Visual Molecular Dynamics (VMD)

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Introduction

VMD web page: http://www.ks.uiuc.edu/Research/vmd/
Documentation: http://www.ks.uiuc.edu/Research/vmd/current/docs.html

VMD is designed for modeling, visualization, and analysis of biological systems such as proteins, nucleic acids, lipid bilayer assemblies, etc. It may be used to view more general molecules, as VMD can read standard Protein Data Bank (PDB) files and display the structure contained in these files. VMD 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, cartoon drawings, and others. VMD can be used to animate and analyze the trajectory of a molecular dynamics (MD) simulation. In particular, VMD can act as a graphical front end for an external MD program by displaying and animating a molecule undergoing simulation on a remote computer.

Open VMD

Open the vmd program by typing **vmd** in the terminal (on ARCHIE-WeSt it should be **vglrun vmd**) and then go to **Main menu → File → New molecule → protein.pdb**. The other way to see the protein is type in your terminal commend line **vmd protein.pdb,** the result will be exactly the same.

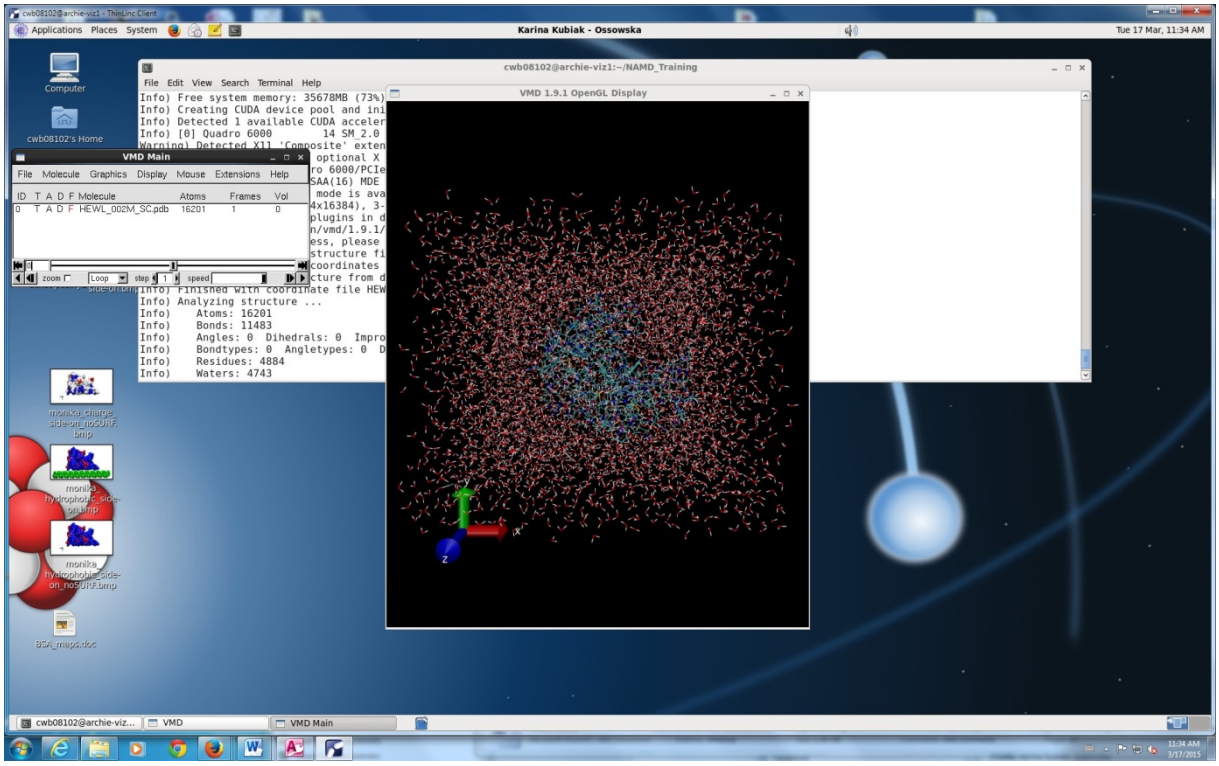


Figure 1. VMD windows

If you have the *psf file you can load this as well:

1) highlight the structure in the Main menu
2) Mouse right click → **Load Data Into Molecule → Browse the file → Load**
3) **Main menu → File → Load Data Into Molecule → Browse → Load**
4) **vmd protein.pdb protein.psf**

Three windows will open: **VMD Main**, **VMD OpenGL** and **vmd console** (terminal window), as it's shown on Fig.1.

1. VMD Main

VMD Main (Fig. 2) is a VMD control window, which contains all sub-menus and also shows all loaded molecules. Each molecule has its **ID**, **T** (Top), **A** (Active), **D** (Displayed) and **F** (Fixed) options, the information about number of atoms in the structure and the number of Frames loaded is given. To change T, A, D and F it is enough to double-click on them. Available values are on or off. If the trajectory is loaded, this window allows the user to play, stop and pause the trajectory, to select a particular frame number to be displayed, to choose if the trajectory should be played once or in a loop or to change the speed (how fast the trajectory is played).

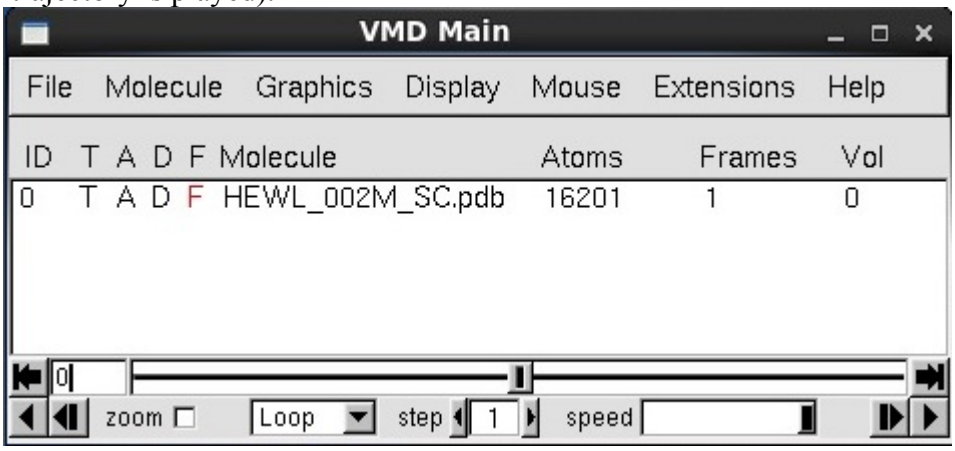


Figure 2. Vmd Main window.

2. VMD OpenGL

The VMD OpenGL (Fig.3) is the main graphic window in which the molecule is displayed. In the VMD OpenGL display window the user can:

1) ***Rotate*** the protein. Press the mouse left key and rotate. If it does not work press **r** on the keyboard to switch-on the rotation mode. Usually the rotation mode is the default
2) ***Translate*** the protein. To switch-on the translation mode press **t** on the keyboard and then press the mouse left key and translate.
3) ***Scale*** the protein. To switch-on the translation mode press **s** on the keyboard and then press the mouse left key and translate. Scaling is also possible in the rotation or translation mode by rolling the middle mouse button. Nevertheless it is more "coarse" scaling.
4) ***Center*** the system on the chosen atom. To switch-on the center mode press **c** on the keyboard and then click left on the chosen atom. If you come back to the rotation mode you will see that now the protein rotates around the chosen atom.
5) ***Select atom***. To switch-on the selection mode press **1** on the keyboard and then click on any atom. The green label containing residue name, number and atom type will appear (Fig. 3). Simultaneously additional information at the vmd console will appear. This will state the molecule id, trajectory frame, atom name, atom type, atom index,

residue name, residue id, chain name, segment name and *x*, *y*, *z* coordinates of the selected atom.

6) ***Select and measure the distance*** between two atoms. To switch-on the selection mode press **2** on the keyboard and then click on any two atoms. The green label for selected atoms and additionally the white label with the distance (in Å) will appear (Fig. 3). Information about the selected atoms will appear also at the vmd console window.
7) ***Select and measure the angle*** between selected atoms. To switch on the selection mode press **3** on the keyboard and then click on any three atoms. The green label for selected atoms and additionally the yellow label with the angle (in degs) will appear (Fig. 3). Information about the selected atoms will appear also at the vmd console window.
8) ***Select and measure the dihedral*** between selected atoms. To switch-on the selection mode press **4** on the keyboard and then click on any four atoms. The cyan green label for the selected atoms and additionally the cyan label with the angle (in degs) will appear (Fig. 3). Information about the selected atoms will appear also at the vmd console window.

Note: to hide atom, bond, angle and dihedral labels it is enough to click again on appropriate atoms. The other option is to use **`Main menu` → `Graphics` → `Labels`** – please see below. For more options please see menu **`Mouse`** below.

