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# Computer simulation of biological interactions and reactivity

J.J. Wendoloski, Z.R. Wasserman and F.R. Salemme

*Central Research and Development Department, E. I. du Pont de Nemours and Co., Inc., Experimental Station 228/320A, Wilmington, DE 19898, U.S.A.*

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## SUMMARY

Computer simulations of molecular motion provide a useful tool for analyzing dynamic aspects of macromolecular structure and function. In many cases, simulations can be compared to experimental results that provide an average estimate of molecular flexibility. For example, variations in computed molecular motions in different regions of a protein structure can be compared to refined B-values obtained from X-ray crystallographic refinement. Such comparisons both provide a detailed view of the motions responsible for crystalline disorder, and allow an evaluation of how crystal packing affects mobility of groups on the protein surface. In these applications, dynamics simulations provide a means of regenerating the temporal dimension of a structure whose average behavior is experimentally well defined in the crystal lattice.

An additional benefit of the detailed and instantaneous view of molecular flexibility offered by simulation methods lies in its potential for exploring infrequent structural fluctuations or dynamic states of molecular association that cannot be examined in detail by X-ray methods, but are suggested on the basis of alternative structural information. For example, studies of the effects of surface chemical modifications on interacting proteins can produce information concerning the sites, if not the exact details, of the intermolecular interactions. The present work describes some applications of molecular dynamics methods to the study of large molecular aggregates whose dynamic properties thus far have precluded detailed structural descriptions. These include simulations of an electrostatically associated electron transfer complex between cytochromes c and $b_5$, some model systems for trans-membrane ion channels, and a phospholipid micelle.

## I. INTRODUCTION

Computer simulations of molecular motion provide a useful tool for analyzing dynamic aspects of macromolecular structure and function [1]. In many cases, simulations can be compared to experimental results that provide an average estimate of molecular flexibility. For example, variations in computed molecular motions in different regions of a protein structure can be compared to refined B-values obtained from X-ray crystallographic refinement. Such comparisons both provide a detailed view of the motions responsible for crystalline disorder, and allow an evaluation of how crystal packing affects mobility of groups on the protein surface [1,2]. In these applications, dynamics simulations provide a means of regenerating the temporal dimension of a structure whose average behavior is experimentally well defined in the crystal lattice.

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## II. METHODS

Simulation of the dynamics of molecular aggregates starts with the application of interactive computer graphics and modelling methods to arrive at one or more initial models for energy minimization and dynamics trajectory computation. Typically, the initial model makes use of any X-ray structural information of the isolated constituent molecules that may be available, together with any other experimental data that may geometrically characterize the aggregate structure. Once having determined an initial model structure, as described in each case below, energy minimization and trajectory integration can proceed using a similar protocol.

The simulations described here were performed using the united atom model and force field from AMBER version 2 [3]. This model explicitly includes only hydrogens on atoms which can potentially participate in hydrogen bonds. Typically, the initial model structures were minimized for 5000 steps prior to dynamics in order to relieve any initial strain. The systems were then heated from 0K to 300K and equilibrated for 25 ps either by coupling to a heat bath [4] with a relaxation constant of 0.1 ps (CRAY runs) or by velocity rescaling (STAR runs). The simulations were run using a 0.001 ps time step and a 9.5 Å cutoff radius for interactions between nonbonded atoms. The typical simulation consisted of 25 ps of initial heating and equilibration followed by 100 ps of data collection at 300K.

One unresolved aspect of this kind of calculation is the approximation of the dielectric constant ($\varepsilon$) used in the potential energy function. Many literature calculations have utilized a distance-dependent approximation in which $\varepsilon$ is replaced by Rij or 4Rij. While this approach provides a useful first level of approximation, it can sometimes lead to disruption of hydrogen bonding networks or other unphysical effects. In the case of aggregates of charged molecules which contain significant charge/charge interactions, a distance-dependent model might also exaggerate the 'through space' intermolecular effects. However, this dielectric model provides a useful reference point for comparison with other calculations and was consequently used in several simulations ($\varepsilon$=4Rij in the micelle, and 1Rij in the electron transfer work) to evaluate its effect. These 'pseudo-gas phase' simulations were followed by calculations that incorporated a detailed solvent model. In the 'solvated' simulations the aggregate structure was surrounded by a 6Å shell of TIP3P [5] water, and a value of $\varepsilon = 1$ was used for the dielectric constant.

