

PyMOL: Introduction

Working with the graphical user interface

Mouse modes, context menu, object menus, pull-down menus

Working with multiple models, sequence display and simple alignments

Working with Wizards

The PyMOL Interface (Mac Aqua) Drop-down Menus

Win/Linux: 2 windows

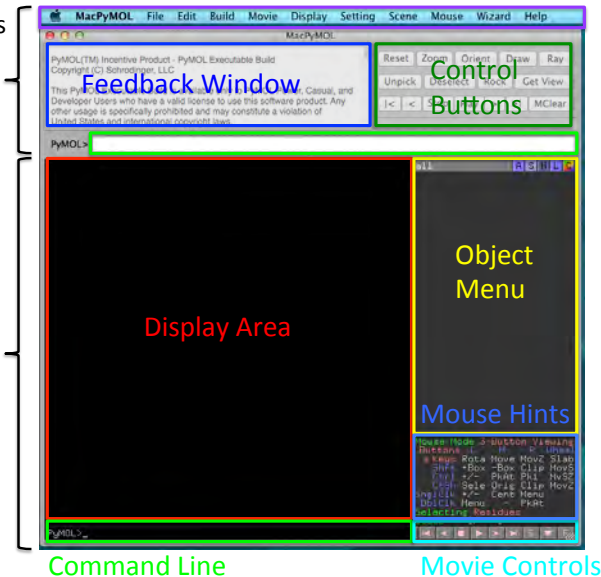
Control window

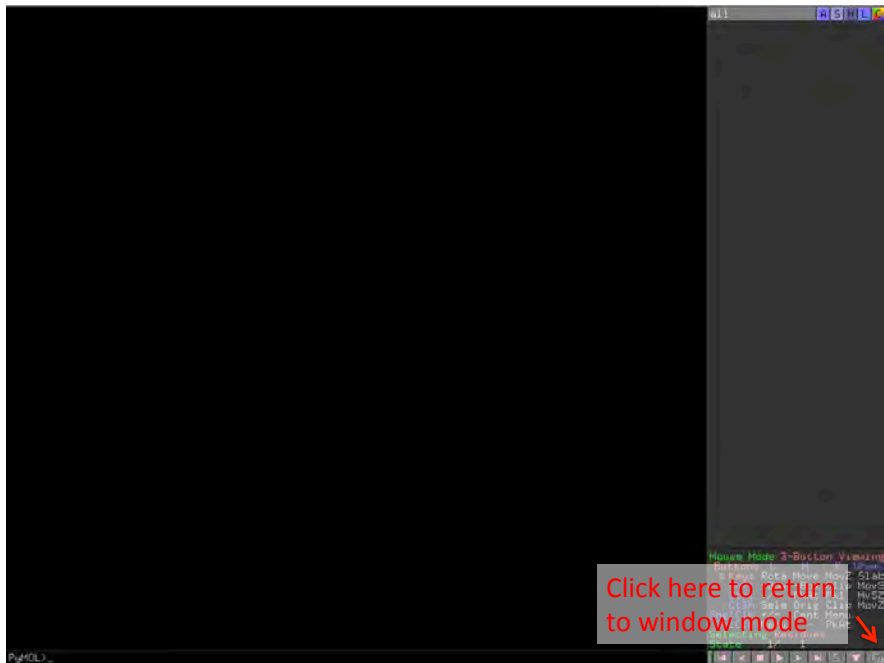
- Pull-down menus
- Command line
- Control Buttons
- Feedback window

Viewing Window

- Display area
- Object menu
- Mouse hints
- Movie controls
- Command Line

DEMO, play along





The PyMOL Interface (Mac X11 Hybrid)

Control window

Drop-down menus

Command line

Control Buttons

Feedback window

Viewing Window

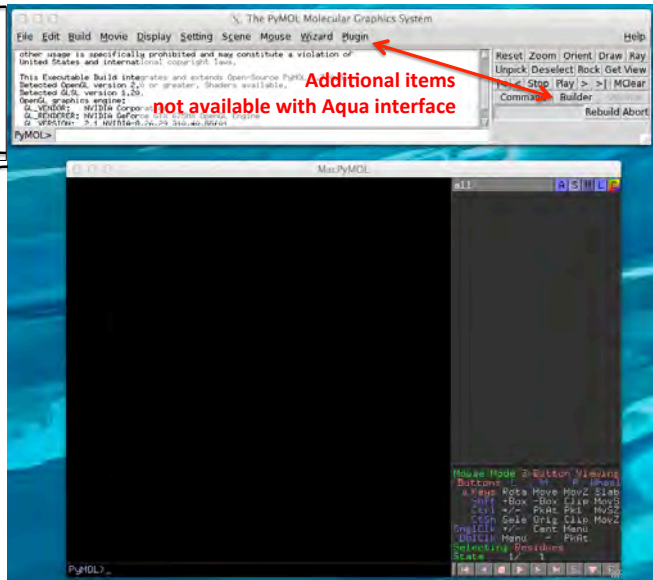
Display area

Object menu

Mouse hints

Movie controls

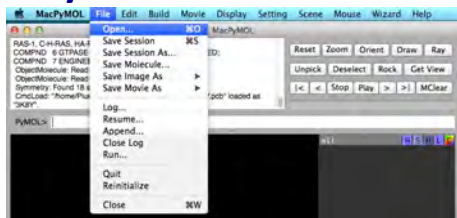
Command Line



Loading a Model into PyMOL

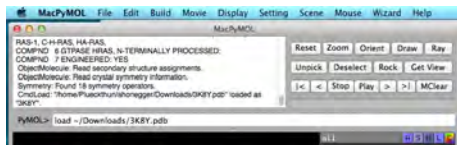
- Using the **Drop-down menu** to select a local file:

~/pymol/pdb/3K8Y.pdb



- Using the **command line** to select a local file:

load ~/pymol/pdb/3K8Y.pdb



- Using the **command line** to fetch a file from the PDB:

fetch 3k8y

- PlugIn: **PDB Loader Service**

- load <http://www.rcsb.org/pdb/files/3k8y.pdb> **This works despite the firewall !**

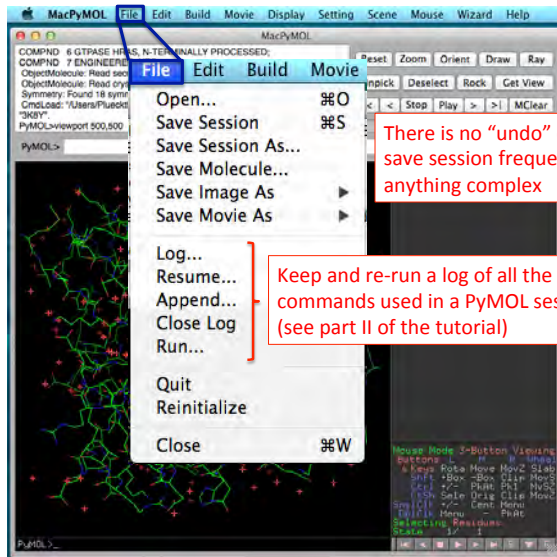
- Mac only?: **Drag-and-drop** pdb file onto the program icon or into the PyMOL display area. If PyMOL is already open, multiple pdb files dropped onto the program icon will open in separate windows. If the program is closed, they will open as separate objects in the same window.

Blocked by the departmental firewall!

Blocked by the departmental firewall!

