

The VMD-XPLOR Visualization Package for NMR Structure Refinement

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Received October 9, 2000; revised January 29, 2001; published online March 21, 2001

In this paper we present the VMD-XPLOR package combining the XPLOR refinement program and the VMD visualization program and including extensions for use in the determination of biomolecular structures from NMR data. The package allows one to pass structures to and to control VMD from the XPLOR scripting level. The VMD graphical interface has been customized for NMR structure refinement, including support to manipulate coordinates interactively while graphically visualizing NMR experimental information in the context of a molecular structure. Finally, the VMD-XPLOR interface is modular so that it is readily transferable to other refinement programs (such as CNS).

1. INTRODUCTION

Visualization of molecular structures is crucial to any NMR macromolecular structure determination, refinement, and analysis. In general, the majority of investigators have examined the ensemble of structures generated by programs such as XPLOR (1), CNS (2), or DYANA (3) using stand-alone graphical viewers (e.g., MOLMOL (4), GRASP (5), RASMOL (6), WHATIF (7), and MIDAS (8)). A tighter integration, however, of refinement and visualization, allowing manual intervention, is highly desirable. Previously, Brünger wrote a primitive, unpublished interface between XPLOR and the Evans & Sutherland PS300 series graphics system which permitted visualization of structure ensembles. This work was subsequently ported to UNIX workstations by means of an interface with the visualization program AVS (9). Unfortunately, this coupling suffered largely due to the proprietary nature of AVS and consequently its capabilities were never extended beyond a bare-bones visualization system. In this paper, we describe a visualization package which combines XPLOR with the open software package VMD (10). In addition to providing an interface by which structures and animation sequences can be passed from XPLOR to VMD, we have developed graphical cues to visualize NMR experimental information as it relates to the molecular structure while simultaneously having the capability of manipulating coordinates interactively (e.g., torsion angles, docking).

XPLOR is a widely used full-featured structure refinement package for NMR (11–14) and X-ray (15) structure determination. Over the past decade, extensions to XPLOR, written at the National Institutes of Health (NIH) (16), have included many newer refinement tools for NMR structure determination including J coupling (17), ^{13}C (18) and ^1H (19, 20) chemical shift, heteronuclear relaxation (21), and dipolar coupling (22, 23) refinement, as well as knowledge-based potentials of mean force (24–26). The base source code for XPLOR (27) as well as all the NIH extensions (28) is freely available for non-profit uses. The follow-on program CNS (2) retains the same high-level language as XPLOR but contains many additional features for X-ray refinement as well as an HTML interface. CNS, however, does not contain any NMR functionalities not present in XPLOR, and indeed the latest NMR features are only present in the NIH version of XPLOR (although porting source code modules between XPLOR and CNS is straightforward). Because of the availability of multiple refinement programs, attention has been paid to making the current interface modular and thus transferable to another package at a future date.

VMD is a visualization package developed at the University of Illinois which allows the viewing and manipulation of large molecules. It supports many rendering techniques from simple lines drawn between atom positions up to van der Waal surfaces and protein cartoon forms which are (or are nearly) publication quality. VMD possesses a Tcl/Tk (29) scripting interface, making it easy to add graphical user interface (GUI) elements. Furthermore, this scripting interface readily allows connections to remote programs running on the local or on remote machines. The VMD development team is accessible and open to suggestion for modifications and additions. Crucial to the current effort was the availability of the VMD source code: when we found the existing text (label) facilities inadequate we found it relatively straightforward to reimplement this portion of VMD—and found the VMD team quite receptive to incorporating the new code into its distributed version.

In the next section we give an overview of the new facilities added to XPLOR and VMD. In Section 3 we discuss future plans

for the software. The VMD-XPLOR package itself is available online at <http://vmd-xplor.cit.nih.gov/>.