The number of atoms in these simulations ranged from about 750 in the ion channel simulation to over 7000 in the case of the solvated micelle. Simulations of this size took tens of hours on a CRAY-XMP using a vectorized version of AMBER, or roughly twice as long on an ST-100 array

processor using Brook's GEMM system [6]. In addition, each system benefitted from preliminary simulations that helped establish the optimum approach for each case. Clearly, adequate computer time is a prerequiste for calculations such as these.

Simulation results were initially examined graphically using MOLEDITOR, an interactive graphics program implemented on an Evans & Sutherland PS 300 graphics system. This program allows flexible graphical display of portions of the dynamics trajectory, along with simultaneous real-time structure manipulations such as translations, rotations, clipping, generation of 1-, 2-, and 3-dimensional periodic images, and others. Although complemented by many numerical analyses of radius of gyration, dihedral angle correlations and the like, visual scanning of the dynamics trajectory was a preeminent means of identifying dynamic features of special interest.

## III. DYNAMICS OF A CYTOCHROME ELECTRON TRANSFER COMPLEX

The flow and conservation of electrochemical energy in living systems is controlled by specific electron transfer between proteins. Since the complexes formed between reversibly binding electron transport proteins are not readily trapped in crystalline form, many questions remain about the geometrical requirements for efficient electron transfer. However, X-ray structures have been determined for many of the individual proteins, and have allowed the generation of computer models of the complexes by interactive docking of the respective protein X-ray structures. Although such studies have provided useful insight, potentially important conformational changes and alterations in molecular packing cannot be realistically evaluated by simple rigid body manipulations of the individual X-ray structures. Molecular dynamics simulation of the model complex provides a means to evaluate the effects of intermolecular interactions on complex structure and dynamics.

One system that has been extensively studied experimentally is the reversible reaction complex formed between mitochondrial cytochrome c and microsomal cytochrome $b_5$ [7]. Examination of a docked model based on the X-ray structures for these two proteins showed that the heme groups for both were nearly coplanar, that good surface complementarity existed between the two proteins, and that the complex was potentially stabilized by several charge-pair interactions between lysine residues on cytochrome c and carboxylic acid groups on cytochrome $b_5$ [8]. The docked complex produced a closest approach distance of 8 Å between $\pi$-bonded porphyrin atoms, a distance similar to theoretical estimates of the distance required for electron transfer by a short-range tunneling mechanism [9].

In order to evaluate the impact of conformational and packing changes arising from the dynamics of complex formation, simulations were performed using pseudo gas phase and solvated methods described earlier. Any initial strain present in the model structure was removed by energy minimization of the starting complex coordinates. The minimized complex (Fig. 1a) does not change substantially from the starting structure, since minimization converges to the nearest local minimum. Molecular dynamics, in contrast, samples a much broader range of conformations than are energetically accessible by the complex. Fig. 1b shows a superposition of several of these conformations around the 46 ps point in the pseudo gas-phase trajectory. This figure illustrates the extensive flexibility of the molecular motions in the complex, which produces substantial motion of the two proteins relative to each other during the course of the simulation.

Local flexibility is also evident in the region between the two hemes. Following the initial few

