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Contents

The Resource has focused its recent activities on computational structural biology and seeks to extend the horizon of modelling techniques to assemblies of biopolymers and to interactive modelling. Assemblies, such as membranes, protein aggregates or complexes of proteins with membrane or DNA, require simulations involving several ten-thousand to several hundred-thousand particles. Through a choice of relevant and challenging projects, the development of molecular graphics tools, fast algorithms and effective simulation on parallel machines, as well as through configuration of an extremely cost-effective, fiber-optic-linked workstation cluster, the Resource has become the acknowledged leader in large scale modelling as evidenced by its series of published accomplishments. These achievements are demonstrated in the advances made in modelling biological membranes as shown in Fig 1: a 27,000 atom bilayer membrane had been described in [1] after a three year simulation period on a 60 processor machine; a 32,800 atom membrane was described in [2]; this simulation provided a monolayer to which the protein phospholipase A2 could be complexed in a 12,600 atom simulation, building the first model of a protein at the water-membrane interface [3]. Recently, Resource researchers have initiated a simulation in which a 30,000 atom model of an annexin XII hexamer [4] is embedded into a lipid bilayer in order to study how annexins modify biological membranes.

Viewing static and dynamic structures of biopolymers is the source of many insights and discoveries in structural biology and allows one to avoid many pitfalls; computational tools provide more than structural information, e.g., they can depict electrostatic fields, atomic flexibilities and other types of susceptibilities. The size and complexity of biopolymer aggregates put even more stringent requirements on molecular graphics tools and on control of and interaction with simulations. Accordingly, the Resource has invested a significant fraction of its effort to develop a visual computing environment for structural biology, MDScope [5], a new version of which has been completed recently. The MDScope environment combines (1) the program VMD for interactive visualization and analysis of molecular structures, (2) the program NAMD for parallel distributed memory molecular dynamics, and (3) MDComm a protocol and library which functions as a communication agent between VMD and NAMD. The package is available to the biomedical research community free of charge and is already being used in over a hundred laboratories.

MDScope offers interactive control with immediate visual feedback to a researcher running molecular dynamics (MD) simulations through VMD. The user can interact with the simulation by applying external forces to selected atoms. Although the speed at which MD simulations can be calculated remains a severe limitation, interactive MD in combination with fast parallel computers promises to become a valuable tool for structure building, docking of drugs, building aggregate structures, and other modelling problems. VMD has been greatly enhanced by the addition of an extension language and analysis routines. Key features have been added to NAMD and a restructuring is in progress to improve performance.

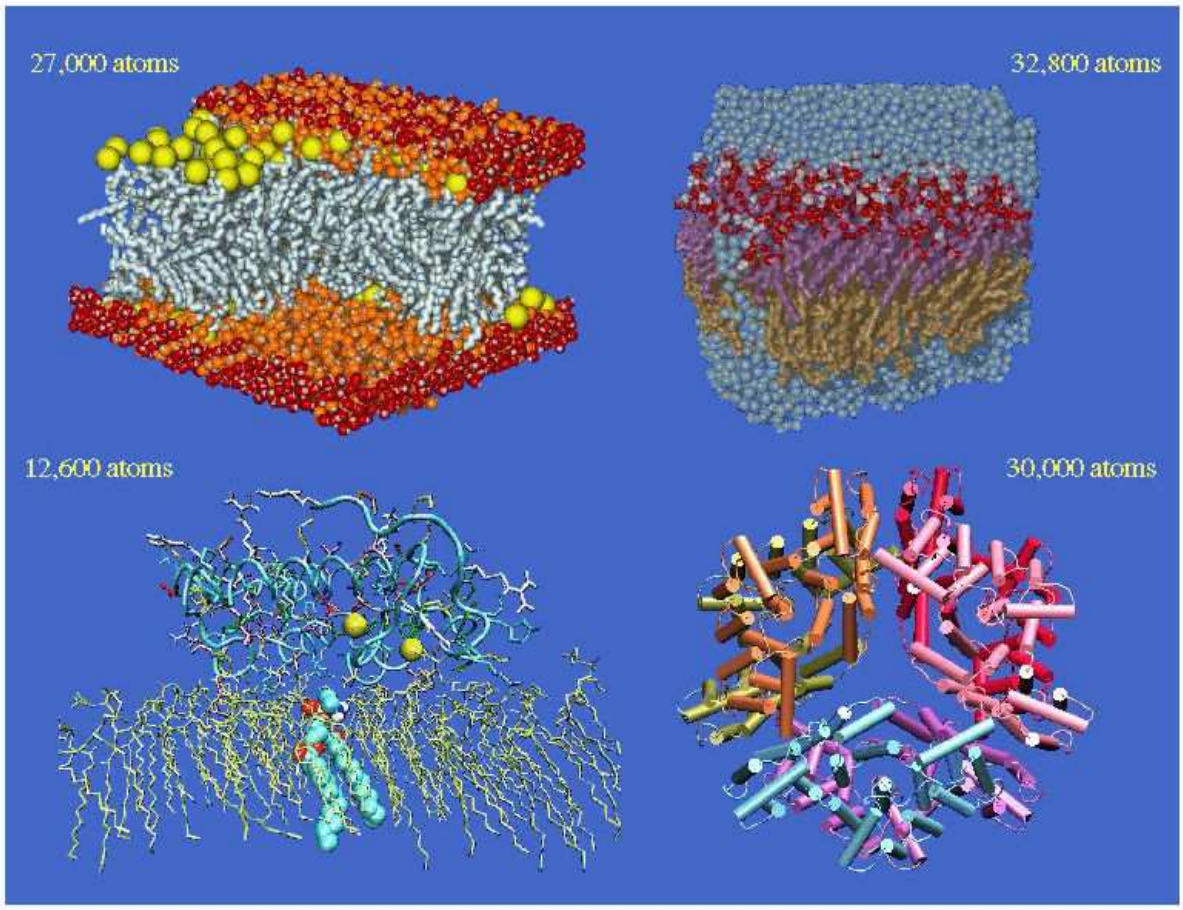

Figure 1: Advances in membrane simulations. Upper left: POPC [1] bilayer; Upper right: DLPE [2] bilayer; lower left: Phospholipase A_2 bound to a membrane [3]; lower right: Annexin XII [4].

For its extremely challenging simulation projects the Resource relies on computer time allocated through a major grant from the National Science Foundation (NSF) Centers where it is one of the major recipients of a Metacenter allocation. In addition, the Resource seeks to experiment with and capitalize on cost-effective computing with equipment available to many biomedical researchers, namely clusters of workstations. Since 1993 the Resource has operated, with great success, a cluster of 12 Hewlett-Packard (HP) workstations coupled by an ATM switch. Recently, clusters of workstations have been widely adopted as a computing platform. The Resource, recognized as a pioneer in cluster computing, consequently received new funding from NSF to acquire four additional Hewlett-Packard K-series multi-processor servers last December. The machines feature four symmetric processors sharing 512MB of memory and are connected to each other and to the existing cluster via ATM. The fast ATM network allows parallel programs to run on more than four processors employing a mixture of shared and distributed memory. In the past twelve months computational power available to the Resource has more than

doubled, and this has been a great boost for all research activities.

In the realm of computational modelling, the Resource focused its efforts both on methodological developments, and on biomolecular systems for which very large scale modelling is essential. A major methodological advance has been achieved in the area of X-ray structure determination. Structure determination through X-ray crystallography requires knowledge of both intensities and phases. A conventional solution of the phase problem requires either crystallizing heavy metal derivatives of the protein, which is time consuming and not always successful, or knowledge of another homologous structure. Resource researchers were able to determine the structure of the light-harvesting complex II (LH-II) of *Rs. molischianum* by means of a computer prediction as the homologous probe (see research highlights below). This is the first structure obtained through a combination of computer modelling for phase determination and X-ray diffraction data and is considered an important event in computational structural biology.

Resource staff also discovered that so-called rupture force measurements, in which two biomolecules, e.g., the protein avidin and its substrate biotin, are pulled apart with an atomic force microscope apparatus, lend themselves to similar and revealing computational experiments. Employing the interactive modelling capacity of the program MDScope as well as conventional molecular dynamics simulations with added external forces, ligand-protein systems were ruptured and the unbinding-rebinding events monitored. Respective modelling calculations reveal the forces engaged in ligand-protein complexes and other aggregates. However, the short time scale available for modelling necessitates a thorough understanding of irreversible processes. For this purpose the work focused on applications, e.g., on the unbinding of retinal from bacteriorhodopsin and on the rupture of biotin from avidin, as well as on theoretical work estimating the amount of irreversible work involved in finite time processes. In particular, hysteresis processes were investigated, for example in the case of dielectric reactions [6].

Finally, the Resource has reexamined to what extent essential degrees of freedom can be identified in protein motion, and whether this information can possibly be employed to reduce the number of degrees of freedom along which protein motion is numerically integrated. For this purpose the method of principal component analysis, also known as quasi-harmonic analysis, has been investigated [7]. Extended simulations of the protein F-actin revealed that the stated method does not allow one to reduce computational work and that the goal of long-time integration of proteins is still elusive: the modes identified in a principal component analysis drift significantly in time and therefore need to be continuously re-established. Resource scientists also explored the method of reduced dynamics in which protein motion is confined to few, mainly dihedral, degrees of freedom. A respective algorithm has been developed and tested, but the approach requires development of an effective force field to yield realistic behaviour.

The ultimate test and justification of the methods developed at the Resource are applications to specific and demanding biomolecular systems which are difficult or even impossible to investigate without the methodological advances achieved. Through continuing collaborations with outside, mainly experimental, laboratories as well as through new projects the researchers at the Resource and guests have exploited the ability of large scale simulations and extensive sampling. The extended search process for the structure of the light harvesting complex mentioned above led to a protein structure which is an aggregate of sixteen helices, 24 chlorophylls and 8 lycopenes in which the chromophores form a ring-like supra-molecular complex with ideal properties for energy transfer. The properties of this complex and its femtosecond dynamics attract biochemists, physical chemists and quantum physicists alike, and will likely become the focal point of intense studies for many researchers.

Simulations of lipid bilayers at the Resource, requiring several years of computing, culminated last year in a study of the protein phospholipase A2 which, when tightly bound to the water-headgroup interface of a biological membrane, becomes activated and cleaves off the lipid head groups. Modelling indicates convincingly that the activation of the enzyme actually involves better access of lipid head groups to the active site due to desolvation [3].

Extremely challenging, yet highly desirable, are better models of DNA-protein complexes for which structures have still not been observed. The challenge arises due to the necessity of solvating such complexes sufficiently in salt water and properly describing long range Coulomb forces governing these systems; the great interest in such models stems from the important biomedical role of proteins involved in DNA expression and synthesis which is still poorly understood. The Resource continued its past research emphasis and recently achieved promising models of HMG-D group proteins bound to DNA (see research highlight below). These proteins bind non-specifically to DNA and bend it to facilitate packing in the nucleus; the proteins are related to sequence-specific systems.

The Resource has recently completed its first simulation cycle of the protein G-actin, an ATPase. The simulations require solvation of the protein to account for the essential role of water. Reactant and product states were studied in the presence of Mg^{++} and Ca^{++} ions. The long range project on the retinal protein bacteriorhodopsin has been continued as well. Presently, the femtosecond dynamics of the protein after light absorption is investigated on multiple electronic potential surfaces, namely, the ground and two excited states.

Exciting new projects have been started at the Resource during the past year. The structure of the protein cytochrome c oxydase of *Paracoccus denitrificans* [8] has been made available to us. Initial studies have placed water into the protein at the various redox states. Oxygen transport has been simulated and the proton conduction pathways

searched in detail. Quantum chemical simulations of the protein's binuclear center are being undertaken with M. Zerner as well as with M. Parrinello. First results have been obtained for simulations of a large segment of the capsid of polio virus containing about 40,000 atoms. In another collaboration an attempt is made to predict the structure of the apolipoprotein A-I, the availability of which should improve dramatically our understanding of the important function of this protein. Finally, the Resource expects to engage in another X-ray structure determination project in which refraction data are phased computationally. Such an undertaking would help us to develop the approach developed in [9] into a general tool.

Following the suggestions of advisory board member B. Alder the Resource will review the key numerical algorithms employed presently in the molecular dynamics program NAMD. For the Coulomb force field algorithm the so-called P^3M method will be explored and multiple time scale methods will extend those in current use based on [10]. In response to recommendations made by P. Arzberger, another board member, the Resource has conducted its first software survey to identify active MDScope users.

All application projects have benefited directly from the graphics program VMD and in a few cases already from NAMD. Most of the projects would have been impossible without the tools available at the Resource.

The Resource has organized a workshop "Computational / Experimental Approaches in Structural Biology" which brought together experimental and computational researchers, most of them involved in projects at the Resource. The workshop successfully bridged the gap between the two groups and provided experimentalists with an opportunity to state problems for which they seek computational solutions. This workshop and other contacts with the biomedical research community led to exciting new projects and collaborations at the Resource.

MDScope

The Resource has continued the development of MDScope [5], a visual computing environment for molecular dynamics and modelling. It consists of three software components which together facilitate interactive studies of biomolecular systems by combining molecular graphics systems and high performance processors over a distributed network of computers. The three components are

- NAMD, an efficient parallel molecular dynamics simulation engine;
- VMD, a molecular visualization and analysis tool;
- MDComm, a communications package which connects VMD and NAMD.

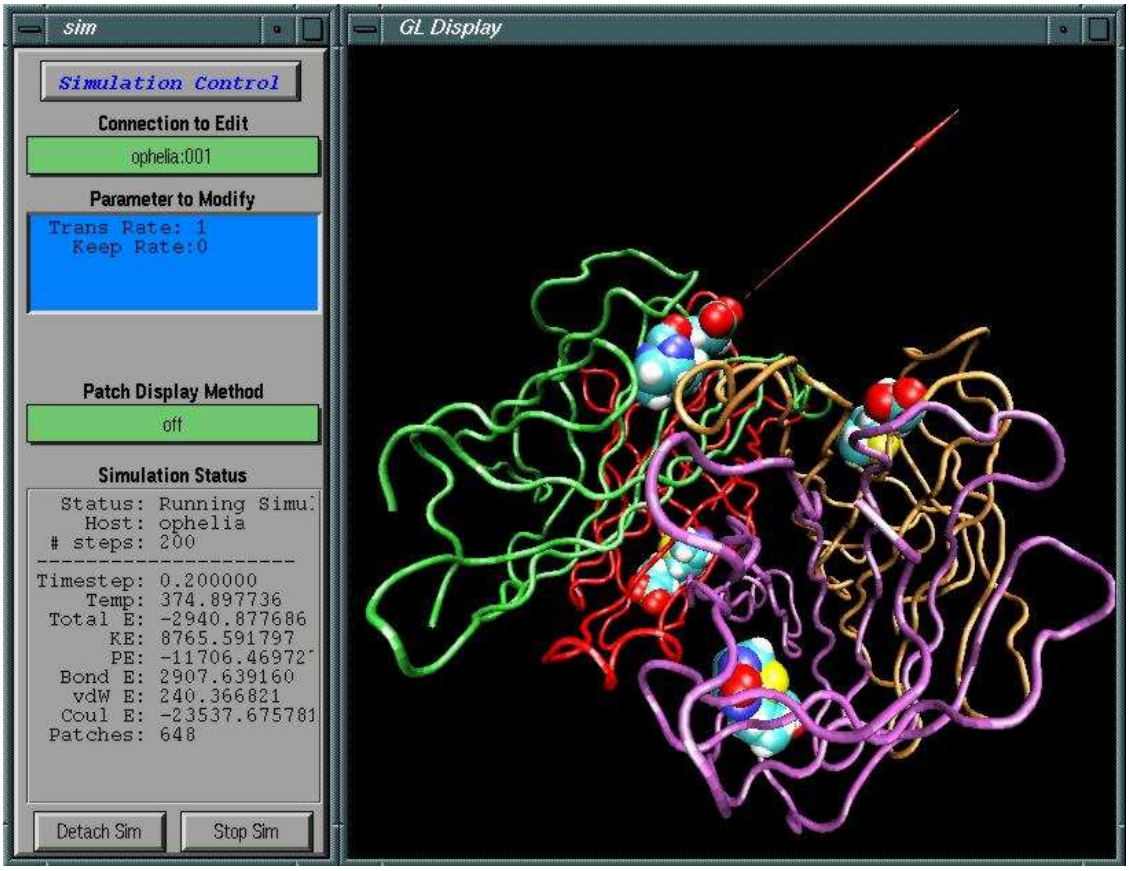

Figure 2: Illustration of an MDScope session in which the substrate biotin is pulled away from the protein avidin. The arrow shows the direction of the applied force. The menu on the left displays information about the simulation.

