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Biocomplexity Faculty Search Committee,
c/o Prof. Rob de Ruyter van Steveninck,
Biocomplexity Institute,
Indiana University,
Swain Hall West 117,
Bloomington, IN 47405-7105.

Dear Sir or Madam,

I wish to apply for the position of Assistant Professor in the Biocomplexity Institute, as listed in Physics Today. Enclosed are my curriculum vitae, list of publications, a description of my research and the names of people who have agreed to support this application. Reprints of two recent articles have also been attached. My research plan describes a multidisciplinary approach to an important problem in computational biology. The goal of this work is to understand the role played by membrane composition in the regulation of protein function. I outline a computational philosophy that is designed to offer quantitative calculations, and mechanistic insight that will enhance experimental observation.

Thank you for your consideration,

Toby William Allen, Ph.D.

I have arranged for the following people to send letters of recommendation:

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RESEARCH HISTORY

After my initial training in computational chemistry and theoretical physics, my postdoctoral research centered on the structure and biological function of membrane proteins, and the mechanisms of ion channel permeation using computational approaches. My research has been interdisciplinary, bringing expertise in physics, chemistry, mathematics, computing and biology to important questions. Computer simulation has become an essential tool in the study of many biologically important problems, because improved force-fields and computer power now allow mechanistic insights to be derived from newly discovered protein structures. My goal has been to apply sound computational methods to pursue quantitative agreement with experimental studies, and to elucidate the mechanisms underlying these processes.

In my first postdoctoral position, with Dr. Shin-Ho Chung (The Australian National University), I investigated ion permeation through potassium, calcium, gramicidin A (gA), acetylcholine receptor and generalized model channels. Using a combination of methods, including molecular dynamics (MD), continuum theories, Monte Carlo (MC), and Brownian dynamics (BD), we showed that it was possible to go from ion channel structure to measurable ion conductance properties that could be directly compared to electrophysiology (Allen and Chung, 2001). In some cases predictions were made about the structure of the ion channel using these techniques (Corry et al., 2001). One of the critical advances in understanding ion permeation was to obtain insight into the properties of ions and water in the confined spaces of biological ion channels (Allen et al., 1999a). We were the first to study the KcsA potassium channel with MD simulation (Allen et al., 1999b; Allen et al., 2000), and to combine these results with three-dimensional BD calculations of channel conductance (Allen and Chung, 2001). Studies at various levels of approximation were invoked to elucidate the critical attributes of ion permeation, with implications for the diversity of potassium channels existent in nature (Chung et al., 2002). Calculations on the gA channel provided the first quantitative examination of the ability of computer simulation to model ion permeation in this channel (Allen et al., 2003a), which initiated a new series of studies into improved computational methods. Throughout this period I performed all of the MD simulations for the group, and made significant contributions to the programming of BD, MD and other code for supercomputers. I also and assisted in the supervision of Ph.D. and undergraduate students.

To further extend my expertise in theory and computation, and to get a better appreciation of the problem from an experimentalist's point of view, I continued my postdoctoral training at Cornell University (Weill Medical College, New York), with Profs. Olaf Andersen and Benoit Roux. My studies at Cornell continued to focus on ion channel permeation, including structural refinement using Nuclear Magnetic Resonance (NMR) data, and understanding the interplay between membrane proteins and lipid bilayers. Fully atomic systems consisting of a gA channel and bilayers of various composition (see Figure 1) were created by developing improved membrane building techniques. We used MD simulation, of these systems, to remove ambiguities in experimental structures (Allen et al., 2003b). In an excellent example of the use of computer simulation to aid experiment, sampling the dynamic ensemble of protein configurations in the membrane, instead of relying on a single protein structure, enabled us to determine the correct structure, and dynamics, that is in agreement with all solid and liquid state NMR, X-ray diffraction and fluorescence spectroscopic observations.

We have raised the standards for quantitative calculation of ion conduction at Cornell. The potential of mean force (PMF) is an important statistical mechanical quantity for describing the free energy of biological processes (Figure 2). Using a combination of umbrella sampling (Torrie et al., 1977) and free energy perturbation (Kollman, 1993) techniques, we have obtained better agreement with experiment than ever before, and provided a systematic approach to attaining quantitative accuracy in the near future (Allen et al., 2003c). Furthermore, these studies have explained how ion binding sites emerge, by means of a breakdown of the free-energy components of the system, and explained the roles of desolvation and vicinal water arrangements in ion permeation. The development of improved computational methods for studying membrane and membrane-protein function has opened the door for future studies where computer simulation can be used to understand the mechanisms responsible for many problems of chemical, biological and medical significance.

