CRYStallize: A crystallographic symmetry display and handling subpackage in TOM/FRODO

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We have implemented in TOM/FRODO a protein crystallographic symmetry display and handling package, called CRYStallize. This package is designed as an aid in solving protein structures by molecular replacement methods. It allows the rotation/translation solutions provided by molecular replacement programs to be checked in a fast and easy way. Using CRYStallize, approximate solutions can also be improved by manual modifications. Symmetry-related objects, represented as surfaces, can be generated and handled in the same way as the reference molecules, thus permitting an efficient analysis of crystal packing and site accessibility. This program is available in the TOM/FRODO software release, which runs on the Silicon-Graphics workstations.

Keywords: crystallographic symmetry, molecular replacement, crystal packing

In the course of the past three years, we have continuously developed our Silicon-Graphics version of FRODO,[1] which is called TOM/FRODO.[2] In addition to the classical FRODO modelling options and map handling, we have implemented other subpackages developed more recently by Jones and Thirup: the skeletal map representation BONES, and the diagonal search algorithm.[3] Our molecular energy-fitting program was also installed in TOM/FRODO.[4] Moreover, the program has been extensively modified throughout, especially in the coloring modes and in the molecular background representation part MOL. Connolly molecular surfaces[5] as well as the derived spline-lines surfaces[6] can be represented. The program has been distributed to more than 60 sites.

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Received 22 November 1989; accepted 12 December 1989

Due to the increasing impact of the molecular replacement methods[7] and the increasing interest in crystal growth understanding and crystal packing analysis,[8] we have written a new subpackage in TOM/FRODO, called CRYStallize, which has the following capabilities:

- Symmetry-related molecule generation
- Symmetry-related background object generation (surfaces etc.)
- Symmetry-related object identification and edition
- Symmetry-correlated movements of symmetry-related objects
- Fast color-coded representation of packing contacts

In this paper, we will describe the subpackage options and some applications from current work in the laboratory.

HARDWARE REQUIREMENTS

The program TOM/FRODO runs on all versions of the Silicon-Graphics display stations that have sufficient hardware for 3D work. This implies that at least 24 color planes should be available. The displayed objects can be handled by either a dial-and-button box or pseudo-dials. The dial-and-button box makes display work much easier, especially when using CRYStallize. The button box allows us to turn on and off any object quickly, thus permitting us to choose a desired orientation with a simplified figure, e.g., using a smaller number of vectors, and to recall the large objects for the final representation almost immediately. For some options, such as symmetry-related object identification and edition (see program description), the button box is mandatory.

PROGRAM DESCRIPTION

All versions of FRODO already have a symmetry generation option. This option allows for the representation of a limited number of symmetry generated atoms in the SPHERE display mode. However, this option does not fullfill the requirements for the studies mentioned above. Still, it is useful in current crystallographic work, when locally fitting a model into a map. Therefore, it has been kept in TOM/FRODO.

Crystallographic information storage

In FRODO, crystallographic information about cell-dimensions and symmetry cards are entered via SAM, and stored in a special entry of the DSN2 molecular coordinate file. This information is lost each time a new DSN2 is created, which can happen frequently during protein refinement. In case of high-symmetry space groups, entering the symmetry cards can be a unreliable and tedious task. Therefore, we have designed a small interface that, upon entering the space group name, generates the appropriate symmetry cards. Fifty-nine space groups among the 65 relevant to protein crystallography have been compiled. For instance, typing C2221 allows us to get the $C222_1$ space group symmetry cards. The initial interface has been kept to account for nonstandard space groups.

CRYStallize menu description

Subpackage CRYStallize is called from the main conversational routine, CHAT. Several options are available, and can be used alone or combined. In this menu, BOX refers to the crystallographic cell box, and CELL to the molecular content of the cell.

- ONE-BOX, 27-BOXES, NO-BOX, allow the user to display 1 or 27 boxes, or to cancel box representation, respectively.
- ONE-CELL, 27-CELLS, NO-CELL, allow the user to display 1 or 27 cells, or to cancel cell-content representation, respectively.
- NEIGHBOR allows the user to represent only those symmetry-related molecules that lie within a chosen radius from the center of mass of the origin molecule. NO-NEIG cancels it.
- NEIGH-RAD allows the user to change the radius value of the NEIGHBOR option. A first pass indicates the calculated default value, and the new value can then be chosen.
- CA-ATOMS, ALL-ATOMS allow the user to display either the Cα backbone or all the atoms.
- ZONE allows the user to select a zone of residues.
- STATUS gives the present option status of CRYStallize.
- GO returns to CHAT.

