

Electrostatically-Directed Self-Assembly of Cylindrical Peptide Amphiphile Nanostructures

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We present theoretical studies of peptide amphiphile nanostructures created by Stupp and co-workers [Hartgerink, J. D.; Beniash, E.; Stupp, S. I. *Science* **2001**, 294, 1684] and show that these amphiphiles exhibit *attractive* electrostatic interactions between their hydrophilic headgroups. These interactions prevail in their competition with the hydrophobic attraction between the amphiphile “tails” for the shape of the self-assembly, leading to cylindrical micelles of nanoscale dimension. The theory is supported by Monte Carlo simulations which show that in the absence of the directional electrostatic interactions between the headgroups the amphiphiles self-assemble into spherical micelles, in accord with our recent formal calculations [Tsonchev, S.; Schatz, G. C.; Ratner, M. A. *Nano Lett.* **2003**, 3, 623], whereas inclusion of the electrostatic interactions leads to cylindrical nanostructures.

1. Introduction

Nanoscience has made great advances in recent years, mostly due to the development of new experimental techniques for fabrication of new materials based on supramolecular structures of nanoscale dimension.^{1,2} The fabrication of these materials uses the so-called “bottom-up” approach, building the material through self-assembly of the constituent nanoparticles into structures with a specific desired shape, unlike the traditional approach of building fine structures by starting from bulk material and manipulating its shape to the smallest possible dimension. The new approach is similar to the way in which biological tissues are formed, and it can, therefore, be of great importance in the fabrication of materials for use in medicine and biotechnology, e.g., for tissue repair or as templates for creation of other materials with a specific biological function. Thus, it is very important to understand how the molecular characteristics of the constituent elements of a given structure predetermine its final shape and properties.

Despite the increasing number of newly reported nanomaterials with important and useful properties, however, theoretical studies of the relationship between the characteristics of the starting molecules at certain conditions, and the final shape and dimension of the self-assembly are in a nascent stage. The lack of theoretical methods to study such self-assembly phenomena has forced experimentalists working in the field to rely mostly on their previous experience and chemical intuition when synthesizing a new nanomaterial. In this fashion, a remarkable set of new materials has been synthesized by Stupp and co-workers.^{3–5} The materials were designed to template bone nanostructure and were created by self-assembly of peptide amphiphiles (PA; shown in Figure 1) into cylindrical fibers, which after stabilization through covalent bonding are subsequently able to direct the mineralization of hydroxyapatite in the same alignment as observed between collagen fibrils and hydroxyapatite crystals in bone. Stupp and co-workers expected the formation of the cylindrical fibers based on geometric

packing considerations developed in earlier theoretical work,^{6,7} where the geometric packing problem was addressed by some approximations. However, recently, we showed that a rigorous solution of the geometric packing problem is possible and leads to the conclusion that the spherical micelle would be the minimum-energy structure of the self-assembly of particles with the shape of the PAs used in the above experiments, within the simple constraints of the geometric packing picture.⁸ Thus, the important question remained: Why do the PAs used in the above experiments form cylindrical, rather than spherical, micelles? Here, we will show that this is due to a peculiar directional electrostatic interaction between the headgroups of the PAs, which is *attractive*, unlike the case of most micelles, where the hydrophilic headgroups repel. In the case of the PAs considered here, the electrostatic interaction between the hydrophilic heads competes with the hydrophobic attraction between the tails of the amphiphiles. In this competition, the electrostatic interaction prevails, leading to a cylindrically shaped micelle.

