TUTORIAL FOR RUNNING AND ANALYZING ADEPTH RESULTS

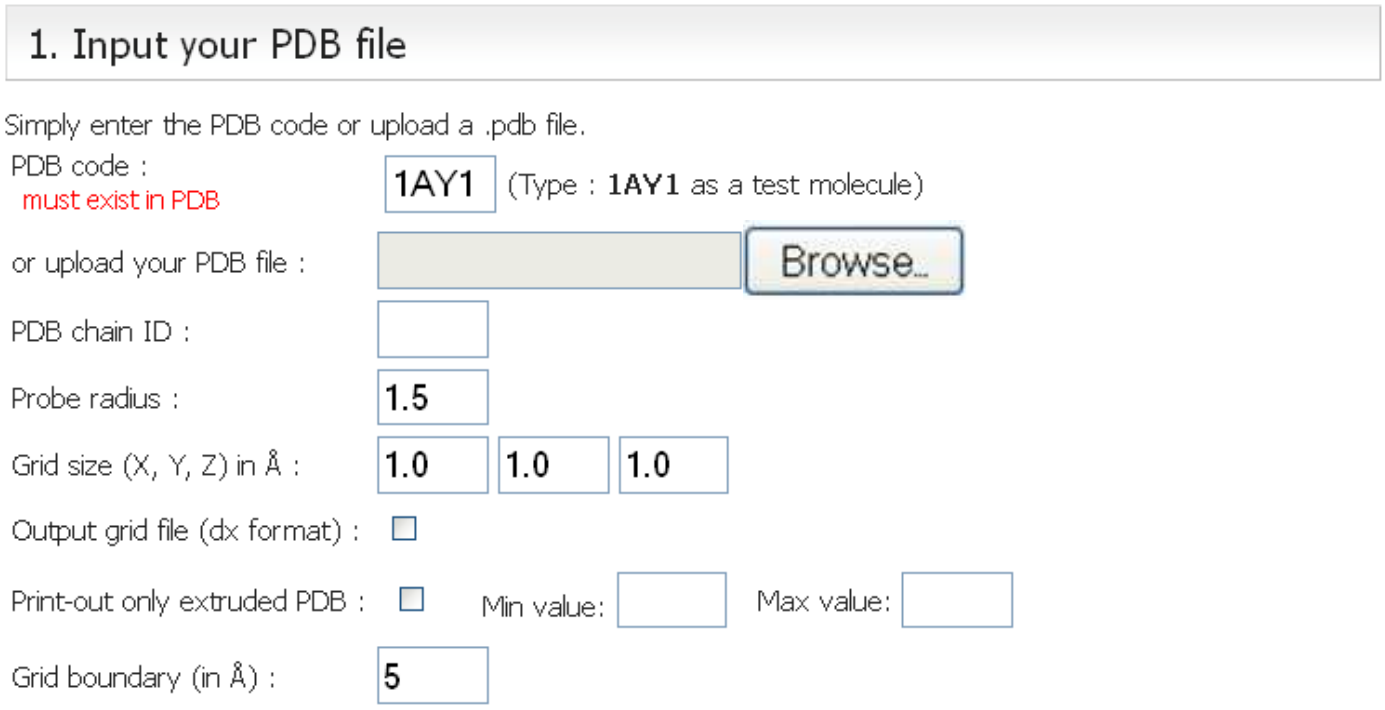
SWW Chen and JL Pellequer

Version 0.3

Updated on: 05-DEC-2012

1) Adepth web-server address

2) Select your PDB file



- Enter the PDB code if available from the PDB; otherwise, select the Browse button to load a structure from your local computer.

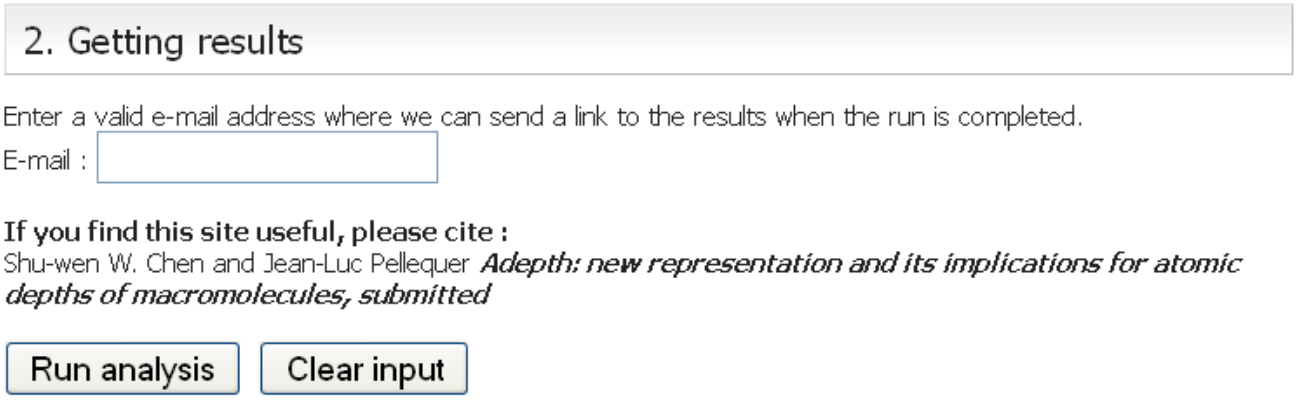
- The grid dimension is automatically computed using both the user input "Grid size (X, Y, Z) in Å" and the size of the macromolecule. The max number of elements in the grid is 300 in one direction. Thus, users MUST ADJUST the "Grid size" values in function of their macromolecule size.

- In the above selection, the "Output grid file (dx format)" has been checked. It will provide the necessary data to display an iso-potential map of the atomic depth with VMD. Small grid sizes will output very large DX files (~200 Mb).

- If one wants only the skin layer of a molecule, you have to check the box "Print-out extruded PDB", then choose the min and max depth values.

