Computational Investigations of Biological Nanosystems

Roy J. Carver Charitable Trust
Final Report

Professor Klaus Schulten, Principal Investigator
Department of Physics and Beckman Institute
University of Illinois at Urbana-Champaign
405 N. Mathews
Urbana IL 61801
http://www.ks.uiuc.edu

July 17, 2000

Contents

1 Overview

Understanding life from its molecular foundation to the cellular, organ, and organism levels complements the practice of medicine. In answering the most basic questions about organisms, biomedical researchers import methods and concepts from the physical sciences that encompass novel experiments and mathematical descriptions. Likewise, motivated by biomedically relevant problems and collaborating closely with experimental laboratories, the Theoretical Biophysics Group at the Beckman Institute of the U. of Illinois at Urbana-Champaign exploits advances in physical theory and computing to model organisms across many levels of organization, from molecules to cells to networks. During the past decade, the group has pioneered the modeling of very large biomolecular structures that are responsible for key processes in living cells, ranging from metabolism to signaling to cell motion.

This report describes our research, supported by the Carver Charitable Trust, during the last year of a total funding cycle of nine years. Our group has derived from the Craver funds great benefits and we feel compelled to explain in a section *Carver Charitable Trust funds built an important science interface* why the support from the Carver Charitable Trust has been important and so extraordinarily successful.

Through the Carver support we continued to advance studies in cellular biology, bridging a gap between laboratories where large biomolecular structures are discovered and measured, and computational laboratories where the expertise for very large scale molecular modeling resides. The group has engaged in relevant and demanding collaborations on cellular nanosystems that required large scale modeling and that reached significant results. Our group has pioneered also the use of so-called steered molecular dynamics in which external forces are applied to test reaction pathways and mechanical properties of biopolymers as well as analyze the results. The Carver funded collaborators applied steered molecular dynamics in several new exciting research projects, for example, to understand the expression of genetic information through mechanical control of DNA, the harvesting of sunlight that fuels nearly all life on earth, the transformation of light energy into electrical energy in the form of a proton gradient, useful to the cell, or to the control of the electrical potential of neurons through ion selective membrane channels. All these systems are fundamental and basic to life, yet involve large molecular systems containing ten thousand to hundred thousand atoms that can be handled computationally by very few research groups.

Research advances achieved through its unique modeling capabilities are a primary measure of our group's success. Through Carver funds our group collaborates on a number of ongoing projects beyond those already mentioned: designing proteins that induce the growth of gold crystals by carrying out novel simulations of an oligopeptide at crystal surfaces; explaining how membrane tension opens channels by molecular dynamics simulation of the stress-controlled gating of the ion channel MscL. Experimental groups at the Mayo Clinic and at the Bionegineering Department of the University of Washington in Seattle have continued their successful collaboration with the Resource on the mechanical properties of proteins in muscle (titin) and the extracellular matrix (fibronectin) using modeling in combination with observations to explain previously unobserved prestretching phases in these proteins. This ideal combination of single molecule experiments and theoretical modeling is regarded by leading researchers as one of the most fruitful areas of mechanistic molecular biology.

To illustrate our recent work following are a few examples that are described in more detail later in this report.

The purple membrane in *Halobacterium salinarium* of the archaea kingdom is a fascinating cellular structure, highly ordered and fully functional as a light-driven proton pump fueling the cellular metabolism. On the basis of electron microscopy and crystallographic data, our group has used new features of its program NAMD to develop a complete atomic level model of the purple membrane, including proteins, all lipids, ions, and bulk water. For the first time a complete structure of a cellular machine, including its native environment, is available.

Cellular structures often exceed the size that is amenable to molecular modeling calculation, even for the most advanced programs. Cases in point include complexes of proteins and DNA, as in the case of multi-nucleosomal systems involved in genetic regulation and often in its disfunction. Using the theory of elasticity, the DNA component of such systems is readily modeled, but effective solution methods and testable predictions must be developed as well. Working alongside other successful computational biologists in this field, in the past year our group made an important prediction of the loop geometry of DNA clamped by the *lac* repressor relying on our own methodological contribution. The *lac* repressor was one of the first regulatory proteins studied and is an archetype system in regard to protein-DNA interactions. The predicted loop form was found to be in remarkable agreement with many observations and has revealed insights into straightforward mechanical strategies of DNA regulation.

A blue-light photoreceptor found in nerve layers of the eyes and brains is another subject of a collaboration our Carver-funded researchers have established. The receptor, cryptochrome, is known to play a prominent role regulating an animal's day-and-night rhythm. Our research suggests that cryptochrome may be the site of a neurochemical reaction that lets birds, for example, process visual clues from the magnetic field and stay on course. Typical biomolecules interact with Earth's magnetic field too weakly to alter the course of their chemical reactions. Earlier experiments had shown, however, that certain chemical reactions involving so-called photo-induced radical pairs can be influenced by weak magnetic fields. The work in our group provided theoretical evidence that a biochemical reaction involving cryptochromes can be influenced by geomagnetic fields. If radical-pair reactions in cryptochromes were connected by photoreception to the vision of animals, the magnetic field would modulate visual sensitivity. Behavioral biologists tested the proposed theory and found, among other surprising agreements, that many magnetic responses in animals require light.

The growing success and recognition of our group's research and development activities and the efforts to sustain high productivity and quality levels have resulted in new federal and other prestigious awards granted to the Resource. Additional spaces allocated to the facility at the Beckman Institute enabled us to increase the number of researchers, developers and visitors. We are very grateful to the Carver Charitable Trust in this regard, but we feel that even more important for us is the role the Carver Charitable Trust played in educating the next generation of scientists in biomedicine. We like to explain this critical contribution in more detail.

Carver Charitable Trust Funds Built an Important Science Interface

The ongoing sequencing of the genomes of diverse organisms provides the inventory of the molecular constituents of life through huge databases. Their use and, more importantly, their integration into the study of signaling and metabolic circuitry of cells and organisms in the postgenomics phase of biology will require routine and all-pervasive use of computing. The far-sighted support that the Theoretical Biophysics Group at the Beckman Institute of the University of Illinois at Urbana-Champaign has received from the Carver Charitable Trust has lead during the past six years to

the recruitment of young researchers who were individually trained in computational-experimental projects. The UIUC campus, with its outstanding departmental programs and units linking the physical and biological sciences, offers an ideal infrastructure and a fertile ground for training at the Biology/Physics interface frontier. The Carver funds provided an essential and well-timed element to maximize the intellectual impact of these programs and to strengthen the relationship between them.

The assignment of a well-defined research project to incoming Carver-funded fellows with a dual link to both computational and experimental groups proved to benefit their training experience tremendously. We believe that typically, students in the physical sciences encounter a vastness of information hard to comprehend by a novice scientist. This barrier can be successfully overcome through a focus on a specific research area. A focus that could ease the steep initial learning curve while still giving trainees a sense of the life science culture and hands-on practices. A given project, adopted early on by a student, could provide a seed around which broader knowledge would be acquired and crystallize and further developed. Once a trainee is comfortable with one biological area, this knowledge would serve as a gateway to other areas. Moreover, having a research project that requires the cooperation of computational and experimental groups would link trainees from the outset to relevant subjects at the forefront of life science. In other words, our approach ensures concrete yet integrative training that encompass theoretical, experimental and computational teaching as well as a close exposure to and appreciation of information technology and its promise. We are convinced, and our research accomplishments and the success of our researchers support our conviction, that linking theory and experiment from the outset is optimal for training a generation of researchers that will have to master information technologies in future life science work. Placing multi-level and cross-disciplinary experience at the beginning of one's studies in a context that is both relevant and practical is more important now than ever before.

The Carver funds have allowed our group to develop ample experience in computational-experimental collaborations. Further, with the support from the Carver Charitable Trust we have established a great training environment for the next generation of bioscientists.

Figure 1: Theoretical Biophysics Group in the garden of Beckman Institute.

2 Research Highlights

2.1 Modeling DNA loops using the theory of elasticity

TB Researcher: Alexander Balaeff
Collaborating investigator: L. Mahadevan, Professor, Department of of Mechanical Engineering, MIT
URL: `http://www.ks.uiuc.edu/Research/pro_DNA/elastic/`

Summary

During the past two years we developed a method for coarse-grained modeling of DNA loops. The method employs the classical theory of elasticity to model the DNA as an electrically charged elastic ribbon. The ensemble of the equilibrium conformations of this ribbon is obtained by solving the classical equations of elasticity. Modifications to the equations produce a very realistic DNA model and permit one to manipulate the DNA structure.

The modeling method was developed and tested in the framework of studies of a DNA loop clamped by the *lac* repressor protein. Several possible structures of the loop were predicted and analyzed; the binding of the catabolite gene activator protein (CAP) within the repressor-clamped DNA loop was simulated and the experimentally observed cooperation between the two proteins was explained.

Introduction

Formation of DNA loops is a common motif of protein-DNA interactions [52, 73]. The loops either have their termini clamped by several protein molecules, or wind around large multiprotein aggregates. DNA loops, shown to play an important role in the regulation of bacterial genomes [52, 73], should also be common structural elements of the condensed protein-DNA media of eucaryotic nuclei [2]. Understanding of the structure and dynamics of DNA loops is vital for studying the organization and function of eucaryotic genomes. To efficiently study the DNA loops, which may reach hundreds of base pairs (bps) in size, it is imperative to use fast coarse-grained modeling methods.

The theory of elasticity provides a powerful tool for the coarse-grained DNA modeling [60, 74, 83]. The DNA is modeled as a continuous electrically charged elastic ribbon. The thermal-average properties of such a ribbon may be obtained in Monte Carlo simulations employing a combined elastic/electrostatic energy function [60, 74, 83]. Alternatively, one can solve the equations of elasticity [40, 43] and obtain the set of equilibrium conformations the ribbon adopts when its ends are fixed or are subjected to an external force [11, 78]. The second approach is computationally much faster and is preferred over the first one for predicting the global properties of the studied DNA structure, *e.g.*, the topology of the DNA loop or the forces of the protein-DNA interactions.

The classical Kirchhoff equations of elasticity [43, 49] have to be modified in order to achieve a realistic description of DNA. The modified model should capture such well-known DNA properties as its intrinsic twist and curvature, its sequence-dependent anisotropic flexibility, and its electric charge [60]. Such modifications render the equations of elasticity more complicated and their

solution more intricate. The main goal of our collaborative project was to derive the new equations and to develop numerical algorithms for solving them.

As a test system for the developed method, we chose the regulatory DNA loop of *E. coli* clamped by the *lac* repressor, a celebrated protein studied since the first days of modern molecular biology [54, 80]. The repressor shuts down a set of bacterial genes responsible for lactose consumption when lactose is scarce. The repressor grabs two out of three recognized sites on the bacterial DNA, forcing the DNA between these sites to form a loop [59]. The crystal structure of the complex between the *lac* repressor and DNA is known [42], but lacks the DNA loop which is so large (at least 75 bp) that it can hardly be crystallized. However, the crystal structure of the *lac* repressor without the DNA loop provides boundary conditions for the DNA loop, thus allowing us to predict the loop structure and to study how the structure is influenced by the physical characteristicies of DNA.

The clamped DNA loop contains a binding site for another *E. coli* regulatory protein called the catabolite gene activator (CAP) [33, 42]. The CAP is a transcription activator, which surprisingly cooperates with the *lac* repressor in suppressing the *lac* operon genes [33]. In one suggested mechanism [42] of the cooperation, the CAP binds within the clamped loop so that two DNA kinks induced by the CAP [62, 77] may stabilize the loop. The CAP binding within the loop can be mimicked by our modeling method, namely, by enforcing the "intrinsic" curvature at the DNA steps kinked by the CAP. The results suggest that the CAP binding within the loop is indeed possible; however, the cooperation is likely to result from entropic effects rather than directly from stabilization of the bent loop by the CAP.