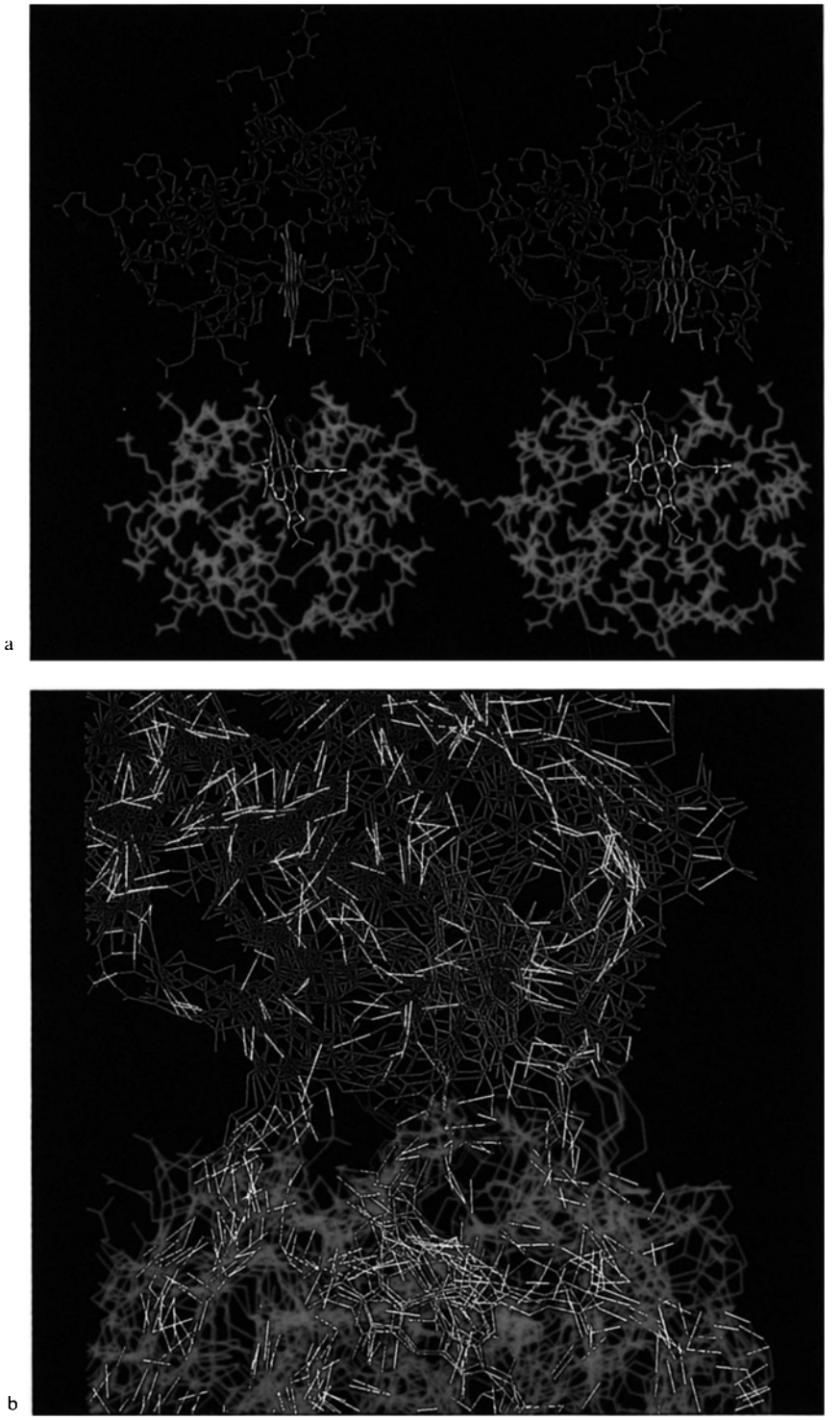


Fig. 1. Views of the cytochrome c-$b_5$ complex. Part a shows a stereoview of the initial energy minimized complex. Part b shows a closeup of the heme region illustrating the motion of cytochrome c phenylalanine 82 (red). This residue lies between the two heme ligands where it can potentially facilitate interheme electron transfer.

picoseconds of the simulation, phenylalanine 82 (F82) of cytochrome c moves out from its initial position in a pocket near the cytochrome c heme, to a position where it roughly bridges the heme groups of the two proteins (Fig. 1b). Concurrently, the distance between the heme iron atoms drops from an initial value of 17.8 Å to about 15.7 Å for the remainder of the trajectory. Both the closer heme iron distances and the bridging orientation of the cytochrome c F82 ring might facilitate interheme electron transfer relative to the initial model complex [7].

While the pseudo gas phase trajectory indicates substantial flexibility and geometric rearrangement, it is important to evaluate how solvent effects might mediate the electrostatic interactions between the proteins in ways not adequately modeled by the $\varepsilon = Rij$ dielectric model. Accordingly, the simulations were repeated with an explicitly hydrated complex. The results of this solvated model simulation were generally similar to the pseudo gas phase model, although the time scale required for them to occur was much longer. In the pseudo gas phase model, the F82 bridging occurred after the first few ps, while in the solvated model it took 98 ps for the bridging conformation to occur. The iron-iron compression was also less, dropping from 17.8 Å initially to 16.7 Å for the remainder of the simulation.

These results illustrate that molecular dynamics can simulate conformational changes in reaction partners that result in the formation of complexes substantially different from those obtained using rigidly docked crystal structures. The equilibrated complex has iron-iron distances from 1.1 to 2.1 Å closer than the average 17.8 Å static distance. In addition, a fairly wide range of interheme orientations and distances are sampled over the lifetime of the complex. The results argue for importance of conformational sampling both in the actual intermolecular electron transfer step, and their realistic modeling based on isolated X-ray crystal structures [8].

