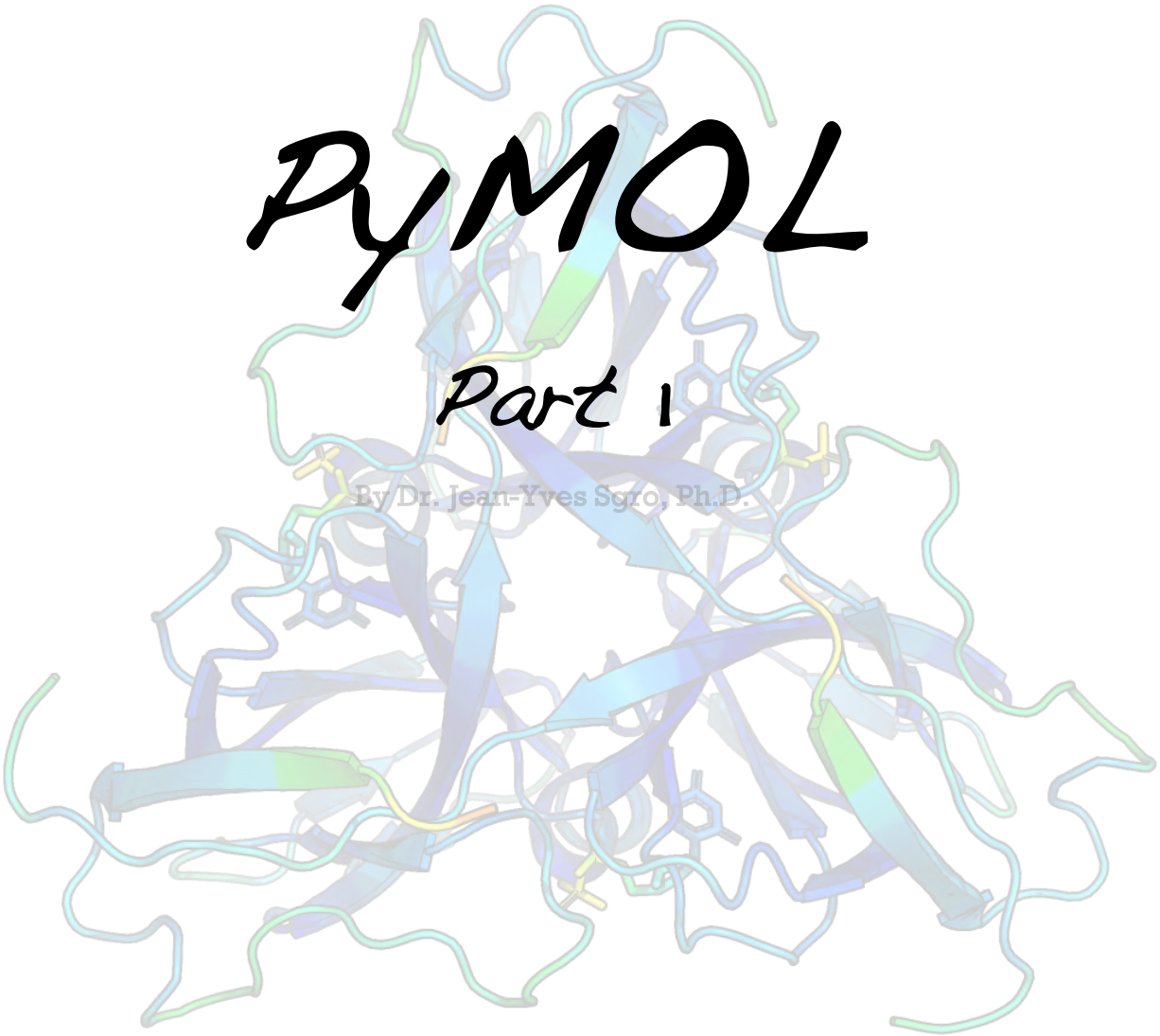
Book 2

PyMOL

Part 1



By Dr. Jean-Yves Sgro, Ph.D.

JYS

Cover: PyMOL rendering of PDB 1DUD (*Crystal structure of the Escherichia coli dUTPase in complex with a substrate analogue (dUDP).* Larsson, G., Svensson, L.A., Nyman, P.O. (1996) Nat.Struct.Mol.Biol. **3**: 532-538)

Preamble

For many years I participated in the teaching of Prof. Ann Palmenberg classes, in particular teaching about molecular graphics on the Desktop at a time when computer use in the classroom was not yet preeminent. This class was a useful complement to the main topic of Sequence Analysis and Evolution also using computers.

I personally used what was called "Unix Workstations" (Silicon Graphics, sgi) which had powerful graphics and rather beautiful "photorealistic" renderings. For teaching molecular graphics on the Desktop, I first used Rasmol, which was an amazing software fitting in about half of a 3.5" floppy disk or about 500 kilobytes. Rasmol included a sophisticated line command language but lacked the beautiful "photorealistic" renderings of the workstations. This was remedied by adding two other software in the course, one for the "publication quality renderings" (VMD) and the other for the modeling abilities of side-chain mutations and automated 3D superimposition of structures (Swiss PDB viewer later called DeepView.)

When PyMOL was still in preliminary development at version 0.99 I spent one intense week porting all the class material to PyMOL. Now, rather than using three different software, all was possible with only PyMOL. Over the years I extended and updated the PyMOL course material.

The UW-Madison Biochemistry students were the primary audience for these classes in courses Biochem 660 and 712, and occasionally in Biochem 511. I offered the PyMOL class in Biochem 660 for over a decade. The PyMOL tutorial and preliminary molecular graphics and file format introduction were part of a very large, made-to-order physical copy of a class book of about 500 pages that also contained tutorials on using other software. The PyMOL section was about 200 pages.

2017 was the last year that Biochem 660 was offered. I have therefore decided to release the complete PyMOL tutorial which you will find split in multiple PDF files. In this final revision, I had updated all web pages, and added links to archived pages when web site were defunct to keep the text as relevant as possible for future use.

I hope that it will be useful to you in accomplishing your molecular graphics goals.

Sincerely,

Jean-Yves Sgro, Ph.D.
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PyMOL Overview

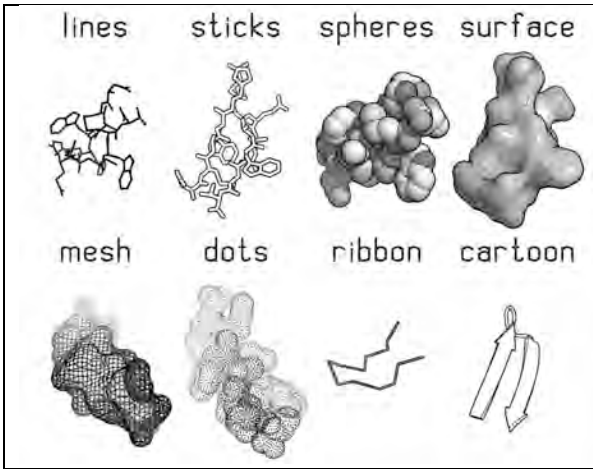
1. Preface

PyMOL *was created in an efficient and pragmatic manner, with heavy emphasis on delivering powerful features to end-users. […] PyMOL is about getting the job done now, as fast as possible, by whatever means were available.* [User's guide preface.][1]

PyMOL has become a *de facto* standard in publications containing molecular graphic figures.

According to the official web site www.pymol.org PyMOL can read **30 different file formats** and offers **more than 600 settings** and **20 representations** to provide users with precise and powerful control.

[1] http://pymol.sourceforge.net/newman/userman.pdf *or* http://pymol.sourceforge.net/newman/user/toc.html



"Using PyMOL, data can be represented in nearly 20 different ways.

Spheres provides a CPK-like view, **surface** and **mesh** provide more volumetric views, **lines** and **sticks** put the emphasis on bond connectivity, and **ribbon** and **cartoon** are popular representations for identifying secondary structure and topology."
(*Scene from menu: Wizard* > *Demo* > *Representations*)

Go further with the web

PyMOL: An Open-Source Molecular Graphics Tool, Warren L. DeLano, Ph.D.
http://www.ccp4.ac.uk/newsletters/newsletter40/11_pymol.html
[http://bit.ly/MfELjP] [archived: http://bit.ly/2c1yJJu]

Video: Understanding the PyMOL User Interface (3:15 min) (split interface)
http://youtu.be/vROwrMqX-0Q

Video: PyMOL (SBGrid presentation) (43:03min) (includes volume rendering)
http://youtu.be/UHOUFJmj_fM

Quick Tutorials: (underlined = archived version for defunct links)

- **http://www.pymolwiki.org/index.php/Practical_Pymol_for_Beginners**
- http://www.carlyhuitema.com/pymol_tutorial.html (2002/2010 – *Carly Huitema*) [*defunct but available at Archive.org*: **http://bit.ly/1tXrJ3x**]
- **http://www.bio.ph.ic.ac.uk/~scurry/pdfs/PyMOL_tutorial.pdf**
  archived **http://bit.ly/1qzZYII** (2009 –*Stephen Curry*)
- **http://www.bio.ph.ic.ac.uk/~scurry/pdfs/PyMOL_movies.pdf**
  archived **http://bit.ly/1BdrWQC** (2009–*Stephen Curry*)
- http://www.psb-grenoble.eu/IMG/pdf/pymol_tutorial.pdf (2010 – *Manjasetty, Dian & Quintero Bernabeu*) - *archived*: **http://bit.ly/1OoLtYi**

Web Resource for users

PyMOL Wiki: http://www.pymolwiki.org/

The Mail Archive:
http://www.mail-archive.com/pymol-users@lists.sourceforge.net/
[http://bit.ly/NeM6lq]

Posting to Forum/Archive:
http://www.mail-archive.com/pymol-users@lists.sourceforge.net/info.html
[http://bit.ly/RYteKw]

Alternate Archive:
http://sourceforge.net/mailarchive/forum.php?forum_name=pymol-users
[http://bit.ly/M18kKc]

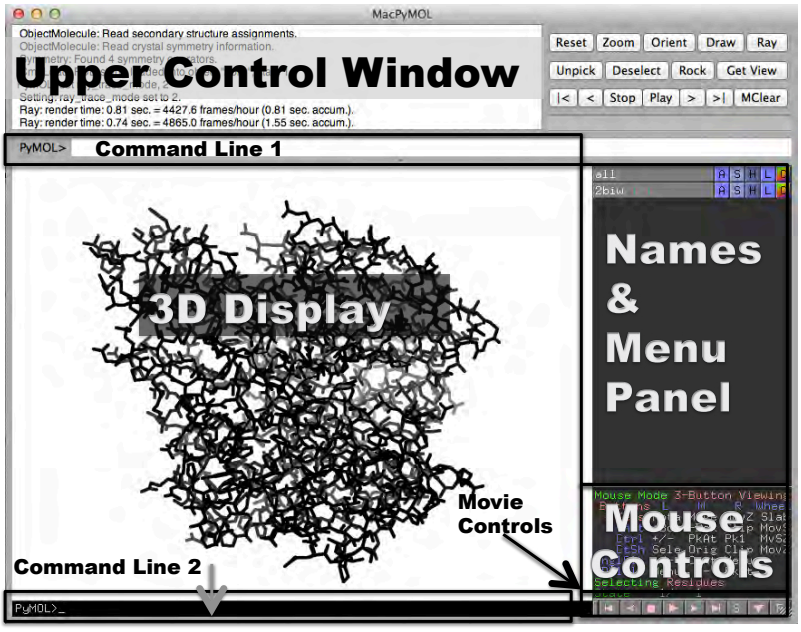
PDB + PyMOL Tutorial from UNC - Video (YouTube Playlist):
https://www.youtube.com/watch?v=bHXJgE_alF8&list=PLr_d6VlHdTSNdv8qtiWDHJnBdBervg_Eo
[http://bit.ly/1vBECkQ]

2. PyMOL interface

WITH ONLY MINOR differences PyMOL works in the same way on all supported platforms for both the Graphical User Interface (GUI) and the command line.

2.1 PyMOL Graphical Interface (GUI)

THE PyMOL INTERFACE is split between an Upper Control Window and a lower Viewer Window; they appear as 2 separate windows (Windows/Linux) or a single, combined window (Mac, as illustrated below.)



Upper Control Window with quick menu buttons and a command line that accepts pasted text and can be copied from.

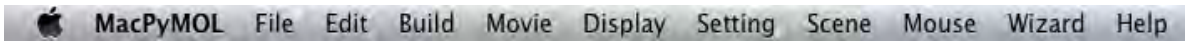
Lower Viewer Window is split into functional sections:

- **3D view** display (640 x 480 default size.)

- **Names Panel**: each file opened will have an entry.

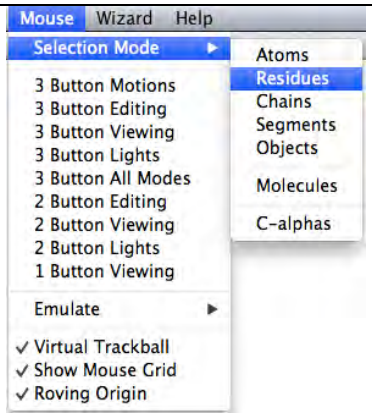
- **ASHLC** (**A**ction, **S**how, **H**ide, **L**abel and **C**olor) **menus** apply independently to each name entry, or to all with the "all" top entry.
- **Mouse Controls**: a reminder of the functions for each button.
- Movie Controls: VCR style button controls for animations.
- **Command Line 2**: commands are accepted here too.

Note: The software menus appear at the top of the screen (Mac) or at the top of the separate Upper Control Window (Win/Lin) and offer amongst others the familiar **File** and **Edit** menus present on all Macintosh software since 1984.

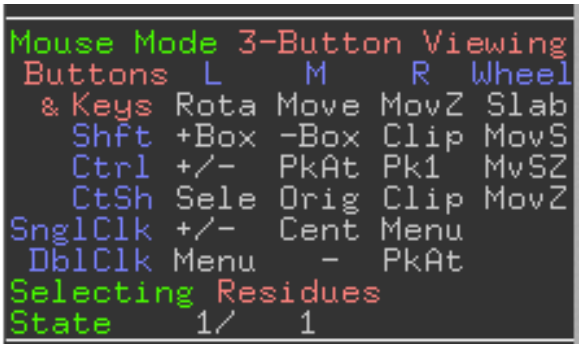


2.2 Mouse Controls overview

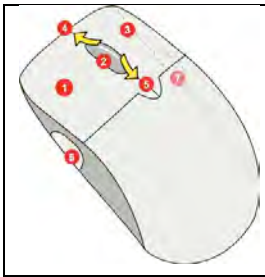
PyMOL SUPPORTS MULTIPLE MOUSE TYPES and track-pads. At launch the default is set to a 3-button mouse, selecting the multiple atoms of a residue when clicking on any of its atoms within the 3D view panel. The default functions can be changed with the mouse menu and the current setting is reflecting within the Mouse Controls sections at the bottom right.



Mouse menu items



Mouse Controls, bottom right.
Buttons are labled as L(eft), M(iddle) ,R(ight) and Wheel



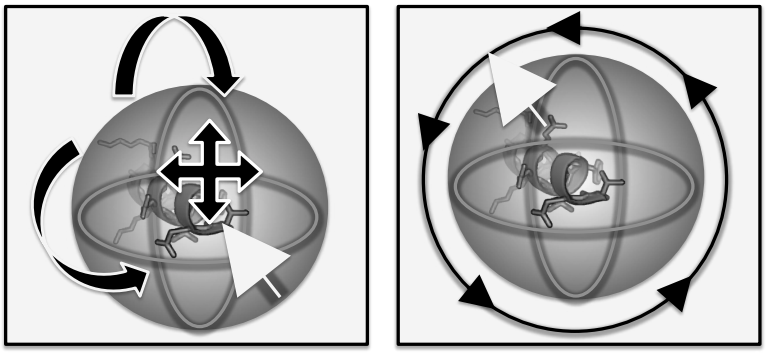
Modern mouse devices have 7 buttons

1 Click
2 Middle Click
3 Right Click
4 Scroll Up (considered

It is relatively easy to ignore buttons 6 and 7.

The actions performed when clicking on button 2 (**M**iddle button) would be different than

	button 4) *5 Scroll Down (considered button 5)* *6 Back button* *7 Forward button*	if the button is scrolled if a wheel is present.



Within the PyMOL viewer, represented here as a black rectangle, the movements of the mouse are interpreted as that of a virtual trackball:

• when the mouse (shown as a white arrow below) is **ON** the displayed molecule, the molecule can rotate freely in all directions.

• when the mouse is clicked **OUTSIDE** of the rotation "sphere", *e.g.* on the top left area as shown, the rotation is limited to around the Z axis, which points outward from the screen.

Clipping planes are useful to focus on specific part of large molecules. Scrolling the wheel can make part of the molecule disappear. Exercises will help better understand this concept.

Go further with the web

Getting Started with Mouse Controls:
http://PyMOL.sourceforge.net/newman/user/S0200start.html
[http://bit.ly/MklUKD] [archived: http://bit.ly/2fDU8Lm]

2.3 Movie Controls, Sequence Display and Full Screen

THE VCR-like Movie Controls panel appears at the very bottom-right of the screen and controls the animation of displayed models.

The last three buttons on the right have the following meaning:

- **S**: display the structure sequence (will appear as a line at the top of the 3D viewer)
- Downward Triangle: rock the molecule back and forth
- **F**: switch to full-screen mode

2.4 Line Commands and Scripts

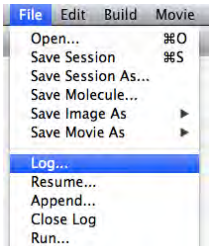
PyMOL ACCEPTS TEXT COMMANDS typed on the upper or lower command-line areas or pasted within the top command-line area (see GUI figure above.)

- Commands are case sensitive, but all commands are currently written within PyMOL as lower-case. Therefore **all commands should be written as lower-case**.
- As of this writing there are **191 commands** listed within the PyMOL Wiki[2].
- Point-and-click (mouse) menu options typically have a line-command equivalent.
- Scripts: all commands can be summarized line-by-line within a text file and run all at once.

While learning line commands and scripting them do take time, this is part of the "user power" afforded by PyMOL for recording, reproducing and sharing images in the form of scripts.

2.4.1 Recording Commands: Log Files

All the commands, typed or clicked, can be recorded in a log file for later replay. For good practice the file name extension should be `.pml`.



Point-and-click method

```
SYNTAX

    log_open log-file-name

EXAMPLE

    PyMOL> log_open log1.pml
```

Command-line method

[2] http://www.pymolwiki.org/index.php/Category:Commands

To stop recording use menu "**File > Close Log**" or type **log_close** in the command line area. If you don't close before you exit PyMOL, your log-file will still be saved to disk.

