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ICM-Bio User Guide v.3.8

by Ruben Abagyan, Andrew Orry, Eugene Raush, and Maxim Totrov
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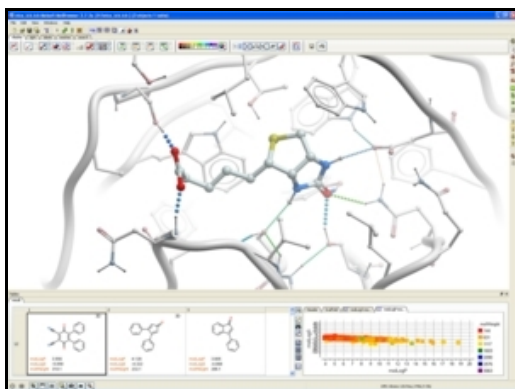
Sep 13 2018

Feedback.

1 Help Videos on YouTube

You can view webinars and how-to videos on many of the functions within ICM on YouTube.

- `Link` to our YouTube Channel.
- `Link` to our Webinar Archives on YouTube.

2 Reference Guide

Chapter Contents:

ICM-Browser Reference Guide

ICM-Browser-Pro Reference Guide

ActiveICM Reference Guide - Create 3D Molecular Documents for the Web and PowerPoint

Menu and Tab Reference Guide:

Menu Option Guide

Tab Guide

2.1 Graphics Reference Guide

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Chemist | ICM-Pro

For instructions on how to use ICM-Browser to make fully-interactive 3D slides and publish them in PowerPoint and the web please see the ActiveICM User Guide. ActiveICM is a free plugin for Windows PowerPoint and web browsers. Other related tutorials include:

- Graphical Display: Molecule Representation, Coloring, Labeling and Annotation
- Graphical Selections Tutorial
- Creating Fully Interactive Slides for PowerPoint and the Web Tutorial

2.1.1 Download and Install ICM-Browser

Getting Started: Download and Install ICM-Browser and ActiveICM.		
Download ICM-Browser Distribution.	Download	video
Install ICM-Browser Instructions.	Windows Linux Mac	
Download ActiveICM Distribution.	Download	video
Install ActiveICM.	Windows Linux Mac	

2.1.2 How to use the Graphical Display

'; winRef.document.write(str); }

How to use the Graphical Display	

How to search the PDB.	`HTML GUI Manual`	`video`
How to Move a Structure in the Graphical Display.	`HTML GUI Manual`	`video`
How to use the Graphics window controls.	`HTML GUI Manual`	
How to use the ICM Workspace Panel	`HTML GUI Manual`	`video`
How to Display a Molecule.	`HTML GUI Manual`	`video`
How to Change Protein Representation.	`HTML GUI Manual`	`video`
How to Change Ribbon Representation.	`HTML GUI Manual`	`video`
How to color wire or xstick carbon atoms.	`HTML GUI Manual`	`video`
How to Display the Residues Surrounding the Ligand Binding Pocket.	`HTML GUI Manual`	`video`
How to remove chain breaks (dotted lines).	`HTML GUI Manual`	`video`
How to Color.	`HTML GUI Manual`	`video`
How to Change the Color of Molecule Representations.	`HTML GUI Manual`	`video`
How to Change the Background Color.	`HTML GUI Manual`	`video`
How to Display a Binding Pocket Surface	`HTML GUI Manual`	`video`
How to (Un)Display Hydrogens.	`HTML GUI Manual`	`video`
How to Save an ICM Object.	`HTML GUI Manual`	`video`
How to Save an ICM Project File.	`HTML GUI Manual`	`video`
How to Drag and Drop.	`HTML GUI Manual`	
How to: Right Click Options.	`HTML GUI Manual`	
How to Move Windows.	`HTML GUI Manual`	`video`

How to Arrange Windows	HTML GUI Manual	video

2.1.3 How to make Graphical Selections

How to Make Selections.	HTML GUI Manual	
How to Select an Object	HTML GUI Manual	video
How to Select a Molecule	HTML GUI Manual	video
How to Select Residues	HTML GUI Manual	video
How to Select Atoms	HTML GUI Manual	video
How to Make a Spherical Selection.	HTML GUI Manual	video
How to Invert a Selection.	HTML GUI Manual	video
How to Remove a Selection.	HTML GUI Manual	video
How to Change the Selection Level and Mode.	HTML GUI Manual	video
How to Check What is Selected.	HTML GUI Manual	

2.1.4 How to Convert Proteins, Display Hydrogens and Ligand Binding Pocket.

Convert Protein, Display Hydrogens and Ligand Binding Pocket.		
How to Convert a PDB Structure into an ICM Object.	HTML GUI Manual	video
How to Display Ligand Binding Pocket.	HTML GUI Manual	video
How to Display Hydrogen Bonds.	HTML GUI Manual	video

2.1.5 How to change Graphics Effects

How to change Graphics Effects		
How to display the FOG effect.	HTML GUI Manual	video
How to display side-by-side stereo.	HTML GUI Manual	
How to display Anaglyph stereo.	HTML GUI Manual	
How to toggle full screen mode.	HTML GUI Manual	video
How to adjust perspective.	HTML GUI Manual	

How to change the lighting.	HTML GUI Manual	video
How to display sketch accents.	HTML GUI Manual	video
How to display elegant ribbon and ligand sketch.	HTML GUI Manual	video

2.1.6 How to add Labels and Annotations

How to add Labels and Annotations		
How to Label Residues.	HTML GUI Manual	video
How to Label Atoms.	HTML GUI Manual	video
How to Label Variables.	HTML GUI Manual	video
How to Display and Undisplay Sites.	HTML GUI Manual	
How to Make and Display Sites (Annotations).	HTML GUI Manual	
How to Make and Display 2D and 3D Labels.	HTML GUI Manual	video

2.1.7 How to Make High Quality Publication Images

How to Make High Quality Publication Images		
How to Toggle High Quality Display	HTML GUI Manual	video
How to Toggle Antialiasing.	HTML GUI Manual	video
How to Copy Image to ClipBoard	HTML GUI Manual	video
How to Write an Image.	HTML GUI Manual	video
How to Use the Advanced Write Image Options.	HTML GUI Manual	video
How to Add an Image to the ICM Photo Album.	HTML GUI Manual	video

2.1.8 How to Superimpose Protein Structures.

How to Superimpose Protein Structures.		
How to Superimpose Two or More Protein Structures.	HTML GUI Manual	video

2.1.9 How to Measure Distances and Angles.

How to Measure Distances and Angles.		

How to Measure Distances Between Two Atoms.	HTML GUI Manual	video
How to Measure Distances From One Atom to Many.	HTML GUI Manual	video
How to Show Corresponding Distances in Two Objects.	HTML GUI Manual	video
How to Display the Ruler Bar.	HTML GUI Manual	video

2.2 ICM-Browser-Pro Reference Guide

NOTE: ICM-Browser-Pro contains all the features in ICM-Browser. Click here for the ICM-Browser Reference Guide.

2.2.1 Download and Install ICM-Browser-Pro

Getting Started: Download and Install ICM-Browser-Pro	
Download ICM-Browser-Pro Distribution.	Download
Install ICM-Browser-Pro.	Windows Linux Mac

2.2.2 Graphics

Graphics and Movie Making	
How to generate the shadow effect.	HTML GUI Manual
How to make a screenshot movie.	HTML GUI Manual
How to make a movie from a set of slides.	HTML GUI Manual
How to move a molecule independently of the other display objects (Connect).	HTML GUI Manual

2.2.3 Protein Structure Analysis

Protein Structure Analysis	
How to calculate contact areas between molecules.	HTML GUI Manual
How to identify closed cavities.	HTML GUI Manual
How to calculate surface area.	HTML GUI Manual

How to generate interactive Ramachandran plots.	`HTML GUI Manual`

2.2.4 Surfaces

Surfaces	
How to generate electrostatic and binding property surfaces.	`HTML GUI Manual`
How to connect and rotate/translate surface (mesh).	`HTML GUI Manual`
How to crop a mesh/surface.	`HTML GUI Manual`
How to save a mesh/surface.	`HTML GUI Manual`

2.2.5 Superimpose Proteins

Superimpose Proteins	
How to superimpose proteins based on 3D by visible atoms, C-alpha, backbone or heavy atoms.	`HTML GUI Manual`
How to superimpose multiple proteins based on aligned residues..	`HTML GUI Manual`

2.2.6 Crystallographic Tools

Crystallographic Tools	
How to contour electron density.	`HTML GUI Manual`
How to display crystallographic cell.	`HTML GUI Manual`
How to display crystallographic symmetry.	`HTML GUI Manual`
How to convert x-ray density to a grid.	`HTML GUI Manual`

2.2.7 Sequence Analysis

Sequence Analysis	
How to annotate an alignment - box and shade.	`HTML GUI Manual`
How to annotate an alignment with text.	`HTML GUI Manual`
How to display secondary structure in an alignment.	`HTML GUI Manual`
How to extract sequences from pdb files.	

	HTML GUI Manual
How to assign secondary structure.	HTML GUI Manual
How to link sequence, alignments, and structures.	HTML GUI Manual
How to save as image, print, and delete sequences and alignments.	HTML GUI Manual

2.2.8 Plotting Tools

Plotting Tools	
Make fully interactive colorful X-Y plots and histograms with up to 4 dimensions.	HTML GUI Manual
Save plot and histogram as image.	HTML GUI Manual

2.3 ActiveICM Reference Guide - Create 3D Molecular Documents for the Web and PowerPoint

This guide is focused on how to make fully interactive 3D documents for Windows PowerPoint and the Web. For more information on the other features in ICM-Browser please see the ICM-Browser User Guide.

Creating 3D Documents Is Straightforward

Creating fully interactive 3D documents for PowerPoint, the web, and standalone browser is straightforward.

1. Download ICM-Browser and the ActiveICM plugin. They are completely free! [video]
2. Open the ICM-Browser and make a series of animated fully-interactive slides showing different colored and rendered views of your molecules. [video]
3. Add hyperlinked HTML text to annotate and link to your slides. [video]
4. Save your file in ICM-Browser and then insert into PowerPoint or the web using the ActiveICM plugin. You can also share your documents in the standalone ICM-Browser. [video - powerpoint] [video -web browser]

'; winRef.document.write(str); }

2.3.1 Getting Started

Getting Started: Download and Install ICM-Browser and ActiveICM.		
Download ICM-Browser Distribution.	Download	video

Install ICM-Browser Instructions.	`Windows Linux Mac`

Download ActiveICM Distribution.	`Download`	`video`

Install ActiveICM.	`Windows Linux Mac`

2.3.2 How to Create a Series of Fully-Interactive 3D Slides.

Creating Slides How to Create a Series of Fully-Interactive 3D Slides.	`video`
How to Make Fully Interactive 3D Slides	`HTML GUI Manual`
How to Animate Slides	`HTML GUI Manual`
How to View and Navigate Slides in the ICM-Browser.	`HTML GUI Manual`
How to Edit Slides.	`HTML GUI Manual`
How to Add Smooth Blending and Transition Effects Between Slides.	`HTML GUI Manual`

2.3.3 How to Create Molecular Documents

How to Create Molecular Documents: Linking Slides to HTML Text.	`video`
How to Create an HTML Document.	`HTML GUI Manual`
How to Edit an HTML Document.	`HTML GUI Manual`
How to Make a Hyperlink Between HTML Text and a Slide.	`HTML GUI Manual`

2.3.4 How to Display Molecular Documents in PowerPoint

How to Display Molecular Documents in PowerPoint	`video`
How to Embed in Microsoft PowerPoint 2003	`HTML GUI Manual`
How to Embed in Microsoft PowerPoint 2007	`HTML GUI Manual`
How to Use ActiveICM in PowerPoint	`HTML GUI Manual`

How to Change ActiveICM Component Properties in PowerPoint	HTML GUI Manual
Advanced use of ActiveICM: Macros to direct visualisation changes.	HTML GUI Manual

2.3.5 How to Display Molecular Documents on the Web

How to Display Molecular Documents in Web Browsers	video
How to Display Molecular Documents in Web Browsers	HTML GUI Manual

2.4 ICM-Chemist Reference Guide

2.4.1 How to Import, Sketch, and Edit Chemicals

How to Import, Sketch, and Edit Chemicals		
How to load the ICM Molecular Editor.	HTML GUI Manual	video
How to sketch chemicals in the ICM Molecular Editor.	HTML GUI Manual	video
How to save a 2D sketch into a chemical spreadsheet.	HTML GUI Manual	video
How to save a 2D sketch in mol format.	HTML GUI Manual	video
How to use SMILES strings to sketch a chemical.	HTML GUI Manual	video
How to load a mol, sdf or mol2 file.	HTML GUI Manual	video
How to extract a 2D sketch of a ligand in complex with a PDB structure.	HTML GUI Manual	video

2.4.2 How to Work with Chemical Spreadsheets

Working with Chemical Spreadsheets		
How to add columns into a chemical spreadsheet.	HTML GUI Manual	video
How to sort a column(s) in a chemical spreadsheet.	HTML GUI Manual	video
How to change the view of a chemical spreadsheet - form, table and grid.	HTML GUI Manual	video

How to copy, cut and paste columns and rows in a chemical spreadsheet.	HTML GUI Manual	video
How to show and hide columns and rows in a chemical spreadsheet.	HTML GUI Manual	video
How to save a chemical spreadsheet in sdf format.	HTML GUI Manual	video
How to export your chemical spreadsheet into Excel.	HTML GUI Manual	video
How to print a chemical spreadsheet.	HTML GUI Manual	video
How to filter columns in a chemical spreadsheet.	HTML GUI Manual	video
How to use find and replace in a chemical spreadsheet.	HTML GUI Manual	video
How to mark and label rows in a chemical spreadsheet.	HTML GUI Manual	video
How to insert hyperlinks to the PDB, PubMed, and Uniprot.	HTML GUI Manual	video
How to copy and paste 2D chemicals.	HTML GUI Manual	video
How to edit data inside a chemical spreadsheet.	HTML GUI Manual	video
How to remove salts, explicit hydrogens and standardize chemical groups.	HTML GUI Manual	video
How to calculate chemical properties in a chemical spreadsheet.	HTML GUI Manual	video
How to identify duplicate chemicals in a chemical spreadsheet.	HTML GUI Manual	video
How to compare two chemical spreadsheets.	HTML GUI Manual	video
How to merge two chemical spreadsheets.	HTML GUI Manual	video

2.4.3 How to Undertake a Chemical Search

How to Perform Chemical Searching		
How to setup a chemical search.	HTML GUI Manual	video
How to draw a chemical search query.	HTML GUI Manual	video

How to add conditions to your chemical search.	HTML GUI Manual	video
How to search chemical spreadsheets, local databases and MolCart.	HTML GUI Manual	video
How to send a chemical search query.	HTML GUI Manual	video

2.4.4 How to Work with Pharmacophores

How to Work with Pharmacophores		
How to draw 2D pharmacophore.	HTML GUI Manual	video
How to search a 2D pharmacophore.	HTML GUI Manual	video
How to draw 3D pharmacophore.	HTML GUI Manual	video
How to extract a 3D pharmacophore from a ligand.	HTML GUI Manual	video
How to edit a 3D pharmacophore.	HTML GUI Manual	video
How to send a 3D pharmacophore search query.	HTML GUI Manual	video
How to color a 2D chemical sketch by pharmacophore feature.	HTML GUI Manual	video

2.4.5 How to Perform Chemical Clustering

How to Perform Chemical Clustering		
How to perform chemical clustering.	HTML GUI Manual	video
How to select representative centers from a tree.	HTML GUI Manual	video
How to reorder branches and change the distance of trees.	HTML GUI Manual	video
How to edit the tree - labels, spacing and coloring.	HTML GUI Manual	video

2.4.6 How to Generate Stereoisomers and Tautomers

How to Generate Stereoisomers and Tautomers		
How to generate stereoisomers.	`HTML GUI Manual`	`video`
How to generate tautomers.	`HTML GUI Manual`	`video`

2.4.7 How to Generate Combinatorial Libraries

How to Generate Combinatorial Libraries		
How to enumerate a Markush library.	`HTML GUI Manual`	`video`
How to decompose a library based on a Markush structure.	`HTML GUI Manual`	`video`
How to create a Markush structure.	`HTML GUI Manual`	`video`
How to enumerate a chemical library by reaction.	`HTML GUI Manual`	`video`

2.4.8 How to Generate Plots and Histograms

How to Generate Plots and Histograms		
How to make a histogram.	HTML GUI Manual	`video`
How to make an X-Y scatter plot.	HTML GUI Manual	`video`

2.5 Menu Option Guide

Note: Click **Next** (top right hand corner) to navigate through this chapter. Headings are listed on the left hand side (web version) or by clicking the **Contents** button on the left-hand-side of the help window in the graphical user interface.

Here we describe all the options in the drop down graphical user interface menus.

2.5.1 File Menu

2.5.1.1 New

This option allows you to create new peptides, dna, sequences etc... and is described in the `Create New Objects` chapter.

2.5.1.2 New ICM Session

File/New ICM Session

This option allows you to generate a new clear ICM session or clone the current one.

2.5.1.3 Open

File/Open

This option is described in the `Open and Read` chapter.

2.5.1.4 Open with Password

File/Open with Password

This option is described in the `Open and Read` chapter.

2.5.1.5 Extract from ICB

File/Extract from ICB

This option is described in the `Open and Read` chapter.

2.5.1.6 Convert to Local Database

Please see the Local Databases chapter for more information about this option.

2.5.1.7 Load

Options contained within the menu File/Load

PDB - read PDB from FTP, http, and local PDB

From Multiple Object File - A multiple object file will have a file extension *.ob and you can select which member of the multiple object is displayed.

PFam Alignment - PFam is a collection of multiple sequence alignments - enter FASTA ID

SwissProt - Download SwissProt sequence.

All Images from Dir - Read into ICM multiple image files png or jpg.

Electron Density Map - Download electron density map from Uppsala electron density server `http://eds.bmc.uu.se/eds/`

3D Mesh in KMZ or COLLADA Format from Google - see `http://sketchup.google.com/3dwarehouse/` to download KMZ or COLLADA.

2.5.1.8 Save Project

This is described in the `Save File` chapter.

2.5.1.9 Save Project As

This is described in the `Save File` chapter.

2.5.1.10 Save Project Compatible with ICM 3_5

File/Save Project Compatible with ICM 3_5

Use this option to save a version of your ICM project compatible with an older version of ICM. Version 3.5 or older. If you have an ICM license you can update your version of ICM by visiting our support site at www.molsft.com/support

2.5.1.11 Save with Password

To save a project which is protected by a password:

- File/Save with Password
- Enter a file name or browse for a previously saved project.
- Enter a password
- Determine whether you want the file to be **Fully Protected**, **read only** or **Read Only and Allow Comments** .

2.5.1.12 Export as ActiveICM Html

To embed in a web browser.

1. Download ActiveICM from here `http://www.molsoft.com/getbrowser.cgi?product=activeicm&act=list` (it is free!).
2. `Create` an HTML page in ICM (File/New/Html).
3. Add a series of `slides`.
4. File/Export As ActiveICM Html..

2.5.1.13 Close Project

To close a project:

File/Close Project

2.5.1.14 Quick Image

See the `High Quality Publication Image` chapter.

2.5.1.15 Write Image

See the `High Quality Publication Image` chapter.

2.5.1.16 Preferences

Preferences are described in the `Preferences` chapter.

2.5.1.17 Recent Files

Recently viewed projects and files can be easily downloaded from the
"Recent Files" option. To access this:

- Select File/Recent Files.
- Select the desired project by clicking on it once.

2.5.1.18 Recent PDB Codes

Quickly retrieve and display PDB structures that have recently been
viewed.

- Select File/Recent PDB Codes
- Select desired PDB code by clicking on it once and it will be
  loaded into the graphical display.

2.5.1.19 Quit

Need to close down ICM - no problem. You do one of the following:

1. Select **File/Quit.** ICM will quit without saving files.
2. Save and Click **X** at the upper right corner of the ICM window.
3. Type `quit` in the terminal window.

> **NOTE:** You may want to save the icm session as an `ICM Project file`
> before quiting.

2.5.2 Edit Menu

2.5.2.1 Delete

This option will delete anything that is `selected`.

2.5.2.2 Delete All

This option will delete everything e.g. sequences, structures, tables
... Use with care!

2.5.2.3 Select All

This option will select everything e.g. sequences, structures, tables...

2.5.2.4 Search in Workspace

This option allow you to search for a particular text in the workspace

2.5.2.5 Selection

This option allows you to make a precise selection either by neighbors
or specifying a particular atom or neighbor. Click on the tabs to jump
between selection levels.

2.5.2.6 Invert Selection

This option will select everything that is not currently selected.

2.5.2.7 Clear Selection

This option will remove all selections. For more information on selections see the `Making Selections Chapter`.

2.5.2.8 Neighbor Selection

This option will allow you to select neighboring atoms. For more information see the `Select Neighbors` section in the Selections Chapter.

2.5.2.9 Undo

Due to the complexities of working in an internal coordinates environment not everything can be undone or redone. Certain things like coloring and representations can be undone or redone.

2.5.2.10 Redo

Due to the complexities of working in an internal coordinates environment not everything can be undone or redone. Certain things like coloring and representations can be undone or redone.

2.5.2.11 Restore Recent Backup

ICM periodically makes a backup of your ICM project. If for whatever reason you lose an ICM session and you want to load the backup for the file use:

Edit/Restore Recent Backup

2.5.2.12 PDB Search

See `PDB Search Tab`

2.5.2.13 PDB Search by Field

See `PDB Search Tab`

2.5.2.14 PDB Search by Identity

See `PDB Search Tab`

2.5.2.15 PDB Search by Homology

See `PDB Search Tab`

2.5.2.16 PDB Search with External Seqeuence

See `PDB Search Tab`

2.5.2.17 Ligand Tools

See the ligand editor section of the manual.

2.5.2.18 Ligand Editor Preferences

See the ligand editor section of the manual.

2.5.3 View Menu
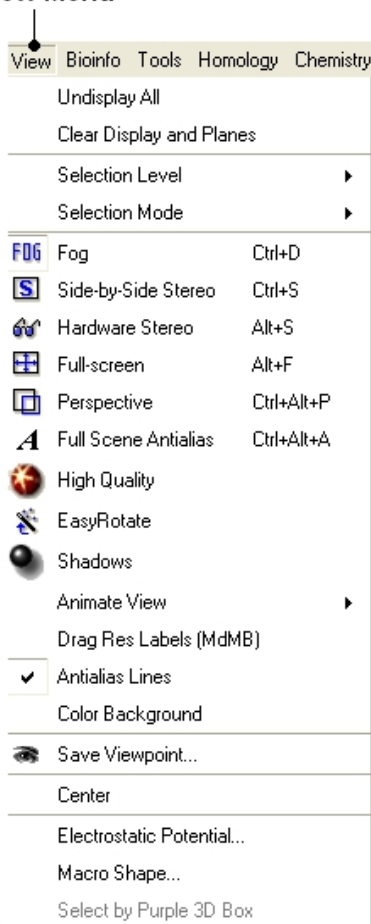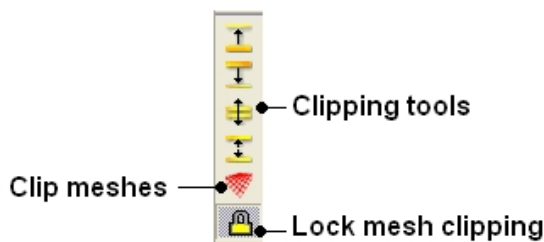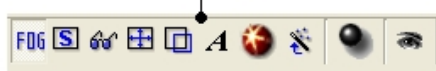
2.5.3.1 Undisplay All

To undisplay everything currently displayed in the graphical display

- View/Undisplay All

Note For more details on displaying structures please see the `GUI Overview` chapter.

2.5.3.2 Clear Display & Planes

To clear the display and planes

- View/Clear Display and Planes

NOTE: For more details on planes please see the sections on `clipping tools` and `mesh clipping`.

2.5.3.3 Selection Level

There are four levels of selection - atom, residue, molecule and object.
For more details on selections please see the `Making Selections` section.

2.5.3.4 Selection Mode

There are four different ways to make selections - new, add, remove and
toggle. For more details on selections please see the `Making Selections`
section.

2.5.3.5 Fog

Fog Toggle(Ctrl + D) : this feature creates a fog-like environment for
your object, so that the part of your structure that is closer appears
clear and the distant parts are faded as if they are in fog. The
`clipping planes` control the point at which the fog begins.

- View/Fog

2.5.3.6 Side-by-Side Stereo

Side-by-side stereo toggle(Ctrl + S) : this feature allows you to view
your structure in 3D form without any 3D goggles.

- View/Side-by-Side Stereo

2.5.3.7 Hardware Stereo

Hardware stereo toggle(Alt + S) - if you have 3D goggles and you wish to
view your structure in 3D form, this feature will allow you to do so.

- View/Hardware Stereo

2.5.3.8 Full Screen

Full screen toggle Alt_F - this makes your graphical display fill the
entire screen. If you wish to exit this mode, press escape.

- View/Full Screen

2.5.3.9 Perspective

Toggle perspective Ctrl_P this will add perspective to your structure,
enhancing depth in the graphical display.

- View/Perspective

2.5.3.10 Full Scene Antialias

Anti-aliasing is the technique of minimizing the distortion artifacts
known as aliasing when representing a high-resolution signal at a lower
resolution. Always use this option before making `high resolution images`.

- View/Full Scene Antialias

2.5.3.11 High Quality

Toggle High Quality: this option will give your ICM object better resolution and higher quality. The change in quality is most visible at a high magnification. However, if your object is very large, this feature could slow down your program.

Always use this option before making `high resolution images`.

- View/High Quality

2.5.3.12 Easy Rotate

Toggle easy rotation: this feature is necessary if your structure is very large or perhaps your computer cannot quickly `rotate` it. It will prevent your structure from fully loading each time you rotate it, therefore speeding up the process.

- View/Easy Rotate

2.5.3.13 Shadows

- View/Shadows

See `Graphics Effects` chapter.

2.5.3.14 Sketch Accents

To make images as shown below use:

- View/Sketch Accents

See `Graphics Effects` chapter.

2.5.3.15 Animate View

This tool is described in more detail in the `Molecular Animations and Transitions` section.

2.5.3.16 Drag Res Labels

To change the location of your residue label:

- Select View/Drag res labels.
- If your mouse has a middle mouse button, then click on handle (as shown) of the label you wish to move, and drag it to your desired area.

- If your mouse does not have a middle mouse button, then click on the Translation icon on the toolbar, and click on the handle (as shown) of the label you wish to move, and drag it to your desired area.

The +/- buttons on the side of the Residue and Atom buttons will shift the label. There are also other **residue label move** options available

when you click and hold the residue label button. These options include **Shift to Sidechain Tips**, **Shift to Calphas**, and **Restore Positions**

2.5.3.17 Antialias Lines

Use this option to activate antialias lines. It is recommended to leave this option selected.

- View/Antialias Lines

2.5.3.18 Color Background

To change the background color

- View/Color Background
- Select a color from the panel and press OK.

This option is also in the more convenient `display tab`.

2.5.3.19 Save Viewpoint

It is possible to store a current view using the button shown below.
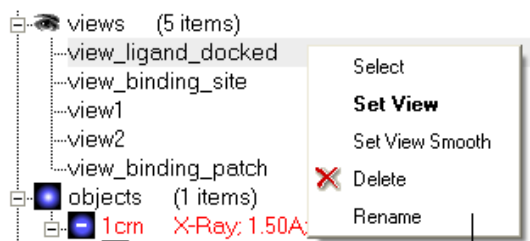

Store Current View

Click on the button and the current view will be stored so that you can view it later. A data entry box will be displayed asking you to name the view. All stored views can be found in the ICM workspace as shown below.

- Double click on the view in the ICM Workspace to display it.

A number of view display options are available by right clicking on the view in the ICM workspace as shown below.

Store current view right click menu

The option in the right click menu called "set view smooth" returns to the view slowly showing the trajectory between the original view and the current one.

2.5.3.20 Center

**To center on an object displayed in the graphical display**

- Make a selection on the region on which you wish to center on.
- Tools/Center (or use the `center` button on the right hand-side of the graphical display).

2.5.3.21 Electrostatic potential

This option generates the skin representation of the molecular surface colored according to the electrostatic potential calculated by the REBEL method (hydrogen atoms are ignored). REBEL is a method to solve the Poisson equation for a molecule. REBEL is a powerful implementation of the boundary element method with analytical molecular surface as dielectric boundary. This method is fast (takes seconds for a protein) and accurate. REBEL stands for Rapid Exact-Boundary ELectrostatics. The energy calculated by this method consists of the Coulomb energy and the solvation energy

In order to color the skin of your molecule by electrostatic potential:

- Select **View/Electrostatic** potential.
- Enter the potential scale value. This is the local electrostatic potential in kcal/e.u.charge units at which the surface element is colored by extreme red or extreme blue. All higher values will have the same color. This absolute scaling is convenient to develop a feeling of electrostatic properties of molecular surfaces.
- Areas colored blue represent positive areas and red represents negative areas.
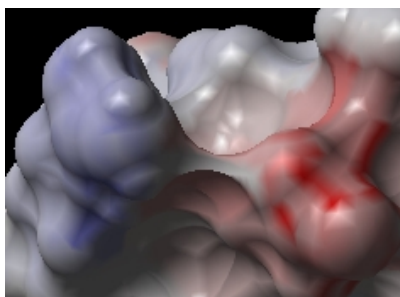
2.5.3.22 Macro Shape

A macroshape allows easy viewing and manipulation of a structure. A macroshape representation is ideal for large structures which allows the user to easily identify important regions of the structure and facilitate the return to the 'standard' view of a particular molecule. The level of detail displayed in the macroshape can be controled by changing the number of harmonics, gridStep, and, contour level.
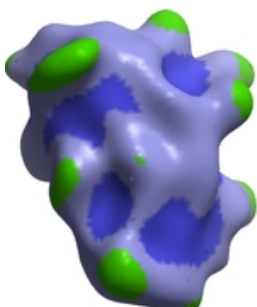
- View/Macro Shape

2.5.3.23 Select by Purple 3D Box

An alternative way to make a make-selection{selection} is to use the
purple 3D box. To do this:

- Select the **display** tab and the purple box button
- View/ Select by Purple 3D Box
- The atoms contained within the purple box will be selected.

2.5.4 Bioinfo Menu

The tools in this menu are described in the `Bioinfo Menu` chapter.

2.5.5 Tools Menu - Xray

The options in the menu are described in the `Crystallographic Analysis`
chapter.

2.5.6 Tools Menu - 3D Predict

The options in this menu are described in the 3D Predict chapter.

2.5.7 Tools Menu - Analysis

The options in this menu are described in the `Protein Structure Analysis`
chapter.

2.5.8 Tools Menu - Superimpose

The options in this menu are described in the `Protein Superposition`
chapter.

2.5.9 Tools Menu - Extras

2.5.9.1 Plot Function

To plot a function:

- Tools/Extras/Plot Function
- Enter the Function(x) eg Sin(x)
- Enter the starting value of x (From).
- Enter the end point of x (To).
- Enter the number of points (N points).
- Click OK and your plot will be displayed next to a table of values
  for your function.

2.5.10 Tools Menu - Table

2.5.10.1 Build Prediction Model

Learn and Predict tools are described here.

2.5.10.2 Predict

Learn and Predict tools are described here.

2.5.10.3 Cluster Set

This is described in the cluster section of the Working with Tables Chapter.

2.5.10.4 Sort Table

There are a couple of ways to sort a chemical table. You can right click on the a column header and select sort or you can use the option in the menu Chemistry/Sort Table.

- Read a chemical table into ICM.
- Select the columns by which you wish to sort by as shown below.
- Select **Ascending** or **Descending** and for each **sort by** option and then click OK
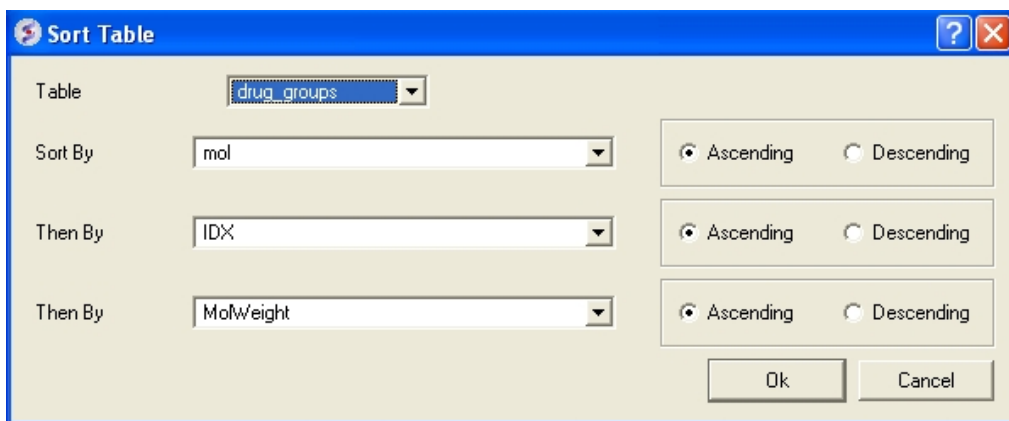
2.5.10.5 Merge Two Sets

To merge two tables:

- Read the two tables into ICM.
- Tools/Table/Merge Two Sets
- Select the first table from the drop down list (Table A) and the column you wish to use to merge the table by.
- Select merge method 1. **inner** - only molecules present in BOTH A and B tables are kept; or 2. **left** ALL rows of A are kept ; or 3. **right** ALL rows of B are kept.
- Select the second table from the drop down list (Table B) and the column you wish to use to mergethe table by.
- Enter a name for the output table.
- Click OK and a new table will be displayed.
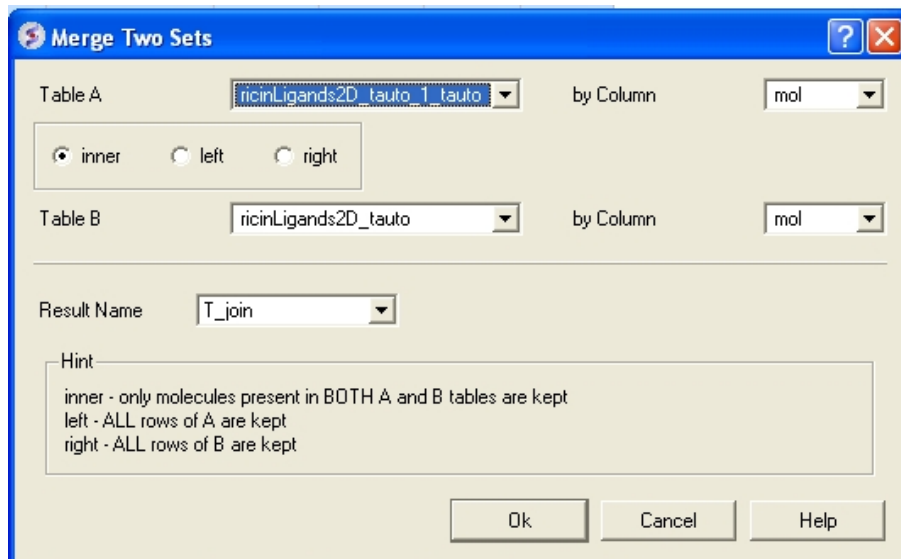
2.5.10.6 Add External Columns

To add external columns to a table (e.g. based on a merging key):

- Read at least two tables into ICM - the table you want to add to and the table you want to add the column from.
- Tools/Table/Add External Columns
- Enter the target table name and the column you wish to match each table by.
- Enter the source of the new column (Other table and column name)
- Choose to add "all the columns" from the source or "overwrite matching columns" or select the columns you want to add by selecting the "choose column" option.
- Select the columns you wish to add.

2.5.10.7 Append Rows

To append rows from one table to another one:

- Read at least two tables into ICM - the table you want to add to and the table you want to add the column from.
- Tools/Table/Append Rows
- Enter the name of the Target Table (where you will append).
- Enter the name of the Source Table (where you will append from).
- Select whether to Merge Columns
- Check whether you would like to **Select New Rows**. If you do this you can easily highlight the new rows using the mark row option.
- Check whether you would like to **Calculate Columns with Formulas**.

2.5.11 Tools Menu - Chemical Search

Chemical searching is described in the Chemistry chapter here.

2.5.12 Tools Menu - Molecular Editor

The molecular editor is described in the Chemistry chapter here.

2.5.13 Homology Menu

The options in this menu are described in the Homology Modeling Chapter.

2.5.14 Chemistry Menu

The tools in the **Chemistry** menu are described here.

2.5.15 Docking Menu

The tools in the **Docking** menu are described in the Docking chapter.

2.5.16 MolMechanics Menu

The options in this menu are described in the Molecular Mechanics
chapter.

2.5.17 Windows Menu

This menu allows you to choose the windows you wish to display. The
windows which open automatically when you first open GUI are shown in
the `Getting Started` section. Other windows can be displayed by selecting
the windows menu. For example, if you have loaded a table but cannot see
it in the GUI it may be because the Tables option in the window menu
hasnt been selected.

To add or remove windows from the GUI display select the 'window menu'.
Other windows not included in the default display such as tables and
alignments can be added.

I have windows open everywhere - Please bring some order.
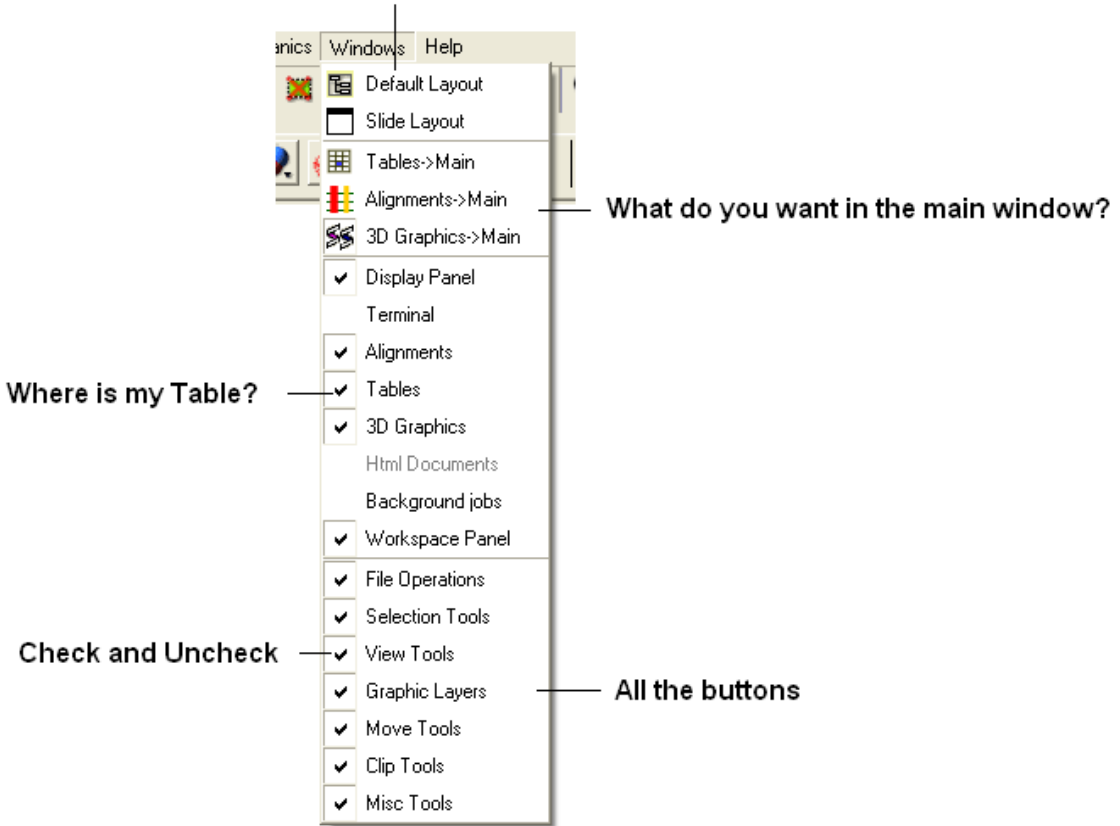
What do you want in the main window?

