

Improving presentations with the program 'Visual Molecular Dynamics' by controlling the display with button navigation windows

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Introduction

A picture tells more than a thousand words, and this applies even more to three dimensional presentations. With VMD [Humphrey et al.(1996), Stone et al.(2001)] and related programs like Rasmol, Pymol, Swiss Pdb Viewer, Chimera etc. and plug-in's to internet browsers like Chime and Jmol, one can basically get a 3D -presentation of biological molecules on a computer screen. Since the function of biomolecules is, to a large extend, determined by structure, such a representations helps very much in the understanding of the molecular basis of the biochemical function. Therefore, if the structure of a new biomolecule is published, a standardized structure file (*.pdb) is deposited at the RCSB protein data bank, that is publicly accessible via the internet [Berman et al.(2000)]:

<http://www.rcsb.org/pdb/>

The program 'Visual Molecular Dynamics' is -among other features, a fairly sophisticated tool for displaying biomolecules. Nice presentations of proteins or nucleic acids can be created even by unexperienced users in a fairly short time from protein data bank files. By taking advantage of the scripting capability of VMD, user controlled navigation windows can be added, that contain buttons for switching between different presentations and selections of the protein displayed. This feature is particularly useful for talks and classroom demonstrations. There are much more sophisticated scripts for VMD around, but the script code used in the provided examples is designed to be easily adaptable to your needs.

This is not supposed to be a thorough introduction into VMD or script programming in general, but merely a description of the example script files which are provided and how to quickly adapt these or create them on your own. However, to give you a quick start, some very basic steps concerning the usage of VMD are also described. If you have problems with these instructions, please seek advice from the manuals, that are provided with the program. VMD automatically creates script files that contain your presentation, and on this level you do not have to bother with the syntax of these files. However, if you want to add control windows, for example with navigation buttons, or if you want to include animations, you have to add code manually. As far as possible, I tried to keep the syntax of this code as readable as possible, and if you stick to the system provided, you may transfer the code to your own examples without really knowing

much about programming or the used scripting language TCL/TK. However a basic understanding about the structure of higher computer languages is of considerable advantage.

How to get started

To get a quick introduction into the visualization of biomolecules, I included example script files ready to use with VMD. Most of these scripts produce additional windows with navigation buttons or explanation parts to facilitate their use in the classroom. The basic idea is to show different preprepared biomolecule representations of one or more biomolecules by just selecting the appropriate button. By pressing a particular button, many of the representations defined in the first step can be switched on and off at once. The main advantage of the navigation buttons and other tools provided here is to quickly change between different, even complex composite presentations of your system very quickly and without worrying about getting lost. An analogue method of depicting biomolecules and to provide an e-learning environment was first applied in connection with the browser plugin Chime. Some biochemistry and biophysics teaching resources and the fifth edition of the biochemistry book by Berg, Tymoczko and Stryer use chime to visualize biomolecules. However, the usability of chime is limited to the windows operating system and often on unsecure or outdated browser versions. There are unofficial ways of installing it with Netscape 8, Mozilla and Firefox, and even under Linux [?], but these methods are not fool-proof. In contrast, VMD works as a standalone program under Linux, Mac and Windows, and the script files included here correspondingly work on all these operating systems .

The selection of protein and nucleotide structures chosen as examples is biased and is not in any way representative. The selection was merely based on

- personal taste and interest
- the content of the lecture Biophysical Chemistry in the Department of Biochemistry in Jena, where some of the examples have been used
- biomolecules, that demonstrate particularly well the usage of certain molecular representations

To start with, you have to download VMD from

<http://www.ks.uiuc.edu/Research/vmd/> .

VMD can be used without charge in academia and education, but please check the licence agreement! Note, that you have to register on the UIUC - website prior to downloading. There are also user manuals [Caddigan et al.(2005)] available, although much of the program is self explaining. VMD is available for various flavours of Unix, including Linux, and for Mac OS and Windows ! The script files provided with this text can be used without limitation, in particular you are encouraged to alter the files to fit your demands. I would appreciate getting a notification, if and how you use the program.

After installation you are ready to use VMD. You can load pdb files under File – New Molecule, and create your own protein presentation starting from the pdb-file. A good start would be to go to Graphics – Presentations and then play around with the options. You can create multiple representations each of which is listed in one line and switch them on and off as required to visualize different aspects of the protein. For example, on one hand you may wish to show the overall structure of the protein, on some other stage, you want to show the cofactors or a specific enzymatic site of the proteins in detail. This can be done by switching the respective representations on and off. Many other parameters can also be changed, like colors, background colors, the perspective and even the number and angle of lights that illuminate the protein.

The presentations can be saved as automatically created script files: File – Save State, which creates a *.vmd -file. The file can be reloaded by using File – Load State. The code is written in a standard scripting language called Tool Command Language (TCL) [Ousterhout(1995)] with extensions specifically designed for use with VMD. Alternatively, python can also be used. See the VMD manual [Caddigan et al.(2005)] for details. The script files are pure ASCII code and can be modified, for example by including the above-mentioned button navigation window.

The script files distributed with this text are provided in the compressed file `vmdexamples.tar.gz` resp. `vmdexamples.zip` (identical content). By uncompressing the file you get a folder `vmdlecture` with the vmd -script files, and a subfolder with the required pdb -files. Copy the folder `vmdlecture` to your own folder structure. The name of the vmd-file should give you information about the featured protein. If you are uncertain, you can check the corresponding pdb -file.

To start with, go to File – Load State. You can certainly change the presentation and save your own *.vmd -script file. Most of the vmd- scripts have button windows included, which is indicated by the name like `gfpbutton.vmd`, or provide animations indicated correspondingly. As a test system, the Photosystem II is used to demonstrate the usability of different schemes like hypertext or radiobuttons with a longer explaining text. This concept resembles the one often used in connection with Chime.

