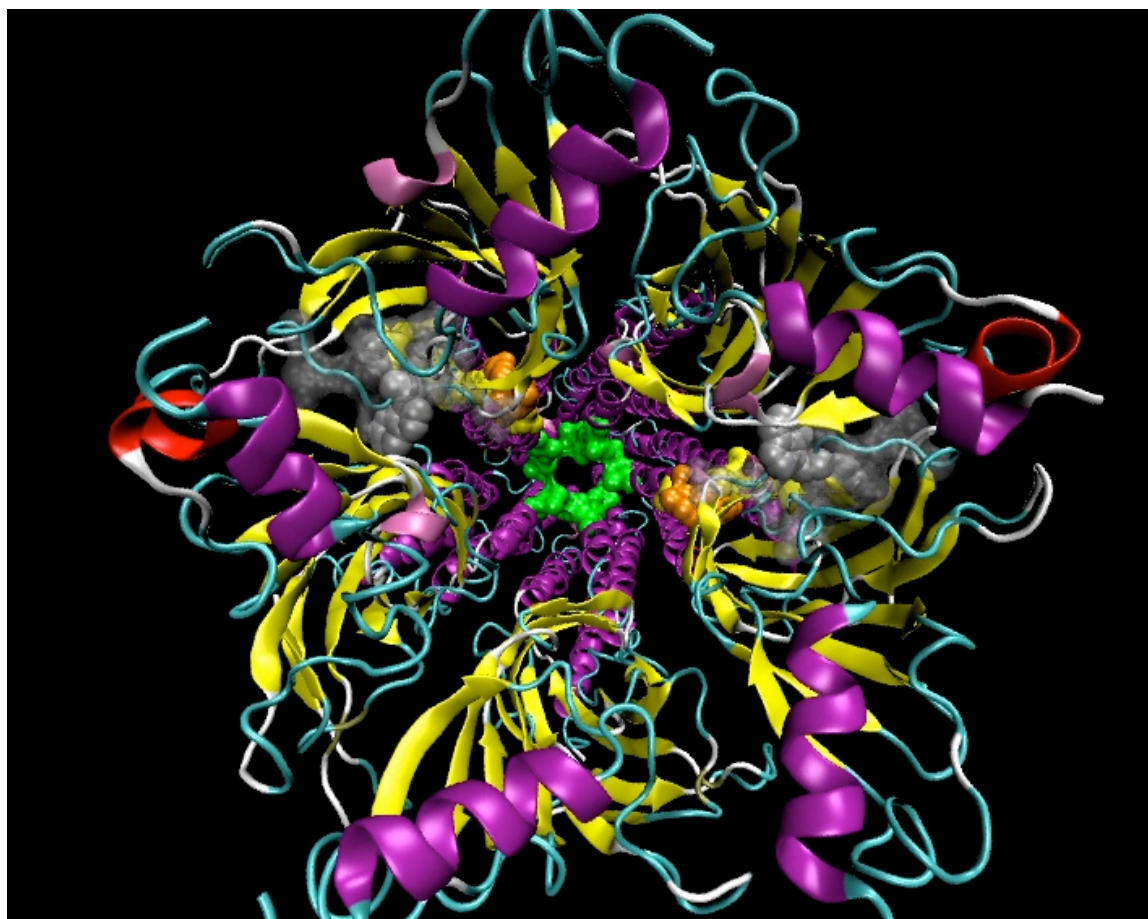


VISUALIZING MOLECULES WITH **VMD**



Aim of the course

After completing this course, you will be comfortable and proficient with VMD. You will learn to display and manipulate molecules, change display styles for all or parts of the molecule and add annotations with the overall goal to create an image of your molecule ready for publication in print or online or for use in slideware.

Some VMD Features

- No limits on the number of molecules, atoms, residues, except available memory.
- Many (many!) molecular rendering and coloring methods.
- Stereo display.
- Extensive atom selection syntax for application of display styles.
- Reads most molecular file formats.
- High quality output through interface to popular ray tracing packages, including POV-Ray, Tachyon, and Raster3D.
- Can be scripted with Tcl/Tk or Python. A selection of scripts for VMD is available at http://www.ks.uiuc.edu/Research/vmd/script_library/
- VMD runs on Windows, MacOSX, and most Unix variants.
- Available for free at <http://www.ks.uiuc.edu/Research/vmd/>.

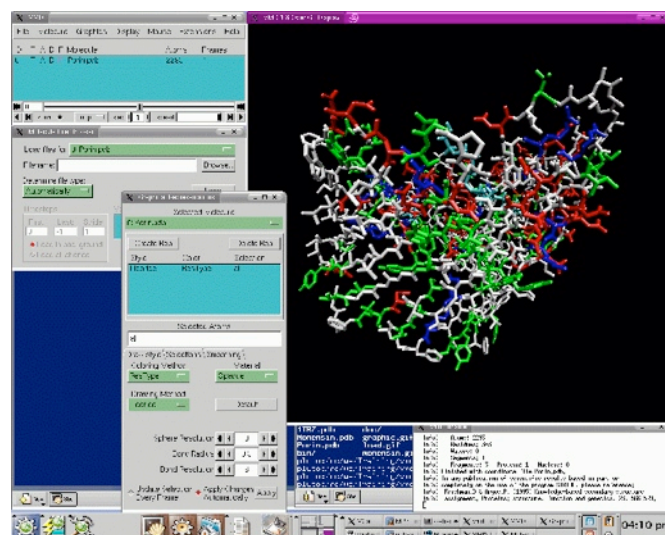
VMD Layout

VMD opens multiple windows on your desktop. Initially, you get the main graphics window, the VMD console and the main form. VMD refers to its menus and dialog boxes as forms, so we'll use this term also.

In the image on the right, you can see the graphics window on the top right, the VMD console below it, and the main form on the top left. Also open are the Molecule File Browser and the Graphical Representations forms, along the left edge. The rest are various icons and windows from the Linux KDE desktop and are not related to VMD.

From the main form you can access all graphical interface features of VMD, i.e. open other forms which will let you drive VMD.

From the console window, you can issue VMD text commands and/or run scripts (as well as save them).



Background Information

VMD Philosophy

It is important to note that while VMD can display any type of molecule, it is especially geared towards display of macromolecules. For smallish molecules it lacks good support to display double and triple bonds.

An central design feature of VMD is that you work with multiple graphical representations of your molecule. For example, you may display the whole molecule as lines (wireframe) and then display selected residues as space-filling balls. These representations are independent of each other, but hierarchical, i.e. the last one overdraws previous ones, if applicable.