2. DESCRIPTION OF THE SOFTWARE

2.1. Extensions to the XPLOR Interface

The XPLOR command language has been extended such that one accesses the VMD-XPLOR interface via the PS command, so named for compatibility with the previous AVS-XPLOR interface. One can send selected portions of structures and labels from this prompt. Further, one can send animations generated either by a dynamics run or by repeatedly appending structure snapshots. Also, one can send native Tcl commands to VMD, accessing its full capabilities. The scripting ability of XPLOR and VMD is crucial in structure refinement, allowing all manipulations to be faithfully reproduced. For instance, it is not unusual to compute hundreds of structures during the refinement process. With scripting, one can easily and reproducibly load and view all of these structures simultaneously in what would otherwise be a tedious typing and clicking experience. An example of the XPLOR syntax for loading the structure and labels in Fig. 2 is shown in Fig. 1.

The execution of XPLOR and VMD on separate hosts requires network communication and a Tcl socket mechanism is employed by VMD-XPLOR for this purpose. If the package is run on a single host, files can be shared. Otherwise they are transmitted over the communication layer. Within XPLOR, separate threads are used for communications with VMD and for computation, minimizing the effect of network latencies on compute-intensive structure calculations.

```
XPLOR>ps
PS>define x
DEFINE>bonds (name ca or name c or name n)
DEFINE>end
PS>define xs
DEFINE>bonds (not hydro and
not (name c or name n or name o) and
not resname ile)
DEFINE>end
PS>define label
DEFINE>label
LABEL>size=1
LABEL>resid=t
LABEL>resname=f
LABEL>name=f
LABEL>select (name ca)
LABEL>end end
PS>
```

FIG. 1. The XPLOR syntax for loading the structure and labels as seen in Fig. 2. The commands define three VMD objects based on the currently loaded structure in XPLOR: one object consisting of the bonds between the backbone atoms of the current structure, another consisting of bonds between the side chain atoms excluding isoleucine, and a third consisting of labels displaying the residue number next to each α -carbon.

Initially, when structures are sent to VMD, they are displayed in the simple line representation and in a single color (which is chosen by cycling through the VMD color map). The user can then change the color, coloring method, and drawing style. However, the line representation is probably the most useful for the purpose of simultaneously overlaying and comparing many structures.

2.2. Extensions to VMD

The Tcl/Tk GUI windows which VMD-XPLOR provides largely replace the native VMD GUI windows. These new windows, which provide an interface specialized for NMR structure refinement, will hence forward be referred to as forms. VMD-XPLOR provides forms to control molecule display, control mouse action, and edit the structure and forms corresponding to specific NMR experiments. However, whenever necessary or convenient, all of the native VMD forms are also accessible.

2.2.1. The molecule form. The molecule form allows easy manipulation of hundreds of structures, with simple controls to toggle on/off the display of individual structures, to change the molecule characteristics, and to focus in on a particular structure. The control for each molecule is coded by that molecule's color, easing the task of manipulating many structures. It is also possible to load and save structures using this form. Examples of the molecule form are shown in Figs. 3–5.

2.2.2. The mouse form. With the mouse form, one controls the action of the pointer in the VMD OpenGL rendering window, be it to rotate/translate/scale the scene containing the structures or to allow a VMD picking action such as drawing atom labels or displaying bond distances. Additionally, the action of the mouse has been extended using the Shift- and Ctrl- modifier keys such that all of the rotate/scale/translate functions are simultaneously available without any interaction with the mouse form, thus reducing the need to leave the VMD OpenGL window.

2.2.3. Edit form. With this form one selects a structure to rotate and translate relative to the other molecules in the scene. An optional collision detection feature prevents the structure's atoms from overlapping those from adjacent molecules. A fit option automates the process of overlaying structures. For editing atomic positions, the user can use the native VMD facility for dragging atoms and fragments about in Cartesian coordinates. VMD-XPLOR adds the torsion form, a facility for editing torsion angles, with a simple mechanism for choosing all the backbone or side chain atoms of a particular residue. A snapshot of the edit and torsion forms is shown in Fig. 2. On IRIX platforms, the editing functions can be performed using a dialbox, if one is available. The dialbox option is particularly convenient for editing torsion angles.