Specific Aims

Our goal was to create a robust modeling method based on the elasticity model of DNA. In a first step we derived the equations of equilibrium of the elastic ribbon with varying anisotropic flexibility and varying intrinsic twist and curvature. Secondly, the equations were augmented with the force terms accounting for electrostatic and van der Waals interactions. In a third step a robust numerical algorithm for solving these equations was developed.

The modeling method was employed to: (i) build the structure of the DNA loop clamped by the *lac* repressor and to determine the force that the DNA exerts on the protein; (ii) determine what changes in the loop structure result from the binding of CAP and to explain the cooperation between CAP and *lac* repressor in DNA binding.

Methods and Results

Following our previous work [4] we define the conformation of the elastic ribbon in terms of the ribbon centerline $\vec{r}$, the principal curvatures κ_1, κ_2, and the local twist ω. All these values change as functions of the ribbon arclength s measured along the centerline of the loop.

In order to build an adequate DNA model based on the theory of elasticity, we modify the classical theory in the following ways, summarized in Table 1. First, the *intrinsic* components, which do not contribute to the stress within the elastic ribbon, are separated from the total DNA twist and curvature. The intrinsic twist and curvature are functions of the arclength s so that they may be different in different parts of the loop. Second, the DNA flexibility is rendered variable along the loop so that the moduli α and β of bending in two principal directions of the ribbon

Classical equations	Introduced DNA property	New equations
$\vec{k} = \begin{pmatrix} \kappa_1(s) \\ \kappa_2(s) \\ \omega(s) \end{pmatrix}$	Intrinsic twist ω_o and curvatures $\kappa_1^o(s)$, $\kappa_2^o(s)$	$\vec{k} = \begin{pmatrix} K_1(s) \\ K_2(s) \\ \Omega(s) \end{pmatrix} = \begin{pmatrix} \kappa_1(s) + \kappa_1^o(s) \\ \kappa_2(s) + \kappa_2^o(s) \\ \omega(s) + \omega_o(s) \end{pmatrix}$
$\alpha = const$ $\beta = const$ $\gamma = 1$	Varying flexibility	$\alpha = \alpha(s)$ $\beta = \beta(s)$ $\gamma = \gamma(s)$

Table 1: Parameters of the Kirchhoff equations, modified in order to adequately model the DNA properties. Notation: $\vec{k}$ - the vector of strains, κ_i - the principal components of the ribbon curvature, ω - the twist of the ribbon, $\alpha = A_1/C_o$, $\beta = A_2/C_o$ - the moduli of the ribbon bending in the principal directions, normalized by the twisting modulus, $\gamma = C(s)/C_o$ - the normalized modulus of the ribbon twisting.

cross-section[1] also become functions of the arclength s. (The moduli are considered constant in the classical theory.) The flexibility is rendered anisotropic, that is, $\alpha \neq \beta$. All these changes affect the cornerstone equation of the theory of elastic ribbons, the Bernoulli-Euler approximation, which relates the elastic torque $\vec{m}(s)$ within the ribbon to the local curvatures and twist:

$$\vec{m}(s) = \begin{pmatrix} \alpha\kappa_1(s) \\ \beta\kappa_2(s) \\ \omega \end{pmatrix} \qquad \text{becomes} \qquad \vec{m}(s) = \begin{pmatrix} \alpha(s)\ (K_1(s) - \kappa_1^o(s)) \\ \beta(s)\ (K_2(s) - \kappa_2^o(s)) \\ \gamma(s)\ (\Omega(s) - \omega_o(s)) \end{pmatrix}$$

(the variables are defined in Table 1).

The computational algorithm used for solving the modified equations is based on the one employed in our earlier work [4]. One starts with the set of boundary conditions and the other problem parameters for which a known solution exists and gradually changes the parameters in a series of iteration cycles until the desired conditions are met. The equations are re-solved at each step of the iteration cycles after some of the parameters were slightly changed. The new modifications, introduced to the equations, simply require running through additional iteration cycles. First the elastic moduli are changed from constant to the varying values, then the intrinsic curvatures are turned on.

The electric charge of DNA phosphates presents a special algorithmic challenge. The charge is reproduced in our model through the continuous charge density $\sigma(s)$ spread along the centerline of the ribbon with sharp maxima between the base pairs, where the phosphates lie. The electric field $\vec{E}(\vec{r})$, created by the external electric charges and the DNA loop itself, results in the force $\vec{f}(s)$ that must be equilibrated by the elastic force $\vec{n}(s)$ at each point of the loop:

$$\dot{\vec{n}}(s) = -\dot{\vec{f}}(s) = -\sigma(s) \left(\vec{E}_{ext}(\vec{r}(s)) + \vec{E}_{DNA}(\vec{r}(s)) \right)$$

[1] The principal axes x and y of the cross-section point towards the DNA backbone and major groove, respectively.

Before including the electric field, the equations of elasticity were ordinary differential equations; now they become integro-differential because the electric field $\vec{E}_{DNA}$ of the DNA loop itself depends on the entire loop conformation. To solve these equations, the following algorithm was invented. Each step of the iteration cycles unfolds into its own iterative sub-process which repeatedly solves the equations of elasticity until the conformation of the loop converges to an equilibrium state. The forces of self-repulsion are re-computed at the beginning of each round of this sub-process. To enforce convergence, the actual forces of self-repulsion are weight-averaged with those used in the previous round:

$$\vec{f}_i(s) = w\ \vec{f}_{i,actual}(s) + (1-w)\vec{f}_{i-1}$$

While this approach may conceivably fail for some intricate ribbon conformations, it works extremely well for the studied case of the DNA loop clamped by the *lac* repressor.

The developed equations were numerically solved for the boundary conditions obtained from the crystal structure of the *lac* repressor-DNA complex. The modeled DNA had the natural intrinsic twist of 34.6 °/bp and no intrinsic curvature, anisotropic but constant flexibility, and the charge of $-0.5\,\mathrm{q}_{electron}$/bp, as compensated by the Manning counterion condensation. Two loops of length 75 bp and 384 bp that the *lac* repressor may induce in *E. coli* were considered. Two different solutions, labeled as the odd and the even [4], were obtained for the 75 bp loop, and four solutions were obtained for the 385 bp loop (Fig. 2). The obtained solutions differ in each case by the amount of the excess (or deficit) twist in the loop. A simple theoretical estimate shows that an additional twist would significantly increase the energy of the loop; accordingly, the other possible solutions have much higher energy and were not found by our procedure.

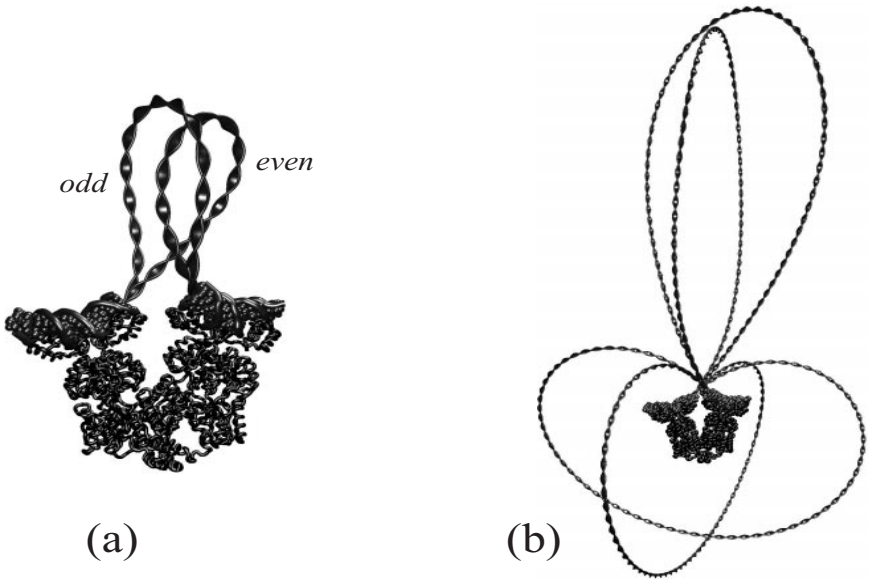

Figure 2: Solutions of the modified Kirchhoff equations for (a) 75 bp DNA loop, (b) 384 bp DNA loop, induced by the *lac* repressor; (a) and (b) are drawn to different scale.

The obtained DNA conformations are likely to be realistic. Their calculated energies (20 to 30 $k\,T$ for the short loop, below 20 $k\,T$ for the long loop) are comparable with those estimated from experiment [28]. The calculated force of the DNA resistance to bending is not exceeding 10 pN, which is well within the range of forces typical for protein-DNA interaction [53]. This force is unlikely to significantly disrupt the structures of the protein domains or the DNA double helix, but

may produce certain structural changes in the *lac* repressor, *e.g.*, cause the rotation of the repressor headgroup domains, bound to DNA.

The DNA elastic moduli and charge were broadly varied in order to assess their influence on the obtained loop structures. The odd solution for the 75 bp loop served as a test case. Changes in bending anisotropy mainly effected the energy, but not the overall geometry of the loop. For α/β ratios varied between 1/20 and 20, the axis of the loop did not deviate from the course of the physiological ($\alpha/\beta = 4$) solution by more than 15 Å or just 6% of the length of the loop (Fig. 3a). At the same time, the energy of the loop decreased by half (Fig. 3b). Such behavior is due to the high intrinsic twist of DNA: the tightly wound elastic ribbon is stiff and can not easily change its global shape but small oscillations of the local twist can save significant amount of energy by adjusting the local structure of the ribbon [4].

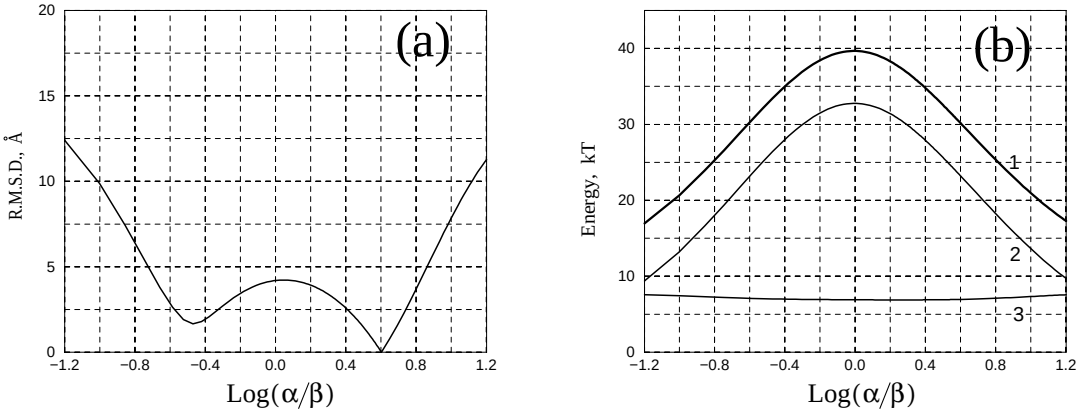

Figure 3: The effect of the anisotropy of bending on the odd solution for the 75 bp loop. (a) Root-mean-square deviation from the solution at $\alpha/\beta = 4$. (b) Energy: 1 - total, 2 - elastic, 3 - electrostatic.

The electrostatic interactions tend to increase the separation between different segments of the loop. The electrostatic effect, however, is reduced due to screening by counterions and is significant only when the loop exhibits a near self-crossing, so that some of its segments approach each other nearer than the Debye radius. Electrostatics pushes such segments of the loop apart. For example, the even solution for the 75 bp loop has a near self-crossing and the self-repulsion moves the centerline of this loop by 30 Å on average. On the contrary, the centerline of the odd solution, which has an open shape without self-crossings, moves only by 3 Å on average after electrostatics is turned on. (These data were obtained for the 25 mM concentration of Na^+ counterions.)

The conclusion of our studies is that the bending anisotropy is essential for a correct account of the energies and forces of protein-DNA interactions, but may be neglected in a quick study of the shape of a DNA loop. The electrostatics is prerequisite for correct treatment of the points of near self-crossing and becomes important for longer DNA loops when the self-crossings are more likely to appear.