Later the file can be run with the menu "**File > Run…**" or from the command line by adding the symbol @ with no space as a prefix. Example: **PyMOL>** `@log1.pml`

2.4.2 Help

To see the list of available commands with help entries type `help` (and press return) within either of command line areas. There are about 80 commands with descriptive help and examples.

```
SYNTAX
   help keyword

EXAMPLE
   PyMOL> help load
```

2.4.3 Loading Data

PyMOL can read and automatically recognize data format from the filename extension, which can be overridden with the appropriate line command. PDB files with extension `.pdb` will be commonly used in class and other formats will not be explored.

Atomic coordinates in PDB format can be read into PyMOL from a file present on the hard drive with the **`load`** command or with the "**File > Open...**" point-and-click menu.
By default the root name (without `.pdb`) of the file will become the object name as a line entry within the Names & Manu Panel for further GUI manipulations. However, one can specify a chosen name if the file is opened by line-command. # is the comment character.

```
SYNTAX

   load data-file-name, object-name

EXAMPLES

   PyMOL> load pept.pdb         # The object is named "pept"
   PyMOL> load pept.pdb, test   # The object is named "test".
```

PyMOL will read files from the HOME directory specific to the operating system used. Alternatively the working directory can be changed, or a path to the file into another directory can be specified e.g. with the `cd` line command.

It might be advantageous or efficient to read the data directly from the Internet with the line-command `fetch`. Furthermore the `fetch` command can be used to download the biological unit instead simply by adding the qualifier `type` and specifying the biological unit desired such as `type=pdb1`, `type=pdb2` etc. An alternate object name is only allowed if downloading the original data.

```
SYNTAX
      fetch PDB-code, type=[pdb1,pdb2,...]

EXAMPLES
      PyMOL> fetch 1DUD, myTest       # The object is named "myTest 1/1"
      PyMOL> fetch 1DUD, type=pdb1    # The object is named "1DUD   1/3"
```

Biological units are encoded as separate "**models**" within the PDB file but are called "**states**" once read into PyMOL. The Names Panel entry reflects the number of models in the file. Models represent the same atomic coordinates in a series of alternate configurations and are numbered consecutively. Such files result from NMR experiments, molecular dynamics calculations, animation or morphing software, or due to the multimeric nature of the structure(s) such as virus capsids. By default only the first model/state is shown in the 3D viewer. To see all models the user has 2 options: show all models at once, or split the models into independent entry copies listed separately on the Names Panel. While these could be considered "advanced" commands they are essential for biologists.

Showing all models at once is an on/off switch setting:

```
SYNTAX
      set all_states, on
      set all_states, off
```

There will be no change in the Names Panel and all models would move together when rotating the mouse.

When making a copy of the individual models into separate object-names the original entry remains present and can be later deleted or hidden. The new copies will be numbered object-name_001, _002, etc.

```
SYNTAX
       split_states object-name

EXAMPLE
      PyMOL> fetch 1DUD, type=pdb1  # The object is named "1DUD   1/3"
      PyMOL> split_states 1DUD      # split 1DUD into 3 new entries
```

```
                                  # numbered 1DUD_001, 1DUD_002, etc.
PyMOL> delete 1DUD                # delete the original fetched entry
PyMOL> delete 1DUD_*              # delete all separate entries
```

Note: the `delete` command is useful for large units such as viruses that would typically yield 60 separate new entries.

2.4.4 Atom Selection: PyMOL Selectors

Atom selections can be made and saved within an object-name listed on the Names Panel. To distinguish these entries form PyMOL keywords a good practice is to start these names with "My" as a way to remember that these are our "creations."

The syntax format is: "***select MyName = criteria***" to create a new object named "MyName" from criteria created with a selection method with the help of one or more "selector" listed in the following table.

Matching Property Selector	Identifier and example
symbol	*chemical-symbol-list* list of 1- or 2-letter chemical symols from the periodic table `PyMOL> select Mypolars , symbol o+n`
name	*atom-name-list* list of up to 4-letter codes for atoms in proteins or nucleic acids `PyMOL> select Mycarbons , name ca+cb+cg+cd`

resn	*residue-name-list*
	list of 3-letter codes for amino acids
	`PyMOL> select Myaas , resn asp+glu+asn+gln`
	
	list of up to 2-letter codes for nucleic acids
	`PyMOL> select Mybases , resn a+g`

resi	*residue-identifier-list*
	list of up to 4-digit residue numbers
	`PyMOL> select Myresidues , resi 1+2+20+8590`
	
	Residue-identifier-range
	`PyMOL> select MyNterm , resi 1-25`

chain	*chain-identifier-list* list of single letter (rarely numbers) of the chain `PyMOL> select MyChain , chain a`
ss	*Secondary-structure-type* list of single letters `PyMOL> select Myalphas , ss hs+l+""`

Single Word Selector	Short Form Selector	Description and examples

all	*	All atoms currently loaded into PyMOL
		`PyMOL> color blue , all`
		`PyMOL> color blue , *`

none	none	No atoms (empty selection)

hydro	h.	All hydrogen atoms currently loaded into PyMOL
		`PyMOL> hide hydro`
		`PyMOL> hide h.`

hetatm	het	All atoms loaded from Protein Data Bank HETATM records
		`PyMOL> show spheres, hetatm`
		`PyMOL> show spheres, het`

visible	v.	All atoms in enabled objects with at least one visible representation
polymer		All atoms on the polymer (not het).
present	pr.	All atoms with defined coordinates in the current state (used in creating movies)

Go further with the web

Getting Started with Commands:
http://PyMOL.sourceforge.net/newman/user/S0210start_cmds.html
[http://bit.ly/N2WeBW] [archived: http://bit.ly/2clELW6]
Command Syntax & Atom Selections:
http://PyMOL.sourceforge.net/newman/user/S0220commands.html
[http://bit.ly/Mncc6y] [archived: http://bit.ly/2c4WvTE]
Property Selectors:
http://www.pymolwiki.org/index.php/Property_Selectors
[http://bit.ly/NrlkVp] [archived: http://bit.ly/2cpZO8w]
Single word selectors:
http://wwww.pymolwiki.org/index.php/Single-word_Selectors
[http://bit.ly/Nk2u4b] [archived: http://bit.ly/2c1GdME]

2.5 Settings

THERE ARE CURRENTLY 604 settings that can modify the behavior of PyMOL, 193 of them are documented within the PyMOL Wiki (references in "Go further" box below.) For example there are 50 settings for ray-tracing, 58 for cartoon representations, 29 for surface etc. A few settings can be changed by mouse within the main "**Setting**" menu.

Other settings can be changed by 2 methods:

- the **`set`** command
- opening a window listing settings: "**Setting > Edit All…**"

Using the line command implies knowing the exact setting name, for example `all_sates` as seen above. The window listing affords browsing and sometimes guessing what the setting could be.

Go further with the web

All Settings: http://www.pymolwiki.org/index.php/Settings
[http://bit.ly/MnKoME] [archived: http://bit.ly/2cy6xkc]

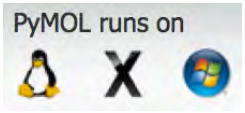
Documented Settings:
http://www.pymolwiki.org/index.php/Category:Settings
[http://bit.ly/LMqIbP] [archived: http://bit.ly/2bVLYfZ]

Gallery of complex images calling on many settings:
http://www.pymolwiki.org/index.php/Gallery
[http://bit.ly/MnNC5n] [archived: http://bit.ly/2bVM2we]

Installing PyMOL

Computer-generated color images are illustrating numerous magazine covers and research articles. Creating 3D representations of molecules is much easier in the 21rst Century than even in the last decade of the 20th Century when these could only be created on (very) expensive workstations.

PyMOL is a *de facto* standard in scientific publications containing molecular graphic figures. The laboratory tutorials appearing in this lab book are self-paced tutorials to understand how to visualize and animate molecular structures in publication-quality graphics and animations for research papers and conference slide shows.



PyMOL is installed on the classroom computers and is the standard molecular graphic software used in the Biochemistry department.

PyMOL download on UW-Campus ?

UW Students, Faculty and Staff can download PyMOL from the **Campus Software Library** (**`software.wisc.edu`**) licensed by the University of Wisconsin-Madison for installation on both UW-owned devices and personally-owned devices.

(Note: **NetID login required**)

SBGrid

Students in labs that access SBGrid in Biochemistry can launch PyMOL from an SBGrid line-command with simply the command line: `pymol &`
Install SBGrid from `https://biochem.wisc.edu/intranet/it/sbgrid`

If you are not affiliated with UW here is some useful information about acquiring PyMOL:

For your personal use, Teachers and Students can request an educational license of PyMOL from the following web page request:
`http://pymol.org/educational/`

> *"PyMOL values scientific progress and understands the opportunity cost that can accompany the pursuit of education. As such, PyMOL offers Educational-use-only PyMOL builds available at no cost to teachers and full-time students for classroom instruction, homework assignments, and to provide students with means for creating professional-grade figures and animations for posters, talks, publications, and dissertations."*

Note: Other free options exist, but require some computer administration:

Precompiled versions for the Windows operating system available at `http://www.pymolwiki.org/index.php/Windows_Install` [archived: `http://bit.ly/2bQMNDr`]

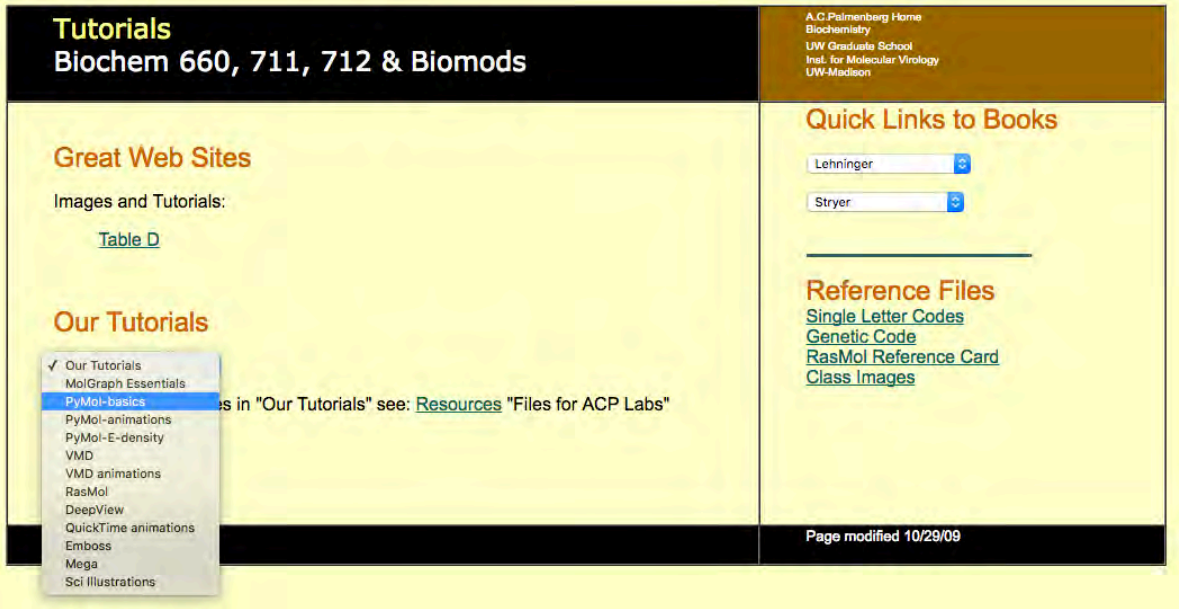
With Mac Port (`http://www.macports.org/`) Macintosh users can install from source code after other requirements have been fulfilled (such as installing Apple's Xcode) with command: `port install pymol`

Another option for Mac users with the same prior requirements is to use *homebrew* (`http://brew.sh`) with the command:
`brew install homebrew/science/pymol`

Linux users can also install from source code with *e.g.*

```
          sudo yum install pymol
   or     sudo apt-get install pymol
```

Tutorials for other molecular graphics software for UW classes are available as PDF online **`http://www.virology.wisc.edu/acp/Classes/tutorials.html`**



These tutorials were previously used in class before or concurrently with PyMOL:

- **VMD** is another free software for creating rendered molecular surfaces and ribbon diagrams and has a method for the automatic generation of animated movies.

- **DeepView** (formely Swiss PDB Viewer) was used for mutating an amino-acid side chain and is also useful to superimpose related structure in three dimensions.

- **Rasmol** is a simple 3D visualizer with a scriptable line command for creating animations. Rasmol provides the basis for quick analyses and can export scripts for other rendering software. Rasmol can be used for creating 3D objects with the Z-print printer as well. Rasmol is free and runs on virtually any platform. Jmol now is used as the Rasmol web plug-in equivalent and offers many of the Rasmol options and line commands.

Before we start with PyMOL – 2biw – carotenoid oxygenase

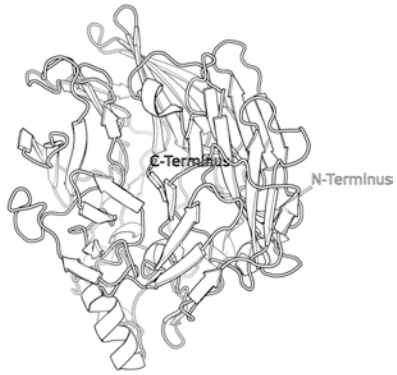
1. Carotenoid oxygenase enzyme

THE EXERCISES IN THE NEXT SECTION concentrate on learning the PyMOL software using a protein example for many exercises. Here is some information about this protein that may be useful:

2biw	Description
Enzyme function description [3]	Carotenoid oxygenase breaks carotenoids (the colorful pigments in carrots) in half, forming vitamin A (retinal). This structure includes the enzyme, a carotenoid molecule, and an iron ion that positions oxygen for the cleavage reaction.
PDB ID number	2biw or 2BIW
Related structure	2BIX (Fe, Free apoenzyme)
Primary citation	Kloer DP, Ruch S, Al-Babili S, Beyer P, Schulz GE. The structure of a retinal-forming carotenoid oxygenase. *Science*. 2005 Apr 8; 308(5719):267-9. - DOI: 10.1126/science.1108965
Abstract: Enzymes that produce retinal and related apocarotenoids constitute a sequence- and thus structure-related family, a member of which was analyzed by x-ray diffraction. This member is an oxygenase and contains an Fe2+-4-His arrangement at the axis of a seven-bladed beta-propeller chain fold covered by a dome formed by six large loops. The Fe2+ is accessible through a long nonpolar tunnel that holds a carotenoid derivative in one of the crystals. On binding, three consecutive double bonds of this carotenoid changed from a straight all-trans to a cranked cis-trans-cis conformation. The remaining trans bond is located at the dioxygen-ligated Fe2+ and cleaved by oxygen.	

[3] Verbatim from PDB 101 molecule of the month (http://bit.ly/qRqNTc)

1.1 Protein fold: seven-bladed ß propeller



PyMOL cartoon representation in similar orientation

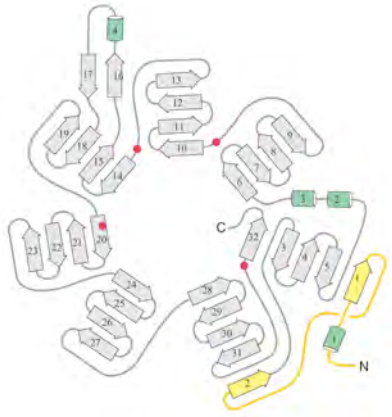


Figure S3 (supplemental material), Kloer et al. *Science paper*

1.2 Reaction

THE REACTION IS DESCRIBED in the Kloer *et al.* Science paper. ACO is short for "apocarotenoid-15,15′-oxygenase"

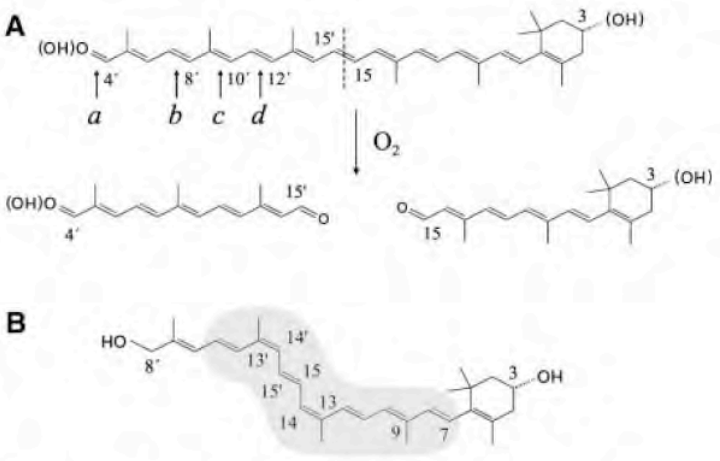
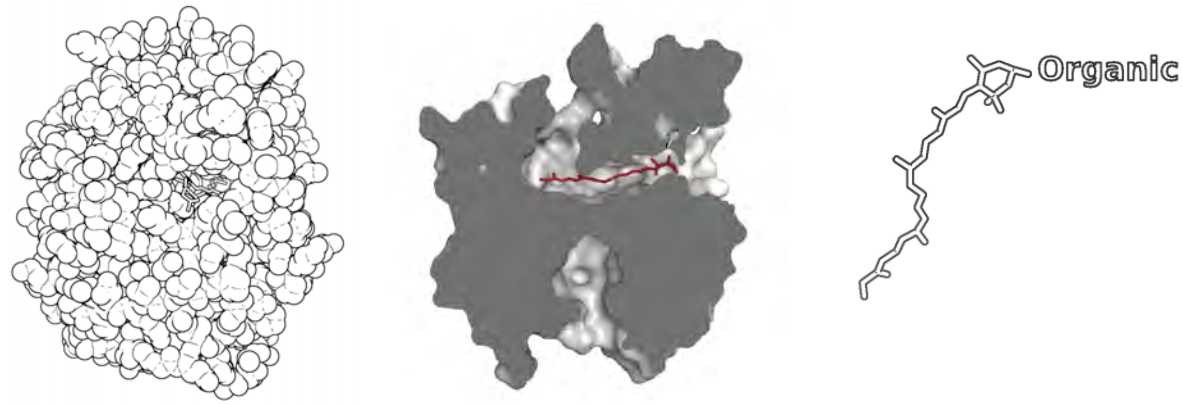
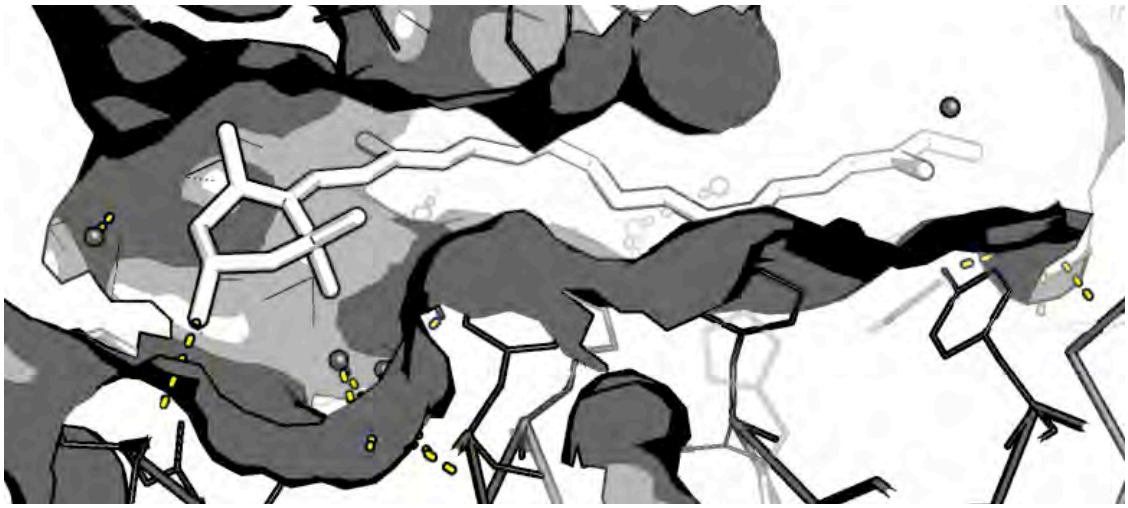


Fig. 1. Enzyme data. (**A**) The reaction catalyzed by ACO (*21*). ACO accepts the all-trans conformations of the homologs *a*, *b*, *c*, and *d* as alcohols or aldehydes with and without the 3-hydroxy group, but it does not accept β-carotene (*21*). (**B**) ACO crystallized in the absence of Fe^{2+} and in the presence of the *b*-type substrate all-*trans*-(3*R*)-3-hydroxy-8′-apo-β-carotenol. The shaded part of the substrate was used to interpret the electron density in a native crystal produced by soaking with Fe^{2+} and subsequent freezing to 100 K. Surprisingly, the all-trans substrate had changed to the 13,14-13′,14′-di-*cis* conformation.