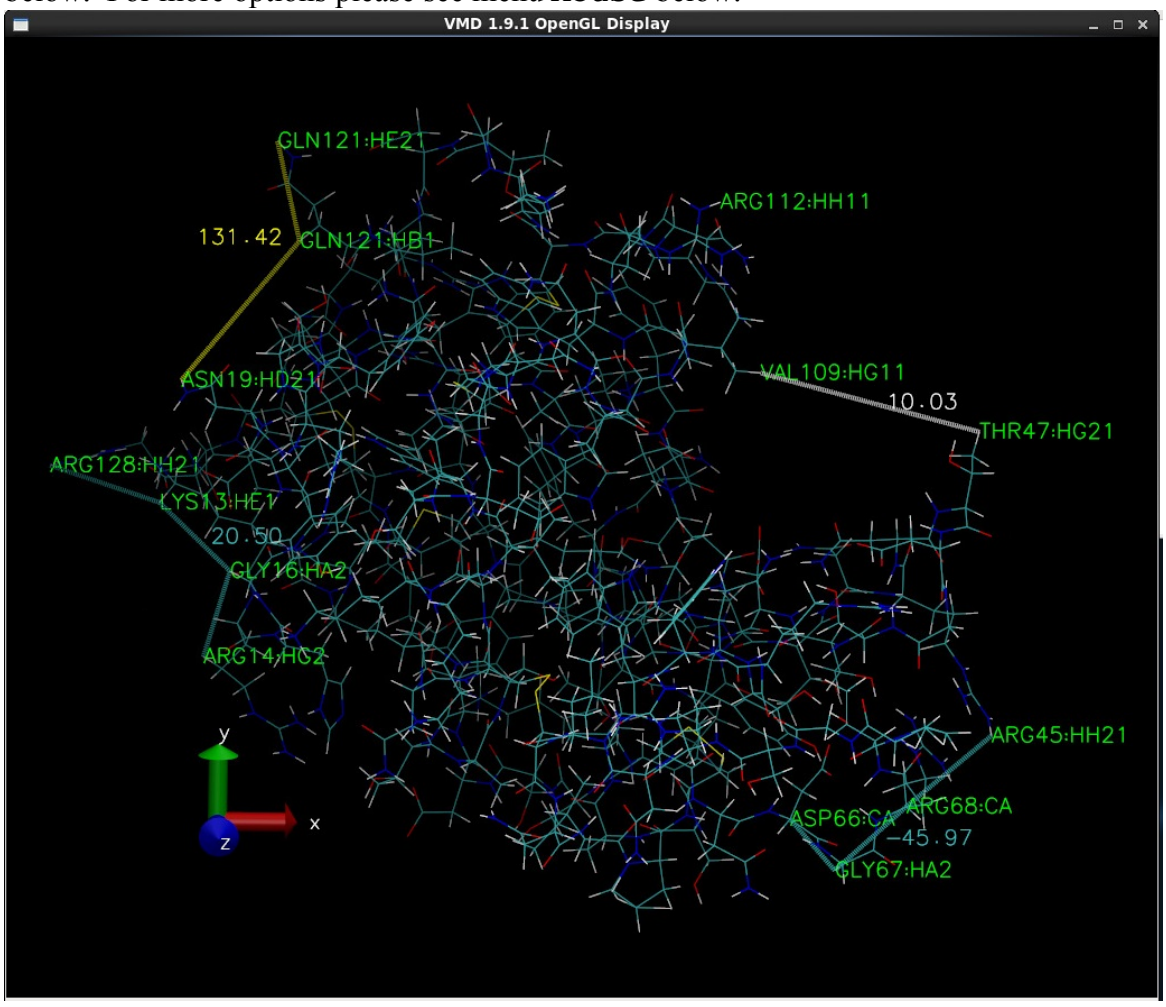


Figure 3. Vmd OpenGL window with atom, bond, angle and dihedral labels shown.

3. vmd console

The vmd console is an information window (Fig. 4).

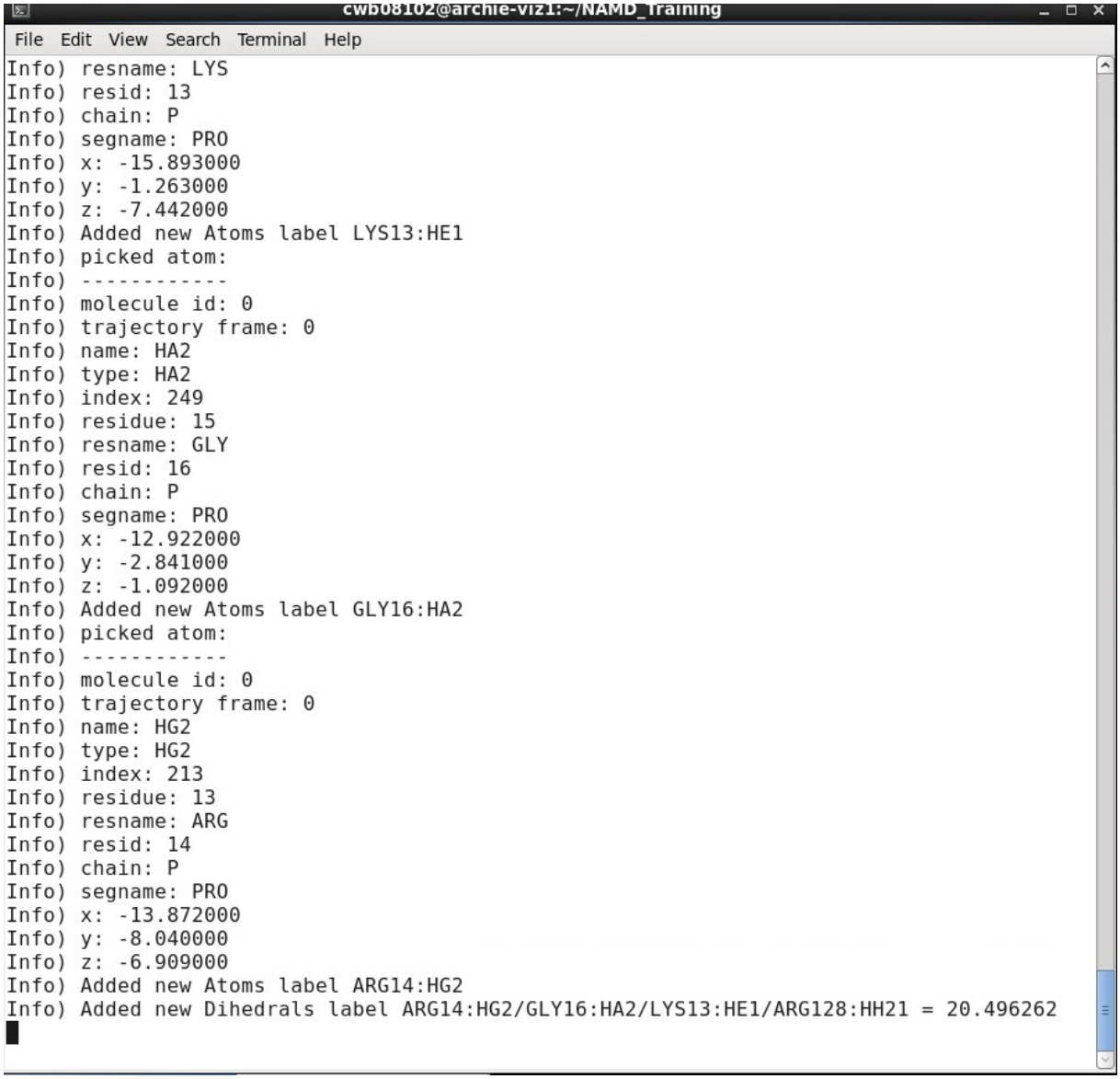
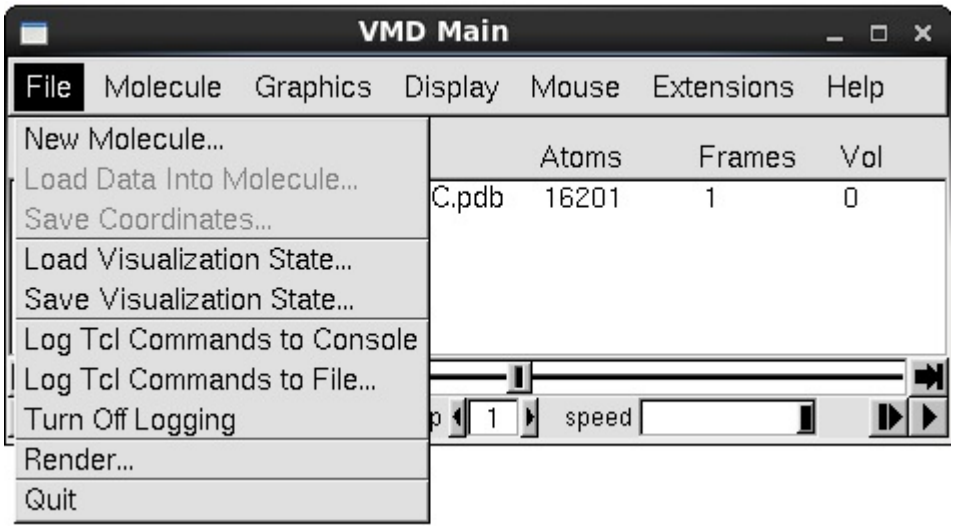