The developed modeling method was used to study the possible binding of the CAP protein inside the DNA loop clamped by the *lac* repressor. The crystal structure of the CAP-DNA complex [62, 77] shows that the CAP induces kinks of about 45° at two DNA steps and unwinds the double helix at those steps by about 20°. The DNA structure between the bends is mainly unperturbed.

To mimic the structure of the CAP-bound DNA, we changed the model parameters along the CAP binding site which lies near the beginning of the 75 bp loop (Fig. 4). The DNA intrinsic

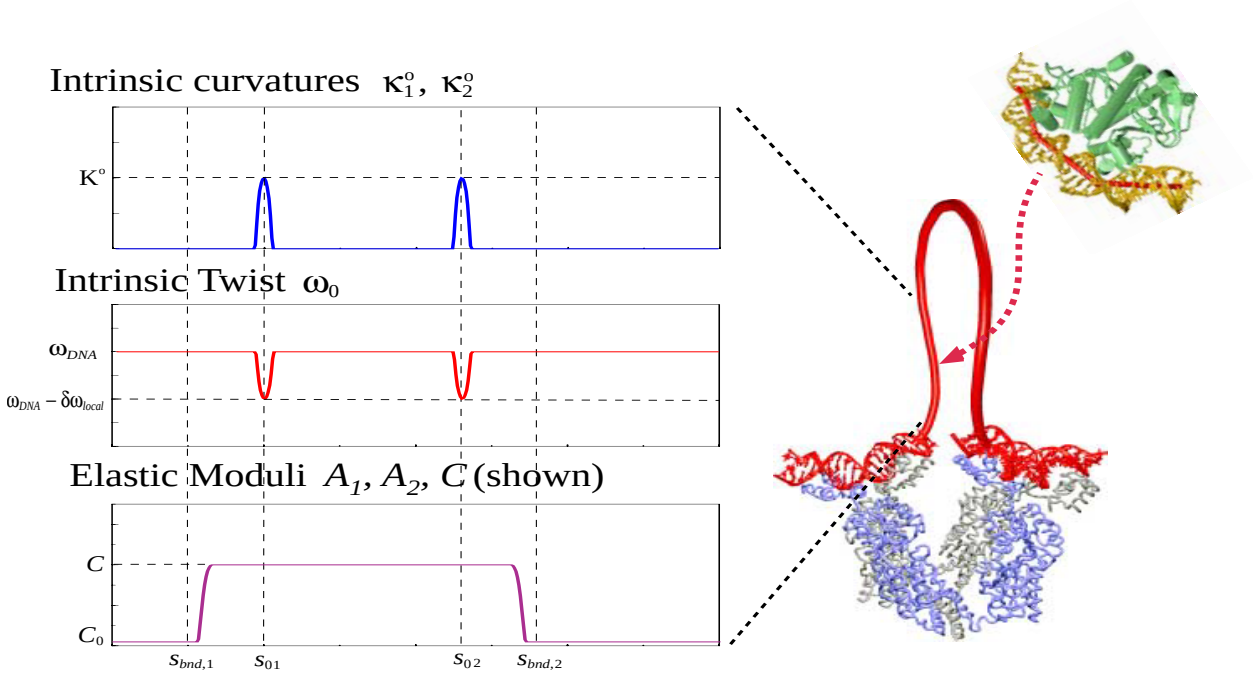

Figure 4: Elasticity parameters that mimic the binding site of the CAP protein within the *lac* repressor-clamped DNA loop. Shown on the right are the crystal structure of the CAP-DNA complex (top) and the crystal structure of the *lac* repressor-DNA complex together with the predicted "odd" structure of the clamped 75 bp DNA loop (bottom).

curvature was raised at the two bent steps so as to result in the experimentally measured bending angles. The intrinsic twist was lowered at these steps to mimic the DNA unwinding by the CAP. Also, the elastic moduli were increased over the CAP binding site in order to make the site "stiff", resistant to any changes from the prescribed geometry.

The Kirchhoff equations with thus modified parameters were iteratively solved starting from the odd and the even conformations of the 75 bp loop. The energy of the resulting odd loop increased by 0.2 kT that is well below the favorable energy of the CAP-DNA interaction [33]. The geometry of the loop is such that the CAP, inserted at the mimicked binding site, can be accomodated with but a small overlap of its hind part with the downstream regions of the loop (Fig. 5a). Such overlap can be avoided after a small adjustment of the loop structure. So, the formation of the ternary complex of the CAP, the *lac* repressor, and the 75 bp DNA loop is likely to be favorable from the energetic point of view. This conclusion is however not final as there must be intensive interaction between the DNA and the hind part of the CAP. If this interaction is unfavorable the predicted structure of the ternary complex would be significantly destabilized.

The structure of the even loop can not accomodate CAP, as the protein would completely overlap with one of the DNA segments bound to the *lac* repressor (Fig. 5b). However, varying the length of the loop has a dramatic influence on the structure (Fig. 5c). Increasing the length of the loop by 5-10 bp results in both a significant drop of the loop energy and a more open loop structure which can easily accomodate CAP (Fig. 5c,d). This result agrees with the experimental observation that binding of the CAP makes the upstream DNA binding domain of the *lac* repressor slide by 6 bp along the DNA [64]. Such relocation of the binding domain is energetically not unfavorable because the upstream binding site has a significant affinity to the *lac* repressor (contrary to the almost optimal downstream binding site). The loss of interaction energy due to the upstream domain relocation should be well compensated by the energy of the CAP binding and the lowered energy of the DNA loop.

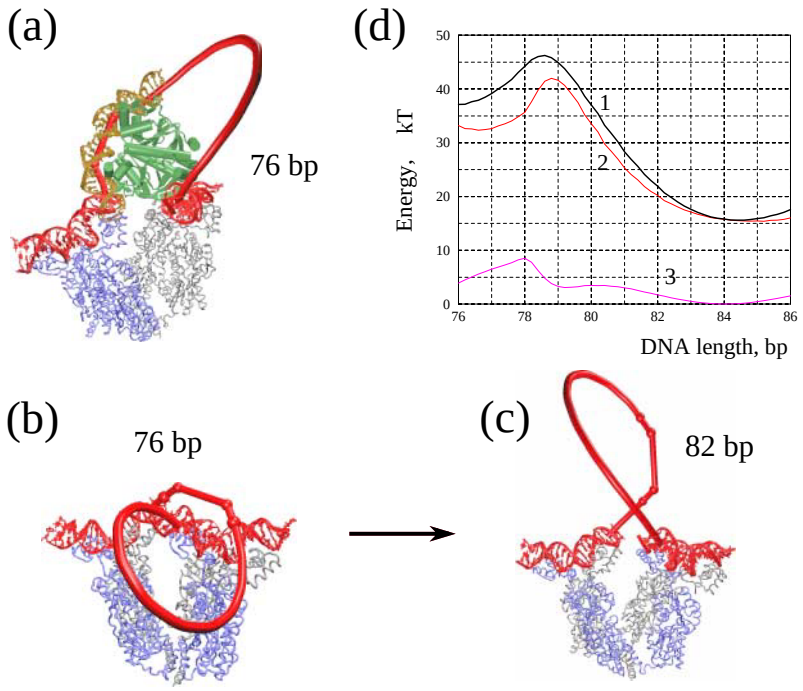

Figure 5: The CAP binding site mimicked within the DNA loop clamped by the *lac* repressor. (a) Crystal structure of the CAP-DNA complex fitted to the "odd" loop. (b) The "even" loop can not allocate the CAP. (c) The structure of the "even" loop can easily allocate the CAP after increasing the loop length. (d) Increasing the loop length results in a significant energy gain (the curves are: 1 - total energy, 2 - bending energy, 3 - twisting energy).

Our results suggest that the cooperation between the CAP and the *lac* repressor involves an *entropic* mechanism. CAP not only binds to the odd conformation of the loop – favorably or slightly unfavorably – but also significantly reduces the energy of the even conformation by inducing sliding of the upstream domain and 6bp loop lengthening. Thus, more conformations become available for the clamped DNA loop which should normally reside only in the low-energy odd conformation. The entropy of the complex increases and the free energy decreases, resulting in the greater stability of the whole system.

Outlook

The development of our method is a significant step towards multi-resolution modeling of protein-DNA interactions. Whenever a simulated protein-DNA complex involves a DNA loop, our approach can be used to rapidly compute the forces which the loop exerts upon the rest of the system. The scope of the time-consuming detailed simulation can thereby be reduced to the rest of the protein-DNA complex. For example, we plan a simulation of the *lac* repressor-DNA complex with the clamped DNA loop represented by the forces acting on the lateral bases of the protein-bound DNA segments. These forces may be re-computed using the elastic ribbon model every time the loop boundaries change their positions in the course of the simulation.

We also plan to apply the developed method to studying other protein-DNA aggregates. Of special interest are the protein-DNA interactions in chromatin, vital for the function of eucaryotic genomes. Our method is now well developed to study the structure of DNA wrapped around single nucleosomes and even multinucleosomal aggregates.

2.2 Photosynthetic Light-Harvesting Complexes

TB Researchers: Ana Damjanović, Thorsten Ritz
Collaborating investigators: X. Hu, Department of Chemistry, University of Toledo
URL: `http://www.ks.uiuc.edu/Research/psu/psu.html`

Summary

In the current funding period we have been studying the light-harvesting role of carotenoids in photosynthetic organisms. The protein that we studied, peridinin-chlorophyll-protein (PCP) of dinoflagellates, is unique among light-harvesting proteins in that it employs predominantly carotenoids (peridinin) as light-absorbers, as opposed to employing predominantly chlorophylls. Through quantum chemistry calculations we investigated the pathways and mechanisms of excitation transfer between carotenoids and chlorophylls in PCP [15], and compared them with those of the predominantly chlorophyll-based light-harvesting complex II (LH-II) of purple bacteria, studied previously by us [13, 71].

In addition we have performed molecular dynamics (MD) simulations of LH-II integrated into a lipid bilayer and water. The information about thermal fluctuations in LH-II emerging from this molecular dynamics study will soon be combined with the quantum mechanical description of electronic excitations in LH-II, developed previously by us [30, 72]. This approach will yield a complete understanding, not only of electronic excitations in LH-II at physiological temperatures, but also of energy transfer processes in the entire photosynthetic unit of purple bacteria.

Introduction

Life on Earth is sustained through photosynthesis. Photosynthetic organisms, i.e., plants, algae, and photosynthetic bacteria, developed very efficient chromophore-protein complexes [known as the photosynthetic unit (PSU)] to harvest the light of the Sun and to utilize its energy to drive photoinduced chemical reactions [29, 31]. Photosynthetic organisms exhibit a great diversity of light-harvesting apparati. For example, dinoflagellates, a class of phytoplankton which causes red tides and fish bite, choose carotenoids as their predominant light absorbers. Figure 6 shows the structure of the major light-harvesting complex of dinoflagellates, PCP [26]. A 4:1 ratio of carotenoids to chlorophylls is found in this light-harvesting protein. On the other hand, purple bacteria, along with most of the other photosynthetic life forms, involve more (bacterio)chlorophylls [(B)Chls] than carotenoids. The structure of the PSU of purple bacteria is shown in Figure 7; ring-shaped light-harvesting complexes (LH-I and LH-II) surround the so-called photosynthetic reaction center (RC), and contain hundreds of individual α-helical segments and thousands of chromophores (BChls and carotenoids). The previously reported crystal structure of the light-harvesting complex LH-II of *Rhodospirillum (Rs.) molischianum* [38] as well as modeled atomic structure of the light-harvesting complex I (LH-I) of *Rhodobacter (Rb.) sphaeroides* [32] which directly surrounds the photosynthetic reaction center [3, 18], yield a complete picture of chromophore organization in the PSU. The resulting model structure of the PSU provides a framework for studying its function: absorption of light and funneling of electronic excitation energy to the photosynthetic reaction center, via a series of energy transfer steps between the chromophores.