2.2.4. NMR experiment forms. Currently, forms are available which support visualization of the following three types of NMR data: distance restraints derived from NOE experiments, torsion angle restraints, and absolute orientation information from residual dipolar coupling experiments. The visual cues

from each experiment type are displayed in the VMD OpenGL window. They can be displayed simultaneously and are updated as molecular coordinates are modified. For each data type, the user has the option of displaying comments as labels in the VMD graphics window at the location of the visual cue. Each comment is associated with a particular XPLOR assignment statement and allows easy referral of the visual cue back to peak assignment information. Also, in each experiment form, subsets of the restraints can be chosen by specifying an appropriate selection using the XPLOR selection language within the form. Finally, the number of satisfied restraints and the total number of restraints are displayed at the bottom of each form.

Figure 3 shows an example of intermolecular NOEs visualized for the complex between the N-terminal domain of enzyme I (EIN) and the histidine-containing phosphocarrier protein HPr (30). Satisfied restraints are displayed as thin white lines, while violated restraints are shown with thick red lines. For the purposes of the figure, the HPr has been moved relative to EIN so as to create the violated NOE restraints shown. The restraint assignments are entered in the format of an XPLOR assignment table.

Each NOE assignment contributes the following term to the NOE energy

$$\begin{cases} k(R_{\text{calc}} - R_{\text{min}})^2, & R_{\text{calc}} < R_{\text{min}} \\ 0, & R_{\text{min}} < R_{\text{calc}} < R_{\text{max}} \\ k(R_{\text{calc}} - R_{\text{max}})^2, & R_{\text{calc}} > R_{\text{max}}, \end{cases} \quad [1]$$

where k is a weighting force constant, R_{min} and R_{max} define the NOE distance range obtained from experiment, and the distance R_{calc} is calculated from the current structure as

$$R_{\text{calc}} = \left(\sum_{ij} R_{ij}^{-6} / n_{\text{mono}} \right)^{-1/6}, \quad [2]$$

where n_{mono} is the number of monomers and is set > 1 for symmetric multimers. In Eq. [2] the sums are over all possible atoms for an observed NOE, allowing for ambiguities. For the case of an ambiguous assignment, the NOE line is drawn between those two atoms that give the dominant contribution to the NOE cost, i.e., those atoms whose separation is closest to R_{obs} , the nominal distance given in the assignment statement.

The torCon form controls the display of torsion restraint information which is visualized as a line between the center two atoms of the four atoms defining the dihedral angle. Again, satisfied and violated restraints are displayed with different widths and colors. Shown in Fig. 4 is an example of the torsion restraint visualization for some side chains of HPr.

Recently, it has been shown that residual dipolar coupling data can make a significant contribution to NMR structure determination, particularly with problems involving long-range orientational order, resulting in improvements in accuracy, reliability, and speed (30–37). The dipCoup form controls the display of

a structure's fit to residual dipolar coupling data. The dipolar coupling energy consists of terms

$$k(\delta_{\text{calc}} - \delta_{\text{obs}})^2, \quad [3]$$

where k is a weighting force constant, δ_{obs} is the observed residual dipolar coupling, and δ_{calc} is calculated from the current structure using the formula

$$\delta_{\text{calc}} = D_a(3v_z^2 - 1) + \frac{3}{2}D_aR(v_x^2 - v_y^2), \quad [4]$$

where

$$D_a = \frac{1}{3} \left[D_{zz} - \frac{1}{2}(D_{xx} + D_{yy}) \right] \quad [5]$$

is the axial component of the molecular alignment tensor, and R is the rhombicity

$$R = \frac{1}{3D_a}(D_{xx} - D_{yy}). \quad [6]$$

v_x , v_y , and v_z are the respective projections of the unit vector between atoms onto the molecular alignment tensor. This is the same formula used by Clore *et al.* (22) in their extension of XPLOR. Here the experiment measures alignment of a vector between two atoms to an overall reference frame. One enters the coefficients in Eq. [4] and then satisfied and violated restraints are again drawn with different color and thickness. Here, however, the restraint line is not drawn directly between the atoms. Instead it is drawn in the direction toward which the vector between the two coupled atoms should be oriented to reduce the dipolar coupling cost. In addition to the dipolar coupling cost, the common metric of root-mean-square (RMS) deviation of calculated and observed dipolar coupling is displayed in the form. An example of dipolar coupling restraint visualization is shown for HPr in fig. 5.