1.3 Pocket

THE PROTEIN WAS CRYSTALLIZED with a retinoic compound that lies within a pocket inside the enzyme as illustrated with the following PyMOL-generated images:





The retinoic compound is labeled "organic" in the illustration and within the PDB file structure the retinol is named: (3R)-3-hydroxy-8'-apocarotenol and abbreviated 3ON.

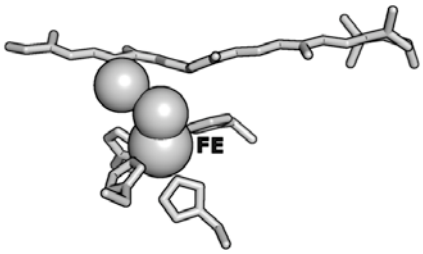
In addition there is an iron atom Fe^2 (see illustration below.)

1.4 Ligand interactions

SOME WATER MOLECULES MAY SOMETIMES PLAY AN IMPORTANT ROLE in the activity of an enzyme. In the case of this protein, two of them are near the position where oxygen is used to cleave the retinol compound. The iron atom (Fe) is held in place by the grip of 4 histidine amino acid side chains. Between the iron sphere and the retinoic compound are 2 oxygen atoms representing water molecules.

Water molecules residues number 2022 and 2079 and iron (Fe) residue number 1492 within the PDB file.

Histidine residues sequence number: 183, 238, 304, 484



2. Membrane-bound

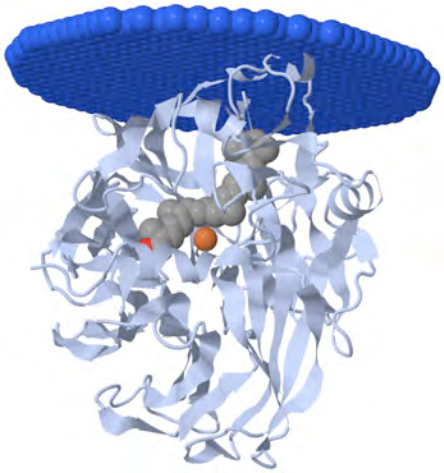
IN THEIR SCIENCE PAPER Kloer et al. propose that "*The surface of ACO contains a nonpolar patch that consists mostly of protruding leucines and phenylalanines (Fig. 3A). We propose that ACO uses this patch to dip into the membrane and extract its nonpolar substrate from there.*"

The link to the web site is:

`http://opm.phar.umich.edu/protein.php?search=2biw`

[`http://bit.ly/Y99eh1`]

The web site "orientations of proteins in membranes" (OPM) offers a set of PDB format coordinates with "DUM" atom entries representing the cytoplasmic side of the membrane boundary.



The coordinates can be downloaded as a PDB text file or viewed with accessory plug-in software *webmol* or *Jmol*.

Shown here is the representation resulting from choosing Jmol.

3. Optional preliminary exercise

Exploring the 3D data file content:

You can display the coordinates of the 2BIW structure by going to the PDB database web site **http://www.rcsb.org**

Enter **2BIW** in the search area.

Next to the large 2BIW on the right hand side choose "Display Files > PDB File"

Scroll up and down the text file and observe the contents.

```
HEADER    OXIDOREDUCTASE                          19-JAN-05   2BIW
TITLE     CRYSTAL STRUCTURE OF APOCAROTENOID CLEAVAGE OXYGENASE FROM
TITLE    2 SYNECHOCYSTIS, NATIVE ENZYME
COMPND    APOCAROTENOID-CLEAVING OXYGENASE
KEYWDS    OXYGENASE,NON-HEME IRON,CAROTENOID CLEAVAGE,RETINAL
KEYWDS   2 FORMATION, OXIDOREDUCTASE, DIOXYGENASE
EXPDTA    X-RAY DIFFRACTION
AUTHOR    D.P.KLOER, S.RUCH, S.AL-BABILI, P.BEYER, G.E.SCHULZ
JRNL        AUTH   D.P.KLOER, S.RUCH, S.AL-BABILI, P.BEYER, G.E.SCHULZ
JRNL        TITL   THE STRUCTURE OF A RETINAL-FORMING CAROTENOID
JRNL        TITL 2 OXYGENASE
JRNL        REF    SCIENCE                       V. 308   267 2005
JRNL        REFN   ASTM SCIEAS  US ISSN 0036-8075
REMARK   1
SEQRES   1 A  490  MET VAL THR SER PRO PRO THR SER SER PRO SER GLN ARG
SEQRES   2 A  490  SER TYR SER PRO GLN ASP TRP LEU ARG GLY TYR GLN SER
[. . . . . ]
MTRIX2   3 -0.756169  0.514705 -0.404088       13.98510    1
MTRIX3   3  0.610627  0.777006 -0.152957      118.86930    1
ATOM      1  N   GLN B  12      16.794 -17.939  28.900  1.00 71.25           N
ATOM      2  CA  GLN B  12      16.091 -17.967  30.218  1.00 71.00           C
ATOM      3  C   GLN B  12      16.877 -17.181  31.273  1.00 69.76           C
[. . . . . ]
ATOM   3766  CG2 THR B 490      47.002  -0.207  59.451  1.00 73.42           C
ATOM   3767  O'' THR B 490      48.633   0.228  56.341  1.00 73.10           O
TER    3768      THR B 490
HETATM 3769 FE    FE B1492      30.259   6.403  38.822  1.00 45.64          FE
HETATM 3770  O1  3ON B1491      26.391  21.062  36.210  1.00100.47           O
HETATM 3771  C5  3ON B1491      25.099  20.591  36.637  1.00100.23           C
[. . . . . ]
CONECT 3799 3798
CONECT 3800 3798 3801
CONECT 3801 3800
MASTER        0    0    0   36  154    0   24   1515742    4   37  152
END
```

PyMOL Tutorials (1)

Note: within the exercises, **Bold** text shows what actions are taken by the user: typing text or clicking the mouse.

Whenever possible a "call for action" icon (√ or ✓) will be shown:

√ or ✓ **TASK**: you have something to do, a task to accomplish!

✓ **READ**: reading this material will help understand the task at hand

✓ **INFO**: additional information that is nice and useful but not critical

✓ **Advanced**: for your information only. Advanced resources are not covered in these exercises and are reserved for expert users.

✓ **NEW:** New feature added in recent version(s)

✓ **FIRST TASK**: Before starting please review "**PyMOL Overview**" in the previous section as a refresher from the slide presentation. Hints will be given along the way as well, and you can raise your hand if you have a question!

1. Open PyMOL and load coordinates

Reminder: Structures are published with a PDB ID code of 4 characters. PDB files are plain text and contain coordinates pertinent to the crystallographic arrangement of the molecules within the crystal. The biological functional entity can be either a multimer of the deposited structure, or just one of multiple copies within the file. In the following example we will obtain one functional biological subunit, in this case a monomer, directly from the Internet. We will specify which of the monomer we want with the use of the qualifier **`type=`** and specifying "**`2`**" will mean that out of the 4 existing monomers making the crystal we want the 2nd one.

PyMOL (or MacPyMOL) should be located within the **Applications folder** on the Macintosh computer.
Your instructor may give you a different location if necessary.

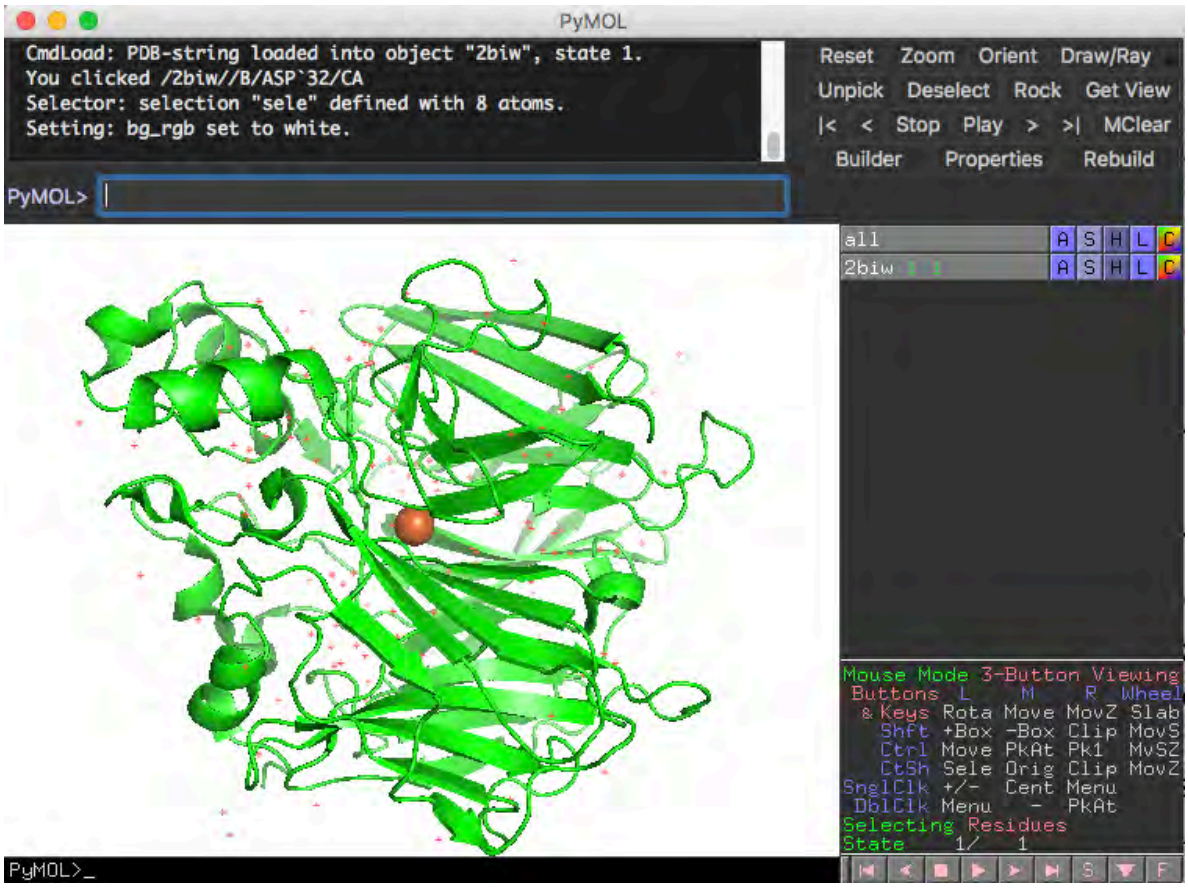
✓ **TASK: double-click on the icon** for **PyMOL** (or MacPyMOL**)** to launch the software.

After the **PyMOL>** prompt within the *top* line command, **type the following command:**

✓ **TASK:** `fetch 2biw, type=pdb2`

This command will instruct PyMOL to connect to the protein data bank (PDB) over the Internet and obtain the 2nd monomer from the original 3D (which contains 4 monomers in total, accessible as type `pdb1`, `pdb3` and `pdb4`.)

The coordinates are downloaded and read into PyMOL (they are also saved within the default user directory.)
Under the Names Panel at right note the new entry: **2biw 1/1**

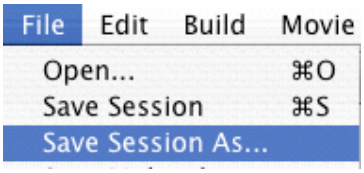


Note: if the molecule moves back and forth you can stop the movement simply by clicking on the top right button Rock. (*Here the background has been changed from the default black to white for clarity*.)

2. Saving a session, Reset, Reinitialize

2.1 Session file

A session file is a binary file containing all the information, settings and graphical displays currently within PyMOL. It is a way to save the "current state"of the software with all that it contains. Later, the file can be opened and everything is restored!



✓ **TASK** From the top menu follow the menu cascade:

File > Save Session As…

In the next window enter a name: **MySession** and save the resulting file on the **Desktop** to easily find it again.

The successful writing of the file will be reported within the text window of the "Upper Window" and a file called `Mysession.pse`
Now quit PyMOL: **File > Quit**

Note: pse files are not always 100% compatible between all PyMOL version numbers (but should be across operating system platforms of same version.)

2.2 Restoring the session file

While it is possible to simply double click on the MySession.pse icon, this could create problems if there are multiple copies or versions of PyMOL within your computer. It is therefore best to **first open the PyMOL software** version you want to utilize.

✓ **TASK** **double click** on the **Applications > MacPyMOL** software to open it.
Within the PyMOL top menu follow the menu cascade: **File > Open…..**
and then **select Mysession.pse** from the Desktop.

All the PyMOL names and selected items are restored as if you had just made them, even if days have passed since you saved the file. How convenient!

Note: the file has a binary format. There is no information within the file that is useful for any other purpose. The file for this exercise should likely be slightly less than 1Mb in size.

2.3 Reset

The line-command `reset` will simply reposition the molecule(s) in the original orientation without changing anything else. The background or the various cartoon representations will not be affected.

2.4 Reinitialize

The top menu "**File > Reinitialize**" will erase everything and reset PyMOL in it's default state as when it was first opened.

This is useful to restart from scratch without re-launching PyMOL.

3. Command GetPDB

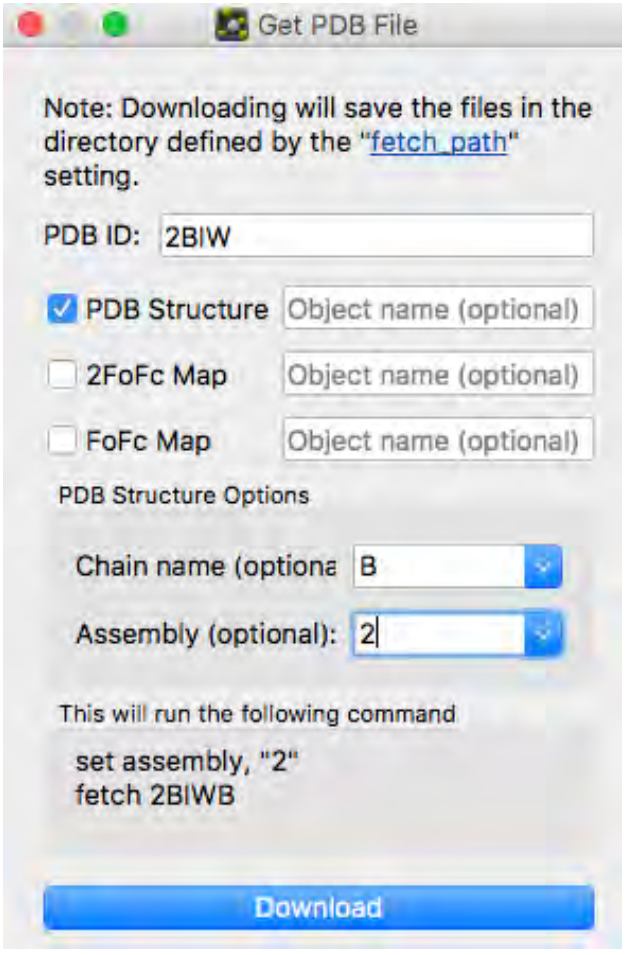
INFO

Starting with **PyMOL 2.0** the command **File > GetPDB** can also help the biological unit of a PDB file through a pop-up window.

However, in some cases it may be necessary to know some prior information.

For example in the case of 2BIW, if we want to call for the 2nd item it is necessary to know that the chain ID name is B. Indeed in the original PDB the four identical biological monomer making up the crystallographic assymetric unit are labeled A, B, C and D.

Therefore the window to load this structure would look like this after calling the menu **File > GetPDB**



Note: the downloaded structure name would be different, in this case it would be 2BIWB.

4. PyMOL – Home Directory & Path

PyMOL will "look" by default within the "Home" directory, typically the directory bearing the user's name.

On the DMC Macintoshes, the HOME directory is called **dmc** and could also be written as `/Users/dmc`

On a Windows system it would depend on the Windows version:

Microsoft Windows 2000, XP and 2003, *e.g.* `C:\Documents and Settings\username`

Microsoft Windows Vista, 7, and 10 *e.g.* `C:\Users\username`

You should be, or become familiar with the operating system of your own computer.

Useful commands:

✓ **TASK** **Type the next commands** after **PyMOL>** within the *top* line command:

PyMOL> **`cd desktop`** Note the echo on the text area above
PyMOL> **`pwd`** This will echo `/Users/DMC/Desktop` or a similar path.

Note: PyMOL uses many Unix-like commands (but this works in all computers!)

`cd` = change directory
`pwd` = present working directory
`ls` = list files

Note: **`cd ~/desktop`** (tilde and forward slash) will always take you home to the desktop if you are "lost" for any reason.

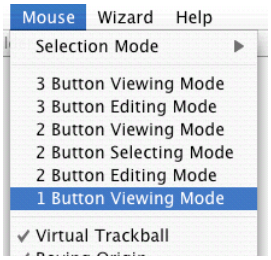
Note: If you have a PDB file on the Desktop that you want to read into PyMOL use either the "File > Open…" menu or the `load filename` line-command.