NAMD

NAMD [11] is the molecular dynamics component of MDScope. It is an object-oriented program specifically designed for parallel computers. It uses spatial decomposition and

message-driven execution to exploit the computational power of scalable distributed-memory parallel computers. Major goals for the program include efficient simulation of very large biological macro-molecules, the ability to easily incorporate new algorithms for development and testing, and interactive molecular dynamics.

During the past year, NAMD has been significantly improved. A major scientific project using NAMD led to a thorough test of the program, the elimination of errors and the addition of new features such as spherical boundary conditions and harmonic constraints. Performance has been enhanced by optimizing sequential code, reducing communication costs via message combining, and achieving higher load balancing through better initial distribution of work to processors. The multiple timestepping scheme has also been improved by adding Verlet-I [10] (also known as reversible RESPA [12]) and Verlet-X methods [13]. The implementation of a stable multiple timestepping algorithm resulted in a 3.5-fold speed improvement in our most demanding application. The recent implementation of rigid bonds between hydrogen and heavy atoms is expected to yield another 2-fold improvement. A restructuring effort to further improve the program's performance is in progress.

Currently four projects in the Resource benefit from NAMD. Two projects are simulations of complexes of nuclear hormone receptors with DNA. These involve very large systems which take advantage of the parallel feature of NAMD to reduce the overall simulation time. Another project, involving the protein bacteriorhodopsin, tests the effect of different molecular mechanics force terms, e.g., hydrogen bonding or multiple potential surfaces during photodynamics. The fourth project tests new methods of integration. The latter two projects benefit from the ease with which NAMD can be modified.

VMD

VMD [14] is the visualization component of MDScope. It is designed particularly to study biological molecules such as proteins, nucleic acids, and membranes, and can be used to visualize a wide variety of molecular systems. It supports both a graphical user interface and a scripting language and is also used to experiment with new types of human–computer interfaces [15].

During the last year we spent much effort on making VMD a mature visualization package by adding features to the existing framework, e.g., new coloring and drawing methods, extensions to the selection language, secondary structure data, and new graphics output formats. A major new development was the incorporation of new commands into the VMD scripting language which access the internal VMD data base. Researchers can build on these commands to develop new analysis routines without recompiling VMD. There are additional commands to add graphics objects to the display and to display

processed simulation data, e.g., local energies or susceptibilities, alongside the rendered structures. New options for static docking, structure alignment, and the interactive force controls needed in the context of MDScope have also been added.

VMD has become the primary visualization tool for members of the Resource. A recent survey conducted by the Resource (discussed on the next page) indicates that VMD is being adopted by other groups for docking studies, structure refinement, trajectory analysis, semiconductor physics, the production of chemistry education videos, and other tasks.

Interactive Molecular Dynamics

MDScope seeks to make MD studies more accessible to researchers by making it easier to visualize the motions in MD simulations and by enabling the user to modify the simulation in real time.

Current generation molecular modelling programs do not allow real-time interaction with the modelled system. When a set of atoms is rearranged, there is usually no feedback to indicate that a given conformation or structure is incorrect. Much time is spent in the cycle of modifying the structure, minimizing its energy to reduce conflicts and viewing the results. By allowing the user to modify the simulation and see the results as they happen, MDScope offers the user a more realistic view of how the molecule is affected by changes and reduces the time required to build a structure.

The interactive capabilities of MDScope are brokered by MDComm (developed by Rick Kufrin at NCSA) which exchanges data between VMD and NAMD. As new simulation coordinates are computed by NAMD, they are transferred to VMD which displays the corresponding structure and optionally performs some analysis on it. Researchers using VMD can modify the simulation using a menu or text interface and have the changes incorporated back into NAMD. At present, MDScope users can move atoms by choosing and applying forces. This has been used for structure building as well as for MD studies of adhesion forces.

The results to date show that while the visualization of molecular trajectories is very mature, interactive modelling is less so. For example, strong localized heating arises when strong forces are applied to induce structural transitions rapidly enough to be viewed in a few seconds of real time.

Distribution

The release of VMD and NAMD was announced on July 1, 1995 . MDComm was released May 1, 1996. There have been over 800 downloads of VMD and 400 of NAMD. As part

of our ongoing efforts to ensure that our programs are up-to-date, useful, and of high quality, we initiated a survey to determine the level of satisfaction and optimally address the needs of our users. Three highly similar questionnaires, with modest adaptations to fit NAMD, VMD and MDScope, were constructed (see enclosed copies) and sent by email to a target population of over 600 addresses which were identified through the records of software downloads from our ftp site. The analysis and interpretation of the responses will be completed soon and Table 1 summarizes some of the preliminary results.

Software package	Number of emails sent	Number of responding sites	Number of users*	Response rate (# responses / # emails)
VMD	364	156	210	43 %
NAMD	120	45	39	38 %
MDScope	130	46	29	36 %
Total	614	247	278	40 %

Table 1: Preliminary results of the MDScope survey

The MDScope web page page is located at `http://www.ks.uiuc.edu/Research/mdscope/`. In a recent search of the World Wide Web, we found that over 50 sites contained direct references to VMD, and 13 to NAMD. Among these sites are the National HPCC Software Exchange, Nan's Parallel Computing page, NIH Molecular Modelling page, and the European Bioinformatics Institute BioCatalog.

*Some responses indicate more than one user at a site

Cluster Computing

This past year the Resource acquired and integrated a new Hewlett-Packard multi-processor server cluster. This cluster more than doubles the computational power available to Resource researchers and offers the Resource staff a new, increasingly common, target platform for their software, namely, a mixture of shared and distributed memory machines. The server cluster expands the concept underlying the workstation cluster which the Resource has operated for over two years. Workstation and server clusters combine standard technical workstations and servers together with off-the-shelf high-speed communication technology (ATM or fibre channel), providing an efficient and cost effective means of computing in molecular biology. Such clusters can be easily implemented at other sites, with technology from various vendors. Our past success with cluster computing has already greatly benefitted the development of parallel computing methods and applications in molecular biology. For example, the Illinois cluster has been essentially duplicated by our collaborators at Yale.

The basic node of the new cluster consists of four symmetric CPUs, which share 512 megabytes (MB) of main memory. Each node has 4 to 12 gigabytes (GB) of disk space, access to high-speed I/O buses, and has a built-in path for processor upgrades which will double the speed of the upgraded machines. We expect to be able to upgrade half of our server cluster with available funds in the near future.

The server cluster nodes are currently interconnected using a 155 Mbit/sec Asynchronous Transfer Mode (ATM) network. This high-speed network is necessary to provide adequate communication between distributed processes of a parallel program running on multiple nodes. In the future we will be able to easily adapt to new technology such as 255 Mbit/sec or 1 Gbit/sec per port FibreChannel switches. We have linked the ATM network between the new server cluster and the workstation cluster, permitting very large parallel computations and supporting high-speed data transfer between all major computational machines. Furthermore, the ATM network is being used to connect additional workstations within the Resource to improve tasks such as remote file access, data analysis and visualization. Figure 3 illustrates our ATM network.

Many systems now feature modest numbers of processors in a shared memory configuration. These systems include such high-end computers as the Silicon Graphics PowerChallenge series and the Convex/HP Exemplar. Even smaller workstations and servers such as ours feature modest shared memory multi-processor configurations. However, scaling these systems to large numbers of processors will still require a clustering type solution. Hence, software developed for of a mixed shared and distributed memory configuration is increasingly important. Our environment can now serve as a testing ground for such mixed configurations.

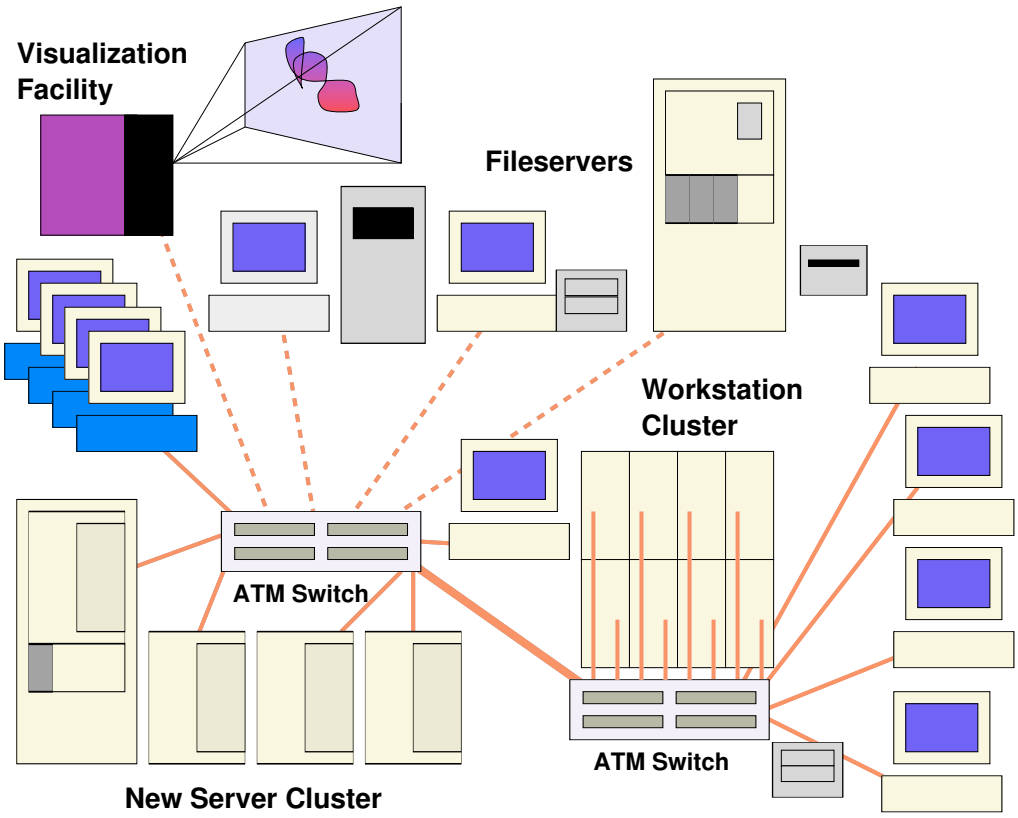

Figure 3: Illustration of the ATM network. Solid lines represent fiber-optic connections between machines and switches. Network connections between ATM switches allow all connected machines to communicate at high-speed. Dotted lines represent possible future expansion of the ATM network to our main visualization computers and fileservers.

Additionally, we are continuing to improve the performance of the overall cluster system by improving important supporting facilities. In the coming year we will be upgrading the central fileserver to allow its connection to our ATM network. We plan to upgrade the network between all our computers to support the increasing data production, analysis and storage. Other plans for the near future include the acquisition of a high-relability disk array to improve the throughput and reliability of large disk farms needed by key applications. Our visualization facility will continue to improve with upgrades to key graphics workstations. Later this year two of the four server cluster machines will undergo processor upgrades which will more than double the computational speed of those machines.

Determination of a Protein Structure by *ab initio* Molecular Replacement

In the current funding period, we have determined the structure of the Light-Harvesting Complex II (LH-II) of *Rhodospirillum molischianum* to 2.4 Å resolution [16, 9]. This is the first structure obtained through a combination of high performance computer modelling (to determine scattering phases) and X-ray diffraction (to determine scattering amplitudes). LH-II is part of the photosynthetic apparatus of purple bacteria, functioning as a light gathering antenna which absorbs sunlight and transfers light energy to the photosynthetic reaction center where the energy is utilized [17]. LH-II is an integral membrane protein involving an aggregate of sixteen independent helical segments as well as 24 chlorophyls and eight lycopenes; as such, the protein matches well the Resource's emphasis on membrane proteins and biomolecular assemblies.

The LH-II complex of *Rs. molischianum* was crystallized by our collaborator (Hartmut Michel , Frankfurt, Germany), and X-ray diffraction data was collected up to 2.4 Å resolution [9, 18]. Resolving a structure from measured diffraction intensities requires knowledge of phases which is unobtainable from a single diffraction experiment. Conventionally, the phase problem is solved by means of the multiple isomorphous replacement method which, in the present case, proved to be unsuccessful due to technical difficulties in making heavy metal derivatives. An alternative solution to the phase problem is to phase the structure by using a homologous structure in a procedure called molecular replacement [19, 20]. In this method, a homologous probe structure is fit into the unit cell of the crystal and used to generate initial scattering phases.

Initially, there existed no structure homologous to LH-II of *Rs. molischianum*. An attempt was made to predict the structure of *Rs. molischianum* and to use it as a probe structure in the molecular replacement method to resolve the 2.4 Å X-ray diffraction data into an atomic structure [16, 21, 22]. We termed the respective method *ab initio* molecular replacement, and a protocol was developed to build a model for LH-II of *Rs. molischianum*. In the first step, a secondary structure analysis assigned helical segments to the two 56- and 45-residue monomers. In the second step, sixteen monomers were aggregated into an octameric complex by means of molecular dynamics simulations and energy minimization. Multiple configurations with different octameric radii were chosen as initial geometries. The resulting structures were employed as search models in the framework of the molecular replacement method as implemented in the program X-PLOR [23]. Due to the complicated crystal packing, four copies of the protein needed to be placed into the unit cell, for which purpose a novel approach involving a combined translational and rotational search was developed [9].

Refinement was carried out using X-PLOR [23] and O [24], achieving a *free* R value

of 23.2% and a conventional R-value of 21.1% for 8 Å–2.4 Å resolution data. Shown in Figure 4 is the 2.4 Å resolution crystal structure of LH-II of *Rs. molischianum* in its entirety. One can recognize an octameric aggregate of two concentric cylinders of sixteen membrane-spanning helical subunits which support two rings of BChl-a molecules, one formed of sixteen BChl-a's perpendicular to the membrane plane and the other of eight BChl-a's nearly parallel to the membrane plane.

While this work was in progress, the crystal structure of LH-II from *Rps. acidophila* determined by the conventional multiple isomorphous replacement method was published by McDermott et al. [25]. LH-II of *Rs. molischianum* is homologous to LH-II of *Rps. acidophila*. However, the size of the aggregate (eighteen vs. sixteen helical segments) differs between the two proteins which prohibits the direct use of a simple homology model in the molecular replacement method.

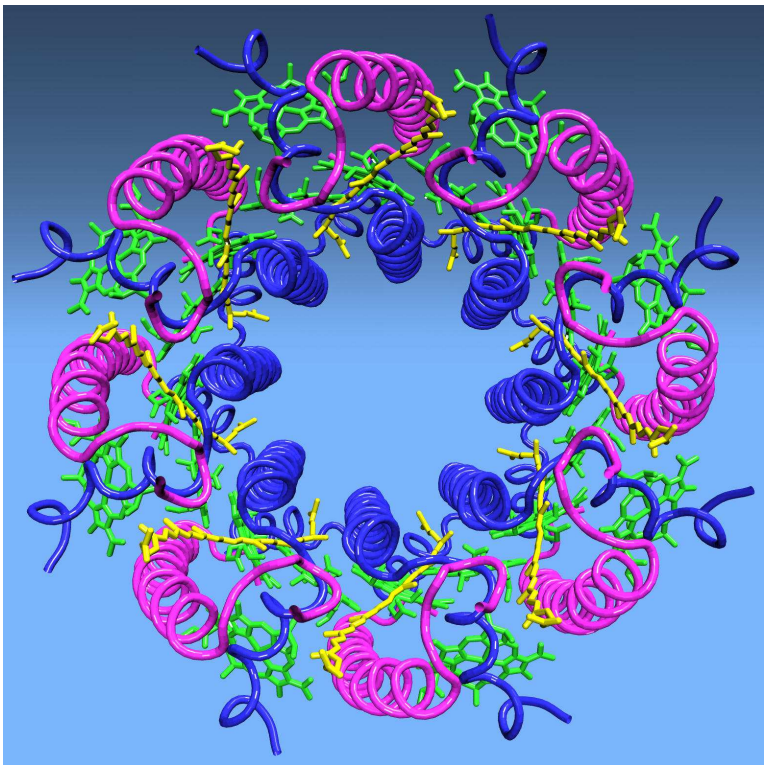

Figure 4: The LH-II octameric complex from *Rs. molischianum*. This image displays a top view with the apoproteins represented as C_α-tracing tubes. The bacteriochlorophyll-a (BChl-a) molecules are shown in green with phytyl tails truncated for clarity. The lycopenes are in yellow.

