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Ideas That Change the World

Hugh Nymeyer
T-10: Theoretical Biology & Biophysics Group
Los Alamos National Laboratory
M.S. K710, T-10
Los Alamos, NM 87545

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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 177
Bloomington, IN 47405-7105

To the Biocomplexity Faculty Search Committee:

Please find enclosed my curriculum vitae with a list of publications, reprints of some representative publications, and a statement of my current research interests. I am providing this as an application for the assistant professor position advertised in *Physics Today* (November, 2003). I have also arranged for three letters of recommendation to be sent to your address.

My background is in biological physics. I wrote my thesis under the direction of Josè Onuchic in the department of physics at UCSD. We used simplified models in an attempt to understand the kinetics of protein folding/unfolding transitions using a small number of reaction coordinates. This work has been highly cited: I currently have more than 254 citations, mostly pertaining to this work.

More recently, I have been focusing on using atomically detailed models to understand the thermodynamics driving conformational change in biological systems. I am actively involved in the development and application of enhanced sampling methods to biochemical systems, an area of increasing prominence and great importance in computational biochemistry. I am also the first person to apply these methods to study the interactions of peptides with solvated lipid bilayers. This provided the first computational demonstration of spontaneous insertion of a hydrophobic peptide into a lipid bilayer and its subsequent folding. It also provided the first reliable computational study of the enthalpic and entropic changes driving insertion as a function of the structure and position of a peptide in the bilayer.

I am looking forward to hearing from you in the future. If you have any questions or would like any additional information, feel free to contact me via my cell phone (505 660-5171) or electronic mail (hugh@lanl.gov).

Sincerely,

Hugh Nymeyer
Directors Funded Postdoctoral Fellow,
Los Alamos National Laboratory

Current Research Interests
Hugh Nymeyer

Computer simulations of biological systems have evolved greatly in the last three decades. Much of the advance has come from simple technological improvements, but improvements in methodology, e.g., temperature control methods and electrostatic calculations, have also been substantial. The most promising methodological advances of the last decade are general sampling algorithms that can speed equilibration by orders of magnitude beyond simple Monte Carlo or molecular dynamics. Most of these methods are based on the multi-canonical [1] or replica exchange algorithms [2], which were created specifically to overcome the critical slowing down seen at discontinuous phase transitions and in glassy systems. Many biological systems, such as proteins, have dynamics with features of both discontinuous phase transitions (the all or non folding transitions seen in protein folding) and glassy systems (frustrated interactions and large enthalpic barriers).

In the last year, I have been involved in applying enhanced sampling methods to study several different biological systems: single helices in aqueous solvent, small helical proteins, and peptides interacting with solvated biological membranes. These methods are allowing us to definitively answer fundamental problems regarding protein thermodynamics and folding and the thermodynamics and kinetics of proteins inserting into and interacting with biological membranes.

Membrane—Protein Interactions

Biological membranes are a quintessential component of autonomous living organisms. In fact, the genesis of life possibly began with the spontaneous formation of the plasma membrane [3]. Cell membranes define the cell and its numerous organelles and maintain concentration differences necessary for storing the energy of photosynthesis and respiration [4]. They are also critical platforms for enzymatic activity: 20 to 30% of all open reading frames are estimated to code for intrinsic membrane proteins [5].

Despite the obvious importance of biological membranes and their resident proteins, much less is known about them then their globular protein cousins. The liquid crystal bilayer structure of membranes and the organization of proteins on the surface of and inside membranes were not understood until the early 1970's [6]. Difficulties in crystallizing intrinsic membrane proteins has meant that the structures of only about one hundred are known to 4Å or better resolution [7] out of more than 22,000 publicly accessible protein structures [8].

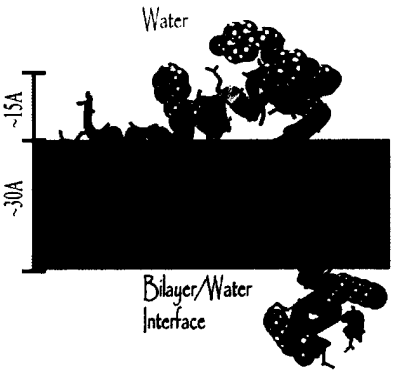

Figure 1: A representation of the fd coat protein [12] modeled into a schematic of a lipid bilayer. This structure clearly shows the two types of helix orientation common in membrane proteins. Aromatic residues, which strongly prefer the interfacial region over both the bilayer interior and water, are rendered as spheres; the backbone is colored according to residue index from red to blue.

