developed by the PIR at NBRF), which allow us to search, with or without mismatches, the residues of an unknown protein fragment within our sequence database. Successful matches are used to generate command procedure files. These command procedures, interfaced with the molecular modeling program MIDAS (Molecular Interactive Display and Simulation, IRIS version, University of California, San Francisco), in turn automatically display the 3D structures of the matched residues and extract their internal parameters. The advantages of this new methodology, as an effective tool, will be illustrated in the modeling of a putative calcium-binding site of (Ca^{2+} + Mg^{2+})-dependent ATPase (Ca^{2+} −ATPase) of rabbit muscle sarcoplasmic reticulum.[1]

We thank K. Ward of Naval Research Laboratory and A.E. Shamoo of University of Maryland for their interest in this work and the Office of Naval Research for financial support.

1 MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. *Nature* 1985, **316**, 696

Molecular Graphics of Lipid Structures

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Molecular graphics display of lipids and lipid aggregates has received only minor attention due to the difficulty of obtaining crystal structures. Reasonable depictions of lipids are crucial, however, for understanding the molecules' physical chemistry. Three-dimensional models are necessary because membrane formation by lipids is dominated by subtle intramolecular forces. Furthermore, the iconographic or schematic representations of the past cannot provide a suitable starting point for subsequent higher-level molecular modeling of lipid/lipid, lipid/small molecule and lipid/protein interactions. Physical CPK models suffice for individual molecules but are too cumbersome for larger structures consisting of lipid arrays (i.e. monolayers and bilayers). In this abstract we describe our strategy for the depiction of lipids in general, some of the problems unique to these systems, and several applications of our methodology.

A database composed of the 14 lipid crystal structures available in the literature has been established. The database is maintained in the PDB format and can be used with a variety of existing tools (MIDAS, FRODO, MOGLI, etc). Modifications were made to the acyl chains when needed to conform to standard hydrocarbon geometry. Novel lipids are constructed by adding or deleting library fragments. Monolayers and bilayers were constructed by propagating the lipid structures in an x-y plane using symmetry operations based on the crystallographic data.

Visualization of lipid superstructures (monolayers and bilayers) is often more difficult than viewing protein models because of the greater packing density and repetitive features of lipid structures. Selective use of color, van der Waal's, and Connolly surfaces was required to differentiate one lipid molecule from another and to examine their interactions. Stereo viewing was not always useful with lipid aggregates because of the planar nature and low depth of field of a monolayer.

A common problem with both protein and lipid depiction is the requirement for hard-copy output for archival and publishing purposes. Color print technology is expensive at best and is often not available. Strictly black-and-white pictures, on the other hand, cannot convey enough information to be useful. Gray scaling is required for meaningful results. One can photograph a black-and-white picture from a color screen, but it is difficult to predict how color intensities will translate into gray scales. Therefore, it is better to photograph a gray-scale monitor. When a photograph is not required, or is not convenient, it is often possible to "print" a plain black-and-white picture from the display program to a PostScript file. PostScript is a page-description language for printers, and PostScript files can be directly modified by a programmer to include gray scaling or to selectively highlight lines by increasing their width. We have found the ability to post-process our images in PostScript extremely valuable in compensating for the lack of color (see Figure 1).

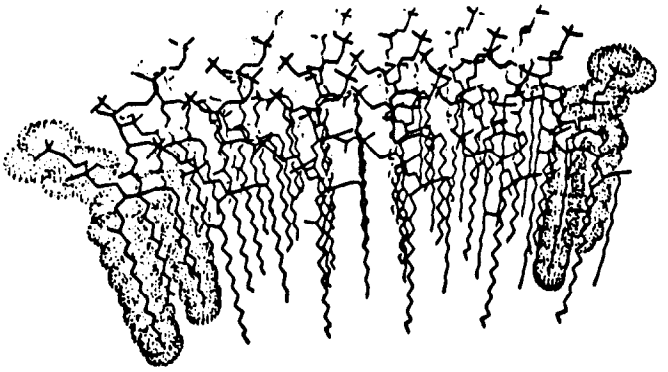

Figure 1. Perspective view of a DMPC monolayer

We have also examined the mechanics of computer graphics display. Our early efforts resulted in the development of SPOCK on a VAX 8650 using a Ram Tek display. SPOCK does not have all of the features of a program such as MIDAS, but it does display our lipid molecules in a timely and attractive manner and is easily altered for either color or gray-scale viewing. With this success, a new program is being implemented on more readily available personal computers. We chose the Apple Macintosh family as our hardware platform because of the high graphics standards, the unique interface, and the availability of a wide range in price/

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.

This work was supported by a contract from the Office of Naval Research.

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.

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.

This work was supported by NIH grants HL07722 (SJW) and HL22654 (RJ).