R. Stroud. A wholly digital version of the device, which allowed coordinates to be both read out and entered through a control console (e.g., for model building from coordinates), was constructed at the University of California at San Diego, and subsequently at the University of Arizona.[4] The motorized versions of this superposition device produce coordinates of comparable precision to the optical and acoustic devices described previously (at least ±1 mm), and similarly allow for very rapid measurements (one atomic position per 30 sec). However, construction of the former device is more complicated than either the AIMS (which can simply be rented) or acoustic coordinate measuring devices.

With the advent of computer graphics and associated software for both map fitting and coordinate readout (this volume [12]), it appears only a matter of time before graphics obviates either building physical models of proteins in optical comparators or devising means for measuring their coordinates. Nevertheless, the techniques described here will undoubtedly continue to prove effective aids in determining crystal structures for some time to come. In addition, many modeling studies of protein conformation and interaction continue to be most readily investigated in their initial stages by the construction of physical models. Subsequent model development and analysis by computer necessitates having accurate coordinates, so that the technical feasibility of such studies depends on having an accurate and easy way of measuring model coordinates.

[4] F. R. Salemme and D. G. Fehr, *J. Mol. Biol.* **70,** 697 (1972).

[12] Interactive Computer Graphics: FRODO

By T. Alwyn Jones

Introduction

Computer graphics provides an elegant method of controlling the protein crystallographer's interaction with his model. The graphics display allows the model to "show" the crystallographer a part of its electron density such that he can decide how a molecular fragment best fits. Once the crystallographer has made his decision, the computer merely does the bookkeeping and minor improvements. The aim of the molecular fitting program, therefore, is to create the necessary environment to allow the crystallographer to decide what atoms he wants in what piece of density.

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A great deal of effort has been spent to develop the necessary control software and, in some cases, to build the hardware. At present there are approximately 10 density-fitting program systems in active use. Most laboratories are not able to build the necessary hardware, but high-performance equipment is available from a number of vendors. The program FRODO[1,2,3,4] is presently implemented on the Vector General 3400 (with DEC, VAX, or PDP-11 computers), MMS-X, Evans & Sutherland PS 2 (PDP-11), MPS (VAX or PDP-11), and PS300.

The man–machine interface in FRODO varies with the available equipment. All systems use a data tablet to pick menu items and to identify atoms shown on the screen. The VG3400 and Evans & Sutherland PS300 systems use analog-to-digital converters to define the view direction, and other picture-related functions such as clipping, zooming, and intensities. Commands that invoke dihedral angle rotations, single atom, and fragment shifts are also coupled to the A-Ds. On the Evans & Sutherland PS2 and MPS equipment, pseudo A-Ds are drawn on the screen and can be activated by the data tablet pen. The MMS-X version uses the standard user control panel.

Program Flow

The following is strictly applicable to only the VG3400 version of FRODO. Other versions have some minor differences.

After starting the program the user must specify a control data set. This data set contains all of the important parameters needed by FRODO. It includes, for example, the data set name for the user's coordinate file and the regularization zone. It is a source file and can be edited (at one's own risk). The user then gets to the CHAT interface, which has a large number of menu items activated from a terminal keyboard. Every exit from CHAT causes an update of the control file. Control normally then passes to the display loop.

To make the system easier to use an effort has been made to keep the display menu options to a minimum, and to a single "page." The data tablet pen position is marked on the screen by a cursor, and a menu item is activated by moving the pen so that the cursor is positioned over the

[1] T. A. Jones, *J. Appl. Crystallogr.* **11**, 268 (1978).
[2] T. A. Jones, *in* "Computational Crystallography" (D. Sayre, ed.), p. 303. Oxford Univ. Press, London and New York, 1982.
[3] B. L. Bush, *in* "Computers & Chemistry," vol. 8, p. 1. Pergamon, Oxford, 1984.
[4] J. W. Pflugrath, M. A. Saper, and F. A. Quicho, *in* "Methods and Applications in Crystallographic Computing" (S. Hall and T. Ashida, eds.), p. 404. Oxford Univ. Press, London and New York, 1984.

item and then pushing the pen into contact with the tablet. In principle, any number of commands can be activated and each is polled in turn. This means, for example, that an atom can be moved and have its contacts updated at the same time. In practice some care has to be taken, since there are a number of mixed options which may be disagreeable to the user. For example, if a group of atoms is being moved and the user activates the SAVE command, then the current fragment coordinates will get written to disk. The active commands can be seen at a glance because each has a star drawn next to it. To exit from the display loop, the user must pick a suitable menu item to either terminate the session or enter one of the utilities. Reentering the display loop causes a new loading of coordinate information from the disk.

The vectors drawn on the display are constructed from three data sets and normally show some of the atoms in the coordinate data set superimposed on a "chicken wire" representation of some sort of electron density.