Figure 8 shows the positions of the chromophores in LH-II. The BChls are named according to

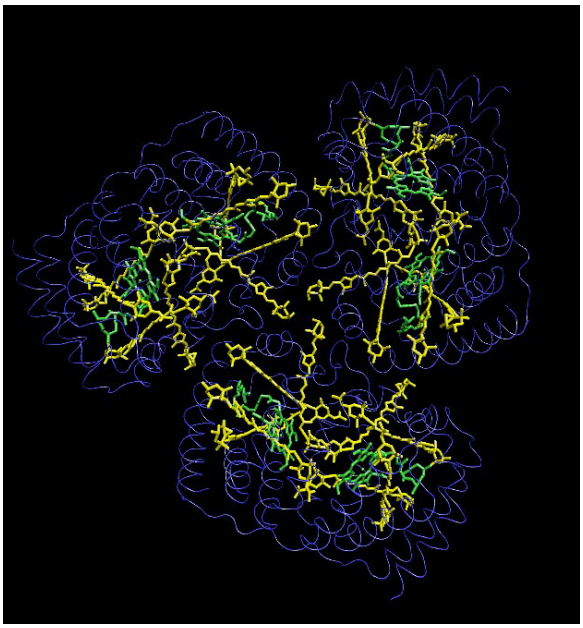

Figure 6: Structure of the PCP trimer of dinoflagellate *A. carterae*. Six chlorophylls (shown in green) and 24 peridinins (shown in yellow) are in licorice representation, while α-helices are represented as blue tubes. The Figure is produced with the program VMD [34].

their absorption maxima as B800 and B850 BChls. Due to the strong coupling between BChls of the B850 ring (the bottom BChl ring in Figure 8), electronic excitation of the B850 BChls is delocalized over several BChls. In the absence of thermal and structural disorder, electronic excitations of B850 BChls would be delocalized over the entire ring, forming coherent superpositions of individual BChl excitations, so-called excitons. The disorder, however, localizes the excitation to fewer BChls. The exact exciton delocalization length, as caused by the thermal and structural disorder, is still a subject of debate [57, 67].

Specific Aims

Our goal is to understand the light-harvesting strategies employed by different photosynthetic organisms. Specifically, we studied the carotenoid-chlorophyll interaction in two light-harvesting complexes (PCP and LH-II) which employ a very different number and type of carotenoids. We developed a quantum mechanical description of electronic excitations and of excitation transfer in closely coupled chromophore aggregates, such as the B850 ring of BChls in LH-II and the carotenoids in the PCP. Ultimately, we plan to study particular steps of energy transfer at physiological temperatures, thus completing the understanding of the energy funnel within the PSU.

Methods and Results

The mechanism of excitation transfer between carotenoids and chlorophylls has been addressed by means of semi-empirical type calculations of the excited electronic states of carotenoids and chlorophylls [13]. The couplings between these states were determined through perturbation theory,

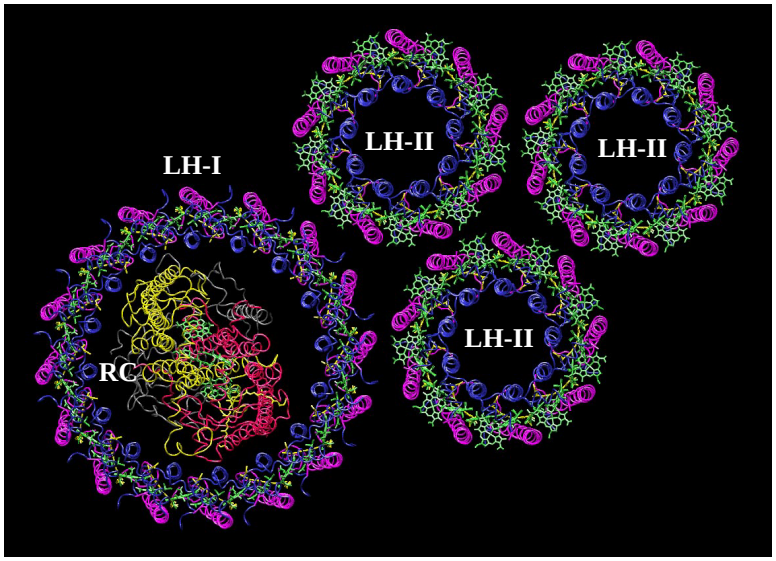

Figure 7: Top view of the modeled photosynthetic unit (PSU) of purple bacteria; ring-shaped light-harvesting complexes (LH-I and LH-II) surround the photosynthetic reaction center (RC). The α-helices are represented as C_α-tracing tubes with α-apoproteins of both LH-I and LH-II in blue and β-apoproteins in magenta, and the L, M, H subunits of RC in yellow, red, gray, respectively. All the BChls are in green, and carotenoids in yellow. (produced with the program VMD [34]).

based on which we calculated the corresponding excitation transfer times, by means of Fermi's golden rule. Based on the agreement of the calculated and experimental transfer times, insights into the pathways and mechanisms of energy transfer were obtained [13, 15, 71], as summarized in the following paragraph.

Carotenoids are chemically related to polyenes, and their relevant electronic excitations are labeled according to polyene symmetries as A_g^- and B_u^+. The absorption takes place into the strongly symmetry allowed B_u^+ state. This state quickly relaxes into the lower lying symmetry forbidden A_g^- state. Our calculations suggest that, due to the choice of carotenoids, the two proteins that we studied, LH-II and PCP, employ very different strategies of energy transfer [71]. Lycopene, a very symmetric carotenoid found in LH-II, uses mostly the allowed B_u^+ state to transfer energy to chlorophyll. However, due to the short lifetime of the B_u^+ state, this transfer pathway is not very efficient. Transfer from the A_g^- state is precluded due to the dipole forbidden character of this state. As a result, the overall energy transfer efficiency for lycopene in LH-II is low [13], as determined also by experiments [68]. On the other hand, PCP uses a very asymmetric carotenoid, peridinin. For this carotenoid, the energy transfer through the A_g^- state is possible, due to non-vanishing transition dipole of this state, acquired through symmetry breaking [15]. Thus, the overall energy transfer efficiency of peridinin in PCP is very high.

Electronic excitations of the strongly coupled aggregate of B850 BChls in LH-II, in the absence of thermal disorder, have been studied by us through quantum chemistry and an effective Hamiltonian [12, 14, 30, 72]. The results provide a detailed description of properties of electronic states (energies, dipole and transition dipole moments) that are relevant to the biological function of BChls in light-harvesting, i.e., light absorption and energy transfer on the sub-picosecond timescale. The need to describe the electronic excitations at physiological temperatures has prompted us to perform a molecular dynamics simulation of LH-II integrated into a lipid bilayer with appropriate

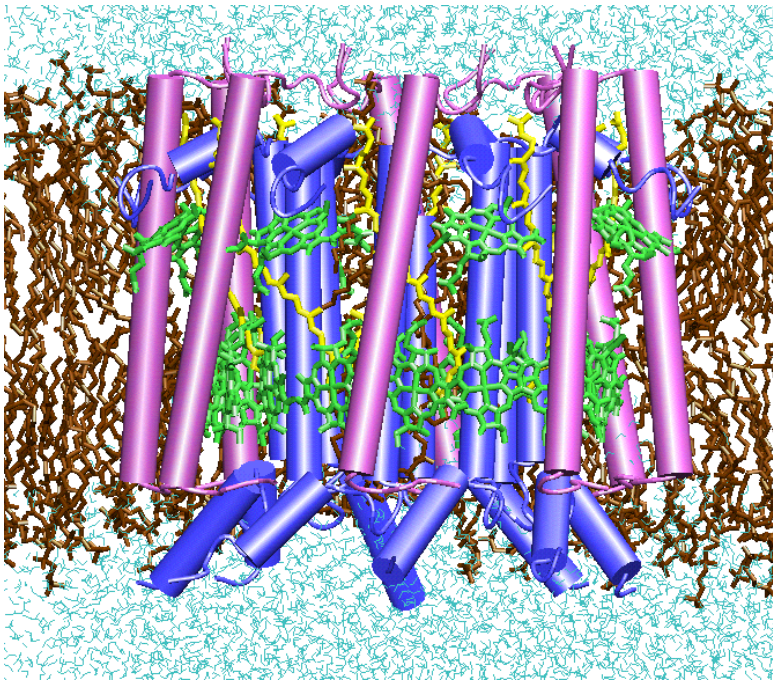

Figure 8: A side view of the octameric LH-II complex of *Rs. molischianum* in its lipid-water environment. The α-helices are in cartoon representation with α-apoproteins shown in blue and β-apoproteins in magenta. All the BChls are in green, and carotenoids in yellow. The bottom BChl ring is denoted as B850, while the top BChl ring is denoted sa B800.

water layers. The modeled system of LH-II embedded into membrane and surrounded by water layers is shown in Figure 8. The system contains about 85,000 atoms. The simulation was performed with the program NAMD [58], using periodic boundary, full electrostatics, and constant pressure and temperature conditions. We are currently developing a theory to integrate the results of the molecular dynamics simulation with the already existing effective Hamiltonian description of the excitonic states of the B850 BChls. The current theory of energy transfer for static structures will also be revised and adapted to dynamics disorder as found at physiological temperatures.

Outlook

The overall good agreement between theoretical description and observation of the femtosecond - picosecond flow of excitation in light-harvesting systems of purple bacteria and dinoflagellates allows us to conclude that the physical mechanism of light-harvesting in these two species is qualitatively understood. Open questions regarding the role of dynamic disorder are being studied through molecular dynamics simulations of the proteins in combination with stochastic quantum mechanical descriptions. The molecular dynamics simulations will allow us to describe the forces that are involved in the formation and stability of the multi-protein light harvesting complexes.

2.3 The Purple Membrane in Halobacteria Fuels the Cells' Metabolism Through Proton Pumping

TB Researchers: Jerome Baudry, Emadeddin Tajkhorshid, Michal Bennun, Ferenc Molnar, James Phillips
URL: `http://www.ks.uiuc.edu/Research/newbr/`

Summary

A key achievement during the past year was the integration of the available bacteriorhodopsin (bR) structures into a model for the entire Purple Membrane (PM). This hexagonally periodic, lamellar model has been hydrated and refined through a constant pressure molecular dynamics simulation. The resulting structure connects extracellular bulk water with water molecules and key side groups in the interior of bR, permitting a seamless overall description of proton conduction and pumping in the PM, from intracellular to extracellular space. For the first time a complex cellular reaction can be accounted for in full atomic detail in its complete native environment.

Introduction

Cells fuel their metabolism by means of a hierarchy of energy conserving steps. The primary step is the generation of a potential across cellular membranes through vectorial transport of protons. In most cells, the machinery involves several types of membrane proteins, but in *Halobacterium salinarium* it involves only a single type of protein, bacteriorhodopsin (bR). This protein is arranged in an ordered, hexagonal array called the purple membrane [17, 76].

Its remarkable simplicity makes the purple membrane an ideal target for studies in bioenergetics. This is compounded by the close relationship between bR and the rhodopsins that act as light detectors in animal vision, as well as a relationship of bR to G-protein coupled receptors, which are important drug targets. Until recently, progress in the study of the purple membrane had been hampered by lack of structural information at the high resolution level necessary to understand the system's function. However, knowledge has improved dramatically through a combination of electron microscopy[20] and crystallographic investigations[6, 19, 48] that yielded the structure of bacteriorhodopsin and some of its associated lipids. Computer modeling has now succeeded in combining all available structural data and building an atomic level model of the purple membrane[5]. For the first time, a structure of an entire cellular apparatus, including all protein components, lipids, ions and water, is available. On this basis the physical mechanism of a key bioenergetic function can be explained in ultimate detail.

Specific Aims

Our goal is to understand how bR converts light energy into a proton gradient, i.e., how free energy stored in the initial photoproduct is funneled into proton translocation and thermal reisomerization under control of the membrane voltage. Fundamental for an understanding of the function of bR are water molecules that participate in proton pumping. Modeling the entire PM enables us to investigate the mechanism of bR's proton pumping by extrapolation from the available static structure of bR in its ground state as well as an interpolation between structurally observed pump

cycle intermediates. The role of the water molecules in the proton pumping process can be elucidated through modeling. The necessary computations are methodologically challenging, both due to problems in describing faithfully lamellar membranes at constant pressure with full electrostatic interactions, and due to the large size of the unit cell of the hydrated PM.