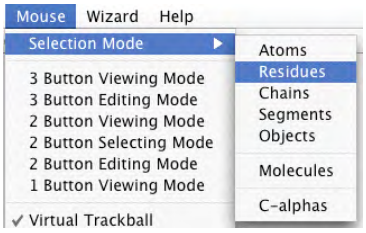
5. PyMOL interface defaults and settings

Interface basics are presented in a previous section named "**PyMOL Overview.**" When PyMOL opens a molecule for the first time all defaults are on (*e.g.* black background, molecule presented as green lines etc.) and PyMOL assumes a 3-button mouse. All of the defaults can easily be altered with a mouse click or a command.

5.1 Default mouse settings and selection

Top menu bar "Mouse" options:





✓ **INFO –** By default **PyMOL assumes that you have a 3-button mouse**.

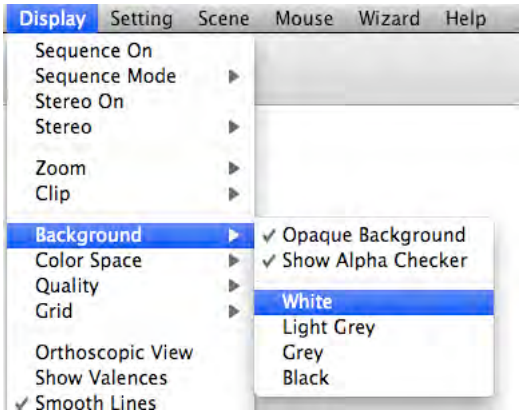
If you have a 2- or one-button mouse you can change the setting accordingly.

✓ **READ –**The **Selection Mode>** submenus define what is selected when one atom is clicked on.
The selection default is Residues (*i.e.* one amino acid or one nucleotide.)

5.2 Background color

Black backgrounds look very nice on the screen but do not print well on paper and do not photocopy well. Changing the background to white is usually very useful:

The "Display" menu within the top menu bar contains most options pertinent to displaying the image within the PyMOL viewer.



TASK

Change the background color to white following this menu cascade:

Display > Background > White

Note: alternatively type the following command: **`bg_color white`**

5.3 ASHLC menus

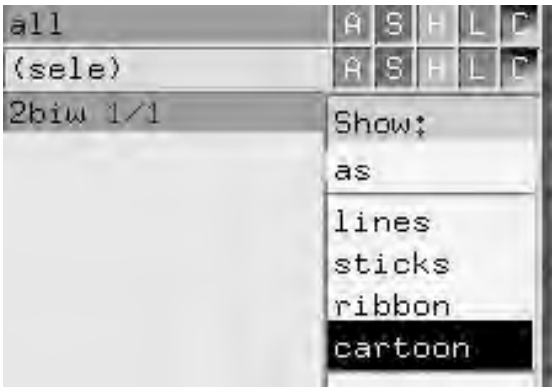
READ PyMOL can open more than one molecule at a time, or separate complex PDB data into individual components. Each one is given a name within the "Names Panel" on the right-hand side. The first name is always "all." Clicking on the name itself will un-display the corresponding molecule(s) (rendering it temporarily invisible.)

The ASHLC menus (A S H L C) are abbreviations for **A**ction, **S**how, **H**ide **L**abel and **C**olor. Some menu items have submenu components. Selections made under the "all" line will affect all listed molecules visible or not.

5.3.1 Show cartoon, Hide lines and waters

TASK Let's first make a cartoon representation of this protein: on the 2biw line within the "Names Panel" perform the mouse-driven changes:

- ✓ **click S**
- ✓ **scroll down** and **select cartoon**.



The molecule is now shown as <u>both</u> cartoon and wireframe.

Remove the wireframe by hiding the lines:

- ✓ **click H**
- ✓ **select lines**.

The remaining red dots are water molecules that are part of the solved crystal structure. We can hide them for now with the mouse command:

✓ TASK

- ✓ **click H** and
- ✓ select **waters**.

<u>Alternatively</u>: use *either* of the following commands (depending on how waters are called within the 3D data.)

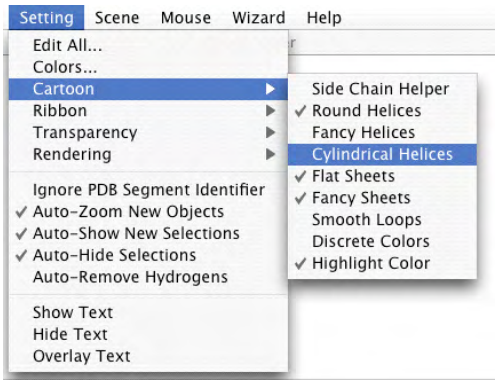
```
hide everything, resn HOH

hide everything, resn WAT
```

5.3.2 Cartoon options and settings

Most options can be set within the "Setting" menu from the top menu bar. For example, it is possible to change the way all alpha helices are rendered.

TASK Follow the menu cascade:



Setting > Cartoon > Cylindrical Helices

*Select this option **again** to remove its effect*

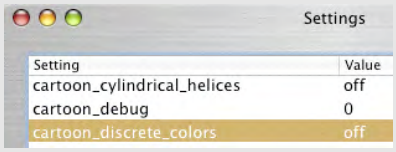
Then do the following:

Setting > Cartoon > Fancy Helices

Testing other cartoon settings: Engaging the option **Smooth Loops** will simplify the drawing. Removing the option **Highlight Color** will make the edges of strands and inside helices surfaces a gray color (default).

INFO – Cartoon Options:

Setting
Edit All...



Turning the variable `cartoon_discrete_colors` "on" makes the helix color end abruptly at the ends of the helix. The default value is off. The change can be done manually with the menu cascade **Setting > Edit All...** or can be given as a typed command:

```
set cartoon_discrete_colors = on
```

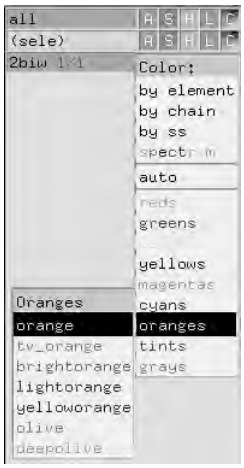
(the blank space on either side of the = sign is optional).

`set cartoon_discrete_colors = off`

`set cartoon_discrete_colors = on`

5.3.3 Changing color

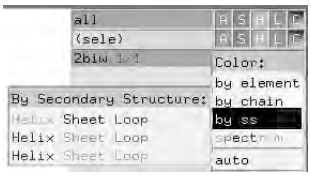
Changing the color of the ribbon is easy with the following cascade menu within the PyMOL "Names Panel" under the C menu as shown in the following menu cascade:



TASK

2biw > C > oranges > orange

Alternatively: you may choose the option to color by secondary structure by following this menu cascade instead:



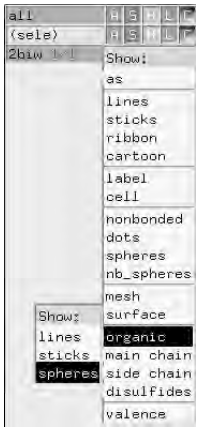
2biw > C > by ss > Helix Sheet Loop

Then choose one of the proposed color options displayed *e.g.* the first option is colored *red-yellow-green*.

5.3.4 Displaying (adding) ligand

When we opted to show the molecule as a cartoon above, one thing happened: the protein was shown as the familiar cartoon representation, but we did not see that the ligand disappear from view; it was shown and "lost" within the many lines shown. Here we will "rescue" the ligand!

The following cascade within the **Show** menu for line 2biw will show the ligand.



TASK

2biw > S > organic > spheres

This cascade will select the carotenoid present within the PDB file.

Note that it is the same color as other parts of the protein, as it is part of the name 2biw line. It will be either orange or the color for loops in the C/ss/Helix-Sheet-Loop color scheme chosen in the step above.

To more easily distinguish the organic (carotenoid) let's color it gray: (see also "mouse selection" section on the next page.)

- ✓ **click on any atom of the organic**
- ✓ on the **(sele) line,** under 2biw**, click C**
- ✓ **choose gray50**.

Alternatively: type the following command: **`color gray50, organic`**

5.3.5 Surface representation

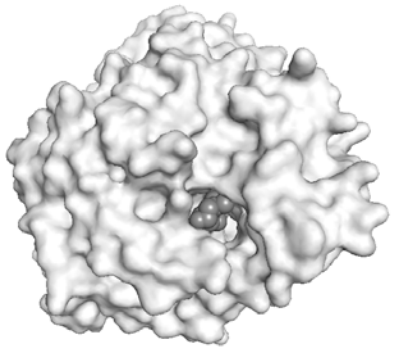
Surface representations used to be extremely computationally intensive. However, PyMOL offers very fast and beautifully rendered surfaces.

TASK

Using the show (S) menu, display the surface of the protein, then hide it with the H menu:

2biw > S > surface

2biw > H > surface

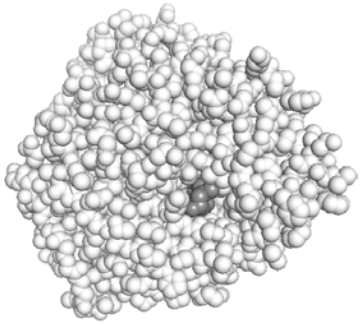


5.3.6 Sphere representation

In the same way, we can ask PyMOL to show all atoms as spheres. Note that the water molecules reappear as they are part of the 2biw structure and need to be hidden again.

TASK

Using the show (S) menu, display as spheres, then hide spheres with the H menu:



2biw > S > spheres

2biw > H > waters

2biw > H > spheres

Note that in doing so, we now loose the organic representation as sphere; we can add it again:

2biw > S > organic > spheres

Making Selections

1. Atom selections

PyMOL offers a few methods for selecting atoms:

- **Mouse clicks**
- **Command line**
- **Sequence viewer**

1.1 Mouse selection

Now that we have made the ligand visible above, it becomes easier to select it with the mouse to make further changes.

1.1.1 Selection by clicking

✓ **TASK** **Click on** one of the **spheres** of the **carotenoid ligand**.

This simple click makes various things happen:

• pink, square dots appear onto all the spheres of the ligand, indicating that it has been selected.
• "(sele)" appears or gets highlighted within the "Names Panel" and is now dedicated to this subset of atoms. Note: clicking more atoms will "add them" within (sele)

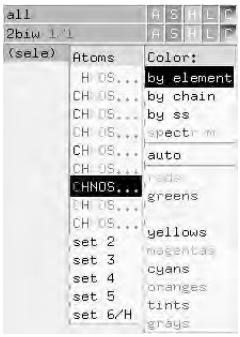
• the name of the atom that was clicked appears within the top text window.

For example:

```
You clicked /2biw//B/3ON`1491/O1
Selector: selection "sele" defined with 32 atoms.
```

✓ **TASK** Alter the atom color by element:

(sele) > C > by element > CHNOS….



Selecting the 6th CHNOS… after HNOS… in the list would display the ligand as gray with one red oxygen.

1.1.2 Selection by dragging

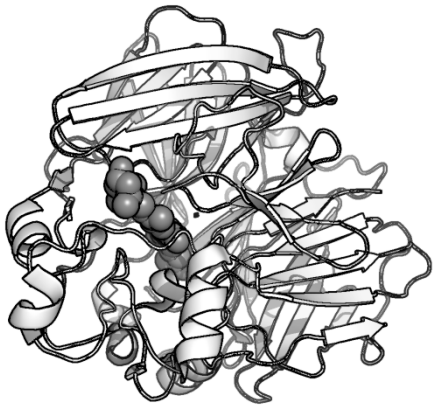
While holding the SHIFT key it is possible to use the left mouse to select a zone (an area) of atoms currently being displayed with a **click and drag motion**.

✓ **TASK: You can try this on your own** then click anywhere in the background to unselect.

1.1.3 Finding and selecting the iron

✓ **READ** Carotenoid oxygenases trap a carotenoid molecule inside a deep pocket and break it into two pieces using oxygen.

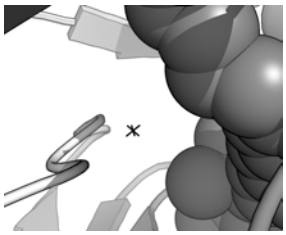
An iron ion assists in the reaction, positioning the oxygen at the proper place and activating it[4].



At the moment, depending on your coloring and 3D rotations, your display should look similar to this:

- protein as ribbons
- ligand as spheres

If you look within the center of the molecule and **zoom in**, you may see that there is an atom that is only shown as a cross-star near the carotenoid compound.



This is the iron atom. Chemical symbol: **`Fe`**

✓ **TASK** Select the iron atom by clicking on the cross-star next to the carotenoid

- ✓ **click anywhere within the background** in order to "empty" (sele)
- ✓ **click on the cross-star**

Alternatively, type the following line command:

```
select IRON, element Fe
```

Then, with the selected achieved either way continue to change its display mode:

(sele) > S > spheres
(sele) > C > oranges > orange

(Note: see Adam Steinberg's hand out for default element colors in PyMOL)

✓ **INFO –** Histidines and bound waters –

Some water molecules play an important role in the activity of an enzyme. In the case of this protein, two of them are near the position where oxygen is used to cleave the retinol/carotenoid/organic compound. The iron atom (Fe) is held in place by the grip of 4 histidine amino acid side chains. Between the iron sphere

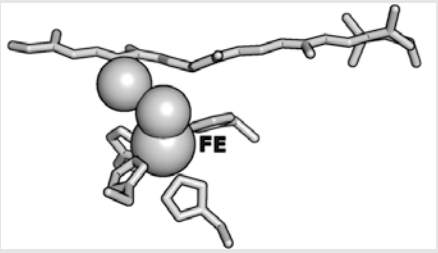
[4] http://www.rcsb.org/pdb/101/motm.do?momID=66 [http://bit.ly/OAXJ50] [archived: http://bit.ly/2yu1yZy]

and the retinoic compound are 2 oxygen atoms representing water molecules.

Water molecules residues number 2022 and 2079 and iron (Fe) residue number 1492 within the PDB file.

Histidine residues sequence number: 183, 238, 304, 484

(See below for atom selection.)



1.2 Atom selection by command-line

While selecting atoms with the mouse may be more intuitive and seems easier, there comes a time when knowing the correct command to type may be the easiest method for atom selection. Commands are created with PyMOL command words called "selectors" which are reviewed in a previous section named "**Presenting PyMOL.**"

✓ **TASK** We will recreate the image shown within the gray box above with line commands.

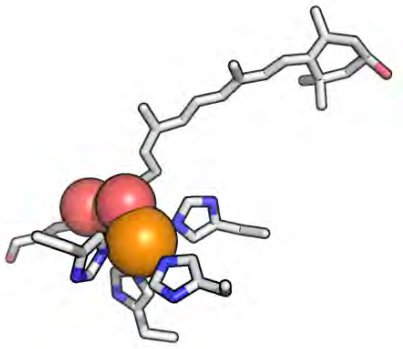
- Reminder: Lines starting with # are comments and need not be typed as they are ignored. Note the important use of the comma (`,`) within the commands.

- `resi` means residue number
- `symbol` is the chemical symbol of an atom
- `name` is the atom name within the PDB data.

Type the following commands within the upper window after the PyMOL> prompt:

Note: lines starting with # are comments are need not be typed.

```
hide all
# color by element, carbons gray
util.cbaw
# the organic compound
show sticks, organic
# the 2 water molecules
show sphere, resi 2022+2079
# the iron
show sphere, symbol fe
color orange, symbol fe
# the histidines
show stick, resi 183+238+304+484
# hide the backbone atoms
hide stick, name n+c+o
```



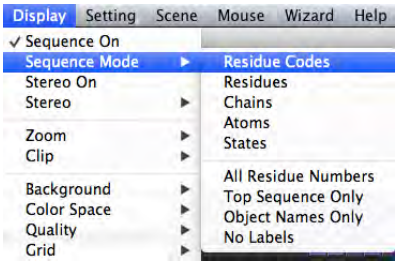
Note: `util.cbaw` is a not well known command utility that means "color by atom" (`cba`) and the 4th letter means something else, here `w` means white/grey. See `http://www.pymolwiki.org/index.php/Advanced_Coloring` for more details and a list of similar commands.

1.3 Atom selection by sequence viewer

The sequence viewer is a feature that can be engaged in 2 ways (see images below:)

- by pressing the **S** button within the VCR viewer (bottom right)
- by using the **Display** Menu

- Pressing the S within the VCR viewer

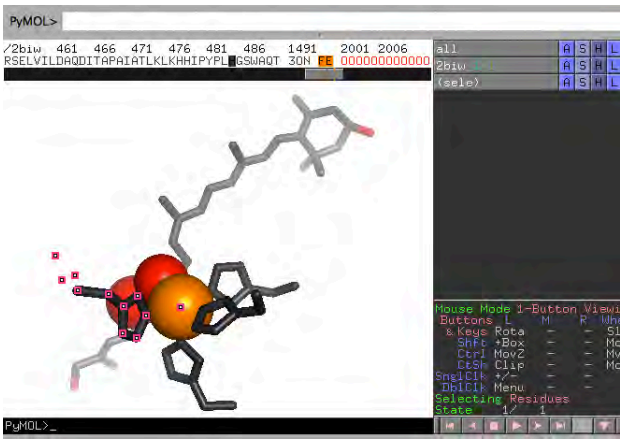


- Using the Display Menu

The default view is to show the one-letter code for amino acids (Residue Codes), while "Residues" would show the 3-letter code.

✓ **TASK** Exercise selecting from the Sequence Viewer.

Show the sequence of the protein using one of the 2 methods described above.



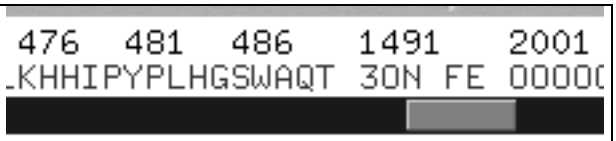
Then **using the scroll bar: go to the end** of the sequence area where you can see the end of the protein sequence.

Click on His 484
Click on Fe

Just note that the pink selection squares appear within the viewer.

The numbering is aligned with the first digit.

Therefore His-484 is either the 3rd after Tyrosine (Y)-481 or 2nd before Serine (S)-486



2. Select residues contacting the ligand

The ligand sits in a pocket and we would like to select amino acids that are in contact with the ligand. For this purpose we can first select the ligand and then modify the selection with a special "modify" menu.