The earliest theories to describe the behavior of lipid bilayers were based on continuum models. These theories treat the bilayer as a layer of insulating bulk material [9] or as a uniformly charged insulating surface [10] that has an intrinsic curvature and bending moments [11]. These models have had some noticeable explanatory power. For example, from the electrostatic interactions of an alanine alpha-helix with the bilayer, represented as a slab of low dielectric, one can observe the existence of two locally stable configurations of the helix [13] which are similar to those observed in many membrane proteins (figure 1).

Also, the free energies for transferring peptides from aqueous solution to a bilayer surface correlate well with transfers from water to bulk non-polar phases (e.g., octanol) [14]. (Reliable measurements of the free energy of transfer from water to the bilayer interior have not to this date been carried out, so a similar comparison here is not possible.) These transfer studies have made it clear that the bilayer/water interface (and most likely the bilayer interior as well) can be considered qualitatively as a bulk phase. This concept applied to the interface is not a priori unreasonable, since diffraction data reveals that the interfacial region of most lipid membranes is on the order of 15 Å thick on each side of the bilayer interior [15], thick enough to accommodate the width of an alpha helix in a parallel arrangement.

In terms of free energy, the interface has properties midway between water and the bilayer interior. The transfer free energy of a hydrophobic molecule from a polar to a non-polar phase depends almost linearly on its surface area [16] with surface tensions for the hydrophobic-water interface between 18 to 28 cal/mol/A^2 [17]. The effective surface tension in the interface of most bilayers appears to be half of the value in water: -12 cal/mol/ A^2 from measurements on tripeptides or –14 cal/mol/ A^2 from a peptides of the form Ac-WL-X-LL [18]. The dielectric constant in the interface is also intermediate between the dielectric constant of the membrane interior (≈2) and bulk water (≈78), having a value close to 18 [19].

However, the same transfer studies also point to deficiencies in thinking of a membrane or membrane interface as a bulk material. For example, although the transfer of hydrophobic molecules from water to the bilayer-water interface has a large negative heat capacity change, which is the hallmark of the hydrophobic effect [20], these transfers occur with unusually large negative changes in enthalpy [21], an effect not seen in transfers from water to bulk non-polar phases that is sometimes referred to as the "non-classical hydrophobic effect" [21,22]. It has been suggested that these large negative enthalpy changes may be created by order or disorder induced in the bilayer lipid molecules upon binding [23], changes that are dependent upon liquid crystalline order not present in most bulk hydrophobic phases.

To understand the origin of the effective surface tensions in the interface and the anomalous enthalpic changes upon transfer, attempts have been made to separate the free energy of solute partitioning into the interior and interface into separate additive terms [13,18a,24]. For example, one may decompose transfer free energies as follows:

$$\Delta G_{transfer} = \Delta G_{np} + \Delta G_{ele} + \Delta G_{lipid} + \Delta G_{imm} + \Delta G_{con} \qquad (1)$$

ΔG_{np} is the non-polar contribution which arises from removing the solute from water, and ΔG_{ele} is the electrostatic interaction with water. These two terms account for desolvation. ΔG_{imm} is the entropy loss due to immobilization of the solute in the bilayer. ΔG_{lipid} is the interaction with the lipids, including the decrease (or increase) in lipid conformational freedom, which could properly be called a measure of the lipophobic effect. ΔG_{con} is to account for any conformational changes in the peptide induced by the water to bilayer transfer.

Experimentally, the separate terms in this equation cannot be determined: only the total transfer free energy is amenable to measurement, and such measurements are problematic. The large free energy penalties involved in desolvating naked (un-hydrogen-bonded) peptide groups [25] leads to a strong coupling between insertion into the membrane and folding [26], a situation sometimes referred to as partitioning-folding coupling [18b]. This coupling makes characterizing a suitable reference state for measuring free energies difficult. This is important, because the terms in the free energy expansion in equation 1 depend strongly on the peptide conformation. Theoretical models have usefully been applied to estimate the various contributions to whole helix insertion but have been limited to continuum treatments of the bilayer [13,24].