DEMO, play along

Drop-Down Menu: File



There is no “undo” command
save session frequently if you do
anything complex

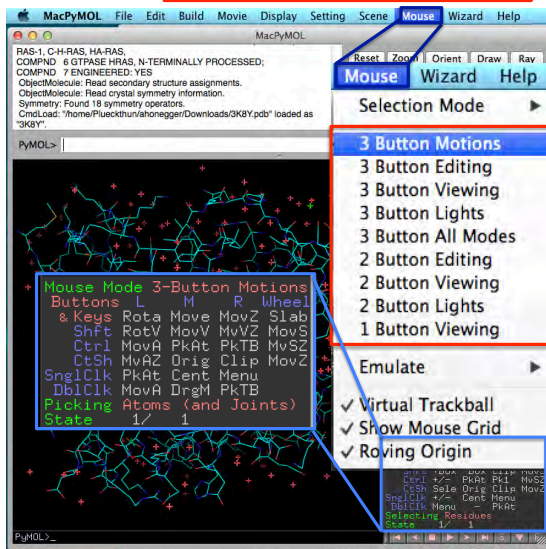
Keep and re-run a log of all the
commands used in a PyMOL session
(see part II of the tutorial)

Using the Mouse

Mouse behavior can be altered using the "Mouse" drop-down menu

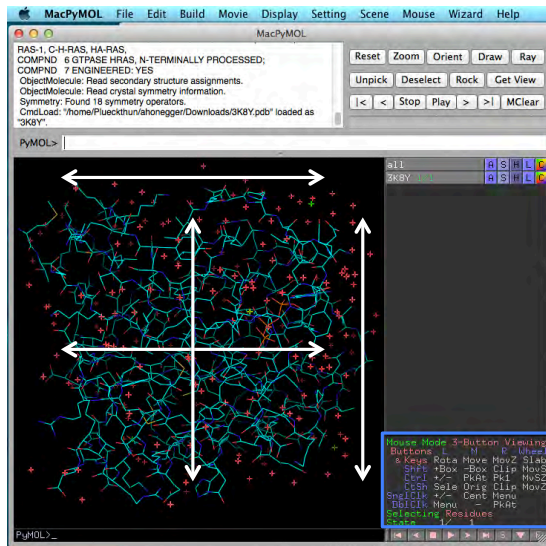
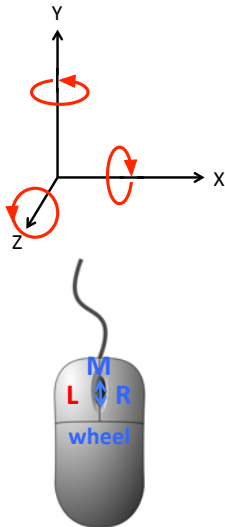


Two buttons and a scroll wheel that can also act as a button

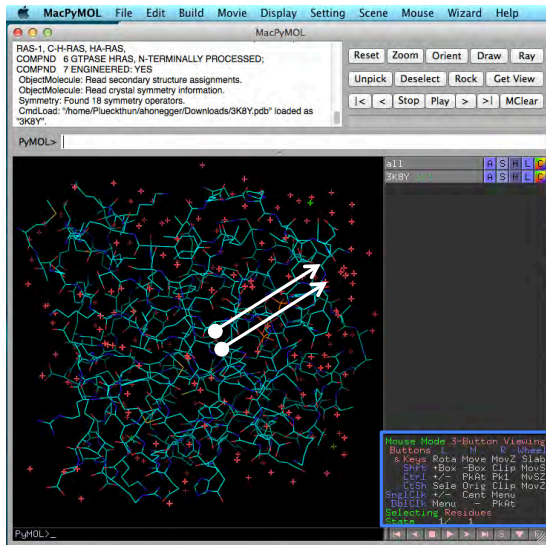
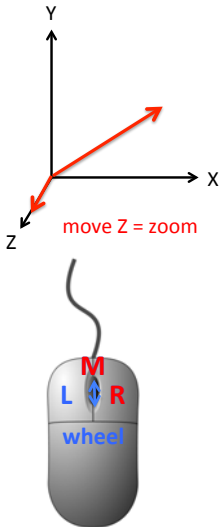


click on mouse hints after altering mouse mode to update panel

Using the Mouse: Rotations



Using the Mouse: Translation



Selecting Atoms and Residues by Mouse-Click



Atoms
Residues
Chains
Segments
Objects
Molecules
C-alfas

Copying or Moving a Selection to a new Object

by cut-and-paste:

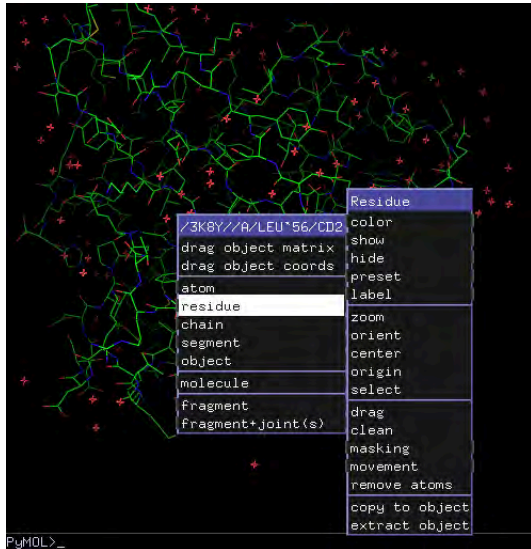
Special Key	Text on the Command Line	Empty Command Line
TAB	Auto-complete text	Display list of all commands
CTRL-A	Position cursor at beginning of line	Select all atoms
CTRL-C		Copy currently selected atoms
CTRL-E	Position cursor at end of line	
CTRL-I		Invert selection
CTRL-K	Delete all text to the right of the cursor	
CTRL-V	Paste into the command line	Paste copied atoms into new object
CTRL-X		Cut atoms
CTRL-Y		Undo
CTRL-Z		Redo

Does not yet work reliably

Context Menu

Normally opened by a single left click on an atom
(dependent on mouse mode, see mouse hints "Menu")

Apply selected action to
individual atom, to the residue,
the whole chain, segment,
object or molecule



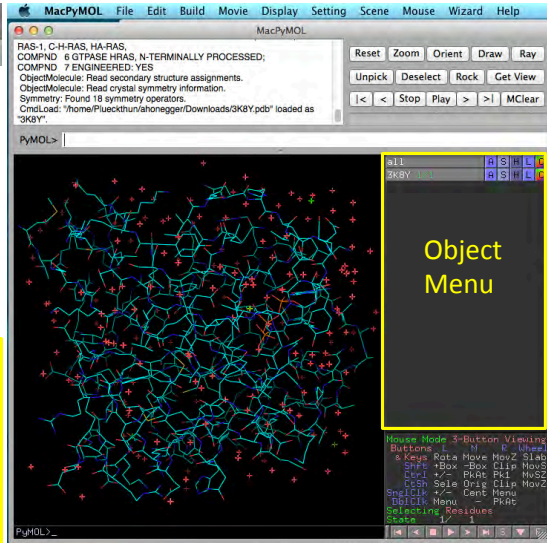
PyMOL Object Menu



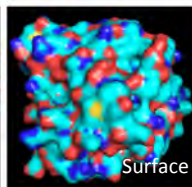
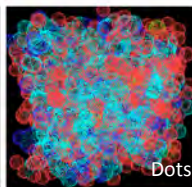
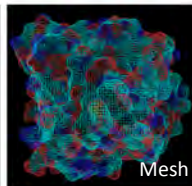
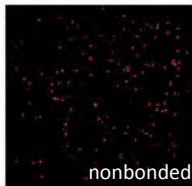
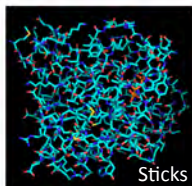
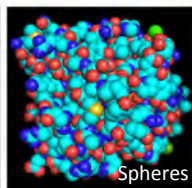
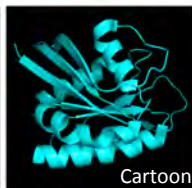
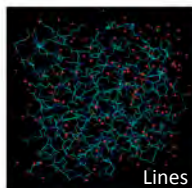
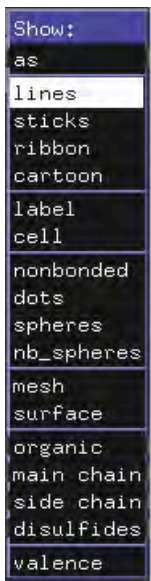
Contains an entry for each object loaded into PyMOL and for each selections. **Display** of an object can be switched off and on by clicking on its name without altering the representation.

