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Computational Validation of Protein Nanotubes

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Abstract

We present a novel generic computational method to assess protein nanotubes with variable diameter sizes, at the atomic level, given their low resolution protomeric structures. The method is based on the symmetrical assembly of a repeating protein subunit into a nanotube. Given the protein unit-cell, and the tube diameter and helicity, we carry out all-atom molecular dynamics (MD) simulations, combined with a unique mathematical transformation. This allows us to mimic nanotubes of even huge sizes, without end or edge effects. All our simulation setups attempt to explicitly adhere to the conditions under which the experiments were conducted. Thus, we are able to obtain high resolution atomic-scale structures at reasonable computational costs. We expect that our approach would prove useful in assessing protein nanotubes, as well as *in silico* constructions of novel nano-biomaterials.

Introduction

In recent years, the growing need for nano-devices and medical applications has accelerated the design of novel bio-nanomaterials. Applications typically include membrane channels, scaffolding tissues for regenerative medicine, and targeted drug delivery systems.^{1,2} Protein building blocks are gradually becoming a choice for such systems due to their physical and chemical diversity, and the feasibility of tailoring them to specific needs.^{3,4} Due to advances in peptide synthesis and molecular engineering techniques, peptide self-assembly can obtain a vast range of nanostructures, such as fibers, tapes, ribbons, vesicles, and tubes.^{5–11}

Efficient modeling algorithms exploring the nanostructures conformational space can considerably shorten the design time. However, modeling methods depend on the system size.¹² As a result, the repertoire of computational validating techniques is limited. Several strategies have been devised to understand and predict the self-assembly mechanism, including geometric decomposition of a viral capsid assembly,¹³ local rules simulation of a viral capsid kinetics,¹⁴ and a prediction of the self-assembly based on Whiteheads Actual Entity idea.¹⁵ Most methods sample the conformational space to obtain the preferred states

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Supporting Information Available. Additional structural analyses of all systems; Illustration of tube-wedge transformations; Illustration of the HIV-1 CA protein P6 symmetry. This material is available free of charge via the Internet at <http://pubs.acs.org>.

of the nanostructures. Since size is a major modeling constraint, some coarse graining modeling techniques were used standalone,¹⁶ or in combination with MD simulations.^{17–20} These have limited resolution, and often neglect important chemical interactions. Other methods used all-atom Monte Carlo simulation to predict amyloid formation,^{21,22} membrane protein assembly,²³ or symmetrical protein assemblies.²⁴ Even though they can mimic the assembly process of several short peptide monomers, these techniques sample a relatively small portion of the macromolecule potential energy surface. Most all-atom sampling methods used MD simulations. These were used for prediction of amyloid formation from short A β segments,^{25,26} or for a nanotube constructed from β -helical motifs.²⁷ Although these methods provide detailed atomic molecular information, they are limited by size.

Peptide nanotubes are computationally challenging, as they are composed of a high number of identical subunits. They can be characterized by one of three possible schemes:²⁸ (1) A cyclic peptide self-assembles in one dimension, along the tube axis; (2) A unit-cell is made of an asymmetric unit that is repeated in two dimensions, both along the tube axis and along its wrapping axis; and (3) A unit-cell is repeated as above, but the nanotube is comprised of several asymmetric units, where each such unit consists of two self-assembling domains. So far, an all-atom approach using MD simulations was applied to the analysis of nanotubes derived from the first scheme only, as these hollow nanotubes are typically self-assembled from relatively short cyclic peptides,^{29,30} and therefore introduce a feasible computational challenge.