Further work is in progress at the Resource to turn *ab initio* molecular replacement into a systematic method for X-ray structure determination of membrane proteins. The approach promises to further extend the applicability of X-ray diffraction methods in structural biology to situations where isomorphous replacement or heavy metal substitution does not succeed, but where structure prediction is possible.

Molecular Dynamics Studies of the Protein Bacteriorhodopsin

Molecular dynamics simulations and *ab-initio* quantum chemical calculations have been used to study the protein bacteriorhodopsin (bR), a light-driven proton pump. Bacteriorhodopsin (shown in Figure 5a) is a seven-α-helix protein which resides in the cell membrane of *Halobacterium halobium*. bR contains an all-*trans* retinal chromophore bound through a protonated Schiff base to a lysine residue; photoexcitation results in an isomerization about the 13-14 bond of retinal initiating a proton pump cycle. This cycle is completed through thermal isomerization back to the initial structure, coupled to a transfer of the Schiff base proton of retinal to side group Asp-85 (from where the proton is released eventually to the extracellular side) and a subsequent replacement of the proton from side group Asp-96 (which retrieves the proton eventually from the cytoplasmic side).

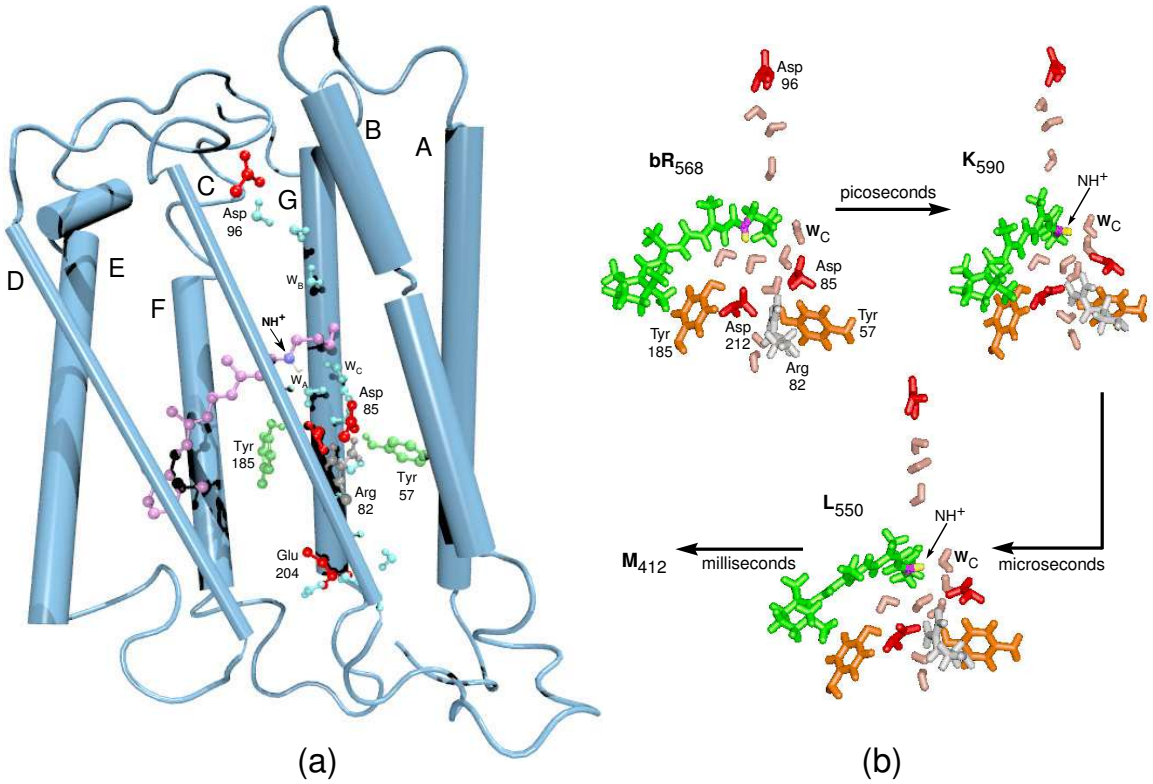

Figure 5: (a) The protein bacteriorhodopsin (bR), showing helices A-G, key residues, and water molecules which participate in the proton pump cycle; (b) suggested structures of the early intermediates in the bR photocycle, as indicated from molecular dynamics simulations.

Experimental studies have revealed many important details of the proton pump cycle, but have not been able to uncover the atomic-level motions responsible for proton translocation. Starting from a structure of bR determined by electron-cryomicroscopy [26], molecular dynamics simulations have refined the structure of the protein, placing, in particular,

internal waters [27]. Molecular dynamics simulations were then used to model the atomic-level motions in bR and to identify the proton pump mechanism [27, 28, 29, 30, 31]. The results of these simulations are reviewed in [32].

The initial phototransformation of retinal consisting of the reactions

$$\mathrm{bR}_{568} \rightsquigarrow \mathrm{bR}^{*}(200\ \mathrm{fs}) \overset{500\ \mathrm{fs}}{\rightarrow} \mathrm{J}_{625} \overset{3\ \mathrm{ps}}{\rightarrow} \mathrm{K}_{590} \overset{10\ \mathrm{ns}}{\rightarrow} \mathrm{L}_{550} \overset{2\ \mu\mathrm{s}}{\rightarrow} \cdots$$

was modelled in [31]. Pump cycle intermediates are denoted J, K, L, ... and are labeled by the corresponding retinal absorption maxima. The J_{625} intermediate is currently thought of as a vibrationally excited form of the K_{590} state [33], but the simulations in [31] suggest that the J_{625} state may instead arise from a dielectric polarization of the protein following retinal excitation, and its decay reflects vibrational cooling of retinal.

The photoisomerization reaction pathway was studied through 50 separate simulations of the initial 5 ps 13-14 photoreaction [29] revealing primarily two different 13-*cis* photoproducts: (1) a product with the retinal proton disconnected from the Asp-85 vicinity and oriented toward Asp-96; (2) a product with retinal connected to Asp-85 via a single water molecule. The latter product most likely represents the functional K_{590} intermediate; its structure was observed to be stable even during subsequent simulated annealing simulations of the longer-lived (2 μs) L_{550} intermediate which directly precedes proton transfer from retinal to Asp-85. Ongoing photoisomerization simulations of non-functional mutants of bR, such as ones with Asp-85 replaced by a neutral residue, displayed a marked decrease in the frequency of product (2)-type systems as compared to simulations of the wild-type. Figure 5b illustrates the conformational changes during the early intermediates which are suggested by the MD simulation results. A key factor in these structures is the role of water, which acts to stabilize the retinal geometry and provide a pathway for proton transfer.

The HMG-D Protein Complexed to DNA

The High Mobility Group 1,2 family (HMG-1,2) includes many proteins from diverse species [34]. A unique feature of the family is that it includes both sequence-specific and non-sequence-specific DNA binding proteins. Although the proteins recognize DNA in two different ways, they have a homologous DNA binding domain called the HMG box. Another interesting feature is their *architecture specificity*, that is an increased affinity for distorted regions of DNA, which can be even larger than the natural affinity of the sequence-specific HMG proteins for their recognition sites.

Our aim has been to determine the structure of the complex of a *Drosophila melanogaster* non-sequence-specific HMG protein (HMG-D) with DNA. It has been proposed that the protein functions as a DNA chaperon facilitating the packing of DNA into nucleosomes [35]. The structure of the HMG-D DNA binding domain has been recently solved by NMR [36], but the structure of its complex with DNA is still unknown. Availability of the structure would provide a better understanding of the interaction of the HMG box with DNA and thus would reveal the mechanisms underlying the architecture specificity. Obtaining the structure would also improve our knowledge of DNA interaction with non-sequence-specific chromosomal proteins and our understanding of DNA packing in chromatin, thereby assisting geneticists in the manipulation of eukaryotic DNA.

Recently, the structures of two sequence-specific HMG proteins, sex reversal Y-factor (SRY) and lymphoid enhancer factor 1 (LEF-1), complexed with DNA, became available [37, 38]. Although the two structures exhibit major similarities, there are also important differences, especially in the shape of the proteins' carboxy termini. Thus, the structure of a non-sequence-specific HMG protein complexed with DNA may exhibit even stronger differences.

In order to predict the structure of the complex of HMG-D with DNA, we have docked the protein's NMR model [36] to various experimentally solved DNA segments. The segment were either a 12-bp DNA pre-bent by a disulfide cross-link [39], or a 12-bp DNA obtained from a complex with the TATA- binding protein [40]. Both DNA segments had a pre-existing bend appropriate for interaction with HMG-D. We positioned the protein on the DNA following various experimental (NMR, footprinting, mutagenesis, etc.) indications that the DNA should be docked on the positively charged concave surface of the L-shaped HMG box and one amino acid residue should intercalate into the DNA minor groove. Three of the best docking structures were selected for further refinement by molecular dynamics (MD) simulations.

Using MD methods successfully employed by the Resource [41], we have performed the simulations in an aqueous environment consisting of a 35 Å-radius sphere of water molecules and 18 Na^+ ions. The model systems, consisting of more than 17,000 atoms

each, were equilibrated for 30 to 50 ps using Langevin temperature coupling. The equilibration was followed by 150 ps of MD without temperature control. The computational demands of these simulations are very high; it takes approximately one day to simulate 6 ps on a single HP735-125MHz workstation. Thus, a complete simulation of each model system required about a month of continuous computing.

We analyzed the dynamics of various structural parameters, such as the rms deviation, the degree of intercalation of Met13, and the DNA geometry (see Fig. 6). This allowed us to determine that the trial complex of HMG-D with TATA-box DNA is the most consistent with experimental data. The complex was simulated for an additional 100 ps that further demonstrated that the structure stabilized itself near a potential energy minimum, that Met13 remained well intercalated during the whole simulation, and that the DNA twist and bend did not exhibit any major changes from equilibrium values.

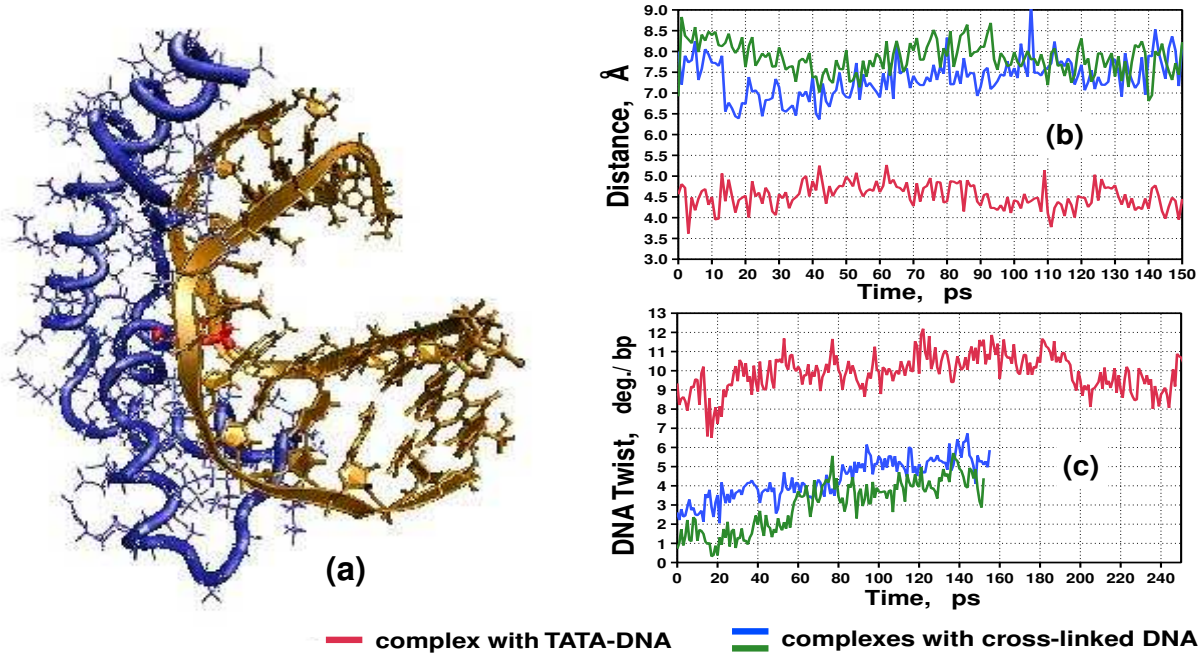

Figure 6: (a) The complex of HMG-D with TATA-DNA, the best candidate for the real structure; protein is in blue, DNA in tan, the intercalating Met13 in red. (b) In this complex, Met13 stays most closely to the DNA bases thus satisfying the experimentally observed intercalation. (c) The DNA geometry in this complex is rather stable whereas the other two complexes continue unwinding towards the former's geometry.

In an attempt to improve our predictions, we are presently using the SRY [38] structure to model the HMG-D–DNA complex. We have performed a homology replacement of amino acids to obtain the sequence of HMG-D on an SRY scaffold. After exploring various simulated annealing and minimization techniques, we improved this hybrid structure and obtained a satisfactory trial complex for MD simulations which are currently in progress. The comparison of the resulting structure with the model previously obtained will allow us to make a better prediction of the structure of the complex of HMG-D with DNA.

Morphogenesis of the Lateral Geniculate Nucleus in Primates

Brain morphology exhibits considerable individual variability. Certain features, however, repeat with strict regularity. This project studies the nature of a particular repeatable quality of a brain structure, the Lateral Geniculate Nucleus (LGN), which is part of the mammalian visual system. The LGN consists of several cellular layers, each mapping in a topographic way part of the visual field. The repeatable quality in the macaque LGN is the co-localization of two features of LGN structure. The first is a singularity, the representation in the LGN of the retinal blind spot as cell-free gaps in some of the layers. The second is an extended, abrupt transition in the lamination structure from six to four layers along a sharply defined surface [42].

In collaboration with the experimental neurobiology group of Joseph Malpeli, Psychology Department at the University of Illinois, we tested the hypothesis that the optic disk gaps in the LGN act as triggers for the laminar transition. In this way, the co-localization between these two morphological aspects is a consequence of their causal relationship. We have developed a three-dimensional model of the LGN morphogenesis [43].

The model includes a wave of development of neuronal properties, and allows one to study the interactions between the gaps and the laminar transition surface in three dimensions. This is probably the first time the three-dimensional morphogenesis of a brain structure has been modeled in a detailed, biologically-realistic framework. Analysis of the underlying dynamics contributes to the understanding of the mechanism of propagation of the developmental wave, as well as the mechanism which allows a localized anomaly to trigger a lamination transition and cause the boundary between the lamination patterns to propagate across the nucleus. In the emerging picture, the gaps destabilize the propagation of the six-layered pattern and trigger a transition to a faster-spreading, four-layered pattern. The resulting boundary between the two patterns is naturally associated with the gaps. The difference of velocities of propagation of the six- and four-layered patterns determines, along with other parameters, the shape of the transition surface. This prediction is tested (and confirmed for a large region of the parameter space) with numerical simulations of the model. The simulations were performed on a single node of the HP cluster operated by the Resource.

The strongest evidence in support of the causal gap-transition relationship in the primate LGN is found in inter-species studies. In two other primate species, chimpanzee and human, the same co-localization between the optic disk gaps and the laminar transition surface in the LGN is suggested by our data. To remove any doubts about the exact morphology, we made a three-dimensional computer reconstruction of human LGN. Figure 7 presents a solid-body view of the LGN as well as three orthogonal sections of the nucleus.

We found the same co-localization between the optic disk gaps and the transition surface

in sections of this computer-reconstructed human LGN and in real tissue sections of human, macaque and chimpanzee LGNs, although the optic disk in the retina and the transition surface in the LGN have different locations in all three primates.