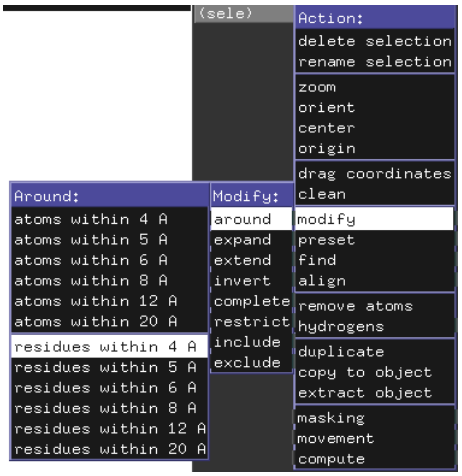
The make the process clearer we'll hide everything and then display only the ligand before searching for the contacts:

✓ **TASK** Hide everything: **all > H > everything**

Now show only the ligand as sticks:

2biw > S > organic > sticks
Click anywhere within the background in order to "empty" (sele)
Click on any atom of the **ligand**

We are now going to modify the selection to find contacting atoms (within 4 Å,) and at the same time select the whole amino acid that owns this atom.
In addition, the ligand itself will be removed from the selection.
The final selection will only contain all the atoms from all the detected amino acids.
This is accomplished with a "modify" menu.
Therefore the ligand serves as a method to find amino acids 'around" it that are close.
In some other cases we may want to "expand" the selection itself with the contacting residues.
For this reason the "modify" menu contains multiple choices.



✓ **TASK** **Click** on the **A** object button of **(sele)** and scroll to find the **modify** menu.

Continue the menu cascade to select around the selected ligand choosing complete residues within 4 Å:

A > modify > around > residues within 4 A

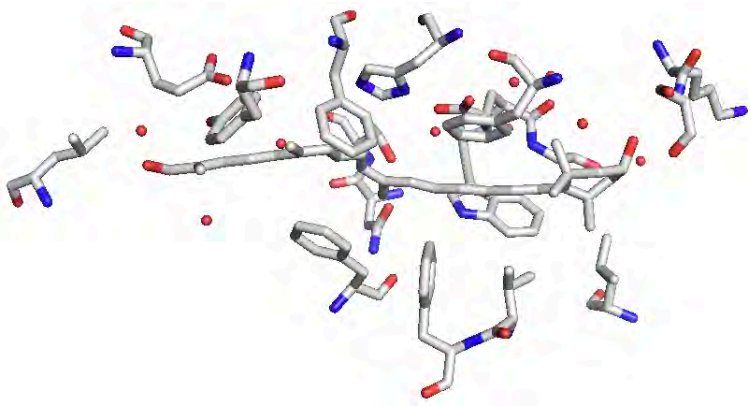
Since we hid everything earlier we can see the selection markers but we need to make the selected elements appear within the view:

(sele) > S > sticks

Note that the ligand is no longer selected.

Now color the new residues, for example:

(sele) > C > byelement



Note that some "floating squares" are water molecules and can be shown as small spheres with:

(sele) > S > nb_sphere

Note: the whole GUI cascade takes time and for repetitive selections a line command can be useful.

The whole thing can be encapsulated in a single line command:

```
select byres 2biw within 4 of organic
```

Making, Saving Images

Images – Ray, Transparency, Size, Saving

✓ **TASK** Display the protein cartoon again:

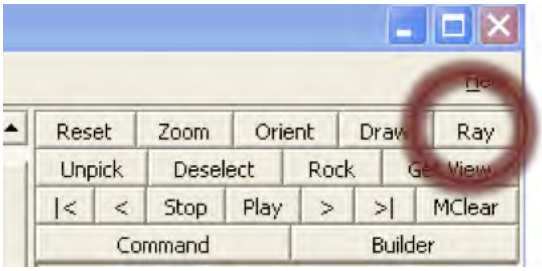
2biw > S > cartoon

1. Ray tracing

PyMOL offers an internal "ray tracer" to create stunning rendered images with a high visual quality much more pleasant to the eye and ideal for publication.

✓ **TASK** To create a standard ray-traced image of the current Viewer scene, **click the "Ray" button** at the top right of the "Upper Window."

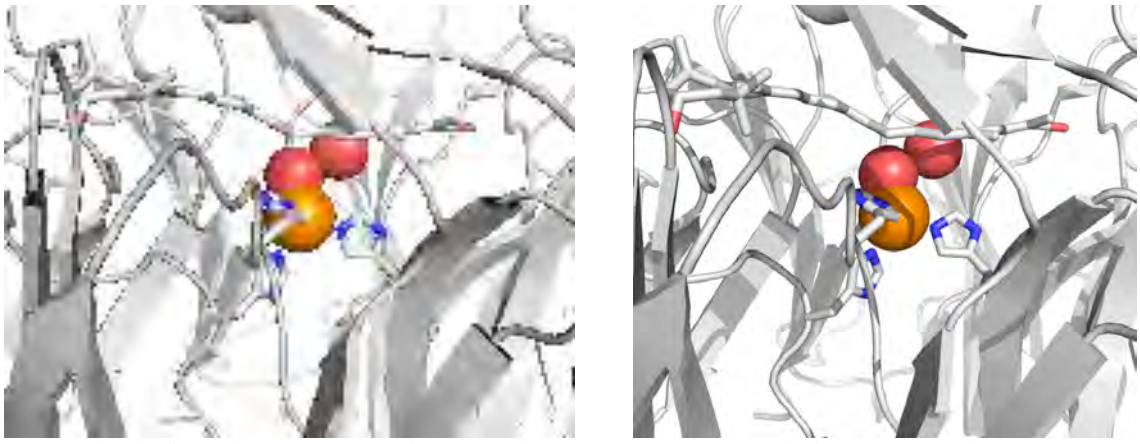
Macintosh



Windows

The rendering will take a few seconds to a few minutes depending on the complexity of the PDB file and the chosen display, and will also depend on the speed of the computer CPU. Once rendered, the image appears within the Viewer.

To save the file, use the save cascade as above: **File > Save Image As…**



Zoomed side-by-side comparison between the PyMOL image and the ray-traced image: note the jagginess of the original image and the smooth appearance of the ray-traced image, with shadows as a bonus. The effect is exaggerated here!

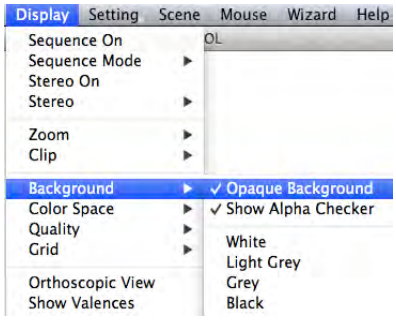
Alternatively: the command `ray` can be used within the command-line area.

2. Transparent background

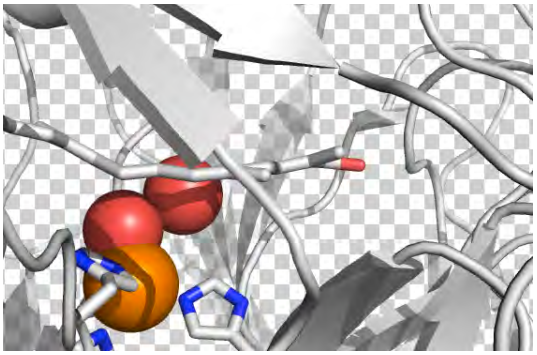
Ray traced images support a transparent background. There are two methods to engage the transparent background for rendered images:

- Uncheck the menu: Display > Background > Opaque Background
- The command line: `set ray_opaque_background, off`

The optional menu "Show Alpha Checker" provides a visual cue that indeed the background is transparent (see image at right.)



Uncheck Opaque Background



Alpha Checker is on by default

Note: the checker background is only shown after the "render" command has been issued.

3. Image size

Screen resolution (72 dpi – *dots-per-inch*) falls short of the needs for paper publication that require a minimum of 300 dpi. For example 1800 pixels would represent 6 inches at 300 dpi.

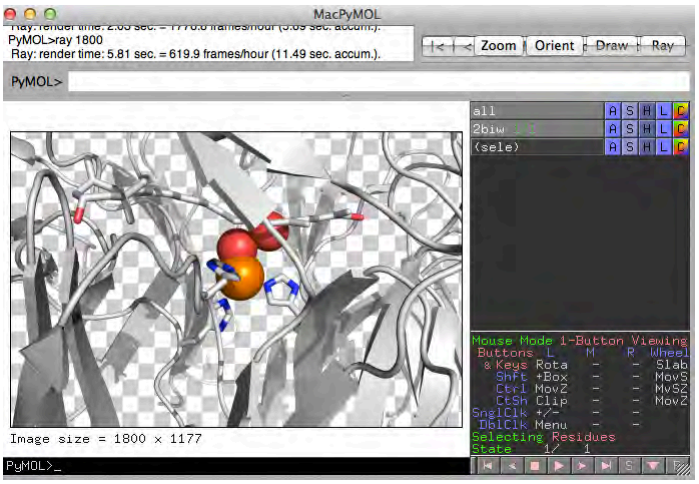
PyMOL can specify the number of pixels for ray-traced images with the `ray` command. The command can be given one or two numbers separated by a comma. If only one number is given the other will be inferred from the current display shape.

TASK

Within the PyMOL> line command type the following command to change the dimensions of the final ray-traced image:

```
ray 1800
```

The resulting image height will be inferred from the current display. (1177 shown here. Results will vary with window shape.)



Examples for "width, height" specified:

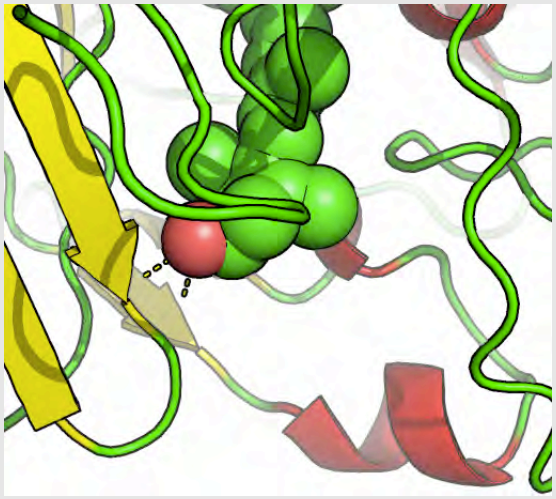
```
ray 1800, 1200
ray 2000, 2000
```

INFO - Ray tracing options:

There are at least 50 editable settings listed in the menu **Setting > Edit All…** for ray tracing! The following 2 are of special interest:

`ray_shadows` is "on" by default and can be switched within the settings window or the line command: `set` **`ray_shadows, on`** or **`set ray_shadows, off`**

ray_trace_mode has a default of 0 and can also adopt values of 1, 2 and 3 to create contour-enhanced images or old-fashion black and white contours in the Jane Richardson style (who used to hand-draw images in the early days!)



`set ray_trace_mode, 1`

`set ray_trace_mode, 2`

To render type **ray** or **click the "Ray"** button

Return to ray trace **defaults**: **`set ray_trace_mode, 0`**

4. Saving an image

Whatever is currently displayed within the 3D viewer (whether ray-trace or displayed) can be saved "as is" into an image file in the PNG[5] format.

4.1 Saving from File menu

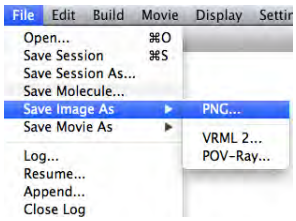
If you still see the selection dots over the ligand from the previous section simply click anywhere on the white background to unselect. Alternatively click on the "Hide-Sele" in the "Names Panel."

[5] PNG = Portable Network Graphics, a compressed raster graphic format expected to replace the Graphics Interchange Format (GIF) owned by Unisys that requires licensing for software development.

TASK **Rotate the molecule** to find a perspective that you deem instructive of the conformation of the protein and its bound ligand.

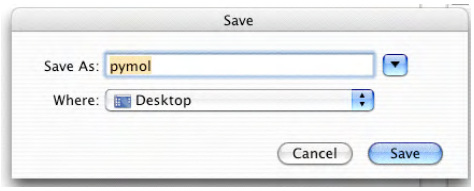
Follow this menu cascade to save the image currently within the Viewer:

File > Save Image As > PNG…



A dialog window will appear.

Replace the default word "PyMOL" to give a name to the file you want to save, *e.g.* image1 or image1.png



In this example the image will be saved as a PNG file on the desktop as indicated by "Where."

INFO - Screen Capture:

If for any reason you cannot save the current image of your PyMOL session, the following info is useful to capture the screen, or a portion of the screen of your computer display:

Macintosh		*Windows*	
Full screen:	⌘ + Shift + 3	Full screen:	Print Screen key
Screen selection:	⌘ + Shift + 4	Screen selection:	Shareware

4.2 Saving and image with command line

INFO

The command `png` *`filename`* will save the image file within the current directory.

```
SYNTAX
     png file-name
```

EXAMPLE

```
PyMOL>  png test.png    # The file is saved by default in home dir
```

Preset menus

Action > preset menus

This is mostly a self-paced exploration of one of the menus that is sure to change over time as PyMOL evolves.

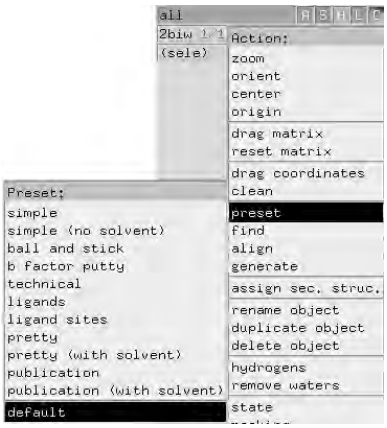
Preliminary If you are not continuing this exercise from the previous exercise do the following tasks:
√ - fetch the structure 2biw, type=pdb2
√ - then click S > Cartoon to be in a similar state as in the previous exercise.

1. Presets: automated complex imaging

The preset menu is part of the Action set of the **ASHLC menus** controlling the aspect of molecules from the "Names Panel."

✓ **TASK** Follow this menu cascade sequence to return to the default view, as when you just opened the molecule. No rotation will occurs:

2biw > A > preset > default



Note: This command has a similar effect but is ***not*** the same as the following cascade:

"hide everything and show lines:"

2biw > H > everything
and
2biw > S > lines

Note: the "preset" options will set some variables that are specific to these views and may change further drawings. To remove the effect of these presets affecting an object representation, use the **A > preset > default** menu cascade reset parameters.

Note: to get back to the original opening view positionsimply type **`reset`** at the PyMOL> line command.

2. Preset options: exploring more

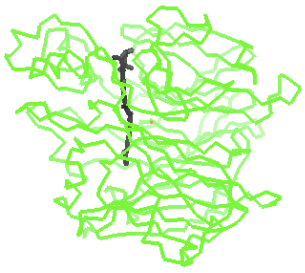
✓ **TASK- Explore the other menus** of this series.

The cascade menu **2biw > A > preset** is assumed in the following commands, just continue with the suggested command.

For example, the first one the complete command would be **2biw > A > preset > simple**

simple

Note: the protein backbone is shown as a line while the organic ligand is shown as sticks.

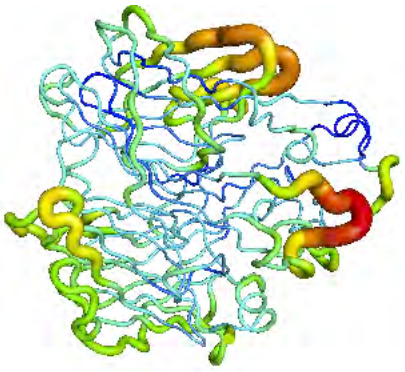


ball and stick

This is not very useful for a large protein such as this.

b factor putty

The segments with the highest temperature factor are shown as thicker cylinders. Regions of better resolution have thinner diameter and are usually found at the core of the protein. Mostly loops in the outside of the protein wobble: the core portions of the proteins usually appear more stable than the external loops. This is mostly useful for crystallographers but is a cool representation.



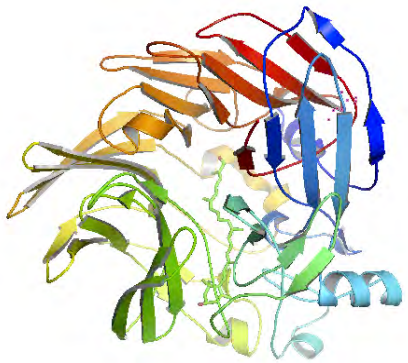
technical

Color domains in separate rainbow colors and shows backbone and side chains.
Note that a subset name appears in the Names Panel (2biw_pol_co) that control the dashed-line hydrogen bonds.

Pretty and ***publication*** create similar images of a rainbow color cartoon with a stick ligand.

Pretty creates the default cartoon setting, while publication creates an image very similar to an image created by Molscript*.

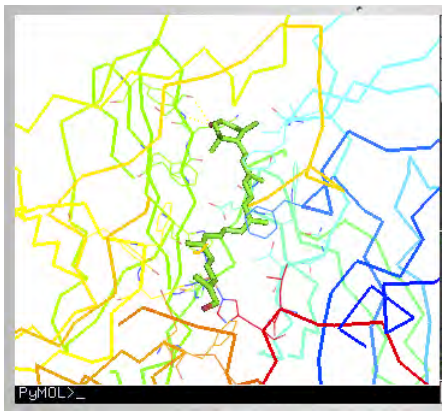
(* Per J. Kraulis 1991: MOLSCRIPT: A Program to Produce Both Detailed and Schematic Plots of Protein Structures. *Journal of Applied Crystallography*. 24: 946-950. **http://www.avatar.se/molscript/)**



ligands

This option will zoom in on the ligand site and show the protein as backbone except in the near vicinity of the ligand where side chains are shown. The ligand is depicted as a thicker cylinder.

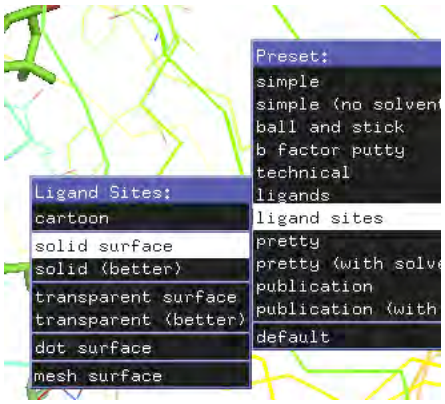
Note: to zoom out, simply click on the A in the ASHLC menu again.



ligand sites

There are a few options available in this submenu, all pertinent to looking closely at the ligand in it's binding pocket.

You can explore a few of them on your own, once you are done then select the following option:
preset > ligand sites > solid surface



You should obtain a centered, zoomed view of the ligand shown as a stick model within a partial molecular surface pocket. The colors are the scheme of previously chosen colors, for example if you tried the "technical" preset earlier the coloring would likely be like a rainbow.