To address the computational validation of various nanotube types and sizes represented by any of the three schemes, we introduce a simple all-atom MD simulations protocol in combination with a unique mathematical transformation. Our approach utilizes the fact that protein nanotubes are assembled from a predefined protomeric unit, according to some symmetrical operation. Hence, throughout the simulation, we store the coordinates of our small protomeric unit-cell only, along with the predetermined transformations of its closest neighboring cells. These are used for the calculation of the unit-cell inter-molecular non-bonded interactions throughout the simulation. This principle was similarly used for analyzing the icosahedral capsid of the Human Rhinovirus.³¹ We demonstrate our approach on the AcV₆D nanotube, a surfactant-like peptide nanotube obtained using the second scheme,²⁸ which was experimentally observed by Zhang S. and his coworkers.³² As this peptide is negatively charged and the experiment was performed in a neutral pH, we analyze and compare the MD simulations of two AcV₆D systems which are neutralized in two distinct manners. We proceed by analyzing the MD simulations of HIV-1 CA protein nanotube derived from the third scheme of nanotubes,²⁸ in which each monomer consists of two self-assembling domains. The HIV-1 CA protein has been observed to self-assemble into nanotubes *in vitro*,^{33,34} and a low-resolution atomistic model of the hexameric unit has recently been obtained.³⁵ Yet, its macromolecular structure has never been addressed at the atomic level, due to its huge size. Hence, using our protocol, we achieve the highest resolution atomic structure of the HIV-1 CA protein nanotube.

Our approach allows an all-atom mimicry of the actual assembly of the nanotube building-block, and provides detailed structural information of the building-block ensembles. Simulating nanotubes represented by the second and third schemes, where each protomeric subunit can consist of a high number of amino acids, suggests that this protocol is capable of analyzing a broad range of nanotube types and diameters. Finally, by running MD simulations in explicit solvent, we can achieve highly accurate results, performed under similar environmental conditions, and at relatively low costs due to our new protocol.

The Concept

Tsai et al.³⁶ defined a procedure for constructing a nanotube, such that a protein subunit is arranged on a planar sheet by a 2D repeating pattern, and this sheet is virtually wrapped onto the surface of the tube. The 2D arrangement of the subunit on the planar sheet can be depicted by three lattice constants $\{a, b, \gamma\}$, which define a unit-cell. Then, the wrapping of the planar sheet is defined by two additional integers $\{n_1, n_2\}$, where n_1 represents the number of unit-cells that complete one full round of the tube along the lattice axis a , and n_2 represents the number of cells shifted along the lattice axis b after one full round (the helicity extent of the tube – Fig. 1).

The radius of the tube is therefore given by the equation:

$$r(\text{tube}) = \sqrt{(n_1 a + n_2 b \cos \gamma)^2 + (n_2 b \sin \gamma)^2} / 2\pi \quad (1)$$

Using these five parameters $\{a, b, \gamma, n_1, n_2\}$, we can define the transformations from a unit-cell to its closest neighboring cells (see Methods). These definitions permit preserving only the full-atomic coordinates of the unit-cell and using the defined transformations to calculate the energy contribution of the inter-cellular non-bonded interactions at each simulation step. Thus, we greatly reduce the computational resources needed to simulate a protein nanotube.

Results

AcV₆D surfactant-like peptide

The AcVVVVVVD is an acetylated seven residue surfactant-like peptide that forms nanotubes and vesicles.³² The peptide has a hydrophobic tail of six valines, and a short hydrophilic head of an aspartic acid. Since the N-terminus is acetylated, the peptide has a charge of -2 (the aspartic side-chain, and the C-terminus). According to the EM image, the peptides are organized in a quasi lipid-bilayer structure, such that the acetylated N-termini of each pair of peptides face each other, and the aspartic acids at the C-termini face the outer and inner surface of the tube. As suggested by Vauthey et al.,³² the initial structure of a single peptide was constructed in a β -strand conformation. To create a chemically stable unit-cell, eight peptides were placed in a bilayer organization, such that four peptides face the tube exterior, and the other four face the tube interior (Fig. 2). The complex was organized in two layers in order to maximize the non-bonded interactions within the cell. Bulk water was added explicitly on the cell exterior and interior.

Following experiment, the system had to be neutralized. Two systems were created and neutralized in a distinct manner, and their simulations were compared. In the first (the “charged system”), two counter-ions (Na^+) per peptide were added to the external and internal bulk of water, neutralizing the negative charges at the C-termini (Fig. 2a). The system final size was 1659 atoms. The second (the “uncharged system”) was neutralized by protonating the aspartic side-chain and by covalently binding N-Methylamide group to the C-terminus of each peptide (Fig. 2b): the final size was 1859 atoms. The chemical groups used to neutralize both systems are shown in Supplementary Fig. S1.