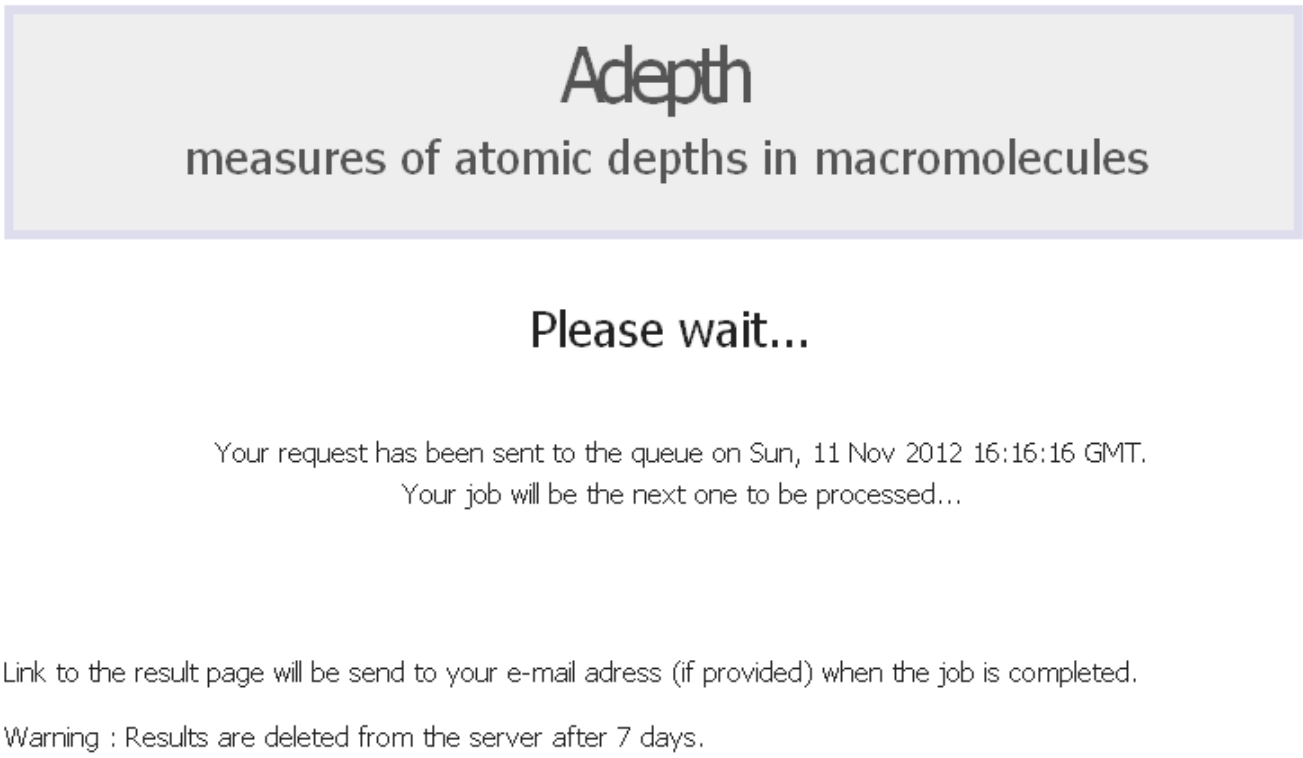
- "Grid boundary" defines the additional space between surface atoms and grid borders. The greater the number the smaller the molecule will appear on the grid.

3) Click on “Run analysis” button to start



The email address is not required. It is only used to send result data in a zip file to your email.

4) Waiting page



If you did not provide your email, wait a few minutes to get the result summary message.

Note: Although Adepth is very fast (a few seconds), the time to obtain results may vary upon the server load or upon the size of output (remember that dx files may be very large for small grid steps).

5) Result output

Results

```
.....................................................
Check the list of WARNINGS:
.....................................................
SDF has been calculated for: 1ay1.pdb
Selected chain Id:
Output PDB file : 1ay1_adepth_sdf.pdb
Probe radius : 1.5 (A)
Grid spacing : 1.0 1.0 1.0  (A in X Y Z)
Grid boundary: 5 (A)
Skin min val : 0.0 (A)
Skin max val : 9999.99 (A)
-----------------------------------------------------------
When using this work, please cite:
SWW Chen and JL Pellequer (2013) Adepth: new representation
and its implication for atomic depths of macromolecules.
Submitted.
-----------------------------------------------------------
```

Result files have been mailed to:
You can also download results.zip with all output files or display output PDB file using JMOL applet...

For additional information please contact the coordinator on the right hand of the screen.

Result file is accessible by clicking on "result.zip", then save the file on your computer.