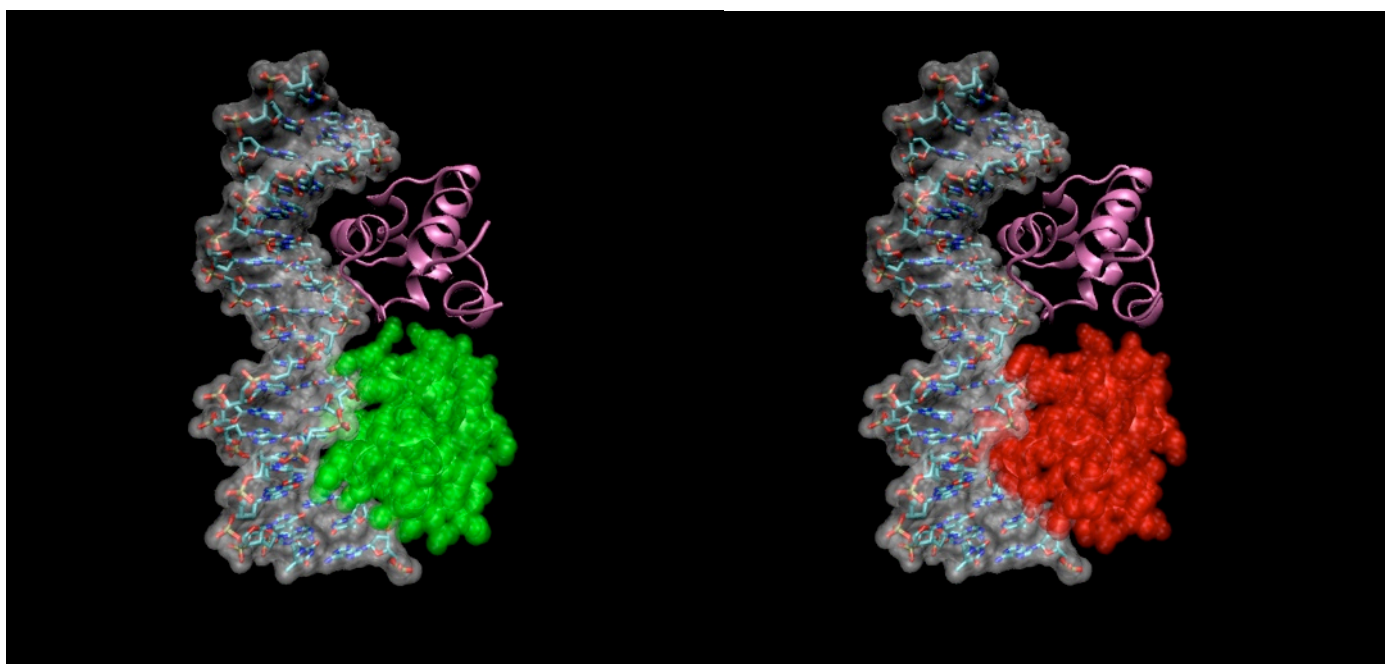
Availability

VMD can be downloaded for free from <http://www.ks.uiuc.edu/Research/vmd/>. It is available for most Unix flavors, Windows and MacOSX.

Getting Help

VMD documentation is on-line at <http://www.ks.uiuc.edu/Research/vmd/current/docs.html>. Also available on-line are quick help topics and a FAQ. In particular, you can select Help on the main form, and this should point your web browser straight to the VMD Quick Help section of the VMD web site.

In addition to web pages, the manual also comes in pdf format so that you can download and print it.



Phage 434 *rho* repressor bound to DNA. Left, green: Bound conformation. Right, red: free conformation. PDB entries 2CRO and 3CRO.

Sources of 3D Molecular Structures

You will need molecular coordinates to load into VMD. Unless you have your own experimental or modeled coordinates, here are a few resources:

Small Molecules

On on-line index of molecular structure resources is at <http://molvis.sdsc.edu/visres/pdbs/titles.jsp>

Proteins and Other Biomacromolecules

The Protein Databank (PDB) is the main resource for 3D coordinates of biological macromolecules. The PDB web site is <http://www.rcsb.org/pdb/> (This site is right here in San Diego at the Supercomputer Center at UCSD.) There are extensive search functions at this site, as well as a host of other informa-

tion. Of particular interest is the Molecule of the Month section at the PDB, which illustrates and explains a different biomacromolecule each month. This feature is written and produced by our institute's David Goodsell and aimed at a broad audience, including science teachers and students at the middle or high school level, as well as college undergrads, graduate students and .

More structural resources are indexed at <http://molvis.sdsc.edu/visres/pdbs/titles.jsp>

Getting Started

A Small Molecule - Proline-Leucine Dipeptide

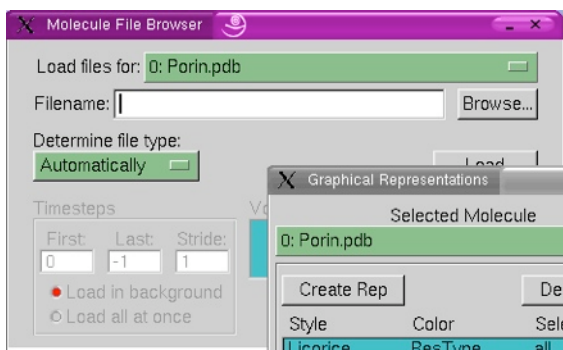
Tasks in this section:

Loading a molecule, rotation, translation, scaling, changing and adding graphical representations, saving application state, rendering output

After starting VMD you will see three windows: The main graphics window, the VMD console and the main menu form. Reposition the windows such that you can see all of the graphics window, all of the main form and most of the console.

Then select *File/Load Molecule* in the main form to open the Molecule File browser form..

Click the Browse button. In the resulting file chooser find and select the file *dipeptide.pdb* and click OK. Now click *Load* in the Molecule File Browser. The structure of the Pro-Leu



dipeptide is displayed in the graphics window. Also, the main form now has a line in its main area indicating various things about the loaded molecule. As is apparent, you can load more than one molecule into VMD. The limit is set by the sum of real and virtual memory of your computer, not by the software.

Now move the mouse pointer into the graphics window. When you hold down the left mouse button and move the mouse you rotate the molecule about the x and y axis. When you hold down the right mouse button and move the mouse you rotate the molecule about the z axis. (If the mouse is in rotate mode, that is.)

Next, select *Mouse* in the main form and survey the options. This menu controls all mouse functions and you'll see that the mouse is in Rotate mode right now. If you select another mode and go back to the graphics window, you will find that the mouse buttons have different functions now. When you start VMD, the mouse is in rotate mode and picking is off as depicted by the absence of a highlighted radio buttons in the *Label*, *Move*, *Force* and *Move Light* section of the menu. It is a good idea to select *Label/Atoms* instead of nothing.

If a pick mode is on and you click a mouse button while hovering over an appropriate part of your molecule, the VMD console will display information about this part of the molecule and a label will be added in the graphics window. You might in fact want to glance at the console regularly to catch up on what VMD is doing.