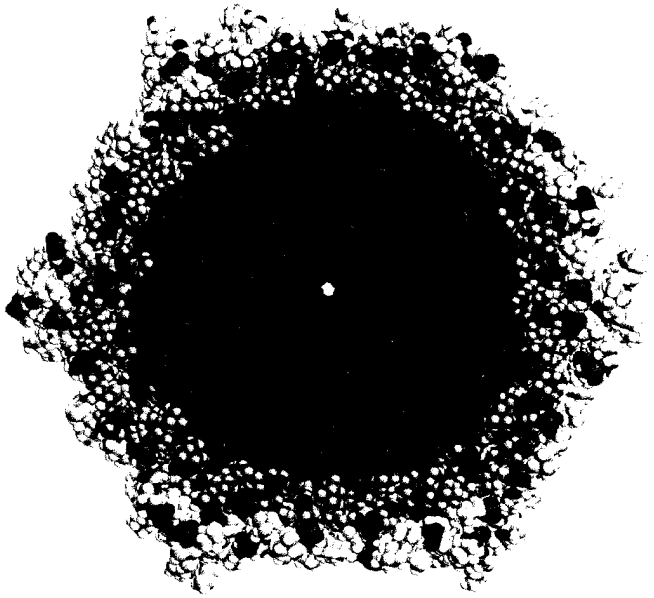

Figure 1 The gA dimer (green) embedded in a bilayer of 230 DPPC molecules with C, O and P atoms as gray, red and yellow balls, respectively. Gray shades represent the 1-5 shells of lipids. Hexagonal periodic boundaries are imposed. The membrane was built using extensions of previous methods (Woolf and Roux, 1996).

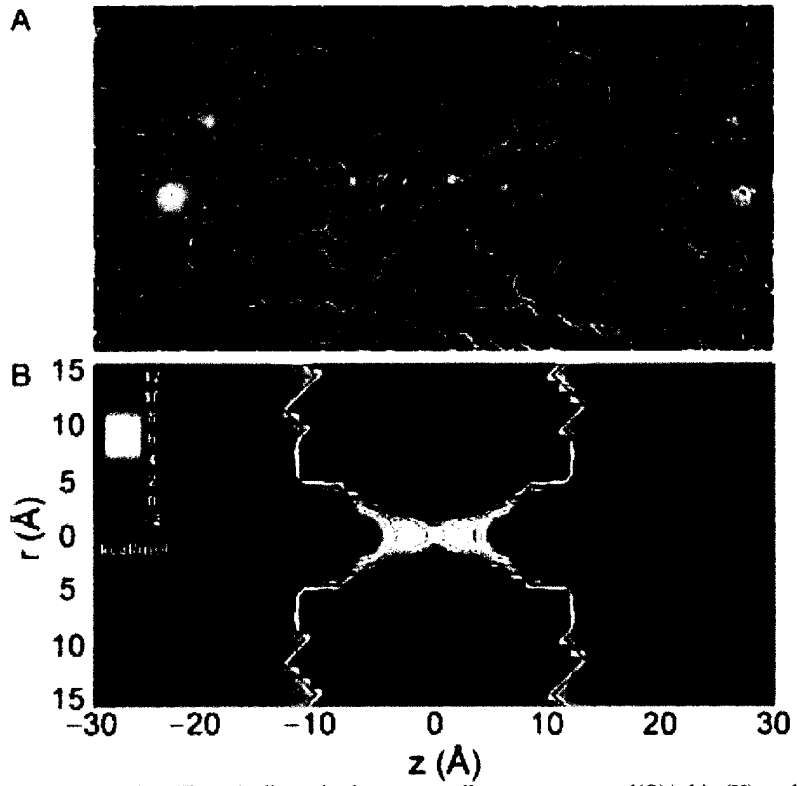

Figure 2 **(A)** The gA dimer is drawn as yellow, water as red(O)/white(H) and DMPC with various color sticks. K^+ and Cl^- ions are green and gray spheres. In the pore, 7 waters and a K^+ ion in one binding site are shown as spheres. **(B)** The 2D PMF of a K^+ ion as a function of axial z and radial r positions. This complete free energy landscape reveals the permeation pathway, binding sites and a barrier within the channel opposing conduction.

