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Probing Peptide Nanotube Self-Assembly at a Liquid-Liquid Interface with Coarse-Grained Molecular Dynamics

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Abstract

Self-assembly at a liquid-liquid interface is a powerful experimental route to novel nanomaterials. We report herein a computational study of peptide nanotube formation at an oil-water interface. We probe interfacial self-assembly and nanotube formation of the cyclic octapeptide, *cyclo* [(-L-Trp-D-Leu-)₄] as an illustrative example. Individual peptide rings are rapidly adsorbed at the liquid-liquid interface where they self-assemble. Monomeric and dimeric peptide rings lie with their molecular planes mostly parallel to the interface. Longer oligomeric nanotubes are increasingly tilted at the interface and grow by an Oswald ripening mechanism to eventually align their tube axis parallel to the interface. The present results on nanotube assembly suggest that computation will be a useful complement to experiment in understanding the nature of self-assembly of nanomaterials at liquid-liquid interfaces.

Introduction

The air-water interface is a traditional medium to prepare nanoscale molecular assemblies such as Langmuir-Blodgett films, as well as ultrathin films of materials for possible device applications.¹ Exciting recent work has drawn attention to the potential of exploiting the liquid-liquid interface to produce novel nanomaterials and ultrathin films of metals, chalcogenides, and oxides.² For example, the toluene-water interface has been employed to make two-dimensional nanostructures in the form of ultra-thin crystalline films of CdSe and CuSe^{3, 4} and monolayers of Ag nanoparticles with diameters in the range of 10-50 nm.⁵ These studies employing the liquid-liquid interface highlight the potential of using non-traditional routes to synthesize nanomaterials. Inspired by these experimental studies, the present work is focused on the possibility of using an oil-water interface as a means to probe the self-assembly mechanism of nano-scale constructs built from biomolecules. The reason for choosing the oil-water interface and biomolecules is that the pallet of the twenty natural amino acids contains both hydrophobic and hydrophilic members allowing for extensive libraries of molecules and a vast array of possible structures. Different kinds of peptide based nanotubes have been shown to be relatively simple, bio-compatible nanodevices.⁶⁻⁹

Herein we focus on the cyclic peptide nanotubes synthesized by the Ghadiri group.¹⁰⁻¹³ These cyclic peptide tubes are extended β -sheet-like structures built from ring-shaped peptide subunits made up of alternating L- and D- amino acid residues. The remarkable stability of these nanotubular structures is attributed to the inter-subunit hydrogen bonds. Steric

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Supporting Information **Available:** Details of the CG peptide force field and movies of self-assembly at the two interfaces. This material is available free of charge via the Internet at <http://pubs.acs.org>.

considerations and the alternating amino acid configuration force all the peptide side chains to lie on the outside of the ensemble, which results in a hollow tubular core, that can transport ions.¹⁴ The inherent design advantages of these tubes are that they can be tailored to specifically assemble in a diverse set of environments with an appropriate choice of amino acid side chains, and their internal diameter can be controlled by the number of amino acids employed. Their ability to transport ions and assemble in diverse set of environments extends their application range from diffusion-limited size-selective sensors⁹ to antibacterial¹⁵ and antiviral agents.¹⁶ Other plausible applications include drug-delivery agents, photo-responsive materials, liquid crystals, tubular polymers, catalysts etc.¹³

Computational Methods

The Ghadiri *cyclo* [(-L-Trp-D-Leu-)₄] rings are a natural choice for a computational study since they are known to self-assemble in lipid bilayers,¹⁷ at the air-water interface,¹⁸ and in organosulfur monolayers on gold films.⁹ However, mechanistic details on the aggregation and growth of the peptide rings into nanotubes are lacking. In previous computational works, the behavior of pre-assembled membrane-bound cyclic peptide nanotubes¹⁹ and bulk solid crystals²⁰ have been studied by classical all atom (AA) molecular dynamics (MD) and *ab initio* CarParrinello density functional based electronic structure methods, respectively. But these studies offered little insight into the mechanism of nanotube formation. We have earlier studied monomers and dimers of *cyclo* [(-L-Trp-D-Leu-)₄] in water, alkane (nonane), and at an alkane-water interface using AA-MD simulations.²¹ It was found that dimers were only stable in the hydrophobic solvent and that in the interfacial system, the peptide rings preferred to reside very close to the alkane-water interface.