ASHLC(M)

- Action
- Show
- Hide
- Label
- Color
- (Movie)

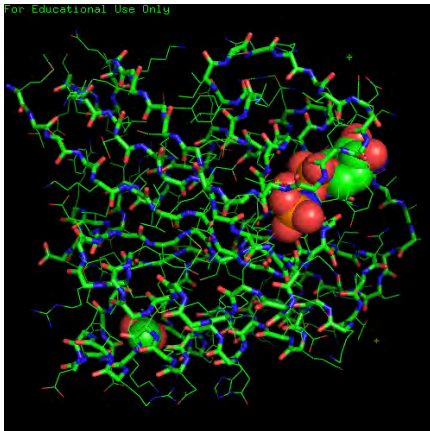


“S” stands for “Show”, “H” stands for “Hide”



Various Representations can be combined

For Educational Use Only



Waters are hidden, side chains shown as lines, the main chain as sticks and the organic molecules as spheres

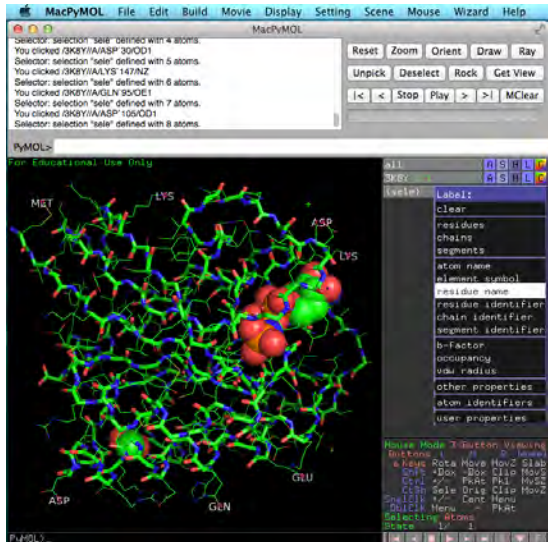
	Show:		Show:		Hide:
	as		as		everything
	lines		lines		lines
	sticks		sticks		sticks
	ribbon		ribbon		ribbon
	cartoon		cartoon		cartoon
	label		label		label
	cell		cell		cell
	nonbonded		nonbonded		nonbonded
	dots		dots		dots
	spheres		spheres		spheres
	nb_spheres		nb_spheres		nb_spheres
	mesh		mesh		mesh
Show:	surface		surface		surface
lines	organic		organic		main chain
sticks	main chain		main chain		side chain
spheres	side chain		side chain		waters
	disulfides		disulfides		hydrogens
	valence		valence		unselected
					valence

Use “L” to Label

If you label 3K8Y, every single atom will be labelled

Whenever you select a subset of the atoms, item (sele) containing this selection appears on the Object Menu

- Mouse: Selection Mode: Atoms
- Pick individual atoms by left clicking then
- (sele): Label: Residue name



MacPyMOL File Edit Build Movie Display Setting Scene Wizard Help

You clicked /KBY//A/ACT 719/CH3
 Selector: selection "sele" defined with 36 atoms.
 You clicked /KBY//A/GLN 22/CA
 Selector: selection "sele" defined with 9 atoms.
 Save: Please wait -- writing session file...
 Save: wrote "/Users/Plueckhuhn/ahonegger/KBY.pse".
 You clicked /KBY//A/HIS 27/CA
 Selector: selection "sele" defined with 19 atoms.

PyMOL> select sele

all
 KBY (sele)
 By Secondary Structure:
 Helix Sheet Loop
 Helix Sheet Loop
 Helix Sheet Loop

Reset Zoom Orient Draw Ray
 Unpick Deselect Rock Get View
 |< < Stop Play > >| MClear

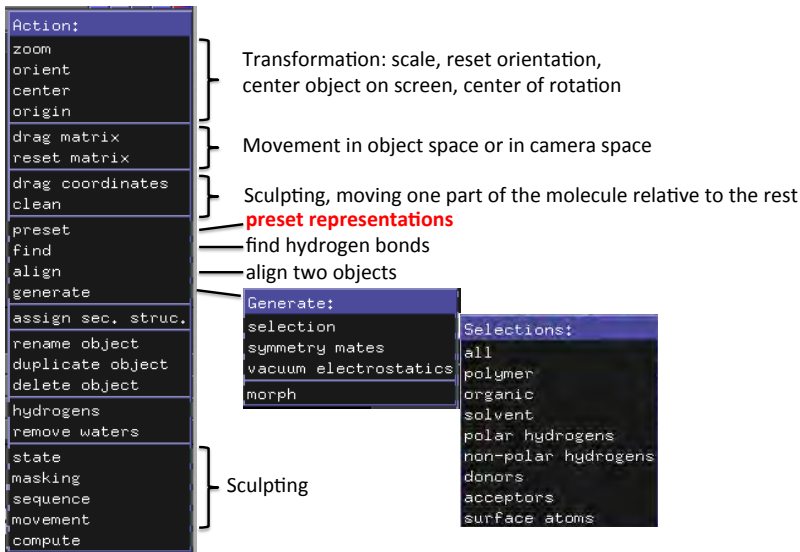
Color:
 by element
 by chain
 by ss
 by rep
 electron
 auto
 red
 greens
 blues
 yellows
 magentas
 cyaans
 oranges
 tints
 grays

Mouse Made 3-Button Vimings
 Buttons: 1 2 3 4 5 6 7 8 9 10 11 12
 * Mouse Rotate Move Move2 Slab
 Shift *Box *Box *Box Clip Move5
 Ctrl */* */* */* */* */* */* */*
 Ctrl Sale Drag Clip Move2
 English */* */* */* */* */* */* */*
 DblClick Menu */* */* */* */*
 Selecting Residues
 State 1/1