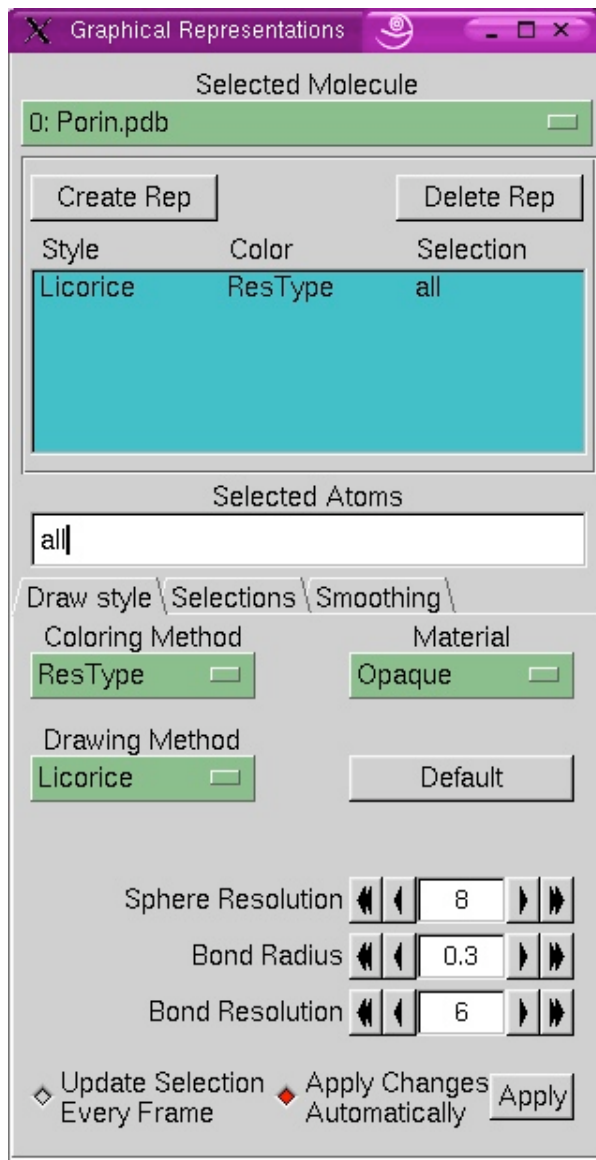
Important: You can cycle between rotate, translate and scale mode with the r, t and s keys instead of going through the Mouse form. This saves a lot of time and will soon become second nature. Experiment with the different mouse functions

now so that you get to know them. Hint: You can delete atom labels when they become too numerous by selecting *Graphics/Labels...* and taking the appropriate action in the resulting form. The Labels form also contains info about the picked / labeled part of the molecule.

Finally, before the desktop gets more crowded, close all forms except the main form by clicking on their close button in the window title bar (usually an X near the top right or a red button near the top left, depending on what operating system you are on).

Changing the display style (graphic representation)

Select *Graphics/Representations...* on the main form. The Graphical Representations form appears. This will most likely be your most used form. It lets you build up sophisticated displays in



successive 'layers'. Right now there is only one representation where all atoms are selected which are drawn as lines and colored as dictated by the atom names.

First, change the Drawing Method to VDW. This draws atoms as balls with the size dictated by their VanderWaals radius, i.e. they are depicted more or less with their 'real' size relative to each other. This gives us a good idea of the total size and overall shape of the molecule, but it dramatically impairs our ability to discern bonded atoms and learn about the architecture of the molecule. If you change the Drawing Method to CPK, the molecule will be drawn with smaller balls at the site of each atom and the balls are connected with bonds if a chemical bond exists at that location. This representation helps us understand the chemical makeup of the molecule. It is important to realize that neither the small balls nor the bonds in this representation represent true physical entities and their dimensions. Atoms are not balls of that size and more importantly, bonds are not sticks. Instead they just *represent* the location of atoms and/or chemical bonds. This level of abstraction is designed to improve our understanding of the molecular structure.

We can take the abstraction one level further by getting rid of balls altogether. Choose *Drawing Licorice*. This draws bonds as round tubes and caps them with half spheres. This is a very nice and informative representation for smallish molecules. We simply keep in mind that every vertex, corner or end point implies the location of an atom. The atoms' identity is encoded by the coloring of the tubes. This representation lets us concentrate solely on the chemical makeup of the molecule. As a side note, the *Bonds* method would be similar, except for the fact that terminal bonds are not capped and vertices are not closed, which makes Bonds virtually useless (in my opinion, anyway).

Now we have a pleasing and informative display of this dipeptide, ready for output. We can now do two things: 1) save the application state so that we can reload the molecule and regenerate the exact view and graphical representation, and 2) save an input file for a raytracing application to get a very high quality rendering of the molecule which can be printed and published (or shown at a seminar).

Saving the application state

In the main VMD menu form, choose *File/Save State* and give the file the name *dipeptide.vmd*. The file name, including extension are completely arbitrary.

To recreate the same view of the dipeptide in your next VMD session, choose *File/Load State* and locate and choose the appropriate state file.

Alternatively you can drive VMD via typed commands. As an example, in the VMD console window, type

```
save_state dipeptide.vmd
```

To recreate the same view of the dipeptide in your next VMD session, you will have to type

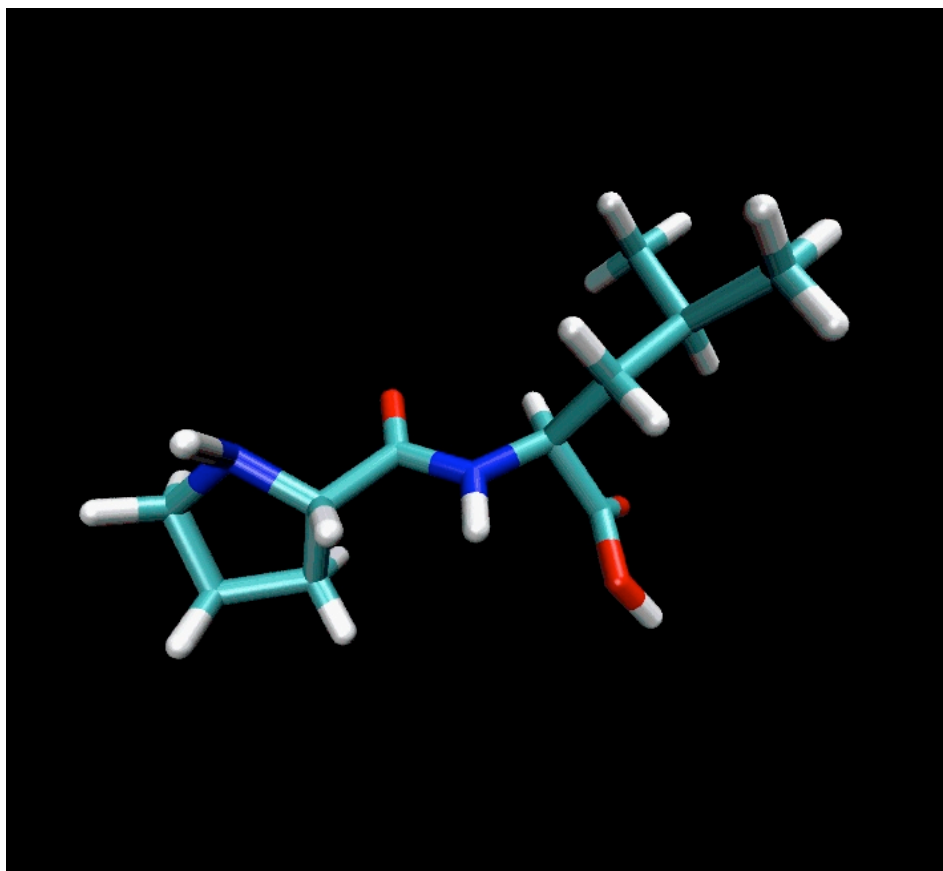
```
play dipeptide.vmd
```

You can also log all or part of your session with the command `logfile <filename>` typed in the vmd console. You can replay the logfile with `play <filename>`.