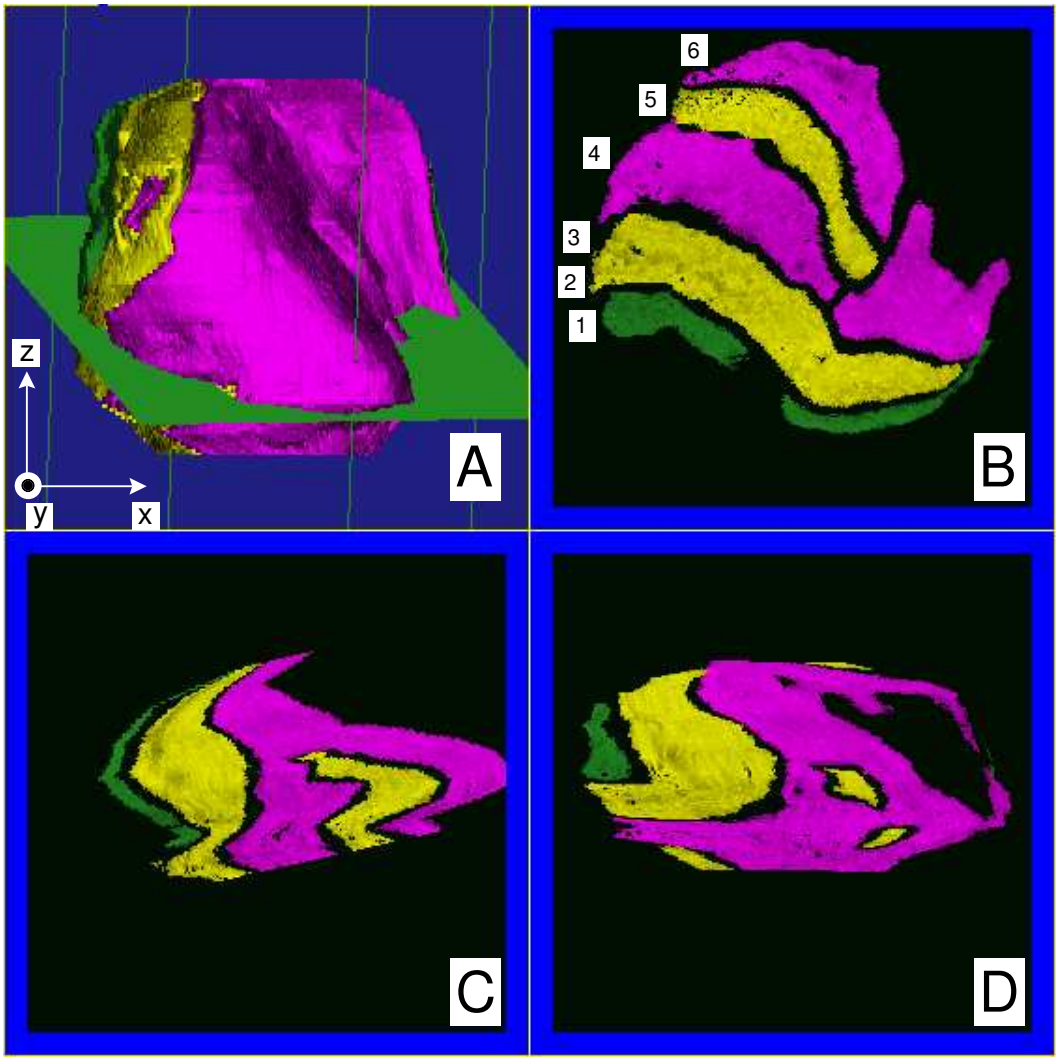

Figure 7: **A**: A solid-body view of the reconstructed LGN. The plane cutting through the LGN is used for the section in **B**. **B**: A look from below at the cut as shown in **A**. Note the alignment of the gaps in the contralateral layers 1, 4 and 6, with the laminar transition. **C** and **D** Sections in planes orthogonal to the plane of **B**. These planes are delineated with the lines running perpendicular to the solid plane in **A**.

The data from the LGNs of three primate species suggests that, and the model helps explain the mechanism through which, the presence of the optic disk gaps triggers an extended laminar transition. Primate LGN morphogenesis provides a rare, but compelling biological example of the well-known physical principle that boundary conditions or singularities may have an extended effect over the state of the entire system. Finally, the three-dimensional computer reconstruction of human LGN can be used as an atlas of a part of the human brain.

BRTP UNIT: D

TITLE: Molecular Visualization: The Program VMD

KEYWORDS: molecular graphics, interactive visualization

AXIS I: 9

AXIS II: 42

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% BRTP $: 13%

ABSTRACT: VMD is an interactive molecular visualization and analysis program currently under development at the Resource. It is the primary visualization tool used by researchers at the facility and has been used by many other groups for a wide variety of tasks including docking studies, structure refinement, trajectory analysis, semiconductor physics, and the production of chemistry education videos.

When used as part of MDScope, VMD is the visual front-end to the molecular dynamics program NAMD, also under development at the Resource. VMD enables researchers to view biomolecules using a wide variety of rendering and coloring methods, as well as to analyze a structure and display the results. When a trajectory is available, either from a data file or via a connection to a running NAMD simulation, the molecules can be animated to visualize the motions. VMD is being used to experiment with new types of input and display mechanisms, and it supports a wide range of devices including a large-screen stereo projection system, the CAVE, and spatial tracking devices. This program, with complete C++ source code, documentation, and precompiled binaries for SGIs, is freely available to the molecular modelling community via anonymous ftp. Additional information on VMD may be found on the VMD web page at `http://www.ks.uiuc.edu/Research/vmd/`.

BRTP UNIT: T

TITLE: Molecular Modelling: The Program NAMD

KEYWORDS: molecular dynamics, spatial decomposition, parallel, interactive

AXIS I: 9

AXIS II: 42 84

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% BRTP $: 13%

ABSTRACT: NAMD, the MD core of MDScope, is a parallel molecular dynamics program for simulations of large biological macro-molecular systems. It is specifically designed for distributed memory parallel computers. It uses spatial decomposition and message-driven execution to exploit the computational power of these machines. Another major strength of NAMD is its modular and object-oriented design that allows researchers to develop and test various new algorithms and to easily incorporate other algorithms such as the distributed parallel multipole tree algorithm (dpmta[44]) for fast full electrostatic force calculations.

The program has been significantly improved in recent months by the addition of new features, including spherical boundary conditions, harmonic constraints, multiple-time-stepping schemes, and rigid bonds to hydrogen. More features such as hydrogen bonds, periodic boundary conditions, and constant pressure dynamics are currently being implemented. The performance has been enhanced by reducing communication costs and improving load balancing techniques. Currently, the

program is being restructured to further improve its performance and exploit newer parallel machines such as clusters of symmetric multiprocessor systems.

As part of MDScope, NAMD is being used for interactive molecular dynamics. Molecular systems currently being studied include protein–DNA systems (37,000 atoms), bacteriorhodopsin (3,800 atoms), and the poliovirus capsid (30,000 atoms).

Another public release of the program (NAMD 1.4) has been recently completed. The program and extensive documentation are available via anonymous ftp. Further information on NAMD is available at `http://www.ks.uiuc.edu/Research/namd/`.

BRTP UNIT: T

TITLE: Longer Time Scales via Torsion Angle Dynamics

KEYWORDS: computer simulation, molecular dynamics, torsion angle dynamics, simulated annealing

AXIS I: 2 9

AXIS II: 74h 84

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ABSTRACT: Traditional molecular dynamics timesteps are limited to one femtosecond by the large force constants associated with bond stretching and angle bending terms in the force field. Fortunately, these motions are of limited interest on longer timescales and, hence, with proper treatment they can be neglected while preserving the other properties of the simulation. Such treatment has made possible computational studies of biopolymers on significantly longer time scales than the present nanosecond range.

A straightforward approach to neglecting bond stretching and angle bending is to mathematically constrain the distances associated with these degrees of freedom and, thus, restrict the system to the slower torsional modes. Iterative methods such as SHAKE [45] are mainly useful for constraining small fractions of the system. For the present case in which the majority of the degrees of freedom are removed from the system direct solution of the associated equations is more appropriate. These methods have been incorporated into NAMD for both normal molecular dynamics and the overdamped dynamics of Grønbech-Jensen and Doniach [46].

While these efforts have been somewhat successful and show promise in combination with interactive molecular dynamics, further work is needed. Particularly, the flexibility lost by constraining many degrees of freedom must be replaced by adjusting the remaining force-field parameters. Also, these methods would benefit greatly from the introduction of a model treating bulk water as a continuum. Success with these methods would allow the simulation of protein folding and vastly improve response times for interactive molecular dynamics.

BRTP UNIT: C

TITLE: Determination of the Structure of an Integral Membrane Protein — the Light-Harvesting Complex II of *Rhodospirillum molischianum*

KEYWORDS: crystal structure, integral membrane protein, light harvesting complex, photosynthesis, *ab initio* molecular replacement

AXIS I: 7a 9

AXIS II: 74h

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INVEST2: Hartmut Michel
DEGREE2: Ph.D.
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% BRTP $: 13%

ABSTRACT: We have determined the structure of the Light-Harvesting Complex–II (LH–II) of *Rhodospirillum molischianum* to 2.4 Å resolution by the molecular replacement method using a computationally modelled probe structure, in collaboration with Hartmut Michel at the Max-Planck-Institut, Frankfurt, Germany [16, 9]. LH–II is part of the photosynthetic apparatus of purple bacteria. The main function of the light-harvesting complex II is to gather light energy and to transfer this energy to the photosynthetic reaction centers for photosynthesis. The LH–II of *Rhodospirillum molischianum* is an octameric aggregate of a basic unit consisting of a pair of polypeptides, commonly referred to as the α- and the β-apoprotein; each basic unit noncovalently binds three bacteriochlorophyll-a and one lycopene molecule. The crystal structure displays two concentric cylinders of sixteen membrane-spanning helical subunits which provide the framework to support two rings of BChl-a molecules, one formed of sixteen B850 BChl-a's perpendicular

to the membrane plane and the other of eight B800 BChl-a's nearly parallel to the membrane plane. This work represents the first successful attempt at using *ab initio* structure prediction to solve the phase problem in X-ray crystallographic structure determination [16, 9]. The approach promises to further extend the applicability of X-ray diffraction methods in the determination of biomolecular structures. All simulations were carried out with the HP workstation cluster at the Resource using X-PLOR and VMD.

BRTP UNIT: C

TITLE: Structure Prediction of a Disk-like Apolipoprotein A-I/lipid System

KEYWORDS: apolipoprotein A-I, rHDL, amphipathic α-helix

AXIS I: 2 6

AXIS II: 74f,h

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% BRTP $: 3%

ABSTRACT: The plasma apolipoproteins are a diverse group of proteins responsible for many functions in lipid metabolism. The apolipoproteins maintain lipoprotein particle structure, act as cofactors for enzymes, and are the ligands for receptors involved in the cellular targeting of lipoprotein particles. Apolipoprotein A-I (28kD, 243 AA) is the major protein component (70%) of plasma high density lipoprotein (HDL) particles [47, 48]. It is a potent activator of the enzyme lecithin cholesterol acyl transferase (LCAT), a key enzyme in the metabolism of cholesterol. Apolipoprotein A-I (apo A-I) does not assume a stable 3D structure in solution. In the presence of phospholipid dispersions, apo A-I binds to the lipid surfaces, markedly increases in α-helical structure, and becomes stabilized [49, 50]. However, no 3D structure of apo A-I has been experimentally determined due to the difficulty of crystalization. The study of reconstituted HDL (rHDL) particles prepared by the sodium cholate dialysis method [51] suggests the formation of discoidal micelles [52] containing two or three apo A-I molecules, each of which contributes up to 8 amphipathic α-helices to form a ring around the lipid bilayer.

Our goal is to predict the 3D structure of apo A-I in a lipid environment, i.e. the discoidal formation of rHDL. The size of the rHDL system is 15,000 to 20,000 atoms (not including waters around the rHDL), and its simulation requires a large amount of time. We have recently finished predicting the secondary structure of apo A-I and assembling the ring-like structure of two apo A-I molecules. Our next goal is to build the entire discoidal system with apo A-I's, lipids, and water.

BRTP UNIT: C

TITLE: Molecular Dynamics Studies of the Protein Bacteriorhodopsin

KEYWORDS: bacteriorhodopsin, membrane protein, retinal, photocycle, molecular dynamics

AXIS I: 2 6 7a

AXIS II: 74h

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% BRTP $: 8%

ABSTRACT: Bacteriorhodopsin (bR) is a membrane protein that functions as a light-driven proton pump in the cell membrane of *Halobacterium halobium*. The function is achieved through a cyclic process initiated by the absorption of a photon. The pump cycle is characterized through a series of intermediates J_{625}, K_{590}, L_{550}, M_{412}, N_{520}, and O_{640}, where the subscripts denote the wavelengths of the respective absorption maxima [53].

Molecular dynamics simulations have been carried out to study the J_{625} and K_{590} intermediates of bR, starting from a refined structure of bR_{568}. The coupling between the electronic states of retinal and the protein matrix is characterized by the

energy difference between the excited state and the ground state to which the protein contributes through the Coulomb interaction. Our simulations indicate that the J_{625} intermediate is related to a polarization of the protein matrix due to the brief (200 fs) change of retinal's charge distribution in going to the excited state and back to the ground state, and that the rise time of the K_{590} intermediate is determined by vibrational cooling of retinal.

Molecular dynamics simulations of wild-type bR and of its D85N, D85T, D212N, and Y57F mutants have also been carried out to investigate possible differences in the photoproducts of these proteins. For each mutant, a series of 50 molecular dynamics simulations of the photoisomerization and subsequent relaxation process were completed, employing the method in [29]. The 13-*cis* photoproducts can be classified into two distinct classes: (1) with the retinal NH^+ bond oriented toward Asp-96; (2) with the retinal NH^+ bond oriented toward Asp-85 and hydrogen-bonded to a water molecule. Simulations of wild-type bR and of its Y57F mutant resulted mainly in class 1 and class 2 products; simulations of D85N, D85T, and D212N mutants resulted almost entirely in class 1 products. These results support the suggestion that only class 2 products initiate a functional pump cycle.

BRTP UNIT: C

TITLE: Quantum Chemistry Studies of the Protein Bacteriorhodopsin

KEYWORDS: bacteriorhodopsin, retinal, quantum chemistry, dark adaptation, spectra, photoisomerization

AXIS I: 2 6 7a

AXIS II: 74h

INVEST1: Ilya Logunov
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INVEST2: Joachim Werner
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% BRTP $: 8%

ABSTRACT: The quantum chemistry packages Gaussian92/94 [54] were employed to study the electronic excitations [55, 56] and the dark adaptation potential surfaces [55, 57] of *in situ* retinal. CASSCF(8,8)/6-31G level *ab initio* calculations were carried out with the protein environment represented through explicit point charges in the electronic Hamiltonian of retinal. The calculations were applied to an ensemble of bacteriorhodopsins generated by molecular dynamics simulations using a CHARMM force field with special parameters for retinal torsions. Our approach resulted in a spectrum of bacteriorhodopsin, with a maximum at 530 nm, a width of 100 nm for the native pigment and a spectrum shifted by 60 nm for the D85N mutant. The calculated rate of dark adaptation at room temperature ($\sim 10^{-5}s^{-1}$) and its enhancement through Asp-85 protonation are in agreement with observation [58].

The excited state potential surface governing the photoreaction of bacteriorhodopsin was determined for a protonated retinal Schiff base analogue containing four double bonds using the quantum chemistry package MOLPRO95 [59, 60]. The calculations confirm that rotation around the 13-14 bond is the predominant photoisomerization pathway. It was found that for retinal geometries close to planar there exists a strong interaction between the first and the second excited states, resulting in an avoided crossing between these two terms. On the other hand, a conical intersection between the first excited and the ground state, occurring at a 90° twist of retinal

around the 13-14 bond, was detected. These results show that accounting for all three electronic terms (the ground, the first excited, and the second excited states) is of crucial importance for the proper description of the retinal photodynamics.

BRTP UNIT: C

TITLE: Cytochrome C Oxidase

KEYWORDS: proton pump, electron transfer, water placement

AXIS I: 2 9 24

AXIS II: 74c 77

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% BRTP $: 5%

ABSTRACT: Cytochrome c oxidase, the terminal enzyme of the respiratory chain, is a membrane protein located in the inner membrane of mitochondria and the plasma membrane of prokaryotes. It accepts electrons from cytochrome c and uses them to reduce oxygen to water at the copper–iron binuclear center. Coupled to the redox reaction, protons are transfered across the membrane, creating a gradient that is used elsewhere, for instance in the synthesis of ATP. Although crystal structures for two cytochrome c oxidases were reported last summer [8, 61], the mechanism of proton pumping remains unclear.

Internal waters are essential for proton pathways, but cannot be resolved in the crystal structure. Using a method developed in Jan Hermans group at UNC [62] we were able to find over a hundred likely water sites in the X-ray structure provided by H. Michel. The placed waters clearly confirm the existence of two proton channels proposed previously.

We have also started molecular dynamics simulations on a 16,000 atom subset of the protein. The calculations are carried out for different stages of the reaction cycle using X-PLOR and recently NAMD. During the simulations polar residues and waters in the proton channels form highly ordered hydrogen bond chains and networks, whose polarization depends critically on the redox state of the binuclear center. Proton conduction is thereby coupled to the state of the reaction center. This is a first step towards understanding the mechanism of the proton pump.