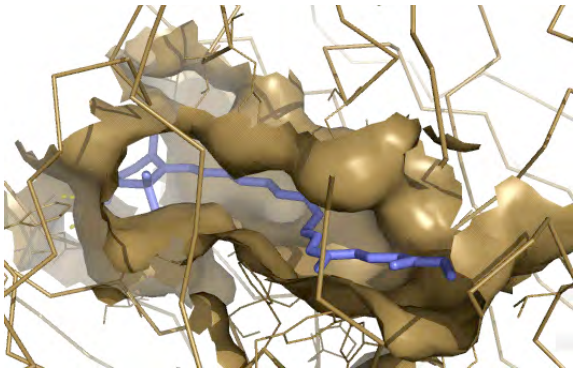
✓ **TASK** This quick change will make a much nicer image:

2biw > C > yellows > sand

Now with the mouse click carefully anywhere on the ligand stick to select it. As before you will have a (sele) name within the Names Panel. You can now change the color with the following menu cascade: **(sele) > C > blues > slate**

Of course you can pick your own colors. Make sure that there is sufficient contrast between the color of the surface and the color of the ligand.

If you have not yet done so, **rotate the molecule** to select a nice viewing angle.



If you want to add a more stunning effect **click on the "ray"** button as we did before to complete your publication quality picture.

Note: If you are preparing a figure for a black and white print publication, it might be advantageous to use the various gray scales, black, and white within the "grays" option under the C menu in the "Names Panel."

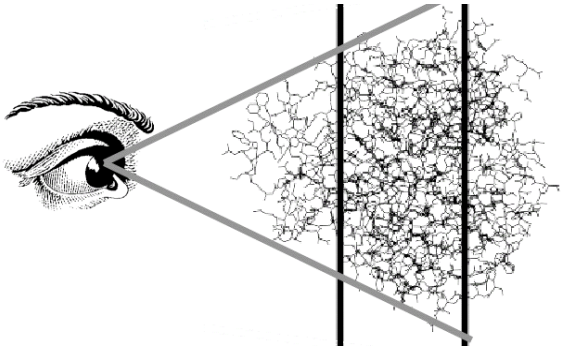
Clipping plane & Surface

1. Clipping planes: near and far

Clipping planes are imaginary planes in the front and back of the molecule. Parts of the molecule that are outside the planes are "clipped" and therefore invisible. This is very useful for complex or large structures.

This image represents the molecule seen side-ways "inside" the computer monitor.

The 2 black lines represent the *yon* (far) and *hither* (near) clipping planes. These are parallel to the flat screen of the computer monitor display. The gray lines converge toward the user's eye who is looking at the molecule on the computer screen.



TASK

The DMC computers are equipped with 3-button mice. To move clipping planes **press shift** and the **right mouse** button **simultaneously** while dragging up and down. The same result can be obtained by using the **middle button scroll wheel**.

As an exercise, try to remove some of the molecular surface covering the ligand to create a picture similar to this:

Note: we have seen previously that the top menu "Mouse" contains all the possible options, with a reminder displayed at the bottom right of the PyMOL window. The method to adjust the clipping plane will depend on the number of buttons on your mouse. Use the "Mouse" menu to adapt to your mouse on your own system.

Regardless of the number of buttons on your mouse, simply look at the bottom right to see the corresponding mouse + keyboard action for the following 2 items: **MovZ** and **Clip**.

MovZ will move the object closer or further from your point of view.

Clip will reduce the size of the box into which the object is represented and increase the contrast with depth-cueing, making parts of the molecule that are further back much darker.

Playing with these 2 items, it is possible to increase the feeling of depth and 3D feeling of any represented object and particularly surfaces with deep pockets.

You need to make sure however that the "depth-cue" option is engaged within the menu "**Display**."

✓ **INFO** Alternatively: the `clip` command can be used with the line-command, as shown in these examples[6]:

```
clip near, -5           # moves near plane away from you by 5 Å
clip far, 10            # moves far plane towards you by 10 Å
clip move, -5           # moves the slab away from you by 5 Å
clip slab, 20           # sets slab thickness to 20 Å
clip slab, 10, resi 11  # clip 10 Å slab about residue 11
clip atoms, 5, pept     # clip atoms in "pept" with a 5 Å buffer
                        # about their current camera positions
```

[6] http://philgrid.asti.dost.gov.ph/index.php/PyMOL#Control_of_clipping_planes [http://bit.ly/O4ckco]

2. Solid, clipped interior surface

IMPORTANT NOTE: the presets can alter default behavior of PyMOL in a way that cannot be (or not easily undone.) Therefore we need to **quit, restart and reload** the structure.

✓ TASK: Quit and restart PyMOL

- Import the 3D data again, type:	`fetch 2biw, type=pdb2`
- Color everything gray	**Display > C > grays > gray70**
or use the typed command:	`color gray70`
- Make background white (top menu):	**Display > Background > White**
or use the typed command:	`bg_color white`
- Hide the water molecules (mouse):	**2biw > H > waters**
or use the typed command:	`hide nonbonded`
- Show as surface:	**2biw > S > surface**
or use the typed command:	`show surface`
- Show ligand as stick	**2biw > S > organic > stick**
or use the typed command:	`show sticks, organic`
- Show iron as sphere, type:	`show sphere, element fe`
- Show 2 waters sphere, type:	`show sphere, resi 2022+2079`
- (re)color all waters red:	`color red, resn HOH`

Now we need to set some parameters that will make the surface look solid by typing the following commands:

```
unset depth_cue
unset ray_shadows
set ray_interior_color, gray80
set opaque_background
```

Now the tricky part will be to rotate the molecule in such a way as to see the interior pocket without clipping the ligand. The 2 waters will most likely be clipped.

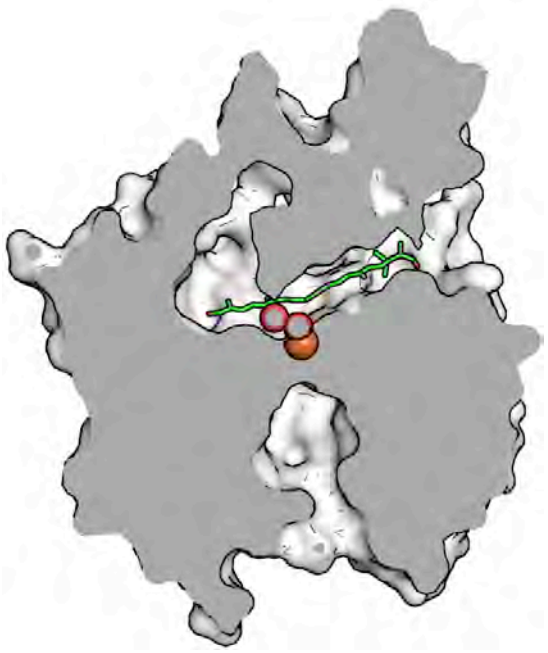
NOTE: if part of the back surface appears black, it means that the far clip plane is too close and need to be placed further. Likely the following command will do the trick, placing the far clipping plane 30 Å further away:

```
clip far, -30
```

We can set the ray trace mode with black highlights:

```
set ray_trace_mode, 1
```

Finally, render the image by pressing the "Ray" button or typing the command **ray**.



✓ **INFO** - Pressing the button "Get View" in the upper right will provide a complex matrix of numbers which, when pasted or copied into PyMOL will give the exact orientation presented above:

```
set_view (\
     0.289975971,   -0.916524768,   -0.275475979,\
     0.937972188,    0.329330176,   -0.108357012,\
     0.190034315,   -0.226969540,    0.955183089,\
    -0.000179246,    0.000244899, -204.606140137,\
    29.327486038,    4.797065735,   31.367763519,\
   195.712844849,  242.537857056,  -20.000000000 )
### cut above here and paste into script ###
```

Distances & Labels

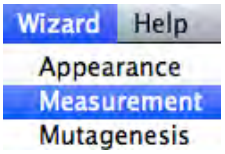
1. Distances: the Measurement Wizard.

(Continues from the previous exercise.)

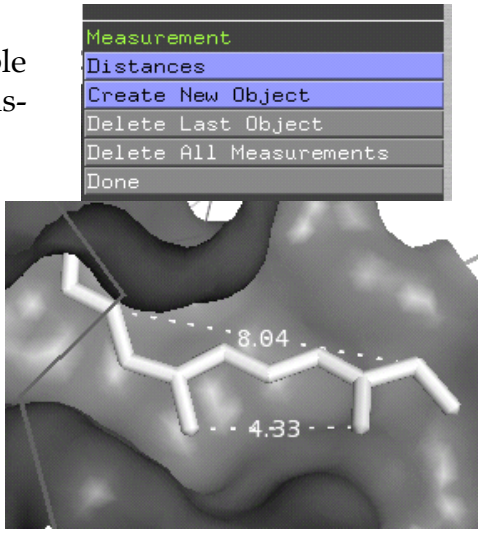
Distances are measured between two atoms and are expressed in the same unit as the XYZ coordinates within the PDB file: Angstroms (1Å = 10^{-10} m).

As an example we shall measure the distance between two atoms within the carotenoid ligand.

Within the top menu select
Wizard > Measurement
(Note: in older PyMOL versions Measurement was called Distance.)
This will create a prompt within the Viewer:
" **Please click on the first atom…**"

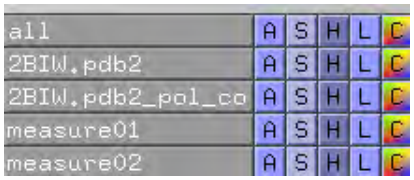


Within the "Internal GUI" a "Measurement" table also appears. It can also be used to remove measurement objects after they are no longer needed.



Click on the **first** atom **on the ligand**
Click on the **second** atom **on the ligand**

(you can select the same atoms as in the examples within the image at right, or select your choice of atoms)



The default color of the measurement object is yellow. This can be easily changed with the familiar ASHLC menu for each measurement, as a new name is entered within the "Names Panel" for each distance, e.g. "measure01."
Change the color to white with the following menu cascade: **measure01 > C > grays > white**

When you are done using the "Measurement" panel on the bottom right **click Done**. If you no longer need to display the distance object, **click Delete All Measurements**. Alternatively you can use the corresponding "A" menu and choose "delete".

INFO - Distances are labels

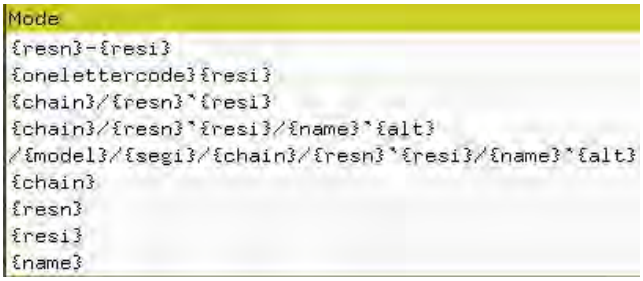
The display of a distance measurement is a label. To alter the number of decimal digits reported change the label setting label_digits from default 1 to the desired value with either the **Setting > Edit All…** menu cascade or the line command:

```
set label_digits, 2
```

2. PyMOL Labels

2.1 Label wizard

The menu **Wizard > Label** can label an amino acid residue by simply clicking. The label choice is by default set on residue name and its number (**resn-resi**) but can be modified by clicking the "Mode" button within the open Wizard menu at bottom right.



To label a residue simply click on one of its atoms.

2.2 Labels

✓ **TASK: Click on TYR 322** to have it within the **(sele)** name.

Alternatively use the command-line to create a selection entry:

```
select TYR322, resi 322
show sticks, TYR322
```

A label containing the residue type and sequence number (*e.g.* TYR 322) can be added to a selected residue with the mouse-driven menu cascade:

(sele) > L > residues

If you have used the line command option the menu would be:

TYR322 > L > residues

By default, labels are created the same color as the atom and placed at the C-alpha carbon position. The next 2 sections will show how to move the label and how to change its color.

2.3 Moving labels with mouse

The default position of the label may not be ideal and it would be nice to move it. This can be done with the "*mouse editing*" mode that can be selected from the Mouse top menu or by line-command. The Mouse menu works regardless of the number of buttons that are actually on the mouse - therefore this works also for laptops with a track-pad. The menus could be

Mouse > 3 Button Editing
Mouse > 2 Button Editing

The line command is simply: `edit_mode`

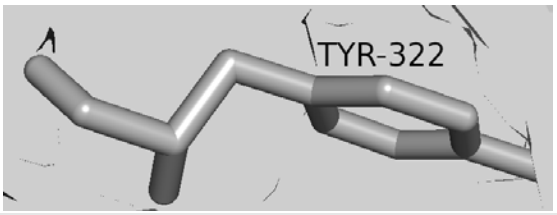
When in edit mode, simply click on the "**control**" button to click and move the label.

TO make it easier to move the label and to see it, we can start by making it a bigger size with the line command:

```
set label_size, 40
```

Then **using the CRTL key move the label** from its default position to a new position:

Before



After

Return to mouse viewing with menu: **Mouse > 3 Button Viewing**

2.4 Label color

Q: "How can I change only the label color without changing the display of the atom or molecule? "

A: You can change the label color to *e.g.* yellow with the following line command:

```
set label_color, yellow, sele
```

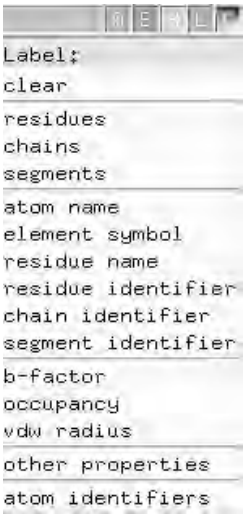
- **yellow** can be changed for other colors (The **C** menu can be a source of inspiration for colors and color names.)
- **sele** represents the current selection. You can alter the command with the name of the object you are working on *e.g.* 2biw
- note the use of the comma (,) within the line command

For publications or slide making, labels could be added with *e.g.* Adobe (PhotoShop, Illustrator) or Microsoft (PowerPoint) graphic editing software.

✓ **TASK**

Explore the L menu, knowing that "clear" will remove the mess that may occur!

(on this image colors were inverted for easier reading.)



2.5 General label Settings

General default settings can also be altered generally:

✓ **TASK**

Open the Setting > Edit All… top menu and change settings as illustrated

Default label values

Setting	Value
isomesh_auto_state	off
label_angle_digits	-1
label_color	default
label_digits	1
label_dihedral_digits	-1
label_distance_digits	-1
label_font_id	5
label_outline_color	default
label_position	[0.00000, 0.00000, 1.750
label_shadow_mode	0
label_size	14.00000
legacy_mouse_zoom	off

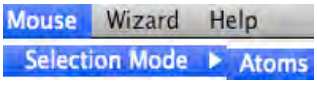
Label values changed

Setting	Value
isomesh_auto_state	off
label_angle_digits	-1
label_color	yellow
label_digits	1
label_dihedral_digits	-1
label_distance_digits	-1
label_font_id	5
label_outline_color	default
label_position	[0.00000, 1.50000, 1.75000]
label_shadow_mode	5
label_size	32.00000
legacy_mouse_zoom	off

2.6 Labeling one atom

Text label can also be added within PyMOL, although as suggested above, adding label within a graphical software might be more appropriate in some cases.

TASK



Change the **mouse selection to Atoms**

Click on one atom within the carotenoid

Pink squares appear.

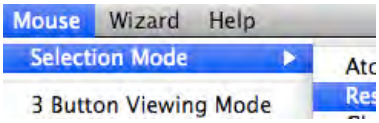
Within the text entry box type the following command:


```
label sele, "Ligand"
```

Note: the word in quotes, here "ligand" can be changed to any other word, such as "my molecule" or "carotenoid" etc.

To clear the label, simply type: `label sele`

TASK



Switch the **selection** mode back to **residues**

2.7 Greek alphabet (OPTIONAL INFO)

Letters from the Greek alphabet can be added to text-entered labels. The commands call on special fonts called Unicode UTF8 fonts. The Greek font is #316, the alpha letter is number 261 and omega is 277 (268 and 269 are not used.)

While alpha and beta might be most useful to label α-helices and β-sheets, the rest of the alphabet might be useful to label specialized items.

OPTIONAL Label the atom with alpha and beta Greek letters and some text:
Type the following commands within the text box:

```
label sele, "\316\261, \316\262: Greek Letters"
<press enter>
set label_size, 40
```

Or write part of the alphabet:

```
set label_size, 22
label sele, "\316\261, \316\262, \316\263, \316\264, \316\265, \316\266,
\316\267, \316\270, \316\271, \316\272, \316\273, \316\274, \316\275,
\316\276, \316\277"
```

2.8 More information

There is a lot more to labeling. Exhaustive information can be found at:

http://wwww.pymolwiki.org/index.php/Label

New objects & automatic zooming

When we showed the surface (above exercise) with the menu cascade **2biw > S > surface** you might have noticed that the carotenoid, organic compound was not shown as part of the surface, which is a useful feature in itself and is a default for all heteroatom entries within the 3D data (see previous section on PDB format.)[7]

The question then arises: Q: "how can one make a surface of that compound?"
The answer is to create a copy of these coordinates and change their type to ATOM

1. Making new objects from selection

Any selection can be copied into an independent object with the **"A" Actions: copy to object** menu cascade. This will create a new entry within the "Names Panel" without the parentheses that becomes independent of the original PDB file. This is also true for the (sele) entry.

1.1 Create a new version of the ligand

TASK

- **Click** on the **background** to clear the selection (sele)
- **Click** on the **ligand** ; (sele) now contains a copy.

[7] Surfaces are reserved for ATOM records only as. Typically ligands are stored as hetero-atom HE-TATM records (but authors of the PDB file may choose otherwise, hence always check within the data!)

- **Click** on the **A** on the (**sele**) line and choose: **copy to object** (a new entry called obj01 appears within Names Panel, and an automatic zooming occurs)
- **Click** on the **S** on the (obj01) line to change the object appearance, *e.g.* as stick.

Note: If you make obj01 invisible by clicking on the obj01 icon, the ligand is still visible because it is also part of the 2biw file!

Note: If the option "extract object" was used instead, the ligand would have been removed from the 2biw object and would no longer be available under that name.

Therefore the 2 options both create a new object, one makes a copy of the original, and the other moves it our of the original data.

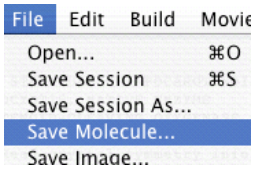
1.2 Changing atom type for the ligand; Make surface for ligand

To change the atom type from HETATM to ATOM we can use the `alter` command on the new object:

```
alter obj01, type="ATOM"
```

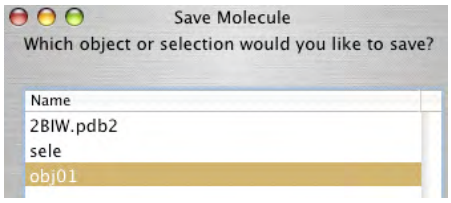
Unfortunately, PyMOL somehow remembers that this is a ligand so one more step is necessary: as a "work around:" save the data in a file and re-open it:

✓ **TASK** Assuming you have created obj01 as described above and changed the atom type:



File > Save Molecule….