Experimentally, the assembly-disassembly of the peptide rings occurs on a time scale of milliseconds to seconds in lipid bilayers, depending on the peptide concentration.¹⁷ Such time scales are currently inaccessible for study with routine AA-MD simulations. However, through a reduction in the number of interacting sites and softer interaction potentials, which are provided by so-called coarse grained (CG) models, MD simulation can be a useful tool to study events on such long time scales²². For example, a lattice CG model has been used to study the process of self-assembly of nanoparticles mediated by solvent vaporization by Brus and co-workers.²³

In the present case, a CG model should be able to capture the essential details of the self-assembly process, if the relative strengths of the interactions between the amino acid residues and the two solvents are captured. In the CG peptide model being used here, the C_αH, CO and NH moieties constitute a single backbone bead (BB), as do both the tryptophan (TRP), and leucine (LEU) side chains. Each peptide ring is thus reduced from 172 AA sites to 16 CG sites: 8 BB, 4 TRP, and 4 LEU. Figure 1 shows the AA to CG mapping of the peptide ring that is employed in the present study. In addition, each solvent nonane molecule consists of 3 CG beads (two terminal CT beads and one middle CM bead), and 3 water molecules are grouped into a single 'water' bead (W). The CG potentials used for nonane and water are taken from Shinoda et al.²⁴ Details of the CG potentials employed for the cyclic peptide beads (BB, TRP, and LEU) are given in the Supporting Information along with the parameters. It is important to note that while the peptide intramolecular parameters were chosen to preserve the ring structure, the non-bonded interactions were parameterized independently. The parameterization approach employed preserves the expected major driving forces for the self-assembly: the relative strengths of interactions between the backbone, side chains and solvents. Specifically, relatively short (~10 ns) CG-MD trajectories of peptide monomers and dimers indeed agree with the results from AA-MD simulations²¹ and thus support the employed CG parameterization.

Self-Assembly via MD

In order to study the self-assembly process, an initial system consisting of 55 peptide rings in a nonane-water two-phase system, with 848 nonane molecules and 3817 CG-water beads (equivalent to 11451 water molecules) was set up. The peptide rings were placed randomly in the system and a tubular stack of five rings was placed in the middle of the alkane region. After ~ 3 ns MD using the NPT ensemble and periodic boundary conditions in three dimensions, the system was replicated in the x- and y- directions to generate a four-times larger system with 220 peptide rings, 3392 nonane molecules and 15268 water beads (Figure 2). A trajectory of the replicated system was then followed for 2.5 μ s with the same simulation conditions as discussed in the Supporting Information under details of the CG peptide force field. The simulation cell dimensions were 17 nm $\times$ 17 nm $\times$ 10 nm. The peptide rings move rapidly to saturate the two interfaces (Figure 2). The four pre-assembled peptide stacks move toward the lower interface (II), and the final concentration of rings is lower at interface II than the upper interface (I) (Figure 3). At $t = 0.050$ μ s, there are 134 rings at interface I and 86 rings at interface II (Figure 3). The varied distributions of the rings at the two interfaces and the subsequent independent evolution of these interfaces effectively allows the study of the system starting from different sets of initial conditions, which likely correspond to two different stages of the self-assembly process (see below).