Color Plates 1, 2, and 5 present some examples of symmetry-generated proteins. Each symmetry card imposes a color for the molecule it generates; molecules translated by lattice symmetry have the same color. In the upper left part of the screen, symmetry operations are displayed with their corresponding colors.

Symmetry-related background object generation

Symmetry operations can be applied to background objects, i.e., nonpickable MOL objects. This is specially useful when applied to molecular surfaces[5] or to their derived spline surfaces[6] (Color Plates 1 and 2). Water channels can easily be identified as well as active site accessibility for diffusing ligands. In Color Plate 1, the original spline surfaces have been loaded via MOL; symmetry generation occurs when activating the display option LNKO (LiNK Object). This option allows us to link any MOL object to the symmetry and translation operations. By default, object colors are determined by the symmetry operations. However, MOL object number 6 has been saved for a specific application: In this case the same color is applied to all the different symmetry-equivalent molecules. This allows us, for example, to distinguish ligands or water molecules from the protein. Such a case is displayed in Color Plate 1, for a trypsin–inhibitor complex. Protein spline surfaces were colored according to their symmetry position, whereas the inhibitor spline surface was colored white for easy identification. In this option, use of the button box is crucial. The number of vectors can be very large (10^5–10^6), which means that a proper view should be chosen by removing the objects by clicking a button and recalling them afterward.

Symmetry-related objects identification and edition

It is often useful to examine contact areas between a few (2–5) symmetry/translation-related molecules. By switching on a button from the button box, 24 buttons of the box are reallocated, each of them permitting us to switch on or off a symmetry/translation-related object. All the different translation operations belonging to the symmetry operations are displayed on the upper part of the screen, with the symmetry color code (Color Plate 5). The translation operation is highlighted when the corresponding object is switched on (default).

Symmetry-correlated translations of symmetry-related objects

This option has been designed as an aid to improve the results of the translation function. When the rotation/translation display option FBRT is activated, symmetry-correlated translations of symmetry objects can be applied. Rotations are not allowed in this mode. They can, however, be applied on the reference molecule using FBRT and then visualized with CRYStallize. The new orientation can be saved.

Fast color-coded representation of packing contacts

This display option, called CSYM, is a fast and condensed way to represent *residue–residue* packing contacts with a blue to red color ramp. This technique uses the same approach as the one used to visualize the B factors in TOM. To take into account the differences in protein sizes and packing interactions, a minimum distance contact and a minimum number of contacts can be assigned in the CHAT option CSYM, to get a proper color range. A larger number of interactions makes the residue redder; a smaller number makes it bluer.

APPLICATIONS

Trypsin active-site accessibility

During a study of trypsin–inhibitor complexes, we have solved the structure of trypsin inhibited by a Markward[9]-type inhibitor.[10] The crystal form was $P\ 2_12_12_1$, with cell

dimensions a = 55.2 Å, b = 57.6 Å, c = 67.3 Å, which already has been described for a trypsin–bezamidin inhibitor.[11] The crystal packing examination indicates that the inhibitor is in close contact with a symmetry-related trypsin molecule (Color Plate 1). A new orthorhombic $P\ 2_12_12_1$ crystal form of a trypsin–benzamidin complex, different from the one previously reported,[11] has been solved recently in the laboratory.[12] Its cell dimensions are somewhat larger (a = 63.4 Å, b = 63.8 Å, c = 69.2 Å). As a result, we can easily see (Color Plate 2) that the active site is more exposed in this case than in the one of the former crystal forms (Color Plate 1). Color Plates 3 and 4 represent these facts in a very compact way.[12] The option CSYM has been applied in both cases. In the first case, the radius was 6 Å, and the redder color indicates the presence of 15 intermolecular contacts. The red residues are distributed over the protein surface, including the surroundings of the active site. In the second case, the radius was set to 10 Å, with a maximum contact count of 25, to get a full color range. This fact by itself indicates a looser[12] packing. The contacts are mainly found in the extremities of the molecule (as seen in Color Plate 4), and the active site is in the middle of a dark blue contact-free area.

Some different lectin packing

During resolution, by the molecular replacement method, of three different *Lathyrus ochrus* lectin–saccharide complexes, we made extensive use of CRYStallize to choose the difference solutions.[13] For example, in the case of the $C2$ crystal form (a = 78.2 Å, b = 76.0 Å, c = 104.1 Å, β = 92°), different solutions for different resolution ranges were examined.[14] The correct packing is displayed in Color Plate 5, in the direction of the diagonal between a and c. The (010) and (001) symmetries have been removed. In this direction, large water channels can be seen.

ACKNOWLEDGMENTS

J. P. Mornon and N. Collo'ch are gratefully acknowledged for making available to us their spline surface calculation program. M. Frey kindly provided us with his preliminary results on the other trypsin–benzamidin crystal form.

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