrophobic attraction (ϵ_{HH}) and hydrogen bonding (ϵ_{β}) –**i.** Free molecules, **ii.** Spherical micelles, **iii.** Micelle corona **iv.** Cylindrical fiber **v.** Stacks of β -sheets **vi.** Single β -sheet layer, and **vii.** Amorphous aggregate. Adapted with permission from ref 8. Copyright (2008) American Chemical Society. **B.** Schematic representation showing the effect of hydrophobic strength in PA organization. With increasing hydrophobicity, first spherical micelles form which later merge to form cylindrical nanofibers and elongated micelles. Adapted with permission from ref 27. Copyright (2015) American Chemical Society.

Figure 4.

A. Time-dependent snapshots showing later stages of self-assembly of Palmitoyl-VVVAEEEE involving micelles merging to form a cylindrical nanofiber. Here t^* is reduced time defined as $t^* = t/\sigma \text{ (K}_B T/m)^{1/2}$, where t is simulation time, K_B is Boltzmann's constant, T is temperature, and σ and m are the average bead diameter and mass respectively. Adapted from ref 24. Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. **B.** Snapshots showing simulation of Lauryl-VVAGERGD nanofibers with increasing temperature. Disassembly was observed to occur at 358K. Adapted from ref 30 by permission of The Royal Society of Chemistry.

Figure 5.

Self-assembly of DAs. Top view **A.** and side view **B.** from molecular dynamics simulation of pre-assembled mCPT-buSS-Tau. **C.** The calculated free energy in the function of distance and dihedral angle between 2 CPTs. Adapted with permission from ref 52. Copyright 2016 American Chemical Society.

Figure 6. CG model for a drug amphiphile. **A.** AA model for 'mCPT-buSS-Tau' in a CPK representation. **B.** CG model for 'mCPT-buSS-Tau' shown with transparent balls, overlapped on the atomistic model. Hydrophobic cancer drug camptothecin (CPT) is in red. The peptide and linker are in yellow. **C.** Top view of the preassembled filament. **D.** Side view of the preassembled filament. Colors follow same representation as A. From ref 95.

Figure 7.

A. A hypothetical perspective that visualizes the dynamics and possible structures of the conjugate drug-peptide amphiphiles (DAs), which can thermodynamically assemble and interact with the phospholipid bilayers (blue-yellow). The amphiphilic peptides (magenta) constitute the cover for the hydrophobic molecules inside (cyan) (e.g., anticancer drugs). **B.** A close-up view shows that the interaction of the DAs and the membrane interferes with the intermolecular forces among the DAs themselves. Thus, the nanotube dissociates into monomers which passively translocate through the membrane. **C.** The interaction of the membrane receptors (blue) and the nanotubes (red-black) stabilizes the DAs in a particular

conformation. Thus, in order to cross the membrane without harm, such a mega-structure should be transported actively (e.g., endocytosis or pinocytosis) into the cell. Adapted from reference 10. Reprinted with permission from AAAS.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

List of simulation studies of PAs.

Peptide Amphiphile	Method	Reference
Palmitoyl-CCCCGGGS(P)RGD	MD	20
Palmitoyl-VVVAAAE-NH ₂	CG - United atom model	8
Palmitoyl-VVVAAAK-NH ₂		
Palmitoyl-SLSLAAEIKVAV	MD - NAMD2	21
Palmitoyl-VVAAAEEEE	MD - NAMD2	22
Palmitoyl-VVVVAAEEEE		
Palmitoyl-SLSLAAEIKVAV	CG - MARTINI	23
Palmitoyl-VVVVAAEEEE	CG - ePRIME	24
Palmitoyl-SLSLAAEIKVAV	CG - MARTINI	25
Palmitoyl-SLSLAAEIKVAV	MD - NAMD2.9	32
Palmitoyl-IAAAEEEE-NH ₂	MD - GROMACS	26
	CG - ePRIME	
Palmitoyl-VVVVAAEEEE	CG - ePRIME	33
Palmitoyl-VVVVAAEEEE	CG - ePRIME	27
Palmitoyl-VVVVAAEEEE	CG - ePRIME	28
Palmitoyl-AAAVVVEEE		
Lauryl-VVAGERGD	CG - United atom model	29
Lauryl-VVAGERGD	CG - United atom model	30
Palmitoyl-KK	Monte Carlo	31