The structure of this paper is as follows. In section 2, we show results from Monte Carlo simulations on a model of the PAs' self-assembly involving hydrophobic interactions between the alkane tails and excluded volume interactions only; these always lead to the formation of spherical micelles, in accord with our recent prediction.⁸ Then, in section 3, we show that under the specific conditions of the experiment,³ namely, the pH of the solution, large dipoles, roughly perpendicular to the backbone of the PA, appear in the hydrophilic heads, and simple calculations regarding the interactions between them lead to the conclusion that the cylindrical micellar shape is the most stable one. This conclusion is strengthened also by considering the possibility for hydrogen bonding between the peptides in the headgroups, which could align with the large dipoles and stabilize the cylindrical shape of the micelle. In section 4, we present results from Monte Carlo simulations on the cylindrical PA self-assembly, including electrostatic, as well as hydrophobic and excluded volume, interactions. The cylindrical micelle is stabilized by the electrostatic interactions, with the PAs' surface dipoles oriented in an antiparallel fashion along the axis of the

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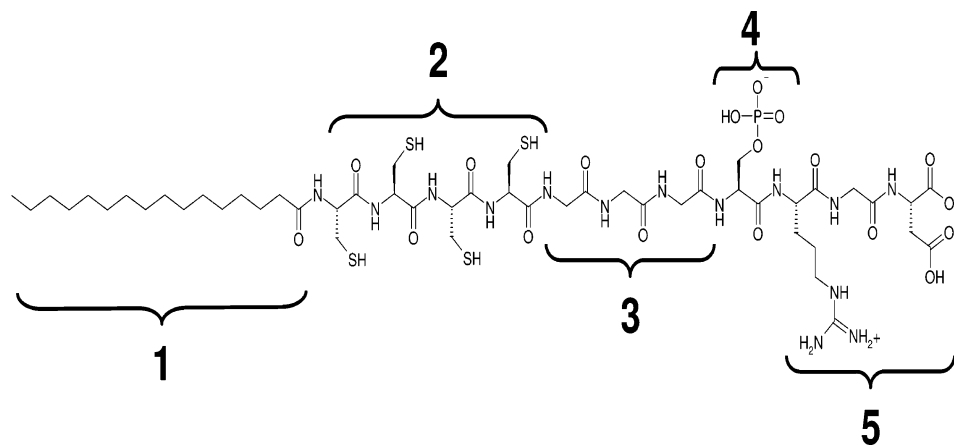


Figure 1. Chemical structure of the peptide amphiphiles from ref 3 used to form cylindrical micelles. Region 1 is the hydrophobic alkane tail, region 2 contains four cysteine residues, which can be oxidized to polymerize the assembly, region 3 provides flexibility of the molecule, region 4 is designed to direct the mineralization of hydroxyapatite, and region 5 is designed to promote cell growth.

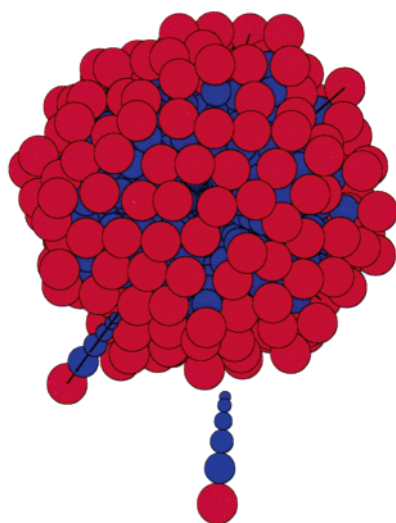


Figure 2. Snapshot of the self-assembled micelle after the structure has equilibrated. The hydrophilic headgroups (red) are distributed on the surface, whereas the hydrophobic tails (blue) form the inner core.

cylinder. In the last section, we outline possible directions and applications of our studies.