BRTP UNIT: C

TITLE: Molecular Dynamics Study of Architecture Specific Protein–DNA Interaction

KEYWORDS: molecular dynamics, DNA, protein–DNA interaction, transcription regulation

AXIS I: 9

AXIS II: 74e,g,h

INVEST1: Alexander Balaeff
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INVEST2: Mair Churchill
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% BRTP $: 3%

ABSTRACT: To study architecture specific but sequence non-specific DNA recognition we simulated the complex of DNA with the chromosomal protein HMG-D of *Drosophila melanogaster*. The protein functions as a DNA chaperon facilitating DNA packing into nucleosomes [35]. We are trying to predict the unknown structure of the complex between HMG-D and DNA. Three possible structures have been suggested by docking the NMR structure of the protein [36] with existing structures of bent DNA. The structures were refined in molecular dynamics simulations using the program X-PLOR [23] and one structure emerged as the most stable and consistent with experimental observations.

The structure of the sex reversal Y-factor (SRY) complexed with DNA has recently been solved [37]. SRY is a sequence and architecture specific DNA binding protein belonging to the same protein family as HMG-D [34]. We are currently simulating the SRY–DNA complex to compare the data to HMG-D simulations. Thus, we will study the difference between sequence-specific and sequence-non-specific DNA recognition by proteins and mechanisms of architecture specificity.

We are also working to verify our theoretical structure of the HMG-D–DNA complex using the SRY–DNA complex. The method of homology replacement has been used to mutate SRY into HMG-D. After molecular dynamics refinement of the mutant structure we will compare it to our former predictions.

BRTP UNIT: T

TITLE: Molecular Dynamics Study of Sequence Specific Protein–DNA Interactions

KEYWORDS: molecular dynamics, DNA, protein–DNA interaction, transcription regulation

AXIS I: 9

AXIS II: 74e,g,h

INVEST1: Thomas C. Bishop
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% BRTP $: 8%

ABSTRACT: The Resource is using molecular dynamics methods to study the interaction of steroid hormone receptors with DNA. Receptors in this class of proteins recognize and bind to specific sequences of DNA and play a crucial role in a variety of biological processes including metabolism, stress response, and the development of secondary sexual characteristics. One member of this class, the estrogen receptor, is also used for the diagnosis and treatment of certain types of breast cancer. The goal of simulations at the Resource is to determine the effects of protein–DNA complex formation on the conformation of the interacting molecules and to gain an understanding of the nature of sequence specific DNA recognition by proteins.

Simulations of the complex of a dimer of estrogen receptor DNA binding domains (ER-DBD), with DNA have been conducted for two sequences of DNA: the palindromic consensus sequence ds(`CAGGTCACAGTGACCTG`) and the non-consensus, yet biologically active, sequence ds(`CAGAACACAGTGACCTG`). Each system consisted of approximately $36,000$ atoms including Na^+ ions for net charge neutrality.

We have utilized the Resource cluster and the program NAMD [5] to compute two 100 ps trajectories, one for each system. Each simulation required 20 days of parallel computation on eight HP workstations. These simulations represent the first production runs of NAMD utilizing the Coulomb force evaluation package dpmta [44] which allows one to efficiently determine electrostatic interactions for

large systems without cut-off. These prototype simulations led to the addition of several user features, the modification of several routines for improved performance and the discovery and correction of several programming errors in NAMD.

A preliminary analysis of the trajectories indicates that the two base pair substitutions altered the protein–DNA recognition and, thus, the global conformation of the complex. This is most readily observed as differences in the conformation of the DNA helical axis and differences in the relative orientation of the ER-DBD's between the two simulations. Complete analysis is being conducted to confirm and quantify these results.

BRTP UNIT: C

TITLE: Molecular Dynamics Simulation of Protein-Ligand Adhesive Forces for the Avidin-Biotin Complex.

KEYWORDS: adhesion forces, ligand binding, AFM, avidin, biotin

AXIS I: 2 9

AXIS II: 74h

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INVEST2: Sergey Stepaniants
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INVEST3: Manel Balsera
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INVEST4: Yoshitsugu Oono
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% BRTP $: 10%

ABSTRACT: Intermolecular forces between macromolecules govern many biological processes. One such process is protein-ligand binding and unbinding. The avidin-biotin complex, known for its extremely high affinity [63], has been experimentally studied more extensively than most other protein-ligand systems. Apart from its biological importance, the avidin-biotin complex is an excellent tool in various experiments such as affinity chromatography, affinity cytochemistry, biosensors, diagnostics, targeted drug delivery [64, 65, 66], and immunoassays [67].

Avidin isolated from hen egg-white [68] is a tetrameric glycoprotein comprising almost 8000 atoms, which can bind up to four molecules of biotin. Biotin is a

32-atom vitamin which acts as a carrier of activated CO_2 in some biochemical reactions. The 2.7 Å resolution crystal structure of the avidin-biotin complex has been reported recently [69, 70, 71]. With the avidin-biotin adhesion force directly measured with atomic force microscope (AFM) techniques [72, 73, 74], the data produced by computer simulations can be compared to experimental observations.

We performed molecular dynamics simulations of the avidin-biotin complex using the program X-PLOR [23] with the CHARMM22 force field and implemented the external force acting on the system through addition of an artificial harmonic potential kx^2 with k increasing linearly in time from zero to some maximum value.

Our simulations demonstrate that the hydrogen bonds yield the major contribution to the binding, the strongest bond of about 10 kcal/mole being formed by Tyr33. However, hydrophobic interactions between biotin and non-polar residues in the binding pocket, three tryptophans and a phenylalanine, contribute about 1/4 of the total avidin-biotin interaction energy.

Currently we are carrying out MD simulations of the avidin-biotin complex rupture for time scales of several hundred picoseconds, which may allow us to extrapolate the forces to the experimental value (millisecond timescale). Related simulations have been recently carried out on streptavidin [75]. We also plan to investigate avidin mutants and compare our results to the data obtained experimentally for streptavidin mutants.

BRTP UNIT: T

TITLE: Molecular Dynamics Simulations of G-Actin

KEYWORDS: actin, back door, ATPase

AXIS I: 9 20

AXIS II: 42 74c 77

INVEST1: Willy R. Wriggers

DEGREE1: M.S.

DEPT1: Physics

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% BRTP $: 2%

ABSTRACT: Actin filaments are dynamic polymers whose assembly and disassembly in the cytoplasm drive shape changes [76], cell locomotion [77] and chemotactic migration [78]. The elongation of the filament in the cell is accompanied by hydrolysis of adenosine triphosphate (ATP), the fundamental carrier of metabolic energy in the cell [79]. We are interested in the role of waters and ions in actin's ATPase activity. We have carried out several MD calculations of the solvated protein (10,000 atoms, including about 1,200 waters) for up to 500 ps of simulation time and have investigated the essential movements [7]. We found two water diffusion channels which we believe serve in the exchange of the metal ion and of nucleotide hydrolysis products. Of special interest is the "back door" diffusion pathway which may allow the release of inorganic phosphate and the exchange of the metal ion without dissociating the nucleotide. We plan to study further the dissociation of the nucleotide and the role of the proposed "back door" for actin's enzymatic activity by means of interactive modelling. If successful, this work will be the first computer simulation to model the exchange of substrates in an ATPase. The methodology developed and the resulting structural prediction of the dissociation mechanism may benefit research on other related enzymes such as the chaperone Hsc70, G-proteins, and ATP-driven motor proteins like myosin and kinesin.

BRTP UNIT: C

TITLE: Molecular Dynamics Simulations of Calmodulin

KEYWORDS: calmodulin, flexible tether, target recognition

AXIS I: 9 20

AXIS II: 42 74c 77

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NONHOST3: CUNY

% BRTP $: 2%

ABSTRACT: Calmodulin (CaM) is a relatively small (148 residues) dumbbell-shaped protein of exceptional versatility that regulates the activity of a large number of cellular proteins. An example of a CaM-activated protein is myosin light chain kinase which regulates smooth muscle contraction [80]. We are especially interested in the conformation of the central α-helix of CaM, which is intact in the crystal structure [80], but has been found to dissolve in solution [80, 81]. The crystal structure of CaM [80] has been placed in a solvent sphere of 44 Å radius, and six Cl^- and 22 Na^+ ions were included to neutralize the system and model a 150 mM salt concentration, for a total simulation size of 32,867 atoms. Less extensive calculations (see [82] for a review) were carried out earlier by our collaborators at CUNY. We have completed 3 ns of simulation time with the program X-PLOR on the Cray T3D at the Pittsburgh Supercomputing Center. The simulation reveals large-scale domain movements on the nanosecond timescale upon relaxation. The central tethering helix bends and unwinds near residue Arg74. The observed flexibility of the long central tethering helix allows CaM to have different binding conformations available for the recognition of various target peptides.

BRTP UNIT: T

TITLE: Poliovirus Coat

KEYWORDS: poliovirus, molecular dynamics

AXIS I: 7b

AXIS II: 66 74f,h

INVEST1: Linsen Bai

DEGREE1: B.S.

DEPT1: Physics

NONHOST1:

% BRTP $: 3%

ABSTRACT: The VP1 protein of the poliovirus capsid contains a β-barrel with a hollow pocket, inside of which resides a lipid-like ligand, modelled as sphingosine. During the normal infection process, the sphingosine may leave the pocket, altering the stability of the virus. This process may be prevented by antiviral drugs which replace sphingosine [83]. At present it is not understood how the various ligands affect the viral stability and we have initiated molecular dynamics (MD) simulations to uncover this mechanism. It is hypothesized that when the sphingosine leaves the pocket the capsid swells, and thereby creates windows through which the viral RNA can exit to infect the cell. In our study we have employed the structure of the poliovirus protomer (P1/Mahony strain [84]) obtained from the Protein Data Bank. The X-ray structure contains four unresolved sections, which we have predicted from homology data. Using NAMD and X-PLOR, we combined the protomers around a five-fold axis to form a pentamer and minimized the structure. To reduce the system size, we cut a 60 Å sphere (containing 30,000 atoms, waters not included) around the five-fold axis. Presently, we are carrying out an MD simulation to observe the possible conformational rearrangement induced by removal of the sphingosine substrate. Also, the capsid structures of poliovirus P3/Sabin strain with and without the drug disoxaril bound have recently become available and can be used to further study the structural changes in the VP1 beta barrel caused by replacing the sphingosine with various drugs. We intend to measure the effect of these changes on the interaction of VP1 with its neighboring proteins.

BRTP UNIT: C

TITLE: Modelling the Morphogenesis of the Lateral Geniculate Nucleus

KEYWORDS: macaque monkey, development, pattern formation, thalamus

AXIS I: 1d,21,25b

AXIS II: 41,60,77

INVEST1: Svilen Tzonev
DEGREE1: Ph.D.
DEPT1: Physics
NONHOST1:

INVEST2: Joseph Malpeli
DEGREE2: Ph.D.
DEPT2: Psychology
NONHOST2:

% BRTP $: 6%

ABSTRACT: The macaque lateral geniculate nucleus (LGN) exhibits an intricate lamination pattern: depending on the visual eccentricity, it has regions with six, four, and two distinct layers. The transition from six to four layers always coincides with the position of small cell-free gaps corresponding to the blind spot in the retina [42]. We have developed a 3-D model in which local cell interactions cause a wave of development of neuronal receptive fields to propagate through the nucleus and establish distinct lamination patterns. The initial (six-layered) pattern is maintained and propagated along the LGN by strict retinotopy, cell interactions promoting clustering of cells with similar functionality, as well as external gradients. The initial pattern gradually becomes unstable and perturbations due to the blind spot gaps induce a sharp transition to a more stable four-layered pattern. Critical factors for the final global lamination pattern are the choice of the initial (foveal) pattern, the cell interaction distances, the size and location of the gaps, and the shape of the developmental wavefront. A simplified version of the model is amenable to analytical treatment, which provides important insights in the behavior of the more general model. This analysis reveals a close similarity of the laminar transition in this biological system with a well understood physical phenomenon, the so called shock-wave effect.

	TECH RES & DEVEL (T)	COLLAB RES & SERVICE (C)	DISSEM & TRAINING (D)	TOTALS
NUMBER OF PUBLICATIONS	23	16	6*	45
NUMBER OF SUBPROJECTS	5	9	1	15
NUMBER OF INVESTIGATORS	8	23	2	31†
PERCENT OF BRTP FUNDS ALLOCATED	29%	58%	13%	100%
SERVICE FEES COLLECTED	0	0	0	0
OTHER FUNDS ($)	640,000	20,000 150,000	–	810,000

*Four of these were published only on our web-site.

†Contibutors to more than one BRTP unit were counted twice.

State or Country	Number of Investigators
IL	21
NY	2
Israel	1
Germany	3

BRTP Unit T

Investigator	Non-Host Institution (Principal Investigator)	Sources of Support	
		TYPE	AGENCY
Bai, Linsen	University of Illinois (Schulten, Klaus)	OTH	
Bishop, Thomas	University of Illinois (Schulten, Klaus)	OTH	
Brunner, Robert	University of Illinois (Kale, Laxmikant)	FED	NIH
Gursoy, Attila	University of Illinois (Kale, Laxmikant)	FED	NSF, NIH
Kosztin, Dorina	University of Illinois (Schulten, Klaus)	OTH	
Nelson, Mark	University of Illinois (Skeel, Robert)	FED OTH	NIH
Phillips, Jim	University of Illinois (Schulten, Klaus)	FED	DOE
Wriggers, Willy	University of Illinois (Schulten, Klaus)	OTH	

BRTP Unit C

Investigator	Non-Host Institution (Principal Investigator)	Sources of Support	
		TYPE	AGENCY
Balaeff, Alexander	University of Illinois (Schulten, Klaus)	OTH	
Balsera, Manel	University of Illinois (Ono, Yoshitsugu)	OTH	
Churchill, Mair	University of Illinois (Churchill, Mair)	OTH	
Hofacker, Ivo	University of Illinois (Schulten, Klaus)	FED	NSF
Hu, Xiche	University of Illinois (Schulten, Klaus)	OTH	
Humphrey, William	University of Illinois (Schulten, Klaus)	FED	NSF, NIH
Izrailev, Sergei	University of Illinois (Schulten, Klaus)	OTH	
Jonas, Ana	University of Illinois (Jonas, Ana)	OTH	
Köpke, Juergen	Max–Planck–Institute for Biophysics, Germany (Michel, Hartmut)	OTH	
Li, Zhigang	University of Illinois (Schulten, Klaus)	OTH	
Logunov, Ilya	University of Illinois (Schulten, Klaus)	FED OTH	NIH
Lu, Hui	University of Illinois (Schulten, Klaus)	OTH	

Investigator	Non-Host Institution (Principal Investigator)	Sources of Support	
		TYPE	AGENCY
Malpelli, Joseph	University of Illinois (Malpelli, Joseph)	FED	NIH
Mehler, Ernest	Mount Sinai School of Medicine, CUNY (Weinstein, Harel)	OTH	
Michel, Hartmut	Max–Planck–Institute for Biophysics, Germany (Michel, Hartmut)	OTH	
Ono, Yoshitsugu	University of Illinois (Ono, Yoshitsugu)	OTH	
Sheves, Mordechai	The Weizmann Institute, Israel (Sheves, Mordechai)	OTH	
Stepaniants, Sergey	University of Illinois (Schulten, Klaus)	OTH	
Tzonev, Svilen	University of Illinois (Schulten, Klaus)	FED	NIH
Weinstein, Harel	Mount Sinai School of Medicine, CUNY (Weinstein, Harel)	OTH	
Werner, Joachim	Sttutgart University, Germany (Werner, Joachim)	OTH	
Wriggers, Willy	University of Illinois (Schulten, Klaus)	OTH	
Xu, Dong	University of Illinois (Schulten, Klaus)	FED	NIH

BRTP Unit D

Investigator	Non-Host Institution (Principal Investigator)	Sources of Support	
		TYPE	AGENCY
Dalke, Andrew	University of Illinois (Schulten, Klaus)	FED	NIH
Humphrey, William	University of Illinois (Schulten, Klaus)	FED	NSF, NIH

BRTP unit: (T)

NUMBER PUBLISHED –
Books: 0 Papers: 14 Abstracts: 0

NUMBER IN PRESS OR SUBMITTED –
Books: 0 Papers: 9 Abstracts: 0

PUBLISHED:

T. Bishop, H. Heller, and K. Schulten: "Molecular Dynamics on Parallel Computers: Applications for Theoretical Biophysics", in R. K. Kalia and P. Vashishta, Eds., "Toward Teraflop Computing and New Grand Challenge Applications", pages 129–138. Nova Science Publishers, Inc., New York, 1995.