Click obj01 within the Save Molecule panel.



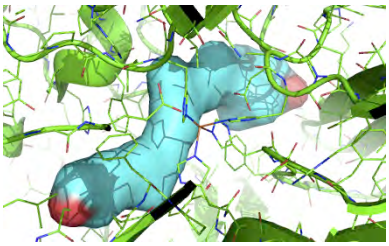
Choose a file **name** and save the file *e.g.* `lig.pdb`.

Save the file on the desktop or within the Home dir.

✓ **TASK** **Open** the PDB **file lig.pdb** with PyMOL:

File> Open…

Alternatively type command: `load ~/Desktop/lig.pdb`

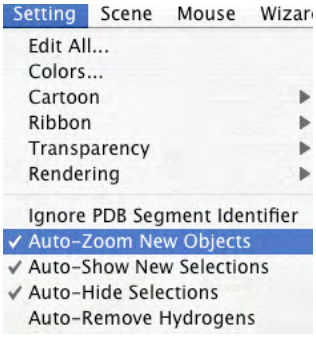


ray-traced example

An entry "lig" now appears in the "Names Panel"

Make a surface:
lig > S > surface

2. New object: turning off automatic zoom



When we created object obj01 the default action of PyMOL was to automatically zoom to the new object and reduce the clipping planes.

That can be annoying in some cases, but could always be reversed with the line command "**`reset`**" or with the menu:

Display > Clip > None or with the
Display > Zoom menu options.

This option is turned off by un-checking the menu item:

Setting > Auto-Zoom New Objects

Displaying hydrogen bonds

✓ **INFO** See also "Hydrogen bonds and Polar Contacts" within the following web page:
https://pymolwiki.org/index.php/Displaying_Biochemical_Properties
[or http://bit.ly/MF3OwT] [archived: http://bit.ly/2xiI9gr]

✓ **TASK** We will display a short helical fragment from 2biw and show H-bonds

Getting prepared:

- The 2biw file should be loaded from the previous exercise. If not use the `fetch` command to import it as in the first exercise.
- Within the Names panel: **2biw > H > everything**

The following is an example to display the hydrogen bonds within the alpha helix consisting of residues 94 to 105 in 2biw:

- First **create object "helix-1"** containing the amino acid residues range forming the helix by **typing the following line** within the line-command area:

```
select helix-1, resi 94-105
```

- Display the selection (use mouse in "Names Panel"): **(helix-1) > S > sticks**
- Add a display of the cartoon: **(helix-1) > S > cartoon**

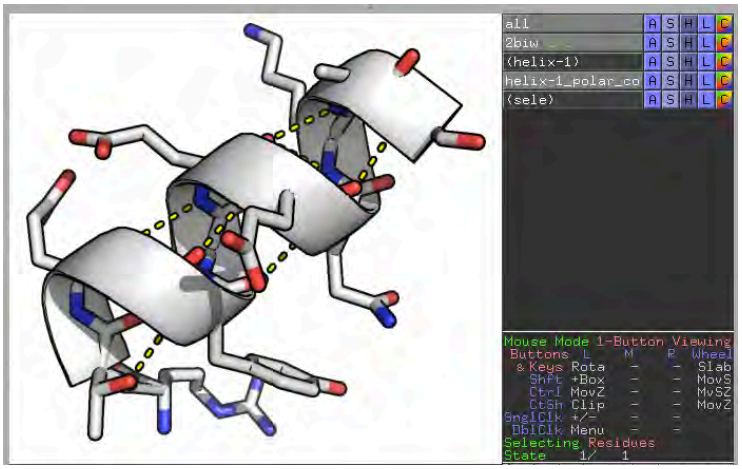
- For easier rotation **type** the line-command: **`center helix-1`**
- Then find the polar contact the menu cascade using the mouse in "Names Panel:"

(helix-1) > A > find > polar contacts > within selection

A new objects **`helix-1_polar_conts`** is created.

The yellow default color for the dashed line is easily changed with the **C** menu for this object.

For example a darker color can be chosen for display against a white background.



Note: choosing the submenu "**to other atoms in object"** would show additional hydrogen bonds of the helix side-chains to other parts of the protein.

Note: for more complex issues regarding hydrogen bonds or adding hydrogen atoms refer to the web link listed above.

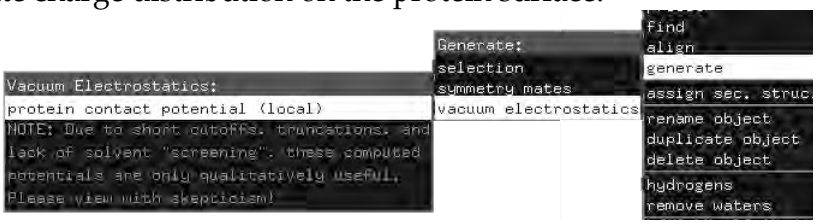
Electrostatic potential

✓ **Advanced:** Alternate methods and resources **not covered in this tutorial**:

The most accurate potential maps can be calculated by external software (Grasp, Delphi, APBS, MEAD) and displayed in PyMOL. A tutorial example for APBS is available online (see section 3.2. PyMOL) at
`http://ibibmem.uchicago.edu/roll-documentation/apbs/tutorial/index.html`
Archived at: **`http://bit.ly/1Bpc1y8`**

New home page for APBS and PDB2PQR:
`https://sites.google.com/a/poissonboltzmann.org/software/home`
Or `http://bit.ly/2c9f4HQ` archived (9/8/2016): `http://bit.ly/2cdDf8J`

✓ **READ** PyMOL offers an approximate map nicknamed "*charge-smoothed*" surface representing approximate charge distribution on the protein surface. Assuming that you have PDB data 2BIW, the potential surface is calculated and displayed with the following menu cascade on the Names Panel:



1. Calculating and displaying a map

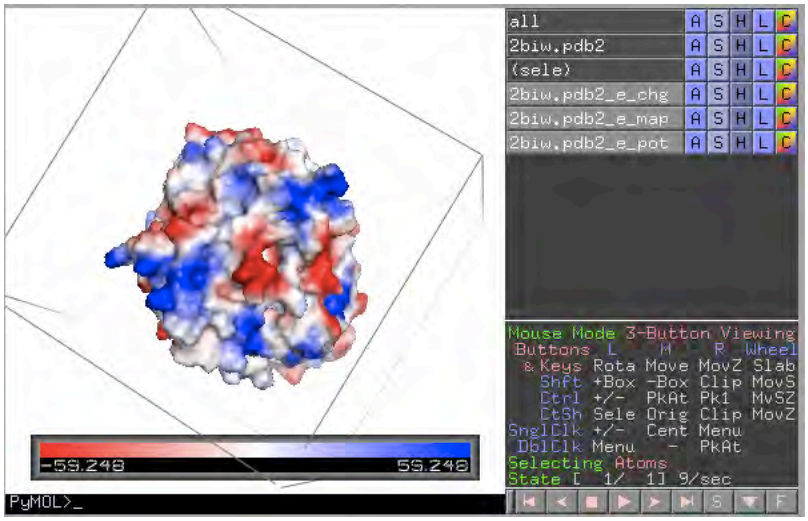
✓ **TASK Reopen the saved session in the previous exercise or start with a new PyMOL session and input the 2BIW data (type=pdb2) as previously.**

Generate an electrostatic potential map display with the following mouse menu cascade:

2biw > A > generate > vacuum electrostatics > protein contact potential (local)

The process creates 3 new entries within the Names Panel:

2biw_e_chg
2biw _e_map, and
2biw_e_pot



2biw_**e_chg** is an object containing the surface, colored red/white/blue and is an approximate map.

2biw_**e_map** is usually not shown (**click on name to show**) and displays the volume boundaries as e.g. a big cube.

2biw_**e_pot** is the object representing the color ramp and value at the bottom of the display.

2. Adjusting color levels with the mouse

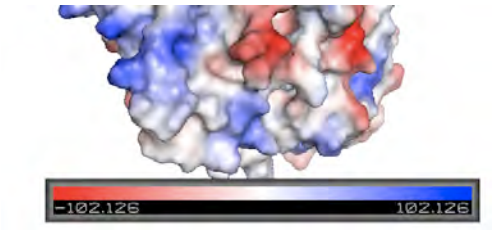
With protein 2BIW the default values for red and blue are -55.248 and +55.248 respectively.

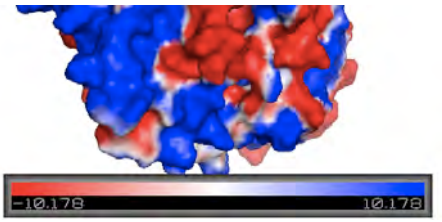
It is possible to adjust the color level with the middle button and the control key.

TASK

- **Place** the **mouse** over the color scale
- **Hold the CTRL** key and **click** on the **middle button**.
- **Adjust** the color level by **sliding the middle button**
- Go first to a **range near +/- 100**
- Then to a **range near +/- 10**

Note: Do not go all the way to **-0 +0** as it then becomes virtually impossible to slide to proper values after that.





3. Adjusting range with line command

If two proteins are present, the process has to be applied to each individual protein, and the same **_e_ objects** are created for each one. The values within the 2biw_e_pot color ramp may be different for each protein. Therefore it is useful to know how to change the values within. Smaller numbers will increase the blue and red strength and contrast within the blend of white surface, while larger numbers will dim the colors.

TASK

To change the color range within the ramp:
(example): **`ramp_new 2biw_e_pot , 2biw_e_map, [-100,0,100]`**

4. Adjusting the display colors

The default colors are red/white/blue, hence the above command could also be rewritten to change ramp values and specify colors:

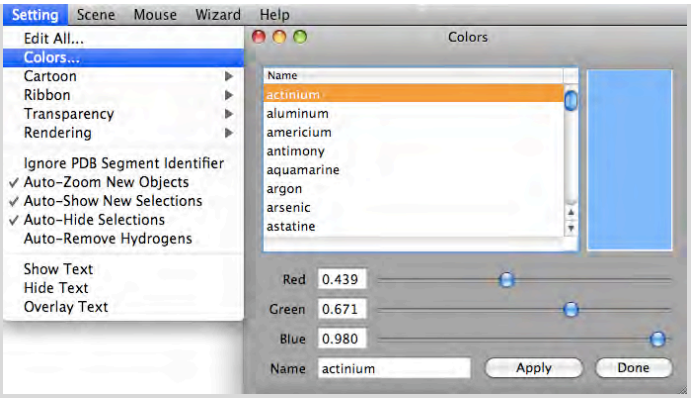
ramp_new 2biw_e_pot , 2biw_e_map, [-100,0,100] , [red, white, blue]
or
ramp_new 2biw_e_pot , 2biw_e_map, [-100,0,100] , [[1,0,0], [1,1,1] , [0,0,1]]

where **`[ [1,0,0], [1,1,1] , [0,0,1] ]`** represents the `[R, G, B]` (red/green/blue channels) values for each of the 3 displayed colors. To change the displayed color, simply change the definition of the colors. For example, the values **`[ [1,1,0], [1,1,1], [0,1,1] ]`** would create a ramp as yellow/white/cyan.

INFO

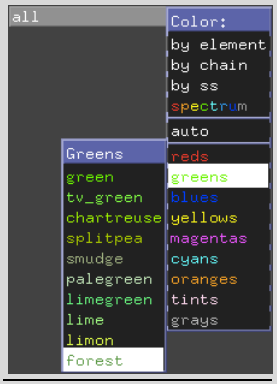
An alphabetical list of all PyMOL-defined color names can be found under the top menu "**Setting > Colors…**"

Within that window the colors can also be edited.



The **C** menu within the Names Panel offers a list of color names grouped by tint.

The name of the tint/color is shown in its own hue.



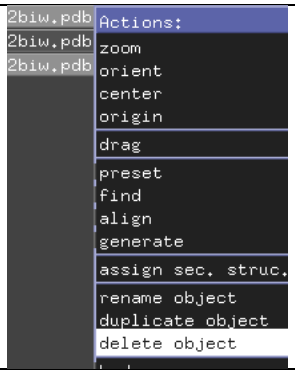
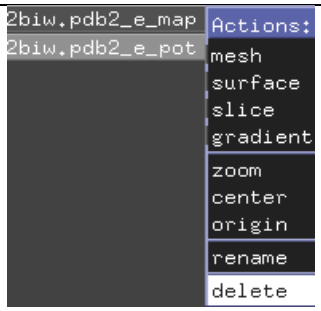
5. Default display

Note that when the calculation first takes place, the surface is shown but the original PDB file is hidden. In the case of 2BIW it means that the carotenoid becomes invisible. To restore it, simply click on button 2biw within the Names Panel: this will restore the display of the protein and ligand as lines or sticks. Since the protein is under the surface it will remain invisible (but some odd side chains might stick out of the surfaces.) To make more complex images it may be useful to create a new object containing the ligand alone (see creating objects below.)

6. Deleting calculated maps

2biw_e_chg, 2biw_e_map, and 2biw_e_pot are PyMOL objects that can be deleted with the A (action) button. Note: the menu will be different for the 3 objects within the **A** menu!

✓ **TASK** **Delete the 3 objects** pertinent to the electrostatic potential :

2biw_e_chg > A > delete	**2biw_e_map > A > delete**	**2biw_e_pot > A > delete**
		

Side chain mutations Wizard

PDB files show the structure of a particular sequence and conformation. However sometimes it is useful to mutate a side chain to see what the effect might be.

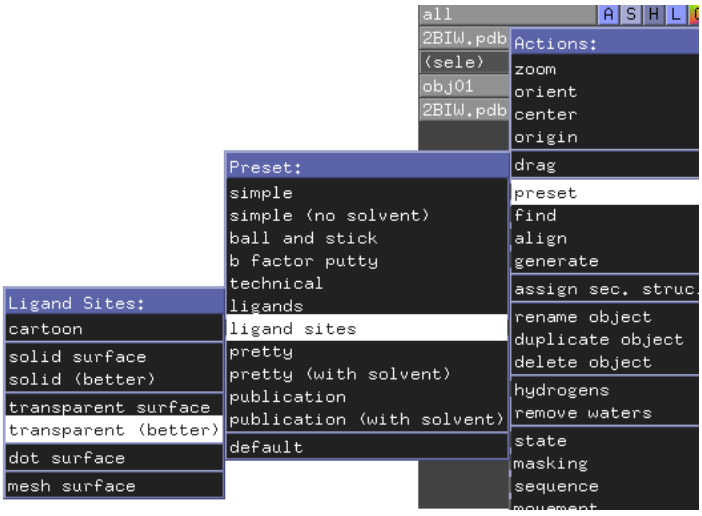
1. Preliminary: create standard view

TASK

- Follow the menu cascades:

2biw > A > preset >ligand sites > transparent (better)

This will create a similar view of the ligand within the pocket with some amino acid sides chains shown as sticks.

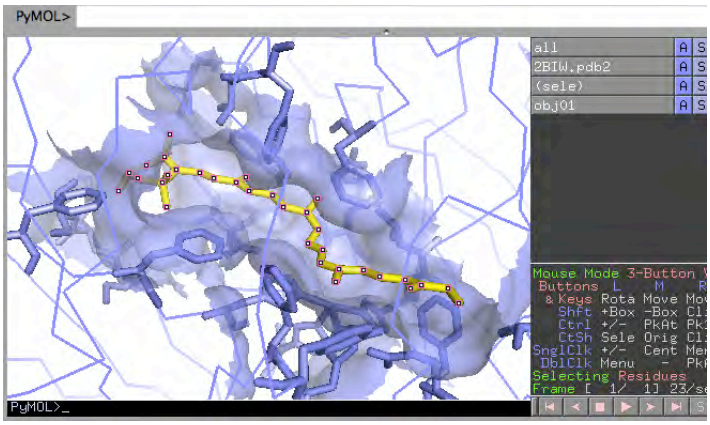


The default colors are rainbow. To make the display clearer, change the colors with the following cascade menus:

- 2biw > C > blues > slate

- Click on the **ligand**

- (sele) > C > yellow



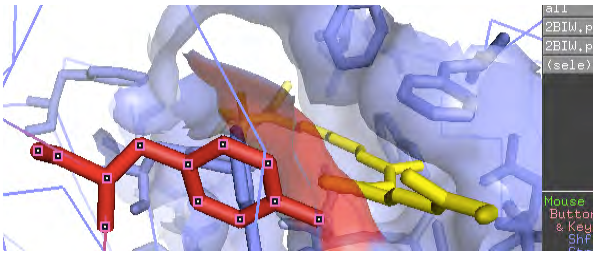
2. Select side chain to mutate

Since we already worked with Tyr 322 above, we shall use this as an example.

✓ **TASK**

- **Rotate** the **view** to **find Tyr 322**
- **Click** within the **blank area** to un-select previous (sele)
- **Click** on **Tyr 322** with the mouse to select it
- Change it to red: **(sele) > C > reds > red**

(note that the surface associated with Tyr 322 will also change color.)



3. Mutagenesis Wizard

The menu cascade Wizard > Mutagenesis opens a new panel below the Names Panel and above the mouse control reminder. Directions will be prompted with text overlaid on the Viewer:

"Pick a residue" and "Select a rotamer, or pick a new residue…"

TASK

Steps to mutate one amino acid side chain on a protein structure:

- Open from the menu **Wizard > Mutagenesis**

- When "Pick a residue" appears at top left, **Click on Tyr 322**

(Note: if Tyr 322 is still selected in (sele it may be necessary to click twice. The first click will unselect Tyr 322, and the second will select it for mutation.)

The text section will echo:

```
You clicked /2BIW.pdb2//B/TYR`322/CG
Selector: selection "sele" defined with 0 atoms.
You clicked /2BIW.pdb2//B/TYR`322/CG
Selector: selection "sele" defined with 12 atoms.
Mutagenesis: 5 rotamers loaded.
```

A new copy of Tyr322 will be displayed in white and corresponds to an ideal rotation. The default is that it is a backbone-dependent rotamer.

Upon selection a slight de-zooming may occur. Zoom back closer with the right-button mouse (Top > Down movement.)

- When "Select a rotamer for TYR`322 or pick a new residue…" appears at the top left: **Click on** the **white Tyr 322**

- Click on the **No Mutation button** and **select** a new amino acid: **PHE**

- **Click** the grey button **Apply**
- **Click** the grey button **Done**

Note: Clicking Done will exit the Mutagenesis Wizard.