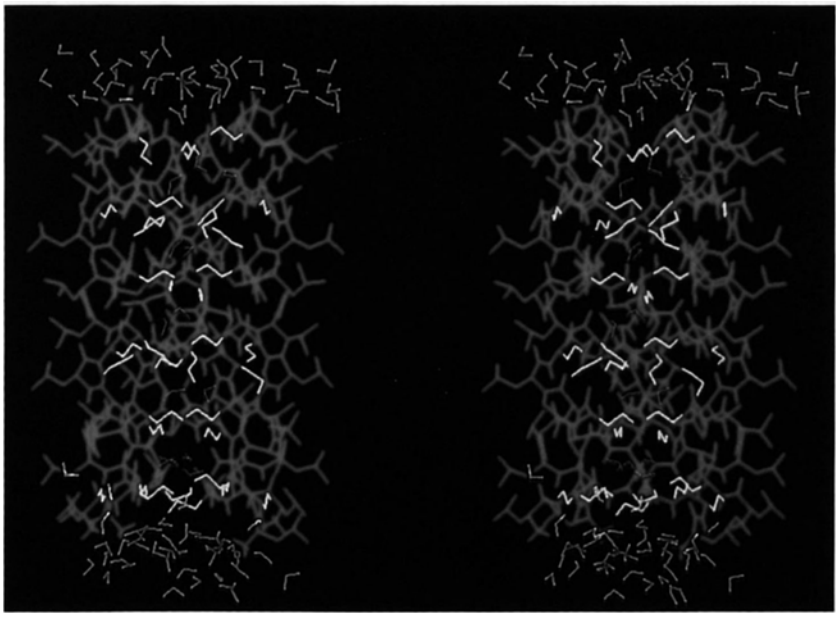


Fig. 2. Stereoview of a tetrameric peptide bundle ion channel with the sequence $(LSSLLSL)_3$. Serine side chains are colored yellow. The channel is hydrated at both ends and contains a few interior waters.

## IV. DYNAMICS OF MEMBRANE ION CHANNELS

Ion channel proteins play a major role in cellular communication by providing a pathway for ion transmission through the otherwise impermeable lipid bilayer. One architecture commonly proposed for transmembrane channels, particularly those assembled from multiple subunits such as alamethecin [10], the acetylcholine receptor [11], and the sodium channel [12], incorporates a bundle of parallel amphiphilic helices whose polar faces form the interior of the channel.

Initial design studies performed in collaboration with synthetic chemists suggested that α-helical sequences composed only of the amino acids serine (S) and leucine (L), respectively providing a means for polar stabilization of the pore interior, and a strong, hydrophobic, α-helix forming capability, might be suitable for the formation of transmembrane pores. Indeed , a model ion channel composed of peptides with the sequence $(LSSLLSL)_3$ seemed to form particularly favorable arrangements, where the 21-residue peptides could potentially fold into amphiphilic helices, and then aggregate to form the channel bundle.

Many alternative helix bundles that might model ion channels were generated and evaluated using interactive graphics and energy minimization techniques. Starting from an initial n-meric bundle with Cn (n = 3, 4, 5 and 6) rotational symmetry, the two techniques were applied alternately in an iterative procedure until no further improvements were made. Conformations were then evaluated according to criteria of low energy and dense packing of side chains between adjacent helices. Although it was not possible to unequivocally determine a best channel geometry, several models had both good local conformational features and interhelical packing.

A partially solvated molecular dynamics simulation was performed on one model, a tetrameric bundle of the $(LSSLLSL)_3$ peptide (Fig.2), in order to evaluate the structure's stability and the interaction of channel solvent molecules with the polar serine side chains in the bundle interior. This model included the 4 α-helices plus a few isolated waters in the polar core and small clusters of water molecules at either end of the channel. Although the mobility of the waters was greater than that of the atoms of the protein itself, they did not drift through the channel. This result is expected since water diffusion through such channels occurs on the nanosecond ($10^{-9}$), rather than picosecond ($10^{-12}$) timescale of the simulation. Nevertheless, the simulation provides valuable insight into channel function. For example, hydrogen bonding between the waters in the channel was found to be very persistent, with stabilities close to those of the helix backbone. In addition, the water-water and water-serine hydroxyl hydrogen bonds formed a continuous network through the channel, affording a potential pathway of dipolar interactions that might stabilize transient ions. Although much further work is required, simulations of membrane channels can complement experimental studies, and potentially assist in the design of novel channel peptides.