Where is my Table?

Check and Uncheck

All the buttons

To return to the default display option select the 'Default layout'
option in the windows menu.

OR

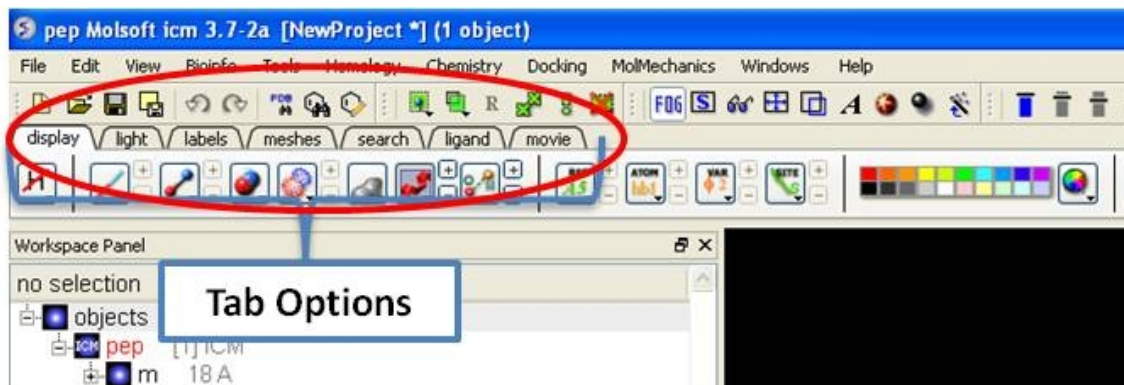Click the default layout icon.

Some options can be accessed at the
bottom of the GUI.

2.6 Tab Guide

In this section we describe the contents of the tabs in the graphical
user interface.

2.6.1 Display Tab

The display tab contains tools for a variety of functions including -
structural `representations`, `coloring`, `labeling` and `superposition`. This
tab is shown below.

2.6.2 Light Tab
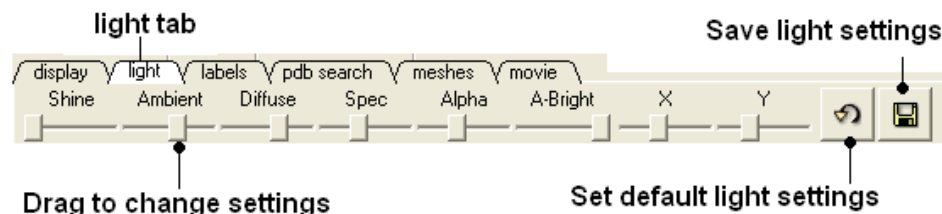
The options in this tab are described in the `Lighting Section`.

2.6.3 Labels Tab
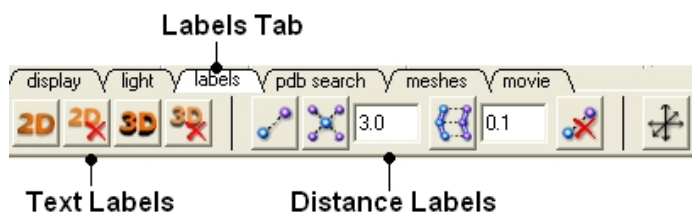
The options in this tab are described in the `labels` section of this
manual.

2.6.4 PDB Search Tab

Instructions on how to use this tab can be found in the `Search PDB`
`section.`

2.6.5 Ligedit Tab

Instructions on how to use the options in this tab can be found in the
How to use the 3D Interactive Ligand Editor section.

2.6.6 Meshes Tab

Click on the tab button entitled **'meshes'** and three different graphical
display tools are available for you to use. The three displays are
surface, meshes and macroshape and are collectively referred to as
meshes.
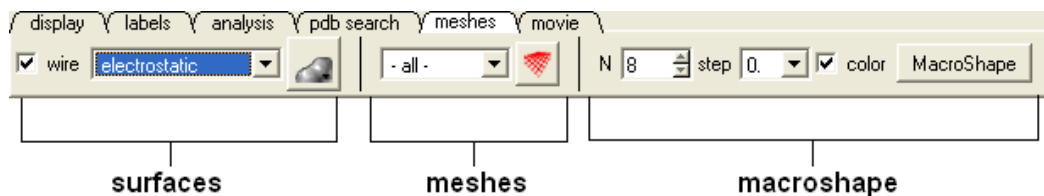
The benefits and applications of each display are described in the
section.

3 Getting Started

Chapter Contents:

- The Basics of the Graphical User Interface
- How to search and Download Protein Structure, Sequences, and Chemicals
- How to create new Objects
- How to open files
- How to save files
- How to Make Selections
- Changing Preferences

3.1 The Basics of the Graphical User Interface

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro | ICM-Chemist

3.1.1 The Components of the GUI

The **Graphical User Interface** (GUI) has many components. When you first use the GUI the default window layout is displayed as shown below.
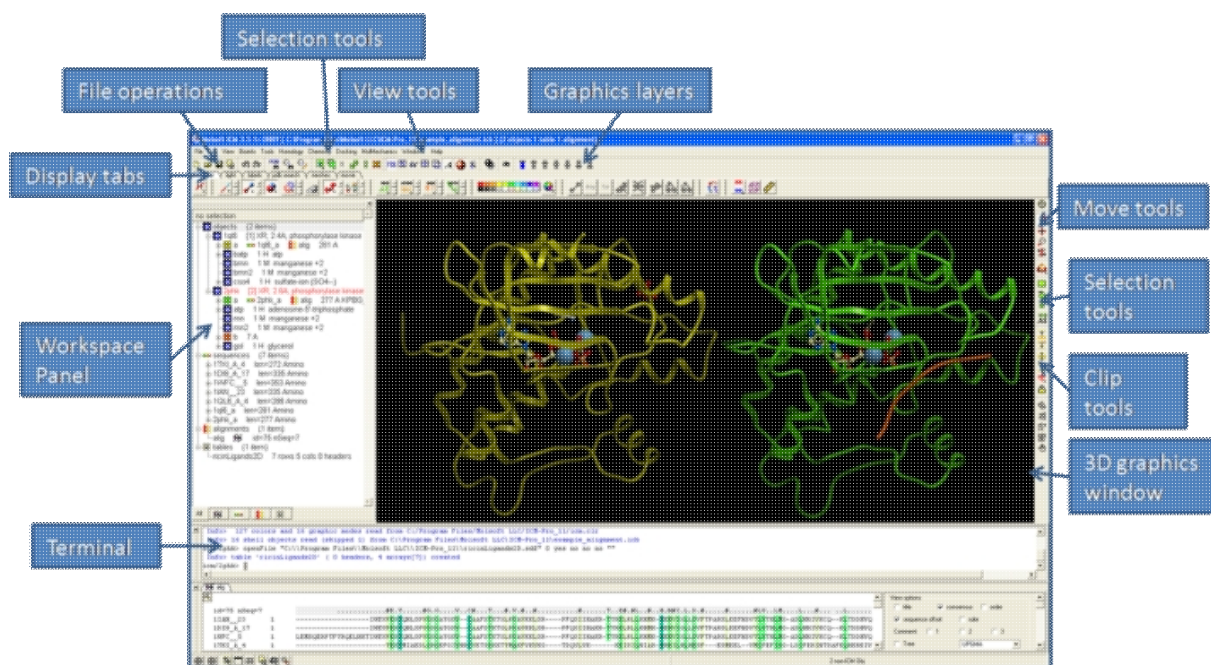
3.1.2 How to load a PDB Structure

There are three main ways to read in a PDB file.

1. Using the command line.
2. Using File/Open button
3. Using the PDB Search tab

Other PDB search options are described in more detail in the PDB Search section of this manual.
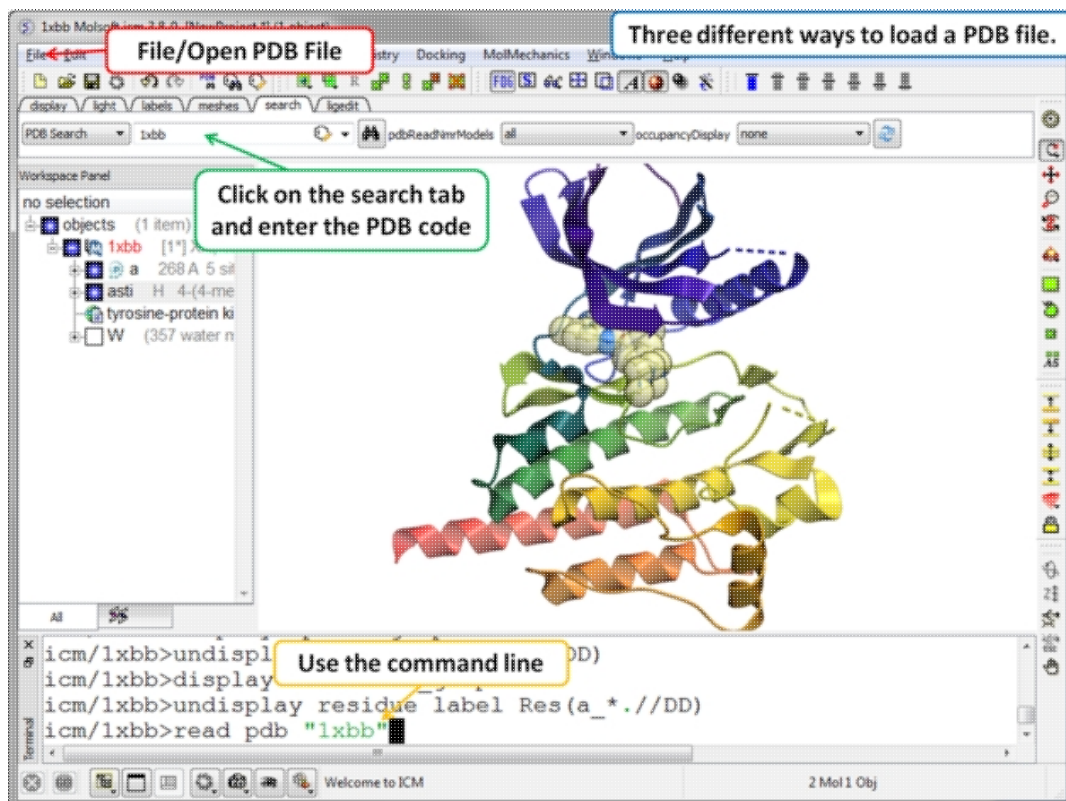
3.1.3 How to Move a Structure in the Graphical Display

Available buttons and options for moving molecules around the graphical display window. This is described in more detail in the section entitled Move Buttons.

How to use the Graphics window controls

In the graphics window you can use various tools described elsewhere but it is helpful to know the following things:

- **Picking a tool**: the left mouse button will function according to the selected tool
- **Popup menus**: right click on an atom gives a pop-up menu
- **Selecting in the rotation mode**: the right mouse button will select atoms
- **Translating in the rotation mode**: the middle mouse button will translate the scene
- **Zooming and moving clipping planes in the rotation mode**: the left, top and right margins of the graphics window are reserved for other actions, zoom, z-rotation, and clipping plains, respectively. That means that even if you are picking atoms, by pressing control you can still rotate your molecule with the left-mouse-button.
- **Rotating in any non-rotation mode**: if you press **Control** in any mouse mode, e.g. zoom, pick etc., it will temporarily switch to **rotation**
- **Escaping from the connect and continuous movement modes**: pressing **Escape** helps to get out of certain modes, such as Full Screen, Continuous rotation or rocking, the Connect mode.
- **Global rotation in the Connected mode**: pressing **Shift** will temporarily switch to the global rotation/translation mode.

## 3.1.4 How to Display Molecules using the ICM Workspace Panel

The workspace panel (located on the left hand side panel of the gui) is an important place within the graphical user interface because it displays which sequences, structures, objects, tables and alignments are currently loaded into ICM. From the ICM Workspace panel you can make graphical selections and display and undisplay molecules.
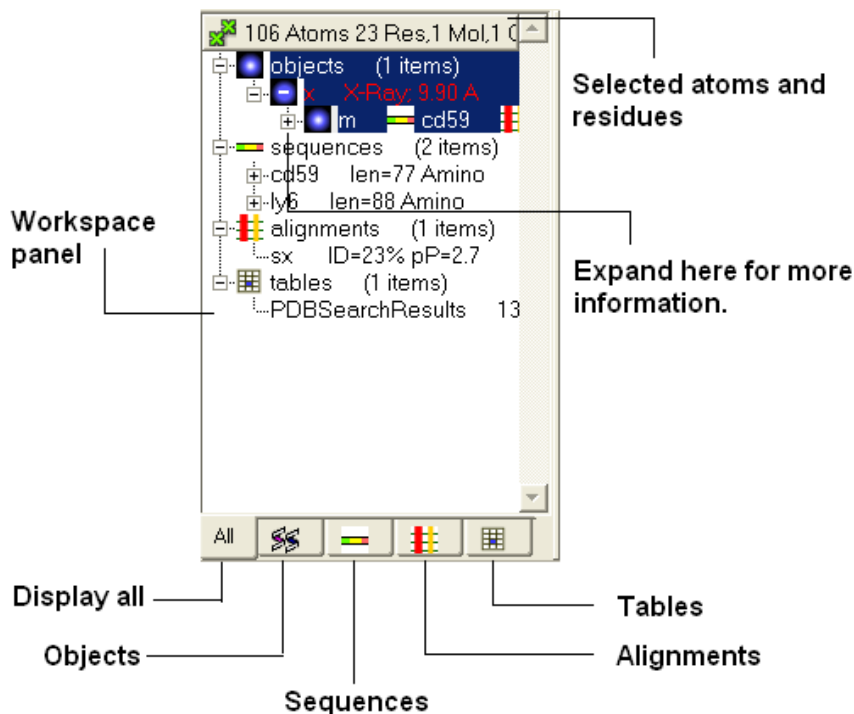
Once a structure has been loaded into ICM the individual components of that structure (i.e. amino acids, metal ions, binding sites etc) are listed in the ICM workspace.

To display every component of the object except for binding sites and water atoms:

- Click on the white box next to the word object at the top of the ICM workspace. This box will be colored blue once the structure is displayed
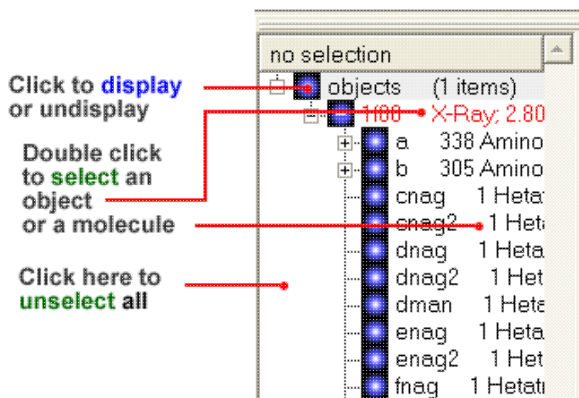
To display the whole structure in wire, ribbon, cpk, skin, surface and xstick representations:

- Right click on the blue box next to the word object. A menu will be displayed.
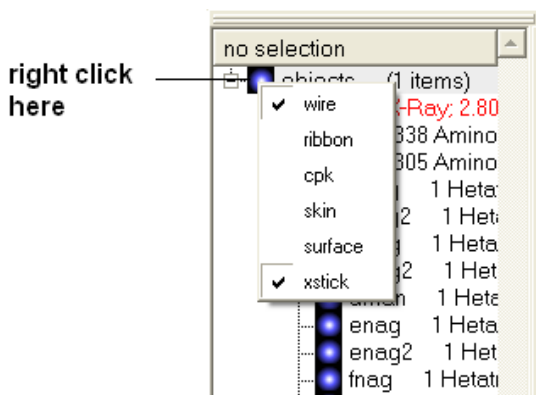
- Select which representation you desire for your structure by clicking on the appropriate word. A check mark indicates the representation currently displayed. To un-display a particular representation click on the word again.

In order to clear your graphical display:

- Select **View/Undisplay All**

If you only wish to display part of the structure then click in the boxes further down the tree in the ICM workspace.

To display selected regions of the molecule in wire, ribbon, cpk, skin, surface and xstick representations:

- Right click on the appropriate box in the **ICM** workspace. A menu will be displayed and select the representation you would like to use (e.g. wire, ribbon etc...)
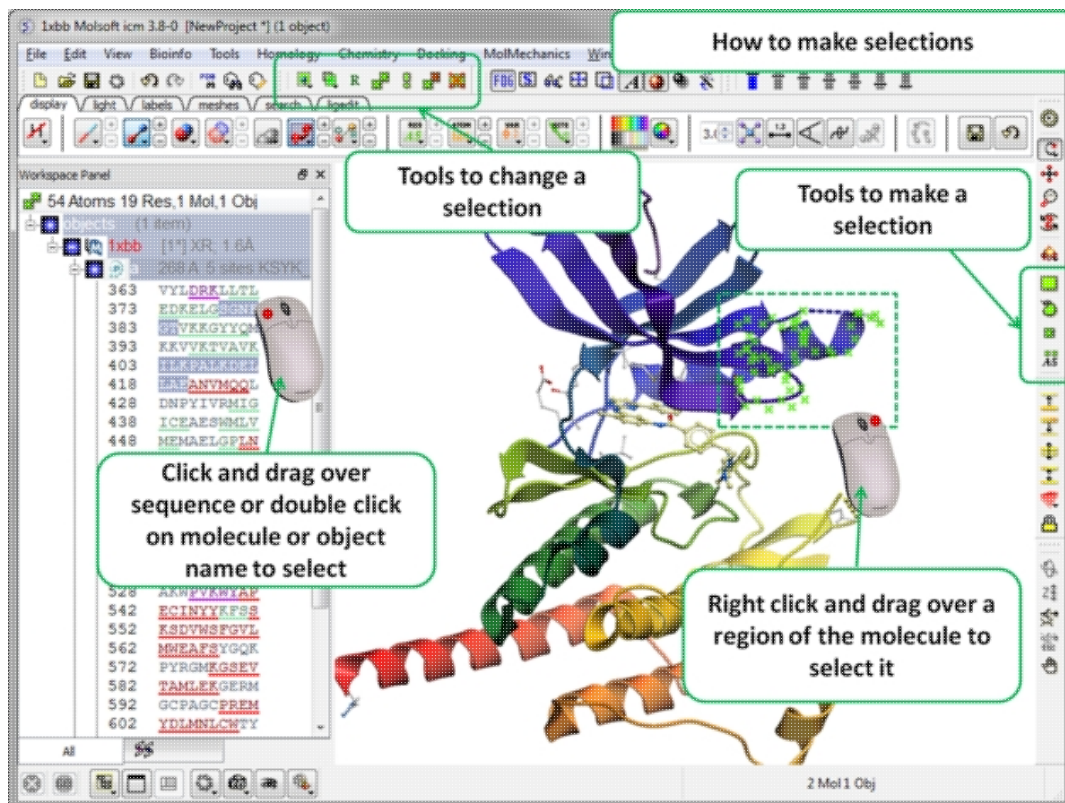
3.1.5 How to Make Selections

Making selections in ICM is an important skill to master (e.g. you may want to select a binding pocket for docking or a region of a molecule for coloring). The four levels of selection are:

1. Atoms
2. Residues
3. Molecules
4. Objects (multiple molecules comprising a PDB entry)

There are several ways of making selection in ICM. The simplest is to interact directly with the graphics window - **right-click, hold and drag** around the area of the screen you want to select. Alternatively, in the workspace window, expand the tree of molecules and chains until the relevant protein sequences is displayed. Then left click and drag to mark residues to form a selection.

See the chapter entitled Making Selections for more information.

How to make selections

Tools to change a selection

Tools to make a selection

Click and drag over sequence or double click on molecule or object name to select

Right click and drag over a region of the molecule to select it

3.1.6 How to Change Protein Representation

To change the representation of the protein, make a selection and then use the tools in the display tab.

There are 6 main types of representation:

- **Wire:** Wires connecting covalently bound atoms of a molecule. This representation has no defined thickness as such will not make shadows. Useful for showing the chemical structure of a small molecule.
- **Xstick:** Covalent bonds are represented as cylinders whilst atoms are represented as small spheres.
- **CPK:** Atoms are represented as spheres with their respective van der Waals radius and coloured according to a standard defined by Corey, Pauling and Kultun.
- **Surface:** Solvent accessible surface. This is the center of water sphere as a water probe rolls over the molecule.
- **Skin:** A Connolly molecular surface over the selection. This is a smooth envelope touching the van der Waals surface of atoms as a water probe rolls over the molecule.
- **Ribbons:** Cartoon representation of protein and DNA secondary structure. Protein residues marked as alpha-helices ('H') are shown as a flat, helical ribbon, those marked as beta-sheets ('E') are shown as a flat ribbon with an arrow-head, and the rest are shown as a cylindrical "worm". If secondary elements are not defined everything will be shown as a cylindrical worm. ICM can automatically assign secondary structure: Tools/3D predict /Assign Helices and Strands
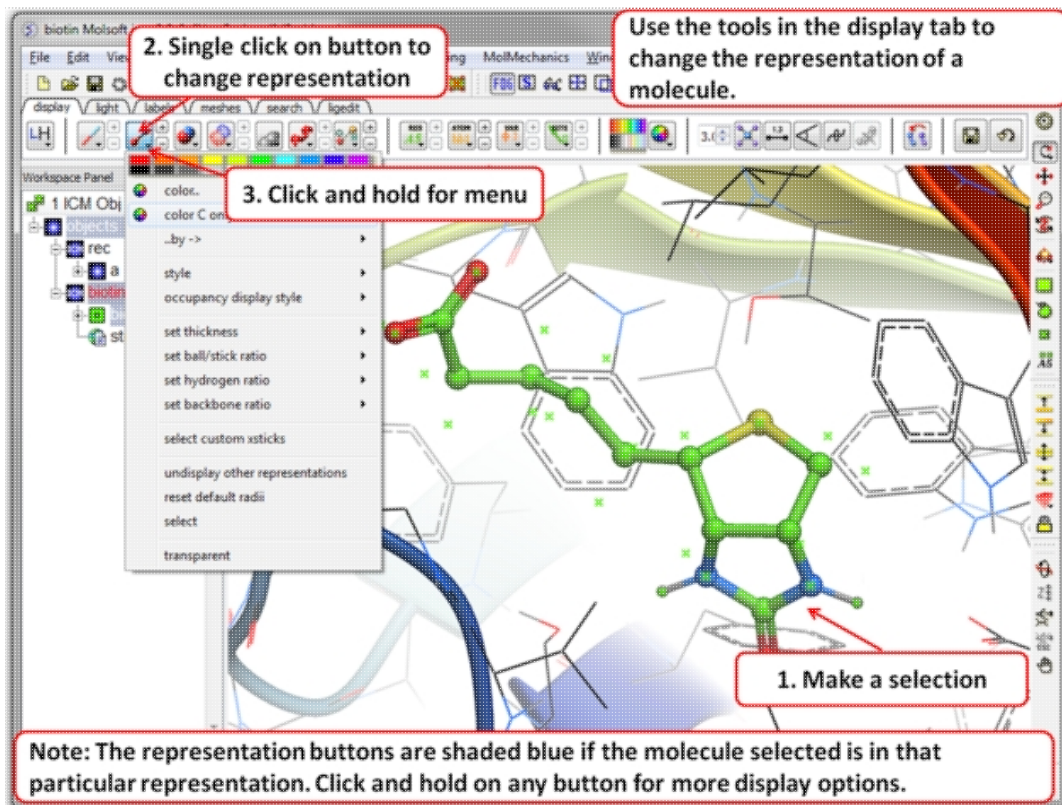
3.1.7 How to Color

To change the color of the representation you need to use the buttons in
the display tab.

Changing the colour of a representation works in much the same way as
displaying the representation itself. The selection rationale is the
same followed by clicking on a colour in the palette in the display tab.
It is also possible to colour different representations of the same
selection independently by clicking and holding on the representation
buttons in the display tab.
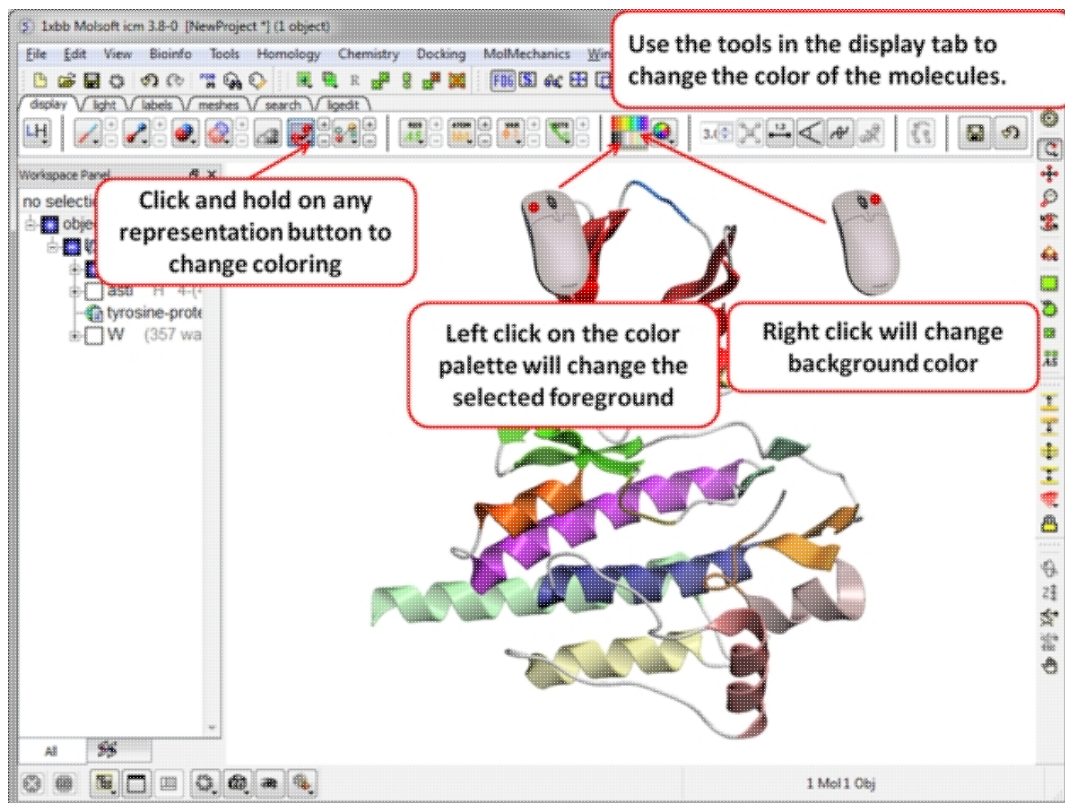
3.1.8 How to Display a Binding Pocket Surface

To display the surface of a small molecule ligand or peptide binding
pocket:

- Load the PDB of interest.
- Convert PDB to ICM object. If you do not convert you will not get
  the properties of the pocket displayed on the surface.
- Right click on the small molecule or peptide in the ICM Workspace
  and select **Ligand Pocket**.

Note: The surface (meshes) and h-bonds can be toggled on and off in the ICM workspace.
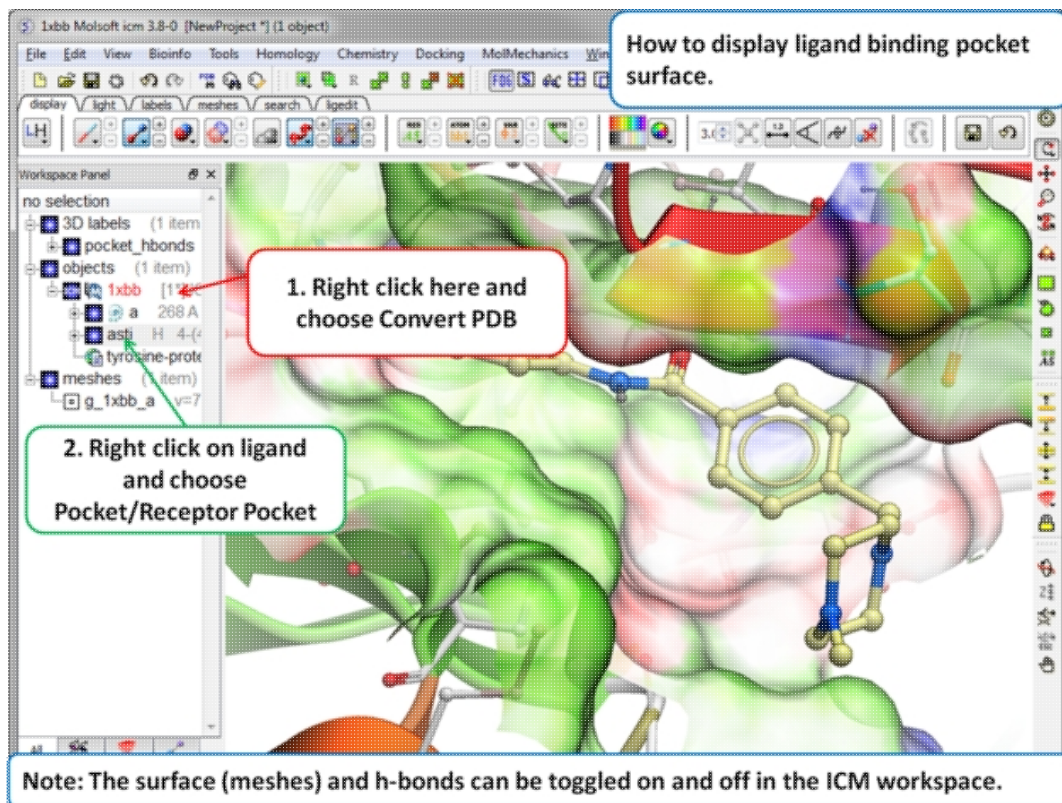
3.1.9 How to Save an ICM Object

Any ICM object such as a structure, sequence, or alignment, can be saved for use at a later time.

To save an object:

- Right click on the object name in the ICM workspace or ICM alignment editor and a menu will be displayed.
- Click on the **Save As...** option.
- Enter the unique name you wish to call your object in the box labeled **File name:**
- Choose which folder or directory you wish to save your object by clicking scrolling down in the box labeled **Save in:**
- Choose which file type you would like to save your object as by scrolling down in the box labeled **Save as type** . ICM structure objects should have the file ending yourfilename.ob and alignments yourfilename.ali
- Once the appropriate information has been entered click on the **Save** button in the bottom right hand section of the window.
- The object is now saved.
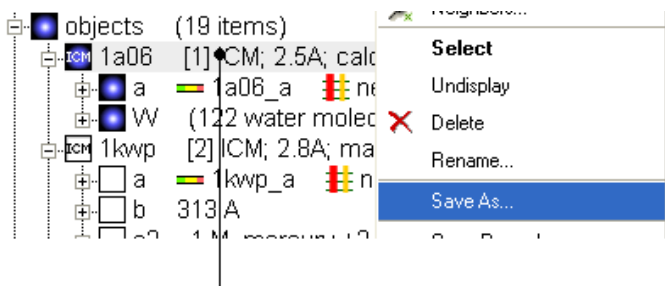
To save an ICM object or PDB
file right click and select
SaveAs..

3.1.10 How to Save an ICM Project File

All objects contained within an ICM session can be saved in a single
file with the extension .icb. The file can then be read into ICM and the
exact layout of the file will be preserved. To save a project file go to
the **File** menu and select **Save Project**.

3.1.11 How to Drag and Drop

NOTE: "Drag and Drop" is a useful way of moving objects and sequences
around the graphical user interface.

Sequences and objects can be moved around the graphical user interface
by dragging and dropping them. All loaded sequences and objects are
always displayed in the workspace panel. Select the desired object or
sequence from the workspace panel by clicking and holding, move the
selection to the desired location and release.

This is a useful application in the graphical user interface. For
example, you may have an alignment displayed and you wish to add another
sequence to the alignment. This can simply be accomplished by dragging a
loaded sequence from the workspace panel into the alignment display
panel. Or, you can quickly view an object by dragging and dropping it
from the workspace panel into the 3D graphics window.

3.1.12 How to: Right Click Options

NOTE: If you right click on any object you will see a new menu of
options related to that object.

The right click mouse option can be used throughout the graphical user
interface. It is a very useful means of opening up a whole new world of
menus and options. Most of these options are described in this book.
However, when using the graphical user interface it is always a good
idea to try right clicking the mouse on an object and seeing which extra
options that are available for you to use.