T. Bishop and K. Schulten: "Molecular Dynamics Study of Glucocorticoid Receptor–DNA Binding", *Proteins*, **24**(1), 115–133 (1996).

E. Erwin, K. Obermayer, and K. Schulten: "A Critical Comparison of Models for Orientation and Ocular Dominance Columns in the Striate Cortex", in G. Tesauro, D. Touretzky, and T. Leen, Eds., "Advances in Neural Information Processing Systems 7", pages 93–100. MIT Press, Cambridge, Mass and London, England, 1995.

C. Kurrer and K. Schulten: "Noise-Induced Neuronal Oscillations", *Phys. Rev. E*, **51**(6), 6213–6218 (1995).

I. Logunov and K. Schulten: "Quantum Chemistry of in situ Retinal: Study of the Spectral Properties and Dark Adaptation of Bacteriorhodopsin", in D. Bicout and M. J. Field, Eds., "Proceedings of the Ecole de Physique des Houches", pages 235–256, Paris, 1995. Les Editions de Physique, Springer.

K.-R. Müller, M. Finke, N. Murata, K. Schulten, and S. Amari: "Large Scale Simulations for Learning Curves", in J.-H. Oh, C. Kwon, and S. Cho, Eds., "Progress in neural Processing Vol. **1** / Neural Networks: The Statistical Mechanics Perspective", pages 73–84. World Scientific, Singapore, 1995.

M. Nelson, W. Humphrey, A. Gursoy, A. Dalke, L. Kalé, R. Skeel, K. Schulten, and R. Kufrin: "MDScope— A Visual Computing Environment for Structural Biology", *Comput. Phys. Commun.*, **91**(1, 2 and 3), 111–134 (1995).

J. Phillips and K. Schulten: "Diffusive hysteresis at high and low driving frequencies", *Phys. Rev. E*, **52**(3), 2473–2477 (1995).

K. Sarkar and K. Schulten: "Topology Representing Network in Robotics", in J. L. van Hemmen, E. Domany, and K. Schulten, Eds., "Models of Neural Networks", Volume 3 of *Physics of Neural Networks*, pages 281–302. Springer–Verlag, New York, 1996.

K. Schulten: "Curve Crossing in a Protein: Coupling of the Elementary Quantum Process to Motions of the Protein", in D. Bicout and M. J. Field, Eds., "Proceedings of the Ecole de Physique des Houches", pages 85–118, Paris, 1995. Les Editions de Physique, Springer.

D. Xu, C. Martin, and K. Schulten: "Molecular Dynamics Study of Early Picosecond Events in the Bacteriorhodopsin Photocycle: Dielectric Response, Vibrational Cooling and the J, K Intermediates", *Biophys. J.*, **70**(1), 453–460 (1996).

D. Xu and K. Schulten: "Velocity Reassignment Echoes in Proteins", *J. Chem. Phys.*, **103**, 3124–3139 (1995).

D. Xu, K. Schulten, O. M. Becker, and M. Karplus: "Temperature Quench Echoes in Proteins", *J. Chem. Phys.*, **103**, 3112–3123 (1995).

F. Zhou and K. Schulten: "Molecular Dynamics Study of the Activation of Phospholipase A_2 on a Membrane Surface", *Proteins*, **25**(1), 12–27 (1996).

In Press or Submitted:

D. Barsky, B. Pütz, and K. Schulten: "Theory of Heterogeneous Relaxation in Compartmentalized Tissues", *Magn. Reson. Med.*, Submitted.

I. Logunov and K. Schulten: "Quantum Chemistry – Molecular Dynamics Study of the Dark Adaptation Process in Bacteriorhodopsin", *J. Am. Chem. Soc.*, Submitted. [Beckman Institute Technical Report TB-95-23].

I. Logunov and K. Schulten: "Quantum Chemistry – Molecular Dynamics Study of the Optical Spectrum of Bacteriorhodopsin", *J. Phys. Chem.*, Submitted. [Beckman Institute Technical Report TB-95-24].

K.-R. Müller, N. Murata, M. Finke, K. Schulten, and S. Amari: "A Numerical Study of Learning Curves in Stochastic Multi-Layer Feed-Forward Networks", *Neural Comp.*, (1995), In press. [University of Tokyo Technical Report METR 95-03].

M. Nelson, W. Humphrey, A. Gursoy, A. Dalke, L. Kale, R. D. Skeel, and K. Schulten: "NAMD— A Parallel, Object-Oriented Molecular Dynamics Program", *J. Supercomputing App.*, (1996), In press.

J. Phillips and K. Schulten: "Modeling AFM Tip Dynamics Through Diffusion in Time-Periodic Potentials", in H. Gaub, Ed., "NATO Advanced Research Workshop on Atomic Force Microscopy", in *NATO ASI Series B*. Plenum Press, New York, 1995, In press. [Beckman Institute Technical Report TB-95-03].

K. Schulten, H. Lu, and L. Bai: "Probing Protein Motion Through Temperature Echoes", in H. Flyvbjerg et al., Eds., "Physics of Biological Systems". Springer's LNP, In press.

K. Schulten and M. Zeller: "Topology Representing Maps and Brain Function", in "Nova Acta Leopoldina". Jahresversammlungsband, 1995, In press.

K. R. Wallace and K. Schulten: "A Model of Cortical Processing During Motor Learning", *Biol. Cybernetics*, Submitted. [Beckman Institute Technical Report TB-94-11].

BRTP unit: (C)

NUMBER PUBLISHED –
Books: 0 Papers: 11 Abstracts: 0

NUMBER IN PRESS OR SUBMITTED –
Books: 0 Papers: 5 Abstracts: 0

PUBLISHED:

M. A. Balsera, W. Wriggers, Y. Oono, and K. Schulten: "Principal Component Analysis and Long Time Protein Dynamics", *J. Phys. Chem.*, **100**(7), 2567–2572 (1996).

J. M. Canfield, R. L. Belford, P. G. Debrunner, and K. Schulten: "A Perturbation Treatment of Oscillating Magnetic Fields in the Radical Pair Mechanism Using the Liouville Equation", *Chem. Phys.*, **195**, 59–69 (1995).

J. M. Canfield, R. L. Belford, P. G. Debrunner, and K. Schulten: "A Perturbation Theory Treatment of Oscillating Magnetic Fields in the Radical Pair Mechanism", *Chem. Phys.*, **191**, 347–347 (1995).

X. Hu, D. Xu, K. Hamer, K. Schulten, J. Köpke, and H. Michel: "Predicting the Structure of the Light-Harvesting Complex II of *Rhodospirillum molischianum*", *Protein Science*, **4**, 1670–1682 (1995).

X. Hu, D. Xu, K. Hamer, K. Schulten, J. Köpke, and H. Michel: "Knowledge-Based Structure Prediction of the Light-Harvesting Complex II of *Rhodospirillum molischianum*", in P. Pardalos, D. Shalloway, and G. Xue, Eds., "Global Minimization of Nonconvex Energy Functions: Molecular Conformation and Protein Folding", pages 97–122. American Mathematical Society, 1996.

W. Humphrey, D. Xu, M. Sheves, and K. Schulten: "Molecular Dynamics Study of the Early Intermediates in the Bacteriorhodopsin Photocycle", *J. Phys. Chem.*, **99**, 14549–14560 (1995).

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).

Q. Sheng, K. Schulten, and C. Pidgeon: "A Molecular Dynamics Simulation of Immobilized Artificial Membranes", *J. Phys. Chem.*, **99**(27), 11018–11027 (1995).

S. Tzonev, J. Malpeli, and K. Schulten: "Morphogenesis of the Lateral Geniculate Nucleus: How Singularities Affect Global Structure", in G. Tesauro, D. Touretzky, and T. Leen, Eds., "Advances in Neural Information Processing Systems 7", pages 133–140, Cambridge, Mass and London, England, 1995. MIT Press, [Beckman Institute Technical Report TB-94-13].

D. Xu, M. Sheves, and K. Schulten: "Molecular Dynamics Study of the M412 Intermediate of Bacteriorhodopsin", *Biophys. J.*, **69**(6), 2745–2760 (1995).

M. Zeller, K. R. Wallace, and K. Schulten: "Biological Visuo-Motor Control of a Pneumatic Robot Arm", in Dagli et al., Eds., "Intelligent Engineering Systems Through Artificial Neural Networks", Volume 5, pages 645–650, New York, 1995. American Society of Mechanical Engineers.

In Press or submitted:

J. Köpke, X. Hu, C. Münke, K. Schulten, and H. Michel: "The Crystal Structure of the Light Harvesting Complex II (B800-850) from *Rhodospirillum molischianum*.", *Structures*, In press. [Beckman Institute Technical Report TB-96-02].

R. Sharma, T. S. Huang, V. I. Pavlovic, K. Schulten, A. Dalke, J. Phillips, M. Zeller, W. Humphrey, Y. Zhao, Z. Lo, and S. Chu: "Speech/gesture Interface to a Visual Computing Environment for Molecular Biologists", in "Proceedings of 13th ICPR 96", Urbana, IL, 61801, USA, 1996, To be published.

D. Xu, J. C. Phillips, and K. Schulten: "Protein Response to External Electric Fields: Relaxation, Hysteresis, Echo", *J. Phys. Chem.*, In press.[Beckman Institute Technical Report TB-95-09].

M. Zeller, R. Sharma, and K. Schulten: "Topology Representing Network for Sensor-Based Robot Motion Planning", in "Proceedings of the 1996 World Congress on Neural Networks", Submitted.

M. Zeller, R. Sharma, and K. Schulten: "Vision-Based Motion Planning of a Pneumatic Robot Using a Topology Representing Neural Network", in "Proceedings of 1996 IEEE Int. Symposium on Intelligent Control", Submitted.

BRTP unit: (D)

NUMBER PUBLISHED –
Books: 0 Papers: 1+4† Abstracts: 0

NUMBER IN PRESS OR SUBMITTED –
Books: 0 Papers: 1 Abstracts: 0

PUBLISHED:

W. F. Humphrey, A. Dalke, and K. Schulten: "VMD – Visual Molecular Dynamics", *J. Mol. Graphics*, **14**(1), 33–38 (1996).

IN PRESS OR SUBMITTED:

I. Kosztin, B. Faber, and K. Schulten: "Introduction to the Diffusion Monte Carlo Method", *Am. J. of Phys.*, In press. [Beckman Institute Technical Report TB-95-06].

WEB DOCUMENTS:

VMD User Guide (Version 1.0)
URL: `http://www.ks.uiuc.edu/Research/vmd/ug/`

VMD Programmer Guide (Version 1.0)
URL: `http://www.ks.uiuc.edu/Research/vmd/pg/`

NAMD User Guide (Version 1.0)
URL: `http://www.ks.uiuc.edu/Research/namd/ug/`

NAMD Programmer Guide (Version 1.0)
URL: `http://www.ks.uiuc.ed/Research/namd/pg/`

†four of these published on our web-site

Advisory committee

The Resource Advisory Committee met on November 13, 1995 (see enclosed agenda).

The following colleagues participated:

Bernie Alder, UC Berkeley, Physics (Computational Science)
Peter Arzberger, San Diego, SC (remotely)
C. William Gear, NEC Research Inst. Inc, Princeton
Attila Szabo, NIH, Chair

The reviewers comments are summarized below:

Software

The completion and distribution of the program MDScope is a major accomplishment. Efforts to document, not only the number of people who ftp-ed the software, but also the number of people who actually use it, should be made by the time of the renewal application.

Researchers

The primary role in the development of this program was played by three students, Schulten graduate students Andrew Dalke and Bill Humphrey in case of the molecular graphics program VMD as well as Skeel graduate student Mark Nelson and Kale postdoc Attila Gursoy. Every effort should be made to hire people of this calibre and interest. At the same time, such individuals should be the yardstick by which other researchers are judged. Apparently, not all computer science graduate students were successful. The recommendation is made by some advisory board members to increase the effectiveness of the interaction between computer science and physics/biophysics graduate students:

students in computer science should only be selected after they have passed their Ph.D. candidacy exam when they are ready to begin their research project and know what their interests are; computer science students should also be selected by the principal investigators in computer science and physics as to investigate a mutually agreeable project.

Computer Administration

As recommended last year, a system administrator, Dr. Ari Shinozaki, has been hired. Shinozaki is evidently an excellent choice and this move freed up researchers to focus on science and program development.

Most Impressive Project

The most impressive single scientific project completed last year is the determination of the structure of the light harvesting complex II of Rs. molischianum. This is judged an extremely important achievement.

Choice of Systems

The systems selected for study, bacteriorhodopsin, cytochrome c oxydase, protein–DNA complexes, actin, membranes and membrane–protein complexes, virus coat proteins, represent the most important and interesting systems studied in molecular biology to date — the group has very good taste!

Collaborations with Experimentalists

A major effort in this direction was made since last year with spectacular results. For virtually every system mentioned above the Resource is collaborating with a leading experimentalist.

Computational Neurobiology Projects

Because researchers working in the area of computational neurobiology could not attend the advisory board meeting this year no presentation of work in this area were made and the committee cannot evaluate the progress achieved. however, the committee understands that the Resource seeks to refocus its activities in the future to computational structural biology, maintaining a smaller fraction of projects in computational neurobiology.

Algorithms Employed in the Molecular Dynamics Program NAMD

The advice is given to evaluate critically the algorithms employed at present and to make every effort to assure that the best numerical approaches are used and are effectively implemented. In particular, the present program for evaluation of long range Coulomb forces, stemming from a collaboration with Board at Duke U., should be compared with results using the P^3M method; the latter approach has been shown to be up to hundred times faster than the dpmta-type method in certain cases.

Likewise, Skeel should re-evaluate the integration schemes used since in this area speed-ups by a significant factor are possible. A compilation of methods by Berendsen (Groningen, Holland) exists. The approach taken by Berne (Columbia U.) should be implemented

and tested. Skeel's own efforts in this regard are to be applauded; it is hoped that his simple model calculations will lead to a practical multi-time scale algorithm that will be incorporated into NAMD.

Original comments of the reviewers may be obtained from the Resource upon request.

Training and Dissemination

The Resource has continued to expand its dissemination and training activities in the past year:

- The principal instrument for the dissemination of software tools and information on prototype modelling projects and related activities is the Resource web site. This site has been significantly improved during the past year and is regularly maintained. The site, accessible to all Internet users, includes both scientific and administrative information such as software available, current research projects, main research accomplishments, publication list, the people in the group, as well as our seminar series, special events organized by the Resource, job announcements, and more. The average number of external accesses per month is about 7,000. The address for our home page is `http://www.ks.uiuc.edu/`.

- Documentation, source code and some precompiled binaries for the MDScope software developed by the group are available from our ftp server `ftp.ks.uiuc.edu`. VMD can be found in the directory `/pub/mdscope/vmd`, NAMD is located in `/pub/mdscope/namd`, and MDComm in `/pub/mdscope/mdcomm`. MDScope's newly designed home page is located at `http://www.ks.uiuc.edu/Research/mdscope/`. At present we are aware of over 50 web sites pointing to VMD and 13 pointing to NAMD. Systematic retrieval records for all software are available.

- To identify and address the needs of our user community we conducted an MDScope survey. The questionnaire was sent electronically to over 600 users who downloaded our software in the past year or so. The response was overwhelming and indicated a genuine interest in our product . By responding to user concerns, comments and ideas, we hope to support better the user base and to further improve our software tools.

- Our two HP workstation clusters are being widely used by on–campus groups such as the Wolynes and Oono groups at UIUC, as well as by off-campus groups such as the Brünger group at Yale, the Board group at Duke, the Pidgeon group at Purdue, and the Michel group at Frankfurt.

- To further enhance collaborations with experimentalists, the Resource organized in November 1995 a workshop "Computational / Experimental Approaches in Structural Biology" (see enclosed agenda). The two day meeting covered a wide range of topics in contemporary Structural Biology. These included protein/nucleic acid interactions, membrane/peptide and protein interactions, light absorbing proteins,

enzyme mechanisms and protein folding. Most of the sessions had both an experimental and a theoretical or computational component and as became evident from the responses we received, the interplay between experimental and computational scientists at the workshop was remarkably constructive.

- The Resource continues to run a popular seminar series in Theoretical Biophysics which serves a wide group of campus researchers and attracted many interesting speakers (see list of speakers and titles in the next section).

- The Resource makes its publications available as preprints and reprints in the form of Beckman Institute Technical Reports. These reports are maintained in a data base accessible to Internet users and are made available upon request.