Some proteins, like those employed in light detection, show a dynamic behavior upon photoexcitation. This is often a cis -trans isomerization via multiple intermediates. The structure of the longer living intermediates can be resolved in time dependent X-Ray and are published in the pdb -data bank. Such a dynamics can be visualized as animation in VMD by aligning the respective pdb files in a movie-like fashion. There are example script files for Bacteriorhodopsin, Photoactive Yellow Protein, Phototropin and a photoswitchable GFP variant included.

A brief introduction, how these files are created is provided, and you may use the scripts and modify for your own purposes.

Note that you can only alter the presentations, which include self-programmed code like buttons, by editing the script source code. If you just save these scripts via File – Save State, you will not keep the button window and the animation features, but just get the file with the presentations that were used prior to adding the code.

Fig. 1 and 2 show screenshots for illustration, more screenshots are available online:

http://www.ipht-jena.de/BEREICH_3/lectures/vmdscreenshots.html

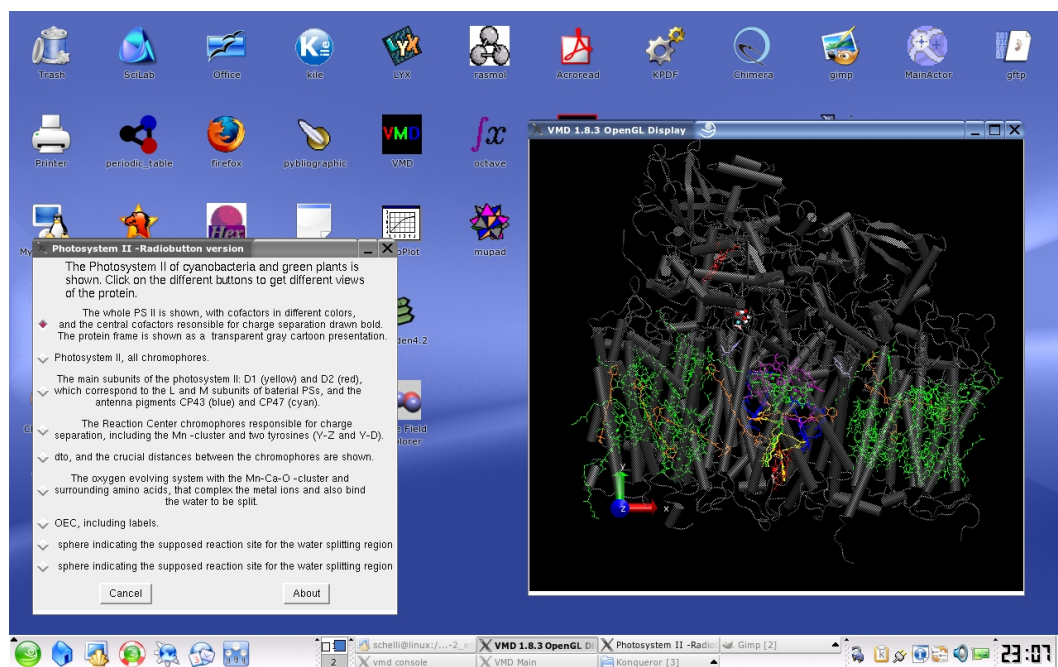


Figure 1. Photosystem II with radiobuttons window

and an example with plain buttons and animation control:

Very important: The script files provided in the folder `vmdlecture` are ready to use under Linux, likely also under other Unix versions. You just have to unzip the compressed file to your folder, to get `/home/myname/vmdlecture`. If you use windows, you have to edit all the `*.vmd` script files and alter all lines in the `vmd`-files that refer to the path to the respective `pdb` files. Often, this is more than one line per scriptfile, in particular, if more than one structure is loaded ! If you have a decent editor, you can load all VMD scripts and use the `replace` command for all of these files at once. Editors with this capability are, for example, the freeware editors `notetab-light` (<http://www.notetab.com>) or `crimson editor` (<http://www.crimsoneditor.com>) and the shareware editor `winedt` (<http://www.winedt.com>). Again, be sure to replace all lines with path informations in the file. Use forward-slashes! For example alter lines like

```
mol new vmdlecture/pdbfiles/1s5l.pdb type pdb waitfor all
```

to

```
mol new C:/users/myname/vmdlecture/pdbfiles/1s5l.pdb type pdb waitfor all
```