After minimization of both systems, the lattice constants a, b, γ were $\{12.0\text{\AA}, 17.0\text{\AA}, 90.0^\circ\}$. These parameters suggest that the AcV₆D unit-cell self-assembles with approximate C2 plane group symmetry to create a nanotube. According to the EM image the AcV₆D nanotubes have outer diameters of 30nm – 50nm. Following the methodology of Tsai et al.,³⁶ the number of unit-cells that wrap around a tube was set to 85 and the helicity extent set to 0. These reflect an outer tube diameter of ~32nm, which is in the above range.

Fig. 3 presents approximately a third of a full round of the uncharged system. The peptides placed in a bilayer organization are surrounded by water molecules in the interior and exterior of the tube. Both systems were simulated for 50ns. To analyze the ability of both nanotube systems to preserve their β -strand conformation, the β -strand order parameter was measured along the simulation³⁸ (Supplementary Fig. S2). This orientation order parameter is defined by equation 2:

$$S_{\beta} = \frac{1}{N} \sum_{k=1}^N \left(\frac{3\cos^2\theta_{\alpha} - 1}{2} \right) \quad (2)$$

Where θ_{α} denotes the angle between the positional vectors that connect two C_{α} atoms, and N denotes the total number of vector pairs. In both systems, the four interior peptides had higher β -strand order parameter values, compared to the four exterior peptides, due to the higher compactness of the peptides towards the center of the tube. However, both interior and exterior peptides of the charged system had less fluctuations of the β -strand order parameter, compared to the uncharged system. This can be explained by the C-terminal neutralizing groups (N-Methylamide) that can create steric clashes. To compare the behavior of the interior and exterior peptides of both systems, the root mean-square fluctuations (RMSF) were measured for each of the seven residues of the eight peptides in the unit-cell (Supplementary Fig. S3). The fluctuations of the interior peptides in both systems decrease from residue 1 to 7, towards the center of the tube. This can be explained by the higher compactness of the interior residues, compared to those of the exterior ones. For this same reason, on average, the RMSF of the exterior peptides tends to increase towards the outside of the tube. The exterior peptides of the uncharged system have higher RMSF values than those of the charged system. This result supports the steric effect of the hydrophobic neutralizing N-Methylamide groups at the C-termini of the uncharged system. To conclude our analysis of both AcV₆D nanotube systems, we measured the radius of gyration (RGYR) of the C_{α} atoms and the C_{α} RMSD of the four interior and the four exterior peptides. RGYR calculates the root mean-square distance of the C_{α} atoms from their center of mass. Therefore it can be used as a qualitative indicator for the conservation of the bilayer structure of the AcV₆D nanotube. Supplementary Fig. S4 presents the RGYR measurements of the uncharged system over the 50ns of simulation. It is evident that the average RGYR of the interior peptides and its deviation are slightly lower than those of the exterior peptides. These findings are compatible with the higher compactness of the interior vs. the exterior peptides. In addition, the steadiness of the total RGYR along the simulation indicates that the exterior peptides maintain their structural position with regard to the interior peptides. Hence, the number of peptides chosen to occupy the unit-cell is optimal. To summarize, the comparison of the two distinct AcV₆D systems at the atomic level shows that both systems maintain structural stability. However, the charged system better preserves its initial β -strand conformation over time, and is therefore considered to maintain stability better than the uncharged system.

HIV-1 CA Protein

The HIV-1 CA protein self-assembles into a cylindrical shape *in vitro*, and a conical shape *in vivo*.³³ Its atomic model is arranged in approximate P6 plane group symmetry. To construct a nanotube wedge, we used the 9Å-resolution atomic model obtained from a cryoEM image by Ganser-Pornillos BK. and coworkers.³⁵ Every chain in this model contains 219 residues. Each monomer has a clearly distinguished N-terminal domain (NTD) and a C-terminal domain (CTD), connected by a short linker of six residues. Six NTDs associate to form a hexameric ring, while the CTD of each monomer connects the hexameric

ring to one of its neighboring rings, forming a dimer³³ (see illustration in Supplementary Fig. S5). We therefore chose a single hexameric ring as a unit-cell for a nanotube.