## V. MICELLE STRUCTURE

Aggregates of phospholipid molecules play an important role in a wide range of biological functions through the formation of membrane and other membrane mimetic structures such as micelles, monolayers, and bilayers. The crysallographic structure for a 'frozen' phospholipid bilayer [13] has been determined, but detailed structural models for more disordered structures such

as micelles cannot be determined by X-ray methods since they do not form crystals. Computer modeling techniques such as molecular dynamics simulations provide an alternative means to begin to study these structures in the context of existing models and experimental data.

Some previous work has been done on modeling micelles, although much of it utilized simplified models for the aggregate molecules along with more traditional statistical mechanical methods [14]. A few authors have reported micelle molecular dynamics simulations using a simplified aggregate molecule model [15], and a simulation of sodium octanoate micelle has also been reported [16]. However, none of these simulations involved models with head groups that form attractive electrostatic or hydrogen bonded interactions typical of many biological systems. Previous work has instead concentrated on detergent micelles which were held together by hydrophobic interactions of the tail regions and destabilized by the repulsive head group (RHG) interactions.

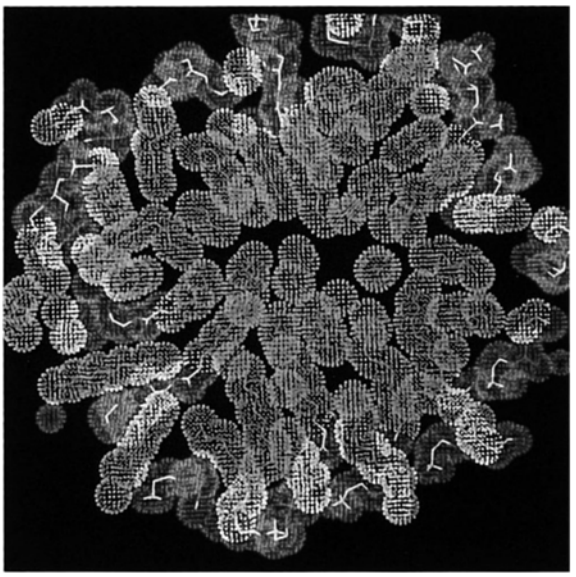
The present work involves molecular dynamics simulations of micelle models based on dilaurylphosphatidylethanolamine (DLPE), a phospholipid with a dipolar, zwitterionic head group. Although DLPE ultimately forms stable bilayer structures in water, we were particularly interested in the properties of the transitional micellar structures formed and, anticipating additional phospholipid simulations, how the dynamics of the constituent molecules change on bilayer formation. Initial micelle structures were built by aligning a single DLPE molecule from the membrane crystal structure [13] on a sphere having a diameter equal to the phosphorous-to-phosphorous distance of the bilayer separation in the membrane crystal structure. Replicates were then distributed uniformly on the sphere so that the surface area of the head groups approximated that of the membrane. This produced a micelle incorportating 85 DLPE molecules.

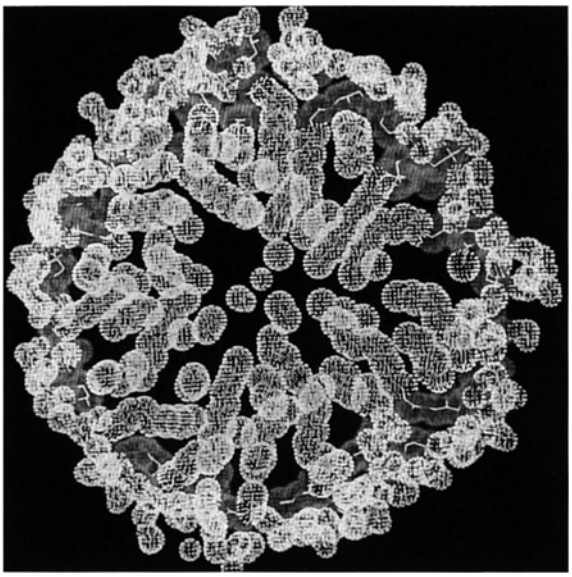
Three separate simulations were performed. The first pseudo gas phase simulation was performed by fixing the DLPE head group phosphorous atoms, while a second simulation allowed all atoms to move. The third, most realistic simulation used the solvated model with all atoms unconstrained. This particular choice of simulation models was designed to help isolate various effects that might contribute to the micelle structure and dynamics, and provide a common perspective for studying alternative phospholipid aggregates such as liposomes and membranes.