Methods and Results

The construction of the purple membrane relied upon our group's molecular dynamics program NAMD into which had been integrated a set of advanced modeling features. NAMD now permits simulation of a periodic hexagonal array under constant pressure and temperature conditions and can account in full for electrostatic interactions important at membrane-water interfaces. The hexagonal unit cell of our purple membrane model contained three monomeric bR molecules, 28 lipid molecules and 2804 water molecules, altogether 23783 atoms. Figure 9 shows a top view of the modeled PM patch including the central unit cell and the surrounding six image cells. The information obtained from several crystal structures of bR was used to assemble all the parts of the unit cell.

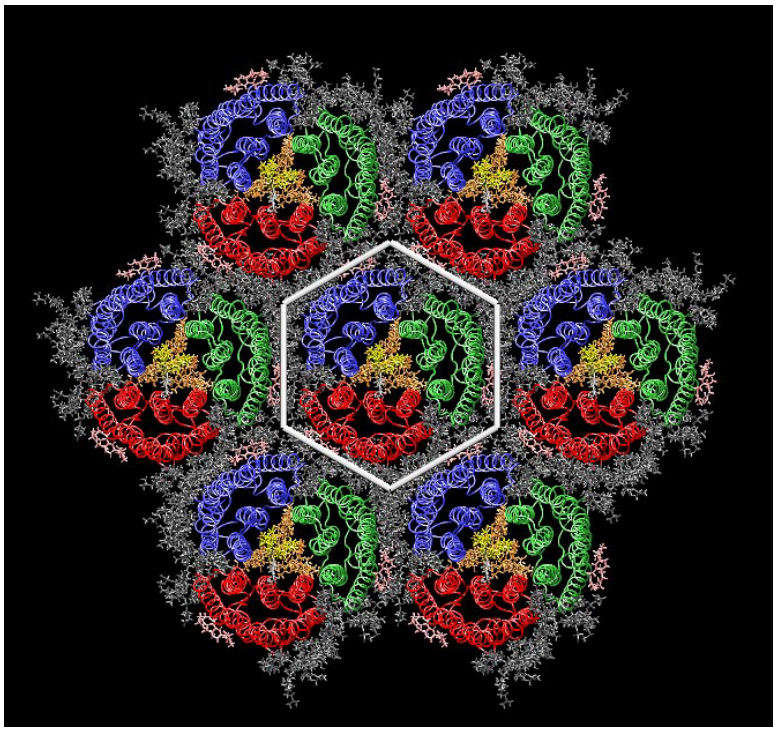

Figure 9: Top view of a purple membrane patch that includes 21 bacteriorhodopsins in seven adjacent unit cells. The hexagonal unit cell is shown in the middle of the patch, its boundary indicated by white lines. (produced with the program VMD [34])

After the PM model had been constructed, molecular dynamics simulations for 1 ns provided the system an opportunity to relax into a stable state and to redistribute in particular its water molecules. The initial model was equilibrated under NVE conditions for the first 650 ps and NpT conditions with a pressure of 1 atm for the remaining 350 ps. During the NpT phase, a significant decrease of the c extension of the unit cell (perpendicular to the membrane plane) was observed.

The reduction of the c extension of the unit cell results from penetration of the external water molecules into inter-protein space of the membrane. The internal water molecules exhibit during the ns simulation significantly different mobilities. The eight water molecules located in the proton channel of the protein, between residues Asp96 and Asp82 and in the vicinity of Arg82, remain in the channel during the simulation. On the other hand, more distal water molecules of the extracellular part of the proton channel were found to diffuse into the extra-membrane environment. In keeping with the stochastic nature of diffusion, the displacement of these water molecules differed between the three bR monomers.

On the time scale of the present simulation, no external water molecules move to the retinal binding site in the proton channel. However, our MD simulations on a monomeric bR model[5] suggest that water molecules could possibly move from a level below Arg82 to the retinal binding site depending on the dynamics of Arg82, and depending on the isomerization state of retinal. Residues Asp96 and Arg82 appear to be acting as gates that define the intra- and extra-membrane regions of the PM and control the penetration of external water molecules in the protein during the pump cycle.

Outlook

Researchers at our group have developed, in anticipation of the outlined development, a description of the potential surfaces of the ground and electronically excited states that govern the photodynamics of the purple membrane's retinal chromophore [56]. They have also improved the simulation of water molecules residing in bacteriorhodopsin[82] and have extended NAMD to describe quantum mechanical dynamics of proteins[7], needed to account properly for the 600 femtosecond phototransformation of retinal that triggers proton pumping. The following tasks need to be accomplished to meet the challenge to eventually describe the function of the PM on theoretical grounds: quantum chemistry calculations of retinal electronic states need to be carried out simultaneously with simulations of the photoprocess; simulations need to identify the nature of the early K intermediates; the time scale of simulations need to be extended to μs to reach the L intermediate and further extended to ms to cover the complete pump cycle; electrostatics of bR needs to be faithfully described in the PM context and pK shifts of the retinal Schiff base and key side groups calculated.

2.4 Steered Molecular Dynamics analysis of the selectivity filter of KcsA

TB Researchers: Justin Gullingsrud, Dorina Kosztin
URL: `http://www.ks.uiuc.edu/Research/Kchannel/`

Summary

The recently-determined structure of the potassium channel from Streptomyces lividans indicates a selectivity filter 12 Å long at the extracellular end of the channel. During the past year we have carried out Steered Molecular Dynamics (SMD) simulations using a fully equilibrated structure containing the KcsA channel embedded in a POPC/PEPG membrane. A moving planar harmonic restraint guided one or two potassium ions through the selectivity filter. The ions were observed to move in discrete steps through the filter. Conformational changes in the selectivity filter backbone during ion permeation can be related to the ion-protein interaction energies. Our results suggest that the filter plays a more active role than might be surmised from examination of the static cystal structure alone.

Introduction

Ion channels are membrane-spanning proteins that form a pathway for the flux of inorganic ions across cell membranes [25]. Among their many functions, ion channels regulate the secretion of hormones into the bloodstream, generate the electrical impulses underlying information transfer in the nervous system, and control muscle contraction [25]. To understand the physical basis of ion channel conduction, one needs to characterize the channel's structural and dynamic properties.

Recently, the crystal structure of the K^+ channel from *Streptomyces lividans* (KcsA channel) was solved [16]. The structure revealed that the KcsA channel is formed by four identical subunits, each subunit containing two α-helices connected by a loop approximately 30 amino acids long extending into the pore region. The channel is approximately 45 Å long and consists of an inner pore (starting from inside the cell), a large cavity near the middle of the pore, and the so-called selectivity filter that separates the cavity from the extracellular solution. The inner pore and the internal cavity are hydrophobic regions; in contrast, the selectivity filter is lined exclusively by polar main chain atoms belonging to the so-called signature sequence. Mutational studies [21, 23] showed that this signature sequence, comprising an eight amino acid sequence motif, is responsible for the channel's 10,000-fold selectivity of potassium over sodium.

An important feature of this channel is its ability to select for potassium over sodium while maintaining nearly diffusion-limited throughput. Intriguingly, the channel appears to rely upon the interactions of multiple ions in the channel to accomplish this. In their report of the KcsA crystal structure [16], the authors proposed the following mechanism. When the channel is gated, ions drift upward through the pore on the cytoplasmic side of the channel to a central cavity. From this point, the ions are attracted to binding sites within the selectivity filter. Mutual repulsion between ions in the cavity and in the filter disrupts the binding of ions in the filter and speeds conduction. The dynamics of this process is not yet understood.

Though the permeation of K^+ ions through the channel only takes around 20 ns, this is still a exceptionally long time for molecular dynamics simulation of systems of this size (38,000 atoms).

The Steered Molecular Dynamics approach [35, 39, 46] accelerates events in biomolecular systems by applying external forces to parts of the system.

Specific Aims

Using SMD, we simulated the permeation of ions through the selectivity filter. Trajectories obtained from free molecular dynamics simulations and from the SMD simulations allowed us to compare the dynamics of the filter in equilibrium with its response to ions passing through the channel.

Methods and Results

The simulated protein-membrane system was based on the crystal structure of the potassium channel of *Streptomyces lividans* at 3.2 Å resolution [16] (entry 1bl8 in the Protein Databank) and an equilibrated, solvated palmitoyl-oleoyl-phosphatidylcholine (POPC) membrane consisting of 100 lipids in each leaflet, generated earlier by our group [24]. Experimental results [22] indicated that the KcsA channel may be functionally reconstituted in liposomes with a mixture of phosphatidyl-ethanolamine (PE) and phosphatidyl-glycerol (PG) headgroups with a ratio of 3:1 PE to PG. Therefore, 25 lipids in each leaflet were converted to PG and the rest to PE. To counterbalance the negative charge of the PG lipids, potassium ions were substituted for the 50 water molecules at the most negative electrostatic potential. This lipid-solvent-ion system was equilibrated for 1 ns in an NpT ensemble.

Missing side chains, heavy atoms, and hydrogen atoms of the KcsA protein were modeled using X-PLOR [10], the structure was then minimized with 1000 iterations of the Powell algorithm to remove unfavorable contacts. The three potassium ions found in the protein crystal structure were held fixed during the minimization. The protein was embedded in the equilibrated POPE/POPG membrane by removing lipids that superimposed with the protein. Due to the conical shape of the protein more lipids were removed from the bottom leaflet than from the top. The membrane-protein-solvent system was equilibrated for 1 ns to allow the lipids to accomodate the protein. The fully equilibrated structure, shown in Fig. 10a, was taken as the starting structure for the free dynamics and the SMD simulations described below.

In the SMD simulations of the potassium channel, a force directed along the channel axis, as shown in Fig. 10b, was applied to a single potassium ion to facilitate its passing across the channel. The applied force had the form

$$F(z,t) = k(vt - z) \tag{1}$$

where z is the coordinate of the ion along the channel, k is the spring constant, v is the pulling speed, and t is the elapsed time of the simulation. Since the channel is essentially cylindrically symmetric about its central axis, steering the ion with a unidirectional force directed the ion along nearly the same path as that taken by a free ion. An applied force of the form of Eq. 1 does not restrain the ion within the plane of the channel. However, if the spring constant is sufficiently stiff, the ion is virtually guaranteed to pass completely through the selectivity filter in time $t = d/v$, where $d \approx 12$ Å is the thickness of the selectivity filter.

Three simulations of 1–2 ns duration were conducted using the program NAMD [37]: the first began with a single K^+ ion placed in the central cavity, while the other two began with one ion in

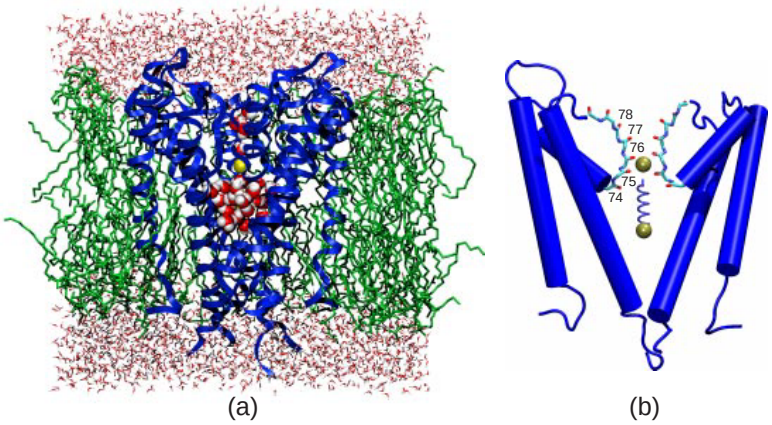

Figure 10: a) Snapshot of the protein-lipid simulated system after 1 ns of equilibration; b) View of the selectivity filter, with residues 74-78 labeled.

this cavity and another in the filter region binding site closest to the cavity. In each simulation, a harmonic restraint was applied to the ion in the central cavity and moved with constant velocity through the filter, resulting in the permeation of both ions through the channel. The SMD module of NAMD was modified to permit a simulation protocol in which ion motion was unbiased within the plane of the membrane, while the progress of the ions along the length of the filter was guided by a harmonic restraint moving at constant velocity. The system, including the lipid bilayer and surrounding water in which the protein was embedded, contained over 38,000 atoms.