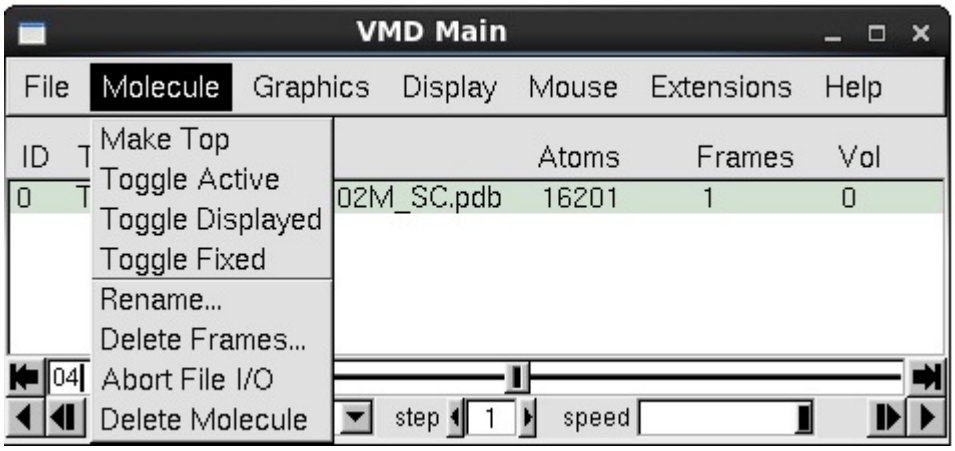
Figure 4. Vmd console window.

Menu File



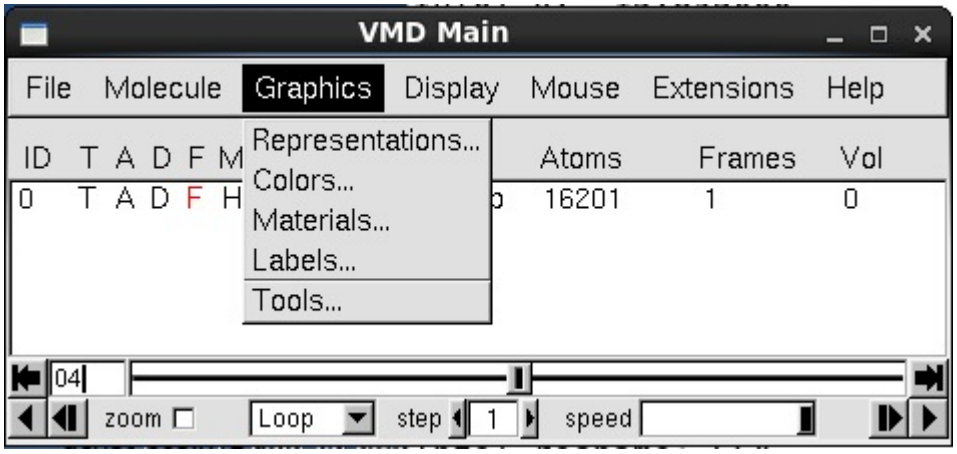
All options are available once any loaded molecule is highlighted. **`New Molecule`** allows a new molecule to be loaded, **`Load Data into Molecule`** allows appropriate data like the psf file or the trajectory file to be loaded. VMD will automatically recognize the file type to be loaded. If the trajectory is loaded it is possible to load each frame (**`Stride`** 1), each second frame (Stride 2) 10th frame (Stride 10) and so on. Loading can start and finish from any chosen frame (options **`First`** and **`Last`**, respectively) . **`Save Coordinates`** allows the user to save the entire trajectory using the same or any other format. It is possible to save the trajectory only for selected atoms and the user can save the entire trajectory, only a part or even a single frame (for example the last one). By changing **`Stride`** it is possible to save for example every 10th frame, every 100th frame etc. By choosing **`Load State`** option from menu **`File`** the user can load previously saved VMD state. For example if the user has a lot of selections, graphical representations, and has loaded many molecules and wishes not to repeat all of that next time, the user can select **`Save State`** and later **`Load State`**. Nevertheless, saving and loading state will change the system orientation in the space. **`Render`** is the last option in the menu **`File`**. It allows user to render current frame as a picture. It is not recommended to change the default values, and in particular the file extension (tga is a default). Nevertheless the file name can be changed. New window with rendered picture will open and the file will be saved in the working trajectory.

Menu Molecule



To make all options active, a molecule in the Main VMD window should be highlighted. First four options: **`Make top`**, **`Toggle Active`**, **`Toggle Displayed`** and **`Toggle Fixed`** are also available at the main control window **`VMD Main`**. Additionally four other options are available. **`Rename`** allows the user to rename the highlighted molecule. If the trajectory is loaded, the user can delete some frames using the **`Delete Frames`** option; it is possible to delete the entire trajectory, or only a part, by specifying **`First`** and **`Last`** frame and the **`Stride`**. **`Abort File I/O`** is useful when the user wants to stop loading the trajectory. To delete the highlighted molecule the option **`Delete Molecule`** should be used.

Menu Graphics



The graphics Menu is one of the most frequently used menus in VMD.

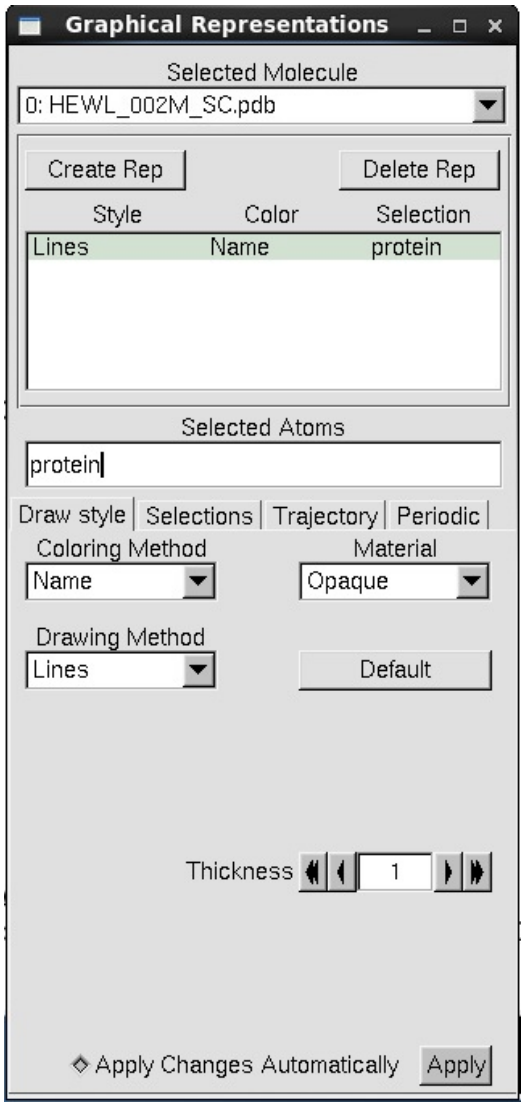
1. Representations

It is possible to change the appearance of the protein, visualize the backbone pattern, highlight the most important part and change colours etc. To do that, chose **`Main menu`** → **`Graphics`** → **`Representations.`** A new window called **`Graphical Representations`** will open (Fig. 5). The default representation shows all the lines colored by the default.

The default colors are:
white for hydrogen
red for oxygen
blue for nitrogen
cyan for carbon
yellow for sulfur and chlorine

Note that the default representation is highlighted. A double-click will switch off the representation. To switch on – again double click.

New representation (`Graphical Representations menu`)



To create a new representation click the button **`Create Rep`**. To remove (delete) the chosen (highlighted) representation click the button **`Delete Rep`**. New representation by default shows everything (all) as lines by default. To change the selection give the new selection in the **`Selected Atoms`** field. If more than one molecule is loaded it is possible to select which molecule the user wish to change the representation by changing the field **`Selected Molecule`**. Please note that molecules IDs are the same as in **`VMD Main`** menu.

Figure 5.Graphical Representations dialog window.

Selections (`Graphical Representations` menu)

`all` – shows all
`resname ARG` – will show all arginine residues
`resid 1` – will show all residues number 1
`protein` – will show only the protein atoms
`water` – will show only water atoms
`all not protein` – will show everything except the protein
`all not water` – will show everything except water
`segname ION` – will show only atoms from the segment called ION
`protein and resid 1` – will show only residue 1 from the protein. Note that numbering residues in each segments is independent, e.g. there is residue1 in segment protein, residue 1 in segment water, residue 1 in segment ION, ect. Only atom index is unique.
`index 10` – will show only the atom number 10
`resid 1 5 9` – will show residues number 1, 5 and 9
`resid from 1 to 9` – will show residues: 1, 2, 3, 4, 5, 6, 7, 8 and 9
`index 1 5 9` – will show atoms number 1, 5 and 9
`index from 1 to 9` – will show atoms number 1, 2, 3, 4, 5, 6, 7, 8 and 9
`resname CYS and resid 1` – will show residue CYS and all residues number 1
`all within 10 of protein` – will show all atoms in 10Å radius from the protein
`all within 10 of protein and resid 4` – will show all atoms in 10Å radius from protein residue number 4
`all water within 10 of protein and resid 4` – will show all water atoms in 10Å radius from protein residue number 4
`all water within 10 of protein and resid from 4 to 8` – will show all water atoms in 10Å radius from protein residues 4, 5, 6, 7 and 8. Note that it is possible to use **`index`** in the same way as **`resid`**

It is possible to give the selection by using the **`Selections`** menu. So far we have used the **`Draw style`** menu (it is the default). In the Selections menu the user can find more selections possibilities.