Generating input for ray-tracing

Click *File/Render* in the main form. From the list of available ray-tracers and rendering programs, select Tachyon and click *Go*. Two files are created: `plot.dat` and `plot.dat.tga`. The former contains instructions for tachyon about the scene to be rendered and can be deleted once you are happy with the result. The latter file, `plot.dat.tga` is a TARGA format image file and can be opened and manipulated with most image editors, e.g. Adobe Photoshop.

Note that other than tachyon, no rendering programs are installed automatically with vmd. You will have to install the one of your choice on your computer. Also make sure that you are happy with the suggested render command and options.



Maximize the Visual Impact of Your Image

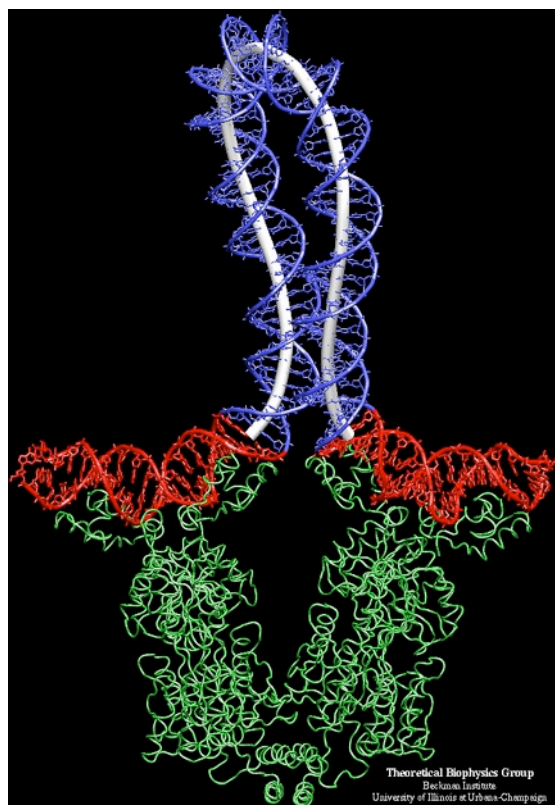
New Tasks in this section:

Direct interaction with raytracer, optimizing molecular representation, changing system colors

Generating a high resolution image

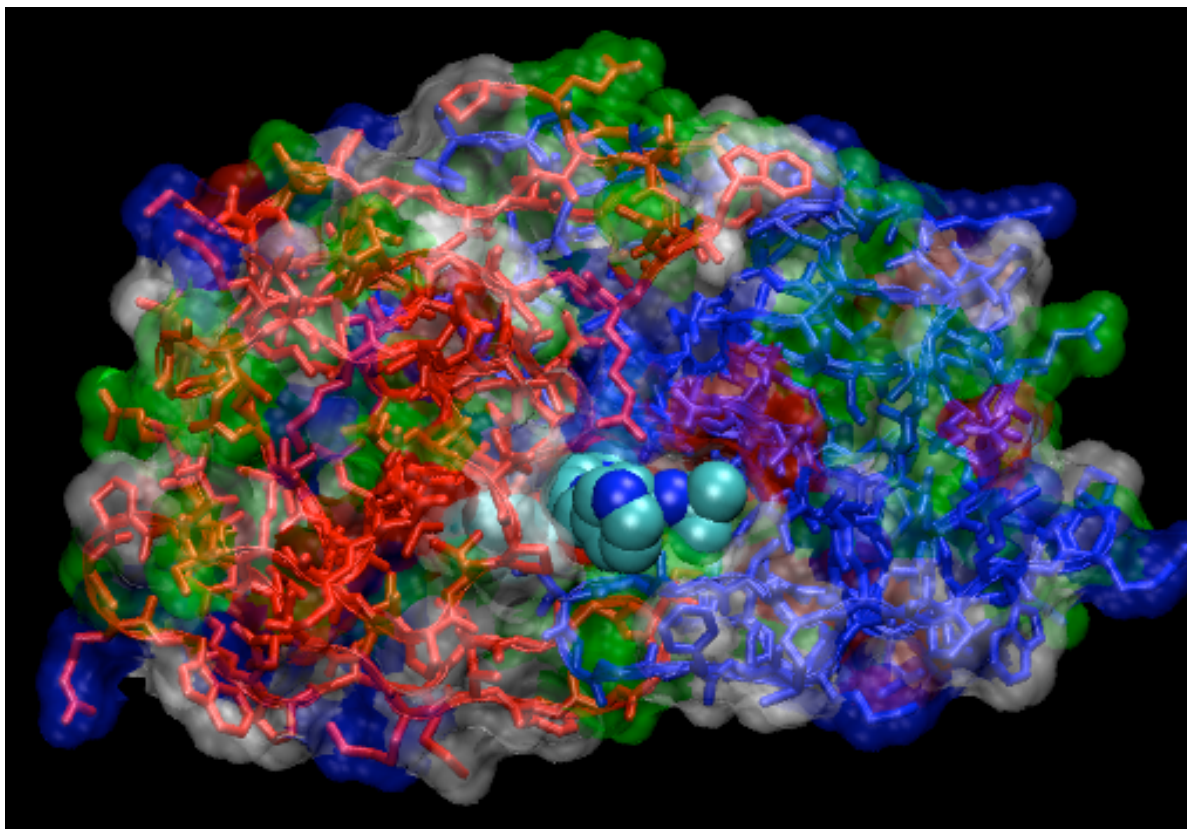
While the image generated before is fine for use in PowerPoint or similar context, you will need a high resolution image for print purposes so that your molecule will look its best. Let's assume that you want your image to span two columns (the full page width) in the journal you are going to publish in, or 6.5 inches, and that the journal's masthead states that images will be printed at 600 dpi. This translates to 6.5 inches * 600 dots per inch = 4000 dots width for your final image. Find the plot.dat file which was generated previously and open it in a text editor (not MS Word!). The second line of the file contains the resolution information and needs to be changed such that the line reads **Resolution 4000 4000**. That's assuming your image has equal width and height, otherwise make sure that the y resolution is scaled by the same factor as the x resolution. Now save the file and exit the text editor. Back in VMD's Render form is a box labeled Render Command. It contains the exact command VMD ran to generate the image. Copy the entire line. In a Terminal window make sure you are in the location where the plot.dat file is - it will generally be your home directory. Then paste the render command into the terminal. Edit `%s` to become `plot.dat` and `%s.tga` to a suitable filename, such as `dipeptide-highres.tga`.