Two important results were obtained from these simulations. First, in all three simulations, the ion or ions moved in a stepwise fashion from binding site to binding site. At each site, the K^+ ions were coordinated by 2–4 carbonyl oxygens of the selectivity filter backbone. The stepwise movement of the ions is evident from a plot of the applied force, as shown in Fig.11.

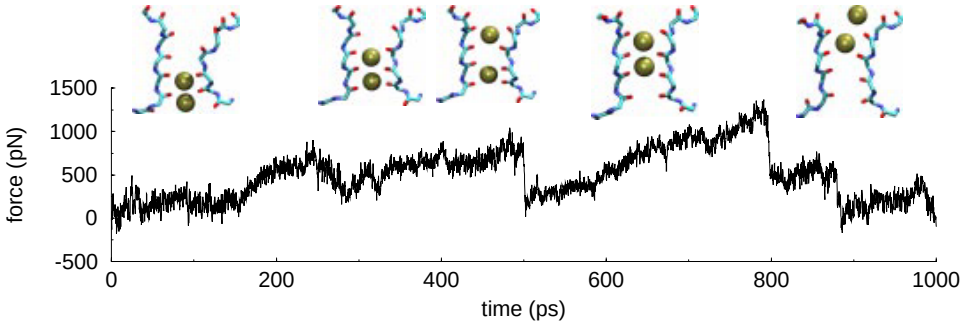

Figure 11: Applied force during a 1 ns SMD simulation of two K^+ ions in the KcsA channel. The peaks in the applied force correspond to discrete hops between ion binding sites. The hops are illustrated by snapshots from the simulation at the corresponding points in time.

The second result concerns the dynamics of the filter during the simulated permeation. During the setup and minimization of the protein structure, several residues in the selectivity filter region assumed a conformation in which the carbonyls were pointing away from the pore. It was observed in each of the SMD simulations that when ions approached the refolded parts of the filter, the carbonyl oxygens swung around to form favorable interactions with the ions. Flexibility of the

selectivity filter backbone has been noted elsewhere [79], but in this study we see that even in a nonequilibrium state, the ions contribute significantly to the stable structure of the protein.

Outlook

The present simulations used a moving restraint to guide the ions through the channel at nearly constant velocity. In the next round of simulations, we will apply a constant force to both ions to simulate the effect of an electric field.

3 Other Scientific Projects

3.1 Magnetic Sensors in Vertebrates

TB researchers: Thorsten Ritz, Salih Adem
Collaborating investigator: John B. Phillips, Department of Biology, Indiana University
URL: `http://www.ks.uiuc.edu/Research/magsense/`

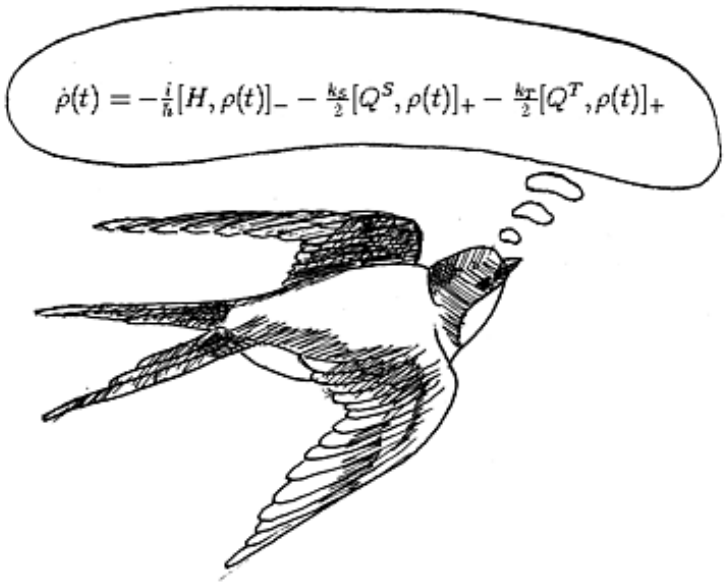

Figure 12: Biophysical Mechanism of Magnetoreception in Vertebrates.

Magnetoreception in vertebrates is one of the few sensory mechanisms for which no receptors have been identified and the biophysical mechanism remains unknown. It has been suggested earlier that magnetoreception can be achieved through a chemical sensor based on molecules engaging in radical-pair processes [75].

In a theoretical study [70], we established a model for a magnetoreception organ, consisting of an orientationally ordered system of molecular substrates undergoing radical-pair processes. Computational modeling showed that a magnetic field as weak as the earth's magnetic field (0.5 Gauss) can produce significant biochemical effects on this model magnetoreception system. The behavioral responses predicted by this model can explain experimental observations, such as the need for blue-green ambient light for magnetic compass orientation of birds and newts [65, 84].

The studies in [70] suggest an involvement of the blue-green photoreceptor cryptochrome in magnetoreception. Cryptochromes have been shown to play a role in regulating the circadian rhythm of animals. The suggestion of cryptochromes as magnetoreceptor candidates is in agreement with neurophysiological and biochemical data and will be studied further in our ongoing collaboration with John Phillips, Indiana University.

Using the behavioral assay developed earlier for fruitflies [66], we will study whether mutant

strains of fruitflies are able to orient in the magnetic field. A failure of orientation that is specific to cryptochrome-deficient mutants, i.e., is not observed in wild-type fruitflies or mutants deficient in other photoreceptors (norpA, eyeless, ocellus, etc), would be a strong support for the suggested involvement of cryptochromes in magnetoreception.

3.2 Gold Binding Protein

TB Researcher: Rosemary Braun

Collaborating investigator: Mehmet Sarikaya, Department of Materials Science and Engineering, University of Washington

URL: `http://www.ks.uiuc.edu/Research/gbp/`

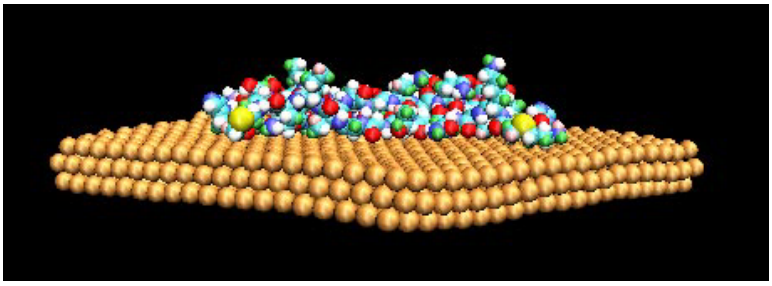

Figure 13: Simulated adsorption of an engineered polypeptide to the {111} Au crystal surface.

The biological control of inorganic crystal morphology is necessary for the formation of biological hard tissue. Sarikaya et al. have developed a genetic system to isolate proteins which control gold crystalization. It was shown[9] that in the presence of gold binding protein (GBP) gold formed large, flat hexagonal crystals displaying the {111} surface. No such crystals were seen to form in the presence of control proteins which do not bind to gold.

It is hypothesized that GBP binds preferentially to the {111} Au surface, and that the covering of the {111} face by the bound GBP plays a role in the mechanism by which GBP alters crystal morphology. Because the GBP sequence does not contain cysteine (known to form a covalent linkage with gold), the mechanism by which GBP adheres to gold is not apparent. It is also unknown why the {111} surface would be prefered to (e.g.) the more sparsely populated {112} face. Both chemisorption (via GBP's methionine sulfurs) and physisorbtion (via polar side-chains) could play a role in the binding.

We have predicted structures for the three GBP sequences available using sequence similarity methods in addition to the Holley-Karplus prediction method [27] implemented in Quanta [55]. Of the three proteins, two are seen to have repeating motifs which may be conducive to binding to a periodic surface. We have begun to carry out ab initio dynamics to study the interaction between the methionine and gold (the experimental liturature is conflicting on whether the methionine sulfur is likely to form the bond). To investigate the role of physisorbtion, we are starting molecular dynamics simulations of GBPs on both the {111} and {112} crystal surfaces, for both the case in which the methionine is bound and for which it is not. The solvated systems ($\sim$ 30000 atoms) are being simulated using NAMD[58].

3.3 Mechanical Unfolding of Titin Immunoglobulin Domains

TB Researchers: Barry Isralewitz, Mu Gao, Hui Lu
Collaborating investigator: Julio Fernandez, Department of Physiology and Biophysics, Mayo Clinic
URL: `http://www.ks.uiuc.edu/Research/titinIg`

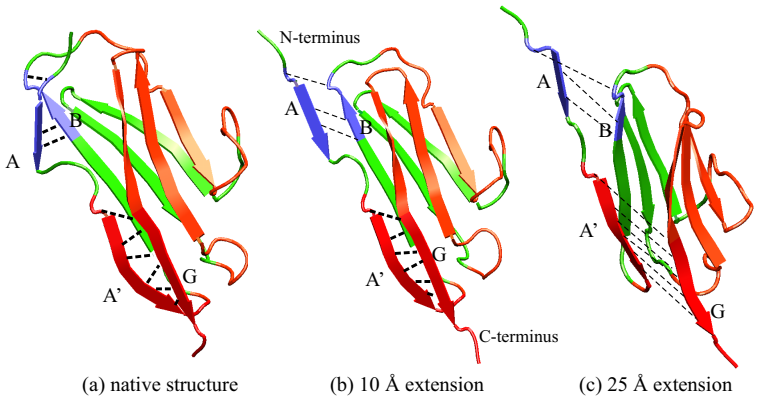

Figure 14: Titin immunoglobulin domain I27 (a) native structure, (b) at 10 Å SMD extension, (c) at 25 Å SMD extension. At 10 Å the hydrogen bonds between β-strands A and B are broken, while those between A' and G are intact. At 25 Å both the A–B and the A'–G bonds are broken.

The modular protein titin, which is responsible for the passive elasticity of muscle, contains roughly 100 immunoglobulin (Ig) domains. Experimental elongation of single titin molecules has suggested that force causes consecutive unfolding of each Ig domain in an all-or-none fashion[69]. We have demonstrated [51], using steered molecular dynamics (SMD) [36] simulations in combination with atomic force microscopy (AFM) experiments, that a fast and reversible unfolding intermediate[45] occurs prior to full domain unfolding. Single proteins were engineered to have multiple copies of single immunoglobulin domains of human cardiac titin. Elongation of these modules in AFM experiments demonstrated an abrupt extension of every domain by $\sim$7 Å prior to the first domain unfolding event. SMD simulations showed [44, 46] that the rupture of a pair of hydrogen bonds near the N-terminus of a protein domain causes a $\sim$6 Å extension, and suggested site-directed mutagenesis experiments that disrupted the predicted hydrogen bonds, and, indeed, eliminated the forced-unfolding intermediate in AFM observations [51].

SMD studies provided new details about the dominant peak that is reflected in the force needed for domain extension; solvating water molecules attacking interstrand hydrogen bonds were found to be essential for the breaking of the six interstrand bonds that provide the domain its chief protection against force-induced unfolding [47]. Water molecules repeatedly interact with the protein backbone atoms, weakening individual interstrand H-bonds until all six H-bonds break simultaneously under the influence of external stretching forces.

Additional site-directed Ig domain mutants have been created to characterize the mechanical details of the main unfolding barrier. AFM extension was performed on Ig mutants designed to have fewer backbone hydrogen bonds; SMD simulations were performed on the corresponding homology-modeled structures. Other SMD simulations examined refolding of native Ig domains: domain extension was halted (applied force set to zero) immediately after a domain entered the pre-unfolding intermediate state, or after it passed the extension at which the dominant force-extension peak is observed. The simulations revealed rapid refolding inducing reformation of most

of the interstrand hydrogen bonds.