3.1.13 How to Move Windows

Is your graphical user interface looking a bit messy? Do you have
tables, alignments, plots all over the place? Here we show you how to
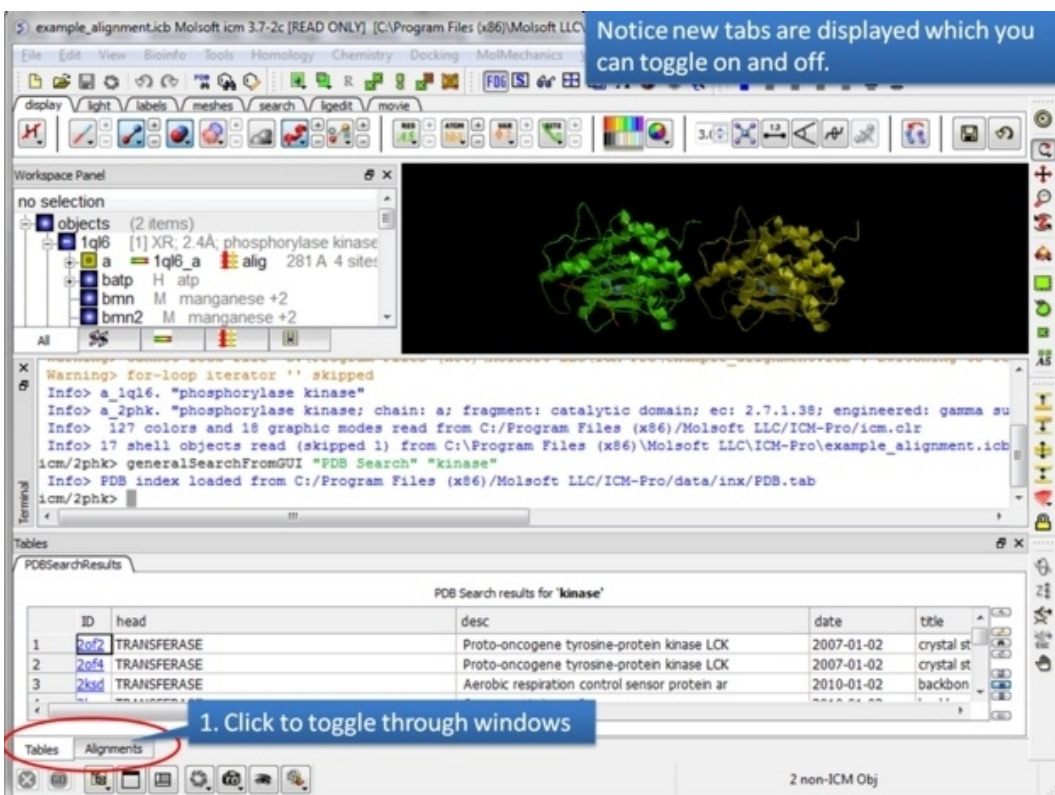arrange everything to make a clearer GUI environment.
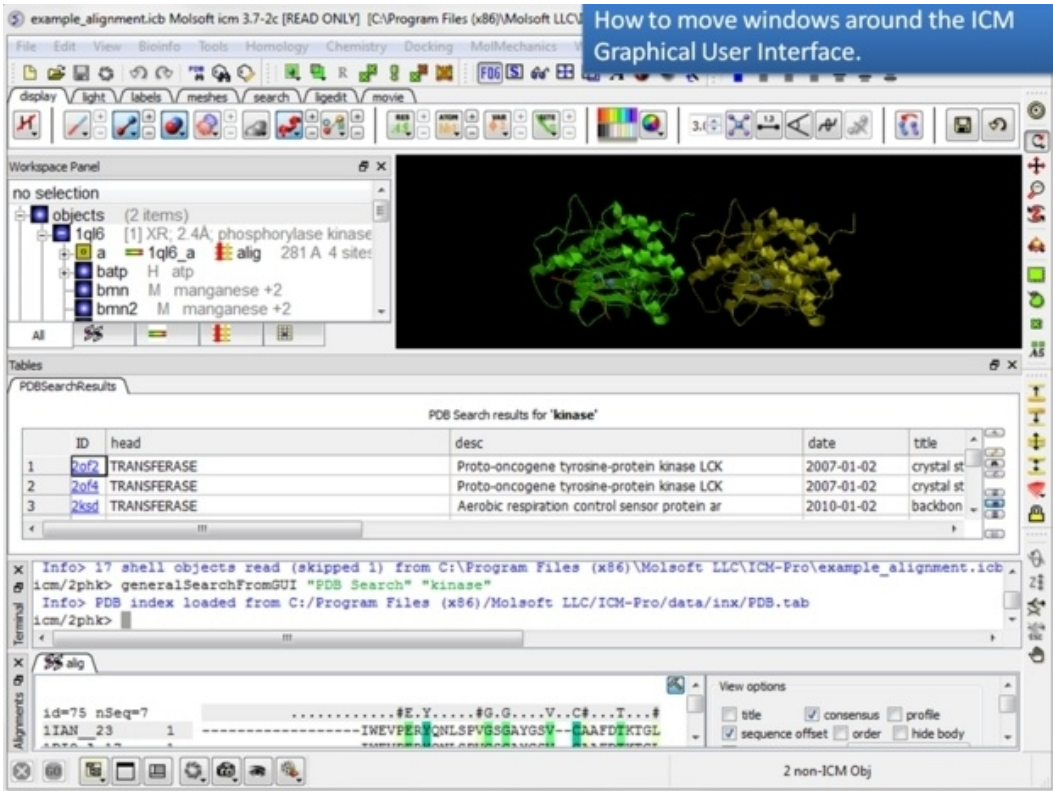
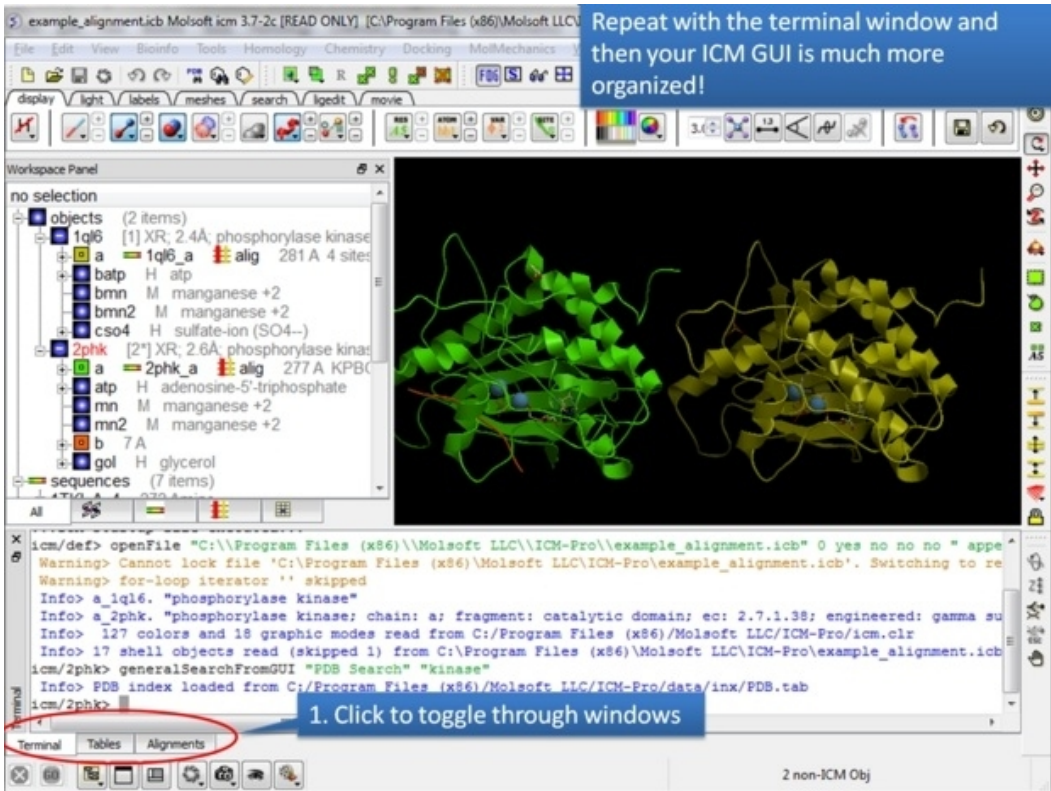**1.** Too many windows in your GUI? You can move them around to make viewing easier.
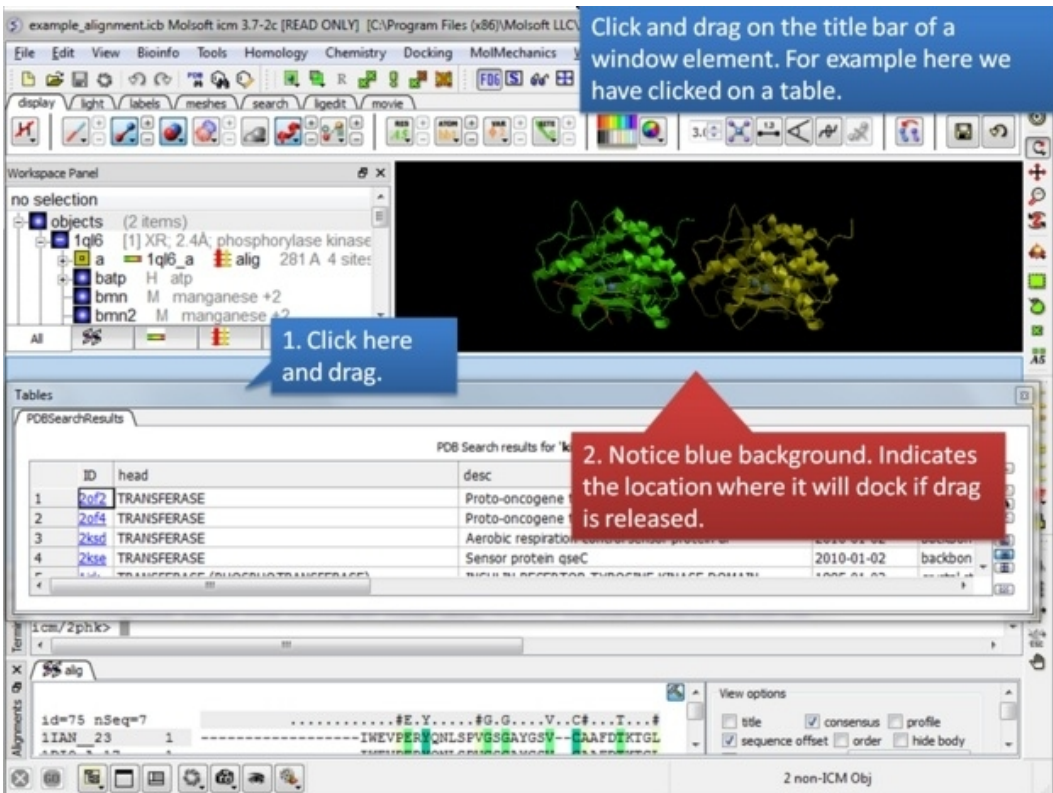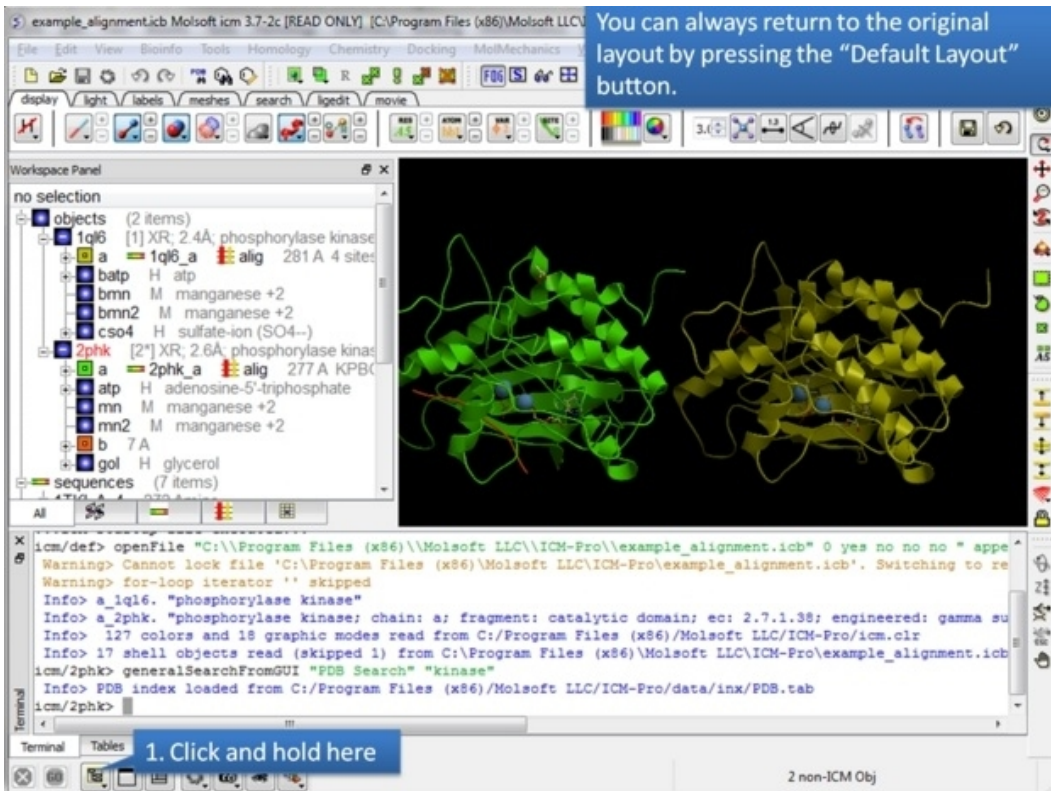

2. Click and drag on a window title bar to move it.
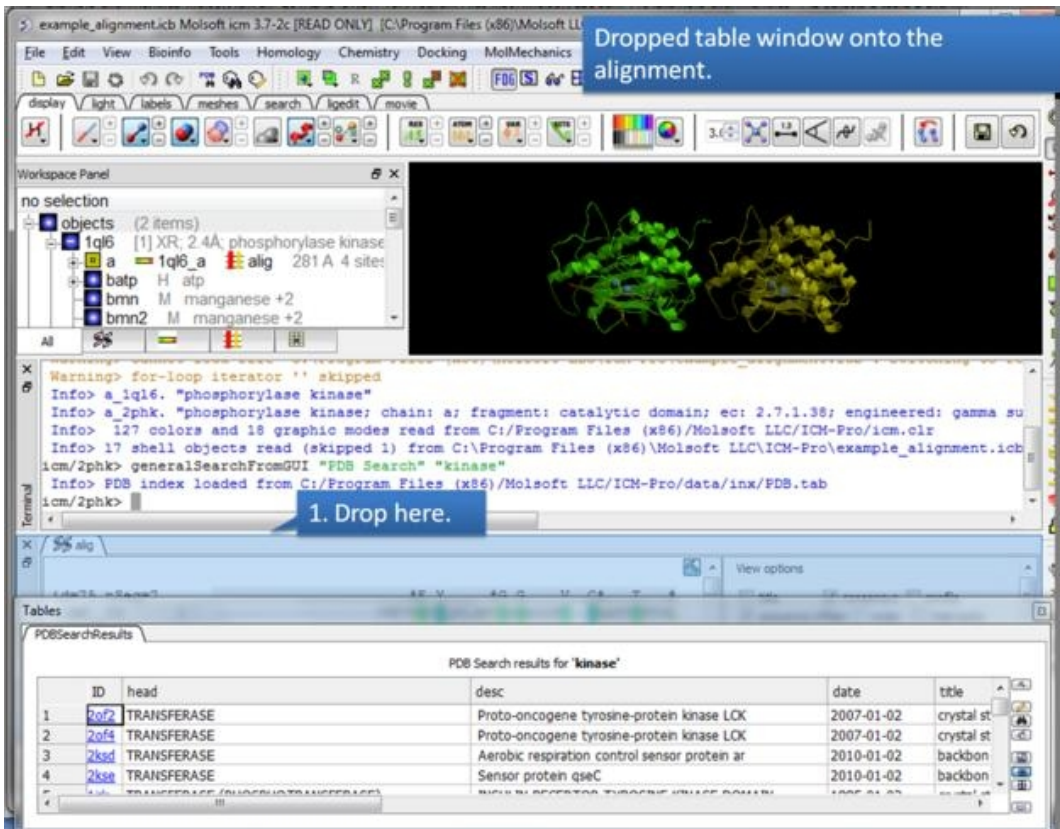
3. Drop one window on another one to dock it.
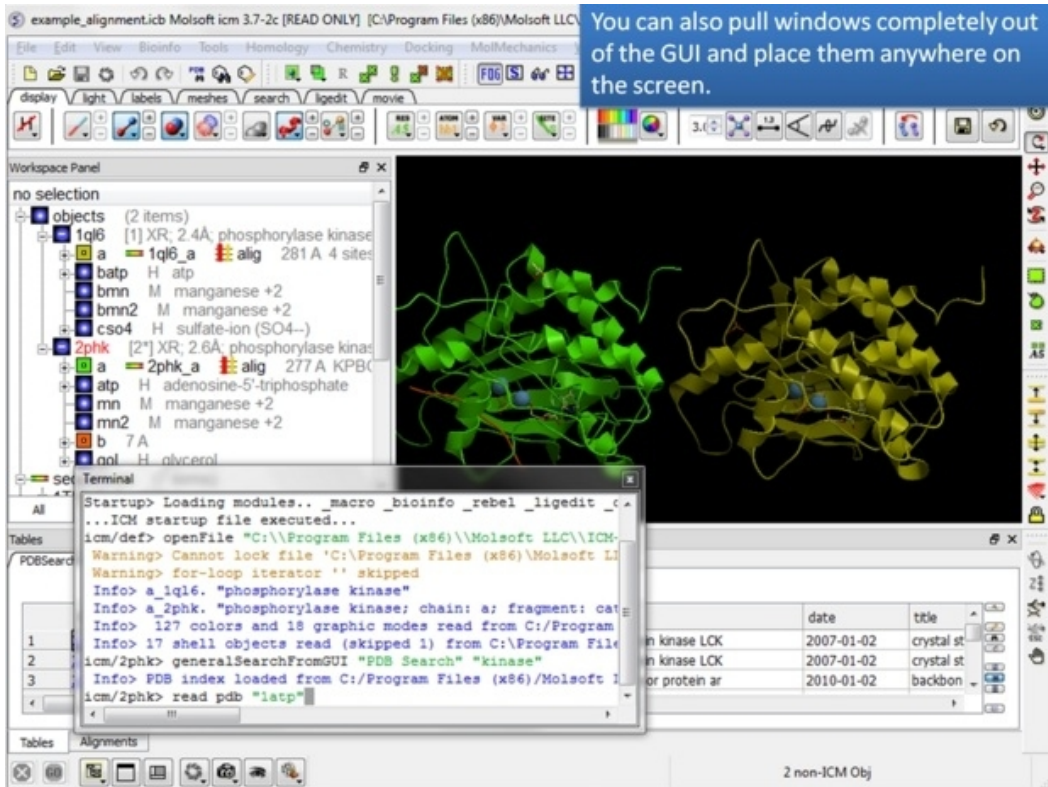

4. You can access each window by clicking on the tabs.

5. In this example we have the table, alignment, and terminal all in the same panel

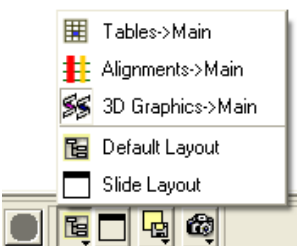**6.** There is a quick way to return the windows to their original location.

7. You can also move a window to any part of your screen.

NOTE: To return to the default display option select the 'Default layout' option in the windows menu.

OR

Click the default layout icon.

Some options can be accessed at the bottom of the GUI.

OR

Double click on the window header.

3.1.13.1 How to Arrange Windows

Sometimes when using ICM you may have many items displayed such as structures, alignments and tables. As a default the graphical display is the largest and centered in the middle of the ICM graphical user interface. However if you wish to work on an alignment or table you can place the alignment or table as the main display by clicking on the buttons shown below. The larger display generally makes it easier to manipulate the alignment or table. There are ofcourse other ways to alter the layout such as `tier` the windows but this option is just a simple click and can sometimes come in useful.
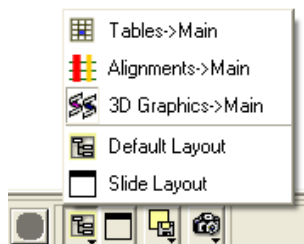
3.1.14 How to Make a Picture

There are several ways of taking a picture of the contents of the 3D
graphical display window `see the write image section`. However the
easiest way is to simply click on the button in the view tools panel
(see image below).
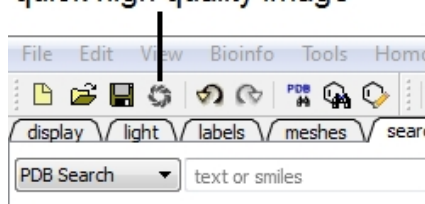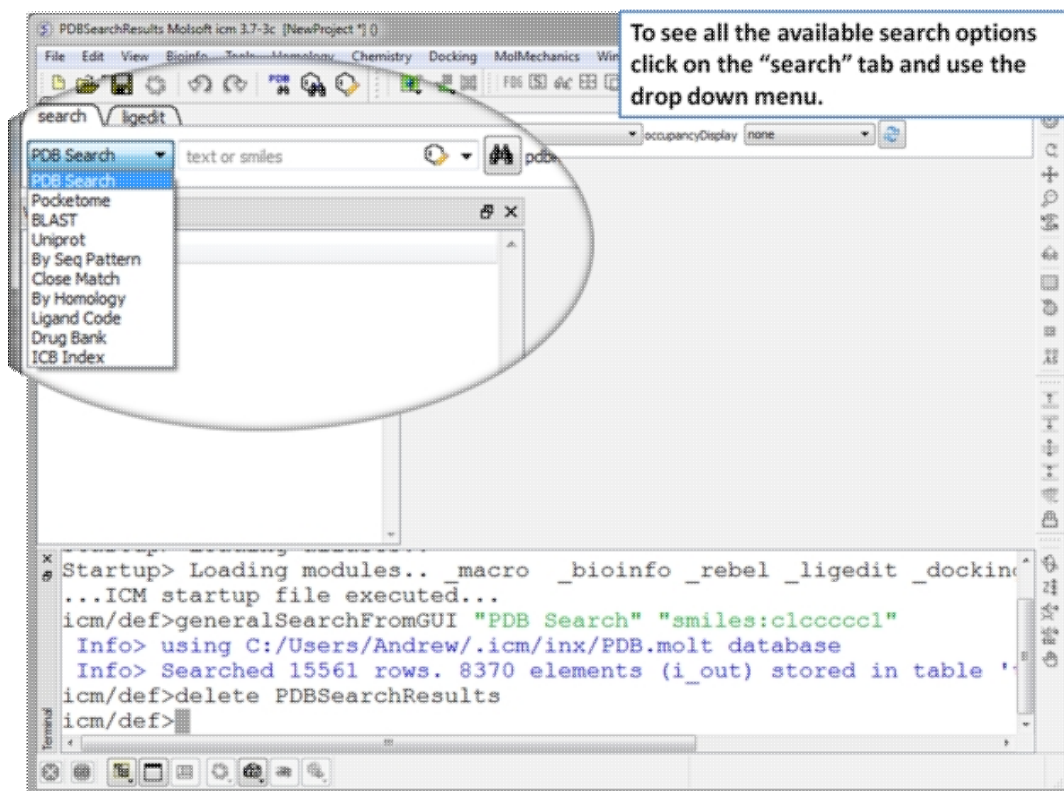

Or select /File/Quick Image

The picture will be automatically saved as a PNG file in the directory
from which you loaded ICM. The default picture name is icm[n].png, where
n is the number of pictures taken in one ICM session. To save in other
picture formats and to change the file name `see the write image section`.

3.2 How to search and download Protein
Structure, Sequences, and Chemicals

Available in the following product(s): `ICM-Browser | ICM-Browser-Pro
| ICM-Pro | ICM-Chemist`

The **Search** tab allows you to search and download content from the
following databases:

- The Protein Databank www.wwpdb.org - search for protein and
  chemical
- The Pocketome Database `http://pocketome.org/`
- BLAST search the NCBI sequence database
- The UniProt Sequence database `http://www.uniprot.org/`
- Search the PDB by ligand code.
- Search Drug Bank `http://www.drugbank.ca/`
- Search PubChem `https://pubchem.ncbi.nlm.nih.gov/`
- Search ChEMBL `https://www.ebi.ac.uk/chembl/`
- Search SureChEMBL `https://www.surechembl.org/search/`
- Search the Crystallography Open Database
 `http://www.crystallography.net/`

Click on the search tab and then use the drop down menu to identify the
database you would like to search and download from.

3.2.1 Search the PDB

How to Search the Protein Databank and Download

The **PDB search tab** provides easy access to the PDB database. You can use
keyword searching or type in the PDB code you are interested in. An
asterisk (*) wildcard can be used to list all the pdb files currently
available in the protein databank. Different fields can be searched by
using the drop down arrow as shown below. More advanced PDB search tools
and how to use the PDB search result table are described in the section
entititled Searching the PDB.

Once a search is complete a table of PDB files relating to your search
query will be displayed. To view the PDB file in 3D in the graphical
display double click on a row in the PDBSearchResults table.
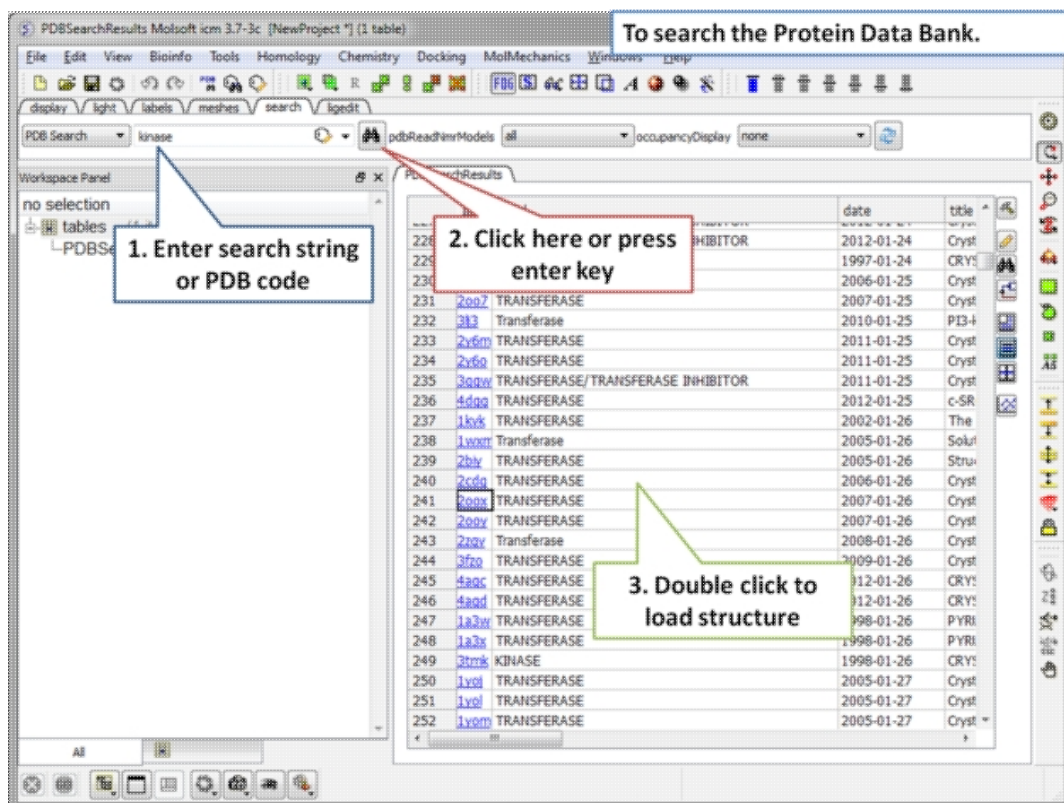
To Search the PDB and Download a Structure:

- Click on the search tab and enter a search string or PDB code.
- A table containing the results will be displayed.
- Double click on a row to load the PDB file. Read more about PDB search here.

NOTE: If you have a PDB structure already saved you can read it into ICM by going to the File Menu and selecting Open. PDB files that have been viewed previously can be loaded using File/ Recent PDB Codes.

3.2.1.1 How to Search the Protein Databank by Chemical Substructure

To search the Protein Data Bank by chemical substructure.

1. Click on the molecule editor button

2. Sketch the substructure

4. Click here or press enter key

3. Close the window

Step 1: Click on the molecule editor button inside the search tab.
Sketch the substructure you are interested in.

Double click on the table to load the PDB structure.

1. Double click to load PDB structure

Step 2: A table containing the results will be displayed. Double click
on a row to load the PDB file.

3.2.1.2 How to Query the PDB by Sequence

There are a number of ways to search the PDB by sequences.

1. BLAST search the NCBI datase using the option "Sequences from PDB".
2. Search by **Sequence Pattern** using the option in the Edit menu.
3. Search by **Identity** using the option in the Edit menu.
4. Search by **Homology** using the option in the Edit menu.

3.2.1.3 How to Query PDB by PDB Field

To query the PDB by field (Author, Compound, PDB Header, Experiment Type, Resolution or Ligand Code

- Select **Edit/PDB** search by field.
- Enter the search string or value
- Click **OK** and a list of related PDB entries based on your search will be displayed in the PDBSearchResults table of the graphical user interface.
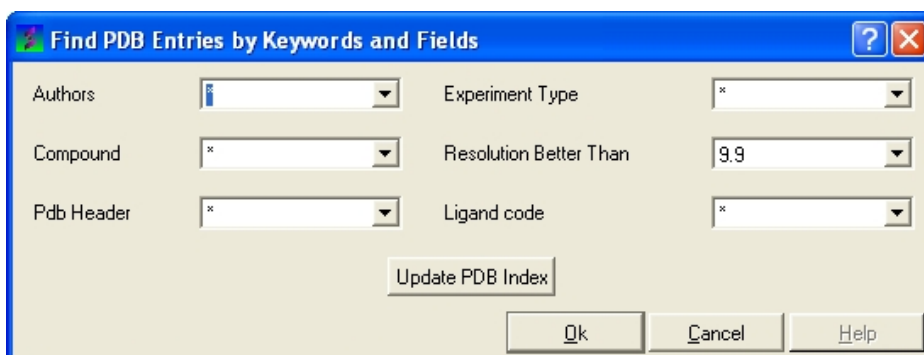
3.2.1.4 Load and Display NMR Structures

Use the PDB Search tab to load NMR structures from the PDB. You can use the drop down button shown below to determine how you want to display your NMR structure. You can choose to display and download the first NMR model, all models in the PDB file or all models in the PDB in a stack. If you choose the stack option the the stack will be stored in the object as described here.

3.2.1.5 Occupancy Display

You can use the options in the PDB Search tab to control if and how the partial or zero atom occupancies are displayed. You can choose to circle or label the poor occupancy atoms.

>>load-pdb-hyperlinks{pdb search hyperlinks} h4-- Hyperlinks to PDB Website and UniProt {Hyperlinks to Databases}

In the PDB Search Results Table you will see blue hyperlinks that will take you directly to the PDB website or Uniprot website.

3.2.1.6 Display PDB Header

To display the PDB Header for a PDB file.

- First load a PDB file into ICM (see Search PDB)
- Double click on the word header in the ICM Workspace.
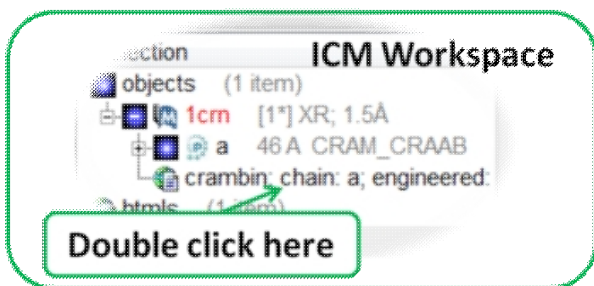
- The PDB Header information will be displayed.
- Click on the blue hyperlinked text to link to external web pages for additional information if needed.

3.2.1.7 Direct link to PubMed

When you search for a PDB file and load it into ICM you will see an icon (shown below) next to your protein name in the ICM Workspace. Click the icon and you will be taken directly to the PubMed primary reference relating to the structure.
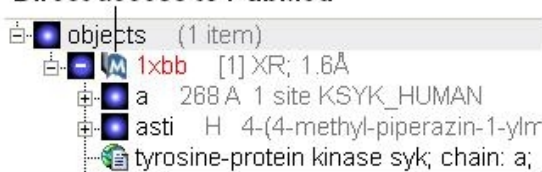
3.2.2 Search Pocketome

The Pocketome (www.pocketome.org) is an encyclopedia of conformational ensembles of all druggable binding sites that can be identified experimentally from co-crystal structures in the Protein Data Bank.
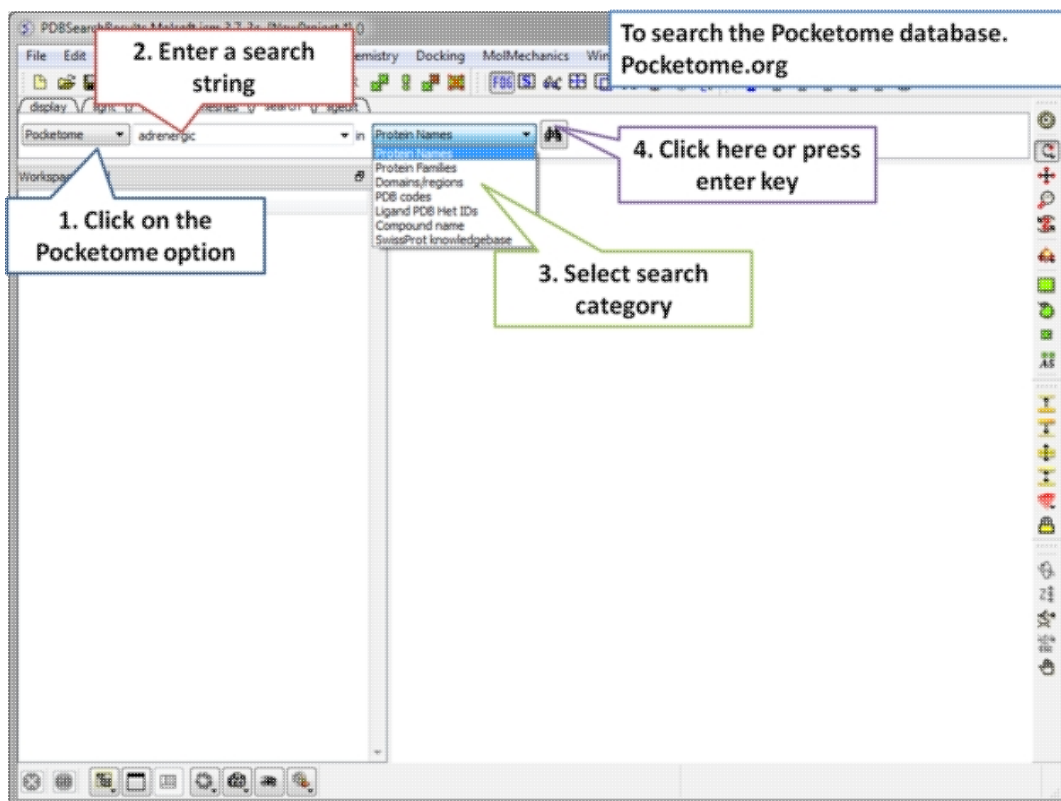
Step 1: Click on the search tab and choose the Pocketome option. Check
a field you would like to search e.g. Protein Name, Family, domain...
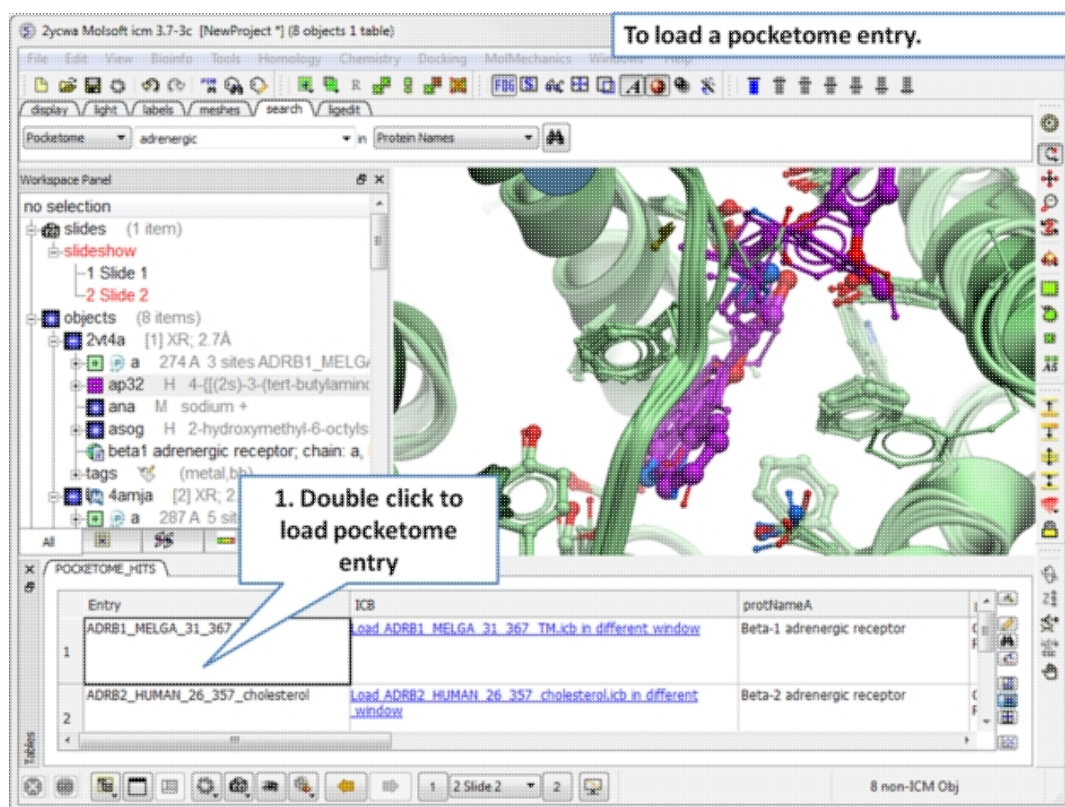
Step 2: A table of pocketome hits will be displayed. Browse the results
and double click to load a pocketome entry.

3.2.3 ChEMBL Search

ChEMBL (https://www.ebi.ac.uk/chembl/) is a manually curated chemical
database of bioactive molecules with drug-like properties. It is
maintained by the European Bioinformatics Institute (EBI), of the
European Molecular Biology Laboratory (EMBL), based at the Wellcome
Trust Genome Campus, Hinxton, UK.

To search and download data from ChEMBL:

- Click on the **Search** tab.
- Choose ChEMBL from the drop down button.
- Enter a search string or search by chemical sketch by clicking on
  the editor button in the panel.
- Select a field to search e.g. Protein Name.
- Select whether you wish to return just activity data or all data.
- Click the search button.

A table will be displayed as shown below.

The table contains the original activity data without merging or
aggregation. For instance,
Chemical 1, Protein 1, Activity 1
Chemical 1, Protein 1, Activity 2
Chemical 1, Protein 2, Activity 1
Chemical 2, Protein 1, Activity 1

Click on the button **Get Best Activity** to display the best activity for
each chemical, protein pair. For instance,

Chemical 1, Protein 1, Best(Activity 1, 2, 3, ...)
Chemical 1, Protein 2, Best(Activity 1, 2, 3, ...)
Chemical 2, Protein 1, Best(Activity 1, 2, 3, ...)

Click on the button **Get Ligand Summary** to display a list of chemicals
with all activities found in ChEMBL. For instance,

Chemical 1, Protein 1, Activity 1

Protein 1, Activity 2
Protein 2, Activity 1
Protein 3, Activity 1

Chemical 2 Protein 1, Activity 1
Protein 2, Activity 1
Protein 2, Activity 2

3.2.4 Search SureChEMBL

SureChEMBL (https://www.surechembl.org/search/) provides free access to
chemical data extracted from the patent literature. To search
SureChEMBL:

- Click on the **Search** tab.
- Choose SureChEMBL from the drop down button.
- Enter a search string or search by chemical sketch by clicking on
  the editor button in the panel.
- Select a field to search e.g. Protein Name.
- Select whether you wish to return just activity data or all data.
- Click the search button.

3.2.5 BLAST Search

To BLAST search the NCBI sequence database:
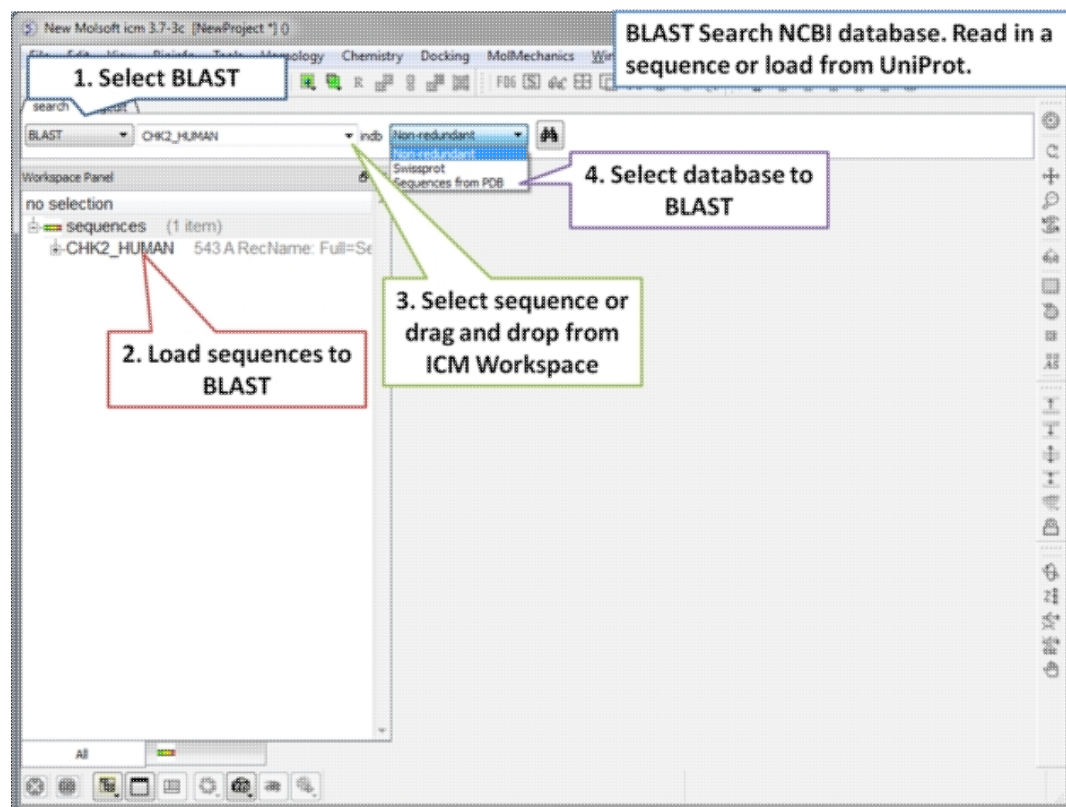
Step 1:   Load a sequence into ICM. Select the Search tab and choose the
BLAST option. Drag and drop the sequence into the search field, use the
drop down menu or type the sequence name.
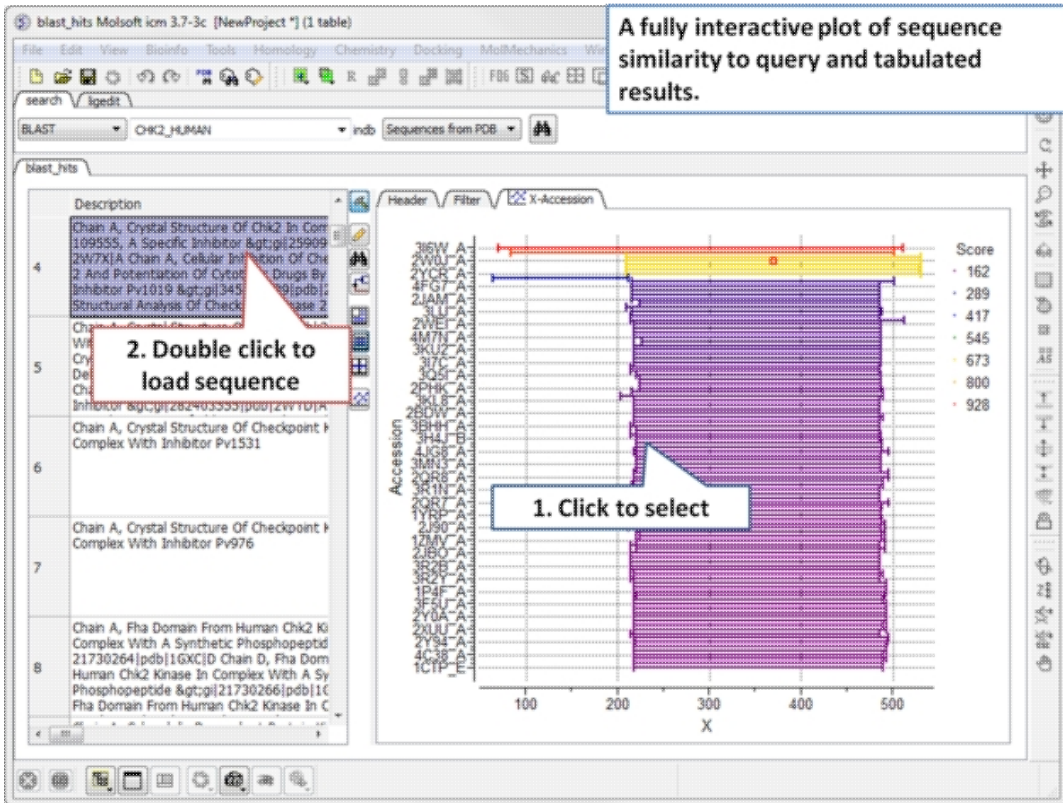
Step 2: A fully interactive table and plot of sequence conservation
will be displayed. Double click to load a sequence.