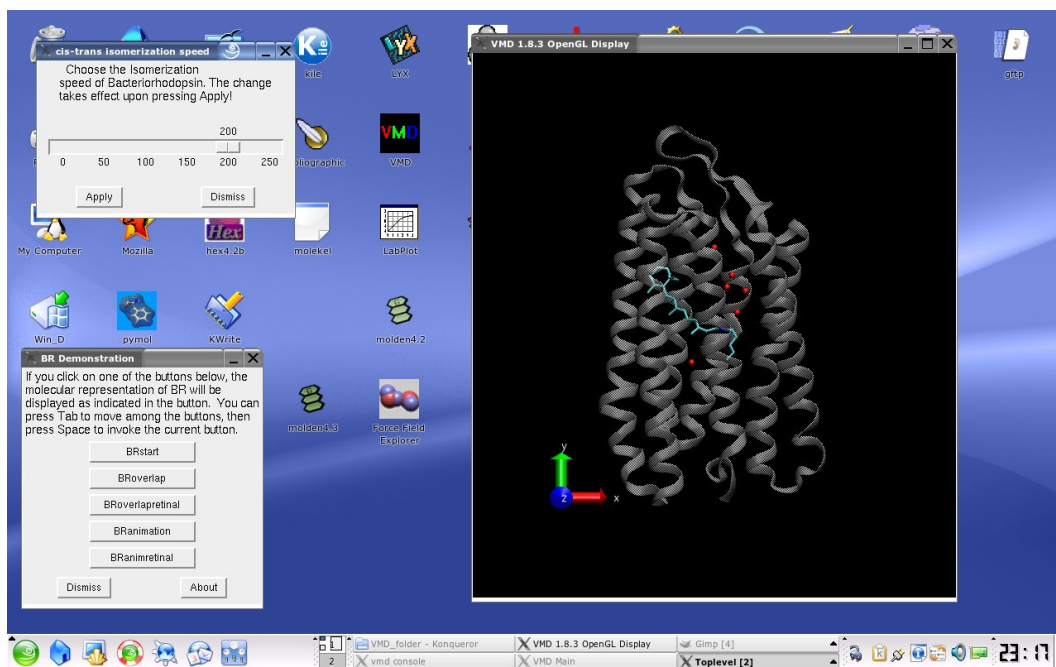


Figure 2. Bacteriorhodopsin with buttons and animation speed window

otherwise the pdbfile will not be found and you get numerous error messages in the terminal window. All and only path lines contain the word `ymdlecture`, you may search for this word. With a Mac, you have to act correspondingly. Sorry for the inconvenience but unlike to Unix, a relative path is not recognized under Windows, and since I do not know how you arranged your folder hierachy, there is no way of giving a generally valid path. If you think, it is worth writing an installation script, you are welcome to do so and I gladly include it into the example files.

The examples

A short description of the used example follows. Usually, the examples contain an additional button window and upon clicking on one of the buttons, a particular presentation of the protein or parts of the proteins becomes visible. The button names are cryptic, but should give you an indication what is presented. There are also some radiobutton and text files include several lines of text for each of the clickable presentations, making it more suitable for self-teaching. This is similar to the system often used in connection with chime. I did not consider it meaningful to give a thorough explanation to each button in this text, since I consider the presentations self-explaining. Just play around !

antibodybutton.vmd:

pdb-files: 1fdl.pdb, ligj.pdb, ligt.pdb, 1ndg.pdb, 1yqv.pdb

Antibodies are the naturally occurring proteins of the immune system. The antibodies bind to specific antigens. To be able to respond to a large variety of antigen structures, the antibodies consist of a hypervariable part which is the loop region at the end of the FAB fragments, and is colored red in this demonstration. Upon starting the script or clicking on the first button, a complete antibody with the heavy and light chains distinguished by color is shown. The next two buttons show an FAB, that binds to a small ligand (digoxin) by partly burying the steroid. The other buttons show three different monoclonal antibody FAB fragments that bind to different epitopes of lysozyme and the last button presents a structural overlap of these three ligands. To align the lysozyme FABs, the RMSD algorithm is employed see alinea 'Overlapping proteins'. In VMD 1.8.3, there was a bug, that prevented the complete antibody structure ligt from being displayed correctly, when employing representations like ribbons or cartoon. A workaround is to save the missing part of the structure in a separate file. This bug has been fixed in VMD 1.8.4., but the special syntax is retained in this example.

L.J.Harris, S.B.Larson, K.W.Hasel, J.Day, A.Greenwood, A.McPherson, *The three-dimensional structure of an intact monoclonal antibody for canine lymphoma*, Nature vol. 360, p. 369, (1992)

A.T.Brunger, *Solution of a FAB (26-10)/digoxin complex by generalized molecular replacement*, Acta Crystallogr.,Sect.A, vol. 47, p. 195, (1991)

T.O.Fischmann, G.A.Bentley, T.N.Bhat, G.Boulot, R.A.Mariuzza, S.E.V.Phillips, D.Tello, R.J.Poljak, *Crystallographic refinement of the three-dimensional structure of the FAB*D1.3-*Lysozyme complex at 2.5-Angstroms resolution*, J.Biol.Chem., vol. 266, p. 12915, (1991)

Y.Li, H.Li, F.Yang, S.J.Smith-Gill, R.A.Mariuzza, *X-ray snapshots of the maturation of an antibody response to a protein antigen*, Nat.Struct.Biol., vol. 10, p. 482, (2003)

aptamerbutton.vmd:

pdb-file: 1hao.pdb

Aptamers are oligonucleotides that are selected in an artificial evolution process for specific properties, like a specific catalysis or to bind to a specific epitope of a protein. The binding of a 15mer DNA-aptamer to Thrombin is shown. There also exists a 29mer aptamer that blocks another exosite of thrombin, for which no structure exists. However

its binding site is indicated by coloring. The energy transfer button shows the aromatic amino acids TRP and TYR, whose fluorescence is quenched to a different extent by the two possible nucleotide aptamers.

K.Brejc, T.K.Sixma, P.A.Kitts, S.R.Kain, R.Y.Tsien, M.Ormo, S.J.Remington, *Structural basis for dual excitation and photoisomerization of the aequeora victoria green fluorescent protein*, Proc.Nat.Acad.Sci.USA, vol. 94, p. 2306, (1997)

M.Ormo, A.B.Cubitt, K.Kallio, L.A.Gross, R.Y.Tsien, S.J.Remington, *Crystal structure of the aequeora victoria green fluorescent protein*, Science, vol. 273, p. 1392, (1996)

atpsynthaseanimation.vmd (contributed by Christopher Bruhn) :

pdb-files: 1c17.pdb, 1e79.pdb, 1l2p.pdb, 2a7u.pdb

ATP-synthase, often referred to as ATPase, is a large trans-membrane complex that exploits a proton gradient across the membrane to produce ATP from ADP. In the frame of the process, parts of the protein rotate in a stepwise fashion in this process. The mechanism was elucidated in the past years, particularly by single molecule methods, where you see a single protein rotating depending on the proton gradient. Some unique presentation techniques are employed in this example.