3.4 Molecular Dynamics Simulation of the Mechanosensitive Ion Channel MscL

TB Researchers: Justin Gullingsrud, Dorina Kosztin
URL: `http://www.ks.uiuc.edu/Research/MscLchannel`

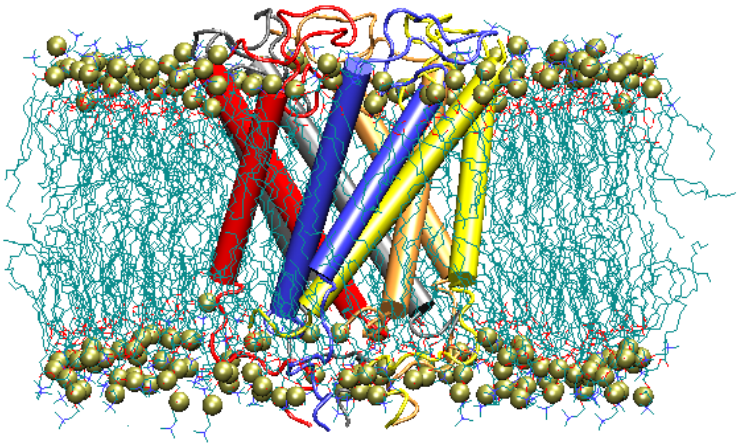

Figure 15: Snapshot of the MscL protein embedded in the POPC bilayer after 1 ns of equilibration.

Mechanosensitive (MS) channels play an important physiological role in living cells of diverse phylogenetic origin. They are ubiquitous in prokaryotes, and have recently been characterized in archaebacteria [41] as well as mammals [50, 63]. In bacteria, a controlled response to the osmolality of the environment is essential for the survival of the cell. In *E. coli*, three MS channels have been identified, and one of these, MscL, has been cloned [81]. Several studies [1, 8, 61] have confirmed the importance of this channel for osmoregulation of the bacterial cell.

The dynamics of MscL within a lipid bilayer environment would help to evaluate models of the gating of MscL in response to membrane tension, or even to suggest new mechanisms for the gating process. Molecular dynamics simulations gave a detailed picture of the dynamics of the protein and membrane on the time scale of a few nanoseconds. While this time is too short to observe channel gating, there are several aspects of channel function that could be addressed by a molecular dynamics analysis. First, examination of specific protein-lipid interactions will shed light on how the protein gate can be controlled by membrane tension alone. Second, the dynamics and environment around specific residues will explain the results of mutagenesis experiments. Finally, the overall rigidity or flexibility of the protein may give us some insight into the gating mechanism of MscL.

Preliminary results have been obtained from a 2 ns simulation of MscL embedded in a POPC lipid bilayer with surrounding water; the simulated system contains over 52,000 atoms. Analysis of the fluctuations of the C_α atoms in the ten transmembrane helices revealed that the protein is most rigid in the gating region formed by the N-terminal part of the first transmembrane helices of each subunit. This result is in agreement with recent electron spin resonance measurements. We plan to analyze correlated movements in the protein, study the role of key residues implicated in

mutagenesis experiments, and examine the interactions between the protein and the surrounding bilayer.

References

[1] B. Ajouz, C. Berrier, A. Garrigues, M. Besnard, and A. Ghazi. Release of thioredoxin via the mechanosensitive channel MscL during osmotic downshock of *Escherichia coli* cells. *J. Biol. Chem.*, 273(41):26670–26674, 1998.

[2] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. *The Cell.* Garland Publishing, Inc., New York & London, 3rd edition, 1994.

[3] M. P. Allen and D. J. Tildesley. *Computer Simulation of Liquids.* Oxford University Press, New York, 1987.

[4] A. Balaeff, L. Mahadevan, and K. Schulten. Elastic rod model of a DNA loop in the *lac* operon. *Phys. Rev. Lett.*, 83(23):4900–4903, 1999.

[5] J. Baudry, E. Tajkhorshid, F. Molnar, J. Phillips, and K. Schulten. Molecular dynamics study of bacteriorhodopsin and the purple membrane. *J. Phys. Chem.*, 2000. Invited feature article. In press.

[6] H. Belrhali, P. Nollert, A. Royant, C. Menzel, J. P. Rosenbusch, E. M. Landau, and E. Pebay-Peyroula. Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 å resolution. *Structure*, 7:909–917, 1999.

[7] M. Ben-Nun, F. Molnar, H. Lu, J. C. Phillips, T. J. Martínez, and K. Schulten. Quantum dynamics of retinal's femtosecond photoisomerization in bacteriorhodopsin. In *Faraday Discussions, No. 110*, pages 447–462. Faraday Publications, December 1998.

[8] P. Blount, M. J. Schroeder, and C. Kung. Mutations in a bacterial mechanosensitive channel change the cellular response to osmotic stress. *J. Biol. Chem.*, 272:32150–32157, 1997.

[9] S. Brown. Metal recognition by repeating polypeptides. *Nature Biotechnology*, 15:269, 1997.

[10] A. T. Brünger. *X-PLOR.* The Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, 1988.

[11] B. D. Coleman, I. Tobias, and D. Swigon. Theory of the influence of end conditions on self-contact in DNA loops. *J. Chem. Phys.*, 103(20):9101–9109, November 1995.

[12] M. G. Cory, M. C. Zerner, X. Hu, and K. Schulten. Electronic excitations in aggregates of bacteriochlorophylls. *J. Phys. Chem. B*, 102(39):7640–7650, 1998.

[13] A. Damjanović, T. Ritz, and K. Schulten. Energy transfer between carotenoids and bacteriochlorophylls in a light harvesting protein. *Phys. Rev. E*, 59:3293–3311, 1999.

[14] A. Damjanović, T. Ritz, and K. Schulten. Excitation energy trapping by the reaction center of *Rhodobacter sphaeroides*. *Int. J. Quantum Chem.*, 77:139–151, 2000.

[15] A. Damjanović, T. Ritz, and K. Schulten. Excitation transfer in the peridinin-chlorophyll-protein of *Amphidinium carterae*. *Biophys. J.*, 2000. In press.

[16] D. A. Doyle, J. M. Cabral, R. A. Pfuetzer, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. MacKinnon. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science*, 280:69–77, 1998.

[17] T. Ebrey. Light energy transduction in bacteriorhodopsin. In M. Jacobson, editor, *Thermodynamics of Membranes, Receptors and Channels*, pages 353–387. CRC Press, New York, 1993.

[18] U. Ermler, G. Fritzsch, S. Buchanan, and H. Michel. Structure of the photosynthetic reaction center from *Rhodobacter sphaeroides* at 2.65 Å resolution: Cofactors and protein-cofactor interactions. *Structure*, 2:925–936, 1994.

[19] L. Essen, R. Siegert, W. Lehmann, and D. Oesterhelt. Lipid patches in membrane protein oligomers: Crystal structure of the bacteriorhodopsin-lipid complex. *Proc. Natl. Acad. Sci. USA*, 95:11673–11678, 1998.

[20] N. Grigorieff, T. Ceska, K. Downing, J. Baldwin, and R. Henderson. Electron-crystallographic refinement of the structure of bacteriorhopsin. *J. Mol. Biol.*, 259:393–421, 1996.

[21] L. Heginbotham, T. Abramson, and R. MacKinnon. A functional connection between the pores of distantly related ion channels as revealed by mutant K^+ channels. *Science*, 258:1152–1155, 1992.

[22] L. Heginbotham, L. Kolmakova-Partensky, and C. Miller. Functional reconstitution of a prokaryotic K^+ channel. *J. Gen. Physiol.*, 111:741–749, June 1998.

[23] L. Heginbotham, Z. Lu, T. Abramson, and R. MacKinnon. Mutations in the K^+ channel signature sequence. *Biophys. J.*, 64:1061–1067, 1994.

[24] H. Heller, M. Schaefer, and K. Schulten. Molecular dynamics simulation of a bilayer of 200 lipids in the gel and in the liquid crystal-phases. *J. Phys. Chem.*, 97:8343–8360, 1993.

[25] B. Hille. *Ionic channels of excitable membranes*. Sinauer Associates, Sunderland, MA, 2nd edition, 1992.

[26] E. Hofmann, P. Wrench, F. Sharples, R. Hiller, W. Welte, and K. Diederichs. Structural basis of light harvesting by carotenoids: peridinin-chlorophyll-protein from *Amphidinium carterae*. *Science*, 272:1788–1791, 1996.

[27] L. H. Holley and M. Karplus. Protein secondary structure prediction with a neural network. *Proc. Natl. Acad. Sci. USA*, 86:152–156, 1989.

[28] W. Hsieh, P. A. Whitson, K. S. Mathews, and R. D. Wells. Influence of sequence and distance between two operators on interaction with the *lac* repressor. *J. Biol. Chem.*, 262:14583–14591, 1987.

[29] X. Hu, A. Damjanović, T. Ritz, and K. Schulten. Architecture and function of the light harvesting apparatus of purple bacteria. *Proc. Natl. Acad. Sci. USA*, 95:5935–5941, 1998.

[30] X. Hu, T. Ritz, A. Damjanović, and K. Schulten. Pigment organization and transfer of electronic excitation in the purple bacteria. *J. Phys. Chem. B*, 101:3854–3871, 1997.

[31] X. Hu and K. Schulten. How nature harvests sunlight. *Physics Today*, 50:28–34, 1997.

[32] X. Hu and K. Schulten. A model for the light-harvesting complex I (B875) of *Rhodobacter sphaeroides*. *Biophys. J.*, 75:683–694, 1998.

[33] J. M. Hudson and M. G. Fried. Co-operative interactions between the catabolite gene activator protein and the *lac* repressor at the lactose promoter. *J. Mol. Biol.*, 214:381–396, 1990.

[34] W. F. Humphrey, A. Dalke, and K. Schulten. VMD – Visual Molecular Dynamics. *J. Mol. Graphics*, 14:33–38, 1996.

[35] S. Izrailev, S. Stepaniants, M. Balsera, Y. Oono, and K. Schulten. Molecular dynamics study of unbinding of the avidin-biotin complex. *Biophys. J.*, 72:1568–1581, 1997.

[36] S. Izrailev, S. Stepaniants, B. Isralewitz, D. Kosztin, H. Lu, F. Molnar, W. Wriggers, and K. Schulten. Steered molecular dynamics. In P. Deuflhard, J. Hermans, B. Leimkuhler, A. E. Mark, S. Reich, and R. D. Skeel, editors, *Computational Molecular Dynamics: Challenges, Methods, Ideas*, volume 4 of *Lecture Notes in Computational Science and Engineering*, pages 39–65. Springer-Verlag, Berlin, 1998.

[37] L. Kalé, R. Skeel, M. Bhandarkar, R. Brunner, A. Gursoy, N. Krawetz, J. Phillips, A. Shinozaki, K. Varadarajan, and K. Schulten. NAMD2: Greater scalability for parallel molecular dynamics. *J. Comp. Phys.*, 151:283–312, 1999.

[38] J. Koepke, X. Hu, C. Münke, K. Schulten, and H. Michel. The crystal structure of the light harvesting complex II (B800-850) from *Rhodospirillum molischianum*. *Structure*, 4:581–597, 1996.

[39] D. Kosztin, S. Izrailev, and K. Schulten. Unbinding of retinoic acid from its receptor studied by steered molecular dynamics. *Biophys. J.*, 76:188–197, 1999.

[40] L. D. Landau and E. M. Lifschitz. *Theory of Elasticity*, volume 7 of *Course of theoretical physics*. Pergamon Press, Oxford, New York, 3 edition, 1986.

[41] A. C. Le Dain, N. Saint, A. Kloda, A. Ghazi, and B. Martinac. Mechanosensitive ion channels of the archaeon *Haloferax volcanii*. *J. Biol. Chem.*, 273(20):12116–12119, 1998.

[42] M. Lewis, G. Chang, N. C. Horton, M. A. Kercher, H. C. Pace, M. A. Schumacher, R. G. Brennan, and P. Lu. Crystal structure of the lactose operon repressor and its complexes with DNA and inducer. *Science*, 271:1247–1254, 1996.