Since the DLPE head group is a zwitterion, the micelle is stabilized both by electrostatic dipolar interactions and hydrogen bonding interactions between the headgroups, as well as attractive hydrophobic interactions between the fatty acid side chains. This forces the micelle to maintain a balance between optimizing head group interactions that define the micelle surface area, and minimizing the overall volume to optimize van der Waals interactions and exclude solvent from the hydrophobic micelle interior. As illustrated in Fig. 3a, fixing the head group phosphorous atoms to maintain the surface area constraints of the bilayer structure, produces a micelle where fatty acid side chains are tightly packed, but where there are voids in the glycerol regions of the head groups.

Fig. 3b shows a section from the simulation where the micelle was unconstrained and hydrated by a 6 Å shell of water molecules. Interestingly, the micelle radius does not appreciably alter when the phosphate constraint is released, so that the average head group area remains similar to bilayer crystal structure. Most of the voids apparent in the constrained simulation are simply filled with water in the hydrated simulation, which in many cases form hydrogen bonds to the semiexposed glycerol hydroxyl groups. Other fluctuating voids in the aliphatic micelle core exclude water on the simulation timescale, and may relate to the metastability of the DLPE micelle.

Some hints of micelle instability are suggested from the unconstrained pseudo gas phase simulation. Previous studies of dynamics simulations with varying dielectric models have suggested that the distance dependent approximation is somewhat disruptive of polar structural interactions, which could have the effect of increasing the frequency of large scale fluctuations in the micelle structure. As illustrated, in Fig. 3c, this 'accelerated' simulation results in a pronounced deformation of the micelle from its initial shape into an ellipsoid with some distinctly bilayer-like structural characteristics. This transition appears to allow better and more extended chain packing interactions between the aliphatic fatty acid side chains, so that the micelle chain packing begins to more closely resemble the parallel chain packing seen in the crystal.





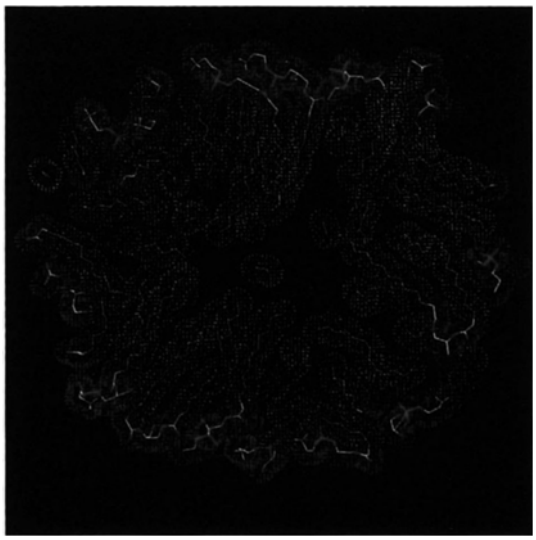


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Fig. 3. Views of the DLPE micelle model. Part a shows a 3 Å cross section of the van der Waals surface of the phosphorous constrained, pseudo gas phase micelle simulation. Part b shows a cross section of solvated simulation. Part c shows a late stage in the unconstrained, pseudo gas phase micelle simulation.

Overall, these initial simulations of phospholipid micelle dynamics present a structural and dynamic picture that is quite different from previous simulations of surfactant micelles or proteins. This is a result of the difference between the strong polar head group interactions that define the micelle surface, and the segmentally mobile motions of the fatty acid side chains on the micelle interior. The combination of strong surface associative interactions and an unstructured interior differentiate phospholipid micelle structure both from proteins, that have more structured interiors, and surfactant micelles, where nondirectional repulsive head group interactions have less influence on internal chain organization.

## VI. CONCLUSIONS

Simulations of motion in transient or dynamically mobile aggregate structures provide an important means of studying detailed properties of functional importance that are not accessible using conventional methods of structure analysis. The emergence of these new tools to investigate and correlate structural information is made feasible by the availability of large scale computational and interactive graphics facilities. These advances parallel developments in biotechnology that allow facile synthesis of new molecules, and improved methods of structure determiniation using synchrotron X-ray sources and 2D NMR methods.

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