3.2.6 Search UniProt
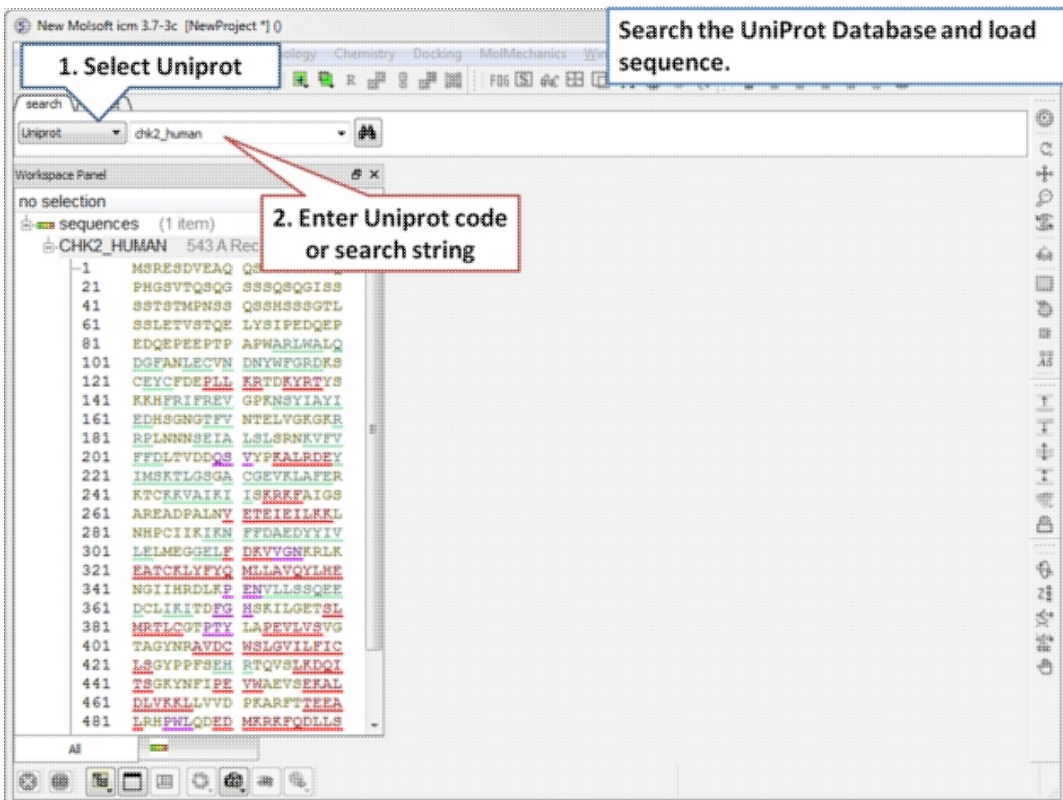
Step 1: Click on the search tab and select **Uniprot** from the drop down menu. Enter the UniProt code. The sequence will be loaded directly into ICM and you will see it in the ICM workspace.

3.2.7 Search PDB by Ligand Code

To find structures containing a particular ligand in the PDB.
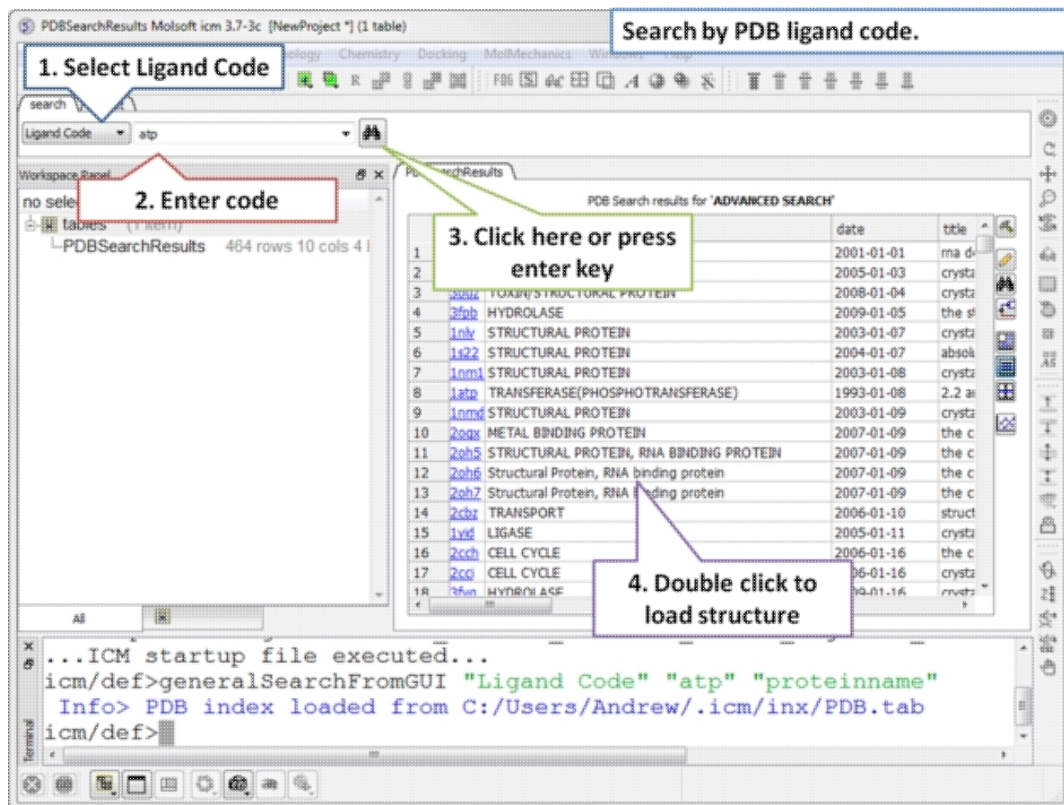
Step 1: Click on the search tab and select **Ligand Code** from the drop down menu. Enter the Ligand code and a table of hits will be displayed.

3.2.8 Search Drug Bank

To search Drug Bank (`http://www.drugbank.ca/`)
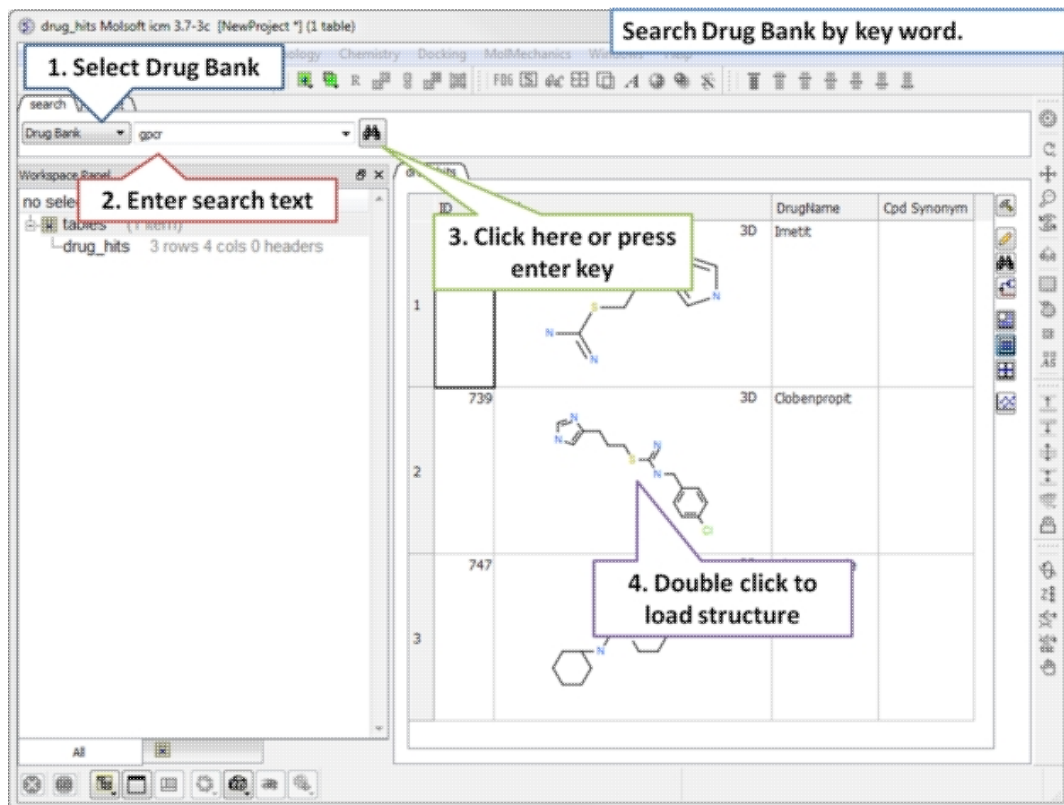
Step 1: Click on the search tab and select **Drug Bank** from the drop down menu. Enter a search string and a table of results will be displayed.

The table contains the original activity data without merging or aggregation. For instance,

Chemical 1, Protein 1, Activity 1
Chemical 1, Protein 1, Activity 2
Chemical 1, Protein 2, Activity 1
Chemical 2, Protein 1, Activity 1

Click on the button **Get Ligand Summary** to display a list of chemicals with all activities found in Drug Bank. The button is located in the extra panel (click on hammer - top right of table panel). For instance,
Chemical 1, Protein 1, Activity 1

Protein 1, Activity 2
Protein 2, Activity 1
Protein 3, Activity 1

Chemical 2 Protein 1, Activity 1
Protein 2, Activity 1
Protein 2, Activity 2

3.2.9 Search PubChem

To search PubChem (https://pubchem.ncbi.nlm.nih.gov/):
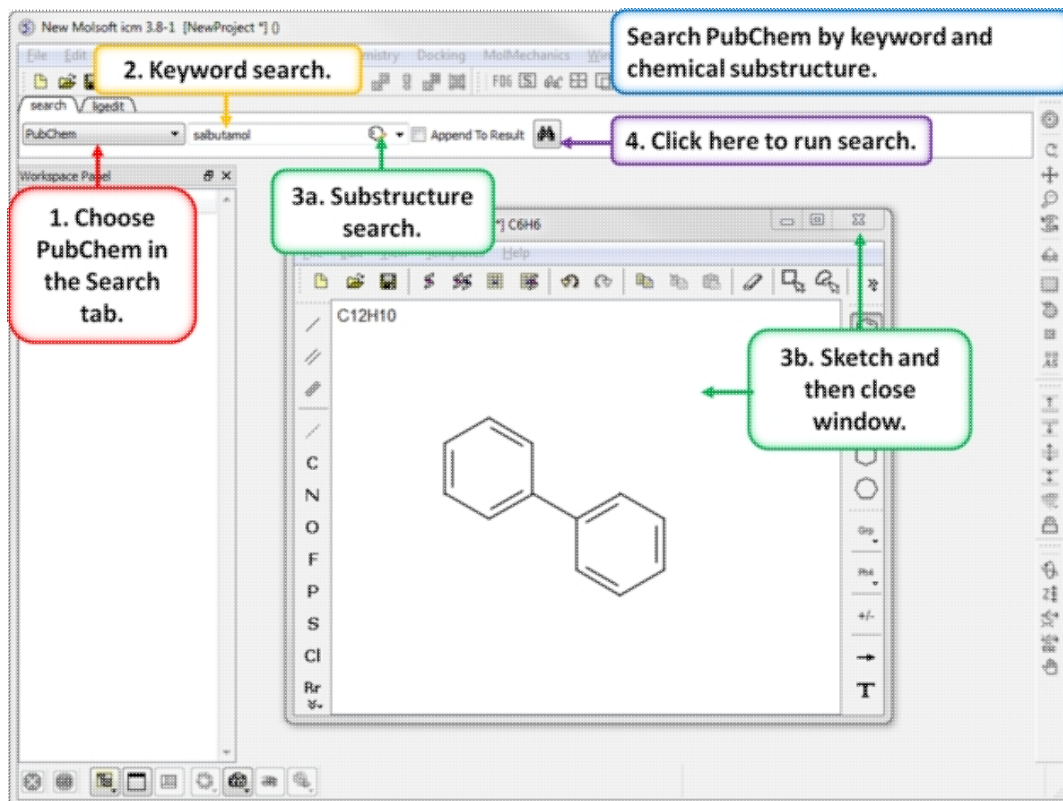
- Click on the search tab and select **PubChem** from the drop down menu.
- Enter a search string and a table of results will be displayed or click on the chemical sketch button and sketch a substructure. When you close the chemical sketch window the smiles string will be added to the keyword search panel.
- Click on the "binocular" button and run the search.
- A table of hits will be displayed.

3.3 Create New Objects

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro | ICM-Chemist

In the File menu there is an option called New. This option can be used to create new objects of the following types:

- Peptide
- Unusual Peptide
- Compound
- DNA and RNA
- Sequence
- Script
- HTML
- Table
- Arrow
- Box
- Sphere/Ellipsoid
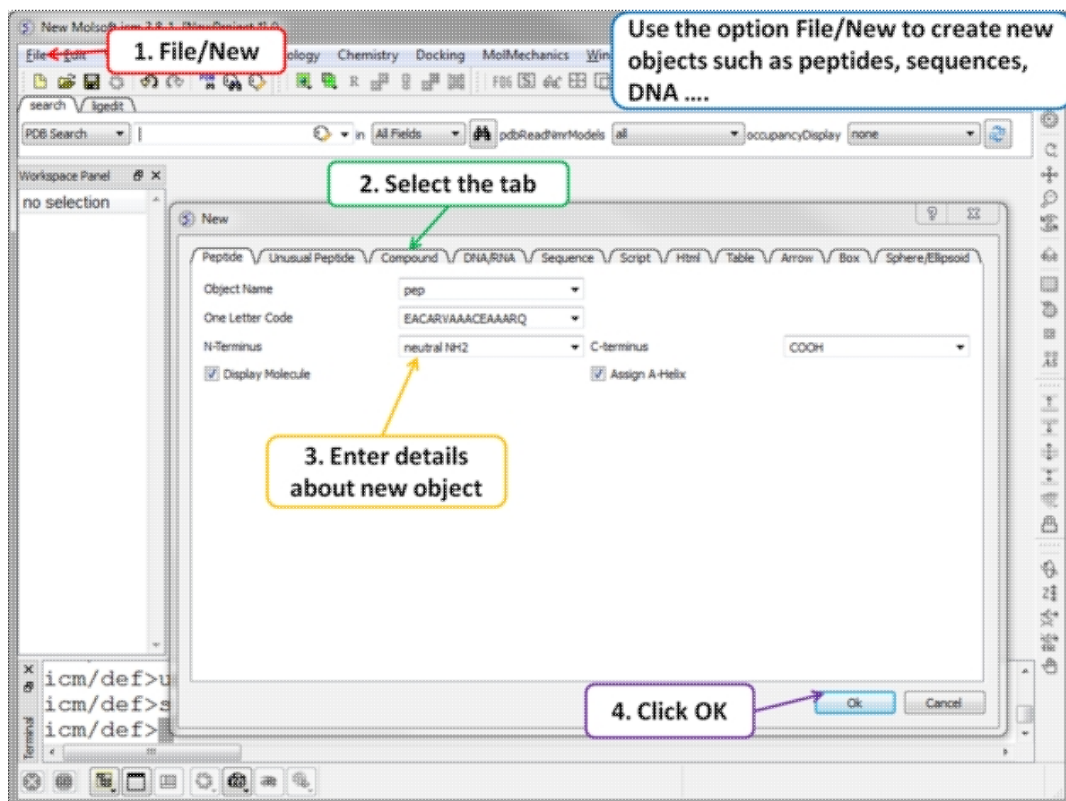
3.4 Open and Read Files

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro | ICM-Chemist

Any file that ICM can understand can be opened by:

* Selecting File/Open.

A file with an extension .icb is an ICM binary file and can be viewed in
the GUI. A .icb file can contain many objects such as sequences, meshes,
protein objects, alignments, tables etc...

3.4.1 Open with Password

To open a file that is password protected:

* File/Open with Password

3.4.2 Extract from icb file

An **icb** file is an icm project file, in some instances you may want to
take objects saved in an icb file and load it in your current ICM
session. This option allows you to view a tabulated list of what a icb
file contains and load individual object files from it.

* File/ Extract from ICB.
* Locate the saved icb file.
* A table as shown below will be displayed
* Double-click on any of the entry to extract that object from the
  icb file.

	name	type	size
2	openFilePRJNAME	string	9
3	1TKI_A_4	sequence	491
4	1DI9_A_17	sequence	509
5	1WFC__5	sequence	538
6	1IAN__23	sequence	415
7	1QL6_A_4	sequence	413
8	1ql6_a	sequence	907
9	2phk_a	sequence	786
10	alig	alignment	10927
11	1ql6	object	231820
12	2phk	object	235471

3.5 Saving Files

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro | ICM-Chemist

Anything you have displayed in an ICM graphics session can be
conveniently stored in a single file called an .icb file. To do this:

- File/Save Project

or

- File/Save Project As

A .icb file can be password protected by:

- File/Save with Password
- Enter a file name or browse for a previously saved project.
- Enter a password
- Determine whether you want the file to be Fully Protected, read
  only or Read Only and Allow Comments .

**Any object (e.g. protein,table, alignment...) inside the ICM GUI can be
saved by:**

- Right click on the name of the object in the ICM Workspace.
- Select "Save As"
- Choose the file format from the "Save as Type" drop down dialog.

To save an object – right click on the name of the object in the ICM Workspace and select Save As...

1. Right click here and choose Save as...

2. Choose the file type from the drop down menu
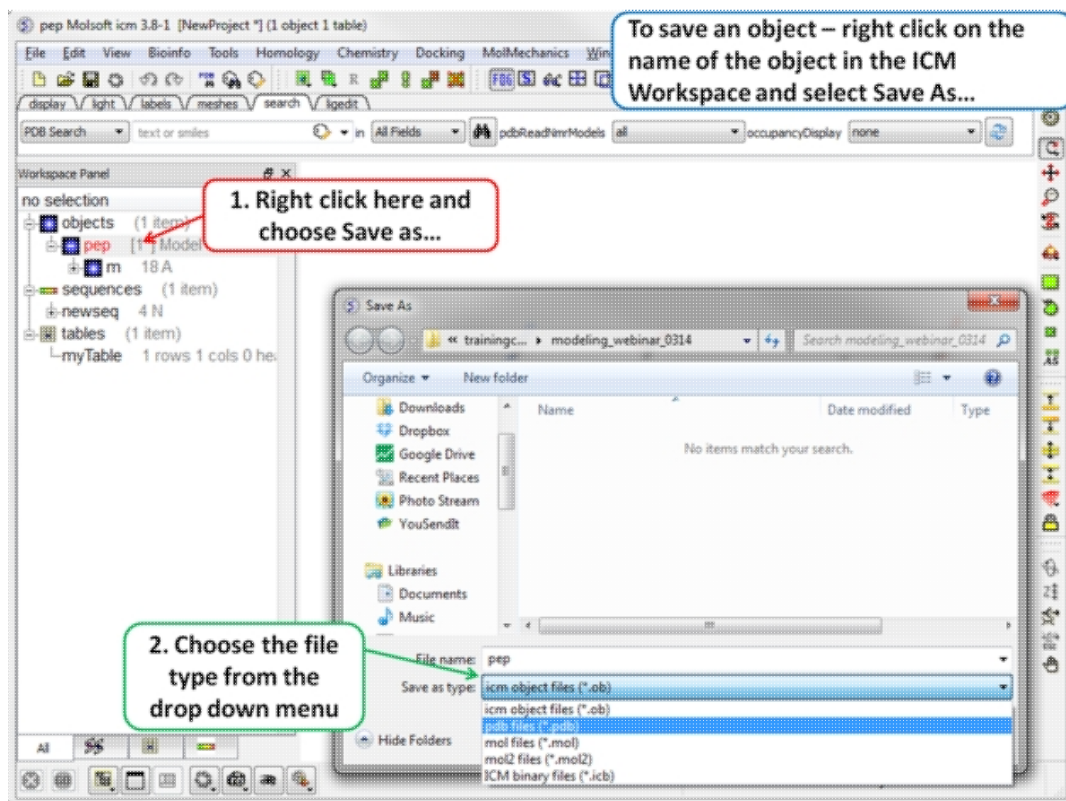
3.6 Making Selections

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro | ICM-Chemist

Note: Click **Next** (top right hand corner) to navigate through this chapter. Headings are listed on the left hand side (web version) or by clicking the **Contents** button on the left-hand-side of the help window in the graphical user interface.

There will be many occasions when you will have to make selections. For example, if you want to display a particular region or molecule contained within your protein structure or if you want to select residues around a binding pocket. If you have a molecule displayed in the graphics window, then selections will be displayed as green crosses. The selection you have made is also displayed at the top of the ICM Workspace. It is always a good idea to keep an eye on what is selected and what isnt.

There are four basic levels of selection

1. Object (eg a PDB structure or ICM object)
2. Molecule
3. Residue
4. Atom

You can make selections in:

- The Graphics Display
- The ICM Workspace (Selections are highlighted in blue)
- Tables
- Sequences
- Plots

- Alignments

3.6.1 Graphical Selection Tools

The following buttons can be used to make a selection once a structure
is displayed.
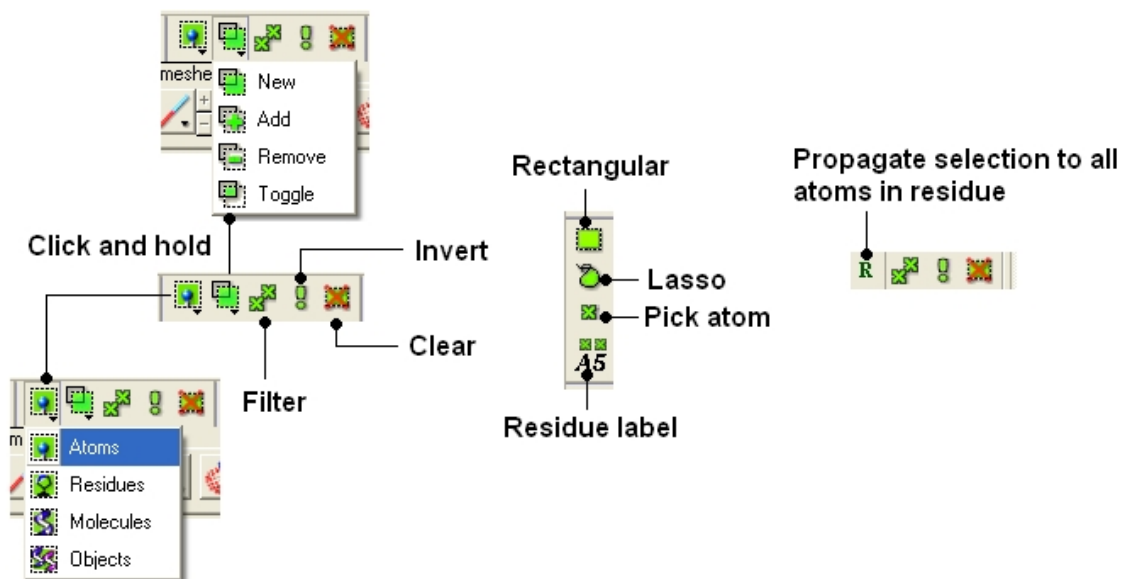
3.6.2 Quick Selection

To make a quick selection the following buttons can be used.
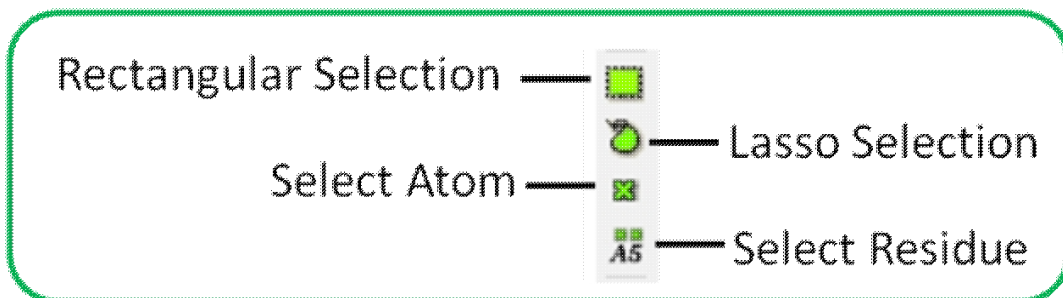
To select parts of your structure:

- Click on the **Rectangular selection icon** and click and drag around
  the part of the structure you wish to select.

OR

- Click on the **Lasso selection icon** and click and drag your mouse
  around the area of the structure you wish to select, forming a
  lasso around it.

To pick individual atoms:

- Click on the 'pick atom' button

3.6.3 How to Change the Selection Level and Mode

It is possible to change the level of selection before or during the building of a selection. The selection level drop-down button can be used to do this (see image below).

For example, a C-alpha of a residue is selected but one would like to select all atoms in the residue. You can change the level to **Residues.** This selection can then be changed into all atoms of the residue by then selecting the **Atoms** level again. Or you can use the **Propagate Selection to all Atoms** button (see image below).

- Click on the **Select** objects , **Select molecules**, **Select residues**, or **Select atoms** icon, depending on which part of the structure you wish to be highlighted.
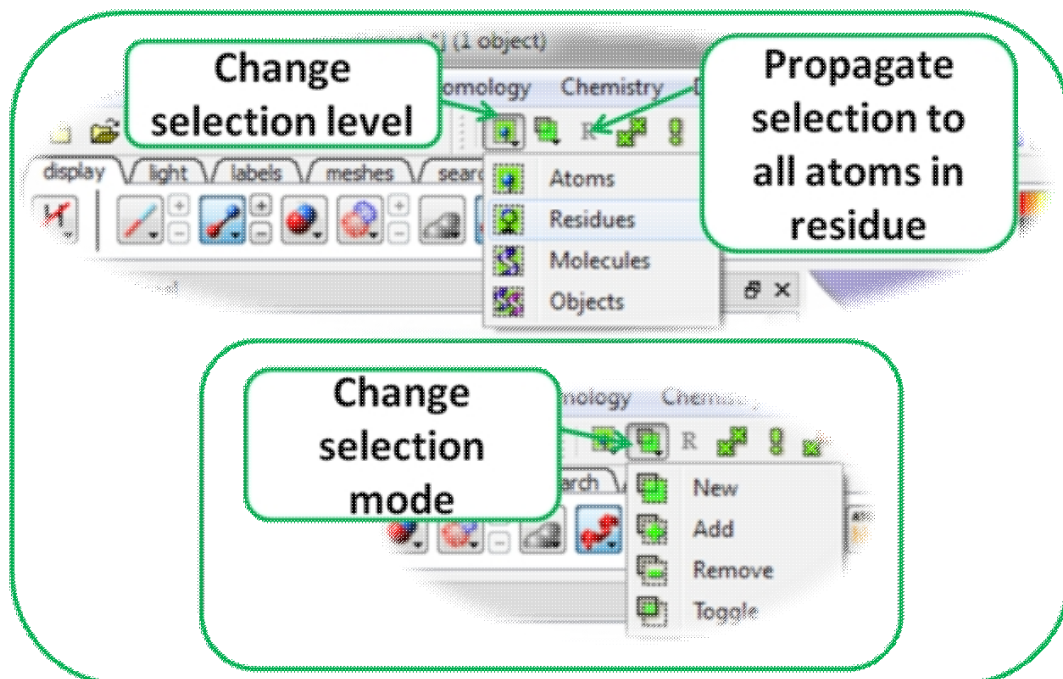
It is also important to observe the selection mode that is being used. There are four modes:

- New: new selection replaces everything selected before
- Add: new selection is added to previous selection(s), if any
- Remove: previously selection (part or whole of it), if included in the new selection will be unselected.
- Toggle: within the new selection, everything that has been selected is unselected and everything that hasnï¿‰t been selected, wilbe selected

3.6.4 How to check what is selected.

All selections are displayed as green crosses in the graphical display and blue in the ICM Workspace. All green selections are returned to a variable called as_graph.

- "Selections are displayed as green crosses in the graphical display and highlighted in blue in the ICM Workspace."
- "Here the selection information is reported"
- "Selection are highlighted in blue in the ICM workspace"
- "Selection shown as green cross in graphics window"
- "All green graphical selections are returned to a variable called as_graph"

Terminal text shown:

```
icm/1xbb>display xstick as_graph
icm/1xbb>as_graph = Atom( Res(Sphere( a_1xbb.asti/1 (Mol( (a_*.*/D
icm/1xbb>undisplay store a_1x
icm/1xbb>display wire  a_1xb
icm/1xbb>undisplay store a_1
icm/1xbb>display xstick  a_1
icm/1xbb>as_graph
```

3.6.5 Orange Selection

Sometimes it is necesary to have two different selections. The Orange selection allows you to do this it is useful for such operations as superposition and more technical procedures such as designing a protein loop. The orange selection is returned to a variable called as2_graph.

Orange selection allows you to copy a green selection to another selection. This allows you to have two different selections.

Click here to transfer a green selection to an orange selection

Orange selection
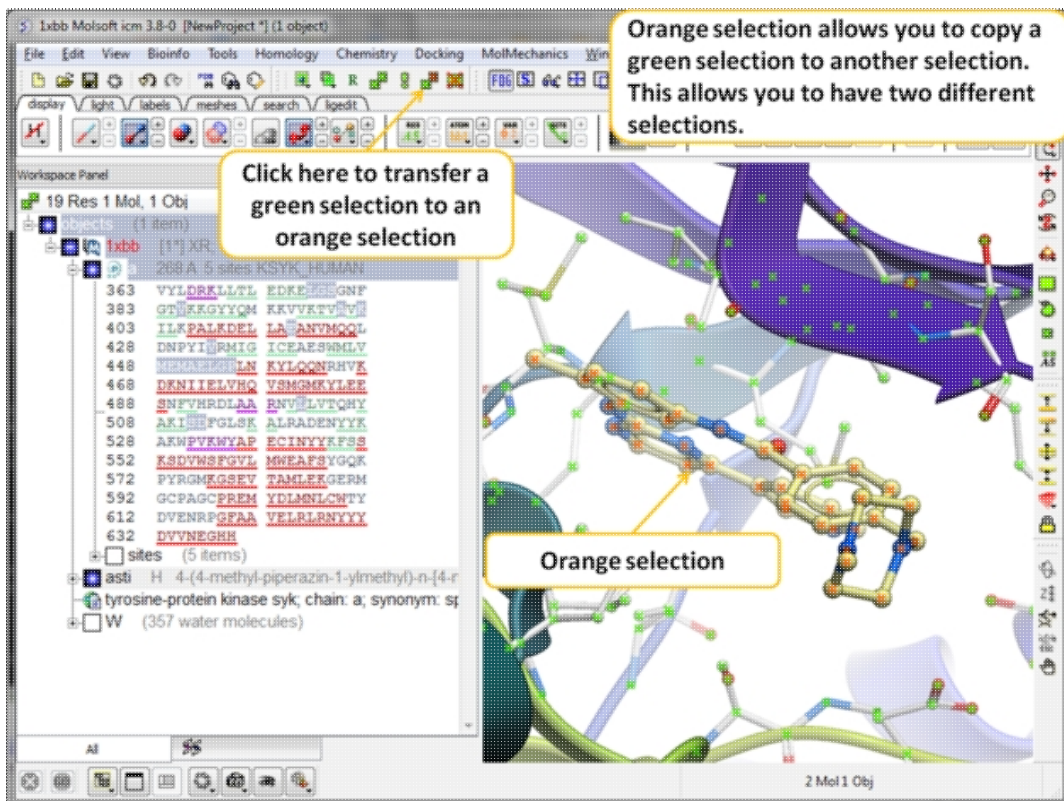
3.6.6 Clear Selection

To unselect everything you have previously selected:

- Simply click on the **Clear Selection** button on the selection toolbar.

OR

- Right click and drag away from the displayed structure.

Two ways to remove a selection.

1. Click here to remove a selection

2. Right click and drag in empty space
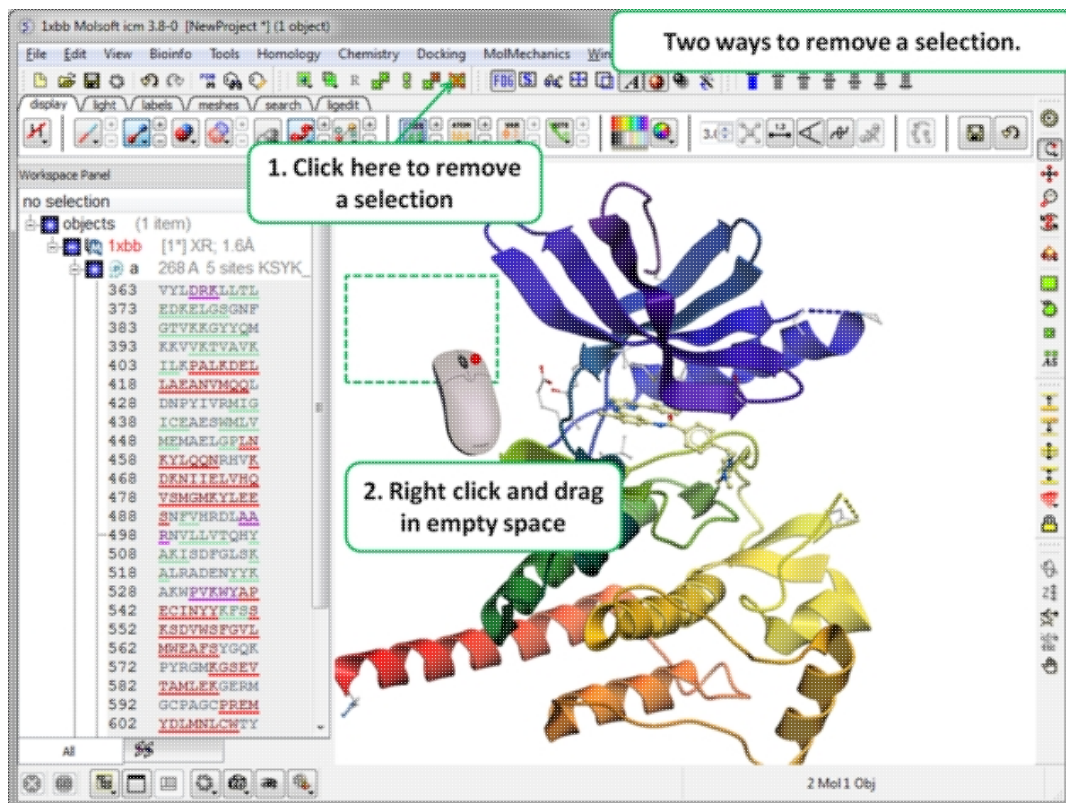
3.6.7 Changing a Selection

Once you have made a selection you may wish to add or remove parts of the selection. The buttons shown below allow you to accomplish this.

Propagate selection to all atoms in residue

Click and hold
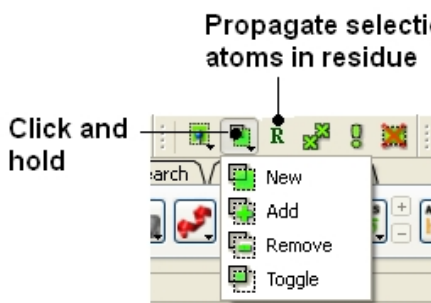
To add or remove from your current selection:

- Click on the **Selection mode: add** or **Selection mode: remove** icon on the toolbar.
- Click and drag around the part of your structure you wish to add or remove.

You may also wish to invert your selection in a specific part of the structure.

The parts that are currently selected will become unselected, and the unselected parts will become selected.

In order to invert a selection:

- Click on the **Invert** icon on the toolbar.

If you wish to select and unselect certain regions of a selection the toggle selection button is very useful.

- Click on the Toggle selection button.
- Right click around the selections you wish to select or unselect.

NOTE: The selection you have made is recorded at the top of the ICM workplace. Any selection is stored in the variable as_graph.

3.6.8 Filter Selection

You may want to be very specific about a selection you want to make. For example you may only want to select protein backbone atoms or you might want to just select the charged residues.

The button shown below enables you to filter your selection:

Or

Right click on a selection and a menu as shown below will be displayed.
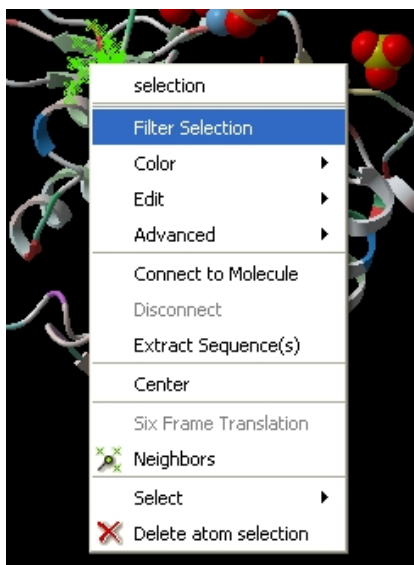
- Select the Filter Selection option.
- A dialog box will be displayed as shown below.
- Select what level of selection you want e.g. atom or residue.

Filter a selection by a variety of selection level properties.