Interface I consists of monomers and dimers at $t = 0.050$ μ s. We observe monomer-monomer self-assembly resulting in dimers, and dimer-monomer assembly resulting in trimers as the simulation proceeds. Figure 4 shows the time evolution of the number of monomers, dimers and higher aggregates (trimers/tetramers) at this interface. Two rings are categorized as a dimer if the distance between their centers is 7 Å or less; the same applies for higher aggregates as well. The cut-off distance of 7 Å is derived from the radial distribution function, $g(r)$ of the peptide ring centers (not shown), which exhibits the first peak at around 4.5 Å and the second peak at around 9 Å. Figure 5 shows snapshots of interface I at $t = 0.050$ μ s and $t = 2.500$ μ s. A closer look at a self-assembly event resulting in the formation of a trimer at interface I is provided in Figure 6. The monomers and dimers are parallel to the interface while the trimers exhibit more non-uniform orientations (Figure 5). Since the alkane-water interface is in the x-y plane, the cosine of the angle (θ) between the normal to the ring plane and the interface normal (i.e., simulation box z-axis) represents the orientation of the ring with respect to the interface. If $\cos(\theta)$ is one, it implies that the ring lies with its molecular plane parallel to the interface, while a value of zero indicates that the ring is tilted with its normal being perpendicular to the z-axis. Figure 9 shows the average orientation of the rings for the last 0.300 μ s of the simulation. Only the absolute value of $\cos(\theta)$ is considered. While the monomers are almost completely parallel to the interface, dimers are slightly tilted, and the trimers and tetramers are significantly tilted with respect to the interface. This interface, with a high initial concentration of monomers and dimers, likely represents the initial aggregation stage of the self-assembly process.

Interface II, on the other hand, consists of 2 hexamers, 2 pentamers and 64 monomers at $t = 0.050$ μ s, and likely represents the self-assembly at a later stage (Figure 7). At $t = 0.800$ μ s, one hexamer and one pentamer join, resulting in a nanotube consisting of 11 rings (undecamer) (Figure 8). At $t = 1.200$ μ s, the second pentamer-hexamer pair also join to give a second stack consisting of 11 rings. Hence, we observe self-assembly of small tubes resulting in longer tubes at interface II. At this interface, the monomers lie with their planes parallel to the interface, but the long nanotubes are aligned parallel to the interface (Figure 7, Figure 8 and Figure 9). Figure 10 shows a snapshot of the simulation cell at $t = 2.500$ μ s with both the interfaces.

Discussion

Based on the multiple self-assembly events discussed above, we propose the following mechanism of nanotube self-assembly. The monomeric rings lie almost exclusively flat at the oil-water interface. They slide horizontally on top of other monomers, dimers and trimers to subsequently form dimers, trimers and tetramers, respectively. Further self-assembly is achieved by recombination of these stacks leading to long nanotubes lying almost parallel to the interface (Figure 9). This change in the orientation is favorable for the accommodation of all the amphiphilic rings in the tubes at the interfacial region, and seems essential for the joining of smaller stacks to create long nanotubes. The observation of the change in orientation of the rings at the oil-water interface is similar to the findings of Rapaport et al.¹⁸ for the air-water interface.

The formation of long nanotubes at interface II is an example of Oswald ripening²⁵ and seems to be governed by strong inter-subunit backbone interactions, while the formation of bundles of smaller tubes would be governed by strong interactions between the side chains. The present CG model of *cyclo* [(-LTrp-D-Leu-)₄] suggests that for this peptide, nanotube growth takes precedence over aggregation of smaller tubes into bundles. The competition between the hydrogen bond driven attachment between the cyclic peptides and the relatively weak interactions between the nanotubes has also been discussed by Rapaport et al.¹⁸ The present simulation study is consistent with these observations, even though the hydrogen bond interactions are only treated in a mean-field-like approach averaged over the backbone fragment of each amino acid. When interpreting the results at interface II, one has to take into account that the final length of the assembled nanotubes at 2.5 μ s turned out to be rather large compared to the total system size, so that finite size effects cannot be excluded.