Clearly, more detailed molecular models would be useful for understanding the forces affecting partitioning; however, simulations of molecularly detailed models of membranes have been hindered by onerous equilibration times. For example, the time for a DPPC (dipalmitoyl-

phosphatidylcholine) lipid to diffuse laterally in a bilayer the length of the average spacing of the head-groups is on the order of 100 ns [27], which is much longer than the time that most classical lipid simulations can be practically integrated forward in time using modern computers.[1]

I have been the first to circumvent these problems by applying enhanced sampling methods to study membrane bilayers[2]. I used the parallel replica exchange molecular dynamics (parallel REMD) method to study a classical model of a bilayer system composed of 36 DPPC molecules, water, and a synthetic peptide (WALP), which was designed and verified [28] to insert into a bilayer in a helical trans-membrane orientation (figure 2). This work was the first demonstration that one could, using enhanced sampling methods, equilibrate a system with a peptide that partitions among the different regions of water, interface, and bilayer interior [29]. Fitting the temperature dependent population of the system as a function of peptide helical content and position also provided the first estimate of the relative changes in energy, enthalpy, and heat capacity upon changes in peptide position and structure. This simulation exhibited the large negative change in enthalpy and heat capacity upon insertion into the bilayer interior that is characteristic of water/bilayer-interface partitioning, *viz.*, the "non-classical hydrophobic effect". It also showed a large positive enthalpy changes due to the desolvation of peptide groups as the WALP peptide was positioned inside the membrane in an unstructured state.

These simulations suggested that the lowest barrier to insertion of the WALP peptide was through a state in which the peptide was inserted yet unstructured. This proposed (and as yet unverified) insertion pathway is contrary to the accepted mechanism for insertion, referred to as the four-stage model [14,30]. In the four-stage model, it is suggested that the large enthalpic cost for desolvating peptide groups prevents peptide insertion without structure formation, consequently, peptide insertion into bilayers only occurs after their helical structure has formed (either in aqueous solution or in the bilayer interface). In our simulations, we see a large and previously unexpected increase in entropy that largely compensates for the insertion of the unstructured peptide into the bilayer interior. Although the peptide does not strongly populate states in which it is non-helical and immersed in the bilayer interior, these states are much more probable than has previously been supposed.

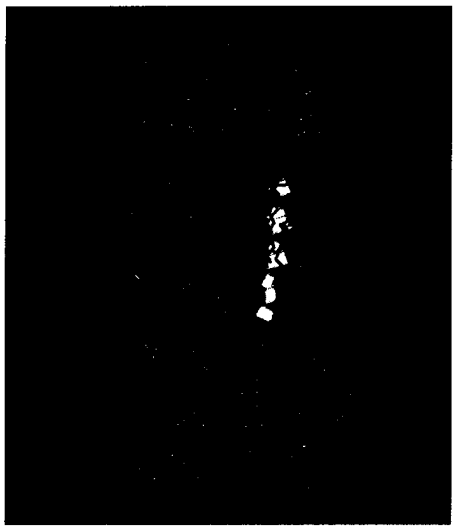

Figure 2: A representation of the unit cell of the mixed bilayer/water/peptide system simulated as described in reference [29]. The peptide (yellow) is shown in its equilibrium conformation in the bilayer interior (cyan). Water flanks both sides.

To gain a better understanding of the enthalpic and entropic compensations involved in peptide-bilayer interactions, I propose using parallel replica exchange and multi-canonical based algorithms to study the interactions of residues and organic molecules with bilayers modeled using all-atom classical force-fields, especially the CHARMM-22 force-field [31]. There are several developments necessary for this to be successful.

1) Other sampling methods must be tested and applied to peptide/bilayer/water systems. We have already demonstrated that the parallel replica exchange molecular dynamics [2f] (parallel REMD) method can dramatically enhance the sampling efficiency in these types of systems by

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[1] Integration time-steps are limited by the frequency of atomic vibrational motion to 2 fs in most instances. Minimal sized membrane systems have on the order of 10^4 atoms. Consequently, a 100 ns membrane simulation requires approximately $5x10^{11}$ atomic force/velocity/position updates.