1. Click on a tab to select desired selection level

2. Click "+" to expand and see options
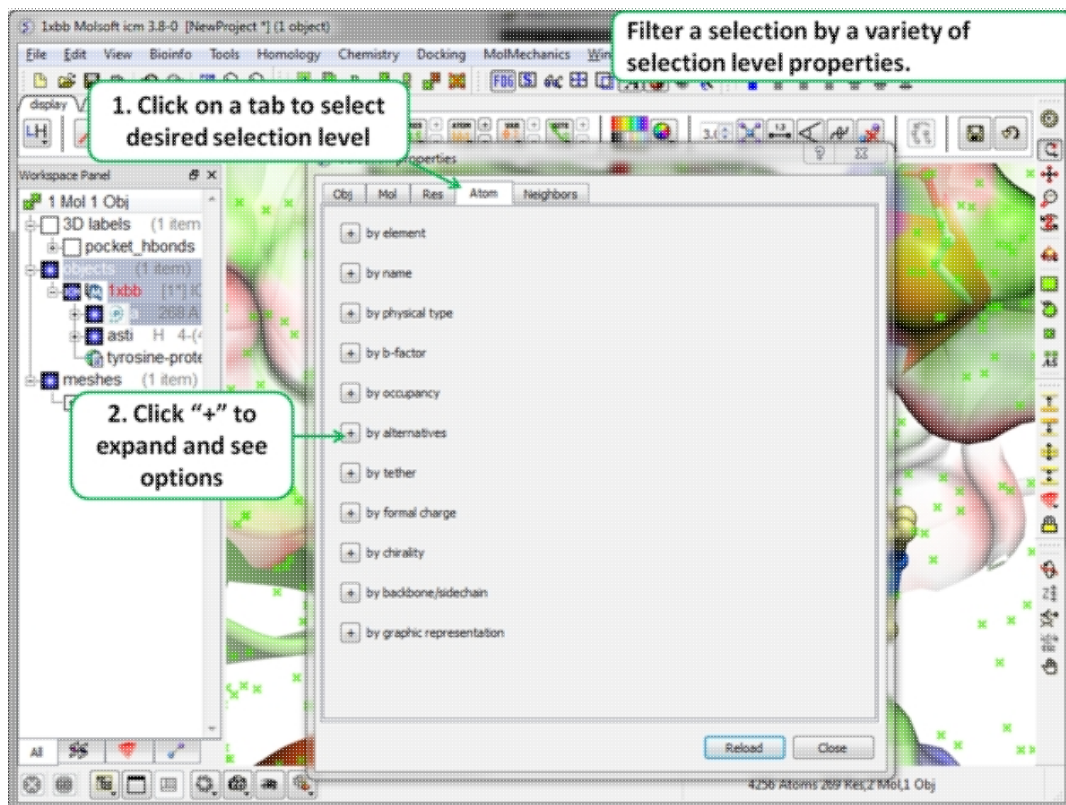
3.6.9 Workspace Selections

In the default GUI layout the workspace panel is located to the left of the 3D graphics display. It is a great tool for keeping track of all your sequences, pdb structures, objects, tables and alignments. As you will see in this section it also provides a way of making selections.

Selected atoms and residues

Workspace panel

Expand here for more information.

Display all

Objects

Sequences

Alignments

Tables
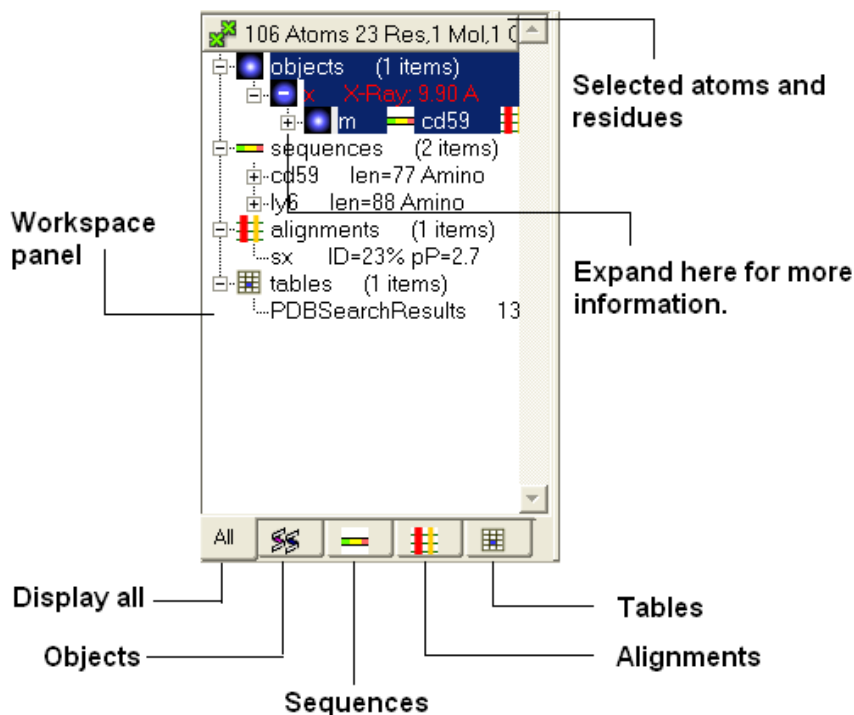
3.6.10 Workspace Navigation

Once you have mastered how to navigate the ICM workspace making a selection will become easier. Each object is divided into 3 levels:

1. Object Level - Shown in red if it is the current object. Holds details about the structure - name, X-ray, NMR, resolution etc. Importantly it will state whether the structure is an ICM object or a structure straight from the PDB. To learn how to convert a PDB into an ICM object go to the section on `converting a PDB`.
2. Molecular Level - Shows the individual subunits, ligands and hetatoms of a molecule.
3. Residue Level - Shows the sequence.
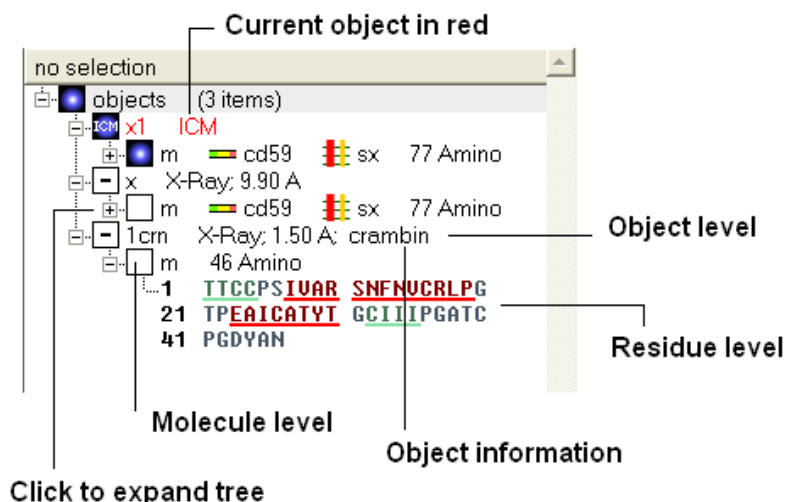
NOTE: You can expand each level of the ICM workspace by clicking the "+" button as shown above.

3.6.11 How to Select an Object

To select the whole object:

- Double click on the object level.
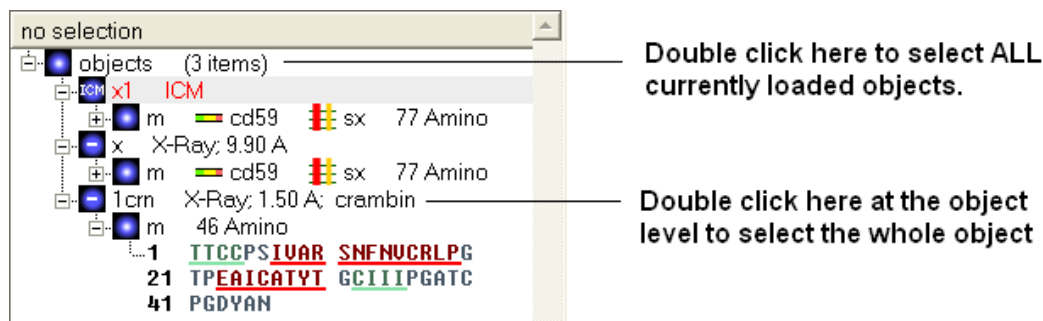
Use the CTRL button to select multiple non-contiguous objects or if they are continuous you can use double click and hold the tab button.

3.6.12 How to select a Molecule

To select a molecule(s):

- Double click on the molecule in the ICM workspace.
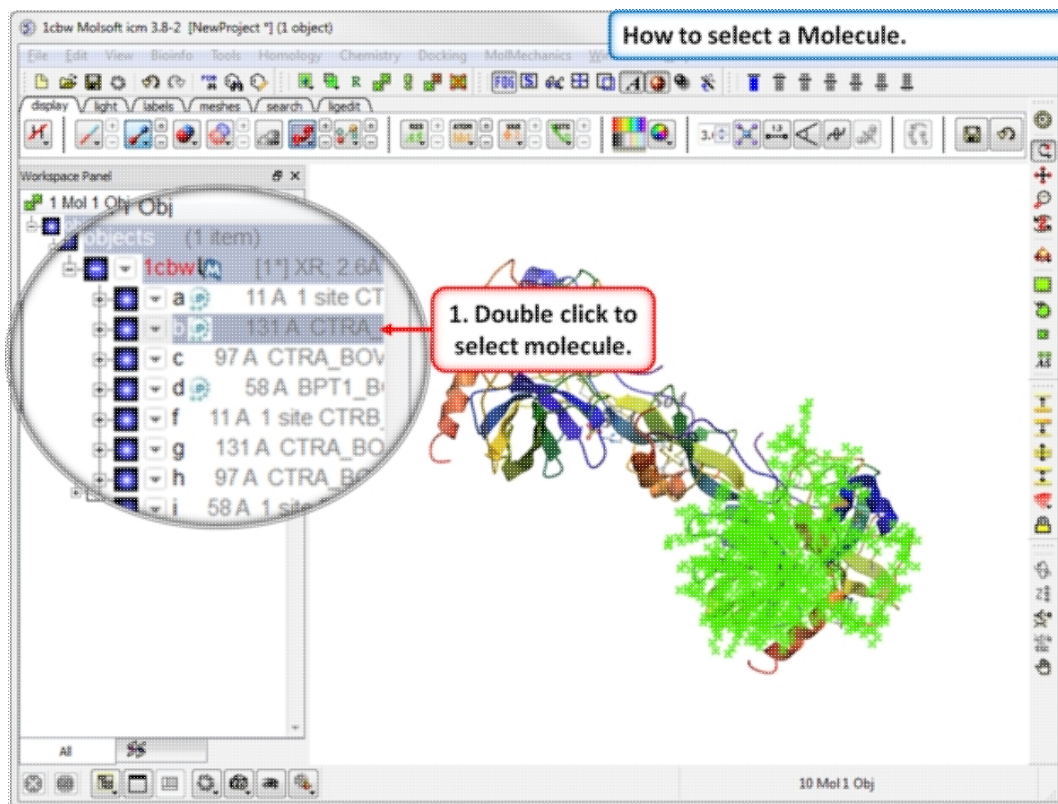
Use the CTRL button to select multiple non-contiguous molecules or if they are continuous you can use double click and hold the tab button.

3.6.13 How to select Residues

There are different options to select residues:

OPTION 1:

- Click and drag over the residues you wish to select in the ICM workspace. Selected residues will be highlighted in dark blue in the workspace and with green crosses in the graphical display.

Selection information is recorded here

Click here to
expand tree to
show amino acid
residues.

Click and drag
over residues
you wish to
select.

Selected
residues will be
highlighted in
the workspace
and graphics
window

184 Atoms 35 Res,1 Mol,1 Obj	

objects   (1 items)
1f88   X-Ray: 2.80 A; rhodopsin
a   338 Amino OPSD_BOVIN

```
  1  MNGTEGPNFY  UPFSNKTGUU
 21  RSPFEAPQYY  LAEPWQFSML
 41  AAYMFLLIML  GFPINFLTLY
 61  UTUQHKKLRT  PLNYILLNLA
 81  UADLFMUFGG  FTTTLYTSLH
101  GYFUFGPTGC  NLEGFFATLG
121  GEIALWSLUU  LAIERYUUUC
141  KPMSNFRFGE  NHAIMGUAFT
161  WUMALACAAP  PLUGWSRYIP
181  EGTQCSCGID  YYTPHEETNN
201  ESFUIYMFUU  HFIIPLIUIF
221  FCYGQLUFTU  KEAAASATTQ
241  KAEKEUTRMU  IIMUIAFLIC
261  WLPYAGUAFY  IFTHQGSDFG
281  PIFMTIPAFF  AKTSAUYNPU
301  IYIMMNKQFR  NCMUTTLCCG
321  KNPSTTUSKT  ETSQUAPA
```

Any selection is highlighted in the workspace as well as in the
3D graphics window if the structure is displayed.

OPTION 2:

- Click on the **rectangular selection** icon or **lasso selection** icon on
  the toolbar.
- Click and drag around the residues you wish to select. Selected
  residues will be displayed by green crosses on the graphical
  display and blue in the ICM workspace.
- Use the `propagate selection to residue level` button.

OPTION 3:

- Right click on the a residue in the graphical display and a menu
  as shown here will be displayed.

selection

Selection Dialog
Advanced   ▶
Residue atoms   ▶
Open with MolEdit
Connect to Molecule
Disconnect
Extract Sequence(s)
Center
Annotate selection
Neighbors
Closed Cavities
Select   ▶
✗ Delete residue selection

- Click on **Select** and a further menu will be displayed.
- Click on **Residue**, **Molecule** or **Object.**
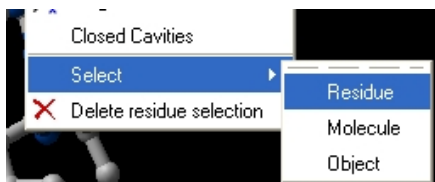
OPTION 4:

Use the select residue button.

3.6.14 Select All

Ctrl + A will select everything in the ICM workspace, and Ctrl + Shift + A will unselect your objects.

> **NOTE:** The selection you have made is always recorded at the top of the ICM workplace. If you are familiar with using the ICM terminal (See language manual) the atoms, residues, molecules or objects selected interactively in the graphics window are automatically s

3.6.15 Selecting Neighbors

In some instances you may only want to display or select only a subset of a structure. For example you may only wish to display the residues surrounding a ligand (as shown below (ligand red; graphical selection green crosses). The "Selecting Neighbors" option selects the residues within a shpere of a defined radius.

There are two ways of selecting neighbours to a particular atom or residue in ICM. Either by right clicking on the atom or residue in the graphical display or by right clicking in the ICM workspace.
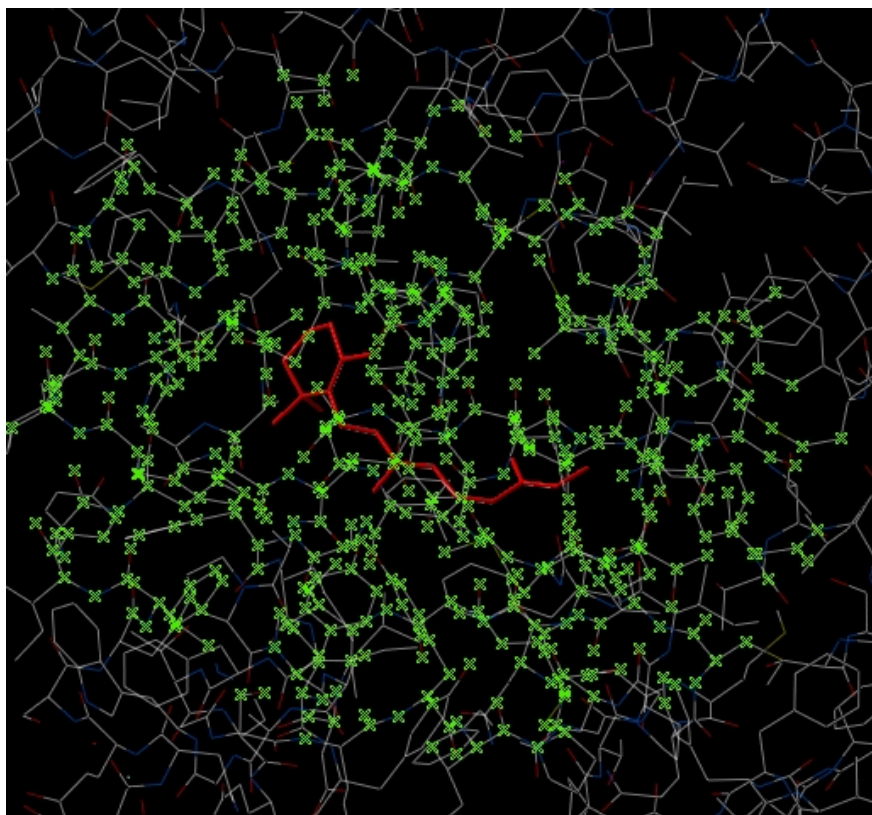
3.6.16 Selecting Neighbors: Graphical

To select neighboring atoms or residues around a sphere of a certain radius:

- First select the residue(s) or atom(s) around which you wish to select neighbors. (See the `Selection Toolbar Section`)
- Right click on the selection and a menu as shown below will be displayed or choose Tools/Geometry/Neighbors.
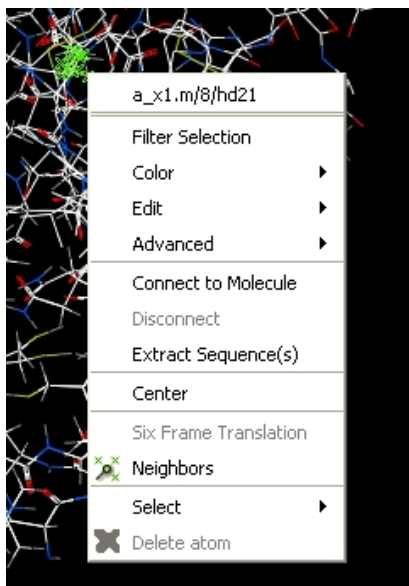
- Select the Neigbors option and a data entry box as shown below will be displayed.

This option will allow you to make a spherical selection.

The window will open as displayed as below:

- Select the molecule you wish to select neighbors around. For example you can `select` a ligand in the `ICM Workspace` and then choose the **Graphical Selection** option in the "Select Neighbors For" dialog entry box. Or alternatively you can select the object by clicking on the drop down button next to the "Select Neighbors For" dialog entry box.
- Enter the radius in Angstroms for the neighbor selection. e.g. 5.
- **Type** - this option is **important.** This option relates to what is going to be selected. For example if you leave this option as **visible** and you only have ribbon representation displayed for your

receptor (e.g. when selecting neighbors for a ligand) then only backbone atoms will be selected.

Selection **Type** option includes:

- **visible** - will select all atoms displayed within the radius selected.
- **visible sidechains** will select all visible side-chains - not backbone atoms.
- **same_object_other_chains** will select all atoms in other chains in the same object as the original selection.
- **other objects** will select atoms in objects other than the original selection.
- **same object** will select atoms in the same object as the original selection.
- **all_objects** will select atoms in all objects
- **choose_from_list** will allow you to select the object you wish to include in the neighbors selection.

- **exclude source** if checked will not include your original selection in the spherical selection.
- **unselect water** if checked will not select water molecules
- **Undisplay Beyond Selection** will only display the atoms selected.

NOTE: The selection you have made is always recorded at the top of the ICM workplace. If you are familiar with using the ICM terminal (See language manual) the atoms, residues, molecules or objects selected interactively in the graphics window are automatically saved in the variable as_graph. Graphical selections are shown in green (crosses) or highlighted in blue in the ICM Workspace.

3.6.17 Selecting Neighbors: Workspace

To select neighboring atoms or residues around a sphere of a certain radius from a residue in the ICM workspace:

- First select the residue in the ICM workspace around which you wish to select neighbors. (See the Residue Selection)
- Right click on the selection and a menu as shown below will be displayed.
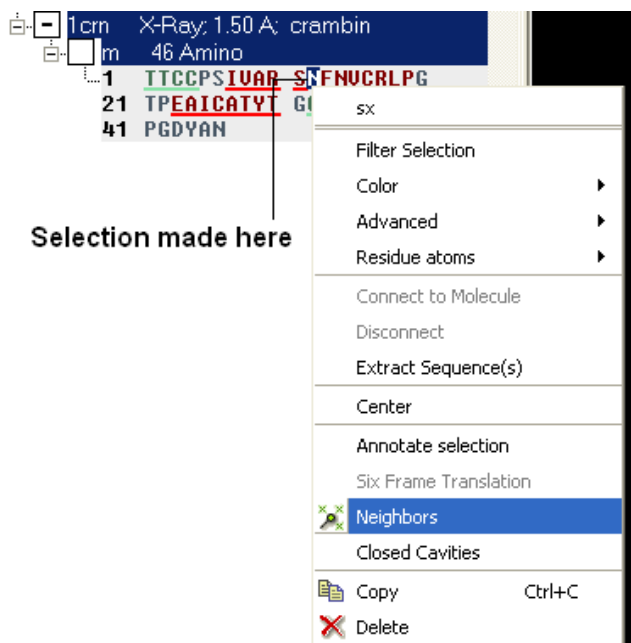
- Select the Neigbors option and a data entry box as shown below will be displayed.
- Follow the instructions in the `previous section`.

> **NOTE:** The selection you have made is always recorded at the top of the ICM workplace. If you are familiar with using the ICM terminal (See language manual) the atoms, residues, molecules or objects selected interactively in the graphics window are automatically s

3.6.18 Select by Residue Number

To select by residue number:

- Right click on the molecule you wish to make the selection in and choose Select/Res Number...
- Enter the residue numbers e.g. 3,7:12,30 Colon indicates range.

3.6.19 Alignment and Table Selections

Descriptions on how to make selections in Alignments and Tables are in the sections entitled `Making Selections in Alignments` and Making Table Selections.

3.6.20 Making Links

It is sometimes necesary to make links between sequences objects and alignments. A link enables you to make selections in one environment such as an alignment and then these selections are transfered to the object such as the PDB structure displayed.

If a link is made then a symbol will be displayed next to the object in the ICM workspace. In the example shown below subunit_a of the X-ray structure 1ql6 is linked to the sequence 1ql6_a and the alignment called 'alig'.
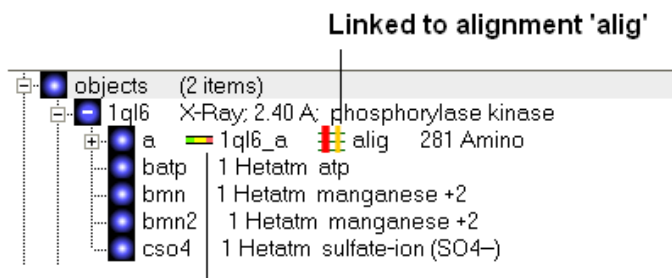
If an object is linked to an alignment a symbol as shown below will be displayed.
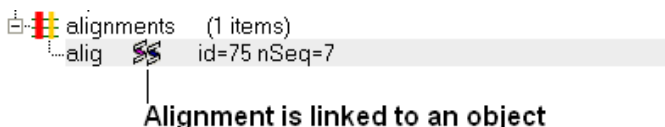
To link a sequence from an object - extract the sequence from the object.

- Right click on the object in the ICM workspace.
- Select extract sequence.

To link a sequence and object to an alignment.

Use the extracted sequence as described above to build your alignment.

In addition a link can be made between a structure and alignment by:

- Bioinfo/Link to Structure.
- Enter alignment name.
- OK

3.6.21 Tags

It is sometimes convenient to tag selections so you can come back and use them at a later date. To do this:
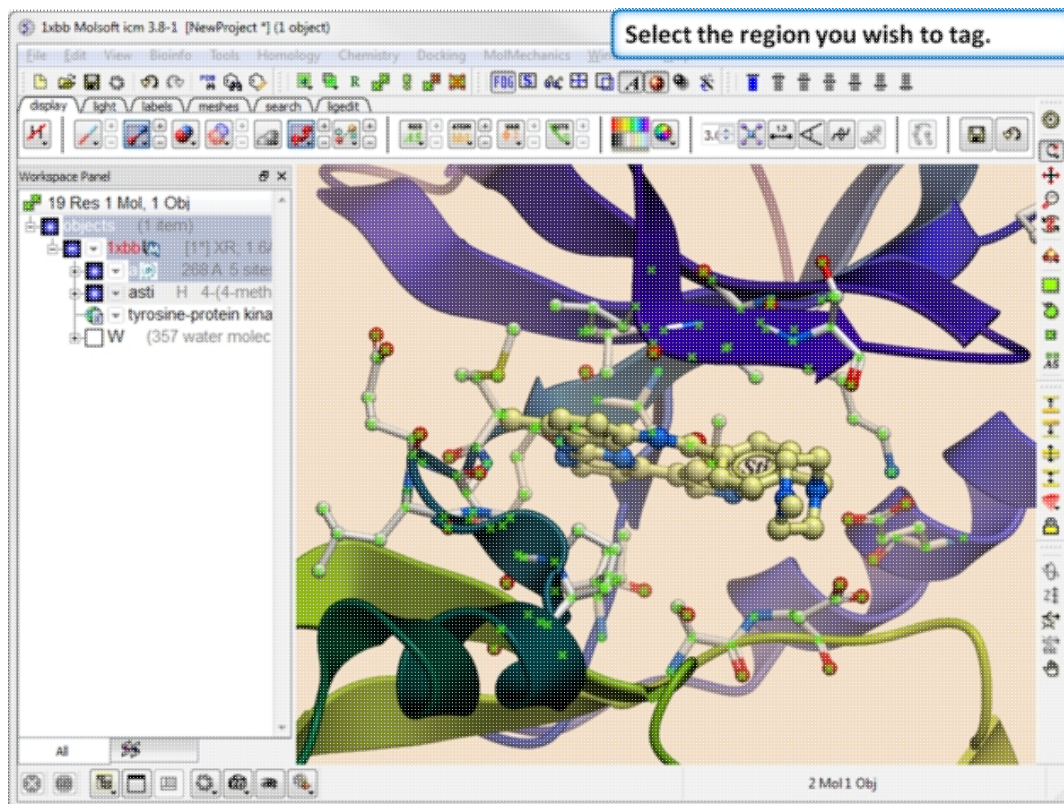
Step 1. Select the region you wish to tag.
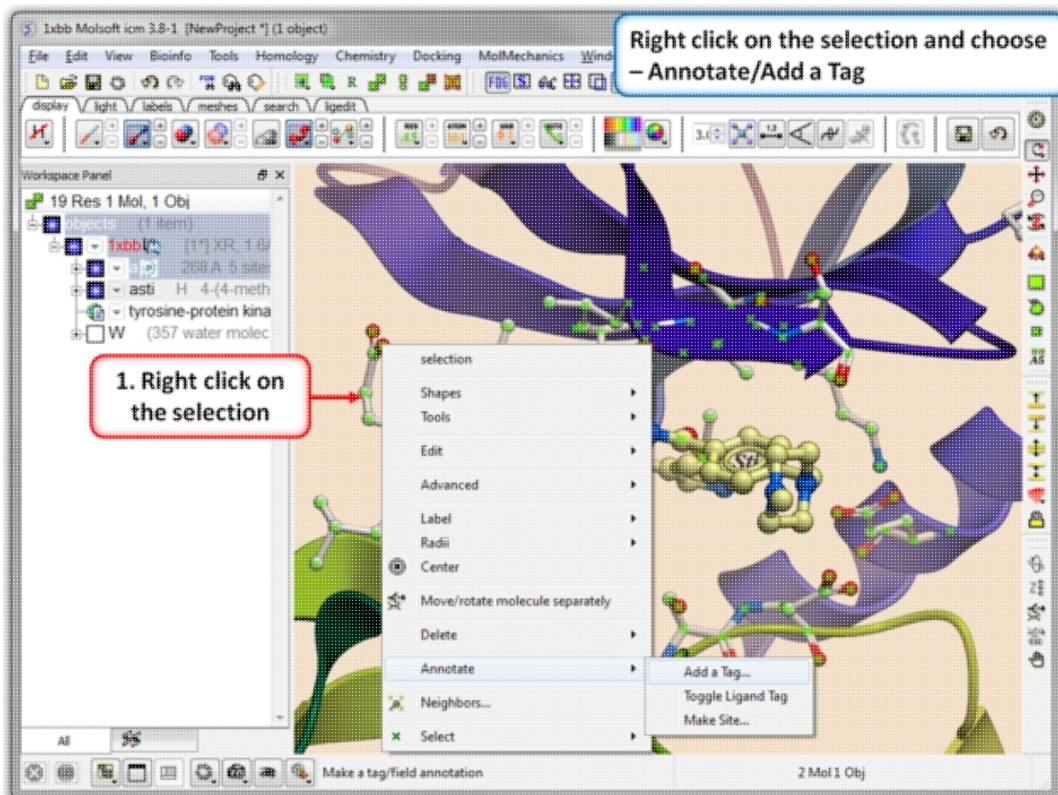
Step 2. Right click on the selection and choose Annotation/Add a Tag.
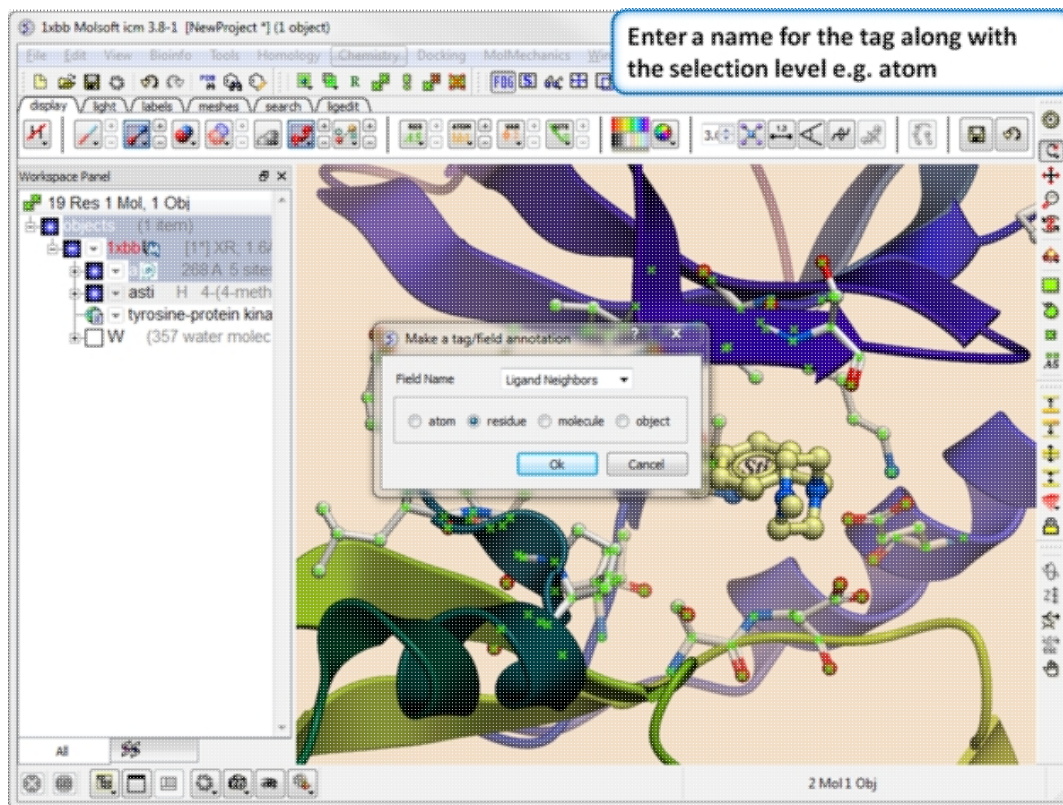
Step 3. Give the tag a name and choose the selection level (atom, residue, molecule, or object).

The tag is stored in the ICM workspace. Double click on it to select.

Double click to select

Toggle the tag on and off
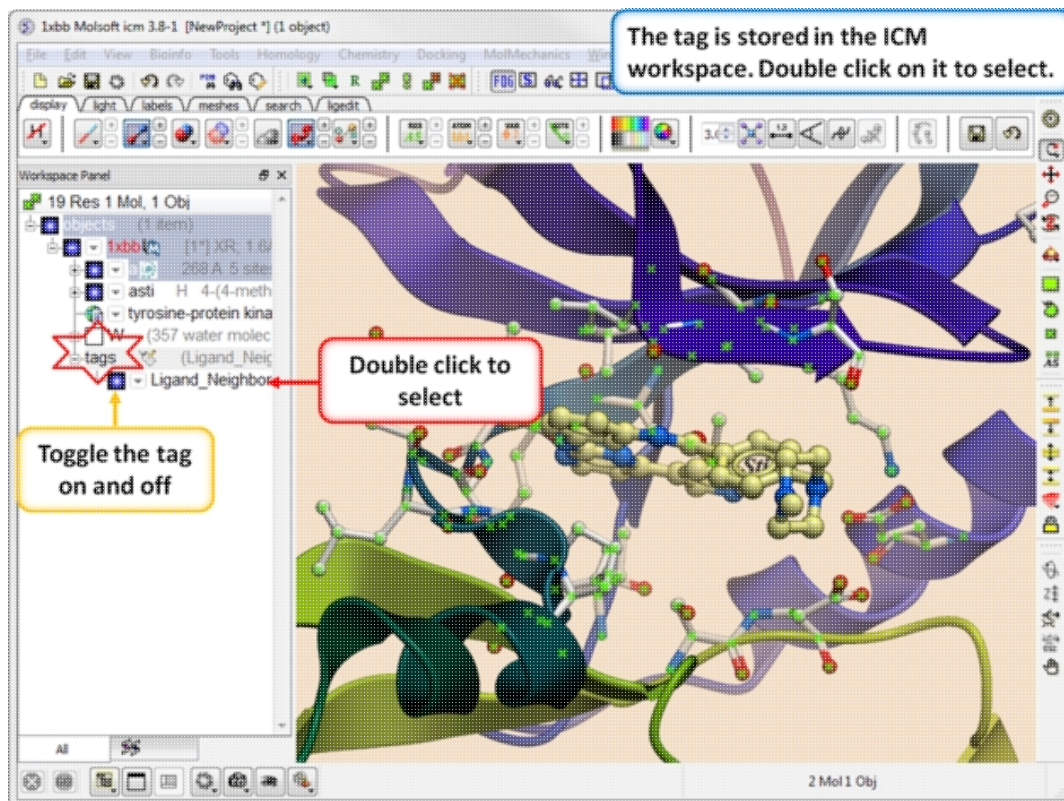
Step 4. You will see the tag in the ICM Workspace. You can toggle the display on or off or select the tag by double clicking on it in the ICM Workspace.

3.7 Preferences

Your ICM preferences can be changed by:

- Select File/Preferences.

NOTE: There is a "Reset to Default" button in case you make any changes you are not happy with and also a search option.
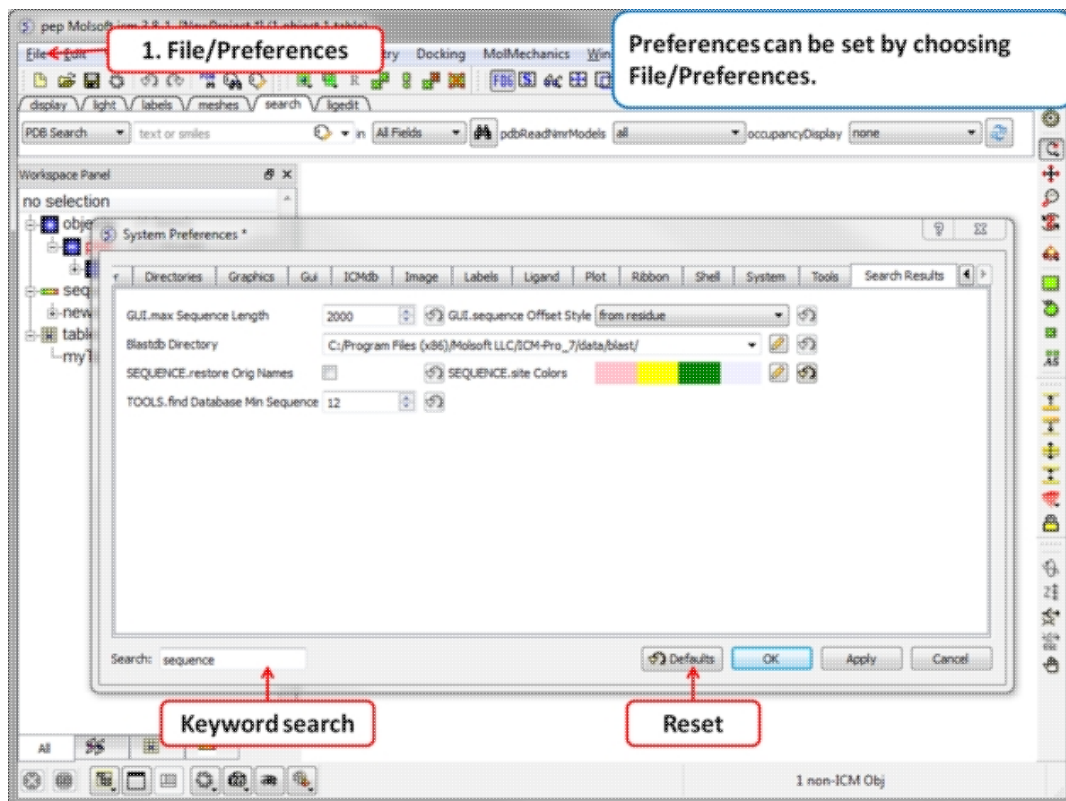
3.7.1 Bonds Preferences

To change Bond Preferences:

- Select File/Preferences.
- Choose the **Bonds** tab.

GRAPHICS.ballStickRatio - A default ratio of ball and stick radii. This ratio is applied when the styles are switched from the GUI xstick toolbar. Default (1.4)

GRAPHICS.hbond Ball Period - Default (3)

GRAPHICS.hbondMinStrength - parameter determines the hbond strength threshold for hbond display. The strength value is between 0. and 2. By changing 1. to 0.2 you will see more weak hydrogen bonds. Default: (1).

GRAPHICS.hbondStyle - determines the style in which hydrogen bonds are displayed. Here hbond-Donor, Hydrogen, and hbond-Acceptor atoms will be referred to as D, H and A, respectively,

GRAPHICS.hetatmZoom - The default ball and stick radii of a ligand can be different by the GRAPHICS.hetatmZoom factor. This makes a better ligand view since the ligand stands out from the surrounding protein atoms.

GRAPHICS.stickRadius - radius (in Angstroms) of a cylinder displayed as a part of stick or xstick graphical representation of a molecule. Individual (residue-wide) control of stick radii.

GRAPHICS.xstick Backbone Ratio - Default (1.2)

GRAPHICS.xstick Style - `xstick` style

wireBondSeparation the distance between two parallel lines representing a chemical double bond if wireStyle = "chemistry". Default (0.2 Angstroms).

GRAPHICS.distance Label Drag - enable distance label dragging

GRAPHICS.hbondAngleSharpness determines how the strength depends on the D-H...A(lone pair) angle. The preference can be found the general Preferences menu Default (1.7)

GRAPHICS.hbond Ball Style even, by atom size, by energy or telescopic

GRAPHICS.hbond Rebuild

GRAPHICS.hbondWidth relative width of a displayed hbond .

GRAPHICS.hydrogenDisplay determines the default hydrogen display mode for the display command.

```
 GRAPHICS.hydrogenDisplay = "polar"
        1 = "all"   # all hydrogens are shown
        2 = "polar" <-- current choice  # polar displayed, the non-polar hidden
        3 = "none"  # no hydrogens are displayed
```

GRAPHICS.wire Width - relative width of wire Default (1)

GRAPHICS.xstick Hydrogen Ratio - Default (0.5)

GRAPHICS.xstick Vw Ratio - Default (0.6)

Wire Style - change the default `wire` style

```
 GRAPHICS.hbondStyle = "dash"
        1 = "wire"       # Just a line
        2 = "chemistry"    # shows different types of chemical bonds.
        3 = "tree"  # shows a directed graph of the ICM-molecular tree
        4 = "aromatic"   #
```

3.7.2 Directories Preferences

DIRECTORIES TAB:

To change Directory Preferences:

- Select File/Preferences.
- Choose the **Directories** tab.