If the selection type "all within …. " is used it will work only for the current frame. Therefore if the trajectory is loaded and if the user wants to upgrade the selection for each frame in the trajectory: go to **`Trajectory`** menu and click **`Upgrade Selection Every Frame`**. It will work only for the highlighted selection.

Drawing Method (`Graphical Representations` menu)

To change the drawing method choose other option under **`Drawing Method`** menu.
The most useful methods are:

- **`Lines`** (everything is represented by lines). It is possible to change the line thickness.
- **`Bonds`** (everything is represented by bonds). The user can change the bond radius and bond resolution
- **`Hbonds`** (only hydrogen bonds are drawn). The user can change Distance and Angle Cutoff (default values are 3Å and 20^0, respectively) as well as the line thickness

- **Points** (everything is represented by points, no bonds are drawn). The point size can be changed.
- **VDW** (atoms are drawn as van der Waals spheres). Sphere size and resolution can be changed.
- **CPK** (atoms are represented as CPKs and bonds are drawn as lines). Sphere size and resolution as well as bond size and resolution can be changed.
- **Licorice** (structure is represented by a licorice). Sphere resolution as well as bond radius and resolution can be changed.
- **NewCartoon** (only protein backbone is shown and secondary structure elements are visible: α-helices indicated by helix, β-sheets are indicated by arrows). Line thickness and resolution can be changed. Changing the Aspect ratio is not recommended.
- **Surface** (only the surface is drawn). Th surface representation method can be changed from solid to wireframe. Changing the probe radius is not recommended.

In order to produce a nice picture ensure that the resolution of each representation has been increased.

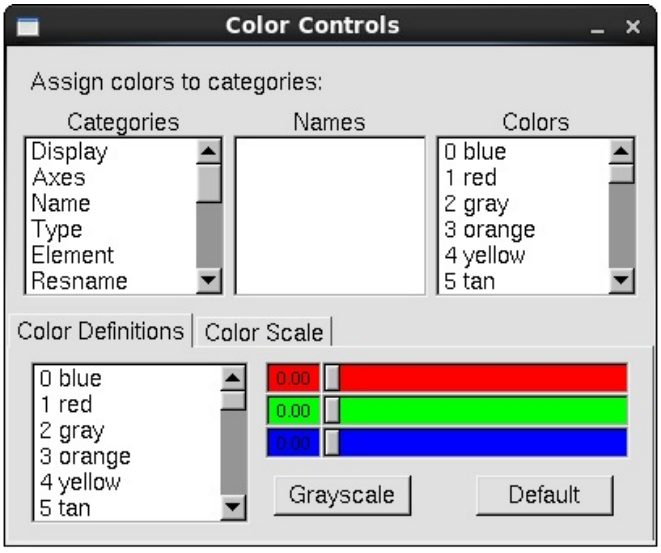
Coloring method (Graphical Representations menu)

To change the coloring method select other option under **Coloring Method** menu. The most useful is coloring by ColorID – once this is selected the new menu appears and the user can chose the color. Note colors are denoted by numbers: 0 for blue, 1 for red, ect.

Material (Graphical Representations menu)

For each drawing method, the material can be changed. The default is opaque, other useful options are: Transparent and Glossy. If the Transparent material is used the display quality is sometimes not very good. To improve this go to **Main menu → Display → Rendermode → GLSL.**

2. Colors



It is possible to change the display colors (but not the representations' colors). To open the appropriate dialog box click: **Main menu → Graphics → Colors,** the new window called **Color Control** will open (Fig. 6). Under **Categories** the user can chose for which category the color should be changed. Then the new color can be chosen from the list or user-defined by moving **Color Definitions** buttons.

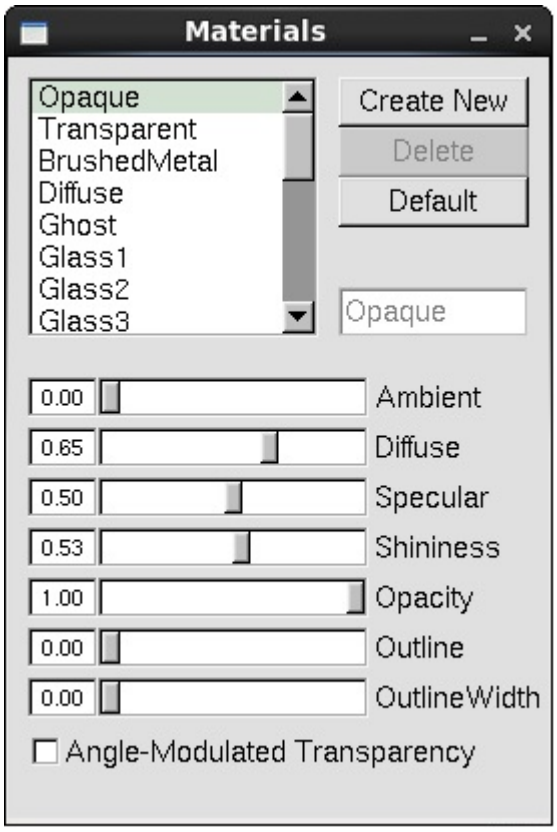
Figure 6.Color Controls dialog window.

Categories (`Color controls` menu)

- **`Background`** – allows the background color to be changed. **`BackgroundTop`** and **`BackgroundBot`** allows the background colors to be changed if the gradient display is chosen (see below for details)
- **`Axes`** – the color of x, y and z axis can be changed as well as labels and origin colors
- **`Name`** – the color of atom names can be changed (used in **`Graphical Representation`** when the coloring method **`Name`** is selected. The same is possible for selections: **`Type`**, **`Element`**, **`Resname`**, **`Restype`**, **`Chain`**, **`SegName`**, **`Conformation`**, **`Molecule`**, **`Structure`**).
- **`Labels`** – allows the label color of **`Atoms`**, **`Bonds`**, **`Angles`**, **`Dihedrals`** and **`Springs`** to be changed.

Note: before producing the figure, it is good practice to change the background color to white, then the axes labels to black. Changing the atom, bonds and angles label color is worth considering as well.

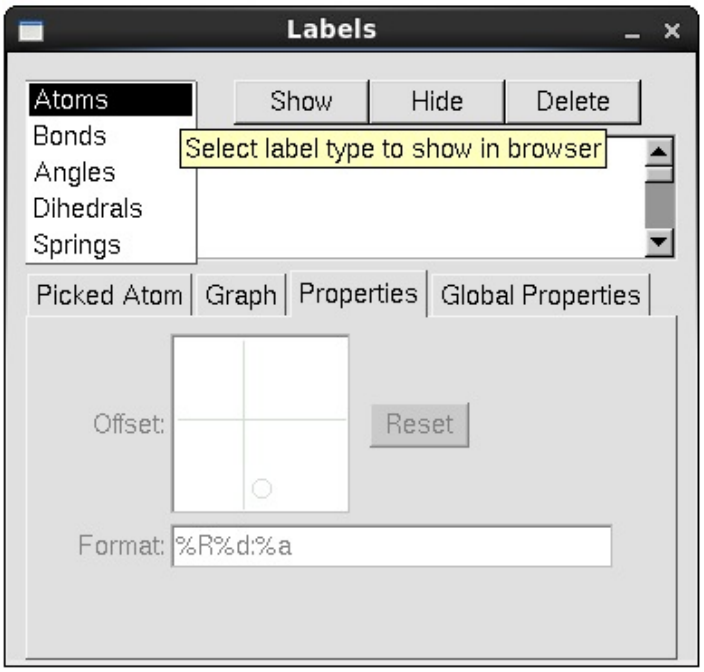
3. Materials



This allows the user to change the materials definition. To open this dialog box click: **`Main menu`** → **`Graphics`** → **`Materials`** (Fig. 7). Navigation under Materials dialog box is self-explanatory.

Figure 7. Materials dialog window.