bacteriorhodopsinanimation.vmd:

pdb-files: 1iw6.pdb, 1ixf.pdb, 1iw9.pdb, 1ucq.pdb

Bacteriorhodopsin is found in certain archaebacteria like *Halobacterium halobium*, that flourish in highly concentrated salt solutions. This system is likely the only photosynthetic protein that is unrelated to the common chlorophyll based photosynthetic systems, but instead has similarities to the rhodopsin proteins of vision processes in animals. Upon light illumination, bacteriorhodopsin pumps protons across the bacterial membrane by isomerization of the Bacteriorhodopsin chromophore. The proton gradient in return drives ATPase. Although the protons are not seen in X-ray diffraction, the displacement of the associated water oxygens during the photocycle can be seen by aligning the X-ray structures from intermediates, that can be identified. The animation with the aligned structures is available by pressing the animation buttons. Note that only the five most significant oxygens are shown in this animation. Similarly to the mechanism in vision pigments like bovine rhodopsin, phytochrome or photoactive yellow protein, a cycle with multiple transition states can be observed. First, the intermediates were observed by transient optical and IR –spectroscopy, and lately, the structure of some of the intermediates were identified by time –resolved X–ray diffraction. These structures drawn in different colors in the script are created by utilizing 1IW6.pdb (white), 1IXF.pdb

(red), 1UCQ.pdb (green) and 1IW9.pdb (blue), corresponding to the spectroscopically distinguished intermediates ground state, K, L and M, respectively.

T.Kouyama, T.Nishikawa, T.Tokuhisa, H.Okumura, *Crystal structure of the L intermediate of bacteriorhodopsin: evidence for vertical translocation of a water molecule during the proton pumping cycle*, J.MOL.BIOL., vol. 335, p. 531, (2004)

K.Takeda, Y.Matsui, N.Kamiya, S.Adachi, H.Okumura, T.Kouyama, *Crystal structure of the M intermediate of bacteriorhodopsin: allosteric structural changes mediated by sliding movement of a transmembrane helix*, J.MOL.BIOL., vol. 341, p. 1023, (2004)

Designerproteinbutton.vmd:

pdb-files: 1qys.pdb

α - β -protein structure, computationally designed novel fold. This example illustrates nicely how small a polypeptid can get and still containing secondary and tertiary structure elements. A calculated density map is also included.

B.Kuhlman, G.Dantas, G.C.Ireton, G.Varani, B.L.Stoddard,D.Baker *Design of a novel globular protein fold with atomic-level accuracy*, Science, vol. 302, p. 1364, (2003)

DielsAlderRibozymebutton.vmd:

pdb-files: 1ykq.pdb, 1ykv.pdb, 1yls.pdb

The structure shows an artificially designed and selected ribozyme, that is optimized for catalysing a particular Diels -Alder reaction, namely the addition of an en to Anthracene. Structures with and without the Anthracene-addition product ligand are shown.

A. Serganov, S. Keiper, L. Malinina, V. Tereshko, E. Skripkin, C. Hobartner, A. Polonskaia, A. T. Phan, R. Wombacher, R. Micura, Z. Dauter, A. Jaschke, D. J. Patel *Structural basis for Diels-Alder ribozyme-catalyzed carbon-carbon bond formation*, Nat.Struct.Mol.Biol. Vol. 12, 218 (2005)

ElongFactortrnabutton.vmd:

pdb-files: 1ttt.pdb

The presentation shows a protein - t-RNA complex, namely the loaded Phe -t-RNA and the EF-TU elongation factor. This example serves to demonstrate how to display

structural features of RNA.

P.Nissen, M.Kjeldgaard, S.Thirup, G.Polekhina, L.Reshetnikova, B.F.Clark, J.Nyborg, *Crystal structure of the ternary complex of Phe-t-RNAPhe, EF-TU, and a GTP analog*, Science, vol. 70, p. 1464, (1995)

gfbutton.vmd:

pdb-files: 1emb.pdb

Green Fluorescent Protein originates from the jellyfish *Aequorea victoria*. Unlike to other chromoproteins, that use separate cofactors, the chromophore in GFP is produced from three adjacent amino acids by an cyclization and oxidation step, and the only necessity to do so is the presence of oxygen. It is easily expressed in *E. coli* and often used as a tandem protein marker: In this case you add the genetic code of GFP to the code of the protein of interest and remove the stop and start codons to get GFP selectively attached to this protein. Due to absorption around 400 nm, the protein can also be used in two-photon microscopy with a 800 nm Titanium -Saphire laser as excitation source. The protein has a barrel like structure consisting of 13 beta sheets and the chromophore fixed in the inside. Today, many mutations exist, that tune the wavelength of the chromophore, and recently there have been similarly structured proteins discovered in sea corals, that extend the color range even to the red of the spectrum. The chromophore is part of an extensive hydrogen bonding network, that is partly responsible for the optical properties of GFP. For example the wt-GFP undergoes an excited state proton transfer (ESPT) that shifts the emission with respect to absorption by more than 100 nm from blue to green.