3. FUTURE WORK

Here we have presented a visualization package specialized for NMR structure determination which combines the XPLOR and VMD programs. This package has been in use for over a year in NMR labs at the NIH and has proven to be an extremely valuable tool in the structure refinement process.

There are popular refinement programs aside from XPLOR, and VMD-XPLOR has been designed with an eye toward implementing interfaces to other programs. In fact, VMD-XPLOR can be run in a stand-alone mode without XPLOR (or any refinement program) while retaining all of its visualization functionality.

In the future, we anticipate the addition of more NMR-specific functionality to VMD-XPLOR, for instance, as new experimental techniques are developed. Also, we anticipate that it will be useful to support the visualization of electron density maps and other probability maps.

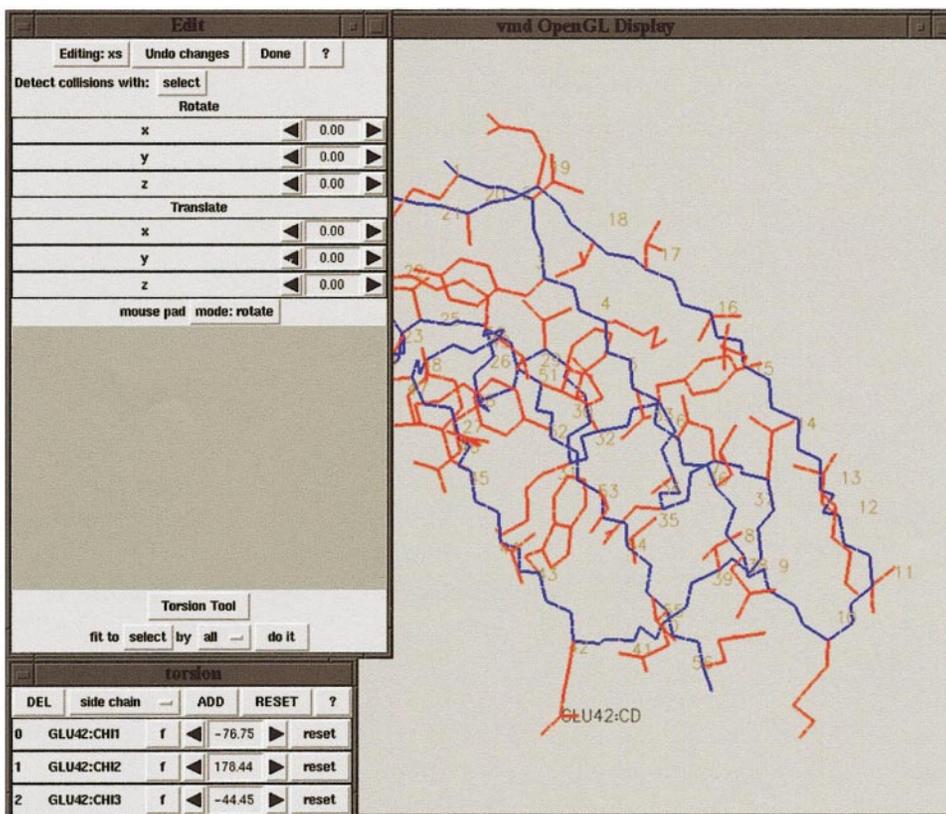


FIG. 2. A screen shot demonstrating the edit capabilities of VMD for protein G. The side chains are depicted in red, while the backbone is blue. The side chain torsion angles of residue 42 have been selected for editing. The ability to rotate/translate the structure becomes useful in docking situations, such as that depicted in Fig. 3.