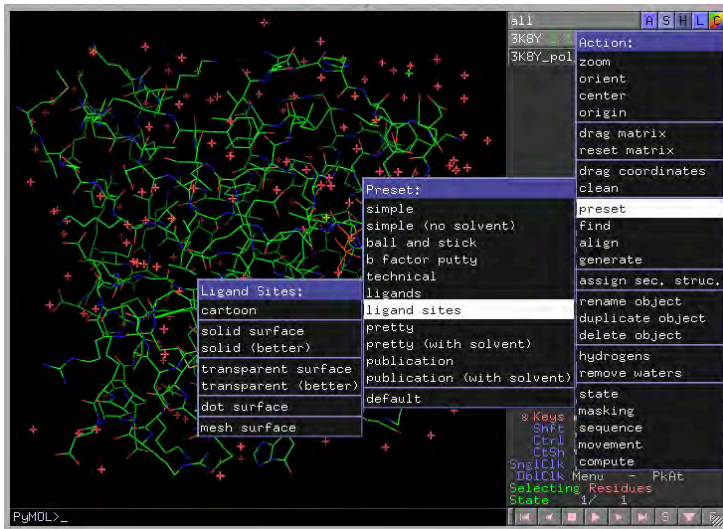
PyMOL>

1

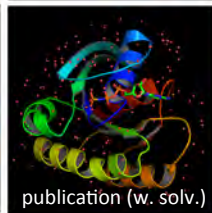
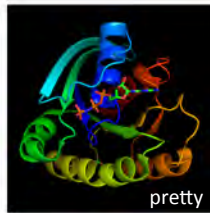
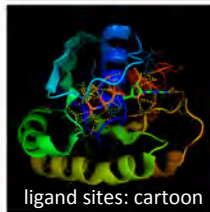
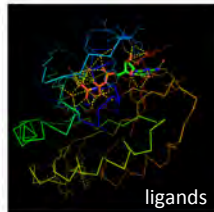
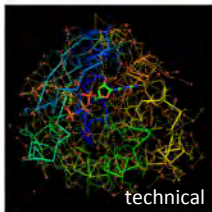
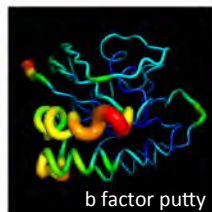
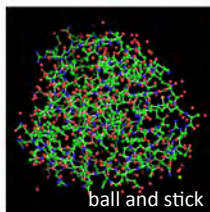
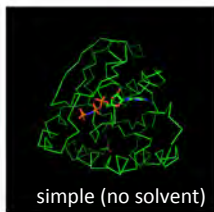
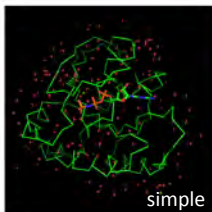
“A” for “Action”: a mixed bag of Options



Choose “A” for “Action”: Presets



Preset representations reset a quick shortcut for various representations



Action: find: polar contacts (~hydrogen bonds)



Combining Different Representations

Download 3K8Y from PDB
<http://www.rcsb.org> and
open 3K8Y with PyMOL

3K8Y: Hide: everything

3K8Y: Show: cartoon

3K8Y: C: spectrum: rainbow */CA

3K8Y: Show: organic : spheres

select these by left click,

sele: C: by element : **C****H****N****O****S**

select C-alpha atoms at either
end of the protein chain

sele: L: residues

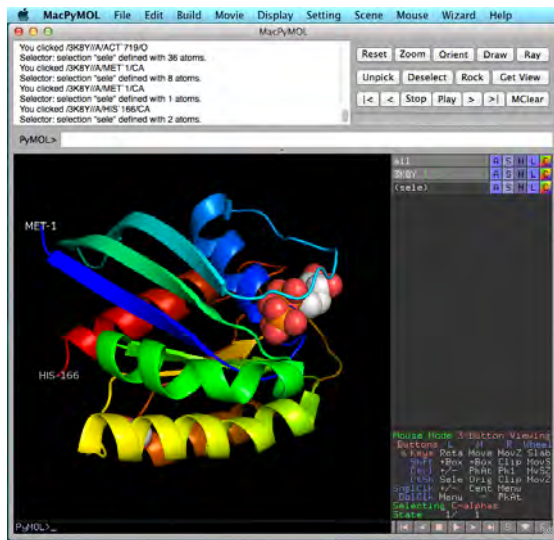
rotate so that both ends of the
chain are clearly visible

For printing:

Display: Background: white

click on the “ray” button

File: save image: png

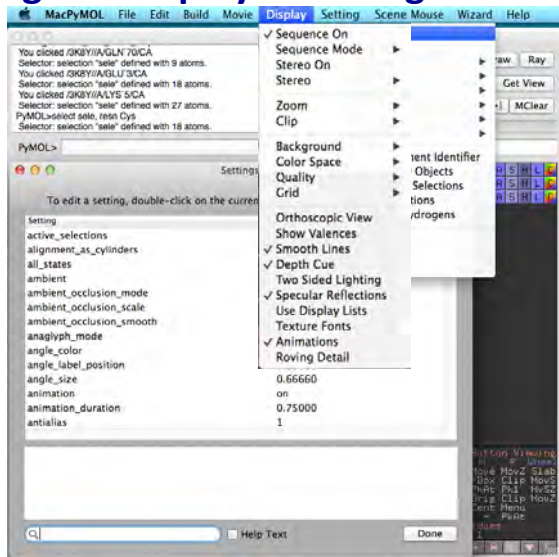


DEMO, play along

Fine-tuning the Figure: Display & Settings

Style and quality of PyMOL representations are controlled by more than 600 different settings.

A few of them can be accessed through the “Settings” and “Display” pull-down menus, others only through the “Edit all” interface or the command line.





Labels are difficult to place precisely – It is often easier to add labels to the image later in a graphics program such as gimp or photoshop.

Saving Images to a File, Image Size

- By default, PyMOL images are produced at the size and resolution of the PyMOL display area. You can set the display area to a precise size with the “viewport” command (command line):

viewport 1200, 900

- This default is overridden if you specify the desired number of pixels when calling the draw or ray command (in the command line):

draw 1200, 900 (fast rendering, lower quality)

or

ray 1200, 900 (slow rendering, high quality, shadow effects)

- The resulting image can be saved through the “File” pull-down menu or with the command line, this can also be used to specify size and resolution:

png filename[, width[, height[, dpi[, ray[, quiet]]]]]

png my_image.png, 1200, 900, 300, 1

Using Named Selections

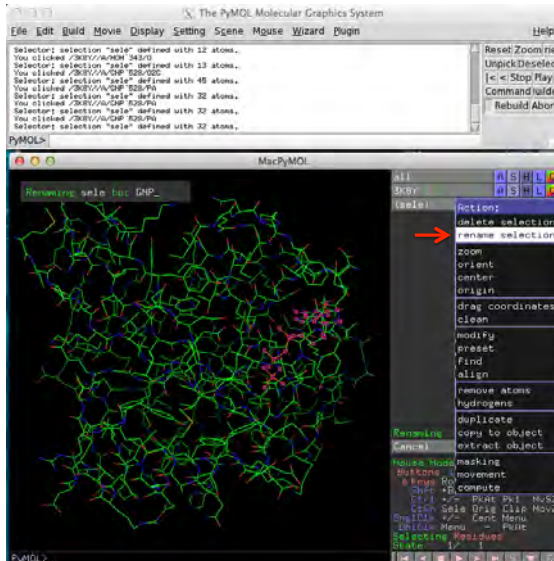
all	A	S	H	L	C
3K8Y	A	S	H	L	C
(sele)	A	S	H	L	C

temporary selection
changes whenever you
select something new

all	A	S	H	L	C
3K8Y	A	S	H	L	C
(GNP)	A	S	H	L	C
(Protein)	A	S	H	L	C

permanent shortcut
to selections of ligand
and protein

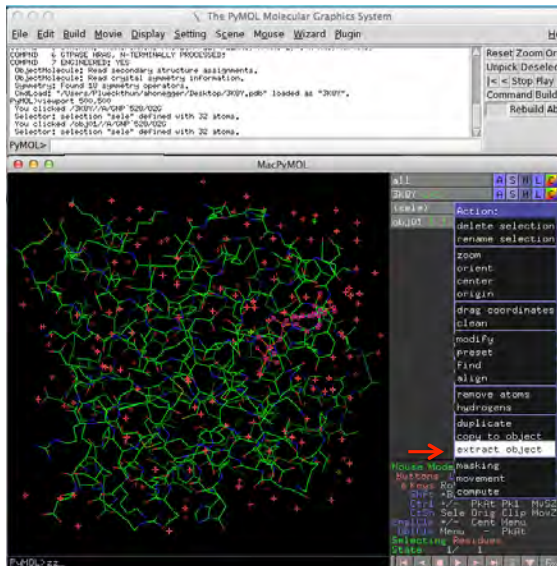
(GNP) and (Protein) are still
part of object 3K8Y