K.Brejč, T.K.Sixma, P.A.Kitts, S.R.Kain, R.Y.Tsien, M.Ormo, S.J.Remington, *Structural basis for dual excitation and photoisomerization of the aequorea victoria green fluorescent protein*, Proc.Nat.Acad.Sci.USA, vol. 94, p. 2306, 1997

M.Ormo, A.B.Cubitt, K.Kallio, L.A.Gross, R.Y.Tsien, S.J.Remington, *Crystal structure of the aequorea victoria green fluorescent protein*, Science, vol. 273, p. 1392, (1996)

gfpswitchanimation.vmd:

pdb-files: 1a53.pdb, 1a56.pdb

This animation shows a GFP analogue protein, extracted from the sea anemone *Anemonia sulcata*. It contains a longer conjugated system, leading to fluorescence in the orange-red part of the spectrum. More unique, it can reversibly be photoswitched between a non-fluorescent and a fluorescent state. The protein usually rests in the non-fluorescent trans state, but upon illumination with green light, it photoisomerizes into a fluorescent cis state.

The back reaction is induced by illumination with blue light. The isomerization process employs a hula-twist motion, similarly to those observed in other biological systems, see pyp or bacteriorhodopsin.

The structure shows the mutant A143S, which has an improved quantum yield for the switching reaction and shows decreased thermal backreaction to the nonfluorescent state compared to the wild type. Such a photoswitchable protein can for example be used for protein tracking or has been used in Ground State Depletion Microscopy, a novel microscopy method circumventing the Abbe resolution limit and producing pictures with a resolution down to 30 nm. The animation included shows the underlying cis-trans isomerization of the GFP variant.

M.Andresen, M.C.Wahl, A.C.Stiel, F.Graeter, L.Schaefer, S.Trowitzsch, G.Weber, C.Eggeling, H.Grubmueller, S.W.Hell, S.Jakobs *Structure and mechanism of the reversible photoswitch of a fluorescent protein*, Proc.Natl.Acad.Sci.USA, vol. 102, p. 13070 (2005)

hollidayjunction.vmd:

pdb-files: 1crx.pdb, 2crx.pdb, 3crx.pdb, 4crx.pdb, 1p4e.pdb

Holliday junction constructs are used for the exchange of genetic material, and it is crucial for generating genetic diversity in a population of molecular diversity in the creation of antibodies. The enzymes supporting the formation of junctions and the cut and cleavages involved are recombinases. As a central step of the reaction, a tyrosine is phosphorylated to the DNA strand, analogue to the mechanism in topoisomerases.

F.Guo, D.N.Gopaul, G.D.Vanduyne *Structure of cre recombinase complexed with dna in a site-specific recombination synapse*, NATURE vol. 389, p. 40 (1997)

P.A.RICE,Y.CHEN *The role of the conserved trp330 in flp-mediated recombination. functional and structural analysis* J.BIOL.CHEM. vol. 278 p. 24800 (2003)

phototropinanimation.vmd:

pdb-files: 1n9l.pdb, 1n9o.pdb

Phototropin is a blue light receptor. Unlike to many other light sensing proteins, the mechanism is not based on a cis-trans isomerization process, but instead on the forming of a covalent bond between the flavin chromophore and a cystein sulfur atom in its neighborhood.

R.Fedorov, I.Schlichting, E.Hartmann, T.Domratcheva, M.Fuhrmann, P.Hegemann, *Crystal structures and molecular mechanism of a light-induced signaling switch: The PHOT-LOV1 domain from CHLAMYDOMONAS REINHARDTII*, Biophys. J., vol. 84,

p. 2474 (2003)

psIIbutton.vmd, psIIradiobutton.vmd, psIIhypertext.vmd:

pdb-files: 1s5l.pdb

These three VMD files show identical representation of PhotosystemII, but are distinguished by the button presentation: psIIbutton shows the common buttons with just a keyword, making it most suitable for classroom demonstrations. The psIIradiobutton.vmd and the psIIhypertext.vmd include several lines of text for each of the clickable presentations, making it more suitable for self-teaching and in an e-learning environment.

Photosystem II is a large protein containing around 100 cofactor units most of which are chlorophylls (green) and carotenoids (orange), and additionally hem groups (red), carbonates (pink) and a manganese–calcium–oxygen cluster. It is one of the two reaction center types of cyanobacteria and green plants (the other one is Photosystem I]. PSII is responsible for oxygen evolution by splitting water. The photosystem consists mostly of 23 transmembrane helices and is embedded in the thylacoid membrane in plants. The chromophores are embedded in this ensemble of helices. The central part, the D₁D₂-fragment colored ocre and red has a similar structure as the bacterial reaction centers and the chromophores are drawn with thicker lines compared to the other chromophores of the photosystem. The special pair is colored in violet, and the monomeric chlorophylls and pheophytins are colored green and blue, resp. The ubiquinones are drawn in yellow. The D₁D₂-fragment is surrounded by antenna pigments, in particular CP43 (blue) and CP47 (cyan) Above the membrane the oxygen evolving system can be found. It contains 4 Mn, 4 O and 1 Ca –atom and is surrounded by mostly acidic amino acids, that complex the ions. Two tyrosin are also crucial for the functioning of the reaction center, in particular TYR 161, chain A, that functions as a transient electron donor / acceptor between the OEC and the special pair. The region indicated by a yellow sphere represents the region in which the water splitting takes place.

