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Graphics for Macromolecular Crystallography and Electron Microscopy

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GRAPHICS FOR MACROMOLECULAR
CRYSTALLOGRAPHY AND ELECTRON MICROSCOPY

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Abstract

In this paper we provide an overview of macromolecular crystallography and electron microscopy methods followed by a survey of existing graphics packages used for molecular modeling. Then we present Tonitza, a graphics package for structural biology developed at Purdue University.

1 Introduction

To gain insight into biological processes scientists need to know where the atoms are located in biological molecules and how they change during biochemical reactions. In his delightful book on protein structure [1], Max Perutz explains how ‘protein crystallography has opened a submicroscopic world were potential drug targets can be mapped out in atomic detail’ and drugs can be made to measure for a variety of diseases from hypertension to HIV, how proteins recognize each other, and how they recognize genes.

Biological molecules differ in their complexity, small proteins have several thousands of atoms while large macromolecules like viruses could have millions of atoms. For example a protein like streptavidin has some 7,000 atoms while the monkey tumor virus has 900,000 non-hydrogen atoms [2]. Structure determination of small molecules has become a routine process nowadays. In contrast, the determination of macromolecular structures such as viruses remains a lengthy and difficult task. One of the main objectives of structure determination is the analysis of biological macromolecular structures, with emphasis on viruses, virus-receptor and virus-antibody complexes. Also of interest is the study of new antiviral compounds that interact with viral capsids, interfere with viral-receptor interaction, or inhibit uncoating. Current studies include rhinoviruses (HRV16), enteroviruses (Coxsackievirus B3), human and animal paraviruses, and the human immunodeficiency virus (HIV).

Structural biology uses nuclear magnetic resonance (NMR), x-ray crystallography, and electron microscopy (EM) methods to gather information about the 3D atomic structure of large macromolecules like proteins and viruses. Then, intricate computations, often involving parallel computers [3], are used to refine the experimental data and produce high resolution electron density maps in the case of x-ray crystallography, or to reconstruct the 3D structure in the case of electron microscopy. Finally, in the model building phase, high resolution electron density maps, information gathered through EM studies, and chemical information allow the structural biologist to determine the 3D atomic structure. According to Hendrickson [2], 481 new atomic level structures were determined in 1991, 352 by x-ray crystallography, 100 by NMR spectroscopy, and 1 each by fiber diffraction and electron microscopy. NMR methods can be used to obtain 3D models of small proteins but cannot be used to obtain detailed information about the arrangement of atoms in large macromolecules. X-ray crystallography is the only method to obtain secondary and tertiary structures and processing of electron microscope images is crucial for the analysis of high resolution structures of biological molecules.

In general, simulations and scientific experiments produce information hard to comprehend, to interpret and to debug. Visualization helps convey this information to the scientist in a form which can be better exploited by the human creative and analytic capabilities. It also enhances the possibility of acquiring new knowledge and understanding by exploring the data space. This is precisely the role of graphics in macromolecular modeling. Graphics plays an important role in the three stages of the structure determination: data collection, data analysis, and model building. Computer graphics is used to display and com-
pare 3-D models of proteins, and to fit structural models to 3-D maps. A more ambitious goal is to predict structure and function and to design useful proteins. The main distinction between graphics for proteins and large macromolecules is the sheer volume of data to be manipulated. A high resolution electron density map of a large virus may contain 500 x 500 x 500 grid points. Any real time transformation (rotation, translation) of such a volume requires efficient computational algorithms and powerful graphics engines.

2 Data Visualization for 3-D Atomic Structure Determination

In this section we present an overview of macromolecular crystallography and electron microscopy methods, followed by a survey of existing software packages for molecular modeling with emphasis on computer graphics and data visualization.

Macromolecular Crystallography.

In 1912 Max von Laue discovered that x-rays are diffracted by crystals and one year later W. L. Bragg founded x-ray analysis [1]. In his Nobel, lecture Bragg explains the foundation of x-ray analysis: "the rays are diffracted by the electrons grouped around the center of each atom. In some directions the atoms conspire to give a strong scattered beam, in others their effects almost annul each other by interference. The exact arrangement of atoms is to be deduced by comparing the strength of the reflections from different faces and different orders."