Within this tab you can select the default directories for:

FILTER.gz, FILTER.uue, FILTER.Z, Filter.zip allows you to read compressed files .gz, .uue, .Z, and, .zip files automatically leaving the compressed file intact.

PDB Directory Style - The style of your Protein Data Bank directory/directories. ICM will understand all of the listed styles, including distributions with compressed *.gz , *.bz2 and *.Z fil es

BlastDB Directory - return directory with Blast-formatted sequence files for ICM sequence searches.You can download Blast formatted databases from here `ftp://ftp.ncbi.nih.gov/blast/db/`

Dock Directory - Default directory for storing docking files.

CCP4 Directory

Editor - Select a default text editor

Inx Directory - location of stored index (*.inx) files.

Log Directory - when you quit an icm-session, a _seslog.icm file is automatically stored. If the s_logDir variable is empty, it is stored to the s_userDir + "/log/" directory. However one can redirect it to the current working directory (".") or any other directory.

Output Directory -

PDB Directory - directory containing the PDB database of 3D structures. These files can also be easily downloaded directly from the PDB site if the variables are set as in the example below. PDB distributions can exist in several styles (all files in the same directory, or divided etc.).

PDB Directory FTP

PDB Directory Web

Projects Directory - Select the default location for storing ICM projects. Save your data in an ICM project. It is a convenient way of keeping all your structures, alignments, tables, docking results etc... in one place. A description on how to save an ICM project is described in the GUI Basics section of this manual.

Prosite Dat - location of the prosite.dat file a dictionary of protein sites and patterns, (Copyright by Amos Bairoch, Medical Biochemistry Department, University of Geneva, Switzerland).

Ps Viewer - Select a postscript viewer

Swissprot Dat - location of swissprot.dat file

Temp Directory - scratch directory for temporary files (some montecarlo files will be saved there).

Uniprot Dat - location of uniprot.dat file

XPDB Directory - Path to the ICM XPDB database of compact binary ICM objects which are annotated with the site information. The advantage of the XPDB database is the speed of reading and smaller size than PDB. XPDB entries are read about 80 times faster!

TOOLS.default ChemDB

TOOLS.eds Directory

TOOLS.pdb Read Nmr Models

```
1. = "first" : reads only one model from a multi-model (e.g. NMR) pdb file
2. = "all" : reads all models from a multi-model (e.g. NMR) pdb file and creates a separate
3. = "all stack" : creates one object and loads all other models as a stored cartesian stac
```

3.7.3 Graphics Preferences

To change Graphics Preferences:

- Select File/Preferences.
- Choose the **Graphics** tab.

Atom Single Style - display style of isolated atoms in the wire mode.

```
1. "tetrahedron"
2. "cross"
3. "dot"
```

GRAPHICS.clash Style - choose clash length, strain or length.

GRAPHICS.clip Grobs - enable grob clipping.

GRAPHICS.clip Static -

GRAPHICS.grobLineWidth - relative width of displayed lines of 3D meshes (grobs). Also affects the interatomic distance display.

GRAPHICS.lightPosition - X ,Y and Z posiion of the light source in the graphics window. The X and Y coordinates are usually slightly@@ beyond the [-1. 1] range where [-1.,1.] is the size of the window, and the Z position is perpendicular to the screen and is set to 2. (do not make it negative).

GRAPHICS.occupancyDisplay preference controlling if and how the partical or zero atom occupancies are displayed. The abnormal occupanices are shown as circles around atoms. These following values are allowed.

```
1. = "none" # nothing is displayed
2. = "circle" # a circle is displayed
3. = "label" # a circle and a lable with the value (zero values are not shown)
```

GRAPHICS.quality - integer parameter controlling quality (density of graphical elements) of such representations as cpk, ball, stick, ribbon . Do not make it larger than about 20 or smaller than 1.

GRAPHICS.ruler Style - change ruler from center to side

GRAPHICS.stereoMode - 1. "up-and-down", 2. "line interleaved" 3. "in-a-window"

- a simple hardware stereo mode for workstations with a horizontal frame splitter.
- In the "up-and-down" mode a longer frame with two stereo images on top of each other is generated and the two halves are then superimposed with the splitter. This mode does not require anything from a graphics card, but does require a frame splitter. A frame splitter box was connected between a monitor and a graphics card output. This mode has an unpleasant side effect, the rest of the screen (beyond the OpenGl window) becomes stretched and the lower part of the screen is superimposed on the top half.
- The "line interleaved" mode can be used with a new type of frame splitter at the line level. In this case the odd lines from one stereo-image are interleaved with the even lines of another. The side-effect of this mode is that the intensity is reduced in half since at each moment one sees only one half of the lines. The splitter device for this mode can be purchased from Virex (www.virex.com). This mode produces a dark stereo image but is easily available (requires stereo goggles, e.g. from Virex).
- The "in-a-window" mode is used in SGI workstations and in a Linux workstation with an advanced graphics card supporting a quad graphics buffer. In this mode the hardware stereo regime applies only to an OpenGl window. This is the best mode but it requires an expensive graphics card (plus the stereo goggles).

GRAPHICS.surfaceDotDensity - Determines the number of dots per square Angstrom on the graphical solvent accessible surface.

GRAPHICS.surfaceProbeRadius - An increment to the van der Waals radii of atoms at thich the dotted atomic surface is calculated. It is used by the display surface command to display dotted van der Waals surface. If the GRAPHICS.surfaceProbeRadius is set to 1.4 the surface becames equivalent to the solvent accessible surface with a probe of 1.4A

GROB.arrowRadius - a real arrow radius in Angstoms used by the Grob("ARROW", R_) function. Default: 0.5.

GROB.contourSigmaIncrement - a real increment in the sigma level used to re-contour an electron density map using the make grob m_eds add r_increment command. This parameter is used in the GUI when plus and minus are pressed.

GROB.relArrow Size - a real ratio of the arrow head radius to the arrow radius. This parameter is used by the Grob("ARROW", R_) function. Default: 3.0.

shineStyle - defines how solid surfaces of cpk , skin and grobs reflect light. Possibilities:

```
1. "white" <- default
2. "color"
```

The first option gives a more shiny and greasy look.

GRAPHICS.center Follows Clipping - determine the function of center button.

GRAPHICS.clashWidth - relative width of a displayed clash .

GRAPHICS.clip Skin - enable skin clipping.

GRAPHICS.displayMapBox - controls if the bounding box of a map is displayed

GRAPHICS.light - a rarray of 13 elements between 0. and 1. which controls the main properties of lighting model in GL.

GRAPHICS.mapLineWidth - relative width of lines and dots of a displayed map.

GRAPHICS.occupancy Radius Ratio - preference controlling the radius of the partical or zero atom occupancies

GRAPHICS.resize Keep Scale

GRAPHICS.selectionStyle - preference for the style in which the graphical selection is shown. The preference may have the following values.

GRAPHIC.store Display - maintains representation and coloring for an object.

GRAPHICS.surfaceDotSize - Determines the size of the dot on the solvent accessible graphical surface.

GRAPHICS.transparency - Two parameters regulating the transparency of grobs.

GROB.atomSphereRadius - default radius (in Angstroms) which is used to select a patch on the surface of a grob.

GROB.relArrowHead - a real ratio of the arrow head radius to the arrow radius.

lineWidth - the real width of lines used to display the wire representation of chemical bonds.

3.7.4 GUI Preferences

GUI TAB:

The options contained within the Preferences/Gui tab are described below.

GRAPHICS.alignment Rainbow - This option controls how alignments are colored by default.

GRAPHICS.NtoC Rainbow - Controls the coloring of structural representation from the N-terminal to the C-terminal

GRAPHICS.rocking - Controls default `rocking motion`.

GRAPHICS.rocking Speed - Controls rocking or rotation `speed`.

GUI.auto Save Interval - Controls auto save period (minutes)

GUI.table Row Mark Colors - Controls colors used for marking tables.

GUI.workspaceTabStyle - Controls the style of ICM-object tabs created in the `workspace panel` of ICM GUI.

Movie.fade Nof Frames - Controls number of frames for the fade out option in `screenshot movie` making.

Movie.quality - Controls the resoltuion of the movie

SEQUENCE.site Colors - Controls coloring of squence sites.

SLIDE.ignore Fog - Fog representations can be ignored in `slide` preparation if desired.

GRAPHICS.discrete Rainbow -

GRAPHICS.rainbow Bar Style - determines if and where the color bar will appear after a molecule is colored by an array.

GRAPHICS.rocking Range - real value of rocking range.

GUI.auto Save - auto save on or off

GUI.max Sequence Length - maximum sequence length displayed in ICM

GUI.workspace Folder Style - `Workspace` folder style.

MOVIE.frame Grab Mode - with `screenshot movie` making you can choose either fixed frame time or real time.

Movie.quality Auto - with `screenshot movie` making you can allow ICM to control the movie resolution.

SLIDE.ignore Background Color - Ignore background color when you are making a `slide`.

3.7.5 GUI Preferences

To change GUI Preferences:

- Select File/Preferences.
- Choose the **GUI** tab.

Quality - controls the quality (density of graphical elements) of such representations as cpk, ball, stick, ribbon . Do not make it larger than about 20 or smaller than 1. We recommend to make this parameter at least 15 if you want to make a high quality image. You can also increase the number of image resolution by making the image window 2,3,4 times larger (in the example below it is 2 times larger) than the displayed window.

Wire Style - Four different wire styles are available.

Hydrogen Display - Select whether you always want all hydorgens displayed or just-polar hydrogens or no hydrogens at all.

Rainbow Scale - determines if and where the color bar will appear after a molecule is colored by an array. Coloring by an array is one of the options of the display and color commands.

```
1. = "left" <- default choice
2. = "right"
3. = "no text"
4. = "no bar"
```

Ball Ratio - The ratio of ball and stick radii. This ratio is applied when the styles are switched to xstick from the GUI xstick toolbar.

Selection Style - Change the graphical display of your selections. Default is a green cross.

Clash Threshold - a clash is defined as an interatomic distance less than a sum of van der Waals radii of two atoms of interest multiplied by the clashThreshold parameter. For hydrogen bonded atoms, the distance threshold is additionally reduced by 20% . Default = 0.82

DotSurfaceRadiusIncrement - adius of a probe sphere used to display a dotted surface of a molecule. All van der Waals radii are expanded by this value. vwExpand=0 corresponds to the CPK surface, vwExpand=1.4 corresponds to the water-accessible surface. Be aware of the difference between the waterRadius and vwExpand parameters: waterRadius is used in

- show energy "sf"
- show [area|volume] skin
- display skin while vwExpand is used in
- show [area|volume] surface
- display surface

Default (1.4).

H Bond Style - How do you wish your H-Bonds to be displayed by default? Dashes, Bond Length, Bond Lenght and Angle.

grobLineWidth - relative width of displayed lines of 3D meshes (grobs). Also affects the interatomic distance display.

general line with - the real width of lines used to display the wire representation of chemical bonds. See also IMAGE.lineWidth parameter which controls line thickness in molecular images generated by the write postscript command, and the PLOT.lineWidth which controls the width for the plot command. Default (1.0)

single atom as - display style of isolated atoms in the wire mode.

```
1. "tetrahedron"
2. "cross"
3. "dot"
The size of the first two representation is controlled by the GRAPHICS.ballRadius parameter an
```

xstickhetatomzZoom - The default ball and stick radii of a ligand can be different. This makes a better ligand view since the ligand stands out from the surrounding protein atoms.

solid shine style - choose either white or color

Stick Radius - radius (in Angstroms) of a cylinder displayed as a part of stick or xstick graphical representation of a molecule. Individual (residue-wide) control of stick radii.

Stereo Mode - Select a default stereo mode

Display Style - A default display style can be chosen using a combination of styles.

Water Radius - radius of water sphere which is used to calculate an analytical molecular surface (referred to as skin) as well as the solvent-accessible surface (centers of water spheres).

clashWidth - relative width of a displayed clash.

hbondWidth - relative width of hydrogen bond display

mapLineWidth - relative width of lines and dots of a displayed map.

3.7.6 Image Preferences

To change Image Preferences:

- Select File/Preferences.
- Choose the **Image** tab.

IMAGE.color - logical to save color or black_and_white ('bw') images.

IMAGE.gammaCorrection - real variable to to lighten or darken the image by changing the gamma parameter. A gamma value that is greater than 1.0 will lighten the printed picture, while a gamma value that is less that 1.0 will darken it.

IMAGE.lineWidth - this real parameter specifies the default line width for the postscript lines.

IMAGE.orientation - image orientation.

IMAGE.previewer - a string parameter to specify the external filter which creates a rough binary (pixmap) postscript preview and adds it to the header of the ICM-generated high resolution bitmap or vectorized postscript files saved by the write image postscript, and write postscript , respectively .

IMAGE.print - unix command for printer.

IMAGE.scale - real variable. If non zero, controls the image scale with respect to the screen image size.

IMAGE.stereoBase - real variable to define the stereo base (separation between two stereo panels) in the write image postscript and write postscript command.

IMAGE.writeScale - an integer parameter used to increase the image resolution in the `Quick Image Write tool`.

IMAGE.bondLength2D - real length of a chemical bond (in inches) in chemical 2D drawings upon the Copy Image command.

IMAGE.compress - logical to toggle simple lossless compression, standard for .tif files. This compression is required to be implemented in all TIFF-reading programs.

IMAGE.generateAlpha - logical to toggle generation of the alpha (opacity) channel for the SGI rgb, tif and png image files to make the pixels of the background color transparent.

IMAGE.lineWidth2D - integer thickness of bonds in chemical 2D drawing upon the Copy Image command. This is useful for cutting and pasting from ICM to external documments.

IMAGE.paper Size - specify paper size.

IMAGE.previewResolution - integer resolution of the rough bitmap preview added to the vectorized postscript file in lines per inch.

IMAGE.printerDPI - this integer parameter the printer resolution in Dot Per Inch (DPI). Important for the write image postscript command.

IMAGE.stereoAngle - real variable to define stereo angle (relative rotation of two stereo images) in the write image postscript and write postscript command.

IMAGE.stereoText - logical to make text labels for only one panel or both panels of the stereo diagram.

3.7.7 Label Preferences

To change Label Preferences:

- Select File/Preferences.
- Choose the **Labels** tab.

atomLabelStyle style of atom labels invoked by clicking on an the `atom label button`.

GRAPHICS.displayLineLabels - enables/disables the display of edge lengths (inter-point distances) of a grob generated with the Grob("distance" ..) function.

GRAPHICS.font Line Spacing - Change the spacing between lines in labels.

GRAPHICS.resLabelDrag - if yes, enables dragging of the displayed residue labels with the middle mouse button.

GRAPHICS. site Arrow - Highlight sites with an arrow yes or no.

Show Res Code In Selection - When you make a selection the icm selection language will be displayed when you right click on the selection.

Res Label Style - Default `residue label` style.

SITE.label Style - Default `label sites` style.

Var Label Style - Default `label variable` style.

GRAPHICS.atomLabelShift - a non-negative integer number of spaces preceding an atom label. This parameter is useful for displaying labels next to a solid representation,

GRAPHICS.fontColor - set font color

GRAPHICS.font Scale - set font size

GRAPHICS.site Label Shift - GRAPHICS.resLabelShift a non-negative integer number of spaces preceding a site label.

GRAPHICS. site Label Drag - if yes, enables dragging of the displayed site labels with the middle mouse button.

Res Label Shift - a non-negative integer number of spaces preceding a residue label. This parameter is useful for displaying residue labels next to a solid

SITE.labelOffset - (default 5. A) the real offset of the site label with respect to the residue label atom.

SITE.wrap Comment - Number of characters per comment line.

3.7.8 Plot Preferences

To change Plot Preferences:

- Select File/Preferences.
- Choose the **Plot** tab.

PLOT.color - logical to generate a color plot. Usually it does not make sense to switch it off because your b/w printer will interpret the color postscript just fine anyway.

PLOT.draw Tics logical yes or no

PLOT.fontSize real font size. Any reasonable number from 3. (1 mm, use a magnifying glass then) to 96.

PLOT.lineWidth - real line width for graphs (not the frame and tics)

PLOT.markSize - real mark size in points. Allowed mark types: line, cross, square, triangle, diamond, circle, star, dstar, bar, dot, SQUARE, TRIANGLE, DIAMOND, CIRCLE, STAR, DSTAR, BAR. Uppercase words indicate filled marks.

PLOT.paper Size - preference to specify plor paper size

PLOT.rainbowStyle - preference defining the color spectrum used by the plot area command.

PLOT.Yratio - real aspect ratio of the ICM plot frame. Using link option of the plot command is equivalent to setting this variable to 1.0. If PLOT.Yratio is set to 0. , the ratio will be set automatically to fill out the available box optimally.

[PLOT.date} - display date on plot

PLOT.font - preference for the title/legend font.

PLOT.labelFont - preference for the data point label font.

PLOT.logo - logical switch for the ICM-logo on the plot.

PLOT.orientation - preference for the plot orientation.

PLOT.previewer - command to local ps viewer

PLOT.seriesLabels - preference to indicate position of a series/color legend inside the plot frame.

3.7.9 Ribbon Preferences

To change Ribbon Preferences:

- Select File/Preferences.
- Choose the **Ribbon** tab.

Combo Display Style - select ribbon-cpk, atoms, ribbon-ligand, chemical

GRAPHICS.dnaRibbonRatio - real ratio of depth to width for the DNA ribbon .

GRAPHICS.dnaRibbonWorm - logical which, if yes, makes the DNA backbone ribbon round, rather than rectangular. Default: no

GRAPHICS.dnaWormRadius - real radius of the worm representing bases in DNA ribbon .

GRAPHICS.ribbonWidth - real width of the protein ribbon .

GRAPHICS.wormRadius - radius of coiled segments (i.e. those where the secondary structure is marked as "_") of a polypeptide chain in ribbon representation. Default (0.3).

Ribbon Style - specifies default style when `ribbon` is displayed.

GRAPHICS.dnaBallRadius - DNA bases in ribbon representation are shown as balls controlled by this real parameter.

GRAPHICS.dnaRibbonWidth - real width (in Angstroms) of the DNA ribbon .

GRAPHICS.dnaStickRadius - real radius of the sticks representing bases in DNA ribbon .

GRAPHICS.ribbonRatio - real ratio of depth to width for the protein ribbon .

GRAPHICS.ribbonWorm - logical parameter, if yes, makes the ribbon round, rather than rectangular.

ribbonColorStyle -

```
- sets the ribbon coloring scheme.
1 = "type"       default. colors by secondary structure type or explicit color
2 = "NtoC"       colors each chain gradually blue-to-red from N- to C- (or from 5' to 3' for DN
3 = "alignment" if there is an alignment linked to a protein, color gapped backbone regions gr
4 = "reliability"       3D gaussian averaging with selectSphereRadius of alignment strength in
If ribbonColorStyle equals to 4, the conserved areas will be colored blue, while the most dive
```

3.7.10 Shell Preferences

To change Shell Preferences:

- Select File/Preferences.
- Choose the **Shell** tab.

Clash Threshold - a clash is defined as an interatomic distance less than a sum of van der Waals radii of two atoms of interest multiplied by the clashThreshold parameter.

Map Sigma Level - (in Rmsd values over the mean value). Margin value used for making graphical objects contouring the 3D density map .

Mnconf - maximal number of conformations in the conformational stack . The stack stops growing after this number is achieved and starts replacing representative conformations with higher energy values by new conformations with superior energies, if the latter are found.

Icm Prompt - defines the ICM-prompt string.

Select Min Grad - default minimal gradient vector length for gradient atom selection (a_//G). This parameter is also used by the montecarlo fast command, which requires a value of 2. to 10. for optimal performance.

Map Atom Margin - Margin in Angstoms around selected atoms. The margin is added to the positional boundaries to define a submap index box in the Map (map_source , as_) function.

maxColorPotential - local electrostatic potential in kcal/e.u.charge units at which the surface element is colored by extreme red or extreme blue. All higher values will have the same color. This absolute scaling is convenient to develop a feeling of electrostatic properties of molecular surfaces.

mnSolutions - this parameter limits the number of hits retained by the program after a search.

Real Format - format of real numbers

Water Radius - radius of water sphere which is used to calculate an analytical molecular surface

3.7.11 System Preferences

To change System Preferences:

- Select File/Preferences.
- Choose the **System** tab.

FTP.createFile -

FTP.proxy - string path to the proxy server for connections through firewall. Default: "" (empty string).

GUI.max Nof Recent Files - maximum number of `recent files` stored.

GUI.splash Screen Image - path to splash image displayed on startup

HTTP. support Cookies - http support cookies yes or no

HTTP.user Agent - client application used within a particular network protocol for www

Beep - warning beep yes or no

Max File Size Mb - Maximu file size in MegaBytes that can be loaded into ICM.

USER.friends

USER.organization

FTP.keep File - (default no). If yes, the temporary file is kept in the s_tempDir directory. Otherwise the file is deleted.

GUI.enumberation Memory Limit - memory limit for enumeration operations.

GUI.splash Screen Delay

HTTP.proxy - string for HTTP server for connection through firewall

HTTP.timeout - timeout in seconds

Http Read Style icm or lynx

Force Auto Bond Typing - yes/no

USER.email, USER.full Name, USER.phone

4 Protein Structure
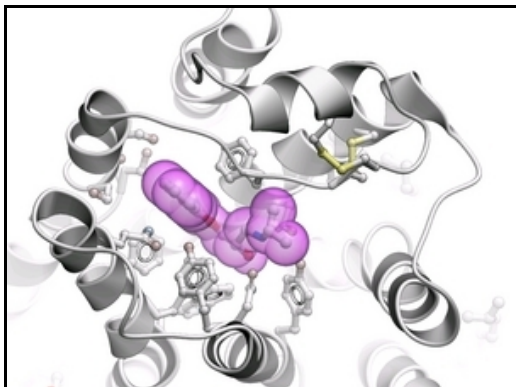
Chapter Contents:

- How to Convert Proteins and Chemicals to ICM Objects.
- Binding Site Display Tools.
- Crystallographic Analysis Tools.
- Protein Superposition.
- Protein Structure Analysis.
- Protein Structure Prediction Tools e.g. ICM Pocket Finder.

4.1 Convert to ICM Object

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro | ICM-Chemist

4.1.1 Load a Protein Structure

There are a couple of ways to read into ICM a protein structure.

1. Search the PDB using the Search Tab.
2. Load in a PDB file that you have saved on your computer using
   File/Open.

4.1.2 Converting PDB Files Into ICM Objects

If you are going to make any type of energy calculation in ICM (eg
docking, display H-bonds, display electrostatic and binding property
surfaces etc..) it is necesary to convert a protein or chemical into an
ICM object. Be aware that upon conversion ICM adds missing side-chain
atoms (but wont try to build missing loops) due to the nature of the
internal-coordinate system. The list of residues/ atoms added is
presented in the command line shell and can be reviewed. For reference,
the original PDB entry is kept in the system. See the command line
manual for a more complete description of what the conversion process
does.

NOTE: Before converting a protein structure to an ICM object make
sure that the chemicals contained within the structure (e.g. ligands)
are correct. If an error is found you can edit the ligand as
described here.

**To convert a PDB structure into an ICM object follow the steps shown
below:**

- Right click on the name of the protein you wish to convert in the `ICM Workspace`.
- A dialog box will be displayed as shown below.
- If you want to delete the water molecules select **Delete Waters**
- If you want to optimize hydrogen atoms (recommended for important work) select **Optimize Hydrogens**. This option performs global optimization of hydrogens to find the best hydrogen bonding network.
- If you want to optimize the orientation of His, Pro, Asn, Gln, Cys residues then choose **optimizeHisProAsnGlnCys.** The following residues will be further optimized: His - three protonation states and two rotations will be tried and the residue will be renamed according to its subtype: hie (epsilon tautomer) or hip (+). Asn and Gln - (a 180 deg. flip will be tried). Cys - in the vicinity of Zn, Cu, Fe and Co to cym.
- If you want to keep a copy of your PDB file uncheck the option **replace original**.
- The converted structure can be displayed immediately by checking **display the result**
- **Uncheck** the box **hide missing side chains** if you want ICM to build missing heavy atoms that are not reported in the PDB (due to the lack of density), they will be added according to the residue name and assigned zero occupancies. **Check** this box if you want residues missing heavy atoms to be hidden.
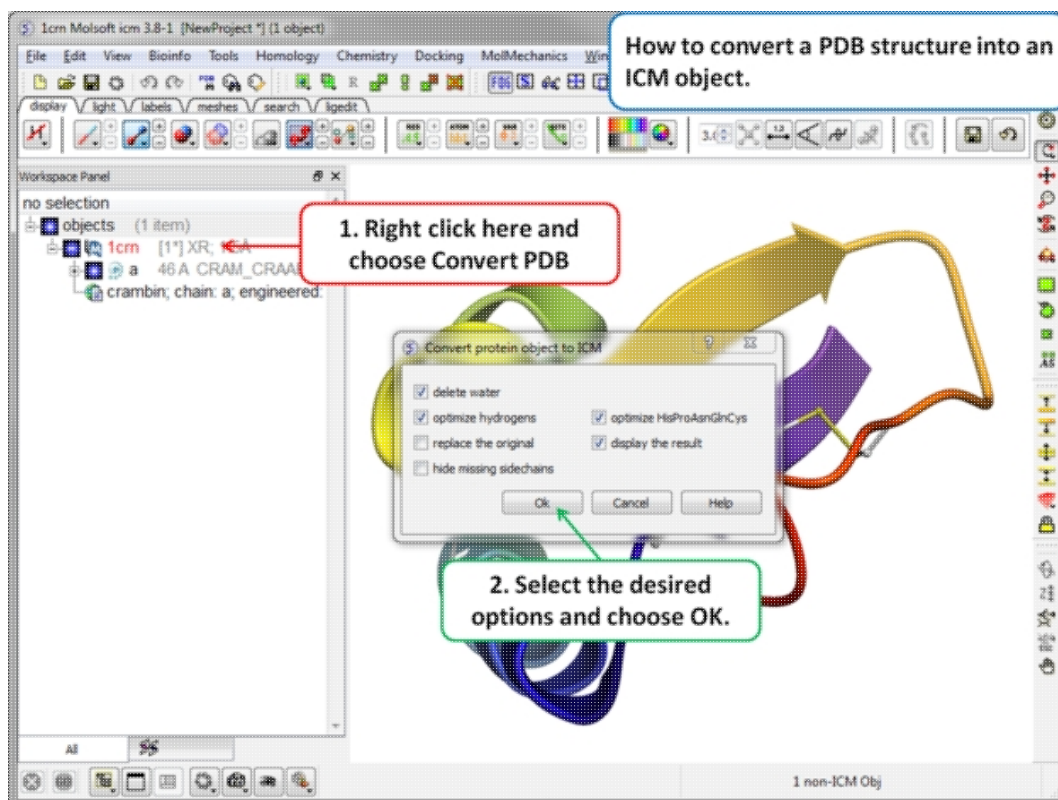
If your object is an ICM object it will display **ICM** next to the molecule in the ICM Workspace.
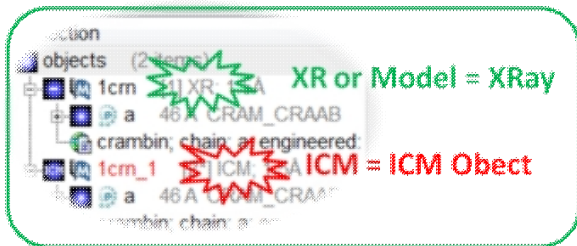
4.2 Pocket Display

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro

4.2.1 Receptor Pocket Surface.

To display the Receptor Pocket Surface:

- As an example we will use the PDB structure 1STP. Type 1STP in the
  pdb search tab and press return.
- Convert the protein to an ICM object. If you do not convert a
  generic surface will be displayed that is not colored by binding
  property.
- Right click on the ligand "abtn" and select **Pocket/Receptor
  Pocket**.
- Select whether you would like to display side-chain hydrogen bonds
  and label.
- The receptor pocket will be displayed colored by binding property
  - White=aromatic lipophilic, Green=non-aromatic other (mostly
  aliphatic) lipophilic surface, Red=hydrogen bonding acceptor
  potential, Blue=hydrogen bond donor potential.
- The surface can be toggled on and off by selecting in the ICM
  Workspace in the **meshes** section.
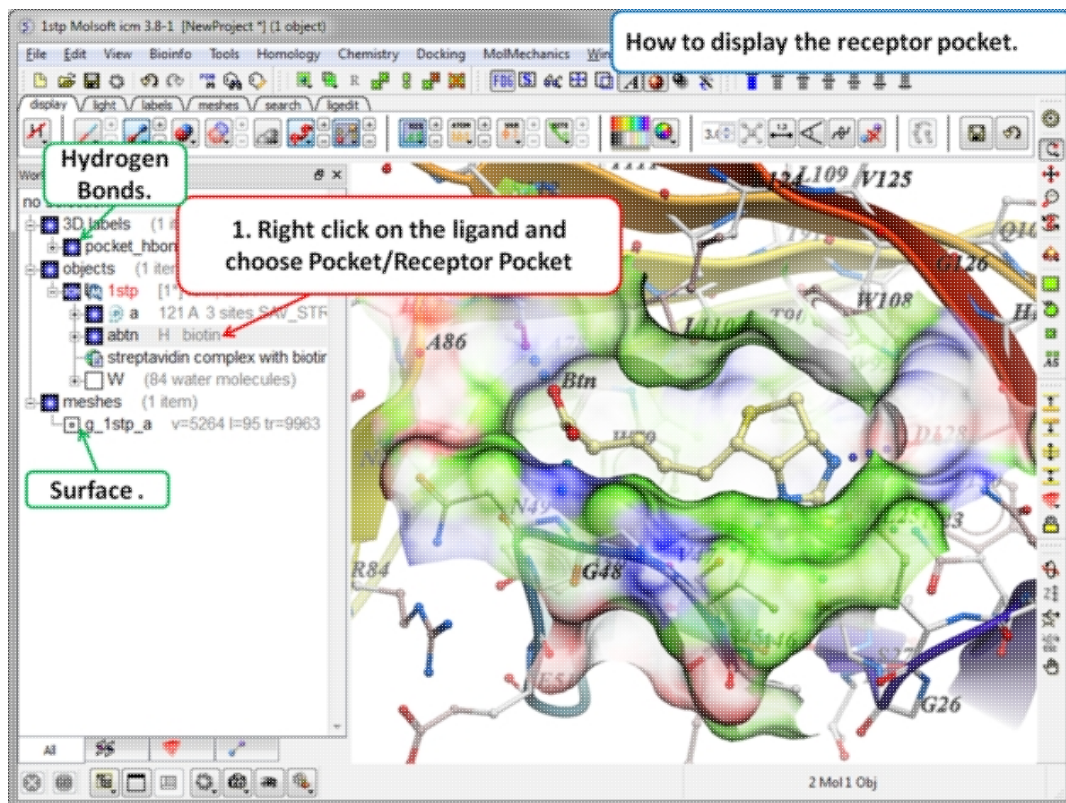
4.2.2 Ligand Surface.

The Ligand Surface option allows you to visualize cavities that are open for ligand modifications. To display the Ligand Surface:

- As an example we will use the PDB structure 1STP. Type 1STP in the `pdb search tab` and press return.
- `Convert` the protein to an ICM object. If you do not convert a generic surface will be displayed that is not colored by binding property.
- Right click on the ligand "abtn" and select **Pocket/Ligand Surface**.
- The Ligand Surface will be displayed colored by binding property - White=aromatic lipophilic, Green=non-aromatic other (mostly aliphatic) lipophilic surface, Red=hydrogen bonding acceptor potential, Blue=hydrogen bond donor potential.

How to display the ligand surface.

1. Right click on the ligand and choose Pocket/Ligand Surface
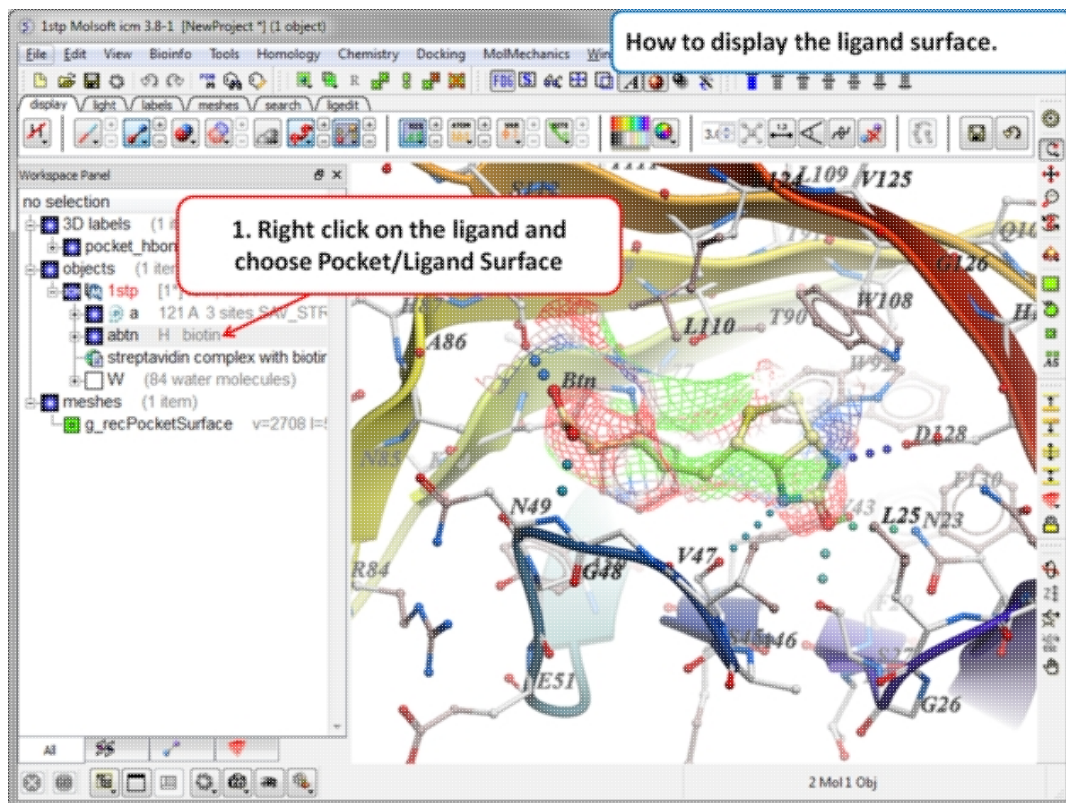
4.2.3 How to Display Hydrogen Bonds

NOTE: The method by which hydrogen bonds are calculated is described here in the command line manual. The GRAPHICS.hbondMinStrength parameter determines the hbond strength threshold for hbond display. The strength value is between 0. and 2. By changing 1. to 0.2 you will see more weak hydrogen bonds.

- In order to display energy related properties we need to convert the PDB file into an ICM object. Convert 1STP into an ICM object. In this example, the option "Replace the Original" was selected.
- Display the receptor in wire format and the ligand in xstick.
- Right click on the ligand and select "Neighbors" - Enter 3 Angstroms and Type = Visible. Do not exclude source (the ligand) therefore remove tick from box entitled "exclude source".
- Select the display tab and then select the Display H-Bond button.
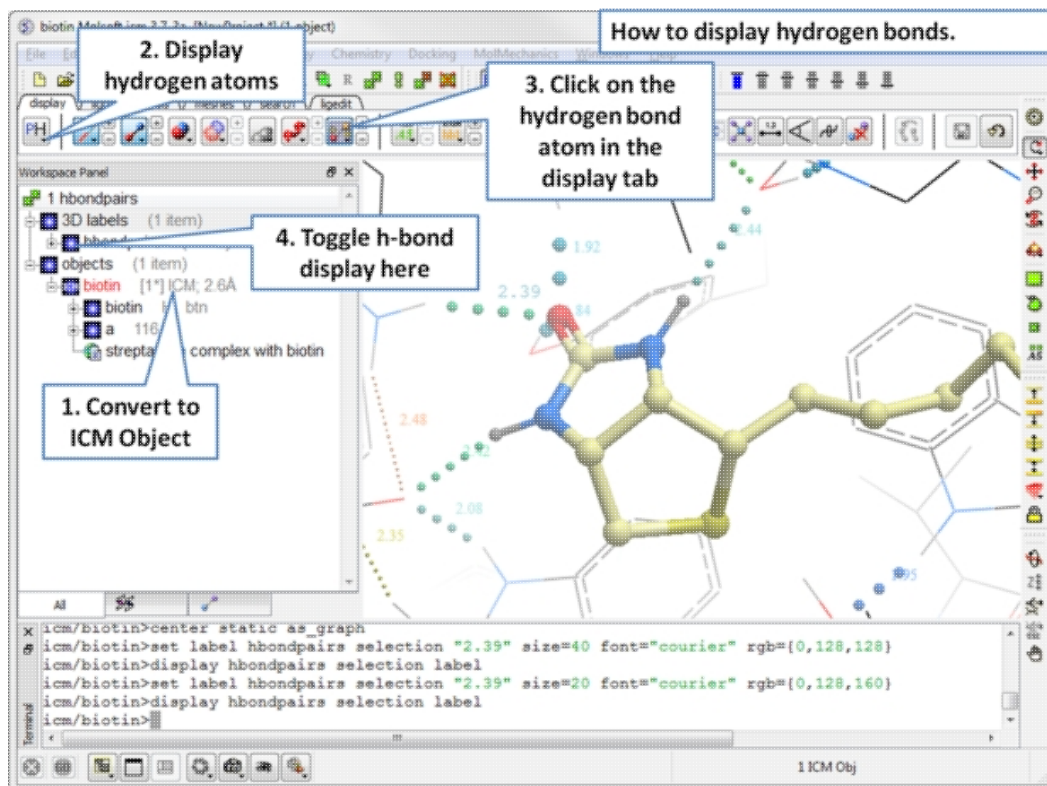
NOTE: Different options for displaying the H-bond can be accessed by clicking and holding on the H-bond button in the "Display" tab. The coloring of the H-bonds are red (strong - thick spheres) to blue (weak - thin spheres). Once the hydrogen bonds have been displayed they can be displayed and undisplayed in the 3D labels section of the ICM Workspace (left hand side of graphical window).

4.2.3.1 Edit Hydrogen Bond Label

To edit a hydrogen bond label

- Right click on the label.
- Choose Edit Label.
- Make changes in the dialog box and press OK.

How to edit a hydrogen bond label.
Right click on the label and choose
"Edit Label".

1. Right click here

3D label hbondpairs[1][20]
✓ text label
✓ distance,[A]
  solid line
  dotted line
✓ spheres

  Edit Label...
  Reset Label Position
  Set Sphere Period...
  Change Color...
✗ Delete

Enter the label details and change font.