RESEARCH PLAN

Biological membranes are comprised of phospholipid bilayers and bilayer-spanning proteins that catalyze the movement of ions and molecules. As an increasing number of structures become available, some common features are beginning to emerge. There is growing evidence that the function of many membrane proteins involves conformational changes in transmembrane (TM) domains. These include: bacterial (Doyle et al., 1998; Jiang et al., 2002) and voltage gated (Jiang et al., 2003a) potassium channels, nicotinic acetylcholine receptor (Miyazawa et al., 2003), chloride channels (Dutzler et al., 2003), Ca^{2+}-ATPase (Toyoshima et al., 2002), ABC transporters (Locher et al., 2002), gap junctions (Unger et al., 1999), mechanosensitive MscL channel (Chang et al., 1998) and gramicidin channels (e.g. Ketchem et al., 1997). The voltage gated potassium channels offer a particularly striking example where a TM unit is thought to pass through the hydrophobic core of the membrane (Jiang et al., 2003b). These conformational changes will be modulated by the properties of the host bilayer, in specific and non-specific ways. While experiment provides ample evidence of this regulation, the underpinning mechanisms remain unclear.

There exist over 200 species of phospholipids in biological cells, with different acyl chain length, saturation and polar head-groups affecting bilayer properties (Gennis, 1989). In addition, biological membranes contain cholesterol, lysophospholipids and various amphipathic molecules and proteins that can affect the mechanical properties of the bilayer (thickness, curvature, fluidity and elasticity). How does the state of the lipid bilayer regulate function? The first, and most direct route, is by specific binding of select molecules and lipids. For example, the KcsA potassium channel has been shown to require activation by negatively charged lipid binding (Valiyaveetil et al, 2002). There is, however, a second, more general mechanism that involves changing bilayer properties. For instance, alamethicin and gramicidin A (gA) channels are altered similarly by changes in bilayer properties (Lundbœk, 1997). Furthermore, many pharmacological compounds are used at concentrations where they alter bilayer properties (Seeman, 1972). The study of the action of diazepam (Amin et al., 1997) and anesthetics (Rehberg et al., 1995) suggests that channel activity is affected by both direct actions on the channel protein and perturbations of the bilayer. Thus, whereas the specific interactions will depend on the three-dimensional protein (and drug) structure, the effects of bilayer modifications are likely to be general, and it becomes important to understand these perturbations to guide the development of specific drugs.

Some membrane proteins are relatively easy to simulate, and which can, in conjunction with experiment, provide insight into the mechanisms of bilayer regulation of function. For instance, the stretch activated MscL channel (containing a pentamer of 2 TM helices) provides an excellent testing ground. MscL gates both when tension is applied (Sukharev et al., 1999), and when the acyl chain-length of the surrounding lipids is reduced (Martinac, et al., 1990). Furthermore, lysophospholipids cause MscL to be stabilized in the open state under tension-free conditions (Perozo et al., 2002). One may conjecture that these molecules stabilize the open state by modifying the hydrophobic matching with the bilayer. Without microscopic trajectories, however, the mechanisms of intervention remain ill-defined. In addition, the gA channel is a good probe for protein-lipid interactions: it is sensitive to the membrane composition (Girshman et al., 1997; Lundbæk et al., 1996) and commonly used drugs (Hwang et al., 2003); is amenable to computer simulation (just 15 residues per subunit); and has been extensively studied structurally and functionally (Andersen and Koeppe, 1992). Finally, some biological processes rely on changes in membrane properties arising from the insertion of relatively small peptides. For example, fusion peptides (spike glycoproteins), consisting of 20-30 amino acids, are thought to promote membrane fusion by inducing bilayer curvature. Liquid state NMR and Electron Spin Resonance (ESR) techniques have provided model structures of the influenza hemagglutinin (HA) fusion peptide (Tamm et al., 2002). These peptides are flexible and likely to be susceptible to bilayer perturbations. The localization of fusion proteins in cholesterol-rich membrane rafts (Chamberlain and Gould, 2002) highlights the significance of studying these perturbations.

A microscopic understanding of the regulation of membrane protein function is my major goal. It is important, however, to first focus on model systems that will test methodologies, provide immediate insight, and permit quantitative comparison with experiment. The orientation and structure of a membrane protein is determined by the minimum in the free energy surface that is a consequence of protein, water and lipid interaction energetics. It is therefore important to obtain a quantitative description of protein-lipid interactions. While the transfer free energies of individual amino acids and polypeptides

from water to the interface of lipid bilayers, and into n-octanol are known (White and Wimley, 1998), experiment cannot determine where in the bilayer these amino acids reside. Microscopic simulation can provide a free energy profile and solvation structure of amino acids that is a function of position. This knowledge will guide models of TM segments, and aid experimental prediction of membrane protein structures from primary sequences. Furthermore, bilayer properties are known to determine the ability of proteins to insert into the membrane (Gennis, 1989; Scotto and Zakim, 1985). Determining changes in the thermodynamics of protein insertion as a result of membrane modifications will help elucidate the mechanisms of protein regulation.