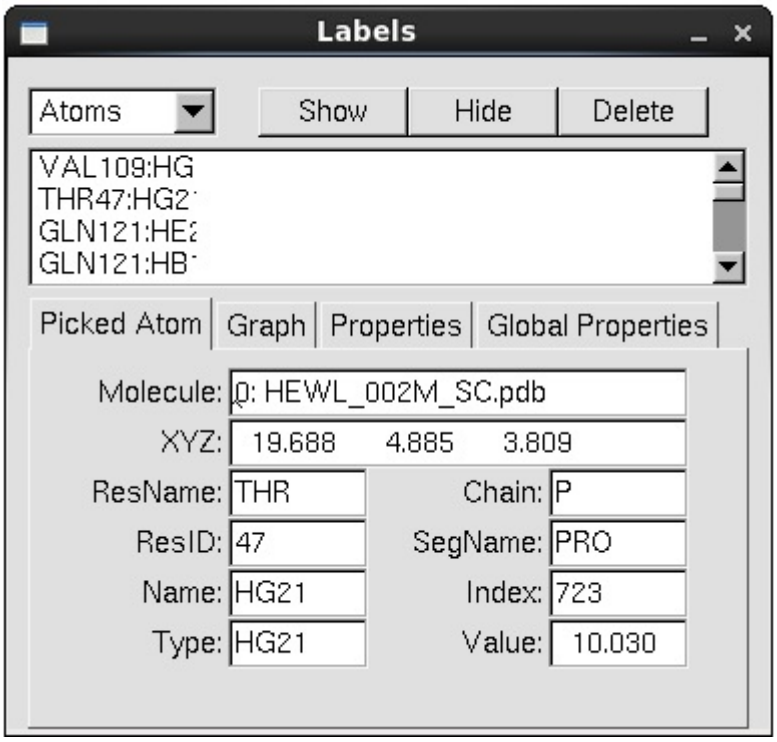
4. Labels



By clicking: **Main menu** → **Graphics** → **Labels** a new dialog box called **Labels** will open (Fig. 8). It allows the user to work on atoms, bonds and angles labels as well as to save plots. To work with Labels, first some atoms, bonds and angles should be selected in the **VMD OpenGL** window as described before. In the scroll-down menu the user can choose the label to work on: the default is Atom. Others are: **Bonds**, **Angles**, **Dihedrals** and **Springs**.

Figure 8. Labels dialog window.

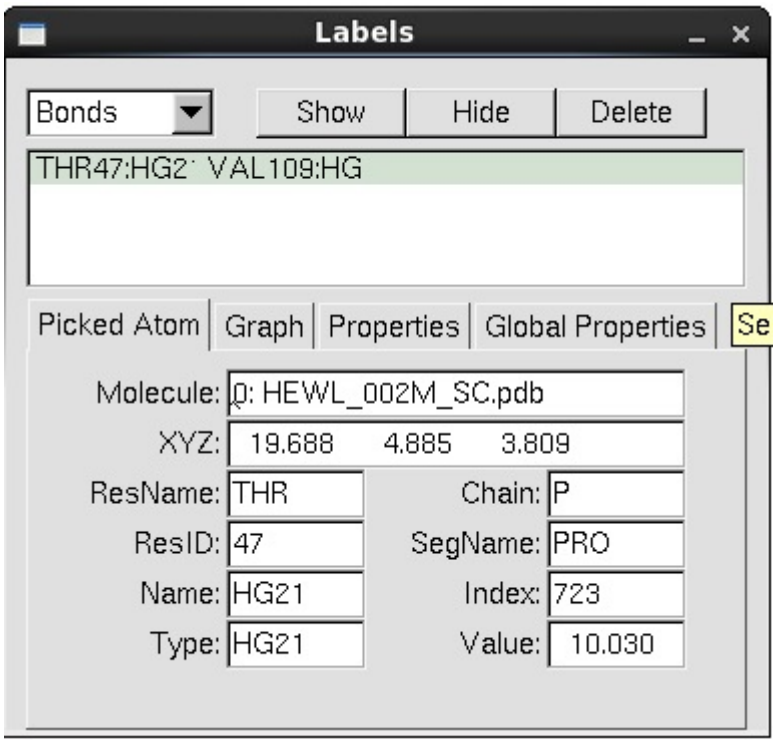
Atoms



The list of selected atoms is available at the navigation window. Once any label is selected the user can show, hide or delete the label by clicking on **Show**, **Hide** or **Delete** buttons. The information about the picked atom is also given (**Picked Atom** sub-menu): the molecule to which the atom belongs to, the (*x*, *y*, *z*) coordinates, residue name (**ResName**) to which the atom belongs to, residue ID (**ResID**), Chain (**Chain**), segment name (**SegName**), atom name (**Name**), atom type (**Type**), index (**Index**). This information is also available form the vmd console window. Finally, the value (Value) is also given. Note that for atoms the value is always 0.000. By clicking the **Graph** tab the user can see and save the plot (**Graph** and **Save** buttons). Note

that for atoms nothing will happen – there is no plot because the value is 0.000. By clicking the tab **Properties** the user can change **Text Size**. Unfortunately it will change the size of all labels. The position of the chosen label can be also changed by changing the **Offset**. Finally, the format of the label can be changed (**Format** window). By default the value of this window is: **%R%d:%a**. It means that the reside name (**%R**), residue ID (**%d**) and the atom type (**%a**) followed by colon (:) is shown. The user can delete the part of the label or the entire label and type their own text here. It will work only for the selected atom label.

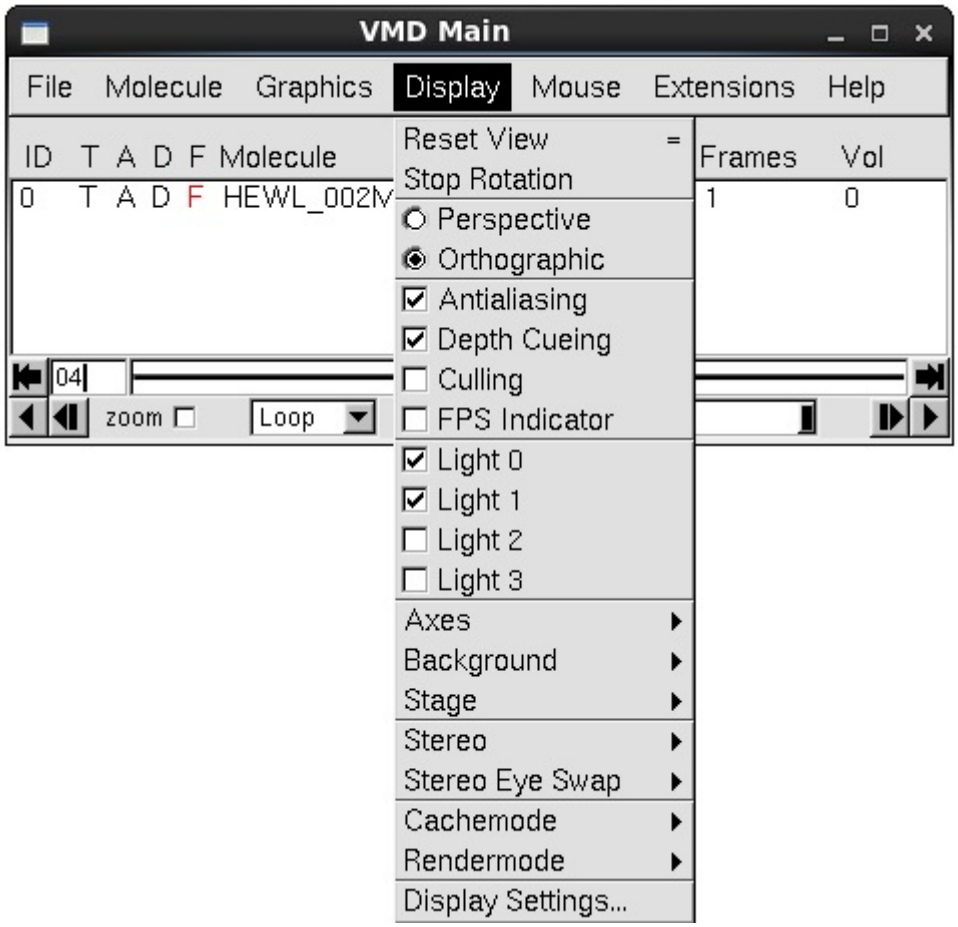
Bonds, Angles, Dihedrals and Springs



The menu options are very similar to the Atom options, the only difference is that the value is not 0.000 and it is possible to see and save the plot. Note that the plot will be empty if the trajectory is not loaded, which is obvious. For only one frame there is only one value. If the trajectory is loaded the value can change in each frame and the plot of value versus Frame can be drawn.