2. Modeling and Monte Carlo Simulations of Self-Assembly of Amphiphiles Involving Hydrophobic and Excluded Volume Interactions

We have shown using geometric constructions that PAs of conical or truncated-conical shape whose self-assembly is hydrophobically driven would form micelles of spherical shape as their most stable structures.⁸ Here we perform Monte Carlo simulations on a model of the PAs created by Stupp and co-workers³ and numerically confirm that this is still the case when entropic effects are included. For computational convenience and speed, we will model the amphiphiles as a string of spheres of decreasing radii starting from the hydrophilic head (rather than cones). From now on, we will call these “lolly pops”, for simplicity. Related “string of beads” models have been used previously by Vekhter and Berry and others to model self-assembly in proteins (see ref 9 and references therein). The decreasing spherical radii are chosen such that each lolly pop fits inside a truncated cone, as can be seen in Figure 2, where the biggest sphere of the hydrophilic headgroups is shown in red, whereas the rest of the spheres are blue. The length of each molecule (about 4 nm) is chosen as the unit length; that is, the

distance between the center of the largest sphere and the tip of each lolly pop is taken to be unity. In these units, the radius of the largest sphere is 0.18 and the radius of the smallest one is 0.05. The hydrophobic tail's length is taken as 0.44, from the tip of the lolly pop. The intervening distance of 0.38 units, represents the cysteine and glycine groups denoted (2,3) in Figure 1. The particles interact via excluded volume and hydrophobic interactions; the former consisting of hard core repulsion between the spheres of each two lolly pops, whereas the later interaction between two particles is modeled by the form⁶

$$E_h = -\gamma e^{-D/\lambda} \quad (1)$$

In the above equation, γ is a surface tension constant which is treated as a free parameter used to calibrate the equation so that it gives the proper hydrophobic energy per particle in the hydrophobic core of the final assembly, which in our case (15 methyl groups in the tail) is about 20 kT at 298 K. After such a calibration, our γ is taken as 0.7787 in units of kT at 298 K. D is the distance between the tails of the lolly pops, defined as the minimum of the average distance between the center of the smallest sphere or the position along the lolly pop axis located 0.44 units from the tip of the lolly pop, and the corresponding points at another lolly pop with which the first one interacts, with the distances starting at the center of the smallest sphere shortened by its radius, and the distances starting from the end of the hydrophobic section shortened by the distance between that point and the surface of the truncated cone around the corresponding lolly pop. λ is a measure of the range of the hydrophobic interaction, and for most systems, it varies between 1 and 2 nm; here we have taken λ to be 0.5, that is, 2 nm. We have also put a cutoff for the hydrophobic interaction for distances $D > 2.5$, above which the interaction energy is negligibly small.

We put a large number, N , of such particles in a cubic box of length $L = 30.0$, distributed at random, except for a fraction of them which are put in the center of the box in close proximity of each other to serve as a nucleation kernel for the micelle. Then the particles are moved one at a time at random, with the motion biased along the length of the particle; this is done in order to speed up the simulation after the anticipated micelle is formed. We run a simulation of 2×10^9 steps, and as expected,⁸ after an equilibration period, we observe the formation of an approximately spherical self-assembly. A snapshot of the equilibrated structure is shown in Figure 2. The assembly

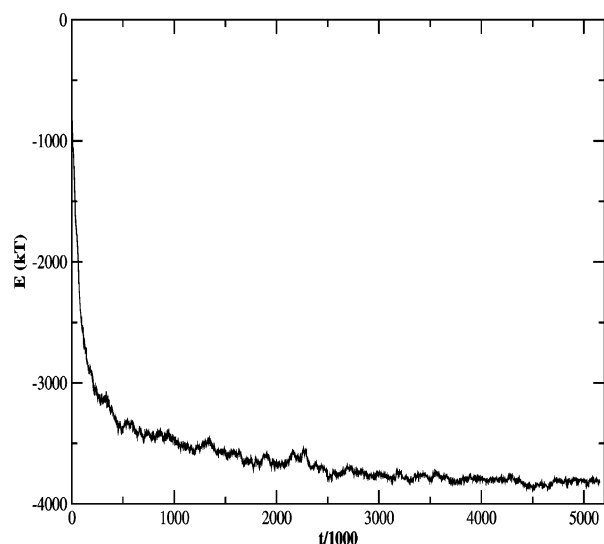


Figure 3. Total energy of the system including hydrophobic and excluded volume interactions only, in units of kT, at 298 K, as a function of the number of simulation sweeps t .