1. Edit label if needed here

2. Change font
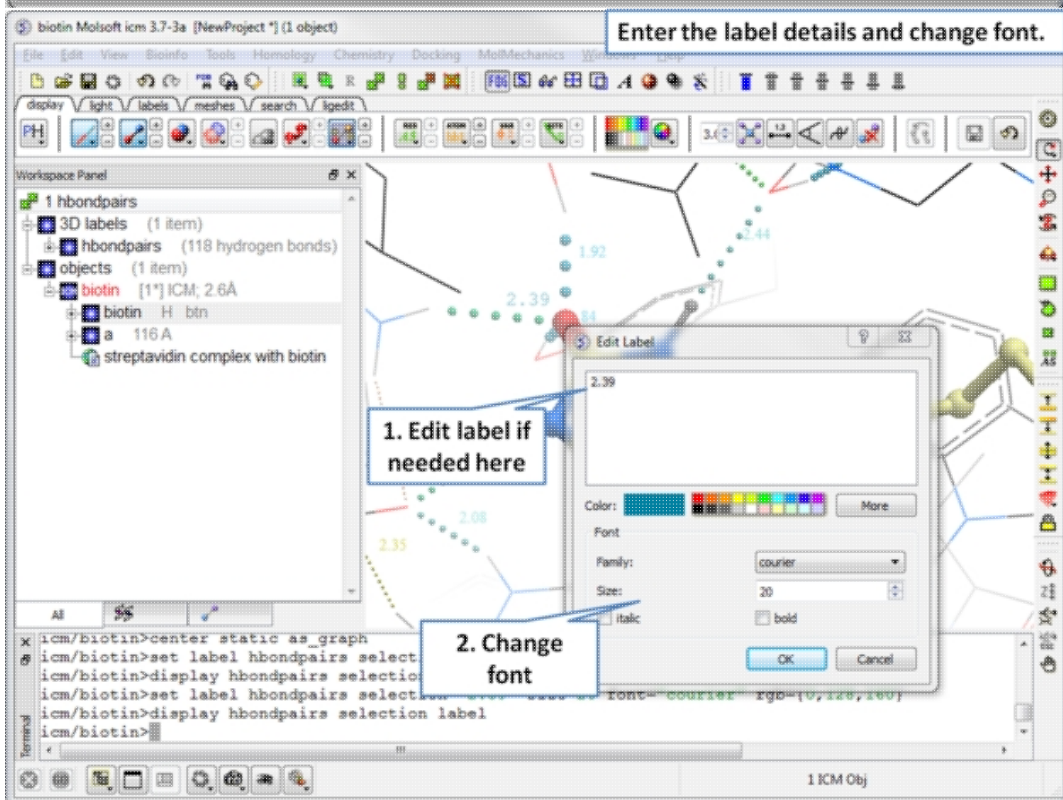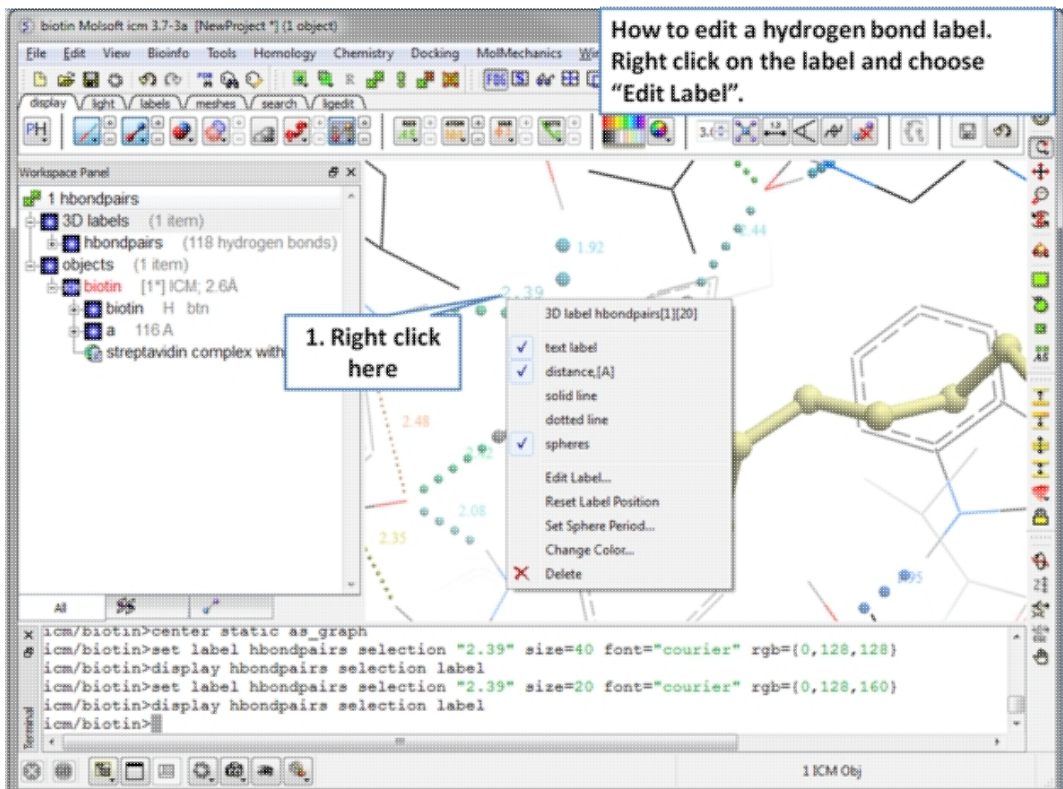
4.2.3.2 Move Hydrogen Bond Label

To move a hydrogen bond label

- Right click on the label.
- Click on the label using the middle mouse button.

- Hold the middle mouse button down and drag.
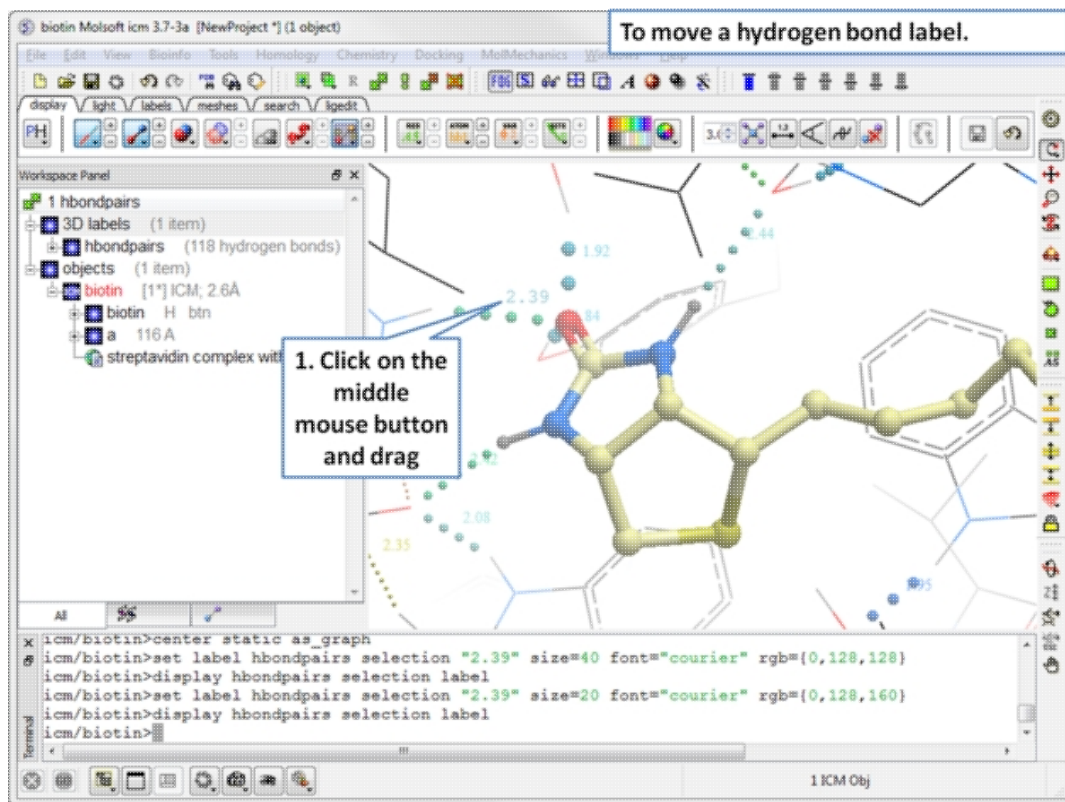
4.2.4 Ligand Pocket Interactions

To view the ligand pocket interactions.

- Right click on the ligand and choose Pocket.
- Choose Ligand Pocket Interactions. Hydrogen bonds, key waters and neighboring residues will be displayed.
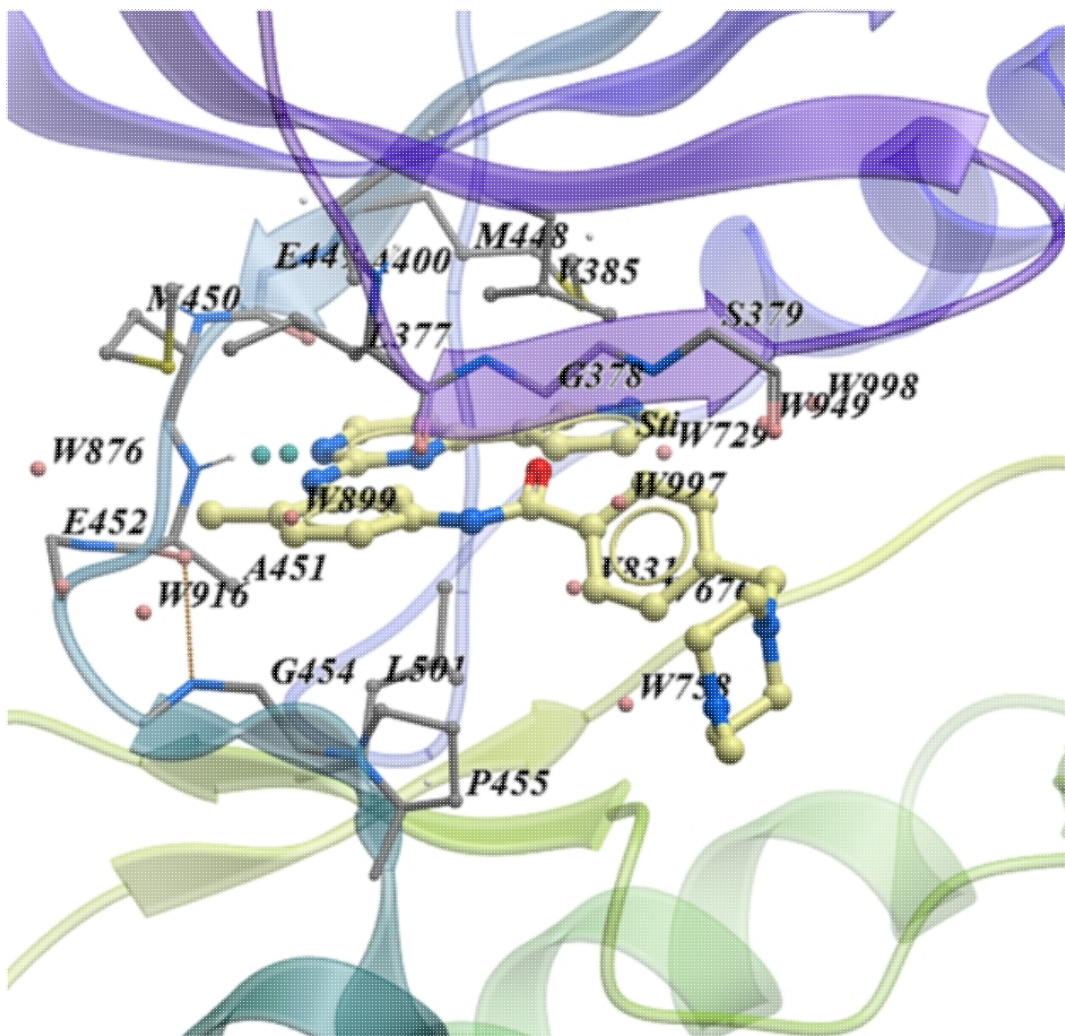
4.3 Crystallographic Analysis

Available in the following product(s): `ICM-Browser-Pro` | `ICM-Pro`

4.3.1 Crystallographic Neighbor

Theory

Molecular objects and 3D density maps may contain information about crystallographic symmetry. It consists of the following parameters:

1. Crystallographic group eg. P2121 that determine N (depends on a group) transformations for the atoms in the asymetric unit.
2. Crystallographic cell parameters A, B, C, Alpha, Beta and Gamma

To generate the coordinates within one cell one needs to apply N transformations and then to generate neigboring cells the content of one cell needs to be translated in space according to the cell position.

ICM has a function which generates crystallographic neighbors for the selected atoms. For large proteins it is impractical to generate neighbors for the whole molecule due to the high number of atoms in all neighboring molecules.

This information allows to generate symmetry related parts of the
density or molecular objects.

To generate symmetry related molecules around a selection of atoms:

- Read a PDB file into ICM. For instruction see the section entitled
  `Search PDB`.
- Display the structure and select the residues around which the
  symmetry will be generated. For information on how to select
  residues see the `Making Graphical Selections` section.
- Select the menu Tools/Xray/Crystallograhic Neighbors.

A data entry box as shown below will be displayed.
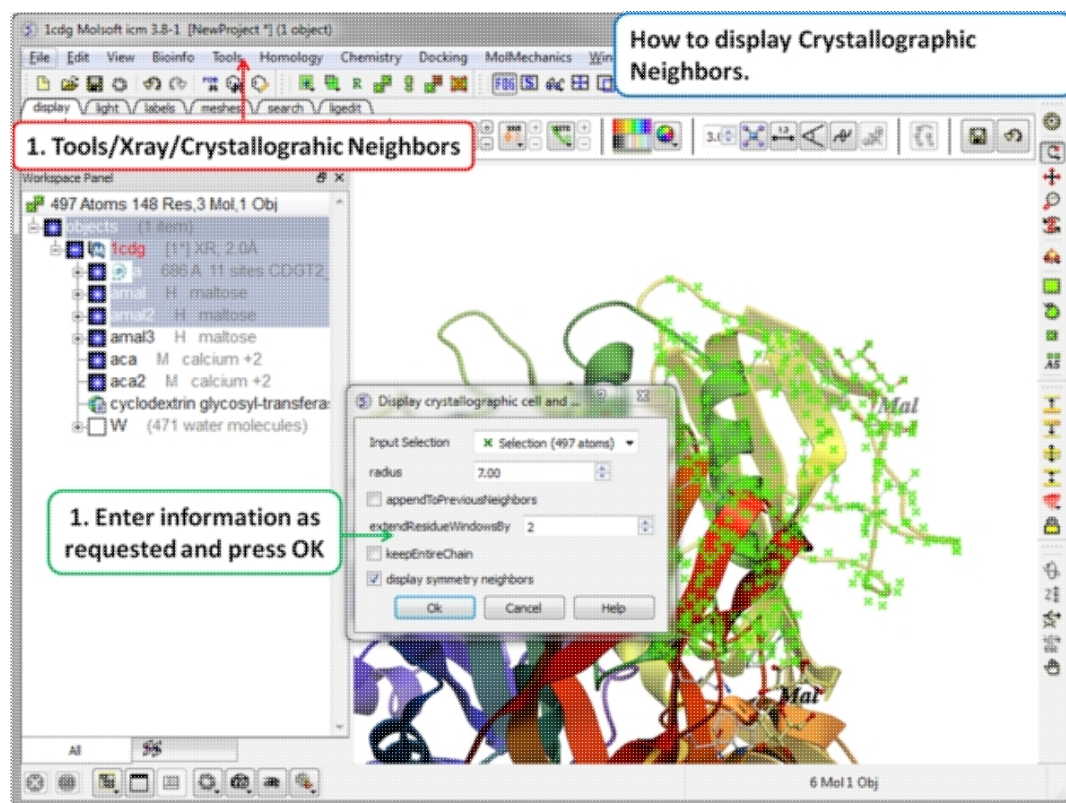
- Check that the selection is correct in the **Input Selection** box.
- Enter the radius around your selction from which you wish to
  construct the symmetry related molecules.
- If you have made symmetry related molecules previously you can
  select **appendToPreviousNeighbors** otherwise leave unchecked.
- The **extendResidueWindowsBy** option will allow a window of residues
  outside of the selection radius selected above to be displayed
- If you leave the **keepEntireChain** unchecked then a fragment of each
  neighbor will be created. If you check this box the full neighbor
  will be generated
- Check display symmmetry neighbors to display them in the graphics
  window. The nearest neighbor residues will be displayed in xstick
  representation and the each neighbor colored by molecule.
- Click OK.

The crystallographic symmetry neighbors will be displayed in the
Workspace. By default the object will have the object name + "Sym" and
each of the neighbors will be individual molecules.

For packing analysis and display you can color each symmetry unit a
different color as described in the `Structural Representations Color`
`section`. This is shown in the picture below.
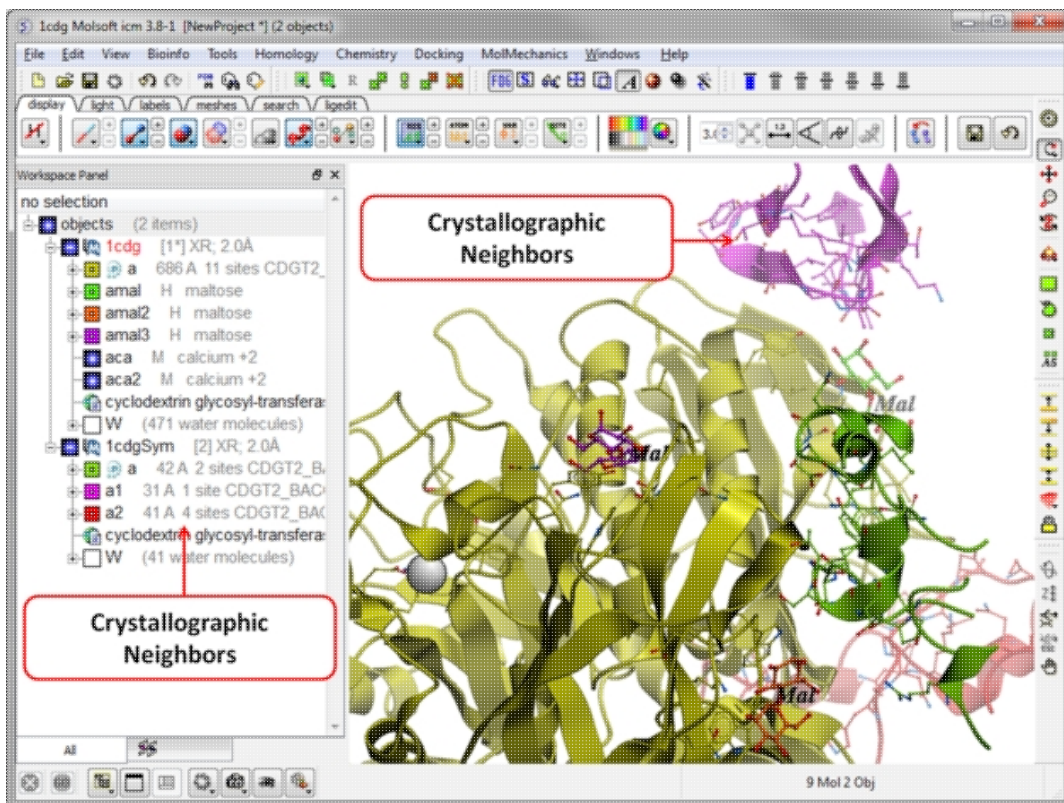
4.3.2 Crystallographic Cell

Theory

The crystal structure of a protein is often discussed in terms of its
unit cell. The unit cell is a box containing one or more motifs, a
spatial arrangement of atoms. The units cells are tiled in
three-dimensional space to describe the crystal. The unit cell is given
by its lattice parameters, the length of the cell edges and the angles
between them, while the positions of the atoms inside the unit cell are
described by the set of atomic positions measured from a lattice point.

To display the crystal cell of a PDB structure:

- Read a PDB file into ICM. For instruction see the section entitled
  `Search PDB`.
- Select the whole object. You can do this by double clicking on the
  name of the structure in the ICM Workspace (a selection is
  highlighted blue in the ICM Workspace and green crosses in the
  graphical display) or you can use the right-click button and drag
  it over the whole structure in the graphical display.
- Select the menu Tools/Xray/Crystallograhic Cell and a data entry
  box will be displayed.
- Click OK

The crystallographic cell will be displayed as a box as shown below.
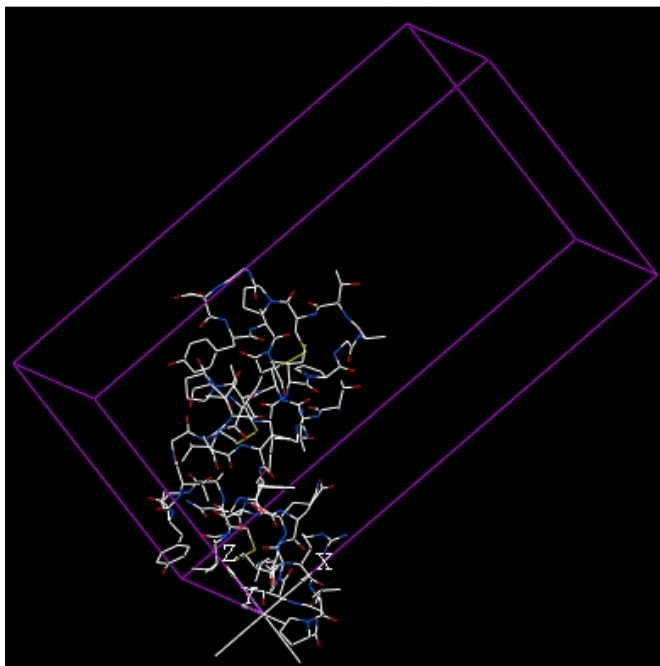
4.3.3 Biomolecule Generator

Theory

It is very useful to know how a protein from the PDB may look in a biological environment. The PDB entries solved by X-ray crystallography and deposited in the PDB contain the information about the crystal structure rather than the biologically relevant structure. For example, for a viral capsid only one instance of capsid protein complex will be deposited and only one or two molecules of haemoglobin that is a tetramer in solution maybe deposited.

In some other cases the asymetric unit may contain more than one copy of a biologically monomeric protein. ICM reads the biological unit information and has a tool to generate a biological unit. Not every PDB entry has the biological unit information.

A gallery of images created using the ICM Biomolecule generator is shown below:
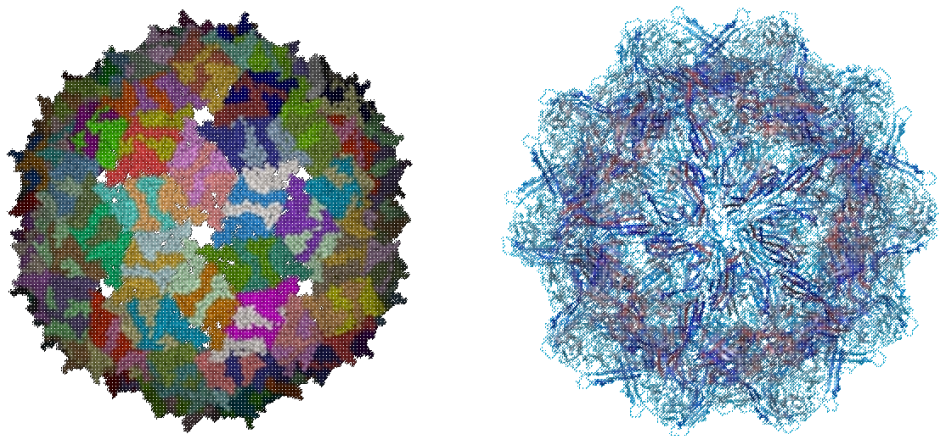
Left: PDB: 1DWN Bacteriophage Pp7 From Pseudomonas Aeruginosa At 3.7 A Resolution **Right:** PDB: 1C8E Feline Panleukopenia Virus Empty Capsid Structure At 3.0 A Resolution
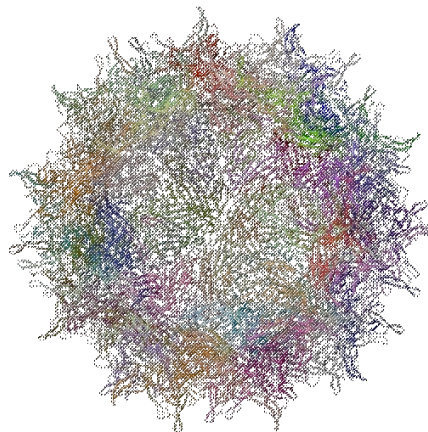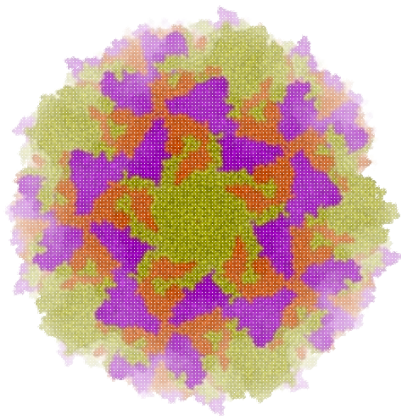
Left: PDB: 1AL2 P1/Mahoney Poliovirus, Single Site Mutant V1160I At 2.9 A Resolution **Right:** PDB: 1LP3 Adeno-Associated Virus (Aav-2), A Vector For Human Gene Therapy At 3.0 A Resolution

> **NOTE:** Right click on a PDB structure in the ICM workspace to determine whether a structure from the PDB has biological unit information. If it does have this information then there will be an option in the menu entitiled "Generate Biomolecules" if not the option will be blanked out.

To generate a biological unit with ICM:

- `Select` the object or PDB file.
- Select the menu Tools/Xray/Biomolecule Generator.

- Tick the **makeAllBiomolecules** box.
- Click OK - with very large molecules the biomolecule generation may take some time.

4.3.4 Get Electron Density Map

Theory

An electron density map is a representation of a crystal structure based on the diffraction data. The map is constructed by a summation of waves of known phase, amplitude and frequency using Fourier transform. The electron density map of a protein can be viewed along with the pdb structure. The easiest way to view the electron density map is to contour and convert it into a graphical object (mesh).

A figure showing the electron density contours surrounding the ATP molecule in pdb entry 1ATP.
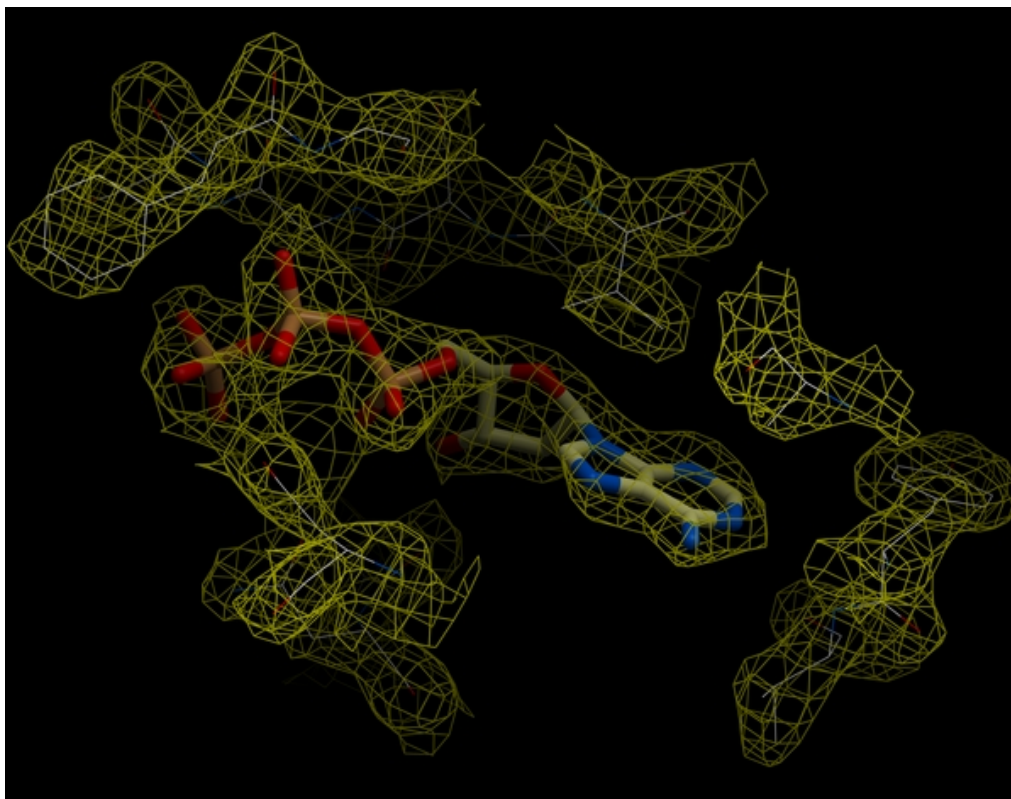
To load an electron density map:

- Tools/Xray/Get Electron Density Map
- Enter the PDB code of the map you would like to view.
- Click OK and the map will be downloaded from the Uppsala Electron Density Server.

The map will be represented in the ICM Workspace as shown below.
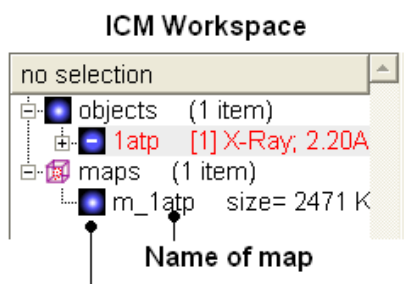
The map can be displayed as shown below however a clearer way of representing the density is to contour the map into a graphical object (mesh) as described in the following section.
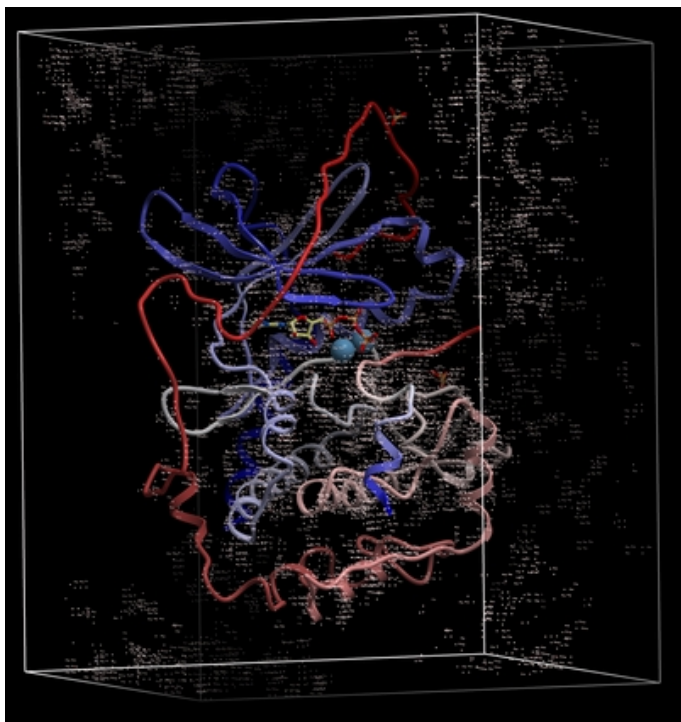
4.3.5 Map's Original Cell

To display the original `crystallographic cell` of an electron density map:

- Tools/Xray/Map's Original Cell
- Enter the name of the map or use the drop-down button to locate it. If you do not know the name of the map the name can be located in the ICM Workspace.
- Click OK and the cell will be displayed. The map can be displayed and undisplayed in the **meshes** section of the ICM Workspace.

4.3.6 Contour Electron Density Map

To contour an electron density map and display as a graphical object:

- Load an electron density map as described earlier in the Load Map section.
- Read in the PDB file - File/Load PDB or use the PDB search tab.
- Tools/Xray/Contour Electron Density.

- Enter the name of the map e.g. m_1cdg - the name of the map is displayed in the ICM Workspace or use the drop down arrow to locate it.

- If nothing is displayed then the whole map will be contoured. If you only want to contour a particular region of the map then you need to `select` the region to be contoured (e.g.the binding pocket) and then select the option **Trim to Selection Vicinity**.
- Enter a sigmaLevel value for more information see: `http://www.molsoft.com/man/reals.html#mapSigmaLevel`. Once the contoured map has been created the sigma level can be changed manually using the +/- buttons in the ICM workspace. Two contour levels at 1 and 2.5 sigma respectively are generated for the 2Fo - Fc map
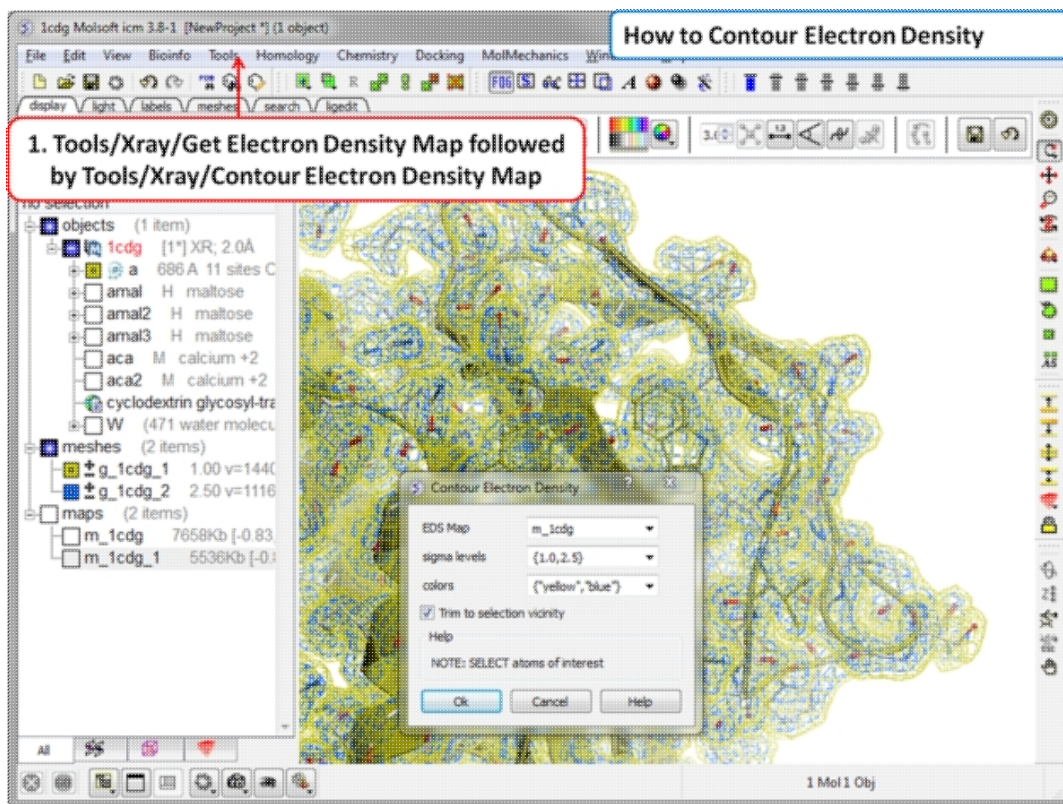
The sigma level can be changed interactively in the ICM workspace as shown below.
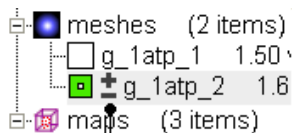
Click here to increase or decrease the sigma level of the contouring

> **NOTE:** Meshes can be cut away using the `mesh clipping tools`.

4.3.7 Convert Xray Density to Grid

For some applications, such as trying to fit a structure to a density map, you may want to extract a sub map and convert to a grid. You can do this by

- First read into ICM a map (eg File/Open or Tools/X-ray/Get Electron Density Map)
- Tools/X-ray/Convert Xray Density to Grid

- Enter the map name or use the drop down list
- Enter a grid size
- Click OK

4.4 Protein Superposition

Available in the following product(s): `ICM-Browser-Pro` | `ICM-Pro`
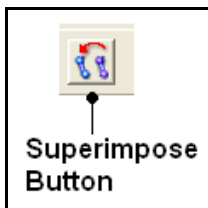
One or more proteins can be superimposed. Simply select the molecules or parts of the molecules you wish to superimpose and then use the selection of protein superimpose tools described in this section. A convenient superimpose button can be found in the `Display tab` (see image of button (left).

Chapter Contents:

- `Select Proteins for Superposition`
- `Superimpose Button`
- `Superimpose by 3D`
- `Superimpose Multiple Proteins`
- `Arrange as Grid`
- `Superimpose Sites by Atomic Property Fields`

4.4.1 Select Proteins for Superposition

Before any superposition operation can be undertaken you need to select the protein structures you wish to superimpose.

One way to do this is by selecting in the ICM workspace. For other selection tools please see the `Making Selections` section of the manual.

- Select both receptors by double clicking on the name of the molecule in the ICM Workspace. To select two molecules use the Ctrl button or use the shift button to select a range of objects in the ICM Workspace. A receptor which is selected will be highlighted in blue in the ICM Workspace and with green crosses in the graphical display.
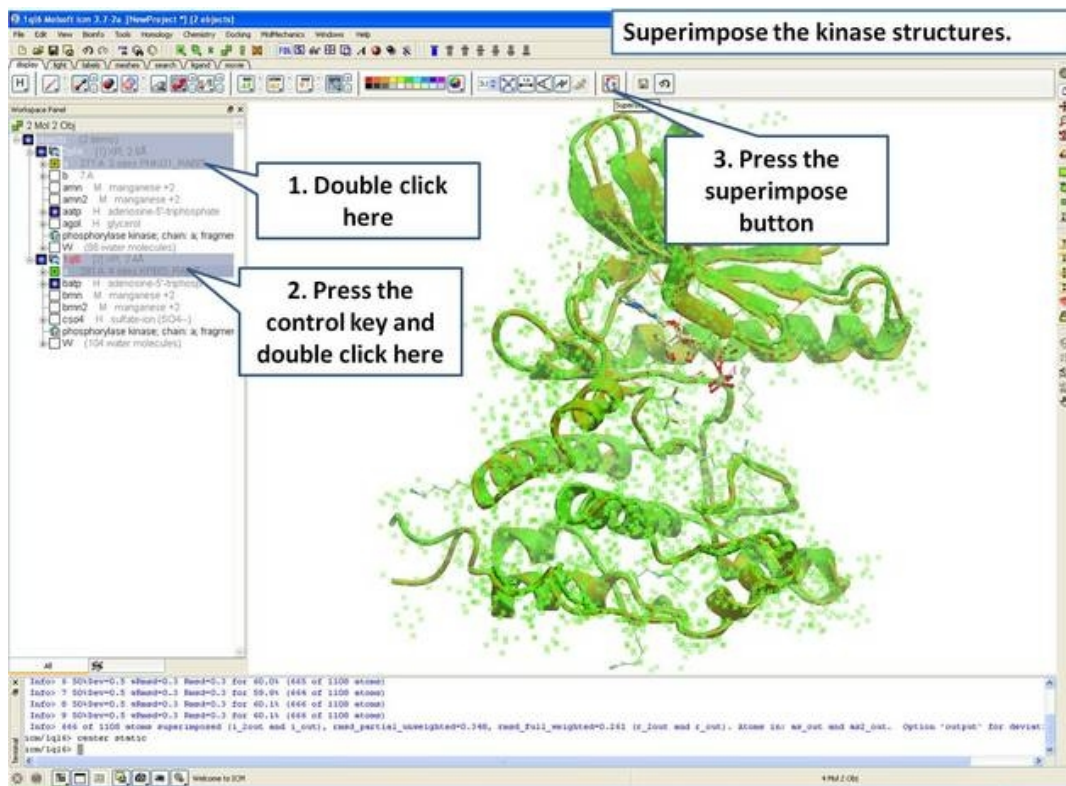
Once the molecules are selected you can then superimpose them using the options described in the next section of this manual.