The coordinate data set contains more than just atomic coordinates. The atoms are grouped together to form a residue. There are residue records to describe the type of residue (e.g., PHE, MPD), the name of the residue (e.g., A2, 10G), the position in the data set of the atom records for this residue, the center of gravity, and the radius of the residue. The residues are grouped together to form a sequence. As far as the display loop is concerned, the sequence is only important when defining viewing zones, i.e., it does not necessarily force any chemical connectivity between residues (although it may exist). The data set can also contain extra information such as lattice type (P, I, R, F, A, B, C), unit cell constants, and crystal symmetry information. This information is optional but may be required for certain commands. The user must decide at the CHAT interface how he wishes to access this data set. There are three possibilities: (1) Define the start and end residues of a zone. The program then displays all the atoms in the residues within the zone as defined by the sequence. (2) Define a point in space and a radius, and then display all of the atoms in the data set which are within the volume. (3) Define a mixture of 10 display zones plus a sphere.

In the sphere mode the user can choose an option to display any symmetry-related atoms which may fall within the volume. Both the sphere and symmetry options make use of the residue center of gravity information to decide what appears in the volume. Another option allows one to define by name which atoms are to be displayed; e.g., one can define just C_α to see the fold of a protein.

After picking which atoms are to be displayed, one must decide on a connectivity, i.e., which atoms should have a line drawn between them.

The usual connectivity scheme in FRODO is based on distance criteria so that if atoms are closer than a certain distance they are joined. In sphere and mix modes all atoms are tested together. In zone mode the connectivity is built up a residue at a time, and a specific link is made between residues. An atom with no connections appears on the display as a three-dimensional cross. If one is displaying just C_α atoms, for example, there is an option to connect the first atom to the second, the second to the third, etc. The initial connectivity does not necessarily represent chemically correct bonds. It is simply there as an initial framework for the crystallographer to decide how he is to change his structure to fit the density.

The second data set consists of linked vectors. It may represent density contoured at a number of different levels, or skeletonized electron density, or guide points, or a vectorized library of molecular data sets. The vectors are arranged in three-dimensional volume elements and in what are called contour commands (C-COMs). If the data set is a vectorized map, each C-COM corresponds to a contour level. In the vectorized molecular library each molecule is equivalent to a C-COM. The user can decide in the CHAT interface which (if any) C-COMs are to be chosen.

The third data set is an electron density map. It is also arranged in three-dimensional volume elements with each density value packed into one byte. This data set is much smaller than an equivalent vectorized map, and has the advantage that the contour levels can be changed at any time. It is, however, slower to work with than the vectorized data set. The map can also be used to automatically fit molecular fragments to the density. One often uses both a map and vector data set where the contour level has been chosen after a brief inspection of the map.

Building an Initial Model

The crystallographer usually knows the rough fold of his molecule before starting work on the display. This is best determined by extensive study of minimaps plotted on stacked plastic sheets. The structure solution of retinol binding protein by Newcomer *et al.*[5] is one of the few exceptions to this rule. There are then four different ways of building the model on the display.

The first method is a relic of working with Kendrew wire models in a Richards box. FRODO has extensive model-making features to produce coordinates from a given sequence which have standard bond lengths and angles and preferred torsion angles. A zone of residues can be made and

[5] M. E. Newcomer, T. A. Jones, J. Åqvist, J. Sundelin, U. Eriksson, L. Rask, and P. A. Peterson, *The EMBO J.* **3:7,** 1451 (1984).

moved to the place in the map where one wishes to begin building (not necessarily the N terminus). The fragment can be translated and rotated by the display menu command FBRT so that the first residue sits close to its density. Up to six consecutive dihedral angles can be varied at a time, and by repeated FBRTs and TORs the fragment may be made to fit the density. These coordinates are written to disk when the user is satisfied by using the SAVE command. The model-making option can be used to extend the zone of residues and an attempt made to fit these. However, it rapidly gets more difficult to do this while maintaining the constrained, rigid geometry.

In method two, by judicious choice of display zones, the user fits a few residues as described above but introduces a discontinuity in a peptide linkage by separately fitting the next zone of residues.

In method three the user introduces discontinuities directly on the screen. The screen connectivity is used to decide which atoms are affected by dihedral rotations and by fragment rotation/translation. Suppose a zone of residues fits the density but the user sees that a side chain in the center of the zone would fit much better if he could change ϕ (around the N–C_α bond) for the residue. If one changes this angle directly, all the other atoms to the end of the zone would be moved out of density. This is prevented by breaking the C_α–C bond of the residue using the menu BOBR command and then rotating around the N–C_α bond. This, of course, distorts the bond angles around the C_α atom. More commonly, the user disconnects the side chain from the main chain and moves the small fragment straight into the desired density. This is illustrated in Fig. 1a (which is drawn on a plotter directly from the picture on our display), where a growing chain has clear density for the phenylalanine side chain. The C_α–C_β bond is broken and the ring moved into the density using FBRT (Fig. 1b). The same coordinates are shown from a different view in Fig. 1c, where one can clearly see density for the carbonyl oxygen. In Fig. 1d the oxygen has been moved into this density and we now have a very distorted residue.

It should be clear that to simplify the fitting process we must introduce errors in bond lengths, angles, and fixed torsion angles. These can be removed by model regularization. To prevent the buildup of errors in particular variables, the regularization should have no built-in rigid constraints (such as fixed bond lengths, for example). FRODO uses the method described by Hermans and McQueen[6], which they call the method of local change. In this method each atom is shifted to minimize a weighted sum of terms representing the shift from its starting positions,

[6] J. Hermans and J. E. McQueen, *Acta. Crystallogr., Sect. A.* **30,** 730 (1974).

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