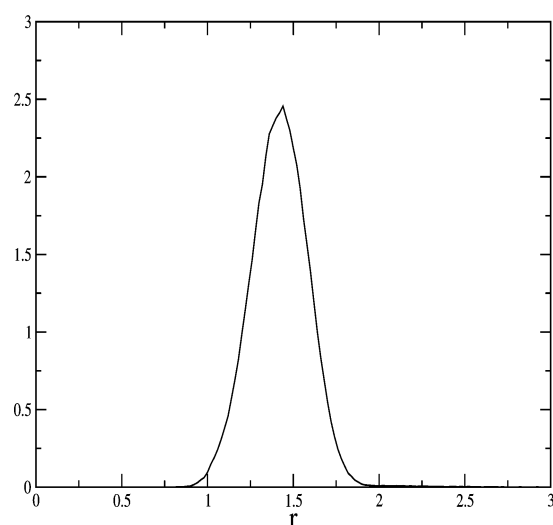


Figure 4. Distribution of distances between the center of each lolly pop head and the center of mass of all lolly pop heads, r , for the equilibrated structures including hydrophobic and excluded volume interactions only, after 4×10^6 sweeps. The narrow width of the distribution demonstrates the spherical shape of the structure.

appears to be a dynamical structure, with particles constantly leaving and entering it, whereas the overall shape of the structure is preserved. In Figure 2, there are a total of $N = 194$ particles in the system, almost all of them being a part of the spherical micelle. In Figure 3, we show the energy of the system as a function of the simulation time, presented in terms of simulation sweeps, where a sweep is defined as N particle moves. To demonstrate the spherical shape of the micelle, in Figure 4, we present a histogram of the distribution of distances between the centers of the largest sphere in each lolly pop and the center of mass of the sum of all such spheres. The narrow distribution of these distances emphasizes the spherical shape of the micelle. We should also note that the Monte Carlo simulations lead to the same kind of a spherical structure even if they start from a cylindrical micelle, such as the one shown in Figure 7, as their initial configuration. Also, starting with a larger number of particles and nucleation kernels leads to the formation of multiple spherical micelles, rather than one single macro-phase, which confirms the contention that the spherical micelle is indeed the thermodynamically most stable structure.

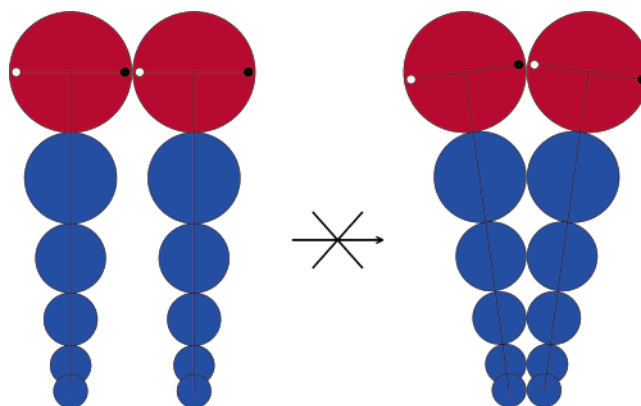


Figure 5. Two parallel zwitterionic amphiphile lolly pops with their dipoles aligned behind each other would remain parallel because the energy stabilization due to the electrostatics in this formation would outweigh the stabilization due to the hydrophobic interaction if the process to the right were to occur. The opposite charges of the dipoles are shown in black and white.