- The 3D projection facility has been extensively used for scientific, dissemination and training purposes, and for meeting community interest. The facility is regularly included on UIUC tours by federal and state officials, and is operated by the Resource personnel with demonstrations being made several times a week. Visitors to the facility in the past year included Dr. T. Bell (Senior Editor, IEEE Spectrum), Prof. M. Bishop (Director of the G. W. Hooper Research Foundation, UCSF), Dr. M. Chaney (Eli Lilly), Prof. M. Feigenbaum (Rockefeller U), Prof. T. Ferrin (UCSF), Prof. R. Freisner (Columbia), Prof. W. Gear (Princeton and NRC), Dr. S. Okinaga (Chairman of the Board of Trustees for the Teikyo University Foundation and President of Teikyo University), Prof. M. Parinello (Max Planck Institute), Prof. K. Patel (Vice Chancellor for Research, UCLA), Prof. R. Penrose (Rouse Ball Professor of Mathematics, Oxford University), Prof. J. Richardson (UNC), Prof. C. Schmidt (Michigan U), Prof. E. Shakhnovich (Harvard), Dr. T. Von Forster (Editor, Springer-Verlag publishing), Prof. J. Widom (Northwestern U), and Prof. P. Woodward (U of Minn., SGI graphics director). Demonstrations were also held for prospective UIUC faculty, high school physics students, our Open House visitors and others.

- The Resource organized a successful Open House on February 29, 1996 (see enclosed announcement and tour guide). The event attracted over 100 on-campus visitors, both students and faculty, and resulted in renewed and increased interest in our research and development efforts.

- The Resource continues to develop its library. There are 16 periodical subscriptions to leading scientific, technology, and software development journals which include titles such as: *Current Opinion in Structural Biology, Trends in Biochemical Sciences, Trends in Neurosciences, Nature, Nature: Structural Biology, Science*, and *Folding & Design*. During the past year we purchased over 100 newly published books in areas such as quantum mechanics, human genetics, mathematics,

biocomputing, protein folding, protein structure, parallel programming, nonlinear dynamics and chaos, system administration, and neurobiology. The library is well catalogued, is available on the web, and has become an important tool for staff members and visitors.

- The Resource has designed a new group brochure that offers an effective vehicle to introduce us to the wider community (see enclosed copy).

- The Resource has published its first comprehensive annual report. The document describes all research activities performed by the group, regardless of sources of support (see enclosed copy). In the future the annual report will be compiled and published every fall.

- The Resource is the center of a so-called Grand Challenge Application Group funded by NSF. This group includes researchers from Duke (J. Board), Yale (A. Brünger), NYU (T. Schlick), and UNC (J. Hermans). The group's goal is to jointly develop and apply algorithms for large scale and long time biomolecular simulations. The group organizes a yearly retreat of all researchers involved. The group's third two-day retreat in November 10-12, 1995 was organized and hosted by the Resource (see enclosed program). About 50 researchers attended. A unique feature of this retreat was the high involvement of students. It was felt throughout that this format led to a very successful meeting. It was also nice to see the young people who have been through more than one retreat maintaining interactions with one another on both professional and personal levels. A team spirit was clearly evident.

- Plans for next year include: the publication of a newsletter; publishing our second internal annual report early next fall; organizing a workshop for the Resource members focusing on the art of presentation and scientific writing and video production; organizing the fourth annual GCAG retreat which is scheduled for September 27-29, 1996; holding a third Open House in the spring; investing in extensive dissemination efforts of MDScope and holding a training workshop to introduce the program to our collaborators and other interested scientists; hosting an X-PLOR workshop in the Summer of '97.

Outside Lectures

The PI presented the following lectures:

- May 2–7, 1995, Workshop on Quantum Mechanical Simulation Methods for Studying Biological Systems, Les Houches, France; Lecture: *Molecular Dynamics and Quantum Chemistry Study of Spectral Properties, Ground and Excited State Dynamics of Retinal in Bacteriorhodopsin*

- June 15–30, 1995, COMET XIV Conference, Kloster Banz, Germany; Lecture: *Fast Photoprocesses in Bacteriorhodopsin*

- July 24–28, 1995, IMA Program "Large Scale Optimization", Week 3, Minneapolis, MN; Lecture: *Modeling the Structure of a Membrane Protein: Secondary Structure Prediction, Aggregation of α-Helices, Energy Optimization Under Constraints, and Verification*

- August 14–27, 1995 Physics of Biological Systems: From Molecules to Species Workshop, Humlebaek, Denmark. Overall Title: *Stochastic Modelling of Biopolymer Behavior*. Lectures:

  1. *From Molecular Dynamics Simulations to Stochastic Models*;
  2. *A Crash Course in Stochastic Differential Equations for Classical and Quantum Systems*;
  3. *Characterizing Biopolymer Dynamics Through Correlation Functions and Susceptibilities*;
  4. *Examples: Spectral Line Shapes, Electron Transfer, Echoes in Various Proteins*.

- September 11, 1995, Rush University, Department of Molecular Biophysics and Physiology, Chicago, IL; Lecture: *Prediction of the Structure of the Light-Harvesting Complex II of Rhodopseudomonas molischianum*

- October 11–12, 1995, Porter Lecture in Living State Physics, Vanderbilt University, Nashville, TN; Lecture: *The Physics of Vision and the Physics of Photosynthesis*

- October 19, 1995, Physical Chemistry Department, Indiana University, Bloomington, IN; Lecture: *The Light-Harvesting Complex-II of Rhodospirillum molischianum*

- October 24, 1995, UIUC Mathematics Department, Urbana, IL; Lecture: *Topology Representing Maps and Brain Function*

- November 7, 1995, Computational / Experimental Approaches in Structural Biology, University of Illinois at Urbana-Champaign; Lecture: *Quantum Chemistry and Molecular Dynamics Studies of Bacteriorhodopsin*

- November 8, 1995, Computational / Experimental Approaches in Structural Biology, University of Illinois at Urbana-Champaign; Lecture: *Modelling the Virus Coat Protein*

- December 11–14, 1995, Function and Structure – Fundamental Concepts in Theoretical Biology, Bad Honnef, Germany; Lecture: *Towards a Theoretical Biology*

- January 2–7, 1996, Aspen Winter Conference on Biophysics Supramolecular Assemblies and Protein Interactions, Aspen, CO; Lecture: *DNA Protein Interactions*

- February 8, 1996, Graduate Student Advisory Committee Seminar, UI; Lecture: *Nanobiology: Biology between 10nm and 1000nm*

- February 13, 1996, Beckman Institute Director's Seminar; Lecture: *Nanobiology: Biomolecular Organization at One to One Thousand Nanometers*

- February 14–15, 1996 NSF Resource Advisory Committee Meeting, UNC, Chapel Hill, NC

- February 21, 1996, Harvard Physical Chemistry Seminars; Lecture: *Quantum Biology - The Bacteria's Tale*

- April 29, 1996, Max Planck Institute for Solid State Research, M. Parinello, host; Lecture: *Bacteria Know Quantum Mechanics: Optimization of Energy, Electron and Proton Conduction in Bioenergetic Proteins*

- May 2, 1996, Dept. of Neuroinformatics, U. of Bochum, Germany; Lecture: *Beauty in the Eye and the Brain of the Beholder - Morphogenesis of the Retinal-LGN-Cortical Pathway*

- May 7, 1996, Dept. of Physics, U. of Frankfurt, Germany; Lecture: *Bacteria Know Quantum Mechanics: Optimization of Energy, Electron and Proton Conduction in Bioenergetic Proteins*

- May 13–17, 1996, 16th Annual CNLS Conference, Los Alamos, NM; Lecture: *Quantum Biology and Energy Landscapes: The Bacteria's Tale*

- May 24, 1996, Dept. of Physics, Technical U. of Munich, Germany; Lecture: *Bacteria Know Quantum Mechanics: Optimization of Energy, Electron and Proton Conduction in Bioenergetic Proteins*

- June 23–28, 1996, 7th International Conference on Retinal Proteins, Zichron Yaacov, Israel; Lecture: *Quantum Chemistry and Molecular Dynamics Studies of the Proton Pump cycle of Bacteriorhodopsin*

- June 12–16, 1996 50th Anniversary ACM meeting on Strategic Directions in Computing Research, Boston, MA

- July 21–26, 1996, Gordon Research Conference, Bioelectrochemistry, Newport, Rhode Island; Lecture: *Recent Developments in the Understanding of Magnetically Sensitive Radical-Pair Reactions*

During the past year the PI served on the following committees:

- Computational Science and Engineering Steering Committee (CSE);
- Beckman Institute Program Advisory Committee;
- National Research Council Committee on the Future of Computer Science;
- Appointment and Promotion Committee, Department of Physics, University of Illinois;
- Beckman Institute Human Computer Interaction Ad Hoc Committee;
- Beckman Institute External Advisory Committee.

During the past year research personnel of the Resource have participated and/or presented contributions at the following meetings and institutions:

- Pfizer-Beckman Institute Conference, Protein Interactions Symposium, Urbana, IL (Kosztin, Bishop, Phillips, Hamer, Balaeff, Humphrey, 1995)
- Summer Conference on Big Brains, Psychology Department, Cornell University, Ithaca, NY (Tzonev 1995)
- Advanced Computing Laboratory, Los Alamos National Laboratory, Los Alamos, NM (Humphrey 1995)
- WCNN '95 Conference, Washington, DC (Zeller 1995)
- Physics of Biological Systems: From Molecules to Species Workshop, Humlebaek, Denmark (Phillips 1995)
- American Chemical Society meeting, Chicago, IL (Logunov 1995)
- IBM Watson Laboratory, Poughkeepsie, New York (Nelson 1995)
- LISA '95 Conference, Monterey, CA (Shinozaki 1995)
- Max-Planck-Institut für Biochemie, Frankfurt, Germany (Hu 1995)
- Universität Stuttgart, Stuttgart, Germany, Guest Scientist (Logonuv 1995)
- Multigrid Tutorial with Applications to Molecular Dynamis, Weizmann Institute, Rehovot, Israel (Skeel 1995)
- SIAM National Meeting, Charlotte, NC (Skeel 1995)

- Minnesota Supercomputing Institute, Minneapolis, MN (Skeel 1995)
- Computational / Experimental Approaches in Structural Biology, University of Illinois at Urbana-Champaign (all Resource members)
- Neurons, Networks and Motor Behavior Conference, Tucson, AZ (Zeller 1995)
- Mathematics and Molecular Biology IV Conference, Santa Fe, NM (Hofacker 1995)
- Artificial Neural Networks in Engineering Conference, St. Louis, MO (Zeller 1995)
- IBM Watson Laboratory, Poughkeepsie, NY (Dalke 1995)
- University of Minnesota, Minneapolis, MN (Humphrey 1995)
- Supercomputing '95, San Diego, CA (Dalke, Gursoy, Humphrey 1995)
- Conference on Dynamical Numerical Analysis, Atlanta, GA (Skeel 1995)
- Max Planck Institut für Biophysik, Frankfurt, Germany (Hofacker 1995)
- University of California-San Francisco Computer Graphics Laboratory, San Francisco, CA (Humphrey 1996)
- UIUC Electromagnetics Seminar, Urbana, IL (Skeel 1996)
- Biophysical Society Meeting, Baltimore, MD (Wriggers, Hu, Kosztin, Hofacker 1996)
- APS meeting, St. Louis, MO (Phillips 1996)
- UIUC Computational Science and Engineering Seminar (Skeel 1996)
- POOMA'96 Conference, Santa, Fe, NM (Humphrey, Kale 1996)
- Jet Propulsion Laboratory, Pasadena, CA (Humphrey 1996)
- NSF Grand Challenge Workshop, Washington, DC (Kale 1996)
- IPPS meeting, Honolulu, HI (Kale 1996)
- Mathematical Sciences, Northern Illinois University, Rockford, IL (Skeel 1996)
- 7th International Conference on Retinal Proteins, Zichron Yaacov, Israel (Humphrey 1996)

Resource Seminar

During the past year the following outside speakers have presented lectures in the Resource seminar series at the Beckman Institute:

- Christoph Schmidt, Department of Physics, University of Michigan, May 15, 1995, Lecture: *Kinesin Stepping of Microtubules - A Motor Protein Observed with Optical Tweezers and Laser Interferometry*
- Hans-Ulrich Bauer, Institute for Theoretical Physics, University of Frankfurt, Germany, June 19, 1995, Lecture: *Variants of Self-Organizing Maps*
- Gomathi Ramachandran, New York University, July 17, 1995, Lecture: *Supercoiled DNA: Structure and Dynamics*
- Michael Chaney, Eli Lilly Corporation, August 7, 1995, Lecture: *Molecular Modeling of the Human Beta Amyloid Peptide Aβ (1-42) and its Oligomerization*
- Thomas E. Ferrin, Computer Graphics Laboratory, University of California at San Francisco, September 25, 1995, Lecture: *The Role of Molecular Graphics in Structure-Based Drug Design*
- Lubos Mitas, National Center for Supercomputing Applications, University of Illinois, October 2, 1995, Lecture: *Quantum Monte Carlo for Electronic Structure of Solids and Clusters*
- Xiche Hu, Beckman Institute, University of Illinois, October 9, 1995, Lecture: *Determination of the Structure of the Light-Harvesting Complex-II of Rhodospirillum molischianum*
- Bob Eisenberg, Rush Medical College, Chicago, IL, October 16, 1995, Lecture: *From Structure to Function in Open Ionic Channels*
- Eugene I. Shakhnovich, Department of Chemistry, Harvard University, November 20, 1995, Lecture: *Unravelling Mechanisms of Protein Folding and Evolution*
- Mitsugu Matsushita, Department of Physics, Chuo University, Tokyo, Japan, November 27, 1995, Lecture: *Spatio-Temporal Patterns Produced by Bacteria*
- Klaus-Robert Mueller, GMD First, Berlin, Germany, December 4, 1995, Lecture: *Learning Curves and Overtraining in Stochastic Multi-Layer Feed-Forward Networks*

- Carolina Cruz-Neira, Computer Science Department, Iowa State University, February 26, 1996, Lecture: *Interactive Visual Supercomputing Immersed in Science and Engineering*

- Helmut Grubmueller, Ludwig-Maximillians-Universitaet Muenchen, Germany, March 4, 1996, Lecture: *Protein Dynamics Simulations: Toward Experimentally Verifiable Predictions*

- Michael C. Zerner, Department of Chemistry, University of Florida, April 1, 1996, Lecture: *On the Initial Photochemical Event in Photosynthesis: A Theorist's View*

- José Onuchic, University of California at San Diego, April 4, 1996, Lecture: *Pathway Tubes as the Building Blocks for Designing Electron Transfer Proteins*

- G.Ludwig Hofacker, Technical University, Munich, Germany, April 4, 1996, Lecture: *Concepts of Biological Information*

Bibliography

[1] 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.

[2] F. Zhou and K. Schulten. Molecular dynamics study of a membrane–water interface. *J. Phys. Chem.*, 99:2194–2208, 1995.

[3] F. Zhou and K. Schulten. Molecular dynamics study of the activation of phospholipase A_2 on a membrane surface. *Proteins*, 25(1):12–27, 1996.

[4] H. Luecke, B. T. Chang, W. S. Mailliard, D. D. Schlaepfer, and H. T. Haigler. Crystal structure of annexin XII hexamer and implications for bilayer insertion. *Nature*, 378(6556):512–515, 1995.

[5] M. Nelson, W. Humphrey, A. Gursoy, A. Dalke, L. Kalé, R. Skeel, K. Schulten, and R. Kufrin. MDScope— A visual computing environment for structural biology. *Comput. Phys. Commun.*, 91(1, 2 and 3):111–134, 1995.

[6] D. Xu, J. C. Phillips, and K. Schulten. Protein response to external electric fields: Relaxation, hysteresis, echo. *J. Phys. Chem.* In press.[Beckman Institute Technical Report TB-95-09].

[7] M. A. Balsera, W. Wriggers, Y. Oono, and K. Schulten. Principal component analysis and long time protein dynamics. *J. Phys. Chem.*, 100(7):2567–2572, 1996.

[8] S. Iwata, C. Ostermeier, B. Ludwig, and H. Michel. Structure at 2.8 Å resolution of cytochrome *c* oxidase from *Paracoccus denitrificans*. *Nature*, 376:660–669, 1995.

[9] J. Köpke, X. Hu, C. Münke, K. Schulten, and H. Michel. The crystal structure of the light harvesting complex II (B800-850) from *Rhodospirillum molischianum*. *Structures*. In press. [Beckman Institute Technical Report TB-96-02].