Then hit the Return key and let the Tachyon renderer generate a glorious high resolution image for you. Add labels and other needed items to the image in Photoshop or a similar program, save as a TIFF file and send this to the journal.



Sample VMD images

Above: Elastic Rod Model of Lactose Operon DNA
Below: HIV protease with bound Indinavir inhibitor. 1HSG



Dealing with Problem Colors

Some images of molecules may contain problematic colors, which tend to disappear against a black background when the image is printed or when it is projected, even though it looked good on your screen and when you printed it yourself. As a rule, your image will be made to look worst when it counts the most, and what is worse, the situation leading to image degradation will often be beyond your control. Therefore you need to take steps to make your image look good under the worst possible circumstances.

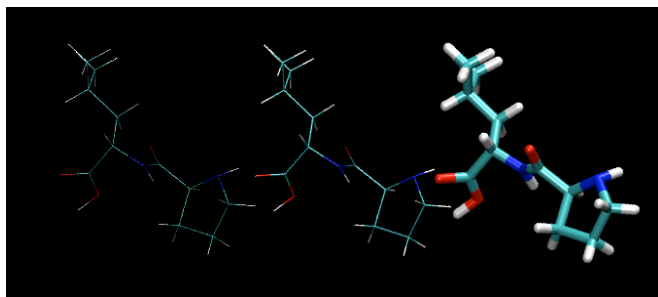
Most molecular graphics are generated with a black background because the dark background will make the molecule “jump out” and increase its visual appeal and 3D appearance. Against a black background saturated blue and red tend to be the most problematic colors, often losing all contrast against the black. The result is that a seminar presenter finds himself apologizing to the audience and explaining that “here you would see residue X in close proximity to residue Y if only the room were darker and the projector of better quality”. What he really means to say is that he couldn’t be bothered to make sure that his image will display properly under non-ideal circumstances and that he probably doesn’t know how anyway.

There are several ways to make sure your image looks great no matter what:

1. Change Graphical Representation and make sure individual details are large enough to be seen from the back row.
2. Change problem colors to lighter ones, or lighten up/redefine problem colors.
3. Change background color.

Change Graphical Representation

The most problematic graphical representation is *Lines* (also known as wireframe). VMD defaults to lines of 1 pixel width. You can simply step up the *Thickness* (line width) to 2, 3 or even 4, and the image will be instantly clearer due to the increased area given to the colored features. The image becomes even clearer when you change *Drawing Method* to *Licorice*. Not only are the colored areas enlarged, but highlights on the cylindrical bonds increase brightness, and the image carries more information because of the improved 3D representation which the lighting and shading affords.

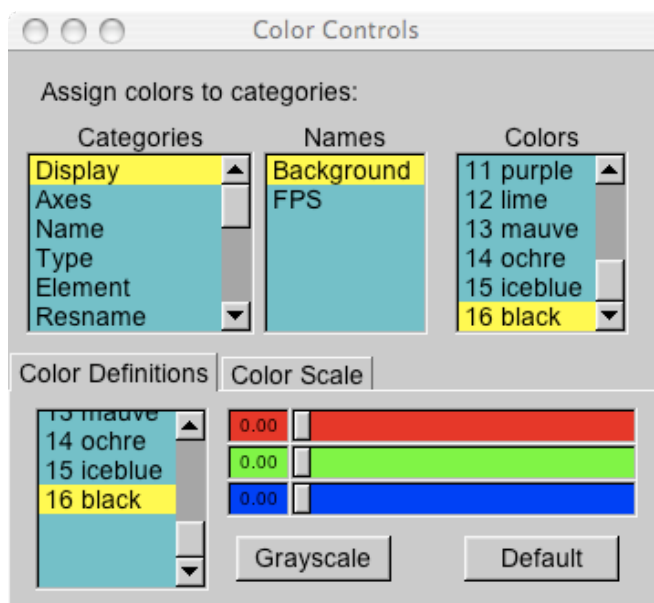


Left: Line representation at thickness 2.
Middle: Line representation at thickness 4.
Right: Licorice representation at bond radius 0.2

Redefining System Colors

In the main VMD form select *Graphics/Colors*. A new window appears and gives you complete control over VMD’s colors. Under *Color Definitions* select *0 blue* and note the position of the three color sliders: Blue is all the way up while red and green are at 0. To brighten the blue, slide up the red and green sliders approx. 25%. You might need a test print or display through a projector in a well lit room, and readjust accordingly.

To adjust the reds select *1 red* under *Color Definitions* and bring up the green and blue sliders to make the red color a little lighter. You may need less adjustment than for blue. Note that the last two adjustments affect all instances of blue and red, whether they be nitrogen or oxygen atoms, Ala residues colored by residue name, basic residues colored by residue type, or whatever else. One adjustment does it all!



If you want to change the background color, select the Category *Display*, find *Background* under *Names* and finally select a color other than black.

Create Complex Displays

A Potein - Porin

New tasks in this section:

Cartoon representation for protein, atom selection by properties, complex atom selection, pick (virtual) bonds

In the main form, select the dipeptide molecule and select *Molecule/Delete Molecule*. Select *File/Load Molecule* in the main form., and load the file **Porin.pdb** by following the same procedure as for the dipeptide. Close the Molecule File browser.

The Porin protein isn't very large as far as proteins go, but looking at it in the default line representation is bewildering, especially for nonspecialists. What we need is another level of abstraction so that we can grasp the structure of Porin.

Open the Graphical Representation form and change the representation for all atoms on porin: Make the Drawing Method *Cartoon* or *New Cartoon* and the Coloring Method *Structure*. You get a secondary structure representation such as below. This representation removes amino acid side chains and concentrates on the protein backbone. Alpha helices are drawn as purple cylinders, beta sheets as yellow arrows, beta turns as cyan ribbons, and irregular structure as white ribbons.



Try and trace the backbone from beginning to end. Hint: The N terminus (the beginning of the protein strand) is at the bottom left in the picture.

Note how beautifully the protein backbone is arranged in this molecule. The beta sheet structure is built up with successive up-and-down strand pairings until we have a complete barrel, fittingly called a beta barrel. Loops of irregular structure, interspersed with the occasional alpha helix make up the

additional parts of the molecule at one end, while the other end is completely devoid of additional features.

With the change to the cartoon representation we lost the two detergent molecules that are part of this crystal structure. To display them, click *Create Rep* in the Graphical Representation form, type in **resname OTE TRS** for atom selection and hit return. Select *Name* for the Coloring method and *VDW* for Drawing Method. Now you can see the two molecules clearly.