Extracting Selection to a Separate Object

all	A	S	H	L	C
3K8Y	A	S	H	L	C
(sele)	A	S	H	L	C
obj01	A	S	H	L	C

The coordinates of the ligand have been made into an independent object, they are no longer part of object 3K8Y



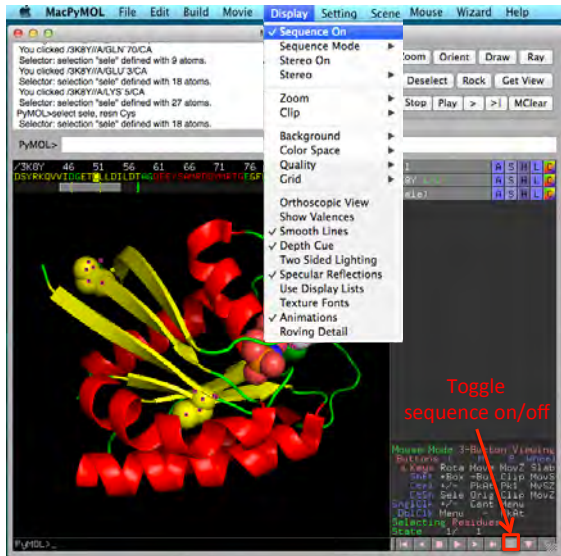
Sequence Display

Coloring of the model is reflected in the sequence

Residues can be selected by left-click in the sequence

Residues selected in the structure are highlighted in the sequence

Vertical bars in the sequence slider indicate the location of selected residues in the sequence



Working with Multiple Models

Load structures **1CTQ.pdb, 1Q21.pdb, 1QRA.pdb, 3K8Y.pdb, 3TGP.pdb** into PyMOL

Click the **“zoom” button** to fit all structures on the screen

For the 3K8Y object, hit **Action:align:all** for a sequence-based structural alignment of the five structures

Click the **“zoom” button** to fit all structures on the screen

Show all structures as **ribbon** only (all – hide:everything, all:show ribbon)

By clicking on the name of each object, you can enable or disable the display of that object without losing its representation

Which of the objects differs most from the others?

If you click on one of the atoms, its full name is listed in the feedback window.

e.g. /3TGP//A/MET`1/CA is the C α in residue 1 (methionine) in chain A of object 3TGP

Now compare the ligands of the different structures (all – show:organic-sticks)

Do you find any difference correlating with the difference in main chain structures?

DEMO, play along

Side-by-side comparison: Arrange Objects in Grid



Demo and Exercise:

- **Find and download the structure of neurotensin receptor type 1**
Seven structures are available, which one should you use?

<http://www.rcsb.org>

- What are the key differences between these structures?
Align and compare the structures.
- Isolate the structure of one receptor/ligand complex
- Prepare a figure that illustrates the ligand in its binding pocket.
- Prepare a figure that illustrates the locations of the cysteine residues within the receptor. Are any involved in disulfide bonds? Which ones are located in a hydrophobic environment, which ones are exposed to the solvent?
- Mutate the free Cys to Ala or Val

Information on the PDB website

- Species / Variant / Link to UniProt sequence annotations
- Type of construct
(domain fusions, stabilizing mutations, loop deletions)
- Resolution, method, refinement statistics, validation report
- Crystallization conditions
- Presence of ligands or inhibitors, proteinacious binding partners
- Biological sequence vs. theoretical sequence vs. residues visible
(missing loops, terminal sequences)
- Biological assembly vs. asymmetric unit
- Related PDB entries
- Literature links

Inspecting a PDB file in a Text Editor

- PDB files are **plain text files** that can be viewed and edited in a text editor.
- They contain a **Header** that contains general information about the molecule: type of molecule, literature reference, resolution and refinement statistics, theoretical sequence (SEQRES), non-standard chemical groups (HETATM), secondary structure, SS-bonds, active sites, crystallographic symmetry parameters.
- **ATOM** (standard protein and nucleic acids) and HETATM (all other chemical compounds) records contain the actual coordinates for each atom of the molecule:

ATOM 1 N ASP L 1 29.392 -8.290 26.733 1.00 42.11 N

- For very high resolution structures, ANISOU records may specify anisotropic temperature factors.

ANISOU 1 N ASP L 1 4820 5485 5696 878 -962 -1205 N

- The most important of these informations are listed on the RCSB page for the structure

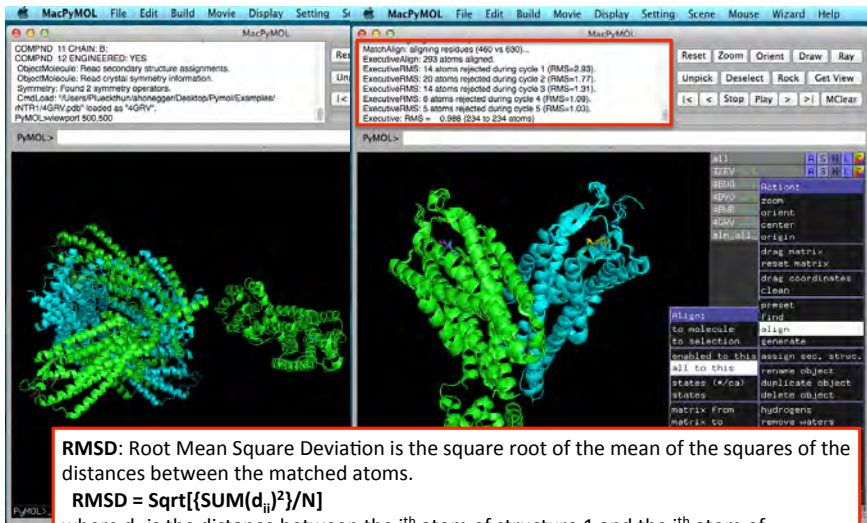
Inspecting a PDB file in PyMOL

- Completeness – entire molecule of interest or isolated domain?
- Additional fused domains to improve production, crystallization
- One or more copies of the molecule in the asymmetric unit?
- One or more chains in the biological unit?
- Overall topography, secondary structure?
- Chain breaks, missing loops and ends?
- Non-protein components: Ligands, Ions, etc.?
- Crystal contacts, b-factors?
- Structural divergence between related structures

Demo and Exercise:

- Find and download the structure of neurotensin receptor type 1
Five structures are available, which one should you use?
<http://www.rcsb.org>
- **What are the key differences between these structures?**
Align the structures in PyMOL and compare.
- Isolate the structure of one receptor/ligand complex
- Prepare a figure that illustrates the ligand in its binding pocket.
- Prepare a figure that illustrates the locations of the cysteine residues within the receptor. Are any involved in disulfide bonds? Which ones are located in a hydrophobic environment, which ones are exposed to the solvent?
- Mutate the free Cys to Ala, Ser or Val