After 250 minimization steps of Steepest Decent and ABNR in vacuum, we solvated our hexameric ring in a truncated hexagonal pyramid-like shape of water box (Fig. 4), and added 24 sodium counter-ions to the bulk of water to neutralize the unit-cell negative charge. As seen from the exterior of the tube, the hexameric ring has a flower-like shape, with a central cavity of radius 8.55Å. The solvated system contains 57507 atoms altogether, and the protein alone contains 20580 atoms. After minimization in explicit solvent, the lattice constants $\{a, b, \gamma\}$ were set to $\{92.7\text{Å}, 92.7\text{Å}, 120.0^\circ\}$ as determined by.³⁵ To coincide with the cryoEM image, the number of unit-cells that wrap around a tube was set to 12 and the helicity extent was set to 0. These parameters reflect an outer tube radius of ~17.7nm. Fig. 5 presents three perspectives of approximately a quarter round of the tube at the atomic level, without the solvent. In Fig. 5a, three rows of hexameric cells are displayed, as seen from the interior and exterior of the tube, and in Fig. 5b, the same cells are seen from the tube axis perspective.

Looking at the tube exterior (right), the hexamers of the NTDs protrude. The tube interior perspective (left) reveals the dimeric interfaces of the CTDs that connect each hexamer to its neighbor.

Fig. 6 presents two perspectives of the HIV-1 CA protein unit-cell along the 10ns simulation at times 1ns, 5ns, and 10ns. It is clear from both angles that the monomers maintain the non-bonded interactions with their neighboring monomers along the simulation. Furthermore, the flower-like structure of the hexameric ring and the 3D structure of the central cavity maintain their shape. Fig. 7 presents the analysis of two structural characteristics that support this behavior.

Neglecting the first 1ns of the simulation, the average C α RMSD measured for all the six monomers of the HIV-1 CA protein unit-cell is 1.00Å (Fig. 7a). In addition, we measured the backbone RMSF for each of the 219 residues of the monomers (Fig. 7b). A significant peak is observed around residues 85–93 in all monomers. This region has a coiled-coil structure in the NTD, and is exposed to the exterior solvent. Previously, it has been identified as the binding site for the human enzyme Cyclophilin A,^{39,40} which *in vivo*, plays an important role in the degradation of the capsid during the HIV infection. The increase in fluctuations in the solvent-exposed termini is also expected. To conclude our structural observations, we counted the number of intra-molecular hydrogen bonds (HB) of the HIV-1 CA protein unit-cell (Supplementary Fig. S6). The average number of HBs of each monomer is 168, and that of the whole unit-cell is 1010, with a standard deviation of 24. These measurements indicate that the structural stability of the HIV-1 CA protein is preserved.

Discussion

We have developed a method that predicts the stability of a protein nanotube, based on the symmetrical assembly of identical subunits. The method performs MD simulations on the full coordinates of a single protein subunit, while maintaining the transformations of its closest neighboring subunits. These transformations allow the calculation of the inter-molecular non-bonded interactions of the subunit at every simulation step. Hence, by simulating only a small subsystem of the nanotube assembly, we are able to greatly reduce the computational complexity of the protein nanotube analysis and make its assessment feasible. Our method has been applied to systems with approximate local C2 and P6 lattice symmetries, but is not restricted to these symmetries and can be extended to assess other

types of symmetrical systems where all subunits are identical, and where these are assumed to self-assemble into a nanotube. Furthermore, the mathematical transformations used can be modified to handle other nanostructures, such as spheres and ellipsoids. Such a new mathematical layer can facilitate the validation of existing bio-macromolecules, such as viral capsids, and vesicles.

Several works have investigated the nature of the HIV-1 CA protein. As it is composed of two domains – NTD and CTD, joined by a short flexible linker, several experimental studies have analyzed the behavior of the wild-type, as well as mutants in the NTD or CTD.^{41,42} To demonstrate the capability of our computational approach, we chose to analyze the full CA protein as a powerful example. Here we use the wild-type protein. Our approach can be further used for analyzing the capsid's mutants at the atomic level, thereby shedding light on their properties and functionality.