Menu Display

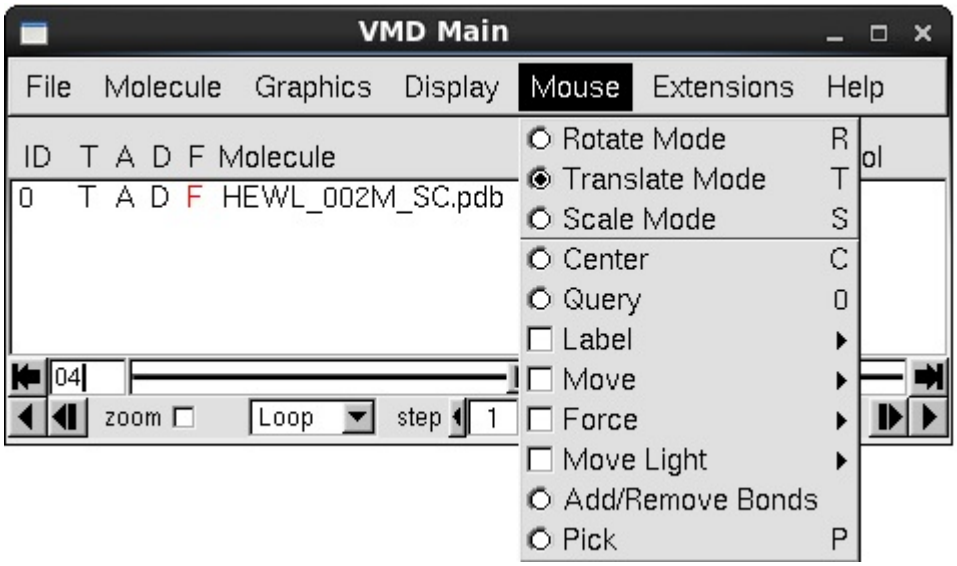
Menu **Display** (**Main menu → Display**) allows user to control the display of the molecule and affects the appearance of the molecule displayed at the VMD OpenGL window.



If the user played a lot with the orientation of the molecule and then would like to come back to the initial view (sometimes the molecule can even disappear from the VMD OpenGL window), the Reset View option (**Main menu → Display → Reset View**) should be chosen. The option **Stop Rotation** will obviously stop the rotation in the VMD OpenGL window. By clicking **Perspective** or **Orthographic** the user can change the view of the molecule from perspective to orthographic and vice-versa (perspective view is a default one but not recommended). Moreover it is possible to switch on/off **Antialiasing**, **Depth Cueing**, **Culling** and **FPS Indicator**. It is not recommended to change the default values except the depth cueing – this allows the user to see what is closer and further to the screen. The user can also switch on/off some lights (**Light 0, 1, 2, 3**). By default Light 0 and Light 1 are on. The next option – **Axes** gives control of the axes, which can be switched on or off, displayed at the origin, lower left or right, upper left or right. The background is displayed by default as a solid color. The control on this is given by **Background**. Once the gradient display is chosen (**Gradient**) the user can change the

default gradient colors (blue and black) via **Main menu → Graphics → Colors → Display → Background** as described above. Changes in **Stage** are not recommended. If the user would like to produce the stereo figure or use stereo glasses, the control on the stereo view is given by the **Stereo** option. Switching on **Cachemode** is not recommended: the default value is off. **Rendermode** is by default **Normal**- change in the render mode can slow down the VMD. If the user uses transparent representation and is going to render the figure, Rendermode should be changed to **GLSL** which gives the best quality picture but slows down VMD. The last option is **Display Settings**. It allows the user to change the near and far clip, screen size and distance or the cue type and its density, nevertheless this control is not very useful.

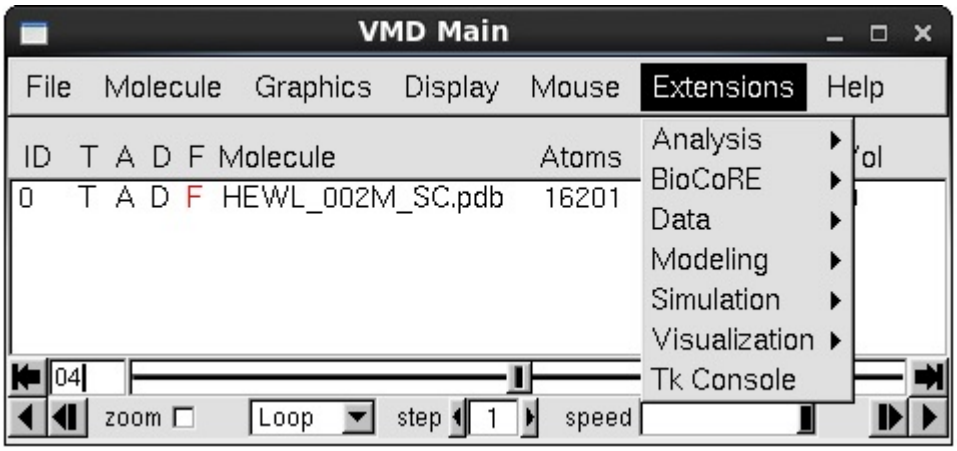
Menu Mouse



Under the mouse menu (**Main menu → Mouse**) the user can change the mouse mode from the default rotate (**Rotate Mode**) to translate (**Translate Mode**) or scale (**Scale Mode**). Other two options are: center (**Center**) and query (**Query**). If Query is on and any atom is picked at the **VMD OpenGL** window, noting will appear. The information about picked atom will be visible only at the **vmd console** window. The **Label** option allows the user to add labels to atoms, bonds, angles and dihedrals as was described above using shortcuts (1, 2, 3 and 4, respectively).

The last four options in the **Mouse** menu allow the user to play with the molecule. It is not recommended to use them for scientific purposes. The user can manually move the selected atom, residue, fragment, molecule or representations (**Main menu → Mouse → Move**), manually add the force to atom, residue or fragment (**Main menu → Mouse → Force**) move the light (**Main menu → Mouse → Move Light**) and add or remove bonds between selected atoms (**Main menu → Mouse → Add/Remove Bonds**).

Menu Extensions



Menu extensions are for more experienced users which are going to use VMD, not only for visualization, but also to analyze the existing MD trajectory and to prepare a new one.

1. Analysis

APBS Electrostatics allows the user to calculate electrostatic potential or solvent accessibility but only when the apbs binary file for the loaded molecule is accessible.

Contact Map allows the user to calculate the distance map between residues within the same or between two molecules. The map is calculated for the current frame only.

It is not recommended to use **MultiSeq** option – vmd can crash and the information given by this option is not very useful.

NAMD Energy can calculate the energy of the molecule using NAMD. The usage is quite complicated, therefore this option is not found to be very useful.

NAMD Plot allows the user to plot information stored in the trajectory output file (*.out not *dcd). Since the NAMD output format is not straightforward for plotting energies "by hand", this option can be very useful. All kinds of energy stored in the output file might be plotted versus time step (TS). First in the NAMD plot window, under the File tab where the output file has to be selected, the user can chose the energy to be plotted and then the plot can be generated by clicking Plot Selected Data in the File tab. The new window containing the plot will open, and the user can save the plot by exporting as a postscript file (new *.ps file will be created in the background) or exporting to the xmgrace program (the *agr file will be created and xmgrace will open).

PME Electrostatics allows the PME potential for the current frame to be calculated. The result will be displayed in the VMD OpenGL window as a new molecule.

Radial Pair Distribution Function g(r) allows g(r) for two given selections to be calculated. g(r) shows the density of atoms around the picked atom as a function of the distance. Selection 1 specifies from which atom, or atoms, g(r) will be calculated. Selection 2 specifies which types of atoms will be shown in the computed g(r). For example if Selection 1 is protien and Selection 2 is all, the g(r) plot will be calculated for all protein atoms and all atoms will be included. If selection 2 is CA, only those atoms will be included in the g(r) plot. Before computing g(r), go to utilities and set unit cell dimensions – Lengths a, b and c should be the same as in *SC* file. Once the g(f) plot is generated, it can be exported as a postscript image or agr file.

IR Spectra Calculator in theory should allow the IR spectra to be calculated. However, this does not seem to work correctly.

Ramachandran Plot allows a Ramachandran plot for any loaded molecule to be produced. The white area on the plot corresponds to disallowed conformations, the blue area represents allowed conformations and classified as α-helix or β-sheets, while green represents all other conformations. If the trajectory is loaded, the Ramachandran plot will be upgraded for each frame automatically. The plot can be saved as a postscript file.

RMSD Calculator allows RMSD to be calculated between all molecules in the memory or only active molecules. The selections for which RMSD is calculated can be freely changed. It is recommended to click Align button and then RMSD. The RMSD will be calculated for current frame only.

RMSD Trajectory Tool can be used to calculate the RMSD for the entire trajectory. The other option (preferred one) is to use tcl script (see below)

Salt Bridges this option allows the user to find salt bridges in the structure.