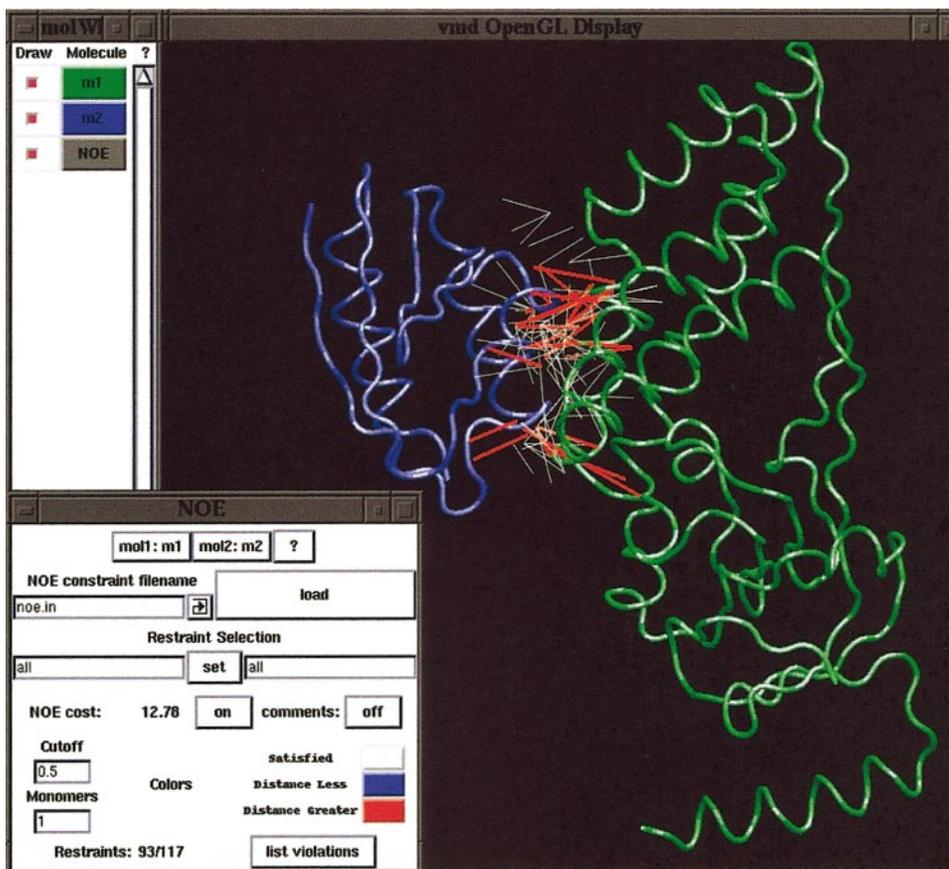


FIG. 3. A screen shot demonstrating the NOE visualization in the protein docking of HPr to enzyme I, which are displayed in blue and green, respectively. Violated NOE restraints are displayed as red lines, while satisfied restraints are white. Also shown are the molecule and NOE forms.