How do we know the name of the residues? You can either look at the PDB file **porin.pdb** - it is a simple text file -, or you can have VMD put labels on them while they are visible (change to a different representation, such as *Lines* of *Licorice* for that) and learn from the labels.

Explorin' porin

Porin is a bacterial outer membrane protein that occurs as a trimer in nature. We are looking at a monomer right now. Let's look at the distribution of hydrophobic and hydrophilic residues in this protein: Create a new representation and type in **hydrophobic or neutral** for atom selection and hit the Return key. Make the Drawing method *Line* or *Licorice* and the Coloring Method *ColorID 3* (orange). Create another new representation, type in **not (hydrophobic or neutral)** for atom selection, and change the color to *15* (light purple). The last two atom selections are examples of more complex selections, utilizing logical expressions. There are far more possibilities in VMD. Just check the Keyword list under the *Selection* tab in the Graphical Representation form.

Look down the central pore of porin and note the light purple colored hydrophilic, and possibly charged residues that line the pore. Then check out how the outer wall of the beta barrel is studded with hydrophobic residues, colored orange. Then look at the role reversal in the domain on top of the barrel and partially inside the pore, which is mostly solvent (water) exposed. Can you also guess where the monomers interact to form a trimer?

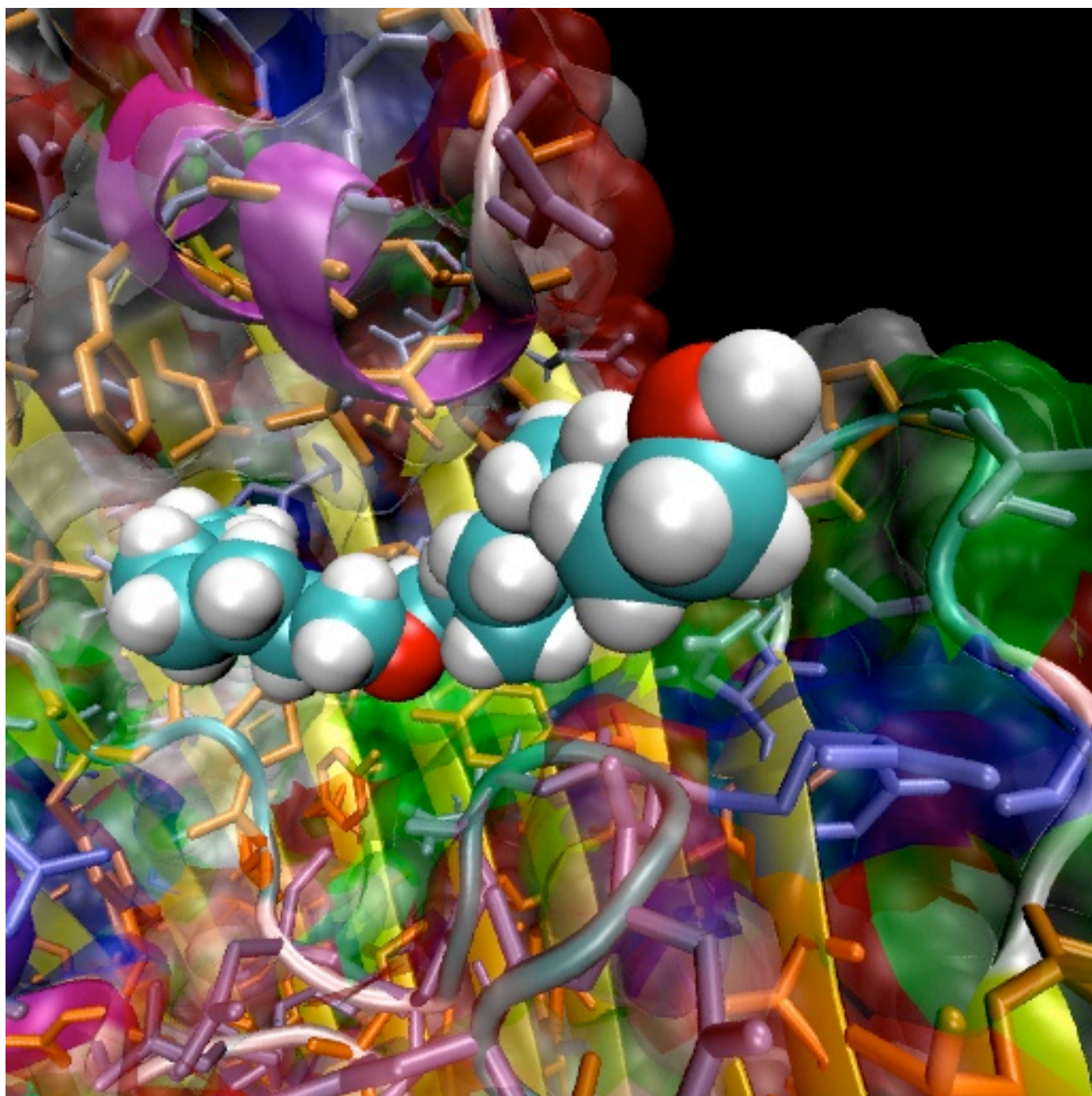
Also check out the binding environment of the detergent molecule near the top of Porin.

Select *Mouse/Label/Bonds*. Find two hydrophobic residues at the top and bottom of the beta barrel and click on one atom in each. A bond will be drawn between them with the distance labeled. The distance will be 19-22 Å, about right for the thickness of a lipid bilayer into which this protein is embedded.

To sum it up, it is very easy to infer from these representations how the protein is built, how it is embedded into the cell membrane, and how the central channel makes for a pore in the cell membrane through which water soluble and possibly charged small molecules can pass into the interior of the bacterial cell.

Finally, create another new graphical representation and select *Surf* for the Drawing Method. The computer will take a few seconds to calculate a molecular surface as 'felt' by a water molecule. When the surface is displayed, choose *ResType* as Coloring Method. This colors acidic amino acids red, basic ones blue, neutral ones white and hydrophilic ones green. (Note: Many older computers will be severely slowed down by this representation. Moving the molecule will be jerky and takes some getting used to. Be patient and turn off this Drawing Method when you've seen what you need.) Now rotate the molecule such that you can see how nicely the detergent molecule near the top fits into a binding pocket that has just the right amino acid side chains exposed to complement the detergent. Tight binding requires both, shape complementarity

and the correct distribution and location of charged, neutral and hydrophilic anchoring points, as illustrated in this example.