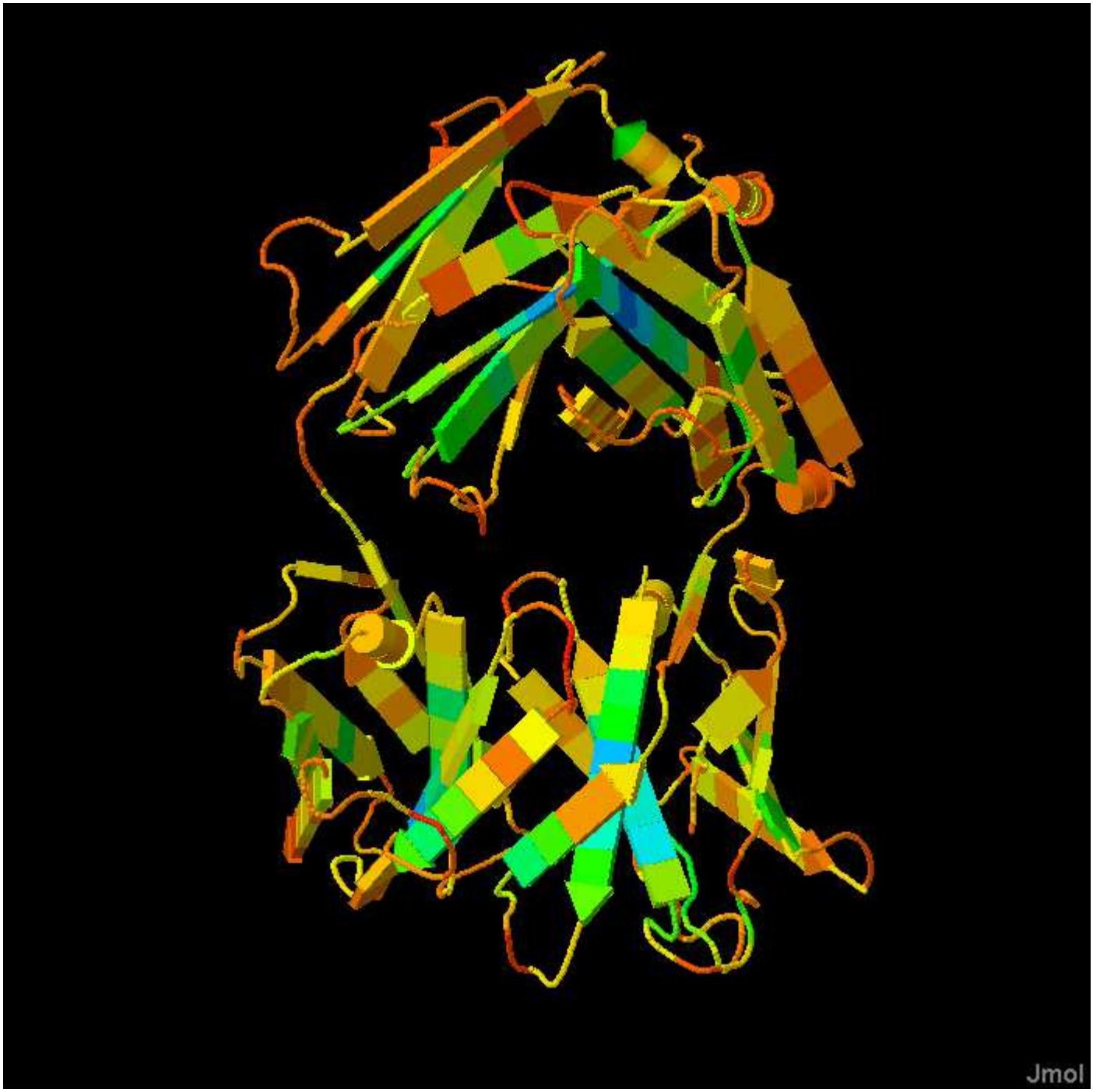
This file contains:

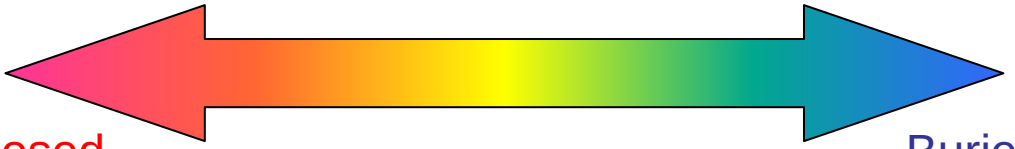
- ***.out** → Log file from the run
- ***.pdb** → Original PDB file
- ***-adepth_sdf.pdb** → Output PDB file with SDF values in b-factor column
- ***.grid** → Output SDF values on the grid§
- ***.dx** → Transformed .grid file into DX format for VMD§

§These files are only present when the "Output grid file" option is selected.

- Display the output PDB file using an atomic-depth colored-coded ribbon by clicking on “display output PDB file”.

- A Jmol applet will display the result in a new window. By default the molecule is shown using the “Rockets” style.





Exposed atoms

Buried atoms

6) Interpretation of results

- The atomic depth value is the distance of the atomic center from the solvent accessible surface
- The header of a sample *_sdf.pdb file is shown below:

```
REMARK: input pdbfile:/opt/appli/temp-day/adepth-41/lay1.pdb
REMARK: PROBE radius: 1.5 (A) ;    GRID spacing x-y-z: ( 1.0 1.0 1.0) (A)
REMARK: sdf range: ( -11.4098  -2.4376 ) (A)
REMARK: COLUMN OCCUPANCY: scaled sdf magnitude
REMARK: COLUMN B-FACTOR: sdf magnitude
ATOM      1  N   ASP L   1      27.504 -17.288  18.937  0.96  3.23 0
ATOM      2  CA  ASP L   1      27.334 -16.029  19.656  1.04  3.82 0
ATOM      3  C   ASP L   1      27.777 -14.855  18.795  0.98  3.62 0
ATOM      4  O   ASP L   1      28.548 -13.993  19.232  0.92  3.04 0
ATOM      5  CB  ASP L   1      28.128 -16.048  20.959  0.89  3.26 0
ATOM      6  CG  ASP L   1      27.644 -15.011  21.948  0.87  3.18 0
ATOM      7  OD1 ASP L   1      26.551 -14.437  21.738  0.85  2.79 0
ATOM      8  OD2 ASP L   1      28.353 -14.782  22.949  0.78  2.56 0
ATOM      9  N   ILE L   2      27.287 -14.850  17.560  1.28  4.27 0
```

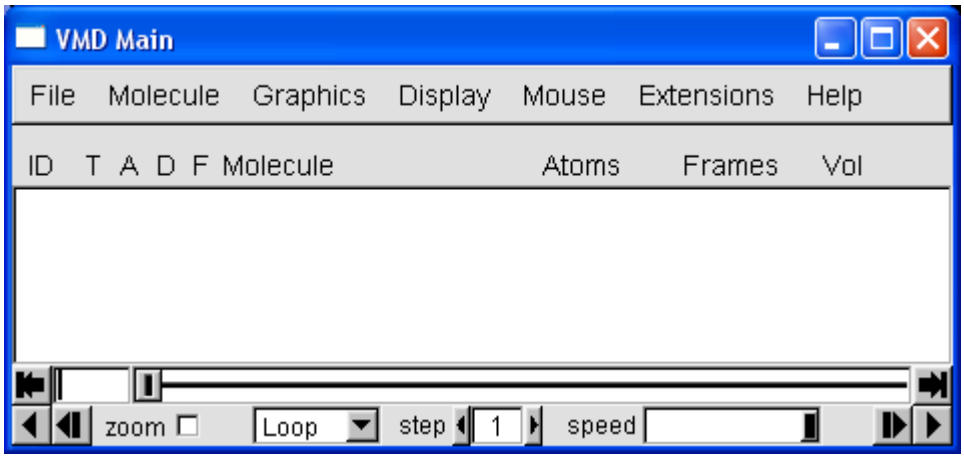
- Red boxes indicate the values for the probe radius and the grid spacing (both in Å)
- Blue box indicates the min and max depth values of macromolecule
- Green box indicates the atomic depth. For respecting the PDB format of the B-factor column, depth values are provided as positive values.

