is screened by ions and by the high dielectric nature of water. The long-range force is calculated by adding the "ion" and the "surface" charges to the solute charge. The short-range electrostatic force is not screened by the ions, but the effect of the dielectric remains. The short-range force is calculated simply by adding the surface charge to the solute charge.

To obtain the ion and the surface charges, we solve the Poisson–Boltzman equation. The divergence of the electrostatic potential is proportional to the sum of the solute and induced charges. We can separate out the induced charge, as the solute charge is given. Then the surface charge is the sum of the induced charges near the surface of the molecule, and the ion charge is the sum of the induced charges outside the solvent-accessible surface. Generally, the induced charge has an opposite sign to the inducing charge, and is smaller in magnitude than the inducing charge. So the calculated electrostatic force is reduced to the screened level. In this method, the water molecule is treated as only dielectric, and the ionic strength is treated as a parameter of the Debye length. So this method can calculate the effects of water and ions, while requiring no water molecules to be treated explicitly in the molecular dynamics simulation. This means that the computational efforts are smaller than those of the conventional periodic boundary method.

This method is tested for relative hydration free energy between GLY and ALA. As a result, 1.14 kcal/mol of the hydration energy is obtained. This is close to the value of 1.18 ~ 1.14 kcal/mol calculated by molecular dynamics simulation with 367 water molecules by Singh et al.

The method can estimate the effects of the ions and the solvent without water molecules explicitly. And this is effective when a macromolecule, which needs many surrounding water molecules, is simulated.

ANALYSIS OF THERMAL FACTOR VARIATION IN PROTEIN STRUCTURE

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A relational database of protein structure, Iditis, has been constructed incorporating all X-ray crystallographically defined structures from the Brookhaven PDB. The database stores descriptions of the protein at all levels of the protein structure hierarchy, and contains many parameters not found in the PDB. Data are stored in tables, and can be accessed through Structured Query Language (SQL) queries. Complex structural motifs can be identified from the database very rapidly.

The database has been used to analyze the thermal factors of protein structures in various ways. Correlations between normalized B-values and residue types, secondary structures, accessibility, stereochemical quality of the structure, and other structural parameters have been performed. Particular attention has been paid to understanding the variation of thermal factors across different regions of a structure, especially disordered regions. It is hoped that future work will lead to a clearer understanding of the nature of flexibility across a structure.

MOLECULAR DYNAMICS SIMULATION OF A MEMBRANE PROTEIN IN A LIPID BILAYER ON A PARALLEL COMPUTER

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Investigations of membrane proteins, e.g., *bacteriorhodopsin* or *Gramicidin*, require an explicit inclusion of their native membrane environment in molecular dynamics simulations to determine the extent to which lipid–protein interactions affect protein structure and function. For this purpose, we constructed a 24,000-atom macromolecular assembly consisting of a bilayer lipid membrane and water, 85 Å × 100 Å in lateral extension and 35 Å in thickness (see Figure 1). The assembly consisted of 200 phosphatidylcholin molecules (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholin, POPC). The phosphatidylcholin molecules are uncharged. Each has a polar head group and two hydrocarbon tails, one of which includes a C=C *cis*-double bond. The two membrane surfaces were covered by a 15-Å layer of water. POPC was chosen as the lipid because it is one of the most prevailing phospholipids in biological membranes. We also replaced POPC by 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholin (DPPC), probably the most studied phospholipid, by altering the C=C *cis*-double bond to a single bond, eliminating two terminal C atoms, and using the POPC membrane's structure to start the respective simulation. We employed stochastic boundaries (boundary thickness 8 Å) to prevent disintegration of the assemblies. In a molecular dynamics run of more than 300 ps, we adjusted

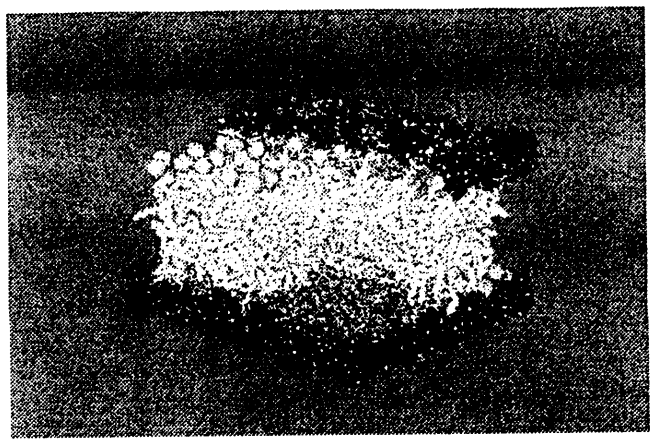

Figure 1. Membrane structure obtained after more than 200 ps simulation of a POPC bilayer. A portion of the water and the lipid molecules has been omitted to provide a view of the core of the membranes: grey, hydrocarbon tails; yellow spheres, head groups; red, restrained water molecules; and orange, unrestrained water molecules.

the equilibrium positions of the stochastic boundary atoms so as to minimize the time-averaged restraint forces on these atoms to achieve a small internal pressure.