Sequence Viewer shows the secondary structure of the protein

Timeline does not seem to be a useful option

VolMap Tool will calculate the volume of the protein and will display the result in the VMD OpenGL window

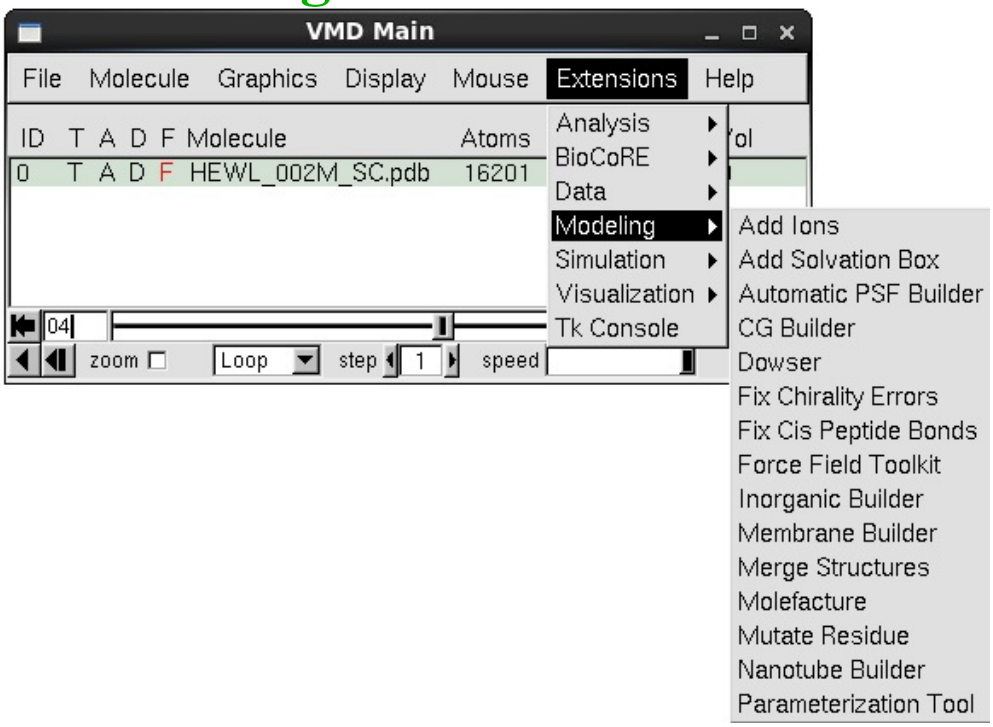
2. BioCoRE

The user has to login to register at VMD web.

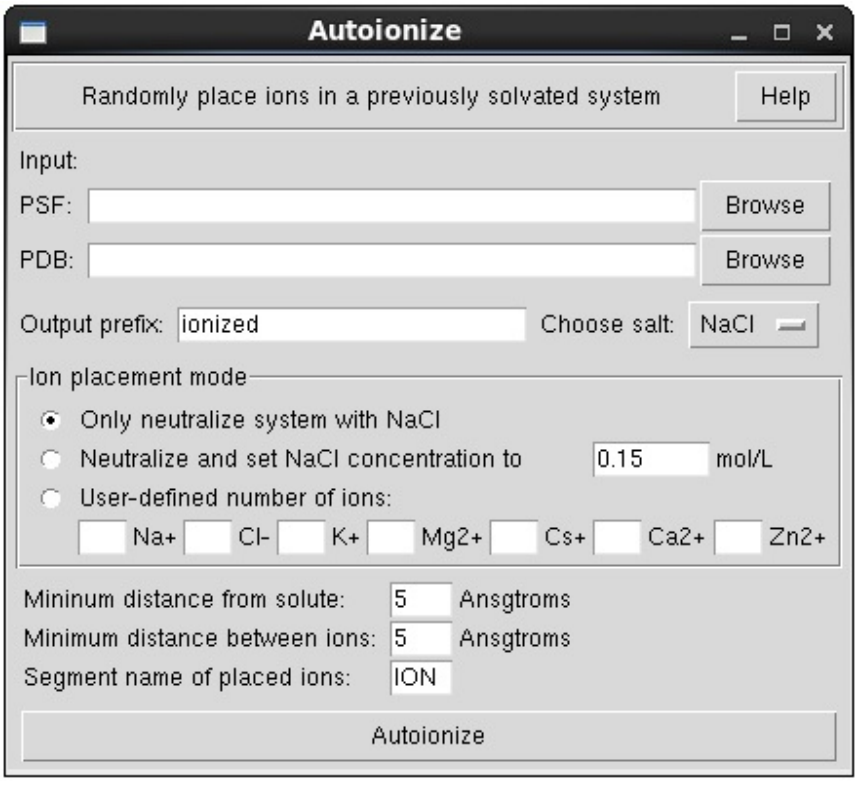
3. Data

The menu is self-explanatory and allows the user to work with a connection to the Protein Data Bank (PDB).

4. Modeling



This menu allows the user to add ions to the structure (**Add Ions**), add the solvation water (**Add Solvation Box**), to build the psf file (**Automatic PSF Builder**), build the coarse grained model (**CG Builder**), add the membrane (**Membrane Builder**), mutate a reside (**Mutate Residue**) or add new parameters to the force-field expressions (**Parametrization Tool**). Only the first option seems to work correctly. The results



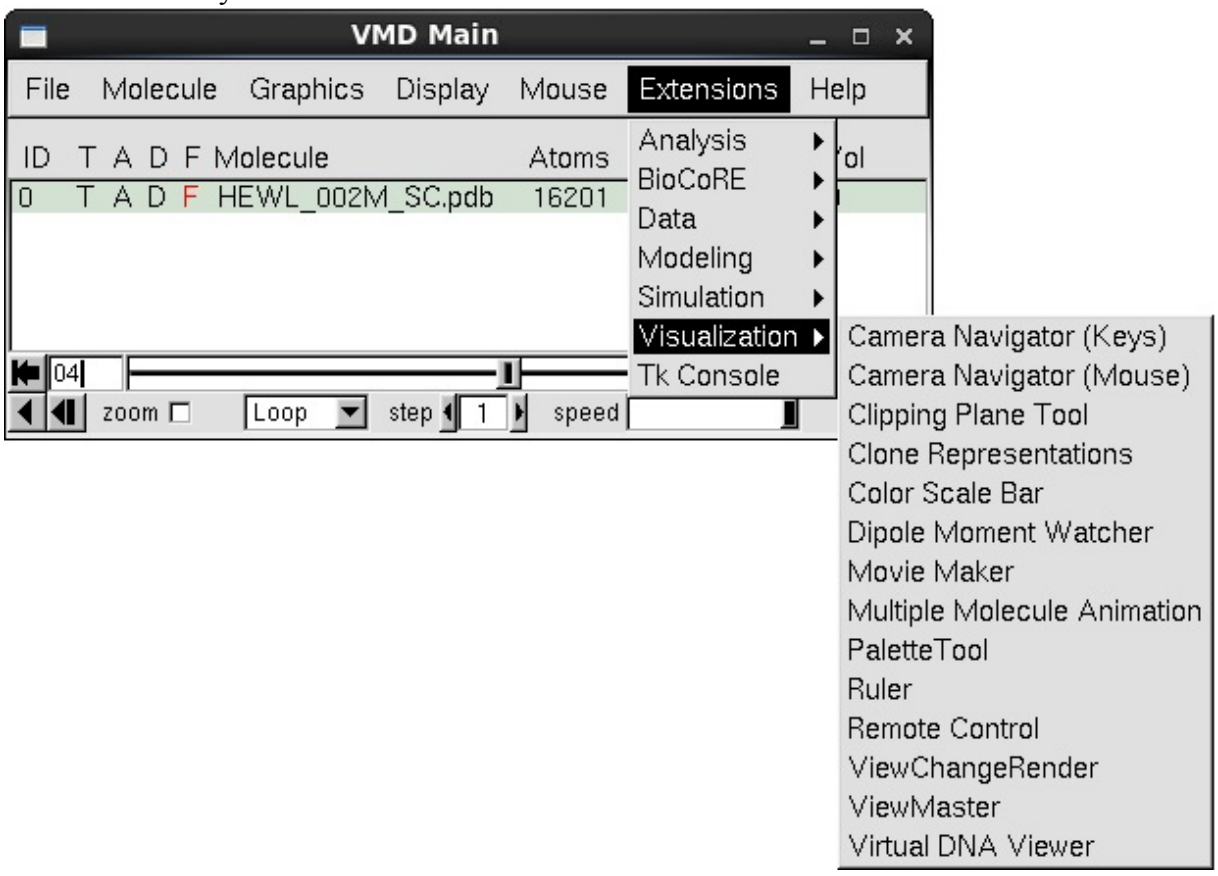
were compared to results obtained by using appropriate scripts. Some of options require additional libraries to be loaded which can be problematic. Most of the above actions can be obtained in other ways, therefore it is recommended that only **`Add Ions`** should be used.

5. Simulation

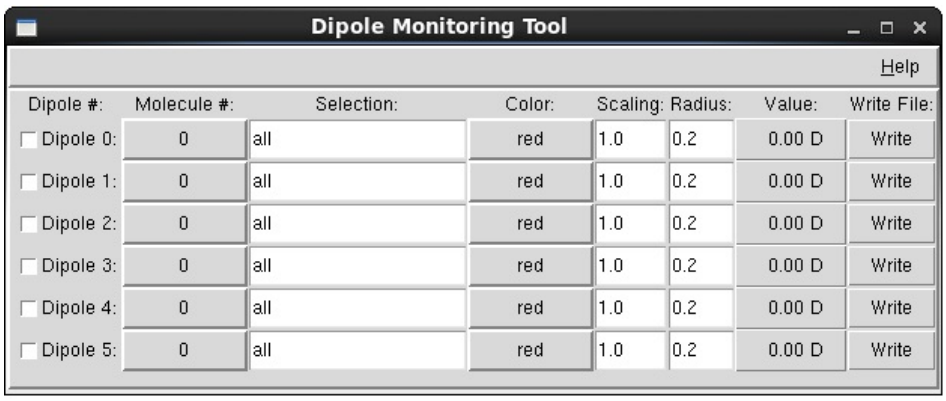
This menu does not seem to be useful. The jobs can be ran from the terminal which allows for better control of the job progress. Maybe it is useful for Windows users?