7) Visualization of results

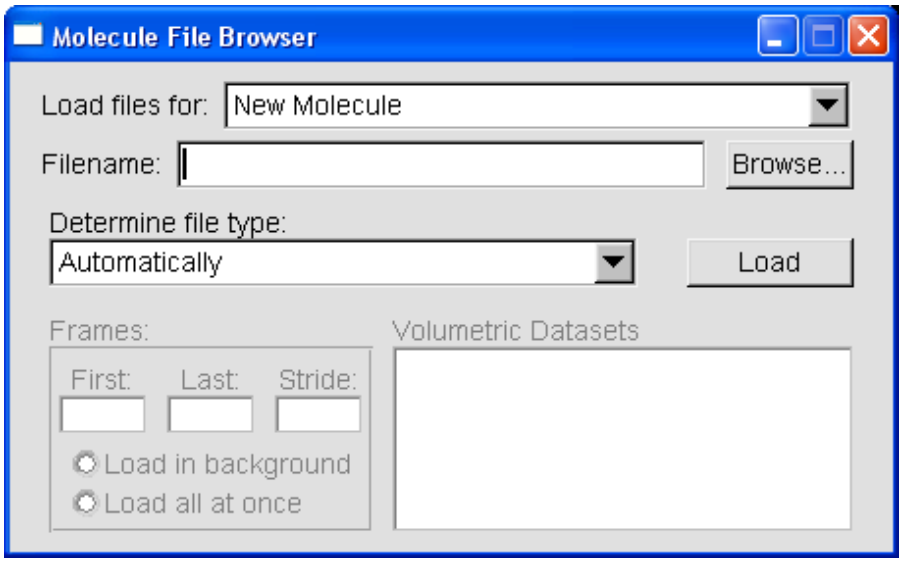
VMD is a molecular visualization program for displaying, animating, and analyzing large biomolecular systems using 3-D graphics and built-in scripting.
Go here to download: http://www.ks.uiuc.edu/Research/vmd/
To cite VMD: Humphrey W, Dalke A and Schulten K (1996) VMD: visual molecular dynamics. *J. Mol. Graph.* **14**: 33-38.

1. Run VMD

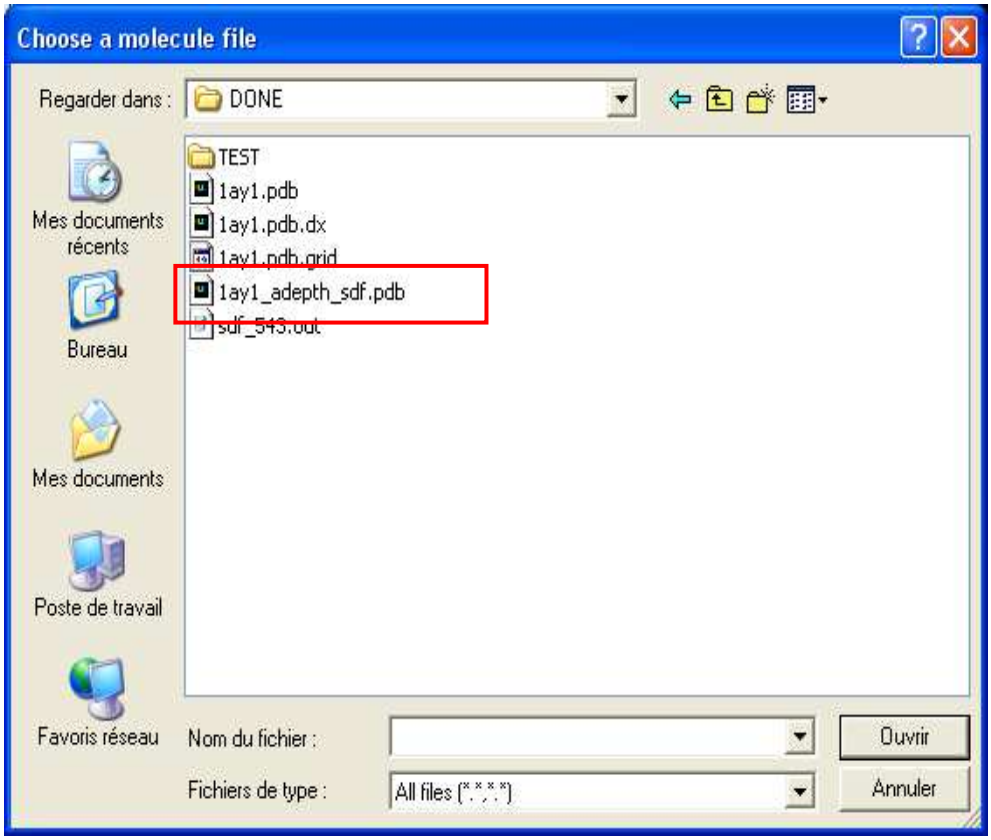
The VMD main menu pop-up.