Since mid 50's x-ray crystallography had a profound impact upon structural biology. The discovery of the double helical structure of the DNA by James Watson and Francis Crick was influenced by the knowledge of the DNA diffraction patterns obtained by Rosalind Franklin and Maurice Wilkins. Max Perutz's studies of the hemoglobin, as well as Michael Rossmann's discovery of the atomic structure of rhinovirus 14 used x-ray diffraction methods. At the present time the impact of x-ray crystallography upon structural biology is accelerating, increasingly more complex macromolecules and biological assemblies are now investigated using new sources of intense x-radiation and new CCD-detectors.

X-ray diffraction provides detailed information about the 3D atomic structure of biological molecules. The wavelength of the x-rays is on the order of interatomic distances and the resulting images can be used to determine the positions of the atoms. Diffraction from crystals of biological materials concentrate the scattering from individual molecules into reflections. If the structure factors \( F(h) \) for all reflections with Miller indices \( h = (h, k, l) \) are known, and if \( r_j = (x_j, y_j, z_j) \) measured in fractions of unit-cell lengths, then the electron density distribution is given by

\[
\rho(r) = \frac{1}{V} \sum_{h} F(h) \exp(-2\pi i h \cdot r)
\]

Macromolecular crystallography requires a sequence of experimental and analytical steps namely crystallization, diffraction measurements, phase evaluation, density map interpretation, and model refinement [2]. Here we discuss only the evaluation of phases, and the model building.

Phase evaluation. The x-rays scattered by biological molecules infer both constructively and destructively, producing a diffraction pattern which can be recorded on a photographic emulsion or using a CCD detector. Structure determination involves measuring the structure factors at many or all points of the diffraction pattern. Macromolecules may produce several million reflections while small proteins typically produce a several tens of thousands reflections. Each structure factor is described by two quantities: an amplitude, which is the strength of interference at a particular point (it is proportional to the square root of the intensity in the recorded pattern), and a phase, which is the relative time of arrival of the scattered radiation at a particular point (e.g. photographic film).

The information about the phases is lost when the diffraction pattern is recorded. Phases cannot, therefore, be measured directly from x-ray diffraction images. Several techniques, including the heavy atom, isomorphous replacement, and molecular replacement methods were devised to solve the phase problem.

Now we describe briefly the molecular replacement method introduced by Michael G. Rossmann and Michael Blow in 1963. In this method one starts with a low resolution model of a virus (e.g. hollow spheres or possibly a related virus with a known structure). This approximate initial solution is then refined taking into account the known symmetry of the virus. Two types of symmetry are of interest for structure determination by molecular replacement. The crystallographic symmetry property implies that an operator applies throughout the whole, infinite crystal. The crystallographic asymmetric unit is the smallest unit from which the crystal can be generated by symmetry operations of its group. The noncrystallographic symmetry is related only to a localized volume within the crystal. The molecular replacement method uti-
izes the similarity (or identity) of structure in different parts of the crystallographic asymmetric unit, caused by the repetition of the same subunit structure in the formation of a whole molecule. It may also use the relationship between different crystal forms of the same or similar molecules. The method consists of three steps. (a) The rotation problem: determine the relative orientation of independent molecules (or subunits of molecules), within one crystal lattice or between different crystal forms. (b) The translation problem: translation of subunits must be determined with respect to the designated crystallographic symmetry elements. (c) The phase problem: determine the phases of the structure factors by solving a set of equations which represent the condition that the electron density distribution within the volume of the unit cell is identical within all subunits related by crystallographic and non-crystallographic symmetry, and it is constant outside these volumes. Solving these equations for the unknown phases is an iterative process, also known as phase refinement and extension. The basic computational procedure for phase refinement and extension is molecular averaging and solvent flattening.

Graphics packages like Tonitza, to be described in the next section, can be used to construct a low resolution model of the virus and to display electron density maps in different stages of phase refinement and extension.

Density map interpretation and model building. The atomic model of a macromolecule can be build using high resolution, 2-3 Å, electron density maps. If phases are accurate, a polypeptide chain can be traced at about 3.5 Å, and individual atoms are isolated at about 1.2 Å. Graphics tools like O (see Table 1) designed by Alwyn Jones are invaluable in this step.

Electron Microscopy.