Objectives

1. Thermodynamics of Protein-Lipid interactions:
   - Calculate the thermodynamics of inserting amino acids/polypeptides into lipid bilayers.
   - Simulate TM segments in lipid bilayers, guided by these partition free energies.
2. Bilayer structure and dynamics:
   - Calculate changes in bilayer structure and dynamics due to the presence of TM segments.
   - Calculate changes in the bilayer properties resulting from the introduction of membrane modifying compounds.
   - Calculate changes in the thermodynamics of inserting amino acids/polypeptides after introducing modifying chemicals.
3. Modulation of membrane protein function (long term goal):
   - Calculate the energetics of membrane protein conformational change in the bilayer.
   - Observe the effects of membrane perturbations on function.

Computational Strategy and Methods

With improved computational resources available today, molecular dynamics (MD) simulation has become an essential tool for investigating a wide range of chemical and biological phenomena (Karplus, 2002). Many studies simulate for long periods and "hope" to see conformational change. This hit and miss approach does not make the best use of computational resources, nor is it reliable as outcomes can depend on initial configurations. A better approach is to calculate a free energy landscape, characterized by identified "relevant" coordinates, that describes the equilibrium properties of the system. Free energy changes can be calculated through thermodynamic integration or umbrella sampling, that enforce configurational sampling by biasing MD trajectories. It was in this fashion that I calculated the potential of mean force (PMF) of Trp side-chain isomerization in gA, guided by observed transitions (Allen et al., 2003b), and that of ion permeation (Allen et al., 2003a).

Improved simulation techniques: Steered, or targeted MD, where conformational changes are induced by application of force, can circumvent the difficulties in sampling large changes in short simulations (e.g. Ma et al., 2002). During this induced conformational change, relevant coordinates for the calculation of PMF - free energy profiles can be identified. Alternatively, multiple sweeps with a constant velocity harmonic force can trace the free energy surface, using Jarzynski's equality (e.g. Jensen et al., 2002). Ultimately, studies of conformational change require the identification of local minima in the free energy surface, and the paths which connect them. The transition path sampling technique (Bolhuis et al., 2000) provides a rigorous statistical mechanical route to identifying the coordinates which drive conformational change.

The method of Replica Exchange (Sugita et al., 2000), where random walks are introduced when parallel simulations at different temperatures are interchanged every few MD steps, may be useful in improving configurational sampling for the calculation of the small energy differences associated with amino acid insertion into the bilayer. In addition, because some bilayer properties manifest on length and time-scales greater than the typical MD simulation, methods that permit extension into large spatial and temporal domains can be investigated. This may be achieved by connecting properties of the microscopic system to a macroscopic simulation in coarse-grained dynamics (Ayton et al., 2002), or by invoking stochastic trajectories.

Finally, protein-lipid interactions may be dependent on the microscopic force-field, because the potential functions involved were not specifically parameterized for this purpose. Quantum mechanical, ab initio calculations can assist in the determination of correct protein-lipid energetics. MD potential functions involve fixed atomic charges that do not incorporate the effects of electronic polarizability.

This effect is especially important for moieties that do not possess a permanent electric dipole; lipid acyl chains and nonpolar side-chains, in particular. To ensure accurate protein-lipid interactions, simulations with Drude-oscillator based polarizable force-fields (e.g. Lamoureux et al., 2003) will be investigated.

Thermodynamics of protein-lipid interactions: The thermodynamics of partitioning of single amino acids and small polypeptides into lipid bilayers will be calculated with these free energy methods. To begin, I will calculate the PMF of single amino acids from bulk water into an n-octanol slab, and compare to experiment. By considering just one residue, the contribution from protein conformational change is minimized. Also, octanol is more fluid and thus easier to simulate than a phospholipid bilayer. A PMF is expected to converge to fraction of a kcal/mol uncertainty within a few days on a 40 node, dual CPU cluster. Once it has been established that the technique is accurate, partitioning of polypeptides will be calculated and compared to experimental values. It is, however, unclear that octanol adequately mimics a membrane environment, as amino acids may remain solvated by a significant amount of water. The partitioning free energies of amino acids and polypeptides into phospholipid bilayers will therefore be calculated, and transfer to interface energies tested against experiment. It will also be useful to calculate the PMF of side chains in a long α-helix that completely spans the bilayer, translated parallel to the membrane normal, thus avoiding terminal-interface interaction effects. The calculation of partitioning thermodynamics will have implications for all membrane proteins. This information will be used to establish models to predict the structure of TM segments from amino acid sequences. Systems including α-helices and β hairpins, for example, will be simulated.