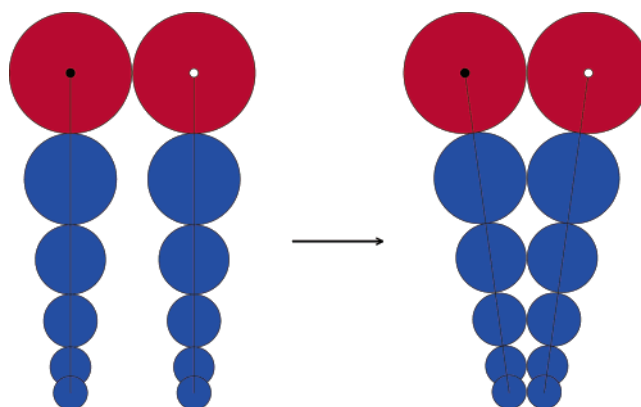


Figure 6. Two parallel zwitterionic amphiphile lolly pops with their dipoles perpendicular to the plane of view would tend to tilt with respect to each other, and the process to the right would occur because of the stabilizing effect of the hydrophobic interaction, which in this case is unopposed by the electrostatics.

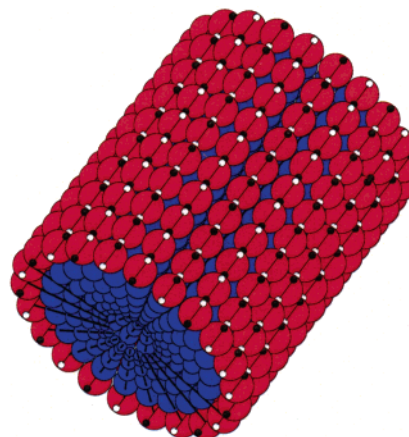


Figure 7. Part of a cylindrical micelle expected to be formed by the amphiphiles at 0 K, based on the considerations illustrated in Figures 5 and 6. The dipoles in the headgroups are shown oriented in an antiparallel fashion on the surface and along the axis of the cylinder.

The formation of a spherical structure is expected also based on the symmetry of the particles, which does not favor different radii of curvature of the formed micelle. Thus, both analytical calculations⁸ and Monte Carlo simulations predict that particles of conical or truncated-conical shape, which interact via hydrophobic and excluded volume interactions only, would form

spherical micelles as their most stable structures. At this point, the question about why the particles synthesized by Stupp and co-workers³ form cylindrical micelles remains open. We offer an answer to that question in the following section.

3. Inclusion of the Electrostatic Interactions between the Amphiphiles

Let us consider more closely the experiment which produced the cylindrical nanofibers discussed here.³ The molecules shown in Figure 1 were synthesized in solution whose pH then had to be lowered to below 4 in order to achieve self-assembly. Looking at parts 4 and 5 of the amphiphile molecule shown in Figure 1, we see that its hydrophilic head has two carboxylic groups, an amino group in the arginine (part 5), and a phosphate group in part 4. The pK_a values for the carboxylic groups are around 4, depending on the molecular residues to which they are attached, whereas the pK_a for the phosphate, a stronger acid, is about 2. Therefore, at a pH above 4, the hydrophilic head would possess a negative net charge, as it would have both the carboxylic groups and the phosphate negatively charged (a charge of -3), whereas the arginine part would be neutral or have a charge of $+1$, depending on the pH. Lowering the pH to about 3, as done in the experiment,¹⁰ leads to a zwitterionic amphiphile headgroup, with the carboxylic groups neutralized, a positive charge in the arginine part, and a negatively charged phosphate group. Not only is the overall charge of the amphiphiles under such conditions zero, but the two charges form a large dipole in the hydrophilic head which is roughly perpendicular to the backbone of the molecule, creating the possibility for attractive interactions between the hydrophilic headgroups in a self-assembly.