In correlation with x-ray diffraction, biochemical, genetic, immunological, and model building studies, image processing of electron micrographs is a powerful tool for investigating the basis of molecular events in living systems. In general, this method gives structural information at very low resolution, usually high enough to reveal the shape of individual subunits, but rarely enough to determine the path of the polypeptide chain within a protein molecule. Efforts are currently being made to bridge the resolution gap between x-ray diffraction and electron microscopy methods by increasing the resolution that can be obtained by electron microscopy methods.

Since 1963 several techniques have been developed and are now routinely used in image processing. These techniques are classified as either real-space (direct) or reciprocal-space (Fourier), depending on whether the micrograph or electron image itself is processed or an intermediate step involving the Fourier transformation of the micrograph or image is required for processing. The first category includes powerful techniques, such as correlation methods, which offer distinct advantages over Fourier techniques for examining certain kinds of specimens (e.g. disordered or non-periodic specimens). Fourier techniques are most effective for the study of crystalline and para-crystalline specimens, although, in principle, they can be applied to the study of any specimen. A wide range of biological specimens such as viruses, muscle proteins, membranes, ribosomes, microtubules, enzymes, etc., have been isolated as regular arrays and have been successfully examined by Fourier-based image processing techniques. Our brief presentation of image processing of electron micrographs concentrates on these techniques.

Fourier processing may be performed optically, on an optical bench, or digitally, on a computer. Digital methods offer distinct advantages because they are quantitative and adaptable. The main disadvantage of such methods comes from the necessity for discrete sampling of data, which leads to aliasing artifacts which can be reduced, but never completely eliminated. A typical digital processing procedure includes several steps [4]. (a) Image selection: after an initial screening by eye (to discard obvious bad images), several micrographs (50 or more) are examined by optical diffraction to select a small subset of the best images in terms of optical quality and specimen preservation (note that optical diffraction is generally unsuitable for selecting images of particles for rotational filtering; the rotational power spectrum must be computed instead). These images will be further examined by digital processing methods. (b) Densitometry: the micrograph is digitized on a scanning densitometer, a computer-controlled device which converts optical densities on the photographic emulsion to a digital image. To minimize aliasing artifacts, images should be scanned at raster settings corresponding to one third or less of the expected pixel resolution in the image. (c) Boxing and floating the digital image: the entire digital image or selected, boxed, areas may be used for subsequent processing steps. Boxing can be conveniently performed interactively, on the image displayed on a graphics monitor. Regions outside the area of interest are zeroed and the numerical image is floated by subtracting the average image intensity around the perimeter of the boxed area from all image.
intensities within the region of interest. Floating suppresses intense diffraction spots generated by the edges of the boxed area. (d) Fourier transformation: the Fourier transform of the numerical image is computed using Fast-Fourier methods. The result of the Fourier transform is an array of structure factors amplitudes and phases. (e) Indexing of two-dimensional lattices: for well-ordered, 2D crystalline biological specimens, the diffraction pattern consists of a series of discrete spots (Bragg reflections) that lie on a reciprocal lattice. Such patterns are usually fairly easy to index. For multilayered or two-sided structures, indexing can be quite tricky, so care must be taken before proceeding to the next step. (f) 2D filtering / 3D reconstruction: once a decision has been made as to what is signal and what is noise, the computed Fourier transform is masked, that is, the amplitudes are zeroed everywhere except at the reciprocal lattice points (i.e. holes centered at the points of an ideal reciprocal lattice). The modified, filtered, diffraction pattern is mathematically back-transformed to reconstruct an averaged image. If the 3D structure of a particle is to be reconstructed, the phases and amplitudes of the structure factors must be determined in three dimensions to fill and generate a complete, 3D Fourier transform. This is accomplished by combining structure factor data from several diffraction patterns of independent views of the crystalline specimen. This method of reconstruction is based on the projection theorem, which states that the two-dimensional Fourier transform of a planar projection of a 3D object is identical with the central section of the three-dimensional transform normal to the viewing direction. The different views may be collected either from a single particle, by using a tilting stage in the microscope, or from a number of particles in different, but identifiable orientations. In general, it is desirable to combine data from different particles so that imperfections can be averaged out.

Survey of software packages for molecular modeling.

A variety of generic as well as specialized software packages for molecular modeling are available today. Generic visualization packages like AVS (Application Visualization System)\(^1\) and IRIS Explorer\(^2\), are suitable for physical sciences and engineering and can be used for molecular modeling. Such packages have a modular design and application specific software can be generated by combining different modules into flow networks. Flow networks are then executed as scripts. A variety of modules for analysis of three dimensional data sets and for importing data into AVS are described in [5].