✓ TASK

- Quit PyMOL

Automatic 3D Structure Superimposition

The example will show how to superimpose 3 viral proteases in 3D:

INFO

PDB ID	*TITLE*
1CQQ	TYPE 2 RHINOVIRUS 3C PROTEASE WITH AG7088 INHIBITOR
2HRV	2A CYSTEINE PROTEINASE FROM HUMAN RHINOVIRUS 2
1L1N	POLIOVIRUS 3C PROTEINASE

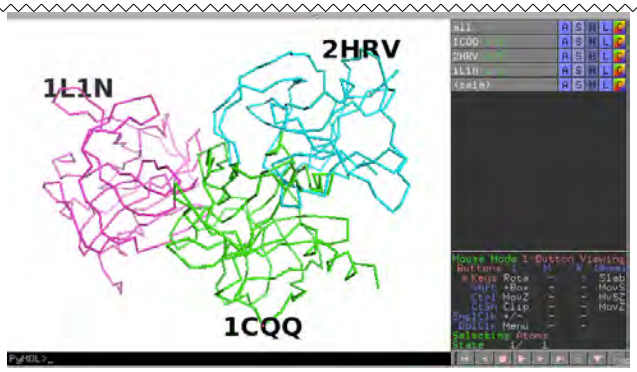
TASK

Preliminary:

- **Open** a new PyMOL (MacPyMOL) session
- fetch 3 PDB entries, for 2 of them we need to specify that we want only one of the biological units. Type the commands in the following order:

```
fetch 1CQQ
fetch 2HRV, type=pdb1
fetch 1L1N, type=pdb1
orient
reset
```

- remove waters with the mouse menu cascade: **all > remove waters**
- Change background to white (**Display > Background > White**)
- Hide everything and show ribbons: (Names Panel)
  all > H > everything
  all > S > ribbon




```
# If you want to add la-
bels:

set label_color, black

set label_size, 32

set label_font_id, 7

# select one atom
# then label it:

label sele, "1L1N"

# repeat for the other 2
```

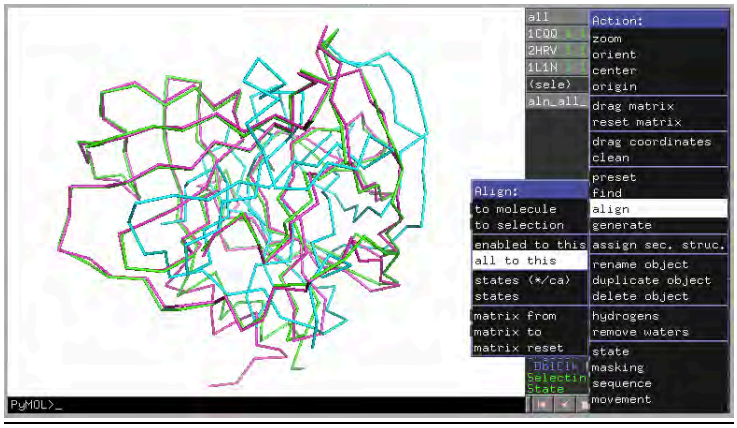
- We are now ready to attempt superimposition.

1. Automatic superimposition: Action menu

TASK

To align all of the objects to 1CQQ, use the following menu cascade:

1CQQ > A > align > all to this



With the automatic superimposition the 3C proteases are best matched together while the 2HRV 2A proteinase does not match as well.

TASK **Repeat** the process this time selecting 2HRV as the reference:

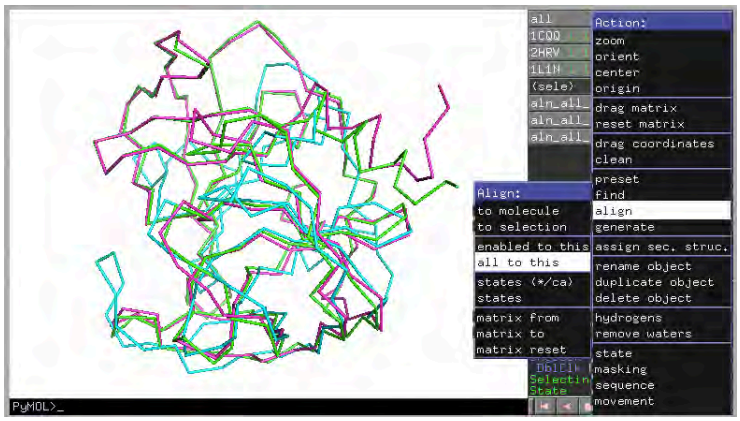
2HRV > A > align > all to this

Is the alignment better, worse or of similar quality?:_____

TASK

Repeat <u>again</u> the process this time selecting 1L1N as the reference:

1L1N > A > align > all to this



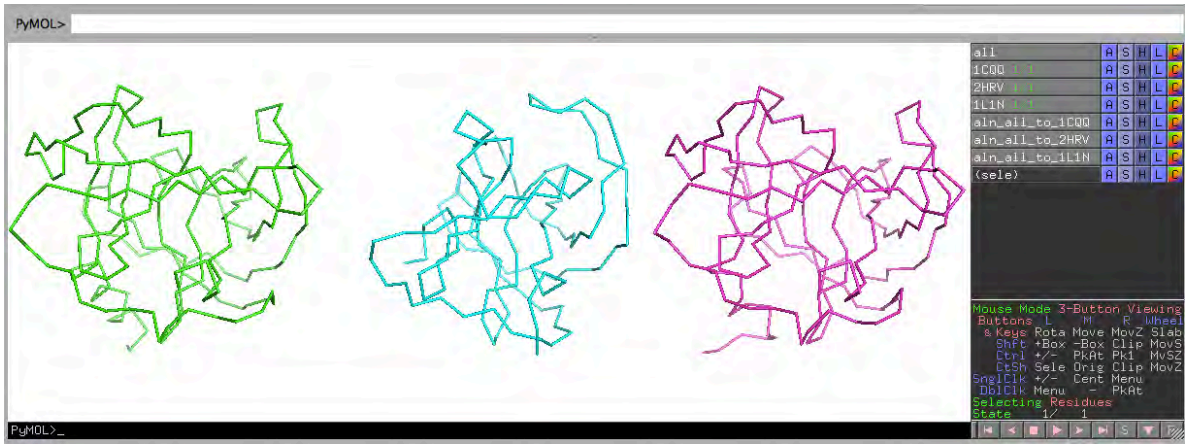
This third attempt aligning all to 1L1L provides the best overall result.

2. Grid view

This is the perfect opportunity to introduce the grid view option where each molecule is given a portion of the viewer screen. It is engaged with the line command:

```
set grid_mode,1
```

Depending on the size or resizing of the PyMOL window the 3 molecules will occupy a 1/4 portion of the screen if the window is roughly square, but will occupy 1/3 of the viewer if the window is stretched:



Molecules will move all at the same time when the mouse is moved.

To return to the normal view simply issue the command: `set grid_mode,0`

3. Pairwise superimposition

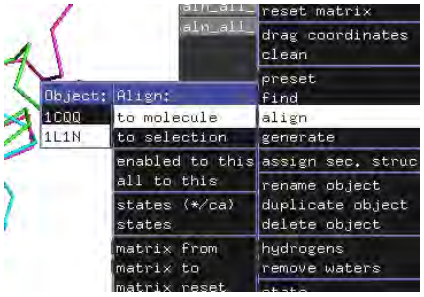
PyMOL will have no problem aligning 2 similar structures. PyMOL firsts creates a sequence alignment then tries to align the structures accordingly.

- mouse method

  The action selected just above (A > align > all to this) aligns all other molecules to the chosen reference. However, each molecule can also be matched to any specified other molecule.

  For example, click on the Action button of 2HRV and follow the cascade:

  2HRV > A > align > to molecule > 1L1N



- line command method

  If 2 proteins are named struct1 and struct2 within the Names Panel, the simple line command will align them: **`align struct1, struct2`** (note the use of the comma after struct1)

  Example: `align 2BIW, 2BIX`

Advanced: Alternate methods and resources not covered in this tutorial:

For more complex alignment questions, see the following web site:
`http://pldserver1.biochem.queensu.ca/~rlc/work/teaching/BCHM823/pymol/alignment/` [archived: `http://bit.ly/2c2YIEx`]

See Pymol commands:
- **Align**: `http://pymolwiki.org/index.php/Align`
- **Fit**: `http://pymolwiki.org/index.php/Fit`

See Pymol extensions:
- **Cealign**: a structure-based alignment (not sequence –based)
  `http://pymolwiki.org/index.php/Cealign`

Model building: modelling a helix

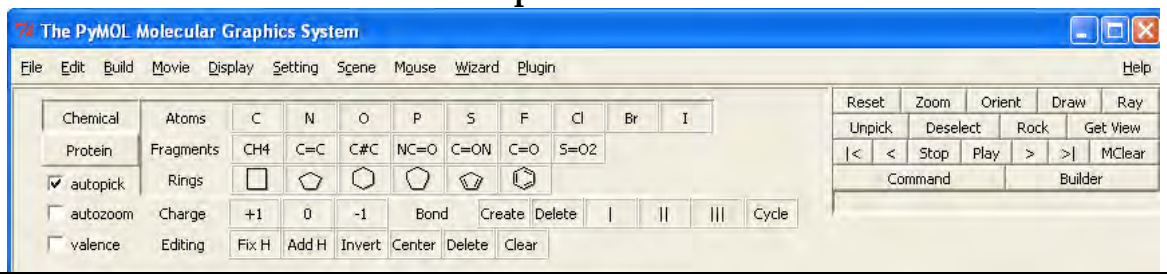
✓ **READ** PyMOL offers modeling options for small molecules and proteins. For example you can build an alpha helix from scratch with a specific sequence.

The "builder" interface to accomplish this is different depending on the operating system.

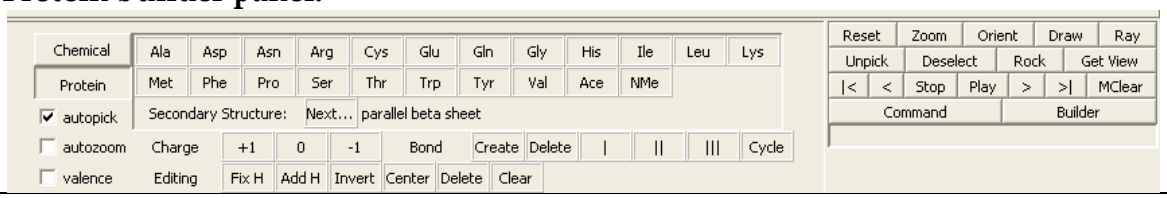
On Windows and X11 (Unix/Linux) versions of PyMOL, the "Upper Window" has an extra set of buttons to build by clicking on the various components to be assembled such as amino acids.

Windows builder interface:

Small Chemical molecules builder panel:



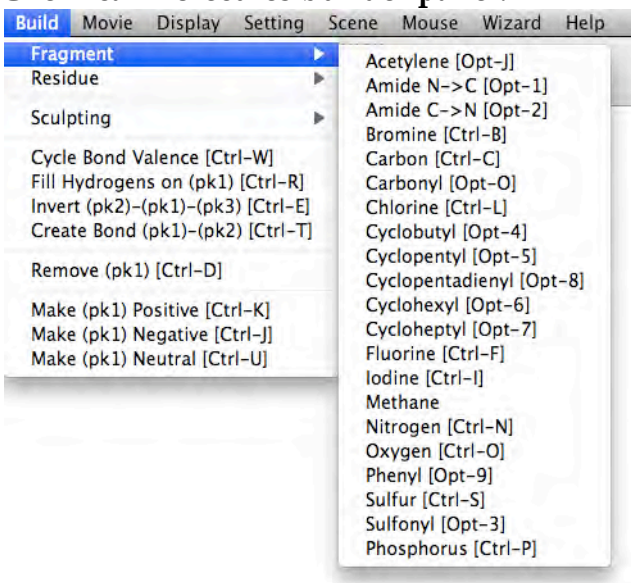
Protein builder panel:



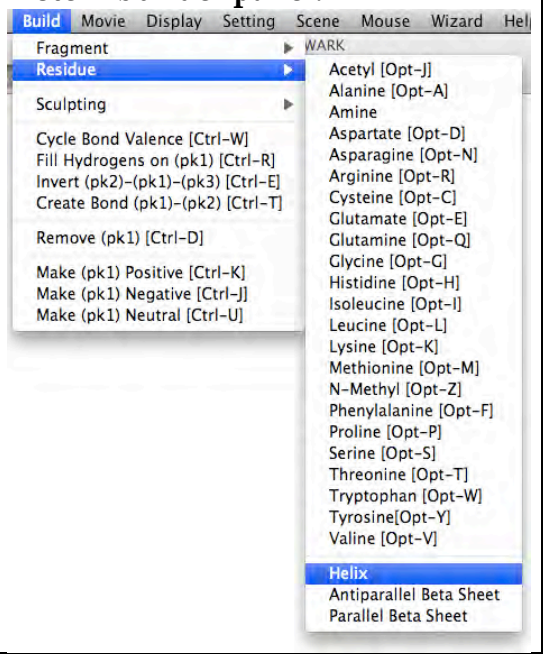
The MacPyMOL version has the same capability but instead of clicking icons menu items are chosen as illustrated below.

MacPyMOL builder interface: is via the Build menus:

Chemical molecules builder panel:



Protein builder panel:



INFO

MacPyMOL is a hybrid version that also contains the X11 version. To access the X11 version simply copy and rename the MacPyMOL application (e.g. on the Desktop) with the menu cascade:

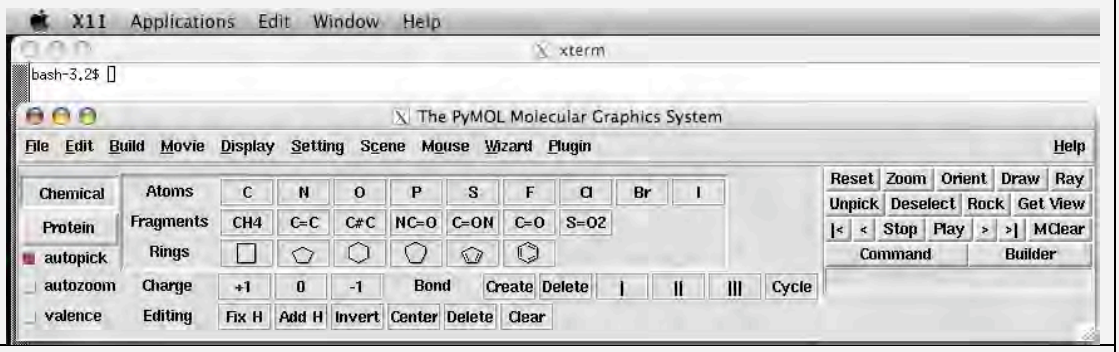
- **Click once** on MacPyMOL
- use the Finder menu: **Edit > Copy**
- **Click** anywhere on the **Desktop** , then **Edit > Paste**
- **Change** the name from "**MacPyMOL Copy**" to "**PyMOLX11Hybrid**"
- The change of name will activate the X11 version embedded within upon double clicking

Note: X11 has to be installed on the Macintosh for this to work. Current versions of the Mac OS install X11 by default.
Note: an Xterm terminal will appear

The X11 version looks very similar to the Windows version, including a split between the upper and lower windows.

X11 builder interface:

Small Chemical molecules builder panel:

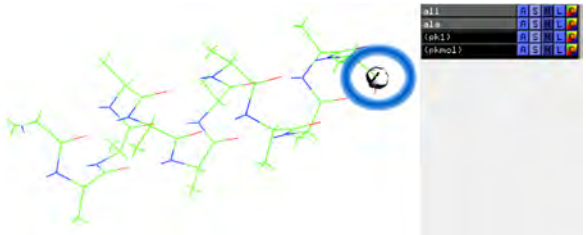


Protein builder panel:

1. Steps to build a poly-ALA model helix

1.1 Macintosh MacPyMOL:

follow the menu cascades:
- Build > Residue > Helix
- Build > Residue > Alanine
- Repeat another 11 times for a total of 12 residues

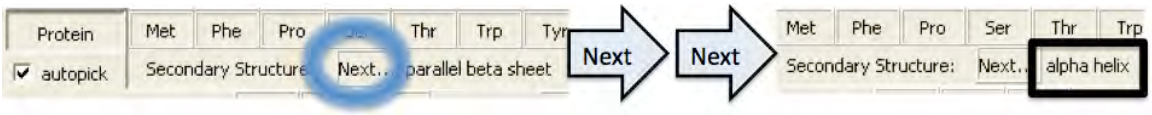


- Alternatively use the **Option+A** keyboard shortcut
- The model will be built within the Viewer and have name "ala" within the Names Panel.

Note: the C carbon to which the next amino-acid is attached is shown by a spherical symbol.

1.2 Windows / X11:

- **Click** on "**Builder**" button (far right)
- **Click** on "**Protein**" button (far left)
- **Change** the **default** parallel-beta sheet to helix: bottom of menu: Click "next" twice

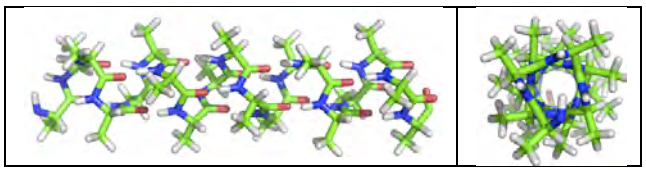


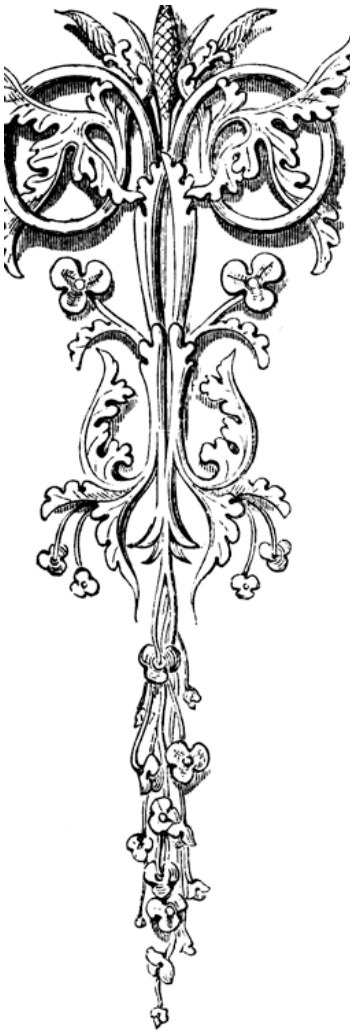
- **Click** on **Ala 12 times**
- The model will be built within the Viewer and have name "ala" within the Names Panel.

1.3 Save the molecule into a file:

- menu **File > Save Molecule** .
- verify that "ala" is selected, or click on ala.
- Click OK
- The default name is the name of the first amino acid used e.g. **ala**.pdb

The alpha helix is created as a "**perfect**" model and contains all hydrogen atoms.





Class notes

http://www.gutenberg.org/files/24518/24518-h/images/image49.png