Sequence-based Structural Superposition



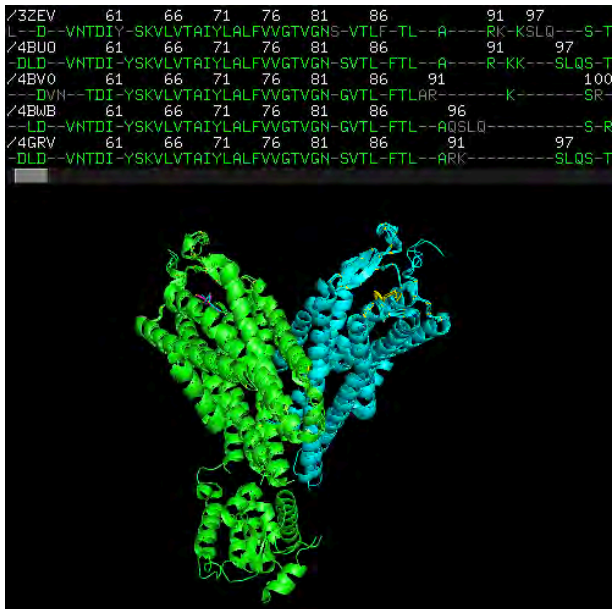
RMSE: Root Mean Square Deviation is the square root of the mean of the squares of the distances between the matched atoms.

$$\text{RMSD} = \text{Sqrt}[\{\text{SUM}(d_{ij})^2\}/N]$$

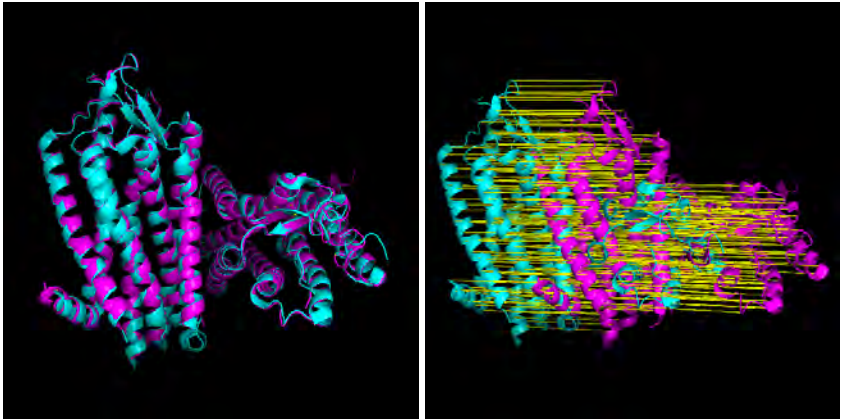
where d_{ii} is the distance between the i^{th} atom of structure 1 and the i^{th} atom of structure 2 and N is the number of atoms matched in each structure.

3D-superposition
based on sequence
alignment

Works well for close
homologs, not so well
for more distant relatives



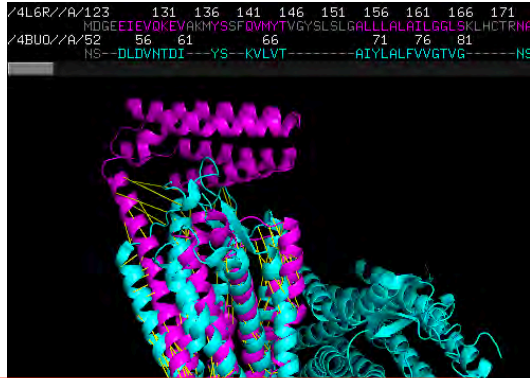
Alignment of structures 4BUO-4BV0



In a good alignment, the lines indicating which atom was superimposed to which only become visible when one moves the aligned structures apart and re-draws the alignment object

If align does not give reasonable results

In this alignment of the glucagon receptor (4L6R) to the rat neurotensin receptor 1 (4BUO) the sequence similarity was too low for a good sequence alignment, resulting in a bad residue pairing for the structural alignment



Other alignment methods exist and can be used through the command line:

“cealign”, “align”, “super”, “pairfit” or “fit” , invoked with defined atom selections for better control over the alignment process

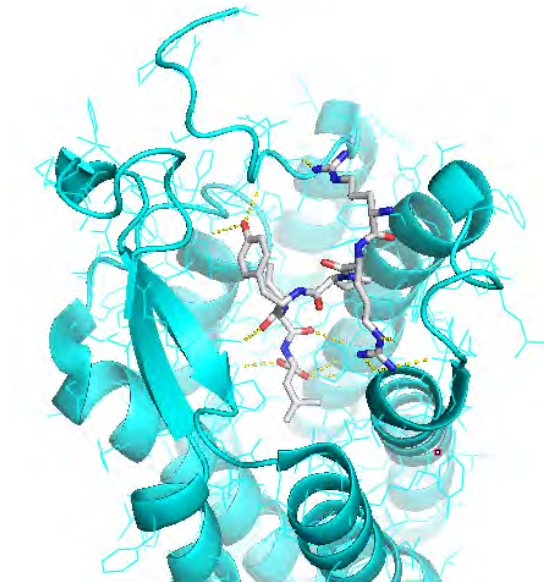
Demo and Exercise:

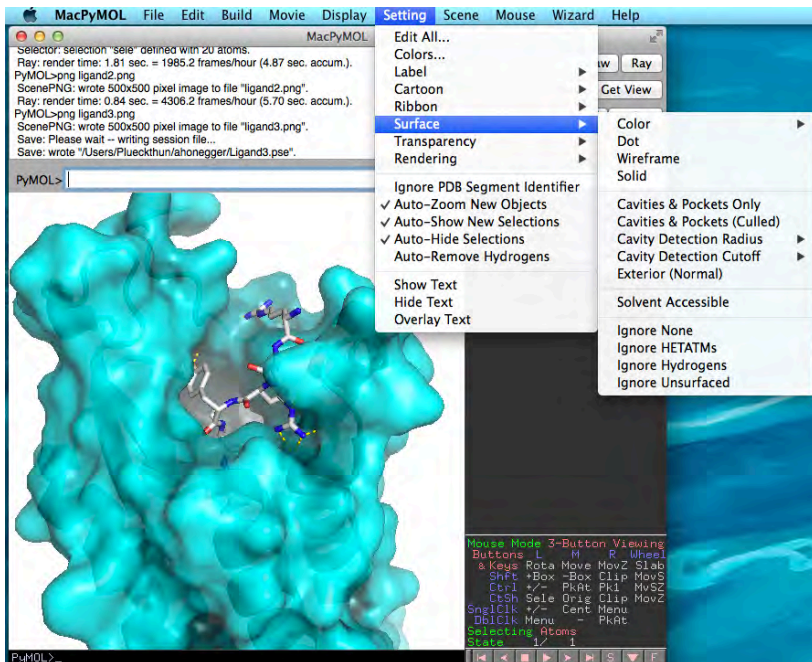
- Find and download the structure of neurotensin receptor type 1
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Align and compare the structures.
- **Isolate the structure of a single receptor/ligand complex**
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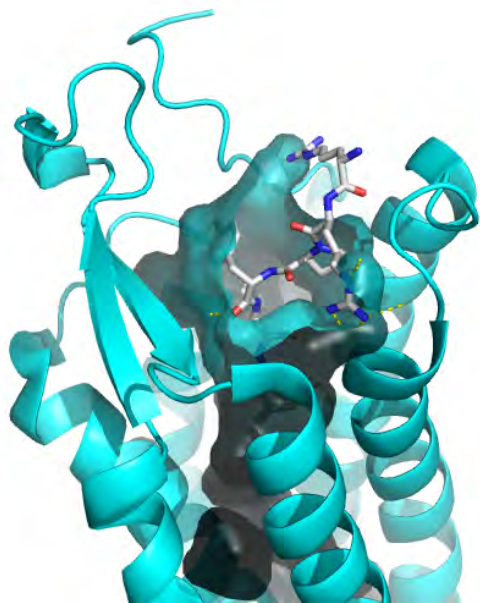
Or you can set selection to chain, select in sequence view, then copy-paste or cut-and-paste to a new object