Create a Movie

New Tasks in this section:

Use of VMD extensions, MoveMaker tool

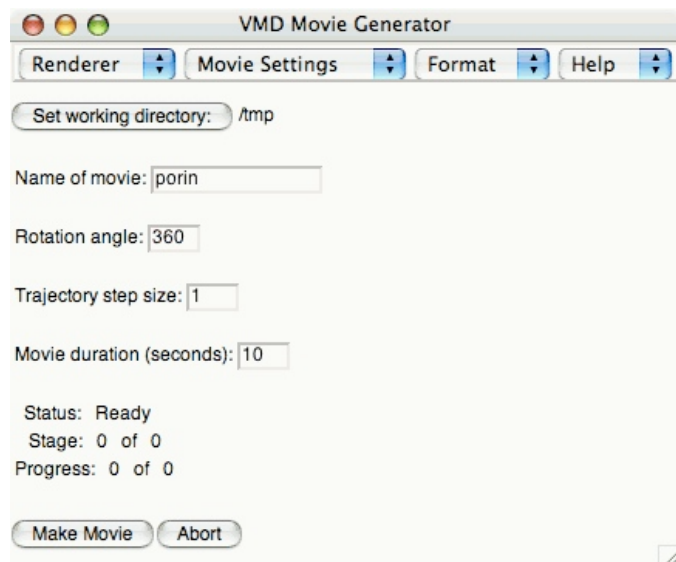
VMD has a series of extensions available under the Extensions menu. Some of these are written by the VMD authors, some are contributed by other people. Please note that many of the extensions rely on extraneous software which needs to be installed on your computer. Specifically, the Movie Maker tool needs movie encoding software; ppmtompeg on MacOSX and Linux/Unix, VideoMach on Windows (see <http://www.ks.uiuc.edu/Research/vmd/plugins/vmdmovie/> for more details). Research Computing can help you with installation of this helper software.

Let's start with the Porin molecule as it is displayed right now. First, resize the VMD OpenGL Display window to the size you want your movie to have. Most likely this will be smaller than what you had used so far. Keep in mind that movie files are large by nature, and therefore, if the movie is to be used on the web or inside PowerPoint you really want to keep the display size as small as you can get away with. Select

Make Movie.

2. Leave the graphics window as is, change the Renderer from Snapshot to *TachyonInternal* and regenerate the movie.

Whatever you decide, generating the improved movie will take a fair bit longer than the simple one before. Finally, import the movie into PowerPoint or play it on its own. Be sure that PowerPoint is configured to copy images and movies into the PowerPoint file.

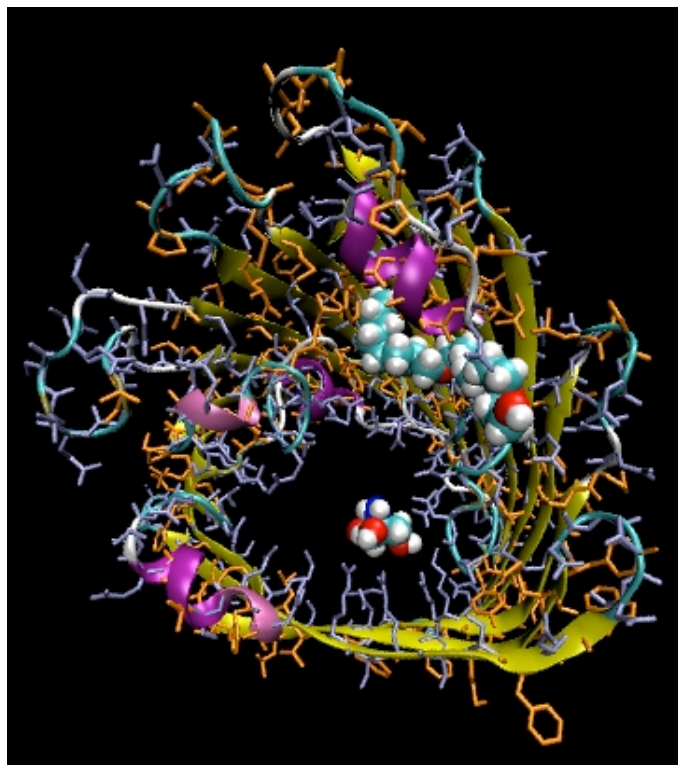


Extensions/Visualization/Movie Maker in the main form. A new window pops up which lets you choose the parameters for your movie. Select *Movie Settings* and change to *Rotation about Y axis*. Give the movie a name, e.g. porin, change the rotation angle to 360, and leave everything else as is. Now click *Make Movie*.

On a modern computer generating the movie takes around a minute. You can follow the progress in the VMD console, and while the screenshots are being taken you will also see the molecule being rotated in the graphics window. Once the movie is complete you can find it in the directory you set as working directory as `porin.mpg` and play it.

Tip: You may want to increase the quality of the movie now and possibly also change the movie duration. For increased quality you have two options:

1. Double the VMD graphics window size and select *Movie Settings/Image Smoothing* and *Movie Settings/Half-size Rescaling*. Then generate the movie again by clicking on



Maltose-binding Protein in bound (green) and free (white) state. Note movement of Gly residue (depicted as orange ball) upon binding. PDB entries 1FQA and 1FQD.



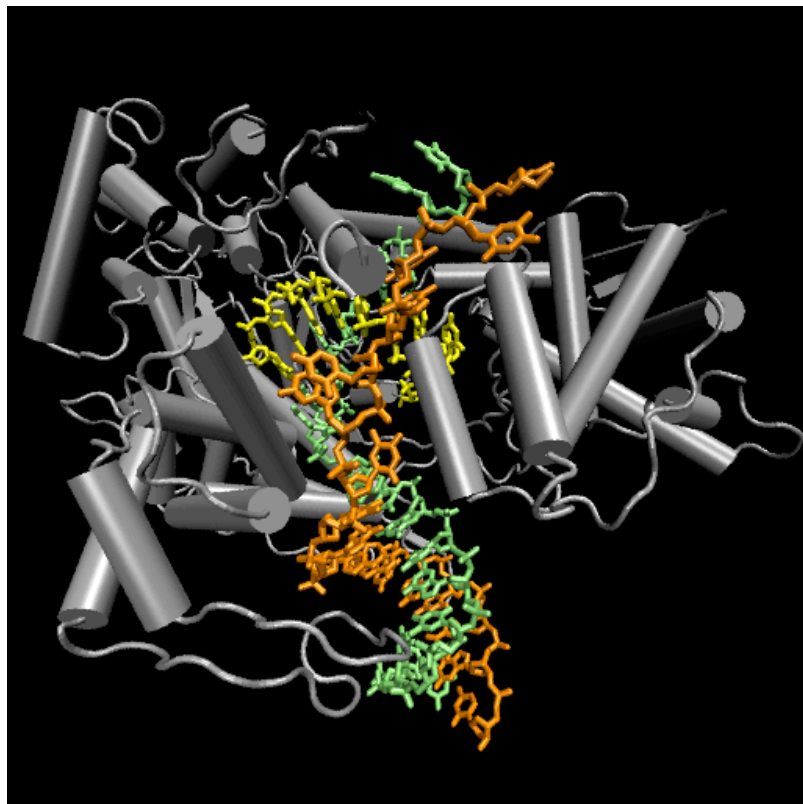
Simplify the Complex

RNA Polymerase

New tasks in this section

More complex atom selection strategies

Delete the Porin molecule. If you don't remember how, check the section on Porin. Then load the file **1MSW.pdb**. This is a bacterial RNA polymerase complexed with a piece of DNA and a nascent RNA strand. Essentially, we are looking at the polymerase caught and stopped in action.