According to [6], the specialized software packages used for molecular modeling can be classified into several categories, based on their functionality (see also Table 1): (a) Structure databases manipulation packages are designed to either search and access the major chemical structure databases (the Brookhaven Protein Databank, the Cambridge Structural Database, the Drug Information System 3D Database) or create, maintain and search local chemical structure databases. PDBtool, for example, is a Protein Data Bank browser that allows querying and verification of macromolecular structure data. It includes a 3D viewer and renderer, and a variety of graphical and textual structure verification tools. It runs on SGI and SUN platforms. (b) Model building packages allow the user to fit structures into electron density maps, and/or compute and display all the molecular graphs that correspond to a given chemical formula, prescribed and forbidden substructures, optional conditions (intervals for possible ring sizes, hybridization of carbon atoms), stereo isomers, etc. An example is O, a general purpose macromolecular modeling environment. The program supports model building and display of macromolecules. Due to specific features such as building and rebuilding of models into electron density maps, and/or compute and display all the molecular graphs that correspond to a given chemical formula, prescribed and forbidden substructures, optional conditions. (c) Structure drawing/visualization packages have revolutionized the publication and presentation of chemistry information. In addition, they can be used to visualize and manipulate structures in various representations and/or to input structures to some molecular modeling programs. RasMol, for example, is a program for the visualization of proteins, nucleic acids and small molecules that it is aimed at displaying, teaching, and generation of publication quality images. The program runs on X-compatible Unix computers, Macintosh, PowerMac, and PCs running Microsoft Windows. Another example for this category is VMD, designed for the visualization of biological systems such as proteins, nucleic acids, and

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\(^1\) AVS is a trademark of Advanced Visual Simulations

\(^2\) Explorer is a trademark of Silicon Graphics, Inc.
l lipid bilayer assemblies. It provides a wide variety of methods for rendering and coloring a molecule: simple points and lines, CPK spheres and cylinders, licorice bonds, backbone tubes and ribbons, and others. It runs on SGI workstations. (d) Molecular mechanics packages include features such as energy minimization (used to optimize initial geometries or to repair poor geometries), template forcing (i.e. evaluating whether a molecule can adopt a template conformation consistent with a given model), and torsion forcing (to obtain Ramachandran-type contour plots for proteins or RIS statistical weights for polymers). (e) Molecular dynamics packages attempt to solve Newton’s equation of motion: $\frac{d^2x_i}{dt^2}(t) = -\nabla_x E_{\text{total}}$, (where $i$ runs through all free atoms and the gradient $\nabla_x E_{\text{total}}$ is derived from the energy function), produce molecular dynamics simulations, store them in trajectory files, etc. (f) Conformational searching packages include algorithms for efficient searching of conformational space defined in terms of torsion angles and multi-molecular translation/rotation, some of which pertain well to parallelization. (g) Distance geometry packages which facilitate molecular structure determination using metric matrix distance geometry. (h) Quantum chemistry packages include various applications of density functional theory. (i) NMR-based structure determination packages allow determination and refinement of structures based on interproton distance estimates, coupling constants measurements, and other information, such as known hydrogen-bonding patterns.

There are many more programs for molecular modeling than those covered by our survey which is focused only on programs widely used by the structural biology community.

### 3 Tonitza

Generic graphics packages for data exploration like IRIS Explorer and AVS offer distinct advantages to scientists and engineers in need of data visualization tools. Yet, when data visualization and very specific computations are tightly coupled together, the use of generic graphics packages looses some of its appeal. For example, it is sometimes necessary to correlate two EM reconstructions of a spherical virus [4]. Such a correlation procedure may include several steps: (a) compute and plot the average electron density as a function of radius and determine spherical annuli within which the correlation is to be performed (one is not interested in correlating densities within the RNA core), (b) determine an optimal magnification factor (as the one that maximizes the correlation coefficient) to scale a map relative to the other, and (c) examine the variation of the correlation coefficient as a function of the radius, using the previously computed optimum magnification factor. For this sequence of computation and visualization steps the integration of data transformations and data visualization into a specialized tool provides distinct advantages: there is a uniform GUI, the efficiency is increased because data manipulation is done in main memory, there is no need for I/O operations.