Demo and Exercise:

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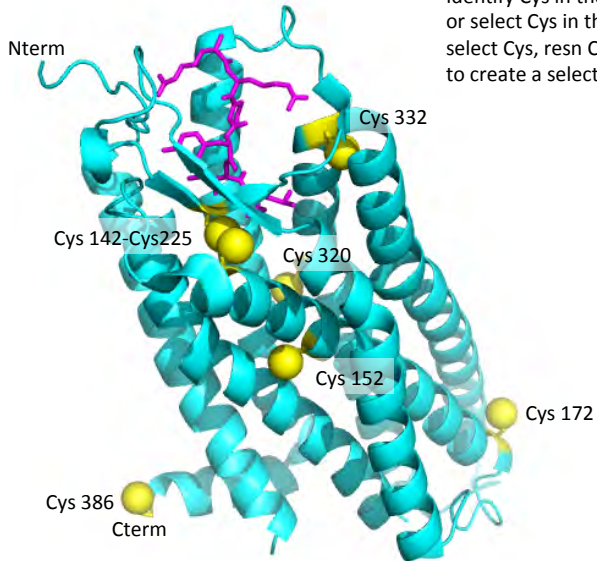




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- Find and download the structure of neurotensin receptor type 1
Five structures are available, which one should you use?
<http://www.rcsb.org>
- What are the key differences between these structures?
Align and compare the structures.
- Isolate the structure of one receptor/ligand complex
- Prepare a figure that illustrates the ligand in its binding pocket.
- **Prepare a figure that illustrates the locations of the cysteine residues within the structure. Are any involved in disulfide bonds? Which ones are located in a hydrophobic environment, which ones are exposed to the solvent?**
- Mutate the free Cys to Ala, Ser or Val

Identify Cys in the sequence display
or select Cys in the command line:
select Cys, resn Cys
to create a selection object called Cys



Demo and Exercise:

- Find and download the structure of neurotensin receptor type 1
Five structures are available, which one should you use?
<http://www.rcsb.org>
- What are the key differences between these structures?
Align and compare the structures.
- Isolate the structure of one receptor/ligand complex
- **Prepare a figure that illustrates the ligand in its binding pocket and the membrane exposed surfaces of the receptor.**
- Prepare a figure that illustrates the locations of the cysteine residues within the structure. Are any involved in disulfide bonds? Which ones are located in a hydrophobic environment, which ones are exposed to the solvent?
- **Mutate the free Cys to Ala, Ser or Val**

Working with Wizards: Mutagenesis

The image displays the PyMOL molecular visualization software interface. The main window shows a protein structure with a specific residue highlighted in yellow. The command line at the top shows the sequence: `4BUO 306 311 316 321 326 331 336 341 346 351 356`. The residue `316` is highlighted in yellow. The command line also shows the sequence: `PGRVQALRRGLVLRRAVIAFVQULPYHVRRLMFCYISDEQNTTFLDFYHYFMYLTHALV`. The right panel shows the **Wizard** menu, which is open, displaying options: **Appearance**, **Measurement**, **Mutagenesis** (highlighted), and **Pair Fitting**. The **Mutagenesis** sub-menu is also open, showing options: **Density**, **Filter**, **Sculpting**, **Label**, **Charge**, and **Demo**. The bottom panel shows the **Mouse Mode** and **Buttons** section, with the **Selecting Residues** section highlighted. The **State** is set to `1/1`.

select the residue you want to change and highlight it with a different color

open the mutagenesis wizard

Wizard Help

- Appearance
- Measurement
- Mutagenesis**
- Pair Fitting

Density

Filter

Sculpting

Label

Charge

Demo ▶

Mouse Mode 3-Button Viewing

Buttons L M R Wheel

& Keys Rota Move MovZ Slab

ShFt +Box -Box Clip MovS

Ctrl +/- PkAt Pk1 MvSZ

CtSh Sele Orig Clip MovZ

SnglClk +/- Cent Menu

DblClk Menu - PkAt

Selecting Residues

State 1/ 1

Working with Wizards: Mutagenesis

Pick a residue...

4BU0 306 311 316 321 326 331 336 341 346 351 356 all A S H L C
PGRVQALRRGVLRRAVIAFVW QLPYHVRRLMFCVISDEQMTTFLDFYHYFYMLTNALV
(NT) A S H L C
(cys) A S H L C
(sele) A S H L C

Select the amino acids to mutate to

Mutagenesis

No Mutation	Mutant
N-Cap: Open	No change
C-Cap: Open	ALA
Hydrogens: Current	ARG...
Show Lines	ASN
Backbone Depen. Rotamers	ASP... ASP
Apply	CYS ASPH
Clear	GLN
Done	GLU...

Mouse Mode 3-Button View
Buttons L M R MH
& Keys Rota Move MovZ S
Shift +Box -Box Clip M
Ctrl +/- PkAt Pk1 M
CtSh Sele Orig Clip M
SnglClk +/- Cent Menu
DblClk Menu - PkAt

Selecting Residues
State 1/ 1

click "apply" to make the change

PyMOL>_

Working with Wizards: Mutagenesis



notice the clash indicators: the larger and the redder they are, the worse the clash!

use forward and back keys to select a rotamer

click "done" to leave the wizard

Clean up the Neighborhood (local minimization)

select mutated residue

(for the selection) Action:Modify:Expand:by 5 Å

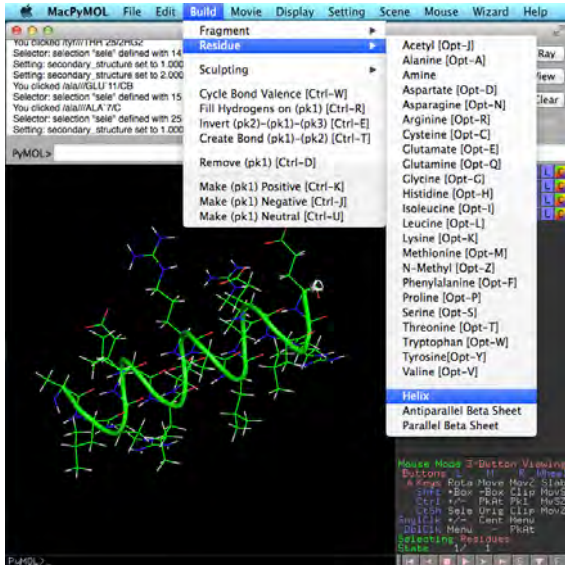
(for the selection) Action:Clean

Uses the Merck Molecular Force Field (MMFF)

The SIB (Swiss Institute of Bioinformatics) offers a PyMOL plugin for download that enables you to mutate any residue of a PDB structure into one of the non-natural L- or D-sidechains of the SwissSidechain database.

<http://www.swissidechain.ch/visualization/pymol.php>

Modelling a Peptide from Scratch



Select the desired secondary structure

e.g. Build:Residue:Helix

Type the desired sequence while keeping the <alt> key pressed

Program bug on Mac:
(CH) <alt>G results in an "@" added to the command line, not in a Gly added to the peptide. Similar problem for <alt>L on German keyboard

Work-around:
Model peptide with Ala, use mutagenesis wizard to substitute Ala by Gly

Working with Wizards

Wizards are PyMOL add-ons that can perform quite complex tasks

The Demo Wizard demonstrates some of the capabilities of PyMOL

The screenshot displays the MacPyMOL application window. The top menu bar includes File, Edit, Build, Movie, Display, Setting, Scene, Mouse, Wizard, and Help. The Wizard menu is open, showing options like Appearance, Measurement, Mutagenesis, Pair Fitting, Density, Filter, Sculpting, Label, and Charge. A red box highlights the 'Demo' option in the Wizard menu. Below the menu, a grid of molecular representations is shown: lines, sticks, spheres, surface, mesh, dots, ribbon, and cartoon. A red box also highlights the 'Demo' button in the 'Demonstrations' section of the interface. The bottom status bar shows 'PyMOL>' and 'From 1/1 State 1'.