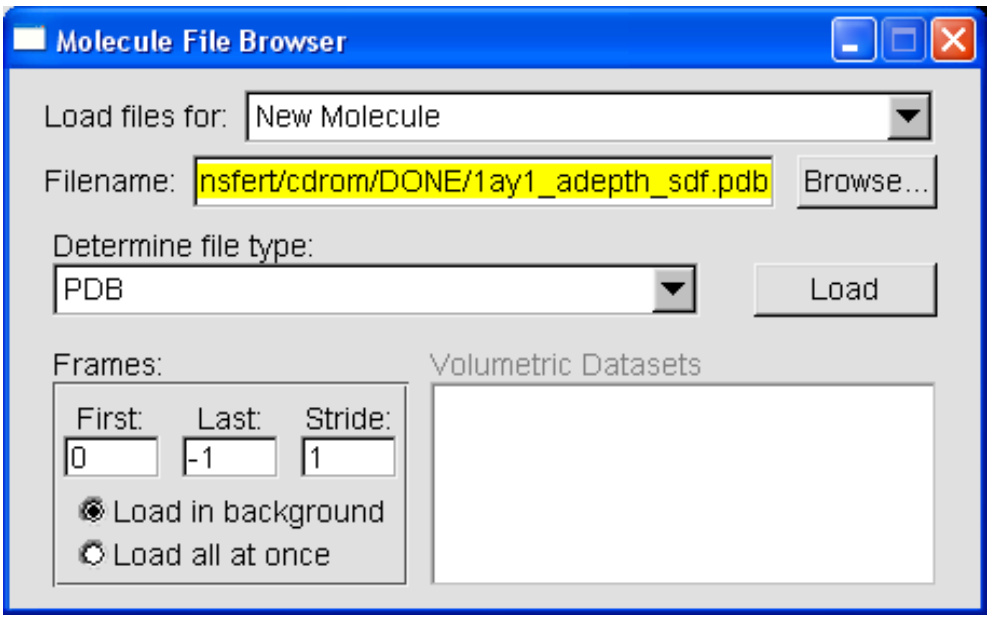


2. Click on <File> and select <New molecule…>.
The "Molecule File Browser" will pop up.

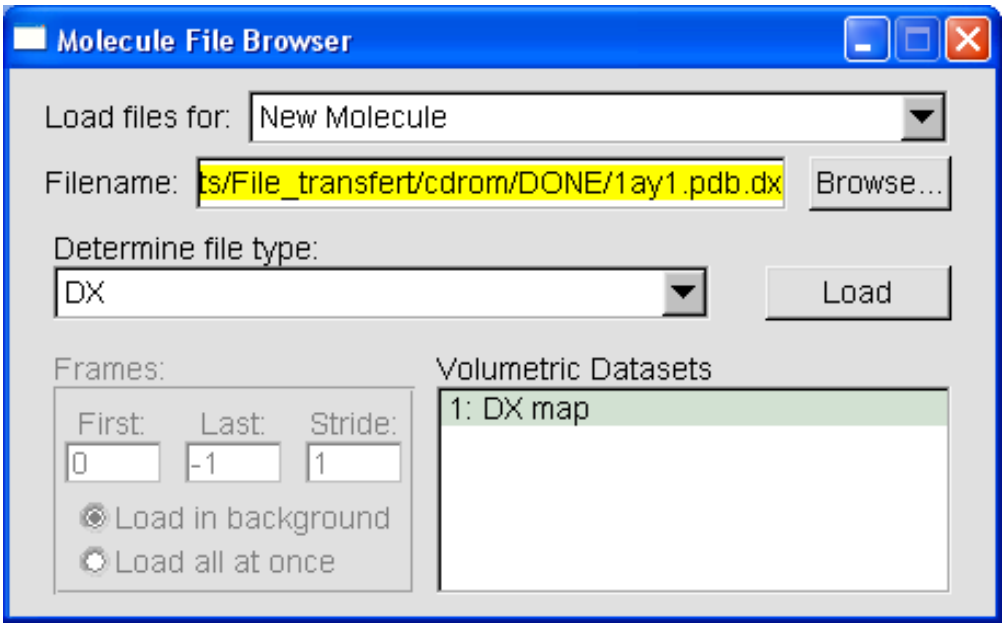


3. Click on "Browse…" button to load your input file. The "Choose a molecule file" menu will pop up, select your molecule and its name will appear in the "Molecule File Browser" highlighted in yellow.





•In the above window, the "Determine file type" has been automatically selected. Click on "Load" to open the output file in PDB format from Adepth,



•Repeat step 3 to open the corresponding DX grid output file.

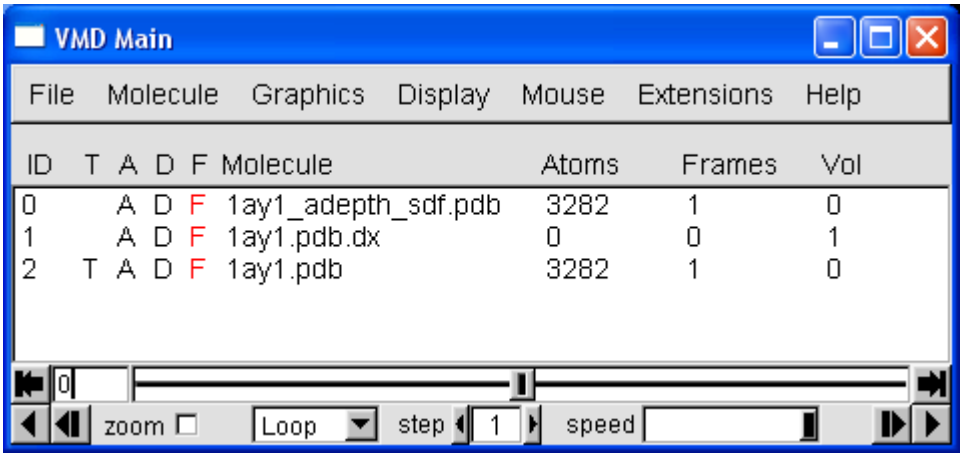
- Careful, when loading multiple files with the above menu, it is necessary to SELECT "Load files for:" New Molecule each time.