6. Visualisation

At this menu only 2 sub-menus seem to be useful.

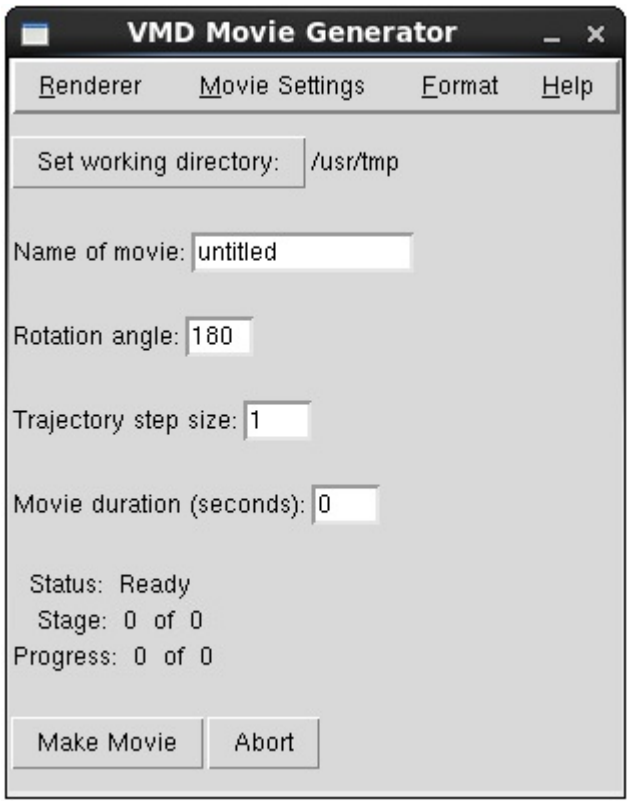


Dipole moment



Dipole Moment Watcher allows the dipole moment to be drawn for the given selection. The dipole moment is displayed in the VMD OpenGL window and upgraded with each trajectory frame. Moreover, the results can be saved to a file containing the frame number, x, y,z coordinates and the value.

Making the movie



To make a movie (*.avi file or animated gif) **Movie Maker** should be chosen. It allows movie to be created from the trajectory, as well as containing only the rotation of the structure. To make the *avi file displayable at Windows ensure that the **VMD OpenGL** window has dimensions 640x480 (or double). Then at the **VMD Movie Generator** dialog window under the **Movie Settings** tab, choose **Trajectory** (if the user wants to make the movie containing the trajectory only), and de-select **Delete image files**. Specify the **Trajectory step size** (it can be understood as stride) and the working directory by clicking **Set working directory**. Finally click the **Make Movie** button at the left bottom corner and … wait. Once all snapshots are rendered and saved to disk. Open the terminal shell and:

1) to change the file types type:
```
for l in *.ppm; do echo $l; convert $l $l.png; done
```

2) to make make the avi file type:
```
mencoder "mf://*.png" -mf fps=2 -o output.avi -ovc lavc -lavcopts vcodec=mpeg4:vbitrate=4000:autoaspect
```
3) to see the movie type:
```
mplayer -vo -x11 output.avi
```

Note: fps parameter has to be 2, othervise the movie will not play under Windows (will play on Linux)

7. Tk Console

Tk Console (Fig. 9) allows to use some tcl scripts and for example to solvate the molecule.

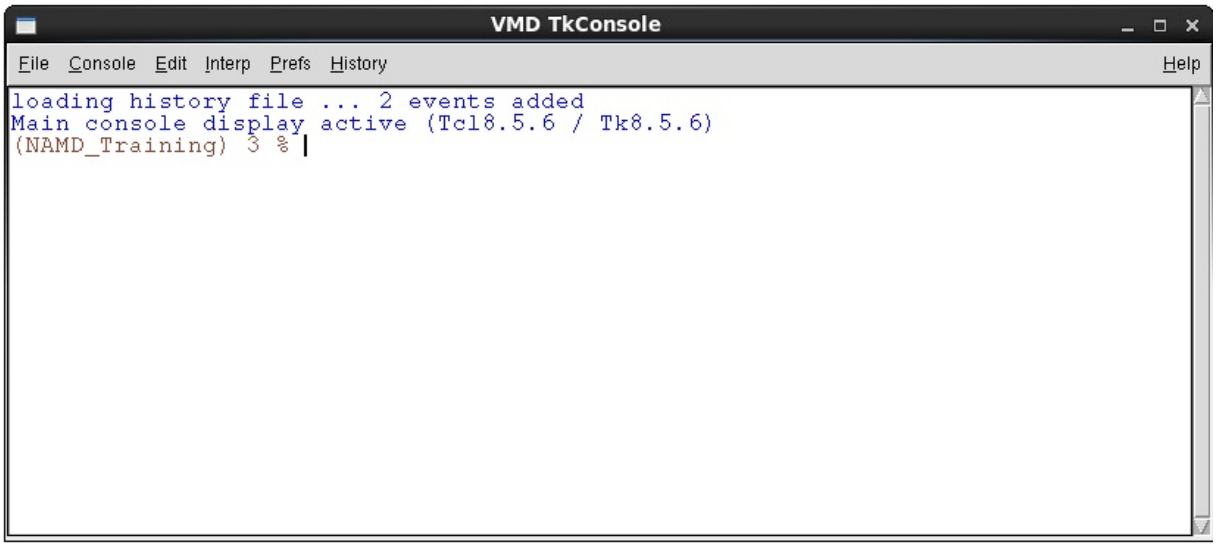


Figure 9. Tk Console dialog window.

Solvation

The commend typed at the Tk console:
```
solvate name.psf name.pdb -t 6 -o name_S
```

will add the solvation box to the given molecule. The distance between any protein atom and the end of the box will be 6 Å (-t 6) and the output prefix will be name_S (-o)

```
solvate name.psf name.pdb -x 1.0 +x 2.0 -y 3.0 +y 4.0 -z 5.0 +z 6.0 -o name_S
```
will add the solvation box to the given molecule. The box will extend from any protein atom by 1 Å in -x direction, 2 Åin +x direction, 3 Å in -y direction, and so on.

Tcl programs

The tcl procedure (rms_ca.tcl) allowing RMSD distances to be created can be downloaded from vmd web. At the TK console type

play rms_ca.tcl

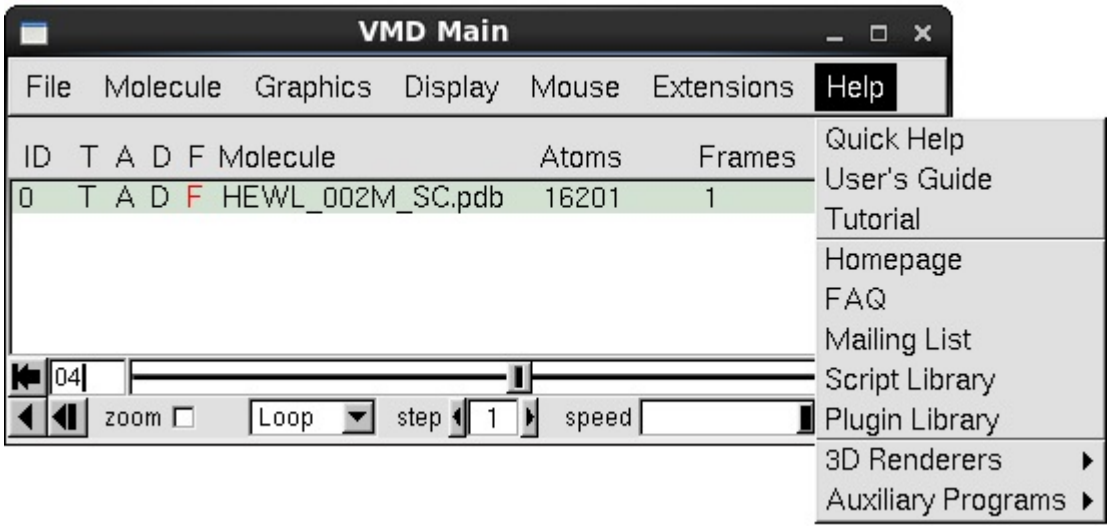
it will load the tcl program to vmd. Then type:

rmsdt_ca

The RMSD will be created and the rms_ca.dat file will be created in background. To change the atom selection or the output file (rms_ca.dat) name, the user should modify the rms_ca.tcl file.

The usage of tcl programs downloaded from the vmd Web is always explained within the tcl file.

Menu Help



If the computer is connected to the Internet it will open the manual or tutorial remotely.