[2] This work was done in collaboration with Angel E. Garcìa at Los Alamos National Laboratory and Thomas B. Woolf at the Johns Hopkins School of Medicine.

several orders of magnitude. But parallel REMD has several drawbacks. Parallel REMD works by running several copies or replicas of the system in parallel. These replicas have different temperatures (or more generally, different Hamiltonians [32]), and periodically these replicas attempt to exchange temperatures with a Monte Carlo move that preserves detailed balance in the whole ensemble of replicas. However, the required number of replicas increases approximately as the square root of the size of the system. For systems with 36 lipids and water, this requires 64 or more separate simulations, which is highly expensive. Methods which combine replica exchange and multi-canonical methods (such as MUCAREM [33a]) have proven more efficient at sampling smaller biological peptides than either replica exchange or multi-canonical methods alone [33b]. These combined methods reduce the number of replicas needed for efficient sampling and increase the sampling efficiency of each replica.

An additional benefit to the reduction in the number of necessary replicas is the possibility of enhancing the sampling along coordinates other that the total energy. This may be done to guarantee that sampling will occur in transition regions that are likely to be poorly sampled at all temperatures. For example, in my prior simulations of the WALP peptide, no sampling occurred along the putative four-stage model insertion pathway, making it impossible to estimate the difference in barrier heights between that pathway and our observed pathway. Flattening the potential of mean force along other coordinates should make sampling more efficient. It might also decrease the necessity of running replicas at very high temperatures, thereby eliminating the need for extensive restraints that prevent the membrane structure from being disrupted at high temperature.

2) I propose to separate the different entropic and enthalpic forces driving peptide insertion and conformational change. Such a separation, along the lines of equation 1, is conceptually useful for understanding the forces driving insertion and folding of peptides in the bilayer, even though these separations are inherently only approximate. The free energy change along a thermodynamically reversible path can be derived from free energy perturbation methods [34], either thermodynamic integration or free energy perturbation through discrete steps. Separating the Hamiltonian of the system into different components (electrostatic interactions between the peptide and water, van der Waals interactions between the peptide and water, etc., etc.) allows one to calculate the free energy change due to these different components. Knowledge of the free energy and average potential energy due to different force components then allows one to deduce the entropic changes due to those components as well.

Computing enthalpies and entropies is more difficult than computing free energies, because free energies are computed as relative differences and enthalpies are usually computed as absolute quantities, making the uncertainties in enthalpies and entropies much greater. Computing the temperature dependence of the free energy and deducing the enthalpies and entropies from a thermodynamic model has been shown to be successful and accurate in the case of the WALP peptide; however, direct computation is only possible if several nano-seconds per replica can be computed.

Initially, it would be good to compare free energy simulations with systems whose partitioning free energy is known experimentally. Two good candidate systems are the ALA-X-ALA-O-*tert*-butyl series of peptides [18a] and the Ac-WL-X-LL series [18b], which are limited to the water and interfacial regions. Although the free energies of transfer of several larger peptides are known, it is better to begin with simpler systems with one variable amino acid position. Of particular interest in these studies is to gain an understanding of tryptophan, which shows an anomalous preference for the interfacial region, presumably due to its shape or complex electronic structure [35].

3) Larger peptides which form regular secondary structure are also interesting targets for study via these methods. There exist many small peptides that spontaneously insert into membranes as alpha helices or small beta sheets. For example, influenza hemagglutinin protein and HIV gp41 [36] both have fusion peptides that serve to anchor their corresponding fusion proteins into

the host cell. Numerous anti-microbial peptides also appear to function by spontaneously inserting into membranes and forming ion channels [37]. The mechanism of insertion of these peptides is unknown. The formation of protein di-mers (such as are seen in Glycophorin A [38]), and the elucidation of the forces driving their formation is also a topic that it should now be possible to study with these new sampling methods. In particular, we do not know the relative importance of weak hydrogen bonds formed with the alpha-carbon protons, side-chain packing, van der Waals interactions, and modification of lipid chain order in driving multi-mer formation.

4) Equally important is understanding the behavior of the membrane in isolation and the accuracy of its empirical force-field. Initial simulations with the WALP peptide in a DPPC bilayer showed a phase transition occurring in the membrane near 400K (possible the L_α-gel phase transition), although this temperature is well above all known phase transition temperatures in pure DPPC bilayers. Multicanonical methods have been used for some time to study first order phase transitions. This should make it possible to study the location of the membrane phase transitions in the classical membrane models, providing an important check of the force-field accuracy. This is important, since membrane-protein interactions (especially entropic and enthalpic terms, rather than total free energy) are sensitive to the phase behavior of the bilayer. Many membrane simulations use DPPC as a model system, and it is highly probably that these simulations are being conducted in regions of state space for which the membrane is in the incorrect phase, which has not previously been recognized due to insufficient equilibration.

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