We have tested our approach on well investigated systems that were previously addressed in the lab.^{32,33} Since EM images exist, we could determine their diameters, and were able to calculate the number of subunits needed to complete one full round of a tube (n_1), as well as the tube's helicity extent (n_2). The results reveal that nanotubes of variable diameters and sizes of asymmetric protomeric subunits can be assessed with this protocol. The approach can be extended to new systems comprised of newly devised oligomers, and can therefore be used to predict the stability of novel self-assembled nanotubes. However, since the diameter of a novel nanotube is unknown, the values of n_1 and n_2 must be predicted, as well. In most cases, nanotubes observed in the lab have a range of possible diameters. Therefore, setting a reasonable value for these wrapping parameters may become a feasible task.

Methods

Infrastructure Definition

To carry out our methodology, we used the Image Facility of CHARMM,³⁷ which enables calculating periodic boundary effects for finite point groups. The initial step of setting the infrastructure for MD simulation of a nanotube was to define the mathematical transformation layer from the computational method of Tsai et al.³⁶ (Fig. 1) to a format that can be interpreted by CHARMM's image facility. Hence, given the five parameters $\{a, b, \gamma, n_1, n_2\}$ we can calculate the transformations of the neighboring images of a unit-cell as follows:

Let θ denote the angle of a tube's wedge. Then $\theta = 2\pi/n_1$. Let $\vec{y}$ be the tube axis, and let $\vec{z}$ be the axis parallel to the tube radius. Since n_2 specifies the helicity of one full round, let δ denote the rotation angle of the cell around axis $\vec{z}$. Then $\delta = 2\pi n_2/n_1$. Now, using angles θ and δ we can define the rotation angle and the translation fraction of b . Both define the transformations of the neighboring images (Supplementary Fig. S7), such that:

$$\{\theta_{left} = -\theta \cos \delta; T_{left} = \sin \delta\} \text{ and } \{\theta_{up} = -\theta \cos(\gamma + \delta); T_{up} = \sin(\gamma + \delta)\},$$

where: θ_{left} , T_{left} denote the rotation angle and translation fraction along the wrapping axis, and

θ_{up} , T_{up} denote the rotation angle and translation fraction along the tube axis.

To demonstrate the capability of our new approach, we chose two well-investigated protein systems which self-assembled in the lab to form nanotubes. Our goal was to construct a system for MD simulation that will coincide with the experiments, and will represent the environmental conditions of the experiment accurately. Therefore, our first step was

surrounding each protein unit-cell explicitly by solvent molecules, such that its assembly with the neighboring images will form a nanotube. An additional constraint was the desired stability of a single wedge of a nanotube during the simulations. Accordingly, the protein unit-cell by itself, considering its inter-atomic and intra-atomic non-bonded interactions, had to be stable. The construction of a unit-cell imposed the values of the lattice constants $\{a, b, c, \alpha, \beta, \gamma\}$. Then the tube's wrapping parameters $\{n_1, n_2\}$ were set according to the tube diameters observed by the corresponding EM images. To optimize the lattice constants, we used the CHARMM program³⁷ with the CHARMM22 Force Field to run "Steepest Decent" followed by "Adopted-Basis Newton-Raphson" method, to minimize the unit-cell, considering the non-bonded interactions with its neighboring images.

After minimization, the systems were gradually heated from 0°K to 300°K, for 30ps, applying harmonic force constraints on all alpha-carbons, and image centering. The SHAKE algorithm was used to restrain the carbon-hydrogen bonds, and a harmonic cylindrical tube constraint was applied both on the outer and inner boundary of the wedge. This potential, from the MMFP facility of CHARMM, was used to restrain the water molecules at the boundaries from escaping to the outside and inside vacuum. The Verlet leap-frog integrator was used with a time-step of 1fs, applying an isotropic periodic sum method for calculating the long-range interactions. The non-bonded lists and the image lists were updated every 20 steps. After heating, the systems were allowed to equilibrate for 140ps, gradually decreasing the harmonic restraints force constant to zero. During the analysis run, the temperature remained constant on average, with occasional velocity scaling. The analysis period was set differently for each system, according to its size and the assumed number of non-bonded interactions, which have the highest cost-effect on the calculations. The CHARMM program³⁷ was also used for heating, equilibration, production run and analysis. Simulating 1ns of the HIV-1 CA protein on the LoBoS supercomputers at the NIH took approximately four days, whereas, simulating 1ns of the AcV₆D systems took approximately two hours. The resulting images of our structures were obtained by VMD.⁴³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A sketch that illustrates the 2D lattice wrapping system. A single 2D cell is highlighted with the angle γ between the two axes, $\vec{a}$ and $\vec{b}$. Using the three lattice constants $\{a, b, \gamma\}$ and the two tube wrapping parameters $\{n_1, n_2\}$, the radius of the tube is given by equation 1. The sketch presents an example of wrapping the planar sheet with parameters $\{n_1 = 7, n_2 = 4\}$. The figure was adapted from ³⁶.