K.N.Ferreira, T.M.Iverson, K.Maghlaoui, J.Barber, S.Iwata, *Architecture of the photosynthetic oxygen-evolving center*, Science vol. 303 p. 1831 (2004)

pypanimation.vmd:

pdb-files: 1s1y.pdb, 1s1z.pdb, 1otb.pdb

Photoactive Yellow Protein (PYP) from *Ectothiorhodospira Halophila* is a relatively small light detection protein that causes positive or negative phototaxis in certain bacteria dependent on the excitation wavelength. The central process is a cis –trans isomerization upon excitation of the chromophore. The isomerization works via a so –called hula–twist motion. This way the dislocation of the aromatic ring is diminished and becomes

possible within the constraints of the protein cage. PYP serves as a model for cis–trans isomerization steps in proteins, and can be compared to the more complex systems like rhodopsin, bacteriorhodopsin or phytochrome. The different structures are overlaid, with the ground state 1otb.pdb (blue), the time resolved X-ray structures taken from 10 to 500 ns 1s1z.pdb (yellow) and the structure taken from 10 μ s to 3 ms 1s1y.pdb (violet)

S.Anderson, S.Crosson, K.Moffat, *Short hydrogen bonds in photoactive yellow protein*, Acta Crystallogr. Sect.D, Vol. 60, p. 1008, (2004)

S.Anderson, V.Srajer, R.Pahl, S.Rajagopal, F.Schotte, P.Anfinrud, M.Wulff, K.Moffat, *Chromophore conformation and the evolution of tertiary structural changes in photoactive yellow protein*, Structure, Vol. 12, p. 1039 (2004)

rclh1button.vmd:

pdb–files: 1pyh.pdb, 1kzu.pdb

This file contains two related proteins, which are structurally very much related. The first protein is bacterial reaction center from *Rhodospseudomonas Palustris* and the surrounding light harvesting complex LC I. The second protein is the LH-II complex from (*Rhodospseudomonas Acidophila*), which surrounds the RC -LH I complex. The chromophores are usually named according to their absorption wavelength as Bacteriochlorophyll B 800, B850 and B875. The first two are present in LHII arranged in two rings, the latter, B875 is present as a one ring arrangement in LHI. The redshift of wavelength from the outside to the inside of the protein assembly is like an energy funnel, in which energy is collected and followingly transferred to the reaction center by Förster Resonance Energy Transfer. For years there was a discussion going on about the nature of a strong coupling of the B850 chromophores in LHII (and accordingly in LHI). In recent single molecule studies, this issue was mostly resolved, and this research gave insight into other interesting topics of general interest for the understanding of proteins.

A.W.Roszak, T.D.Howard, J.Southall, A.T.Gardiner, C.J.Law, N.W.Isaacs, R.J.Cogdell, *Crystal structure of the RC-LH1 core complex from rhodospseudomonas palustris.*, Science vol. 302 1969 (2003)

C. Hofmann, T.J. Aartsma, H. Michel, J. Köhler, *Direct observation of tiers in the energy landscape of a chromoprotein: A single-molecule study*, PNAS vol. 100 ,15534-15538 (2003)

ribosomebutton.vmd:

pdb-files: 1jgp.pdb, 1giy.pdb

The ribosome from *Thermus Thermophilus* is shown, composed of two pdb-entries. The pdb file 1jgp.pdb shows the path of messenger RNA through the ribosome. It contains the 30S ribosome subunit, three t-RNA and one m-RNA molecule. The file 1giy contains the 50S ribosome subunit. The ribosome complex is the largest biomolecular structure resolved so far. The resolution is around 5 Å, not exactly atom-resolution, but still good enough to sketch the structure. It is composed of two subunits, which resemble the function of the head of a tape reader. The biomolecule is composed of several large RNA, called ribosomal RNA, that not only provide for structural integrity, but also act as ribozymes, and of several proteins. In the center between the two subunits, the transformation from m-RNA to protein takes place. A small strip of m-RNA is shown, which is attached to three t-RNAs, which mediate the translation. There is another file that shows a t-RNA in detail.

M.M.Yusupov, G.Z.Yusupova, A.Baucom, K.Lieberman, T.N.Earnest, J.H.D.Cate, H.F.Noller, *Crystal structure of the ribosome at 5.5 Å Resolution*, Science, vol. 292, p. 883, (2001)

G.Z.Yusupova, M.M.Yusupov, J.H.D.Cate, H.F.Noller, *The path of messenger rna through the ribosome*, Cell, vol. 106, p. 233, (2001)

You can find other examples for vmd-presentations in the folder VMD-lecture, that are not connected to a button control window, but are used for demonstrating how to display certain types of molecules.

How to create button controlled presentations

This is a brief introduction, how to create the VMD - files including buttons, animation and other features in a relatively short time. You can quickly produce nice representations for your own use and apply alterations to these presentations easily by switching them on and off in the Graphics Representation window. However, the change between different representations may take too much time in the classroom or during similar demonstrations, while you would rather like to spend this time with explanations. In these cases, the fast change between different presentations by just clicking on buttons is useful. For example, by just pressing one button you can switch back and forth from a general presentations of a protein like a ribbon presentation to a detailed molecular view of a region of interest or to an enhanced view of the cofactors. Furthermore the button based approach allows you to use features like overlying structures from time resolved X-ray. In the following paragraphs, I include a short description how buttons and animations can be included into a VMD script quickly. Briefly, the idea is to first take advantage of the built -in automatic script creation by VMD, and followingly edit the created script to include the button control.

Preparatory Arrangements

First you have to **load all pdb files and produce all presentations** which you want to show at some stage. You have to do this by loading the molecules in the New Molecule dialog and create the presentations in the Graphics Presentations dialog. It does not really matter, which of these you switch to visible or invisible. However, it is a good idea to eventually switch those to active, that you want to be presented directly after loading the script. Save the script and exit VMD. Note, that each loaded pdb-file is given a unique number starting with zero to address this particular structure in the scripts. If you delete on of the loaded pdb-files, this number is not reused, which may lead to confusion later on. In such a case, save your script , exit VMD and restart. The numbering is OK again.

In the second step, you take this automatically created script and add support for the buttons window manually by using an external editor. You may copy and past the program code given in the button scripts provided, add it to your own files and modify it according to your needs. The code in the provided example button files is split into the automatically created part and the part containing the code for the buttons. In the example files, the two parts are strictly separated, which facilitates transfer. The end of the automatically created and the beginning of the edited part of the code is separated by comment lines.