Bilayer structure and dynamics: To begin, model proteins as simple as a hydrophobic cylinder, or a carbon nanotube, will be inserted to perturb the bilayer and induce lipid ordering. I will then study the changes in bilayer structure and dynamics in simulations with secondary structures (α-helix or β hairpin). I plan to also observe the changes in bilayer properties (in particular curvature) following the insertion of influenza HA peptides, which may help us understand the onset of membrane fusion.

To obtain a clear understanding of the mechanisms of membrane regulation of protein function, it is important to investigate the effects of perturbing molecules, first on pure lipid bilayers, and then on membrane-protein systems. Bilayers containing various concentrations of cholesterol, lysophospholipids and drugs such as diazepam, valproic acid or pentobarbital will be created, and structural and dynamical variables (diffusion, area/volume, compressibility, NMR order-parameters, bending modulus) calculated (e.g. Hofsäß et al., 2003), and compared with experiment. I will then recalculate the partitioning free energies of amino acids and polypeptides into lipid bilayers that contain these compounds. This set of PMFs will generate a scale of energetic changes for amino acids and polypeptides that will reveal both common and specific effects of the membrane perturbing compounds. These calculations will guide the study of conformational changes in TM segments due to the inclusion of these perturbing chemicals.

Modulation of membrane protein function (long term goal): The culmination of this work will be to simulate realistic models of membrane proteins, determine the modes of conformational change, and calculate the influence of membrane perturbations on function. MscL gating will be studied using a combination of steered MD and umbrella sampling - PMF calculations that will elucidate the energetics of conformational change. The protein will first be embedded in an implicit membrane with imposed pressure, friction (Langevin dynamics) and dielectric regions, to provide immediate insight into conformational change, without the burden of the lipid bilayer in full atomic detail. These modes of change will then be simulated within the bilayer, and PMFs calculated to quantify the energetics of gating. Calculations will then be repeated with membrane modifying chemicals in the bilayer. Finally, the gA channel provides a direct connection to experiment. MD can be used to identify protein-lipid and protein-chemical interactions responsible for changes in channel lifetimes seen in electro-physiology. Furthermore, established techniques (Allen et al., 2003c) can be used to calculate changes in single-channel conductance, via single ion PMFs, due to the membrane modifying chemicals.

Summary and Career Plan

With the accessibility of inexpensive computer clusters today, improved simulation techniques can be used to elucidate the microscopic mechanisms of important biological phenomena. A quantitative description of the microscopic interactions between proteins and the lipid bilayer will aid the determination of new membrane protein structures from amino acid sequences. Explaining the influence of the bilayer environment on these interactions, and on protein conformational change, is necessary to

bridge the gap between structure and function. Importantly, understanding the specific, and non-specific roles of pharmacological compounds on protein function will aid the drug discovery process.

This research will promote the interplay between computation and experiment. While simulations can help explain microscopic mechanisms, and assist in the development of future experimental studies, experiments can be used to guide computational models. These include: NMR to determine order parameters to monitor structural changes in the lipid bilayer; ESR to determine mean orientation and motional restriction of lipid head-groups adjacent to the protein; and electrophysiology to compliment theoretical determinations of ion channel modulation (Prof. Olaf Andersen, Weill Medical College, has expressed interest in continuing our collaboration).

Teaching: I am planning to develop lecture courses in computational chemistry, physics and biology, with emphasis on rigorous statistical and quantum mechanical methods for studies of important chemical and biological processes. I consider it to be very important that both undergraduate and graduate students are exposed to the different levels of theory for biological computation, and their ranges of validity. This enables those who anticipate future careers, in both computation and experiment, to critically evaluate published articles, and direct their research. I plan to provide a multi-disciplinary approach to teaching, by explaining the physical, chemical and biological aspects of many computational problems. This will include: classical and quantum mechanics; thermodynamics; electromagnetism; applied mathematics; and advanced simulation, modeling and analysis methods to extract meaningful information that may be used in conjunction with experiment.

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