Now, in the main window of VMD, three molecules are loaded:

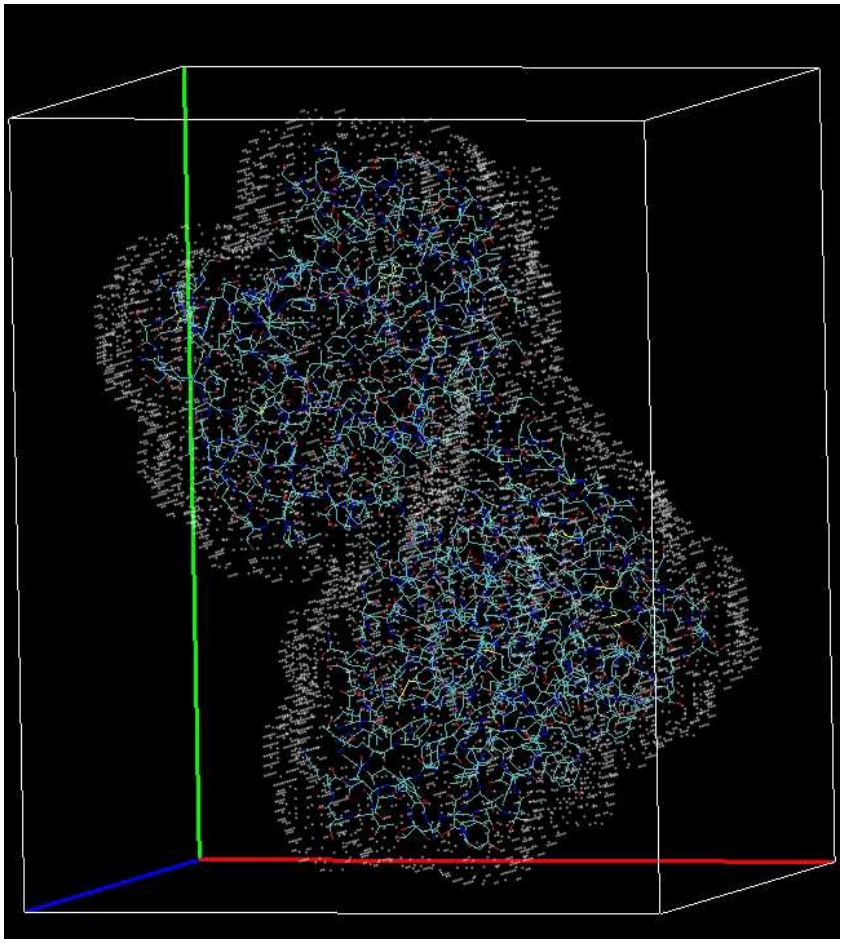
Input PDB file: 1ay1.pdb

Output Adepth PDB file: 1ay1_adepth_sdf.pdb

DX grid file: 1ay1.pdb.dx

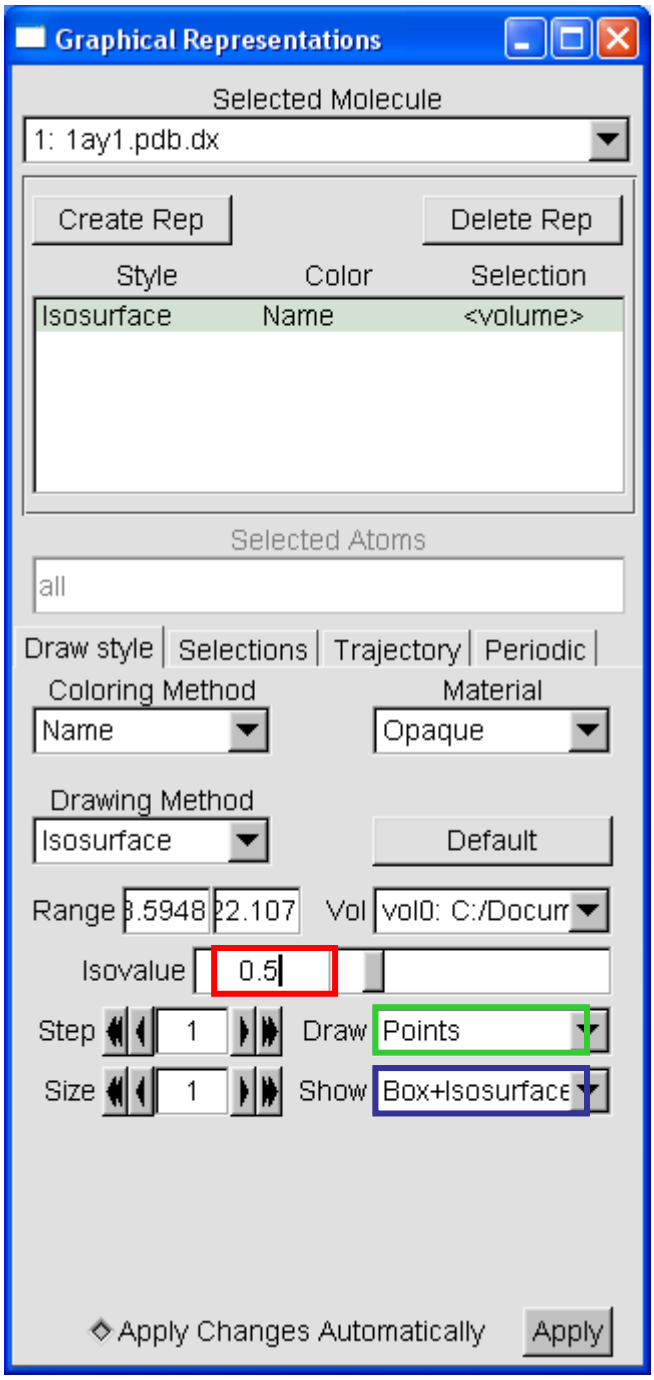


You should see something like this in the Graphics window:



4. Wireframe view of the isosurface

a/ Click on the VMD main window <Graphics> and select <Representations…>. The “Graphical representations” menu will pop up.