[10] H. Grubmüller, H. Heller, A. Windemuth, and K. Schulten. Generalized Verlet algorithm for efficient molecular dynamics simulations with long-range interactions. *Molecular Simulation*, 6:121–142, 1991.

[11] M. Nelson, W. Humphrey, A. Gursoy, A. Dalke, L. Kale, R. D. Skeel, and K. Schulten. NAMD— A parallel, object-oriented molecular dynamics program. *J. Supercomputing App.*, 1996. In press.

[12] M. Tuckerman, B. J. Berne, and G. J. Martyna. Reversible multiple time scale molecular dynamics. *J. Chem. Phys*, 97(3):1990–2001, 1992.

[13] J. J. Biesiadecki and R. D. Skeel. Danger of multiple–time–step methods. *J. Comput. Phys.*, 109:318–328, 1993.

[14] W. F. Humphrey, A. Dalke, and K. Schulten. VMD – Visual molecular dynamics. *J. Mol. Graphics*, 14(1):33–38, 1996.

[15] R. Sharma, T. S. Huang, V. I. Pavlovic, K. Schulten, A. Dalke, J. Phillips, M. Zeller, W. Humphrey, Y. Zhao, Z. Lo, and S. Chu. Speech/gesture interface to a visual computing environment for molecular biologists. In *Proceedings of 13th ICPR 96*, Urbana, IL, 61801, USA, 1996. To be published.

[16] X. Hu, D. Xu, K. Hamer, K. Schulten, J. Köpke, and H. Michel. Predicting the structure of the light-harvesting complex II of *Rhodospirillum molischianum*. *Protein Science*, 4:1670–1682, 1995.

[17] R. van Grondelle and V. Sundstrom. Excitation energy transfer in photosynthesis. In H. Scheer, editor, *Photosynthetic Light–Harvesting Systems*, pages 403–438, Berlin, New York, 1988. Walter de Gruyter & Co.

[18] H. Michel. General and practical aspects of membrane protein crystallization. In H. Michel, editor, *Crystallization of Membrane Proteins*, pages 74–88, Boca Raton, Florida, 1991. CRC Press.

[19] M. Rossmann, editor. *The Molecular Replacement Method*. Gordon and Breach, New York, 1972.

[20] E. Lattman. Diffraction methods for biological macromolecules. Use of the rotation and translation functions. *Methods in Enzymology*, 115:55, 1985.

[21] X. Hu, D. Xu, K. Hamer, K. Schulten, J. Köpke, and H. Michel. Knowledge-based structure prediction of the light-harvesting complex II of *Rhodospirillum molischianum*. In P. Pardalos, D. Shalloway, and G. Xue, editors, *Global Minimization of Nonconvex Energy Functions: Molecular Conformation and Protein Folding*, pages 97–122. American Mathematical Society, 1996.

[22] X. Hu, D. Xu, K. Hamer, K. Schulten, J. Köpke, and H. Michel. Prediction of the structure of an integral membrane protein–the light-harvesting complex II of *Rhodospirillum molischianum*. In K. Merz and B. Roux, editors, *Biological Membranes:*

A Molecular Perspective from Computation and Experiment. Birkhäuser, Cambridge, MA. In press.

[23] A. T. Brünger. *X-PLOR, Version 3.1, A System for X-ray Crystallography and NMR*. The Howard Hughes Medical Institute and Department of Molecular Biophysics and Biochemistry, Yale University, 1992.

[24] A. Jones, J.-Y. Zou, S. Cowan, and M. Kjeldgaard. Improved methods for building protein models in electron density maps and the location of error in these models. *Acta Cryst. A*, 47:110–119, 1991.

[25] G. McDermott, S. Prince, A. Freer, A. Hawthornthwaite-Lawless, M. Papiz, R. Cogdell, and N. Isaacs. Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature*, 367:614–621, 1994.

[26] R. Henderson, J. M. Baldwin, T. A. Ceska, F. Zemlin, E. Beckmann, and K. H. Downing. Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. *J. Mol. Biol.*, 213:899–929, 1990.

[27] W. Humphrey, I. Logunov, K. Schulten, and M. Sheves. Molecular dynamics study of bacteriorhodopsin and artificial pigments. *Biochemistry*, 33:3668–3678, 1994.

[28] I. Logunov, W. Humphrey, K. Schulten, and M. Sheves. Molecular dynamics study of the 13-*cis* form (bR_{548}) of bacteriorhodopsin and its photocycle. *Biophys. J.*, 68:1270–1282, 1995.

[29] W. Humphrey, D. Xu, M. Sheves, and K. Schulten. Molecular dynamics study of the early intermediates in the bacteriorhodopsin photocycle. *J. Phys. Chem.*, 99:14549–14560, 1995.

[30] D. Xu, M. Sheves, and K. Schulten. Molecular dynamics study of the M412 intermediate of bacteriorhodopsin. *Biophys. J.*, 69(6):2745–2760, 1995.

[31] D. Xu, C. Martin, and K. Schulten. Molecular dynamics study of early picosecond events in the bacteriorhodopsin photocycle: Dielectric response, vibrational cooling and the J, K intermediates. *Biophys. J.*, 70(1):453–460, 1996.

[32] 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.

[33] S. J. Doig, P. J. Reid, and R. A. Mathies. Picosecond time-resolved resonance Raman spectroscopy of bacteriorhodopsin's J, K, and KL intermediates. *J. Phys. Chem.*, 95:6372–6379, 1991.

[34] D. Landsman and M. Bustin. A signature for the HMG-1 box DNA binding proteins. *BioEssays*, 15(8):539, 1993.

[35] S. S. Ner and A. A. Travers. HMG-D, the *Drosophila melanogaster* homologue of HMG1 protein, is associated with early embryonic chromatin in the absence of histone H1. *EMBO J.*, 13(8):1817–1822, 1994.

[36] D. N. M. Jones, M. A. Searles, G. L. Shaw, M. E. A. Churchill, S. S. Ner, J. Keeler, A. A. Travers, and D. Neuhaus. The solution structure and dynamics of the DNA binding domain of HMG-D from *Drosophila melanogaster*. *Structure*, 2:609, 1994.

[37] J. Love, X. Li, D. Case, K. Giese, R. Grosschedl, and P. Wright. The HMG box of lymphoid enhancer factor 1 bends DNA and facilitates assembly of functional nucleoprotein structures. *Nature*, 376:791–795, 1995.

[38] M. Werner, J. Huth, A. Gronenborn, and G. Clore. Molecular basis for human 46X,Y sex reversal revealed from the three-dimensional solution structure of the human SRY–DNA complex. *Cell*, 81:705–714, 1995.

[39] M. E. A. Churchill, D. N. M. Jones, T. Glaser, H. Hefner, M. A. Searles, and A. A. Travers. HMG-D is an architecture-specific protein that preferentially binds to DNA containing the dinucleotide TG. *EMBO J.*, 14(7), 1995. In press.

[40] Y. Kim, J. H. Geiger, S. Hahn, and P. B. Sigler. Crystal structure of a yeast TBP/TATA–box complex. *Nature*, 365:512–520, 1993.

[41] T. Bishop and K. Schulten. Molecular dynamics study of glucocorticoid receptor–DNA binding. *Proteins*, 24(1):115–133, 1996.

[42] J. Malpeli and F. H. Baker. The representation of the visual field in the lateral geniculate nucleus of *macaca mulatta*. *J. Comp. Neurol.*, 161:569–594, 1975.

[43] S. Tzonev, J. Malpeli, and K. Schulten. Morphogenesis of the lateral geniculate nucleus: How singularities affect global structure. In G. Tesauro, D. Touretzky, and T. Leen, editors, *Advances in Neural Information Processing Systems 7*, pages 133–140, Cambridge, Mass and London, England, 1995. MIT Press. [Beckman Institute Technical Report TB-94-13].

[44] W. Rankin and J. Board. A portable distributed implementation of the parallel multipole tree algorithm. *IEEE Symposium on High Performance Distributed Computing*, 1995. In press. [Duke University Technical Report 95-002].

[45] J.-P. Ryckaert, G. Ciccotti, and H. J. C. Berendsen. Numerical integration of the Cartesian equations of motion of a system with constraints: Molecular dynamics of *n*-alkanes. *J. Comp. Phys.*, 23:327–341, 1977.

[46] N. Grønbech-Jensen and S. Doniach. Long-time overdamped langevin dynamics of molecular chains. *J. Comp. Chem.*, 15:997–1012, 1994.

[47] H. Brewer, T. Fairwell, A. Larue, R. Ronan, A. Houser, and T. Bronzer. The amino acid sequence of human apoA-I, an apolipoprotein isolated from high density lipoproteins. *Biochem. Biophys. Res. Commun.*, 80:623, 1978.

[48] A. Scanu, J. Toth, C. Edelstein, S. Koga, and E. Stiller. Fractionation of human serum high density lipoprotein in urea solutions. Evidence for polypeptide heterogeniety. *Biochemistry*, 8:3309, 1969.

[49] A. Tall, D. Small, G. Shipley, and R. Lees. Apoprotein stability and lipid-protein interactions in human plasma high density lipoproteins. *Proc. Natl. Acad. Sci. U.S.A.*, 72:4940, 1975.

[50] H. Pownall, F. Hsu, M. Rosseneu, H. Peeters, A. Gotto, and R. Jackson. Thermodynamics of helix formation in the association of high density lipoprotein A-I (Apo A-I) to dimyristoyl phosphatidylcholin. *Biochem. Biophys. Acta*, 488:190, 1977.

[51] A. Jonas. Reconstitution of high-density lipoproteins. *Methods Enzymol.*, 128:553, 1986.

[52] A. Jonas, K. Kezdy, and J. Wald. Defined apolipoprotein A-I conformations in reconstituted high density lipoprotein discs. *J. Biol. Chem.*, 264:4823, 1989.

[53] R. H. Lozier, R. A. Bogomolni, and W. Stoeckenius. Bacteriorhodopsin: A light-driven proton pump in halobacterium halobium. *Biophys. J.*, 15:955–962, 1975.

[54] M. J. Frisch, G. W. Trucks, M. Head-Gordon, P. M. W. Gill, M. W. Wong, J. B. Foresman, B. G. Johnson, H. B. Schlegel, M. A. Robb, E. S. Replogle, R. Gomperts, J. L. Andres, K. Raghavachari, J. S. Binkley, C. Gonzalez, R. L. Martin, D. J. Fox, D. J. Defrees, J. Baker, J. J. P. Stewart, and J. A. Pople. *Gaussian 92, Revision A*. Gaussian Inc., Pittsburgh, PA, 1992.

[55] I. Logunov and K. Schulten. Quantum chemistry of in situ retinal: Study of the spectral properties and dark adaptation of bacteriorhodopsin. In D. Bicout and M. J. Field, editors, *Proceedings of the Ecole de Physique des Houches*, pages 235–256, Paris, 1995. Les Editions de Physique, Springer.

[56] I. Logunov and K. Schulten. Quantum chemistry – molecular dynamics study of the optical spectrum of bacteriorhodopsin. *J. Phys. Chem.* Submitted. [Beckman Institute Technical Report TB-95-24].

[57] I. Logunov and K. Schulten. Quantum chemistry – molecular dynamics study of the dark adaptation process in bacteriorhodopsin. *J. Am. Chem. Soc.* Submitted. [Beckman Institute Technical Report TB-95-23].

[58] S. P. Balashov, R. Govindjee, M. Kono, E. Imasheva, E. Lukashev, T. G. Ebrey, R. K. Crouch, D. R. Menick, and Y. Feng. Effect of the arginine-82 to alanine mutation in bacteriorhodopsin on dark adaptation, proton release, and the photochemical cycle. *Biochemistry*, 32:10331–10343, 1993.

[59] H.-J. Werner and P. J. Knowles. A second order multiconfiguration SCF procedure with optimum convergence. *J. Chem. Phys.*, 82:5053–5063, 1985.

[60] P. J. Knowles and H.-J. Werner. An efficient second-order MC SCF method for long configuration expansions. *Chem. Phys. Lett.*, 115:259–267, 1985.

[61] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinnzawha-Itoh, R. Nakashima, R. Yaono, and S. Yoshikawa. Structures of metal sites of oxidized bovine heart cytochrome c oxidase at 2.8 Å. *Science*, 269:1069, August 1995.

[62] L. Zhang and J. Hermans. Hydrophilicity of cavities in proteins. *Proteins: Structure, Function and Genetics*, 1996, In press.

[63] N. M. Green. Avidin. *Advan. Prot. Chem.*, 29:85–133, 1975.

[64] M. Wilcheck and E. A. Bayer. Avidin-biotin technology ten years on: has it lived up to its expectation? *Trends in Biochemical Sciences*, 14:408–412, 1989.

[65] N. M. Green. Avidin and streptavidin. In *ME*, volume 184, pages 51–67. Academic Press, London, 1990.

[66] E. A. Bayer and M. Wilcheck. The use of avidin-biotin complex as a tool in molecular biology. *Methods Biochem. Anal.*, 26:1–45, 1980.

[67] M. Kanzaki and A. Iwasawa. A sensitive, non-isotopic immunoassay for progesterone using the avidin-biotin system. *Biomedical Research*, 16(6):381–386, 1995.

[68] G. Heney and G. A. Orr. The purification of avidin and its derivatives on 2-immunobiotin-6-aminohexylsepharose 4b. *Anal. Biochem.*, 114:92–96, 1981.

[69] L. Pugliese, A. Coda, M. Malcovati, and M. Bolognesi. Three-dimensional structure of the tetragonal crystal form of egg-white avidin in its functional complex with biotin at 2.7Å resolution. *J. Mol. Biol.*, 231:698–710, 1993.

[70] L. Pugliese, A. Coda, M. Malcovati, and M. Bolognesi. Crystal structure of apo-avidin from hen egg-white. *J. Mol. Biol.*, 235:42–46, 1994.

[71] O. Livnah, E. A. Bayer, M. Wilchek, and J. L. Sussman. Three-dimensional structures of avidin and the avidin-biotin complex. *Biochemistry*, 90:5076–5080, 1993.

[72] E.-L. Florin, V. T. Moy, and H. E. Gaub. Adhesion force between individual ligand-receptor pairs. *Science*, 264:415–417, 1994.

[73] V. T. Moy, E.-L. Florin, and H. E. Gaub. Intermolecular forces and energies between ligands and receptors. *Science*, 266:257–259, 1994.

[74] V. T. Moy, E.-L. Florin, and H. E. Gaub. Adhesive forces between ligand and receptor measured by AFM. *Colloids and Surfaces*, 93:343–348, 1994.

[75] H. Grubmüller, B. Heymann, and P. Tavan. Ligand binding and molecular mechanics calculation of the streptavidin-biotin rupture force. *SCI*, 96.

[76] J. V. Small. Microfilament-based motility in non-muscle cells. *Curr. Opinion Cell Biol.*, 1:75–79, 1989.

[77] J. A. Theriot, T. J. Mitchison, L. G. Tilney, and D. A. Portnoi. The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization. *Nature*, 357:257–260, 1992.

[78] P. N. Devreotes and S. H. Zigmond. Chemotaxis in eukaryotic cells: a focus on leukocytes and *dictyostelium*. *Ann. Rev. Cell Biol.*, 4:649–686, 1988.

[79] M.-F. Carlier. Actin: Protein structure and filament dynamics. *J. Biol. Chem.*, 266:1–4, 1991.

[80] M. Ikura, G. M. Clore, A. M. Gronenborn, G. Zhu, C. B. Klee, and A. Bax. Solution structure of a calmodulin-target peptide complex by multidimensional NMR. *Science*, 256:632–638, 1992.

[81] M. Zhang, T. Tanaka, and M. Ikura. Calcium-induced conformational transition revealed by the solution structure of Apo calmodulin. *Nature Struct. Biol.*, 2:758, 1995.

[82] J.-L. Pascal-Ahuir, E. L. Mehler, and H. Weinstein. Calmodulin structure and function: Implication of arginine in the compaction related to ligand binding. *Molecular Engineering*, 1:231–247, 1991.

[83] R. A. Grant, C. N. Hiremath, D. J. Filman, R. Syed, K. Andries, and J. M. Hogle. Structures of poliovirus complexes with anti-viral drugs: implications for viral stability and drug design. *Curr. Biol.*, 4(6):784–797, 1994.

[84] J. M. Hogle, M. Chow, and D. J. Filman. Three-dimensional structure of poliovirus at 2.9 Å resolution. *Science*, 229:1358, 1985.