Consider the arrangement of two neighboring lolly pops, as shown in Figure 5. When the dipoles of the lolly pops are aligned behind each other, there is a possibility for a very strong attractive interaction between neighboring opposite charges. If, then, the lolly pops were tilted toward each other by rotations around the axes passing through the centers of the largest spheres in their headgroups and perpendicular to the plane of view, as shown in the right-hand side of Figure 5, their tails would come closer together, which would stabilize this formation due to the hydrophobic interaction. However, if such a tilt were to occur, the neighboring opposite charges of the dipoles would have to be separated farther, which would destabilize the structure. A simple calculation shows that if the process shown in Figure 5 were to occur then the electrostatic energy would increase by about 2.0 kT, at 298 K, whereas the hydrophobic energy would be lowered only by about -0.25 kT, as calculated from eq 1 and the Monte Carlo simulation described in section 2. Thus, we have a competition between attractive electrostatic dipolar interactions between the hydrophilic heads, tending to keep the amphiphiles parallel to each other, and attractive hydrophobic interactions between the tails, tending to tilt the amphiphiles toward a circular formation. The electrostatic interactions prevail, leading to the parallel structure, shown on the left-hand side of Figure 5, as the more stable one. It is important to note that this structure would be strengthened even further by the possible formation of hydrogen bonds between the amphiphile headgroups. Part 3 of the molecule, shown in Figure 1, is rather flexible, which allows the hydrogen bonds between the rigid parts 2 of two neighboring molecules to orient in the same direction as their dipoles and thus to further stabilize the micelle in that direction, forming an effective beta-sheet.

Let us now consider the interactions between two lolly pops from a different direction, perpendicular to the previous one,

in which the dipoles are perpendicular to the plane of view, as shown in Figure 6. In this case, rotating the lolly pops around the axes of the dipoles would bring the hydrophobic tails together, leading to stabilization of the tilted structure, just as before; however, the distance between the charges would now remain unchanged, and therefore, the electrostatic energy change would be zero and would not interfere in this process. Thus, as shown in Figure 6, this time the process to the right would occur and the lolly pops would tend to assemble in circular formations in the direction perpendicular to their dipoles.

These considerations suggest that both electrostatic and hydrophobic interactions between the amphiphiles considered here are attractive and thus favor self-assembly; however, they compete for the shape of the resulting nanostructures. The electrostatic dipolar interactions, which favor parallel formations in the direction along the dipoles of the headgroups, win this competition, whereas in the direction perpendicular to the dipoles, the hydrophobic interactions favor the circular assembly, unopposed by the electrostatics. As a result, the most stable micellar structure formed by these amphiphiles would be a cylinder, with an infinite radius of curvature in one direction, and a radius of curvature approximately equal to the length of the amphiphile in the other. Such a structure is shown in Figure 7, where the headgroup dipoles are, naturally, shown oriented in an antiparallel fashion on the surface and along the axis of the cylinder. From an alternative viewpoint, we could also consider the dipoles formed under the right conditions of the experiment as breaking the symmetry of the amphiphiles considered in section 2, thus leading to a preferable direction of self-assembly, in which direction the amphiphiles tend to stay parallel, while forming circular structures in the perpendicular direction, which defines a cylindrical formation.

4. Monte Carlo Simulations of Electrostatically Interacting Amphiphiles in a Cylindrical Self-Assembly

To support the considerations of the previous section, we completed Monte Carlo simulations on electrostatically interacting lolly pops in a cylindrical self-assembly, including also the hydrophobic and excluded volume interactions described in section 2. We start from an initial configuration in which the lolly pops form a cylindrical structure, such as the one shown in Figure 7; however, we distribute the orientations of the dipoles on the surface of the cylinder at random. The lolly pops form circular arrangements perpendicular to the axis of the cylinder containing 22 particles each, whereas the distance between the centers of two such neighboring rings is 0.365. We have taken 10 such circles in total, having 220 particles in the system, which, as in the simulation described in section 2, is placed in a cubic box of length $L = 30.0$. The charges, placed at the edge of the hydrophilic headgroups of the amphiphiles, interact according to

$$E_{\text{el}} = \frac{q_1 q_2 e^{-r/\kappa}}{4\pi\epsilon_0 \epsilon r} \quad (2)$$

where q_1 and q_2 are the two interacting charges separated by a distance r , ϵ_0 is the dielectric permittivity of free space, ϵ is the dielectric constant of the solution, taken as 78.5, and κ is the Debye length, which we have taken as 1.70, that is, 68 Å, which corresponds to a 0.002 M solution of simple monovalent ions. We have introduced a lower limit cutoff for the interaction distance r of 0.005, below which the interaction energy does