Figure 2.

The unit-cells of two systems of AcV₆D that were validated by MD simulations. Each system contains eight peptides of seven amino acids each in a bilayer structure (displayed in ribbon), such that four peptides are in the outer layer, and four peptides are in the inner layer. The layers face each other through the acetylated N-termini, whereas the aspartic acids at the C-termini (in red) face a bulk of water (blue beads). Each peptide is ~2nm in length, so that the width of the bilayer is ~4nm. (a) Each peptide contributes a total charge of -2, due to the aspartic acid at the C-terminus. To neutralize the system, eight counter-ions of Na⁺ (yellow beads) were added to each (exterior/interior) bulk of water. The unit-cell size is 1659 atoms. (b) Each aspartic side-chain is protonated and the C-termini are neutralized by N-methylamide. The unit-cell size is 1859 atoms.

Figure 3. Approximately one third of a full round of the uncharged AcV₆D nanotube. The full atomic coordinates were produced using the “Image Facility” of CHARMM³⁷ after the equilibration phase.

Figure 4.

The HIV-1 CA protein unit-cell solvated in water. The self-assembled unit-cell is comprised of six monomers (highlighted in different colors), solvated in water (blue beads), and neutralized by sodium counter-ions (red beads). Each monomer has 219 residues, so that there are 1314 residues in the whole unit-cell, and a total of 57507 atoms including the water molecules and the counter-ions. The structure is an outcome of the equilibration phase. Two perspectives are shown: (a) as viewed from the exterior of the tube. Since the CTDs point to the interior of the tube, only the NTDs are seen. The N-termini are located on the outer surface of the tube. The surrounding solvent is of hexagonal shape. (b) The sixfold axis is vertical. Both the NTDs and the CTDs of the monomers are seen. An arrow marks the binding site for the human enzyme cyclophilin A.

Figure 5.

Approximately a quarter round of the self-assembled HIV-1 CA protein nanotube. The wrapping parameters are $\{12, 0\}$. These define the outer radius as $\sim 17.7\text{nm}$. The proteins are displayed in licorice, without the solvent. The NTDs of the monomers are in red, whereas the CTDs are in green. The full atomic coordinates were produced after the equilibration phase. (a) Three by three cells are seen from the tube interior (left) and exterior (right) perspectives. The interior view shows the dimeric associations of the CTDs in green, whereas the exterior view shows the hexameric associations of the NTDs in red. An illustration of the perspective angle appears between the two views. (b) A top view illustration of the three by three cells that self-assemble into a quarter round of a nanotube. The NTDs, in red, stick out from the tube exterior, whereas the CTDs, in green, point toward the tube interior.

Figure 6.

Snapshots of HIV-1 CA protein unit-cell taken at simulation times of $t = 1, 5$, and 10 ns. The upper figures are views of the cell from the tube exterior perspective, whereas the lower ones are the corresponding lateral views. The monomers are in ribbon display, and each monomer appears in different color. The water solvent is in blue beads and the sodium counter-ions are in red beads.

Figure 7.

Structural characteristics of the HIV-1 CA protein measured along 10ns of simulations. (a) The C α RMSD. After approximately 1ns simulation the graphs of the monomers reach a plateau. The average C α RMSD, neglecting the first 1ns is 1.00Å. (b) The average RMSF was calculated for each of the 219 amino acids of the monomers. The six identical monomers that build-up the unit-cell are labeled M1 to M6. A significant fluctuations peak is observed around residues 85–93 in all of the monomers. This region has a coiled-coil structure, and is exposed to the outside solvent of the tube. In addition, there is an increase in fluctuations towards the N-terminus and C-terminus regions in all of the monomers.