Considerations like the ones described above provided the motivation for Tonitza, an interactive package for the visualization, analysis, and data manipulation developed in collaboration by scientists in the Computer Sciences Department and Structural Biology Group at Purdue University. This collaboration allows the use of high performance computing [3] and advanced graphics systems for structural biology.

Tonitza is aimed at the field of structural biology, but it can be used to explore any vector field data. It allows processing and display of multivariate gridded data in a variety of representations and it is available on graphics workstations running OpenGL 3.

Tonitza consists of a graphical user interface, and I/O, computation, and visualization modules. On-line context-sensitive help uses hypertext viewers (Mosaic 4 or Netscape 5).

The **user interface** is based on X-Windows 6 and Motif 7. The interface has been designed in a *direct manipulation style* [7] which has proven quite powerful and easy to learn. It consists of a large main window and various smaller auxiliary windows. The main window appears on the screen at the beginning of a Tonitza session, and it remains on the screen for the entire duration of the session. The auxiliary windows are invoked by the application as needed and they are dismissed when their presence is no longer necessary. The main window consists of a menu bar, a drawing area, and an optional command area. The contents of these components is context-sensitive, that is, it changes depending on the function currently being executed. The auxiliary windows are dedicated to specialized dialogs, editors, and display areas. Examples include dialogs for input parameter specifica-

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3 OpenGL is a trademark of Silicon Graphics, Inc.
4 Mosaic is a trademark of University of Illinois
5 Netscape is a trademark of Netscape Communications Corporation
6 the X Window System is a trademark of The Massachusetts Institute of Technology
7 Motif is a trademark of the Open Software Foundation
tion, volume selection, color and material editors, etc. Color plate 1 shows a snapshot of the screen during a Tonitza session. The system can be entirely controlled with the mouse and also provides support for diags, when available.

The I/O module controls the various conversions between internal and external data formats. It is responsible for the automatic recognition of input data formats and handling of I/O errors. Tonitza accepts as input gridded data produced by scientific and engineering simulation software. It provides support for a variety of data formats used in x-ray crystallography and electron microscopy. In addition, it accepts as input image and movie files. The general structure of an input file in Tonitza consists of a header followed by data. The format of the data is automatically detected using the information provided in the file header.

The computation module provides support for data analysis. It can be used independently or in conjunction with the visualization module to obtain information about the contents of the data. The functions included in this module are briefly summarized here. (a) Data rotation allows computation of rotated maps by resampling from the original data. This feature is useful, for example, in the case of 3D electron density maps representing virus particles reconstructed by electron microscopy methods. Such particles usually exhibit high symmetry and it is important to be able to select subvolumes for visualization based on the symmetry elements. (b) Computing the correlation between two data sets allows one to compare the two sets, and to adjust one relative to the other, by resampling based on the variation of a scaling parameter. One example when such a feature proves useful is the 3D reconstruction of a virus from independent 2D views obtained by electron microscopy methods [8]. The input data sets in this case may represent two different views of the same particle in different orientations. The maximization of the correlation between them may be used to determine the orientation of one relative to the other. The quality of the reconstruction may be significantly improved when corrections are made to adjust for slight magnification variations in one of the two data sets. (c) Composite map computation allows one to compute a linear combination of two data sets. This can be subsequently displayed in various representations and may give some insight about the relationship between the two maps. A special case is the difference of two data sets. Color plate 2(a) shows a shaded surface representation of the Ross-River virus [8] in its native form (blue) together with Fab antibody fragments attached to it (yellow). The yellow surface corresponds to a map computed as the difference between data representing the complex form of the virus attached with antibodies and the native virus structure. Plate 2(b) shows a close-up view of one of the spikes shown in plate 2(a). (d) The peak location feature allows determination of regions of maxima or minima in a data file, based on gradient evaluation. This can be particularly useful for detecting peaks of electron density corresponding to positions of atoms or groups of atoms in Structural Biology files. (e) Graphs and histograms can be produced relative to any data file, customized, and displayed interactively.