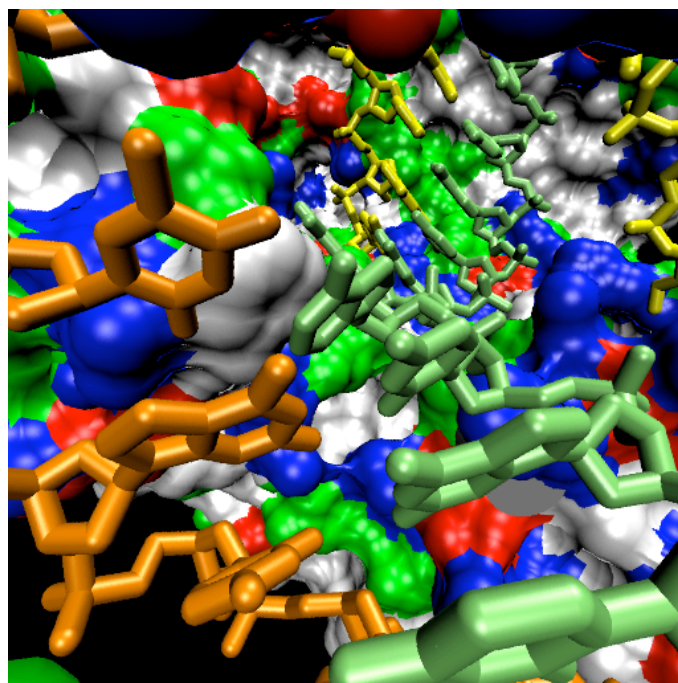
To understand this large molecular assembly, let's simplify the display: In the Graphics Representation form, type in *protein* for the Atom Selection and hit return. Change Coloring Method to *ColorID* and choose color 6 (silver). Choose the Drawing Method as *Cartoon*. This simplifies and visually deemphasizes the protein part of the complex so that we can later concentrate on the nucleic acids.

Now create a second representation with the following parameters: Atom Selection *chain N*, Coloring Method *ColorID 3*, Drawing Method *Licorice*. The result is one of the DNA strands drawn in orange bonds. Create a new representation with these parameters: Atom Selection *chain T*, Coloring Method *ColorID 12*, Drawing Method *Licorice*. The result is the other of the DNA strands drawn in lime green bonds. Create yet another representation with these parameters: Atom Selection *chain R*, Coloring Method *ColorID 4*, Drawing Method *Licorice*. The result is the RNA strand drawn in yellow bonds.

Explore the molecule, trace the DNA and RNA strands and see how they thread through the RNA polymerase protein or along its surface. Note how the coding DNA strand and the RNA strand are base-paired in the active center of the po-

lymerase.

Now reactivate the first representation (the protein) by clicking on it in the Graphics Representation form. Change its Coloring method to *ResType* and change its Drawing Method to *Surf*. It will take a few seconds and rotation will get jerky as before, but it is worth it. Note how the polymerase molecule has 'tunnels' and grooves through which the nucleic acids thread. Turn the polymerase such that you can see the orange, outer DNA strand embedded into a groove on the enzyme surface. Note how some of the phosphates of this strand are interacting with basic (blue) protein patches, while bases are lying against green hydrophobic patches and in some cases have their nitrogen atoms interacting with acidic (red) patches. If you look real close you can see a white phenylalanine ring stacked on top of the last proper DNA base pair, displacing the base of the orange DNA strand which has now swung outward to the surface of the complex. This is the DNA unwinding action, and it takes some real patience to get the view just right.



Exercises

Small Molecules: Estradiol and Testosterone

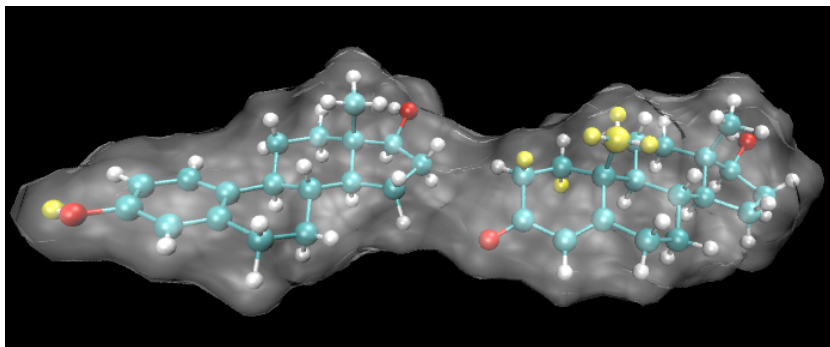
Load the file **sexhormones.pdb** into VMD. On the left you have estradiol, a member of the estrogen family of (female) sex hormones, and on the right you have testosterone, the male sex hormone.

View and compare the two molecules all around. Figure out the differences between them, highlight them with a suitable display style so that you could easily show and explain the differences to an audience. Note that no double bonds or aromatic rings are drawn. You will have to infer these properties by the number of atoms bound to each carbon. Hint: Find atom names and indices through labels and use the *name* and/or *index* keywords for atom selections.

It might help to display a molecular surface and make that surface transparent to emphasize

the steric (volume) difference(s) of the two molecules. Find out how to do this.

Save your work to a postscript file and print it. If your black-and-white print does not highlight the differences enough, you may want to go back and change to colors and/or display styles that will stand out in B/W.



Protein: Insulin

If you want to go all out, start a web browser and go to <http://www.rcsb.org>. Otherwise skip to the next paragraph. Search for human insulin. In the resulting hits, look for an entry at 1.60 Å resolution with the title containing 'crystallographic evidence for dual coordination around zinc'. Download this file by clicking on the Explore link on the right, and then on the Download link on the left of the next page. On the actual download page, select the option to download in PDB format without compression.

Load the file **1TRZ.pdb**. This is human insulin in complex with two zinc ions. Insulin consists of two chains, linked with several disulfide bonds. However, in this coordinate file there are four chains due to the way it crystallized, and there are also a good number of water molecules which got refined into the electron density.

1. Switch off the two additional chains C and D and the waters (chain X) and display only chains A and B.
2. Find a suitable display to highlight the architecture of the insulin backbone and the disulfide bonds linking them. Note that a bug in VMD versions up to 1.8.3 traces ribbons and other, similar backbone representations wrong in this structure. The current version is fine..
3. Display hydrophobic and hydrophilic residues in different colors and note the obvious concentration of the two groups in the core and on the surface, respectively.
4. If you have arrived at a nice display for either backbone architecture or side chain distribution, render it with one of the ray tracing programs available to you.