To modify the button controll panel, you have to alter the name of the buttons and the actions correlated with every individual button. Although I tried to keep the code as readable as possible you may need some knowledge of the general structure of computer languages. Some minimal knowledge of TCL/TK [Ousterhout(1995)] would also be an advantage, especially if you wish to modify your scripts beyond a pure transfer of the examples provided. The button creating code consists of two parts that I tried to keep separated as far as possible. One part creates the buttons window and manages the click events, the other one contains procedures with the actions you want to have performed.

In the button window part these procedures are called by clicking on the corresponding button. In principle you need not really know, what is going on, as long as you stick to the script and just fill in the appropriate button names and actions.

The employed windows

This part consists of several procedures, the first of which (and least important one) is `aboutBox`. This procedure is executed upon pressing the About button in the button window.

```
# aboutBox --
#
# Pops up a message box with an "about" message
#
proc aboutBox {} {
    tk_messageBox -icon info -type ok -title "Reaction Center LH1 complex" \
        -message " This is a message text in the about window  "
}
```

The second procedure (`proc positionWindow w`) positions the button window on your computer screen and the code that follows creates the button window including all the buttons as displayed on your screen

```
proc positionWindow w {
    wm geometry $w +300+300
}

set w .button
catch {destroy $w}
toplevel $w
wm title $w "PSII-LHI -Complex Demonstration"
wm iconname $w "button"
positionWindow $w

label $w.msg -font $font -wraplength 4i -justify left -text "Description."
pack $w.msg -side top

frame $w.buttons
pack $w.buttons -side bottom -fill x -pady 2m
button $w.buttons.dismiss -text Dismiss -command "destroy $w"
button $w.buttons.about -text "About" -command "aboutBox "
pack $w.buttons.dismiss $w.buttons.about -side left -expand 1

button $w.b1 -text "Title of Button" -width 15 \
    -command "proc_to_be_executed "
```

```
button $w.b2 .....  
button $w.b3 .....
```

```
pack $w.b1 $w.b2 $w.b3 -side top -expand yes -pady 2
```

You can certainly include more than the three buttons, but beware of too complicated button menus. If it makes sense you can also include several commands to be executed when a button is pressed, including calls to more than one procedure, definition of variables and conditional structures.

```
button $w.b4 -text "Title of button" -width 15 \  
    -command {  
        set bool_var 0  
        proc1_to_be_executed  
        proc2_to_be_executed  
        if $bool_var {destroy .hscale}  
    }
```

This pattern is employed in connection with animations.

Inclusion of button commands

The procedures called upon pressing buttons are defined in a second section. In these procedures you can switch on and off different graphical representation that you defined before and saved as the abovementioned automatically created VMD script file. An example for the lines of code representing a particular presentation are as follows:

```
mol new vmdlecture/pdbfiles/1otb.pdb type pdb waitfor all  
.....  
mol representation Tube 0.100000 20.000000  
mol color ColorID 0  
mol selection {all}  
mol material Opaque  
mol addrep top  
mol selupdate 2 top 0  
mol colupdate 2 top 0  
mol scaleminmax top 2 0.000000 0.000000  
mol smoothrep top 2 0  
mol drawframes top 2 {now}  
mol showrep top 2 0  
.....  
# done with molecule 1
```


This is the tube presentation of all of protein 1otb.pdb, the protein number is 1 (which means it is the second protein loaded into VMD) and it is presentation number 2. The line `showrep top 2 0` means it is not shown upon starting the script, but instead is switched on at a latter stage when activating the resp. buttons.

You can switch on or off every presentation by referring to the resp. molecule number and presentation number by using the command `mol showrep $i $j on/off`, where `$i` is the structure number and `$j` is the graphical presentation number.

You first define a procedure that switches off everything to Reset your graphical presentation.:

```
proc Reset {} {  
  
    label hide Atoms all  
    label hide Bonds all  
    set j 0  
    while {$j < 32} {  
        mol showrep 0 $j off  
        incr j  
    }  
}
```

In case of additional loaded structures, you need a loop for each one. The purpose of this procedure is to get a well defined starting point prior to switching on presentations according to the procedures invoked upon pressing the resp. button. This way, You than collect all representations that you wish to switch on upon calling the resp. procedure without having to bother about the history prior to invoking the button. In case you altered other parameters like color definition etc. you can also include a reset of these parameters.

There are several types of loops, that are invoked by pressing a button to activate a particular display:

The graphical representations 0 and 4 of structure 1 are switched on. This is convenient if there are only few presentations to be switched on.:

```
proc Proteinstart {} {  
  
    Reset  
    mol showrep 1 0 on  
    mol showrep 1 4 on  
}
```

All graphical representation of structure 0 between 2 and 16 are switched on :

```
proc Proteinmany {} {  
  
    Reset  
    set j 2  
    while {$j < 17} {  
        mol showrep 1 $j on  
        incr j  
    }  
}
```

The graphical representations 3, 5, 6, 9, 10, 12, 13 and 16 of structure 1 are switched on:

```
proc Showchromophores {} {  
  
    Reset  
    foreach i {3 5 6 9 10 12 13 16} {  
        mol showrep 1 $i on  
    }  
}
```

The procedures which switch on the particular ensemble of structures and graphical representations have to be connected to the corresponding buttons as shown above.