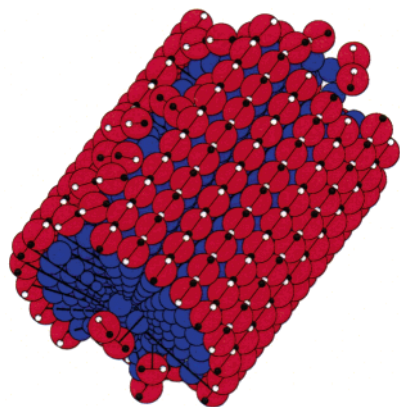


Figure 8. Snapshot of an equilibrium configuration of the cylindrical micelle. Most dipoles are oriented in an antiparallel fashion on the surface and along the axis of the cylinder.

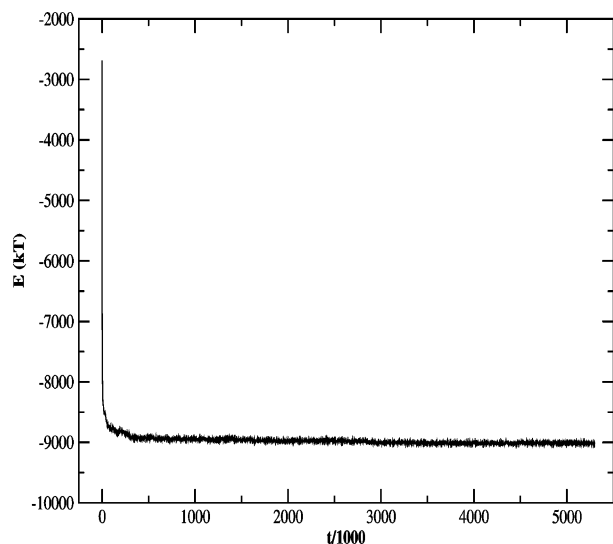


Figure 9. Total energy of the electrostatically interacting system in units of kT, at 298 K, as a function of the number of simulation sweeps t .

not change. The simulation is run for 2×10^9 steps, and, after a period of equilibration, most of the lolly pops remain in the cylindrical formation, as seen in Figure 8, where a snapshot of the equilibrated structure is shown. The total energy of the system as a function of the simulation time, expressed in sweeps, is shown in Figure 9. To demonstrate the cylindrical shape of the equilibrated structure, in Figure 10, we show the distribution of distances between the center of each lolly pop head and the center of mass of all such heads. The wider distribution, as compared to the one in Figure 4, emphasizes the cylindrical shape of the micelle.

From Figure 8, it is seen that most dipoles on the surface are oriented in an antiparallel fashion along the axis of the cylinder, as predicted by the model of section 3; however, there is still some randomness in their order, as well as defects in the structure, especially at the edges, as is to be expected from a self-assembly at finite temperature. A quantitative picture of the dipoles' orientation is presented by the histogram of Figure 11, where the distribution of angles between the dipoles and the axis of the cylinder is shown. The bimodal character of this distribution emphasizes the dipoles' antiparallel orientation along the axis of the cylinder. We would like to point out that the results presented here are in agreement with recent all-atom calculations performed by us on this system, using empirical force fields.¹¹

