performance options. While it has been challenging to write software that conforms to the Macintosh standards, the result is a highly interactive, easy-to-use program.

Using these techniques and tools, we have created structures for, and analyzed the interactions of, a monolayer of DMPC, diacetylenic lipids, and the combination of the disaccharide trehalose and the polar surface of the DMPC monolayer.

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**FUS: A Rule-Based System for the Rapid Evaluation of Folding and Unfolding Strategies**

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In order to study the dynamics of protein and nucleic acid conformations, a molecular folding-unfolding system (FUS) has been implemented. Features of the secondary structure of these molecules, such as helices, β-strands and loops, are graphically represented by simple polygonal objects. Modeling of the unfolding (denaturation) and folding of their three-dimensional structure is made possible by the use of operators that allow displacement of these structural features in space. The system uses two primary operators that allow topological manipulation of the structure; these primary operators can be used in the implementation of higher-level operators. First-order logical rules are used to validate the action of these operators. Rules are stored in a database and can be modified by using a predicate calculus-like language. The user can implement his own algorithms using the default (furnished) rules, user-defined rules or a mixture of both combined with topological operators. For example, a user-defined rule could be constructed to infer the presence of complex structures like triplets in proteins (two parallel β-strands antiparallel to an adjacent helix). Due to this flexibility, FUS is a useful tool for the rapid evaluation of user-defined folding and unfolding strategies. Some of the advantages of such a system are: (1) topological validation based on logical rules is faster than validation based on energy calculations, and (2) logical structures are much closer to the reasoning process of biochemists. As an example, we use the yeast phenylalanine tRNA sequence as input to a secondary structure algorithm. The output is employed to deduce the secondary features that are the input of FUS. Then, a logical strategy can be designed based on a set of topological hypotheses, in order to obtain the final "L" structure of tRNA. Once accomplished, these same rules can be applied to other RNAs to test their generality.

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**Display and Interpretation of Protein Electrostatic Potential Maps**

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A major class of heme containing proteins are the cytochromes c, which are crucial catalysts in electron transport chains. The exact function and position of the cytochromes c in electron transport chains is dependent on the redox potential of the heme protein. The redox potential has been found to vary from −290 mV to 400 mV, a large range for an enzyme with a remarkably conserved primary and tertiary structure.[1] A major objective in the study of cytochromes c is to determine how the protein's amino acid sequence and tertiary structure tunes its redox potential.

We have applied a continuum electrostatic model to describe the protein and surrounding solvent. Electrostatic potentials resulting from the protein's charge distribution and the high dielectric medium are calculated by the finite-difference solution to the Poisson-Boltzmann equation pioneered by Warwicker and Watson[2] and Honig and coworkers.[3,4] We have developed an interactive interface, within the framework provided by the HYDRA[5] molecular graphics package, to explore and interpret the information contained in the electrostatic potential map.

Our menu-driven routines read in calculated electrostatic maps in the format of a 65 × 65 × 65 lattice. From these maps isopotential surfaces, field-lines and solvent-accessible surface potentials can be generated and overlaid on molecular structures. These composite structures can be rotated and manipulated in real time. The field-line option can be used to examine the electrostatic gradient about selected atoms, residues or chain segments. This option is valuable in analyzing and comparing local perturbations to the electric field, resulting from point mutations, counterion binding, solvent interactions and protein conformational changes.

We have mapped the phosphate and carbonate binding sites of tuna cytochrome and shown the dependence of these binding sites on the counterion radius. Additionally, we have found the isopotential surface about the proposed contract region between tuna cytochrome c and its redox partners to be insensitive to changes in ionic strength, pH and iron oxidation state. In contrast, the isopotential surfaces on the "backside" (i.e., opposite the redox contact site) of tuna cytochrome c vary greatly with changes in solution conditions and the iron oxidation state.

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**Developments in the Theory of Protein Folding Simulations**

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I would argue that, on the whole, a wrong approach is being taken to computer-aided protein engineering. The reasons for this controversial view are as follows. For many years we have been developing algorithms for treating the problem of predicting the structure and behavior of molecules. To do this we have sought, borrowed and adapted ideas from several areas of science, especially mathematics. More recently some new developments in mathematics have caused us to think in a rather different way about our algorithms, or, rather, these new developments have permitted us to overcome certain difficulties that we felt were there.

In brief, these new developments are about the behavior of simulation algorithms in general and about how to describe their behavior, possibilities, and limitations in the language of topology. In fact, a great deal of this is already inherent in control theory, but a number of recent extensions of this kind of mathematical area provide a more powerful general approach.

Except for the neglect of quantum mechanical phenomena, molecular dynamics provides us with the most complete description of the conformation and behavior of a system in terms of the phase space. The conformation and behavior of the protein, which is to say the essence of its being, is represented by a trajectory in this phase space, which is a space in three dimensions consisting of three coordinates $(x,y,z)$ and the three momenta conjugate to them $(px,py,pz)$ for every atom. Fundamental principles established from early this century and beyond establish the view that the main ways to envisage problems in the phase space are ways of topology (i.e., of the weaving of threads and sheets in multidimensional space). Modern developments in so-called nonlinear science, chaos theory and the like simply turn out to be in large part the sciences of solving iterative equations and interpreting their effects in terms of topology in multidimensional space. They also explicitly reveal the first glimmerings of the nature of the simulation algorithm, how to use external information from expertise and databases to direct the simulation toward a solution, and what we mean by a "solution" anyway. In short, molecular dynamics provides a route to a unification of the seemingly disparate concepts that the computer-aided protein engineer must employ.

Major advances in our programs for calculating the structures and properties of molecules have been made possible by these developments, and examples of these studies will be presented in the lecture in order to underline the fact that these theoretical advances can quickly find tangible, practical application in biomolecular computation.

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**An Area-Based Algorithm for Cast Shadows on Space-Filling Molecular Models**

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The area-based algorithm for space-filling molecular models has been used to produce full color pictures with realistic shadows. Previous work could only "dim" atoms proportionally to the amount they were obstructed from the light source. This approximation gave soft, penumbra-like shadows, instead of the accurate, "hard-edged" "cast-shadows" that we are now able to achieve without ray tracing. The cost of the visibility calculations is independent of the raster, and the cost of the shadow calculations grows linearly with the resolution.

Space-filling molecular models are built from intersecting spheres with radii equal to the Van der Waals radii of the atoms they represent. The equilibrium distance between two nonbonded atoms under van der Waals force is represented as spheres that just touch. Shadows provide extra visual depth cues for understanding the three-dimensional structure of the molecule when it is rendered in a raster image.

One method to achieve such realism is to repeat the visibility calculations from the point of view of the light source. The visible regions are then transformed back to the final view, where they become the illuminated regions, while the rest remain in shadow. The new algorithm computes the visible portions of the atoms at floating-point precision from both the viewer reference and from the light source view. The information from these two calculations is then merged into a color image of the space-filling molecular model with cast-shadows, self-shadows and highlights.

The output of the program is kept in a flexible format that allows it to be rendered on anything from an 8-bit frame buffer to a 24-bit film recorder. This allows one to view the output on an inexpensive raster device before putting it out to film. In addition, the resolution can