In summary, we have shown that large scale CG-MD can be used to study the self-assembly of cyclic-peptide nanotubes at a liquid-liquid interface: a process currently prohibitively expensive to simulate with AA force fields. The employed CG-parameterization strategy can maintain a high level of specificity despite the loss of atomic detail. Systematic studies investigating the effects of modifying interactions between the side chains (i.e., by changing the peptide residues) are feasible with CG-MD. Given generous amounts of computer time, the CG models could conceivably be used as a design tool for novel nanostructures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Shown is the mapping of the cyclic peptide onto a coarse grained (CG) model. The all-atom (AA) representation is shown on the left and the CG representation on the right. Backbone is shown in blue, Trp side chain in red and Leu side chain in green. Hydrogen atoms have been removed from the AA picture for clarity.

Figure 2.

(a) Snapshot of the initial MD simulation cell created by replication (2×2) of a smaller system in the x- and y- directions, respectively. Peptide sidechains and solvent molecules are not shown for clarity. The solvent arrangement can be seen in (b). Monomeric peptides are shown in yellow, dimers in green and oligomers with more than two rings in red. The same color coding is used in Figures 3, 5, 7, 8 and 10 below. (b) Snapshot at $t = 0.0024 \mu\text{s}$. Half of the nonane (green) and water (blue) has been removed to reveal the peptide rings (yellow). While the peptide stack toward the right is still close to the initial vertical orientation, the stack toward the left is already tilting toward the lower interface (II).

Figure 3. Snapshot showing the two liquid-liquid interfaces at $t = 0.050 \mu\text{s}$. The peptide rings have all moved to the two interfaces, albeit with different concentrations (see Figure 2a for color coding of rings and Figure 2b for the solvent arrangement). Note the orientation of the peptide nanotubes (red) differs from that of the monomers (yellow) and dimers (green).

Figure 4.

Shown is the evolution of the number of peptide monomers, dimers and trimers/tetramers at interface I. At $t = 0.050 \mu\text{s}$, there are 82 monomers and 26 dimers at interface I. The number of monomers decreases and the number of dimers and trimers/tetramers increases, indicating multiple self-assembly events of the rings. After $2.50 \mu\text{s}$, there are 36 monomers, 35 dimers, 8 trimers and 1 tetramer.

Figure 5. Top view of interface I (left) at $t = 0.050 \mu\text{s}$ and $t = 2.500 \mu\text{s}$ (right), respectively. The black border represents the boundary of the periodic simulation box. A comparison of the two images reveals extensive aggregation, including the formation of trimers and tetramers, on the timescale of the simulation.

Figure 6.
Shown is an example of trimer formation at interface I.

Figure 7.

Top view of interface II at $t = 0.800 \mu\text{s}$ (left) and at $t = 1.2 \mu\text{s}$ (right). During this time interval the four short nanotubes (two pentamers and two hexamers) have dimerized to form two undecamer nanotubes. The black border represents the boundary of the periodic simulation box. No further aggregation was seen on the timescale of the simulation.

Figure 8.
Shown is an example of the fusion of a pentamer and a hexamer to produce a longer nanotube at interface II. Note that the time sequence runs clockwise starting at the upper left.

Figure 9.

Orientation distribution, $\cos(\theta)$ of the peptide rings based on their aggregation status for the last 0.3 μs of the MD trajectory, where θ is the angle between the normal to the cyclic peptide ring and z axis (normal to the liquid-liquid interface). The monomers at both the interfaces lie with their ring-planes parallel to the interface (x-y plane). The dimers, trimers and tetramers are at interface I: the trimers/tetramers exhibit significant tilting with respect to the interface. The undecamers (tubes with eleven rings) at interface II are oriented mostly parallel to the interface.

Figure 10. Snapshot of the two interfaces at the end of the MD simulation, $t = 2.5 \mu\text{s}$. The upper interface consists of monomers, dimers, trimers and a tetramer, while the lower interface consists of monomers and two undecamers.