We studied the following properties of the membrane: pressure on all six faces of the simulated volume, displacements of (the center of mass of) lipid molecules and atomic displacements, the deuterium order parameter (S_{CD}) as a function of chain position, fluctuations of chain and head group dihedral angles, penetration of water, surface area per lipid molecule, distribution of molecular groups, and transverse and longitudinal lipid self-diffusion coefficients. Interesting results of the simulations include: the assembly is stable; the lipid head groups tilt towards the membrane plane at an angle of 21°; a phase transition from the gel to the liquid crystal phase could be induced by changing the surface area per lipid molecule; a high mobility of the two double bonded carbon atoms was discovered; and the many investigated membrane properties are in excellent agreement with experimental results. Part of this work has been accepted for publication in *J. Phys. Chem.*[1]

We also succeeded in embedding *bacteriorhodopsin* into our membrane–water system (see Figure 2). We plan to investigate the membrane–protein interactions between *bacteriorhodopsin* and the lipid molecules, e.g., structural changes induced in *bacteriorhodopsin* through contact with the lipid molecules, as well as changes induced in the configuration and dynamics of the lipid molecules in contact with *bacteriorhodopsin*. It is expected that this ongoing research will be completed at the time of the conference.

The large-scale simulation described became possible through the use of a 60-node MIMD type parallel computer with a double ring architecture. Each node of this machine consists of a T800 Transputer processor with 4 MByte DRAM. The computer was designed and built by us[2] (for US-$60,000) in 1988, and achieves Cray 2 processor speed for molecular dynamics runs. The molecular dynamics program EGO, written in occam II, is compatible with CHARMm and X-PLOR. It employs the CHARMm force field, and can be started from X-PLOR restart files. The trajectory files can be analyzed using X-PLOR. EGO achieves a speed proportional to the number of nodes. The program employs a distance class algorithm[3] for the Coulomb forces and provides bond length constraints (SHAKE) for hydrogen atoms.

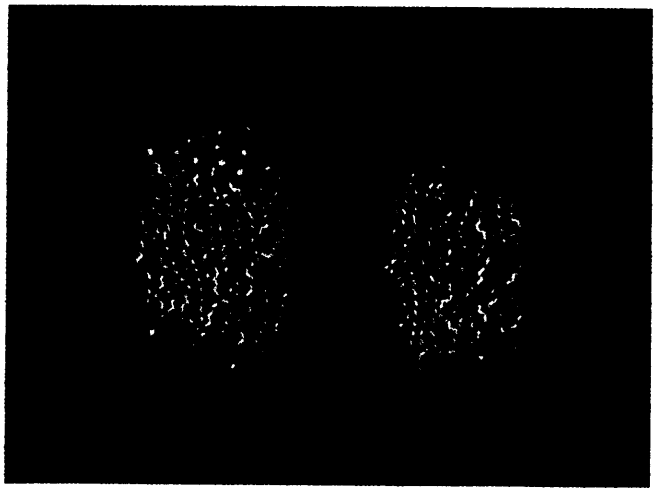

Figure 2. Membrane with embedded bacteriorhodopsin. *A portion of the water and the lipid molecules has been omitted to provide a view of the interior: grey, hydrocarbon tails; yellow spheres, head groups; red, restrained water molecules; orange, unrestrained water molecules; and green, backbone of* bacteriorhodopsin.

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MODELING OF ACTIVE SITE OF CYTOCHROME P-450IA2

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Cytochrome P-450 constitutes a superfamily with more than one hundred different isoenzymes. This family plays an important role in the metabolism of a wide variety of drugs, and in the bioactivation of potent carcinogens.

Although the primary sequence of several mammalian cytochromes P-450 are known, obtaining three-dimensional structures of these cytochromes using homology techniques is difficult because the only crystallographic structure described is that of cytochrome P-450cam, and this prokaryote cytochrome has only 10–20% homology with the mammalian forms.

Our work has focused on building a three-dimensional structure for the active site of cytochrome P-450IA2. This isoform is involved in the metabolism of caffeine, and in the bioactivation of heterocyclic amines to potent carcinogenic agents.

We propose a three-dimensional structure based in three sources of information:

- Homology studies using cytochrome P-450cam as a template.
- Steric and electronic considerations to hold caffeine and other substrates in the active site.
- Our previous work,[1] which describes a particular electrostatic pattern associated with the demethylation of caffeine.