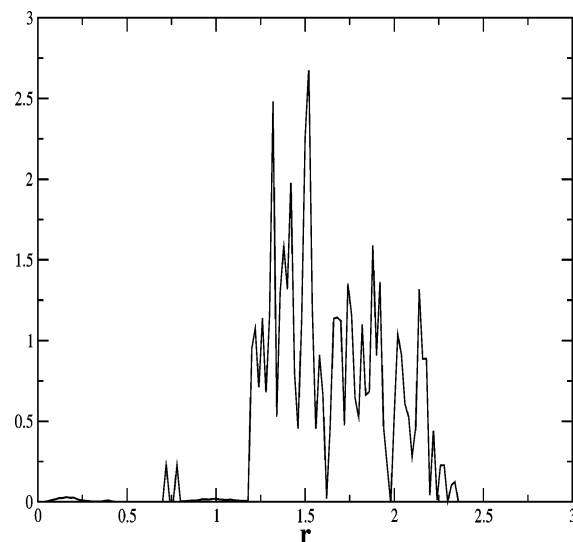


Figure 10. Distribution of distances between the center of each lolly pop head and the center of mass of all such heads, r , in the case of 220 electrostatically interacting lolly pops at 298 K, for the equilibrated structures, after 3×10^6 sweeps.

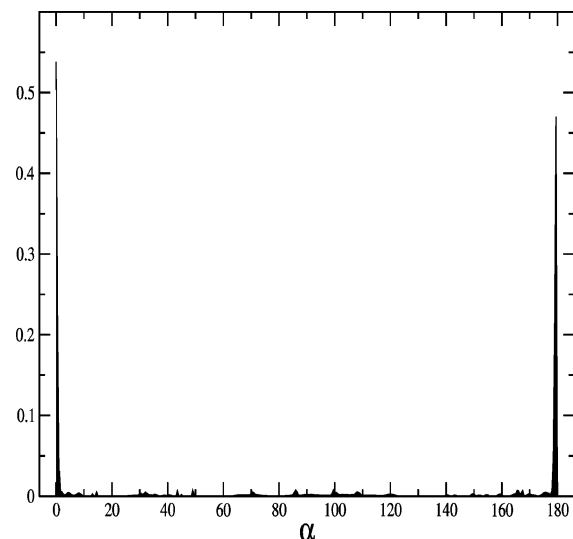


Figure 11. Distribution of angles α between the amphiphile dipoles and the axis of the cylindrical micelle. The dipoles' antiparallel orientation along the axis of the cylinder is evident from the bimodal character of the distribution.

5. Conclusions

We have presented a model for the self-assembly of an important class of PAs of truncated-conical shape, forming cylindrically shaped nanofibers to be used for the fabrication of artificial bone nanostructures. In this study, we have reduced a complex system to a simple object, approximately retaining its shape, symmetry, and major interacting components, namely, the charges in the hydrophilic headgroups, the hydrophobic attraction between the tails, and the excluded volume repulsion between the particles. The model reveals a new and interesting mechanism for self-assembly, which is not only hydrophobically driven, as commonly thought, but also electrostatically driven and directed, exhibiting a competition between attractive electrostatic interactions between the headgroups and attractive hydrophobic interactions between the tails. The competition is won by the electrostatics, leading to cylindrically shaped micelles. We have also noted the possibility for hydrogen bonding between the hydrophilic headgroups, the flexibility of

the molecules along their backbones making it possible for them to form effective beta-sheets which could be oriented in the same direction as their dipoles, so that both dipoles and beta-sheets would direct the growth of the micelle along the cylinder axis. The model presented here is supported by Monte Carlo simulations, which in the absence of electrostatic interactions lead to spherically shaped micelles, in accord with our expectations,⁸ whereas the inclusion of the electrostatics makes the cylindrical structure most stable.

The simplicity of this model makes it very attractive for studying problems of self-assembly at the nanoscale, and it opens the door to a new approach for dealing with such problems, in which the system is reduced to a simple analogue of itself, retaining only its most essential features, such as the dominant interactions and symmetries. Such an approach could be utilized for Monte Carlo simulations of self-assembling systems, as shown here, and could, thus, provide means for directing experiments and predicting their results. In addition, the structural model used for each monomer (superposition of several spheres to reproduce the monomer shape plus point charge electrostatics and exponential hydrophobic inter

action) can easily be adapted to many problems in self-assembly.

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