[43] A. E. H. Love. *A treatise on the mathematical theory of elasticity*. Dover Publications, Inc., New York, 1927.

[44] H. Lu, A. Krammer, B. Isralewitz, V. Vogel, and K. Schulten. Computer modeling of force-induced titin domain unfolding. In J. Pollack and H. Granzier, editors, *Elastic Filaments of the Cell*, chapter 1. Kluwer Academic/Plenum Publishers, New York, NY, 1999. In press.

[45] H. Lu and K. Schulten. Steered molecular dynamics simulation of conformational changes of immunoglobulin domain I27 interpret atomic force microscopy observations. *Chem. Phys.*, 247:141–153, 1999.

[46] H. Lu and K. Schulten. Steered molecular dynamics simulations of force-induced protein domain unfolding. *PROTEINS: Struc., Func., and Genetics*, 35:453–463, 1999.

[47] H. Lu and K. Schulten. The key event in force-induced unfolding of titin's immunoglobulin domains. *Biophys. J.*, 79:51–65, 2000.

[48] H. Lücke, B. Schobert, H. T. Richter, J. P. Cartailler, and J. K. Lanyi. Structure of bacteriorhodopsin at 1.55 å resolution. *J. Mol. Biol.*, 291:899–911, 1999.

[49] L. Mahadevan and J. B. Keller. Coiling of flexible rods. *Proc. R. Soc. Lond. A. (Math. Phys. Sci.)*, 452:1679–1694, 1996.

[50] F. Maingret, M. Fosset, F. Lesage, M. Mazdunski, and E. Honoré. TRAAK is a mammalian neuronal mechano-gated K^+ channel. *J. Biol. Chem.*, 274(3):1381–1387, 1999.

[51] P. E. Marszalek, H. Lu, H. Li, M. Carrion-Vazquez, A. F. Oberhauser, K. Schulten, and J. M. Fernandez. Mechanical unfolding intermediates in titin modules. *Nature*, 402:100–103, 1999.

[52] K. S. Matthews. DNA looping. *Microbiological Reviews*, 56(1):123–136, 1992.

[53] A. D. Mehta, M. Rief, J. A. Spudich, D. A. Smith, and R. M. Simmons. Single molecule biomechanics with optical methods. *Science*, 283:1689–1695, 1999.

[54] J. H. Miller and W. S. Reznikoff, editors. *The Operon.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1980.

[55] Molecular Simulations Inc., Burlington, Massachusetts. *QUANTA 97*, 1997.

[56] F. Molnar, M. Ben-Nun, T. J. Martínez, and K. Schulten. Characterization of a conical intersection between the ground and first excited state for a retinal analog. *Journal of Molecular Structure (THEOCHEM)*, WATOC special issue, 506:169–178, 2000.

[57] R. Monshouwer and R. van Grondelle. Excitations and excitons in bacterial light-harvesting complexes. *Biochim. Biophys. Acta*, 1275:70–75, 1996.

[58] M. Nelson, W. Humphrey, A. Gursoy, A. Dalke, L. Kalé, R. D. Skeel, and K. Schulten. NAMD – A parallel, object-oriented molecular dynamics program. *Int. J. Supercomput. Appl. High Perform. Comput.*, 10:251–268, 1996.

[59] S. Oehler, E. R. Eismann, H. Krämer, and B. Müller-Hill. The three operators of the *lac* operon cooperate in repression. *EMBO J.*, 9(4):973–979, 1990.

[60] W. K. Olson. Simulating DNA at low resolution. *Curr. Opinion Struct. Biol.*, 6:242–256, 1996.

[61] X. Ou, P. Blount, R. J. Hoffman, and C. Kung. One face of a transmembrane helix is crucial in mechanosensitive channel gating. *Proc. Natl. Acad. Sci. USA*, 95:11471–11475, 1998.

[62] G. Parkinson, C. Wilson, A. Gunasekera, Y. W. Ebright, R. E. Ebright, and H. M. Berman. Structure of the CAP-DNA complex at 2.5 Å resolution: A complete picture of the protein-DNA interface. *Proc. Natl. Acad. Sci. USA*, 260:395–408, 1996.

[63] A. J. Patel, E. Honoré, F. Maingret, F. Lesage, M. Fink, F. Duprat, and M. Lazdunski. A mammalian two pore domain mechano-gated S-like K^+ channel. *EMBO J.*, 17(15):4283–4290, 1998.

[64] M. Perros, T. A. Steitz, M. G. Fried, and J. M. Hudson. DNA looping and *Lac* repressor-CAP interaction. *Science*, 274:1929–1931, 1996.

[65] J. Phillips and S. Borland. Behavioral evidence for use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature*, 359:142–144, 1992.

[66] J. Phillips and O. Sayeed. Wavelength-dependent effects of light on magnetic compass orientation in *drosophila melanogaster*. *J. .Comp. Physiol. A*, 172:303–308, 1993.

[67] T. Pullerits, M. Chachisvillis, and V. Sundstrom. Exciton delocalization length in the B850 antenna of *Rhodobacter sphaeroides*. *J. Phys. Chem.*, 100:10787, 1996.

[68] M. Ricci, S. E. Bradforth, R. Jimenez, and G. R. Fleming. Internal conversion and energy transfer dynamics of spheroidene in solution and in the LH-1 and LH-2 light-harvesting complexes. *Chem. Phys. Lett.*, 259:381–390, 1996.

[69] M. Rief, M. Gautel, F. Oesterhelt, J. M. Fernandez, and H. E. Gaub. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science*, 276:1109–1112, 1997.

[70] T. Ritz, S. Adem, and K. Schulten. A model for vision-based magnetoreception in birds. *Biophys. J.*, 78(2):707–718, 2000.

[71] T. Ritz, A. Damjanović, K. Schulten, J. Zhang, and Y. Koyama. Efficient light harvesting through carotenoids. *Photosynthesis Research*, 2000. In press.

[72] T. Ritz, X. Hu, A. Damjanović, and K. Schulten. Excitons and excitation transfer in the photosynthetic unit of purple bacteria. *Journal of Luminescence*, 76-77:310–321, 1998.

[73] R. Schleif. DNA looping. *Ann. Rev. Biochem*, 61:199–223, 1992.

[74] T. Schlick. Modeling superhelical DNA: recent analytical and dynamic approaches. *Curr. Opinion Struct. Biol.*, 5:245–262, 1995.

[75] K. Schulten. Magnetic field effects in chemistry and biology. In J. Treusch, editor, *Festkörperprobleme*, volume 22, pages 61–83. Vieweg, Braunschweig, 1982.

[76] K. Schulten, W. Humphrey, I. Logunov, M. Sheves, and D. Xu. Molecular dynamics studies of bacteriorhodopsin's photocycles. *Israel Journal of Chemistry*, 35:447–464, 1995.

[77] S. C. Schultz, G. C. Schields, and T. A. Steitz. Crystal structure of a CAP-DNA complex: The DNA is bent by 90°. *Science*, 253:1001–1007, 1991.

[78] Y. Shi and J. E. Hearst. The Kirchhoff elastic rod, the nonlinear Schrödinger equation, and DNA supercoiling. *J. Chem. Phys.*, 101(6):5186–5200, September 1994.

[79] I. H. Shrivastava and M. S. P. Sansom. Simulations of ion permeation through a potassium channel: Molecular dynamics of KcsA in a phospholipid bilayer. *Biophys. J.*, 78:557–570, 2000.

[80] L. Stryer. *Biochemistry*. W. H. Freeman and Company, New York, 4th edition, 1995.

[81] S. I. Sukharev, P. Blount, B. Martinac, F. R. Blattner, , and C. Kung. A large-conductance mechanosensitive channel in *E. coli* encoded by *mscL* alone. *Nature*, 368:265–268, 1994.

[82] E. Tajkhorshid, J. Baudry, K. Schulten, and S. Suhai. Molecular dynamics study of the nature and origin of retinal's twisted structure in bacteriorhodopsin. *Biophys. J.*, 78(2):683–693, 2000.

[83] A. V. Vologodskii and N. R. Cozzarelli. Conformational and thermodynamic properties of supercoiled DNA. *Ann. Rev. Biophys. Biomol. Struct.*, 23:609–643, 1994.

[84] W. Wiltschko, U. Munro, H. Ford, and R. Wiltschko. Red light disrupts magnetic orientation of migratory birds. *Nature*, 364:525–527, 1993.

Resulting Publications (5/99 — 5/00)

Alexander Balaeff, L. Mahadevan, and Klaus Schulten. Elastic rod model of a DNA loop in the lac operon. *Physical Review Letters*, 83:4900–4903, 1999.

Jerome Baudry, Emaddin Tajkhorshid, Ferenc Molnar, James Phillips, and Klaus Schulten. Molecular dynamics study of bacteriorhodopsin and the purple membrane. *Journal of Physical Chemistry*, 2000.

Ana Damjanovic, Thorsten Ritz, & Klaus Schulten. Excitation Energy Transfer in the Peridinin-Chlorophyll-Protein of Amphidinum carterae. *Biophys. J.* Submitted, 2000.

Ana Damjanovic, Thorsten Ritz, and Klaus Schulten. Excitation energy trapping by the reaction center of Rhodobacter sphaeroides *Int. J. Quantum Chem.*, 77:139–151, 2000.

Justin Gullingsrud, Rosemary Braun, and Klaus Schulten. Reconstructing potentials of mean force through time series analysis of steered molecular dynamics simulations. *Journal of Computational Physics*, 151:190–211, 1999.

Sergei Izrailev, Antony R. Crofts, Edward A. Berry, and Klaus Schulten. Steered molecular dynamics simulation of the Rieske subunit motion in the cytochrome bc1 complex. *Biophysical Journal*, 77:1753–1768, 1999.

Dorina Kosztin, Richard Gumport, and Klaus Schulten. Probing the role of structural water in a duplex oligodeoxyribonucleotide containing a water-mimicking base analogue. *Nucleic Acids Research*, 27:3550–3556, 1999.

Hui Lu and Klaus Schulten. Steered molecular dynamics simulations of force-induced protein domain unfolding. *PROTEINS: Structure, Function, and Genetics*, 35:453–463, 1999.

Ferenc Molnar, Michal Ben-Nun, Todd J. Martnez, and Klaus Schulten. Characterization of a conical intersection between the ground and first excited state for a retinal analog. *Journal of Molecular Structure* (THEOCHEM), a special WATOC issue, 1999.

Ferenc Molnar, Lawrence S. Norris, and Klaus Schulten. Simulated unbinding and binding of fatty acid substrates in the cyclooxygenase site of prostaglandin H2 synthase-1. *Reaction Kinetics and Mechanisms*, 2000.

Thorsten Ritz, Ana Damjanovic, and Klaus Schulten and Jian-Ping Zhang and Yasushi Koyama. Efficient Light Harvesting Through Carotenoids. *Photosynthesis Research on Emerging Techniques.*

Thorsten Ritz, Salih Adem, and Klaus Schulten. A model for vision-based magnetoreception in birds. *Biophysical Journal*, 78:707–718, 2000.

Klaus Schulten. From simplicity to complexity and back: Function, architecture and mechanism of light harvesting systems in photosynthetic bacteria. In H. Frauenfelder, J. Deisenhofer, and P. G. Wolynes, editors, Simplicity and Complexity in Proteins and Nucleic Acids, pages 227–253, Berlin, 1999. Dahlem University Press.

Rajeev Sharma, Michael Zeller, Vladimir I. Pavlovic, Thomas S. Huang, Zion Lo, Stephen Chu, Yunxin Zhao, James C. Phillips, and Klaus Schulten. Speech/gesture interface to a visual-computing environment. *IEEE Computer Graphics and Applications*, 20:29–37, 2000.

Emadeddin Tajkhorshid, Jerome Baudry, Klaus Schulten, and Sandor Suhai. Molecular dynam-

ics study of the nature and origin of retinal's twisted structure in bacteriorhodopsin. *Biophysical Journal*, 78:683–693, 2000.