The visualization module provides support for 2D and 3D visualization of data. It allows interactive zooming, rotation, translation of the objects displayed, image recording, animation, and printing. Multiple data sets and/or multiple scalar properties can be displayed simultaneously. The following representations are available. (a) Planar sections allow display of data in sections parallel to the planes of coordinates. Sections of different orientations can be displayed simultaneously, at different positions within the data volume. A set of slider bars allows the user to sweep the entire volume with such sections. The data in planes may be represented as a set of contour lines at user-defined levels, as a continuous scale map, by associating data values with colors, or using both representations superimposed. The colors can be interactively edited using a colormap editor. Stacks of contours plots can be created for a set of sections, to allow interpretation from a 3D perspective. Color plate 3(a) shows three orthogonal equatorial sections through a Ross River virus particle with density contoured a three levels: low (blue), medium (green), and high (red). A stack of contours for a Coxsackievirus B3 particle is shown Plate 3(b). Density is contoured at medium (purple) and high (orange) levels. Color plate 4(a) contains a continuous scale representation of an equatorial cross-section of a Ross-River virus reconstruction along a 2-fold axis, with densities mapped from dark (solvent and lipid) to light colors (protein). Plate 4(b) shows three cross-sections through a Coxsackievirus B3 asymmetric unit mask map. Particles are shown with different colors. Note that the data volume in this case is non-orthogonal. (b) Spherical sections can be displayed for cubic data sets and correspond to values obtained by interpolation of the data on spheres centered at the center of the cube. They are represented as continuous scale maps and may be displayed in conjunction with planar sections. The user is allowed to sweep the volume with spherical sections.
by modifying the radius of the sphere. Color plate 5 shows two-, three-, and five-fold views of spherical density distributions of a Ross-River reconstruction at specific radiae. (c) Isosurfaces [10] can be computed and displayed as wireframe or shaded for the entire volume and/or for subvolumes. A material editor allows interactive specification of the lighting and material parameters. Plates 1 and 6 show isosurfaces for data related to a Ross-River virus reconstruction. Plate 6 shows a wireframe molecular envelope of a virus spike.

A few lessons learned during the design and the implementation of Tonitza are discussed next. Tonitza started out as a general interactive scientific visualization tool, running on virtually any workstation. It included 2D and 3D graphics, such as contouring and continuous scale maps, isosurfaces, histograms, and so on. The real challenge came when we attempted to visualize high resolution maps with 20 to 250 million grid points. Using memory mapping of large data files proved to be a significant step to improve the I/O performance. Then there was the need for interactive manipulation of the objects displayed, for viewing selected volumes from arbitrary positions, for animating individual frames [4]. Efficient algorithms were not enough to achieve the real-time effect, given the large data sets. This motivated the first major change in the design of Tonitza: portability was traded for interactivity and we redesigned the package to take full advantage of graphics hardware by using Open GL. Finally, generality was traded for usefulness and ease of use in molecular modeling, which led us to the current version of Tonitza. A considerable fraction of the effort to design and implement this package as it is now has gone into the GUI, the I/O, and the computation modules which are very specific for molecular modeling.

4 Conclusions

Paraphrasing Hamming's renown quote 'The purpose of computing is insight, not numbers' we think that the purpose of graphics should be insight rather than just pretty pictures. We believe that only interdisciplinary collaborations can create the graphics tools that are versatile enough, yet easy to use, general enough yet embed specialized algorithms for a specific application, to be truly useful to a computational scientist.

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References


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Table 1. Selected software packages used in Molecular Modeling.
Color Plates

Plate 1. A snapshot of the screen during a Tonitza session. The main window, a histogram, the colormap editor and the material editor are shown.

Plate 2(a). Shaded isosurface for Ross River reconstruction data. The blue surface represents the native virus structure. The yellow surface corresponds to the antibody fragments attached to it.

Plate 2(b). Close-up view of one of the spikes shown in Plate 2(a).

Plate 3(a). Orthogonal equatorial sections through a Ross River virus with density contoured at three levels: low (blue), medium (green), and high (red).

Plate 3(b). Stack of contours for a Coxsackievirus B3 particle. Density is contoured at medium (purple) and high (orange) levels.

Plate 4(a). Equatorial cross-section through a Ross-River virus reconstruction. Density is shown in a continuous scale representation, varying from dark (solvent and lipid) to light (protein).

Plate 4(b). Three cross-sections through a Coxsackievirus B3 asymmetric unit mask map.

Plate 5. Three spherical sections through a Ross-River particle, viewed along a two-, three-, and five-fold axis, respectively.

Plate 6. Ross-River spike shown in a wireframe representation.
PLATE 3(a)