Overlapping structures

If you want to create an animation file or otherwise want to compare structures, you may want to overlay the different pdb-files. There are several ways to achieve this, and most methods are based on the One way is too keep the original pdb-files and include a procedure in the vmd-script that overlaps the structures. This is done by selecting those parts of the protein, that are identical, and apply the RMSD- algorithm. This way, the algorithm does not consider those parts of the protein that are different, like the hypervariable regions of an antibody or the differently bound antigen. Such a procedure is used in the file `antibody.vmd`:

```
# The following is code to align the three lysozyme antibodies.  
# For details check the VMD user manual!  
#  
  
set sel0 [atomselect 0 "backbone and (residue 5 to 85)"]  
  
set sel1 [atomselect 1 "backbone and (residue 5 to 85)"]  
measure rmsd $sel0 $sel1  
set transformation_matrix [measure fit $sel1 $sel0]
```

```

set move_sel [atomselect 1 "all"]
$move_sel move $transformation_matrix

set sel2 [atomselect 2 "backbone and (residue 5 to 85)"]
measure rmsd $sel0 $sel2
set transformation_matrix [measure fit $sel2 $sel0]
set move_sel [atomselect 2 "all"]
$move_sel move $transformation_matrix

set sel3 [atomselect 3 "backbone and (residue 5 to 85)"]
measure rmsd $sel0 $sel3
set transformation_matrix [measure fit $sel3 $sel0]
set move_sel [atomselect 3 "all"]
$move_sel move $transformation_matrix

```

The other way is to load the pdb-files in question and followingly apply the RMSD algorithm from the VMD -Extensions menuue:

Extensions > Analysis > RMSD calculator or by using

Extensions > Analysis > Multiple Alignment

Upon execution, the pdb-files are rearranged, and you may save these rearranged coordinates by using **File > Save Coordinates** from the VMD-menuue. The files created do not contain header or other information. I always kept the original file and allocated a new name to the file created this way.

There is also the option of overlapping or arranging the different structures manually. In this case, you have to activate the fixed flag in the VMD main panel for all structures but one, followingly move that structure with respect to the fixed structures and then save the rearranged file as above. Similarly, you can also include VMD commands in your code that manipulates the respective structure, like: **rotate**, **translate**, **moveby**. Take a look at the script **atpsynthaseanimation.vmd** by Christopher Bruhn for applications and consult the VMD manual for the syntax.

Including Animations

Some proteins exhibit a specific time -dependent behaviour, for example upon excitation by light. The chromophores of light detection proteins such as Photoactive Yellow Protein or Rhodopsins undergo a cis-trans isomerization upon light excitation, that afflicts the whole protein. For some of these proteins, time resolved X-ray structures are available and one can produce an animation by aligning the pdb-files belonging to the distinct structures. This is done in the script files **pypanimation.vmd**, **bacteriorhodopsinanimation.vmd** and **phototropinanimation.vmd**. The animationspeed in these scripts is set via a user-controlled

horizontal scale in a separate window. There was a specific problem concerning the termination of the animation upon pressing a non-animation button, which is illustrated in the script `bacteriorodopsinanimation_problemdemo.vmd`. The only fix to this problem is to check in every instance, if another button is pressed. To this end, new functions were defined that are functionally equivalent to the standard VMD commands, but check for a changed button. For example, the command `'mol showrep molnr presnr on'` was replaced by the command `'molshowAnim molnr presnr on'` which is defined as:

```
proc molshowAnim {molnr presnr status} {
    global stopanim
    if {! $stopanim} {mol showrep $molnr $presnr $status}
}
```

This ensures synthax compatibility with the original VMD-commands. If you know a better way to solve the problem, please let me know!

Workarounds for bugs in VMD

There are some bugs in VMD, particularly in the versions prior to VMD 1.8.4, that made it necessary to employ workarounds. Sometimes presentations based on the protein backbone recognition like tube, trace, and the ribbons and cartoon presentations do not show the protein structure correctly. In short, part of the structure is not shown. That happens in case of the antibody `ligt` and the ribosome structure. In both cases the workaround is to extract the missing part of the protein resp. RNA structure into a new file (for example `ligt_B_chain.pdb`, `ligt_D_chain.pdb`) and separately load these into VMD. In case of the antibody, the program seemingly got confused at the interchain disulfide bridges, and is fixed in version 1.8.4. The ribosome structure is still not shown correctly.

Another problem encountered in versions prior to VMD 1.8.4 is the handling of alternative structures contained in the protein data bank file, which according to their regulation is the `altloc` definition. This `altloc` definition is not recognized in old VMD versions and bonds are drawn between atoms of separate structures, provided these are within the appropriate radius defined by VMD. For example, Photoactive Yellow Protein contain alternate structures from time -dependent X-ray in one file. The solution in this case is to select all atoms belonging to one or the other structures for each presentation, which is fairly time consuming.

The syntax of script files, that were created prior to the release of VMD 1.8.4 is kept as mentioned above. While their syntax is more complicated and not very elegant in view of the new syntax features, the files work fine under new versions of VMD. However, new files will be implemented to run with VMD 1.8.4 and may not work properly in old versions of VMD.

Some final remarks

Note that you can not save the manually edited script from within VMD, since all the added features are going to be lost. You basically get your starting script back. You can change representations directly by editing the parts by hand, but this is difficult to do.

There are more procedures defined that may be useful to you, for example one that creates a sphere indicating the water splitting region in Photosystem II, or the inclusion of arrows. Just take a look at the examples.

References and Notes

- [Berman et al.(2000)] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, & P.E. Bourne (2000). ‘The protein data’. *Nucleic Acids Research* **28**:235–242.
- [Caddigan et al.(2005)] E. Caddigan, J. Cohen, J. Gullingsrud, & J. Stone (2005). *VMD User’s Guide*. Theoretical Biophysics Group, University of Illinois and Beckman Institute.
- [Humphrey et al.(1996)] William Humphrey, Andrew Dalke, & Klaus Schulten (1996). ‘VMD – Visual Molecular Dynamics’. *Journal of Molecular Graphics* **14**:33–38.
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