MacPyMOL File Edit Build Movie Display Setting Scene Mouse Wizard Help

MacPyMOL

RAS-1, C-H-RAS, HA-RAS, COMPND 6 GTPASE HRAS, N-TERMINALLY PROCESSED; COMPND 7 ENGINEERED: YES
ObjectMolecule: Read secondary structure assignments.
ObjectMolecule: Read crystal symmetry information.
Symmetry: Found 18 symmetry operators.
CmdLoad: "/Users/Plueckthun/ahonegger/Desktop/3K8Y.pdb" loaded as "3K8Y".

PyMOL>

lines sticks spheres surface

mesh dots ribbon cartoon

will 3K8Y Ent
rep1
rep2
rep3
rep4
rep5

Wizard

Appearance
Measurement
Mutagenesis
Pair Fitting
Density
Filter
Sculpting
Label
Charge

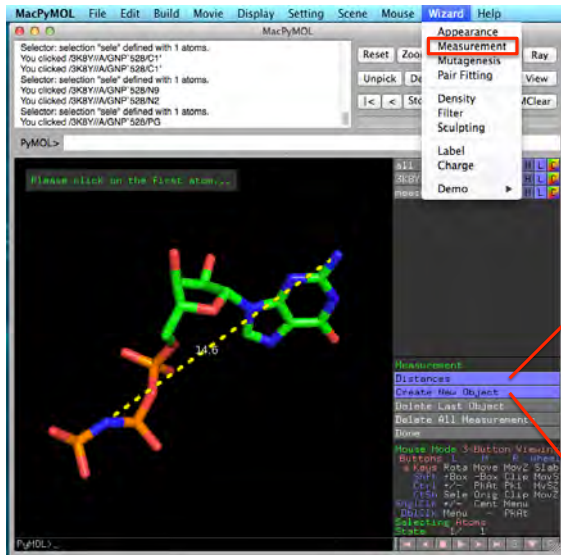
Demo

Demonstrations

Representations
Cartoons
Roving Detail
Roving Density
Transparency
Ray Tracing
Sculpting
Scripted Animation
Electrostatics
Compiled Graphics Objects
Molscript/Raster3D Input
End Demonstration

Try it out!

Working with Wizards: Measurements



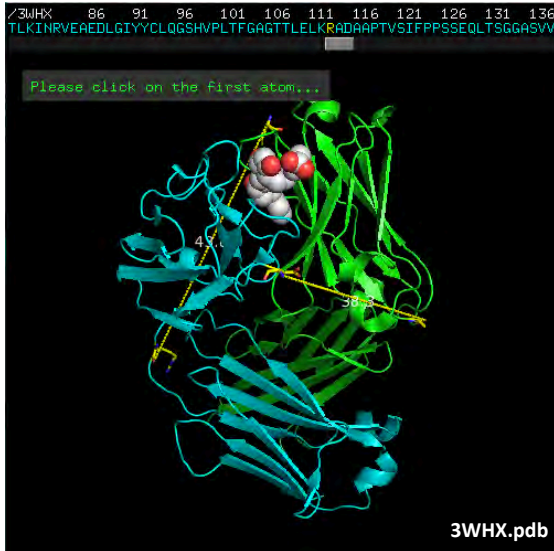
Measurement Mode

Distances
Angles
Dihedrals
Polar Neighbors
Heavy Neighbors
Neighbors
Polar Contacts

New Measurements?

Merge With Previous
Replace Previous
Create New Object

Application: Linker Length



Example:

You want to know how long a peptidic linker you need to either connect the C-terminal end of the VL domain in an antibody Fab fragment to the N-terminal end of VH or vice versa.

Highlight the residues want to connect and measure the

Distance:

Cterm VL – Nterm VH 43.8 Å

Cterm VH – Nterm VL 38.3 Å

repeat for several structures

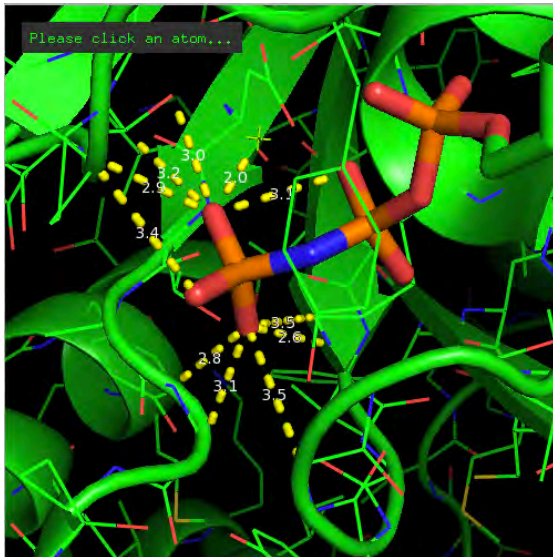
Rule of thumb: Linker needs to be long enough to connect ends in a semi-circle of AA in beta-strand conformation:

$$n \text{ AA} = (d \cdot \pi / 2) / 3.5$$

$$n \text{ AA} = 0.45 \cdot d$$

17-20 Aminosäuren

Working with Wizards: Measurements



Example:

You want to know what atoms make contacts to the gamma phosphate group of GPPNP in the ras structure 3K8W

Measurement

Neighbors

Create New Object

Delete Last Object

Delete All Measurements

Done

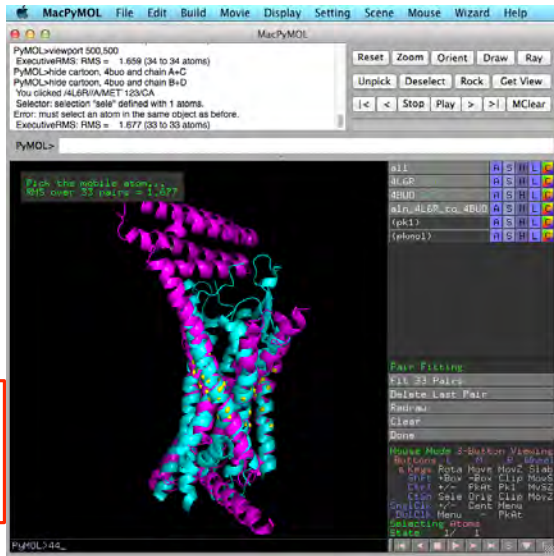
Open measurement wizard
select Neighbors: in same obj.
click on the atoms comprising
the terminal phosphate group

Working with Wizards: Pair fitting

In `pair_fit`, the subset of atoms pairs to be used for the fit are picked by the user.

This way, an iterative improvement of the initial fit is possible, however since the user picks a small subset of atoms to fit, the alignment is more subjective.

Pairfit is particularly useful to align small molecules or for a rough initial alignment of key residues in a protein structure



PyMOL is Not Yet Ready for serious Modelling

Molecular sculpting works like a real-time energy minimizer, except that it isn't minimizing the energy. Instead, its just trying to return local atomic geometries (bonds, angles, chirality, planarity) to the configuration the molecules possess when they were first loaded into PyMOL.

Optimize provides a PyMOL graphical interface to some of the molecular mechanics features available in openbabel, allowing the user to optimize (minimize) the energy of any molecule uploaded on PyMOL.