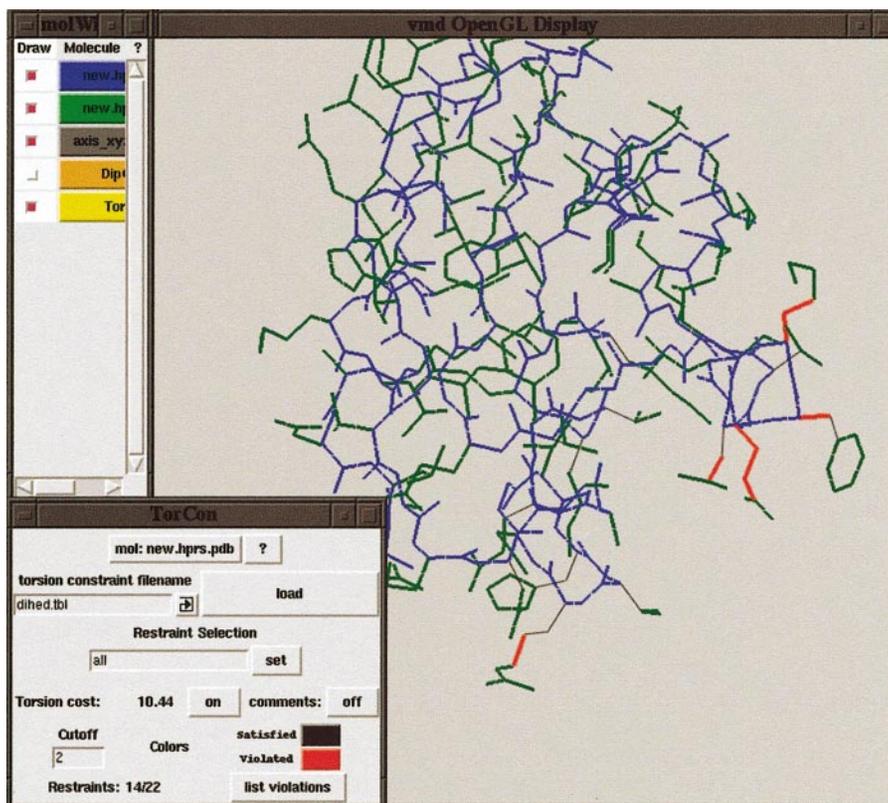


FIG. 4. A screen shot demonstrating the visualization of torsion restraint information for some side chains of the HPr protein. Violated dihedral restraints are displayed in red, while satisfied restraints are thin black lines. Also shown are the molecule and TorCon forms.

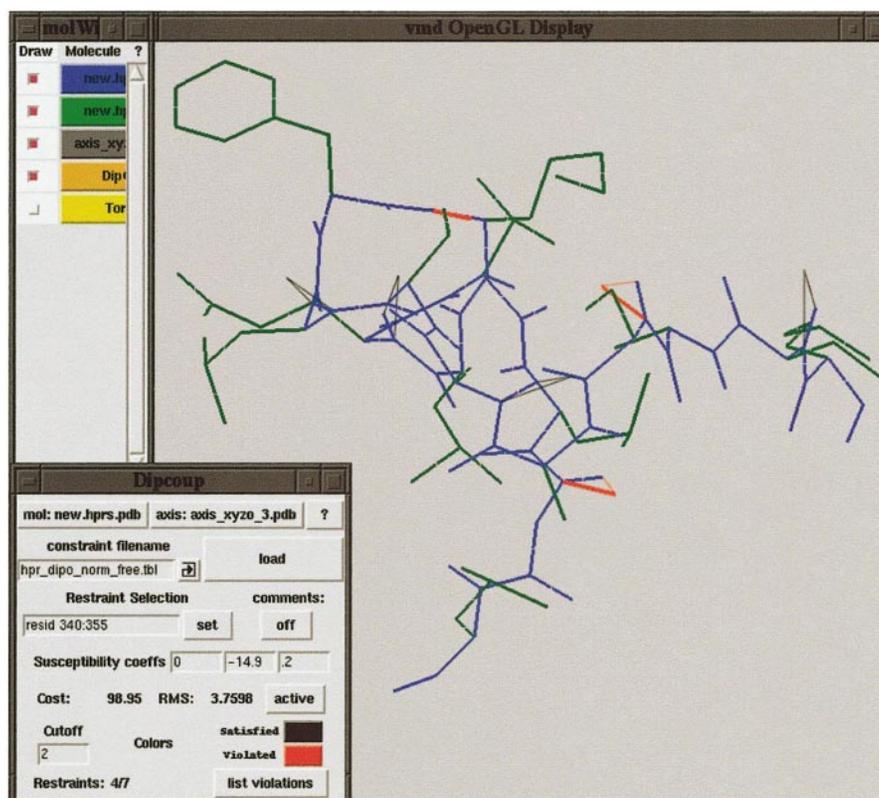


FIG. 5. A screen shot demonstrating dipolar coupling visualization for a subset of residues of the HPr protein. Violated dipolar coupling restraints are displayed in red, while satisfied restraints are black. The visual cue consists of two lines, one from each atom defining the bond. By rotating the bond to make it coincide with the thick line, the energy for that bond is reduced. Also shown are the molecule and dipCoup forms.

ACKNOWLEDGMENTS

We thank John Kuszewski and Chris Lanczycki for interesting and useful discussions.

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