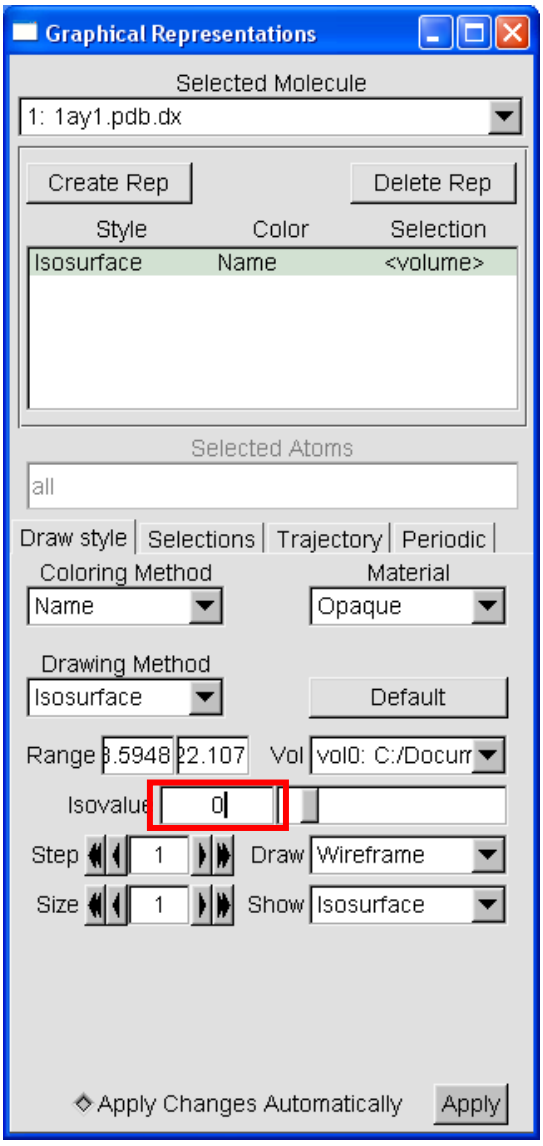


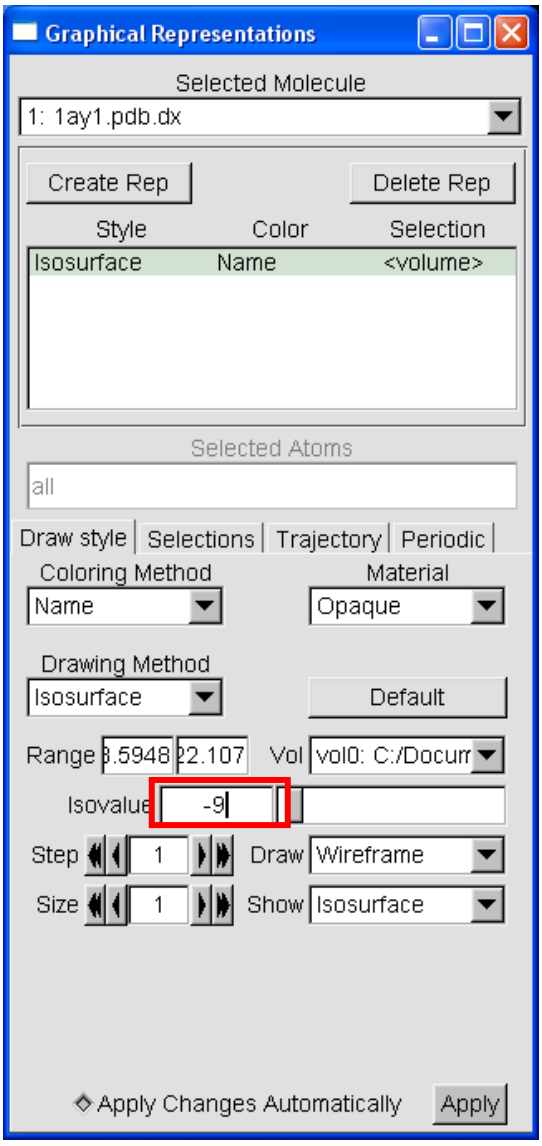
b/ Click on the Draw “green box” and select “Wireframe”.

c/ Click on the Show “blue box” and select “Isosurface”

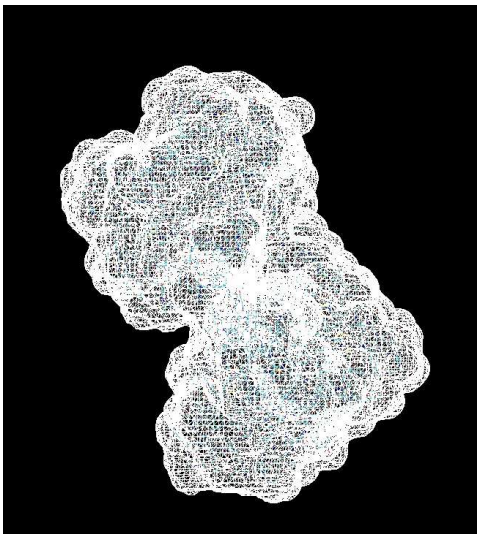
d/ Click in the Isovalue “red box” to change the depth of the isosurface

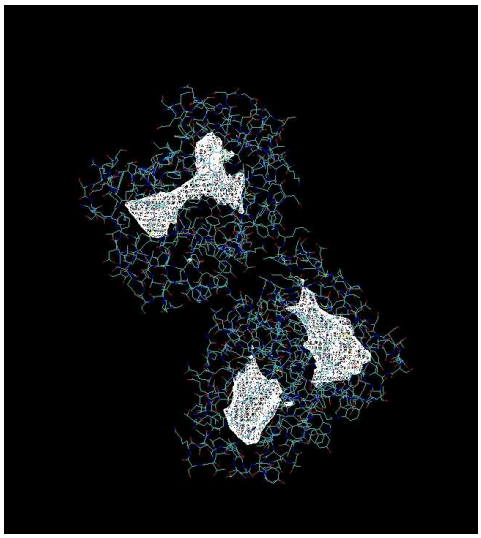
Representation of two different isosurface depths:





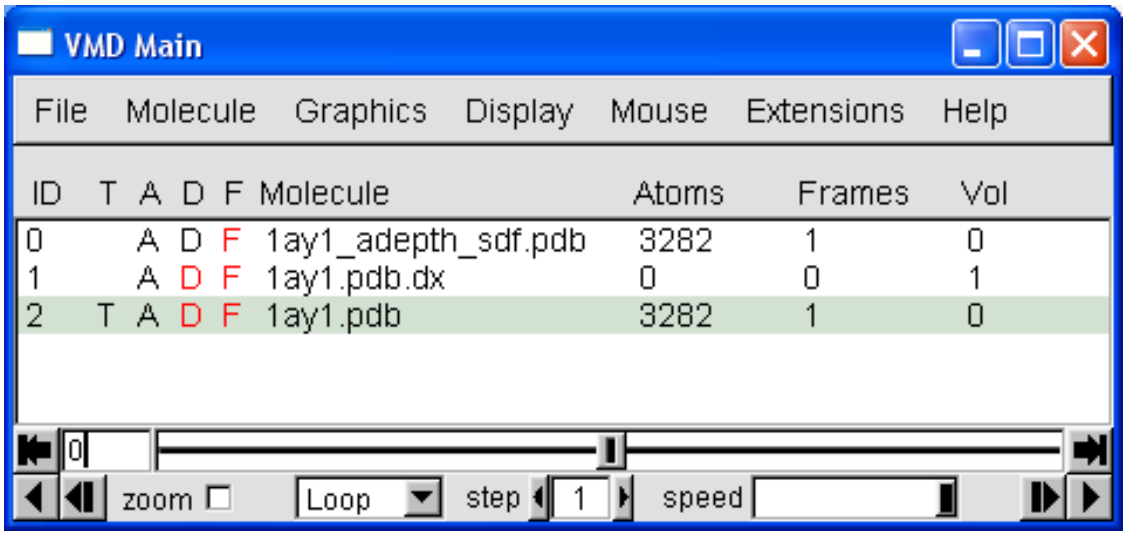
Using the above settings, the graphical window should looks like:





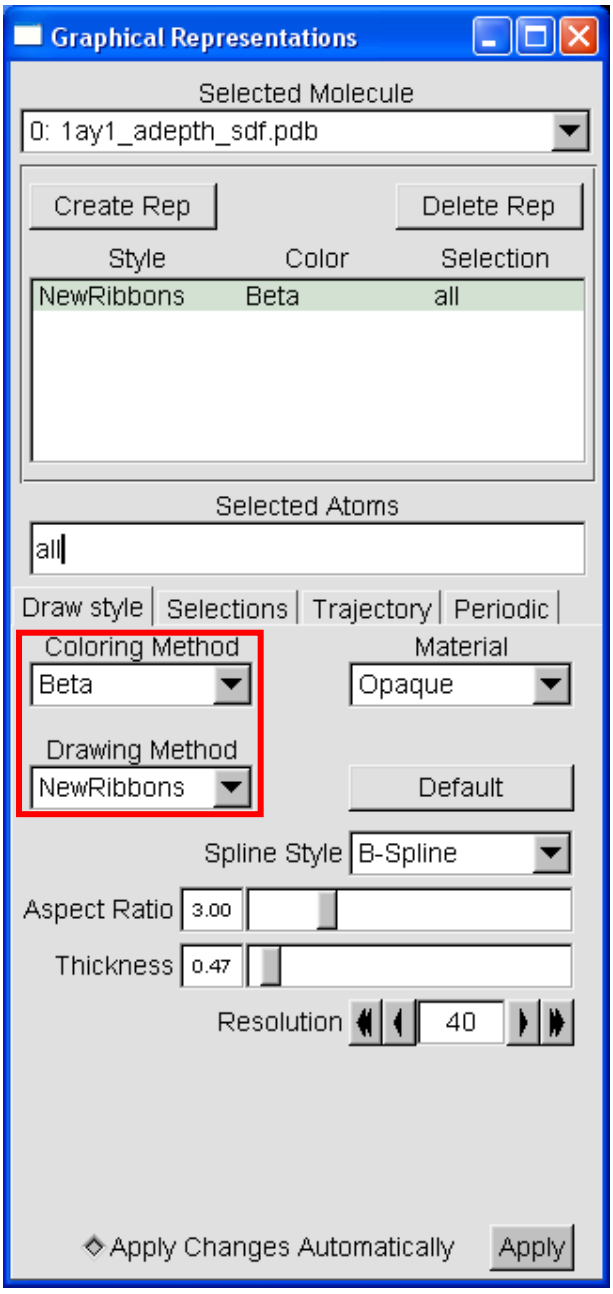
5. Display of atomic depth using VMD ribbons

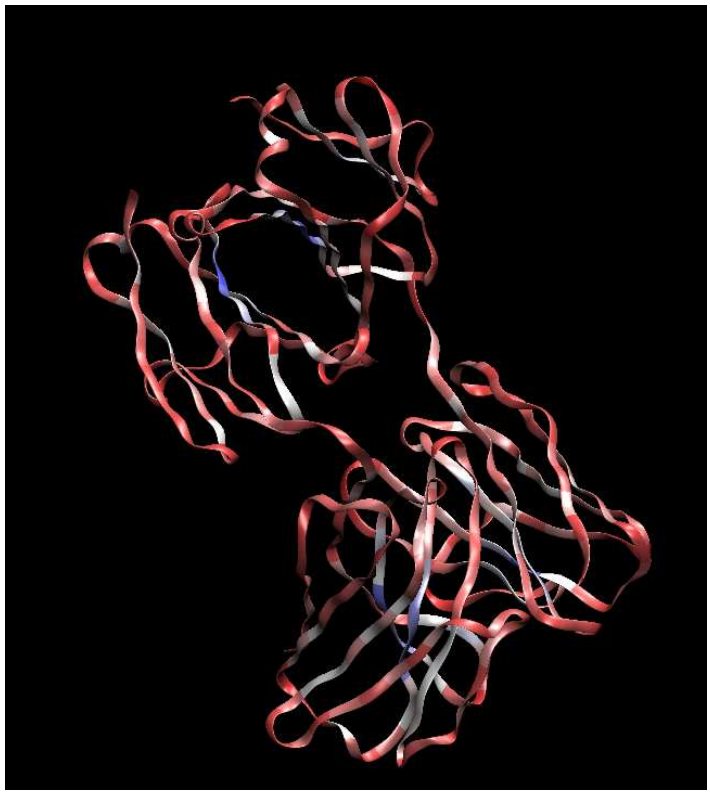
a/ Disable the display of isosurface: use the "VMD main" window and double click on the D letter of the corresponding molecule you wish to disable:



b/ In the above example, both *.pdb and *.dx files have been switched off.

c/ Using the "Graphical Representations" window, it is possible to display the atomic depth values on a ribbon representation of the macromolecule as shown in the below graphics:





The above image shows the “New Ribbon” drawing method colored according to values present in the b-factor column.

In the above window, the selected “Drawing method” is “New Ribbons” and the selected Coloring method is “beta”. This option select a default blue-to-red gradient color scale for values present in the B-factor column of a PDB file.