4.4.2 Superimpose Button

A convenient way to superimpose two molecules is by using the superimpose button in the **display** tab, ICM will calculate the Ca-atom, backbone atom and heavy atom differences between the two structures. More advanced superimpose options can be found in the **Tools/Superimpose** menu.

To superimpose:

- First load the two structures into ICM.
- Select which parts or all of the two structure you wish to superimpose (see the chapter on Selections or the description protein-superposition-select{here}.).
- Select the **display** tab (previously called Advanced tab) at the top of the GUI.
- Select the superimpose button.

Superimpose
Button

The rmsd will be displayed in the terminal window as shown below:

```
INFO> 04 atoms superimposed, rmsu=1.381043
icm/ly6> superimpose ( Res( as_graph ) & a_.//ca,c,n,o ) & Obj( as_graph )[1]
  Warning> [110] skipped 4 atom pairs with zero occupancies
  Info> 64 atoms superimposed, rmsd=1.381643
icm/ly6>
```

RMSD displayed here

NOTE: You do not need to select the whole molecule, the superimpose
button will work on small selections e.g the loop regions or domains.

4.4.3 Superimpose by 3D

To superimpose proteins by 3D:

- First display and select the proteins you wish to superimpose by
  3D.
- Tools/Superimpose/Proteins by 3D
- A window as shown below will be displayed.
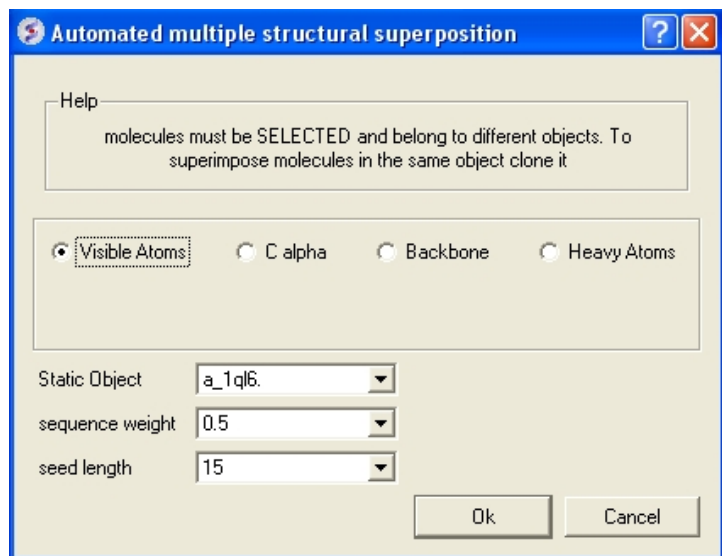
- Select by which atoms you wish to superimpose.
- Enter the ICM selection language description for the protein
  structure you wish to remain static. You can also use the drop
  down arrow button to select it.
- Enter the **sequence weight** Average local sequence alignment score.
- Enter the **seed length** This is the similarity window size.

4.4.4 Superimpose Multiple Proteins

To superimpoe multiple proteins:

- First display and select the proteins you wish to superimpose by
  3D.
- Tools/Superimpose/Multiple Proteins
- A window as shown below will be displayed.
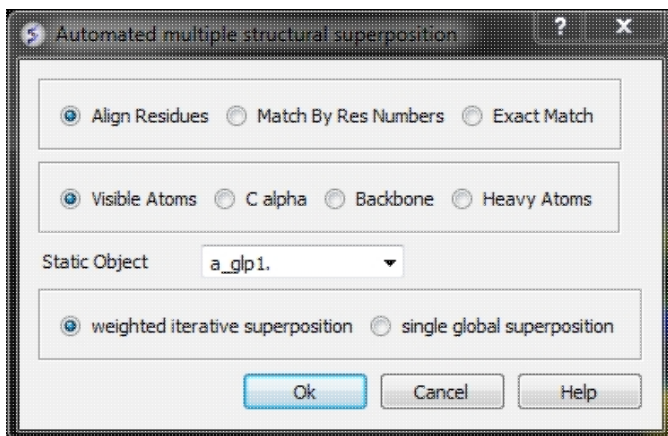
- Select by which method you would like to superimpose

Align Residues - Residue correspondence is established by sequence alignment using the ICM ZEGA alignment Abagyan, Batalov, 1997. Atom alignment: by atom name.

Match by Res Numbers - Residue alignment by residue number.Atom alignment: by atom name for pairs of identical residues or pairs of close residues (F with Y; B with D,N; D with N; E with Q or Z, Q with Z), for other residue pairs only the backbone atoms ca,c,n,o,hn,ha are aligned.

Exact Match - Residue alignment is by the Needleman and Wunsch method. Inside residue atoms are aligned sequentially and regardless of the name.

- Select which atoms you would like to superimpose. Visible Atoms, C alpha, Backbone, or Heavy Atoms.
- Select whether you would like to use **weighted iterative superposition** as described here
  http://www.molsoft.com/man/icm-commands.html#superimpose-minimize
  or non-iterative **single global superposition**.

4.4.5 Arrange as Grid

To separate superimposed proteins:

- Tools/Superimpose/Arrange as Grid

Superimpose

Arrange as grid

4.4.6 Superimpose Sites by Atomic Property Fields

Here we describe how to superimpose and compare a ligand binding site using Atomic Property Fields. This method is described in more detail in this publication.

In this example we superimpose the ligand binding pocket of thiamine diphosphate in the binding sites of pyruvate dehydrogenase (pdb code: 1rp7) and pyruvate decarboxylase (pdb code:1pvd).Even though the sequence identity between both proteins is very low (19%) and the secondary structure surrounding the ligand undergoes considerable displacement you will see that the pockets can still be superimposed very well using the APF method.

Some Comments about Selecting the Pocket APF pocket overlay works by superimposing a layer of receptor atoms around the pocket using APF chemical property fields of these atoms "pocket" of course is something that is not uniquely defined and is somewhat subjective. One way to define it is to use the ligand inside, in which case a layer of receptor atoms within a certain cutoff distance from the ligand is considered in superposition. This is "around selected ligand" mode. It focuses on atoms that directly interact with the ligand while largely disregarding backbone fold, which can be helpful when comparing different pockets binding similar ligands. The other mode uses input selections directly, leaving it up to the user to define the sets of atoms that comprise the two pockets. Depending on the system and degree of similarity, different selections may work better or worse. For instance in the absence of ligand one may want to detect pocket with `pocket finder` and then select residues around the pocket "blob". Regarding the influence of ligand on superposition, if pocket is defined via the ligand it will of course matter whether the ligands extend into certain regions or not. If external pocket definition is used, ligands will have no direct effect on superposition process.
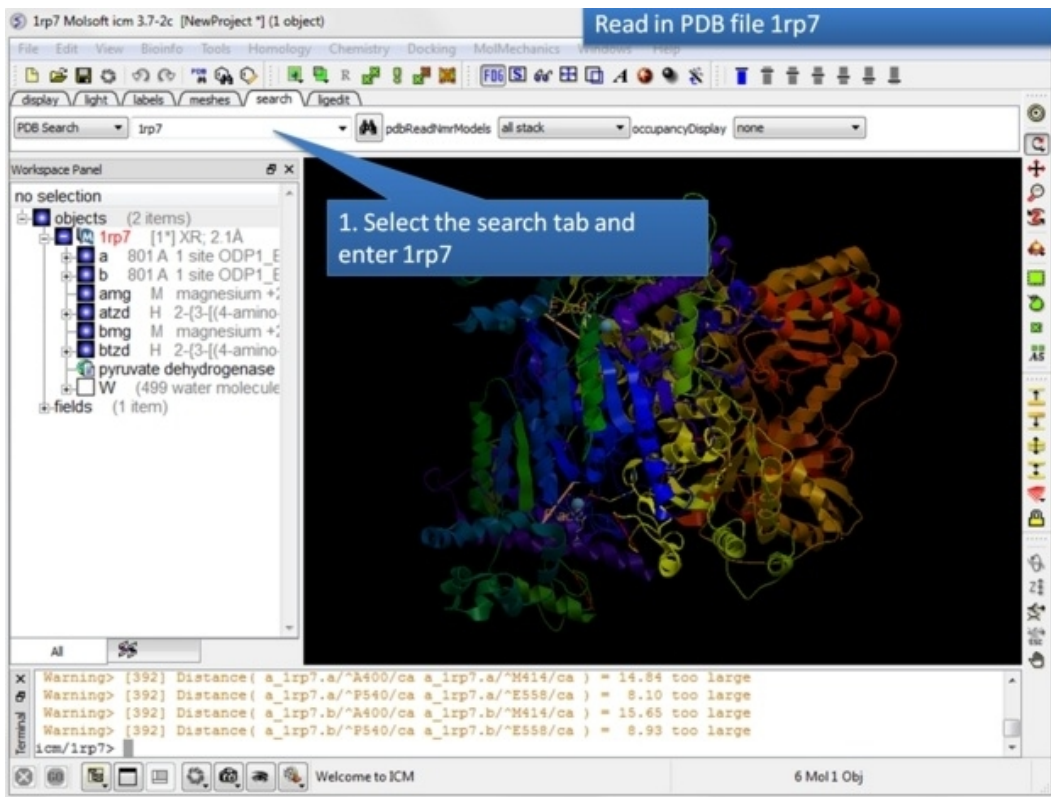
Step 1: Read in the pyruvate dehydrogenase (pdb code: 1rp7) structure.
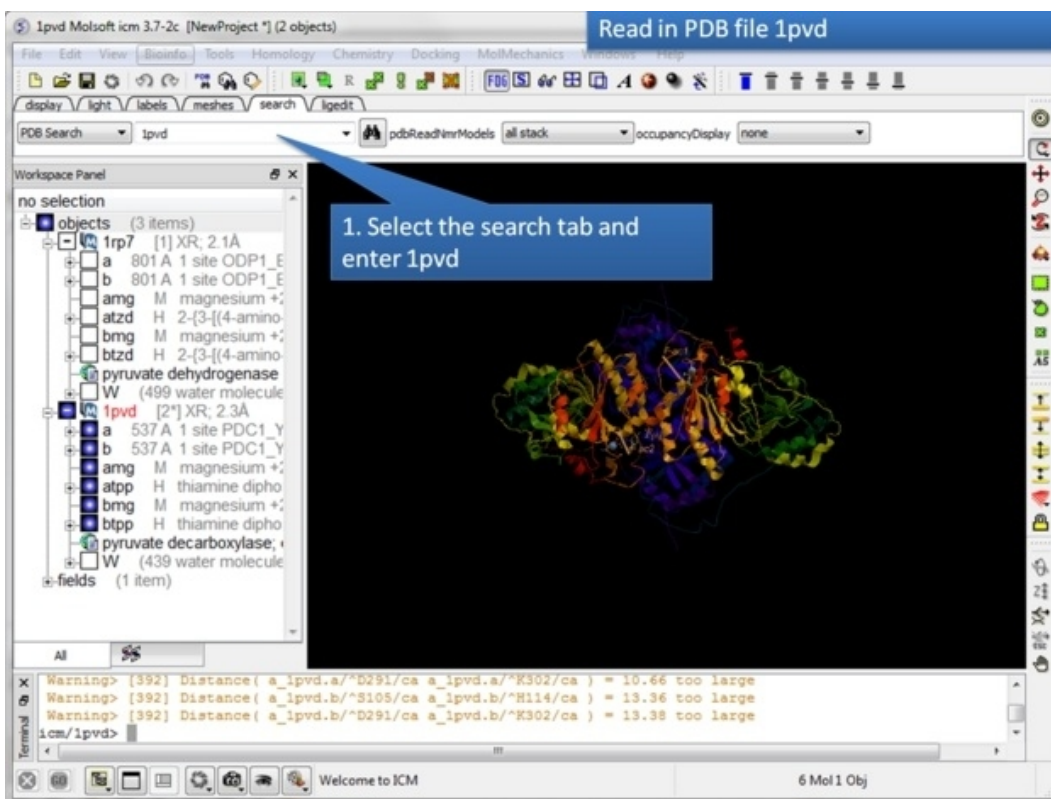
Step 2: Read in the pyruvate decarboxylase (pdb code:1pvd).
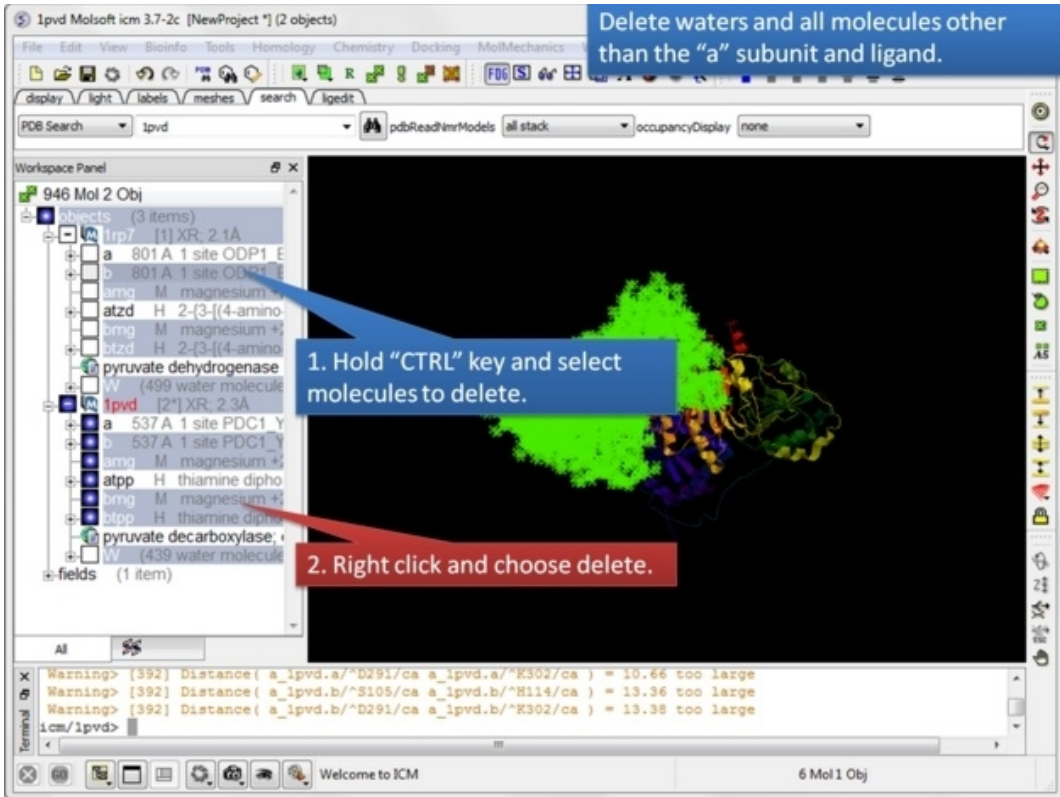
Step 3: Delete unwanted molecules in the b chain and waters.
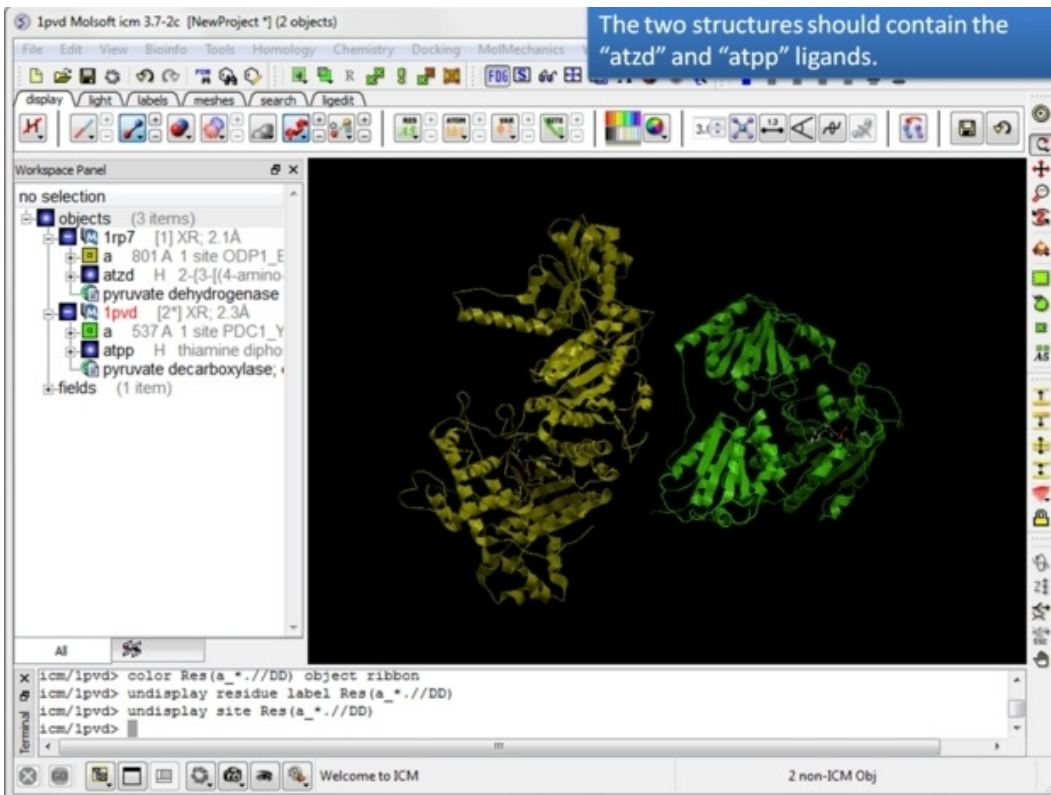
Step 4: You should have two objects containing the protein and ligand.
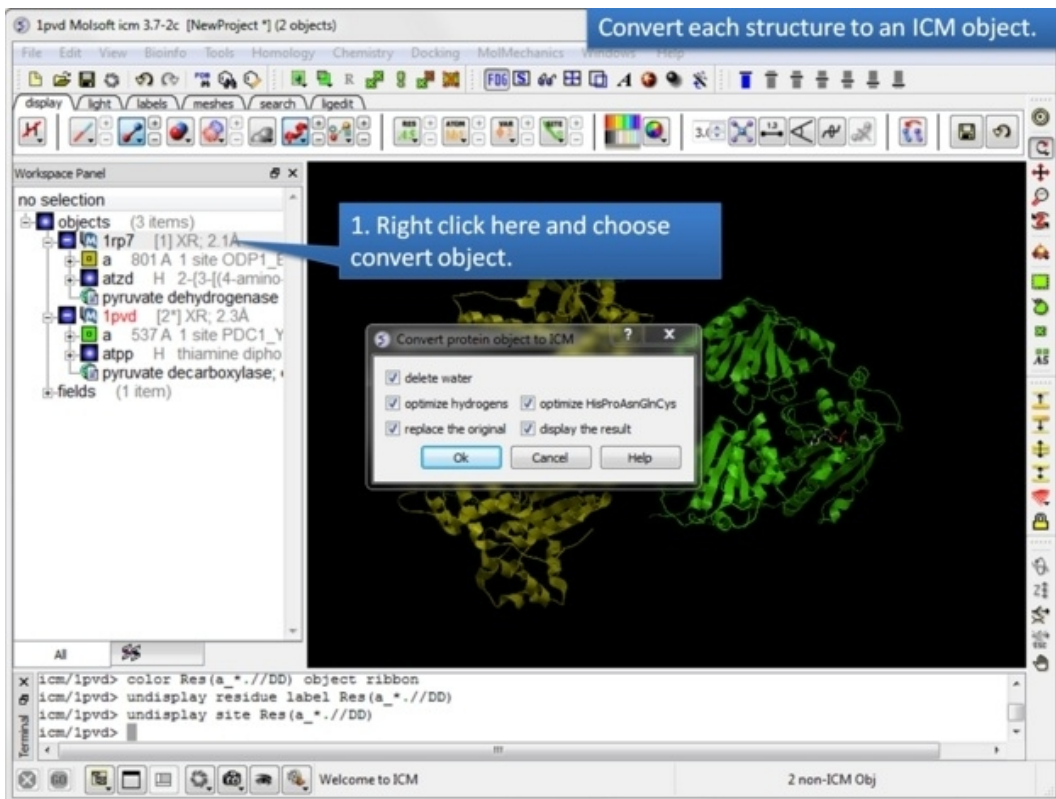
Step 5: Convert both structures to an ICM object.
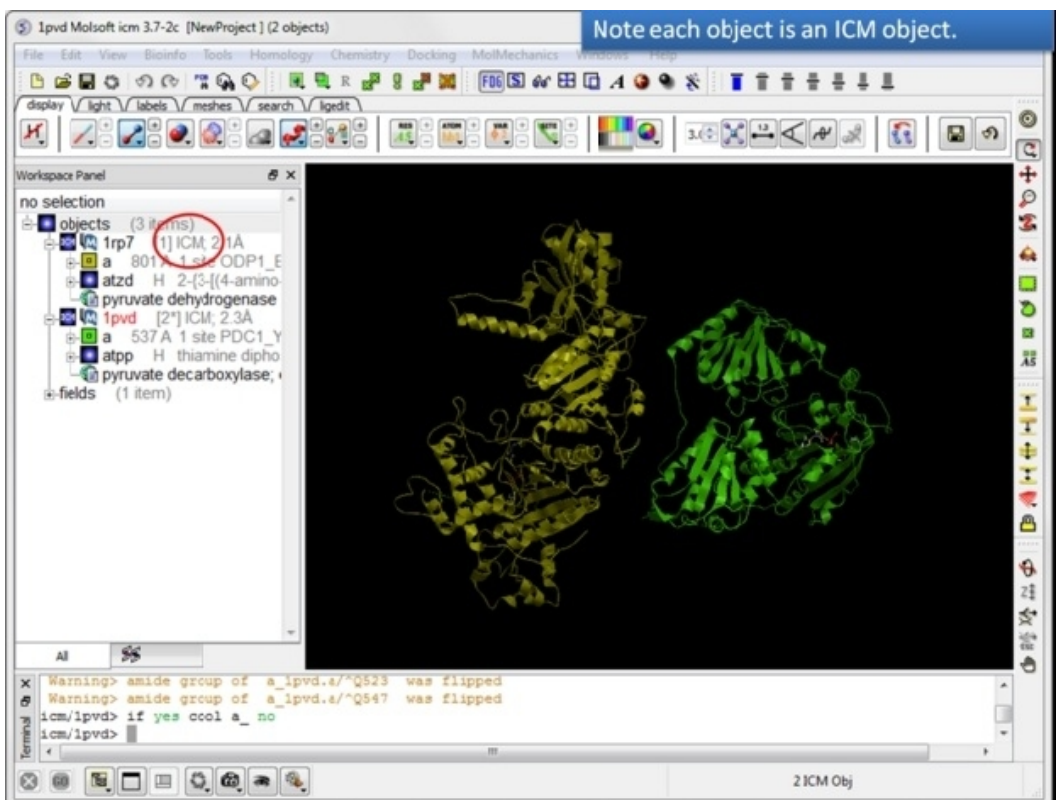
Step 6: If the pdb is converted you will see "ICM" in the workspace.
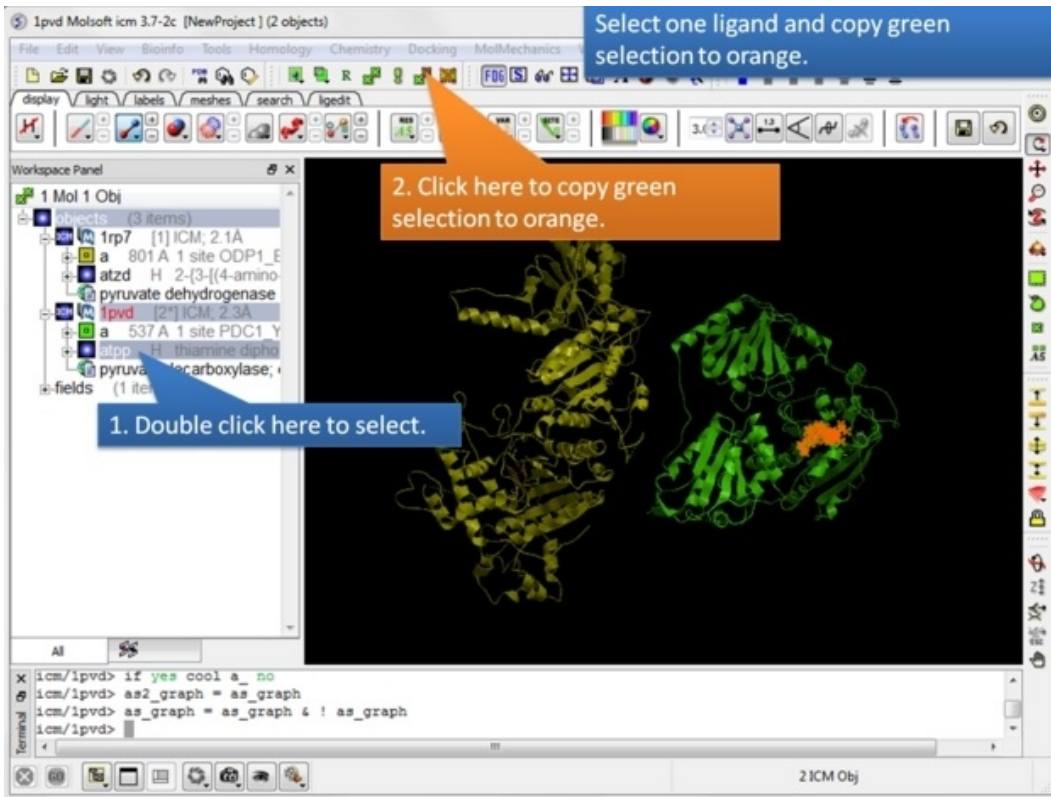
Step 7: Select one ligand and copy the green selection to orange.
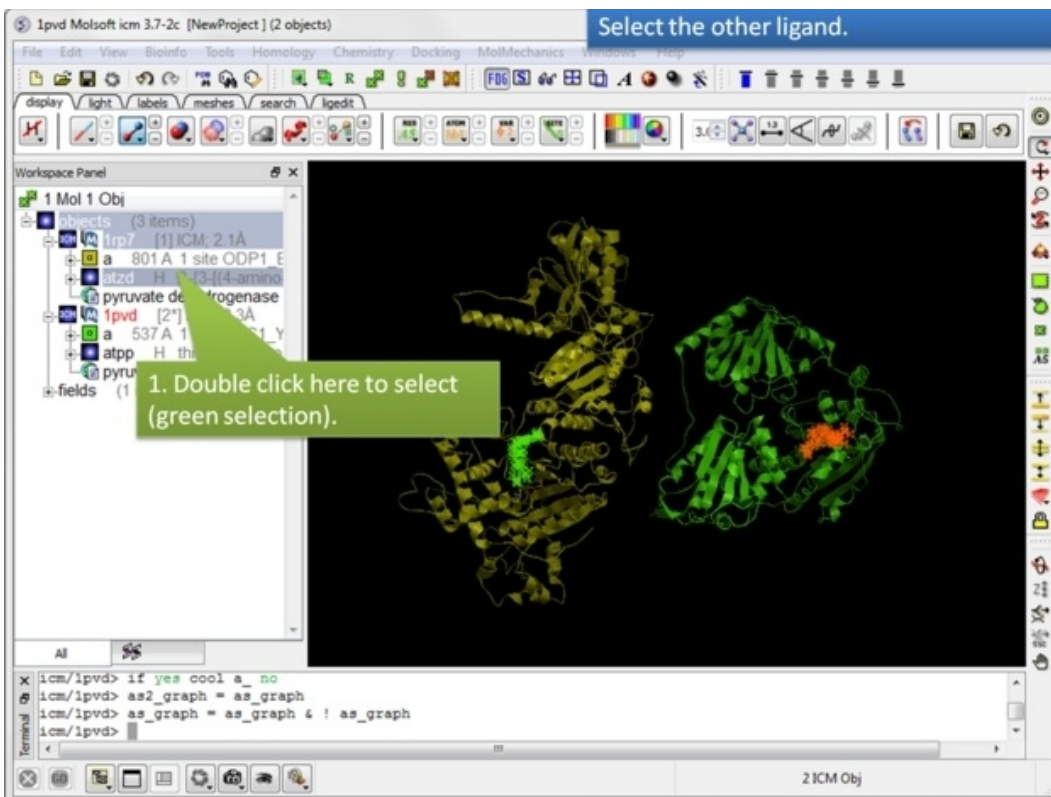
Step 8: Select the other ligand (regular green selection).
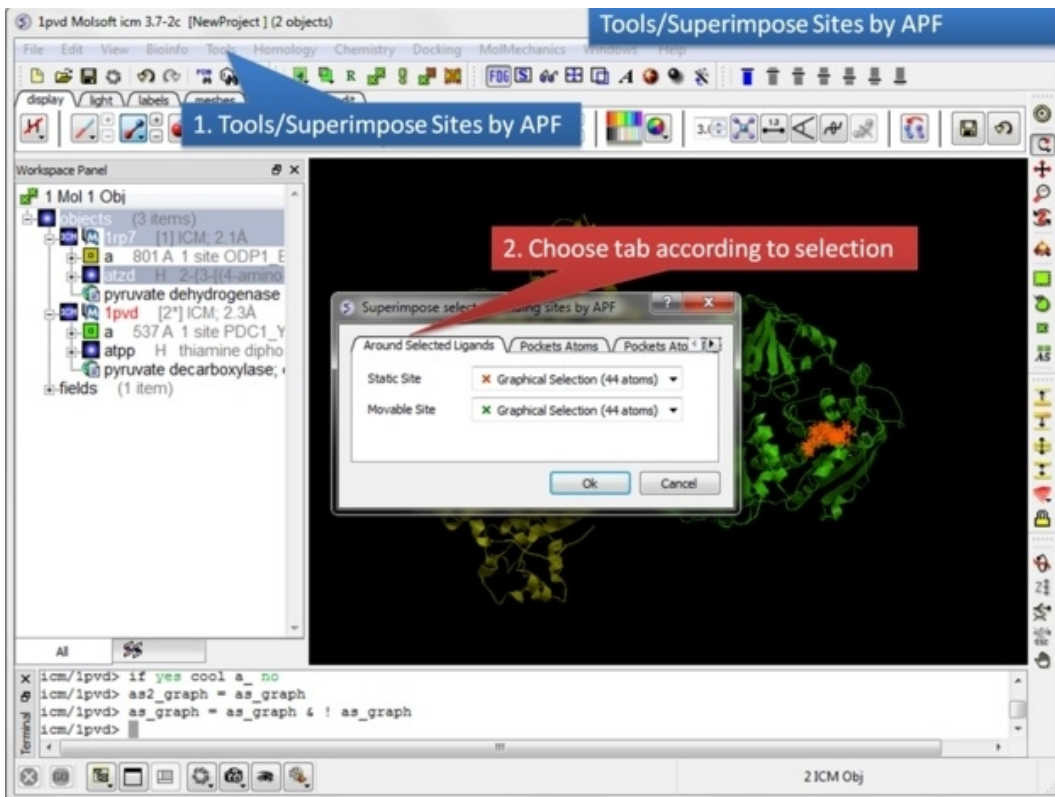
Step 9: Select the option Tools/Superimpose/Sites by APF. Note you can also superimpose pockets - choose the appropriate tabs in the dialog box.
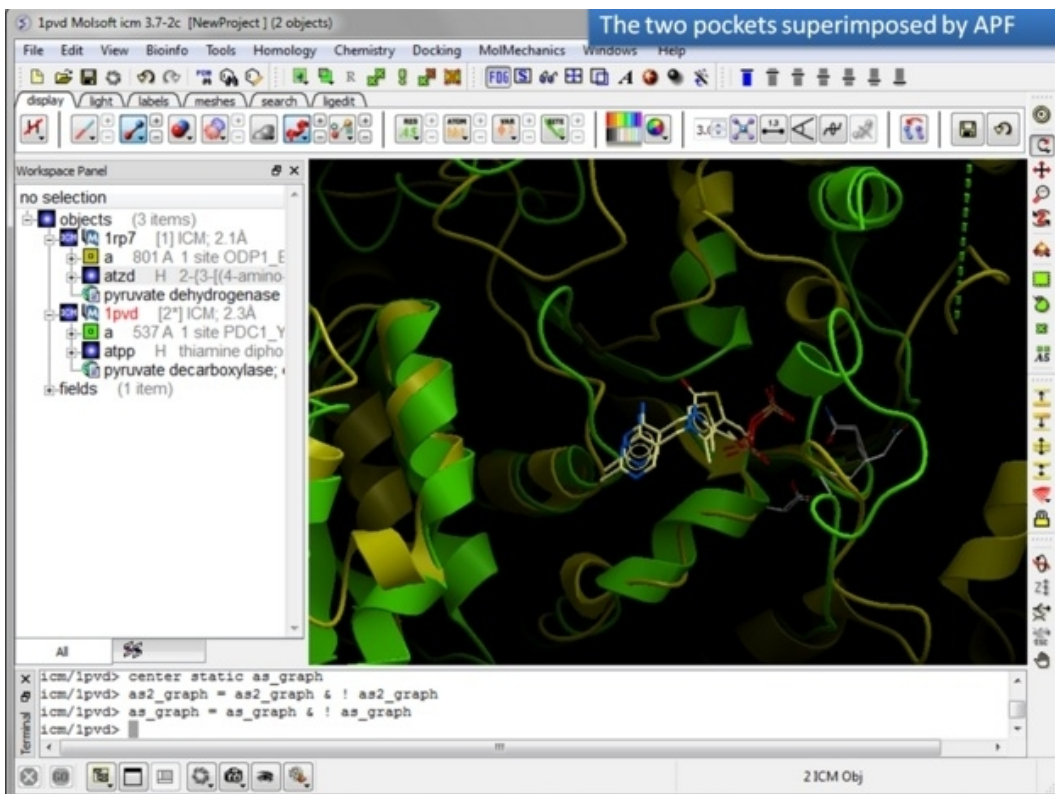
Step 10: Observe the superimposed site.

4.5 Protein Structure Analysis

> Available in the following product(s): `ICM-Browser-Pro` | `ICM-Pro`

In this chapter we describe the tools available for analyzing protein structure. These tools include calculating RMSD, identifying closed cavities, calculating contact and surface area, measuring anlgles and distances, and generating Ramachandran plots.

Chapter Contents:

- Find Related Chains
- Calculate RMSD
- Contact Areas
- Identify Closed Cavities
- Surface Area
- Measure Distances
- Planar Angle
- Dihedral Angle
- Ramachandran Plot Interactive
- Export Ramachandran Plot

4.5.1 Find Related Chains

This option allows you to search the currently loaded PDB files or ICM objects and identify chains which are similar and/or related.

You can do this by:

- `Select` the objects or pdb files you want to compare.
- Tools/Analysis/Find Related Chains
- Click OK to confirm the selection you made
- A table as shown below will be displayed.

name1 = Name of query structure molecule **name2** = Name of hit **len1** = length of query **len2** = length of hit **seqid** = Sequence identity percentage **consensus** = Consensus sequence

4.5.2 Calculate RMSD

> **NOTE:** This option is for protein structures only not for chemical compounds. You can use the command line options RMSD (http://molsoft.com/man/icm-functions.html#Rmsd) and Srmsd (http://molsoft.com/man/icm-functions.html#Srmsd) for chemicals.

To calculate RMSD between two protein structure:

- Read into ICM the two structures (`File/Open` or `PDB Search` or Read in Chemical) you wish to compare.
- `Select` one of the two molecules you wish to compare, you can do this by double clicking on the name of the structure in the ICM Workspace. Convert this selection to an `Orange Selection`.
- Select the second molecule, and then you should have one orange and one green selection in the graphics display.
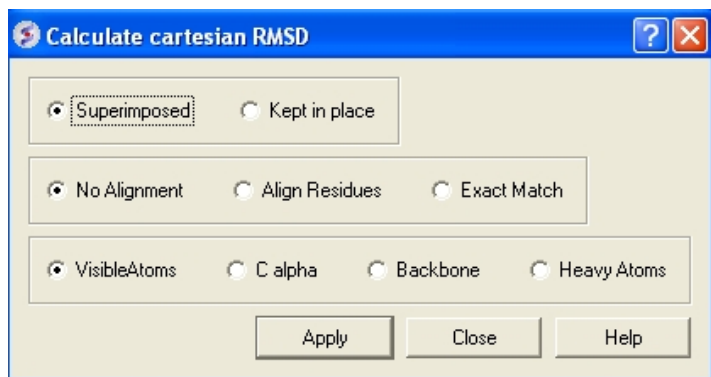
- Select whether you wish the atoms to be superimiposed onto one another or kept in place. The kept in place option would be ideal for comparing docked structures.
- Choose whether you wish to make the superposition by alignment or exactly matching the atom names.
- Select which atom types you wish to superimpose.

The **RMSD** value will be displayed in the terminal window.

4.5.3 Contact Areas

- Read in a protein structure (`File/Open` or `PDB Search`).
- `Select` the region you wish to analyse.
- Tools/Analysis/Contact Areas
- The xstick display in the region will be scaled according to the atom/residue contact area. For example, residues making large contacts with a ligand will be displayed in thicker xstick representation (and colored yellow) than those making less significant contacts.
- A table as shown below will be displayed.The table lists the contact area, exposed area, percentage of contact area compared to exposed, the nearest atom of a residue and the distance.

4.5.4 Identify Closed Cavities

This tool will identify cavities within a molecule which are completely closed,. If you are looking for buried and open pockets then use icmPocketFinder.

- Read in a protein structure (File/Open or PDB Search).
- Convert the protein structure to an ICM object.
- Tools/Analysis/Closed Cavities
- Use the drop down arrow to locate the molecule you are interested in.
- Enter the minimum volume of the cavities you wish to identify.
- Click OK
- The closed cavities will be displayed in the meshes section of the ICM Workspace and a table of the cavities will be displayed. Double click on a row in the table to jump to a particular closed cavity and select the residues surrounding it.

What is the difference between Closed Cavity and `ICMPocketFinder`?
Closed pocket (cavityFinder) is purely geometrical/topological - it
is a part of molecular surface that is completely disconnected from
the exterior surface. It means that a probe sphere of 1.4A radius
(representing a water molecule) can not pass in or out of the cavity
(considering protein as completely rigid of course). icmPocketFinder
identifies pockets that are likely to contain ligands (not
specifically open or closed pockets). Pockets are defined based on
physical interaction rather than geometric criterion. Blobs of
'pocket density' generated by icmPocketFinder represent continuous
regions of space where there is significant favorable van der Waals
interaction with the receptor.

4.5.5 Surface Area

This option calculates solvent accessible area of each selection in
multiple objects and stores it in a table. If a molecule is specified in
a multi-molecular object, the surface area of an isolated molecule is
calculated and other molecules are ignored. The area is reported in
square Anstroms and the probe radius is assumed to be the value set in
the variable waterRadius.

Output: the macro creates table AREA . The empty comment field is added
for user's future use. If the table exists, new rows are appended.

To calculate a surface area:

- Read in a protein structure (File/Open or PDB Search).
- Select the region you wish to analyse.
- Tools/Analysis/Surface Area
- A table will be displayed listing the residues in the selection
  along with the corresponding total surface area.

4.5.6 Measure Distances

There are two approaches to calculating and displaying distances between atoms. You can either use the options in the `Labels` tab or use Tools/Analysis/Distance.

To display **all to all** distances:
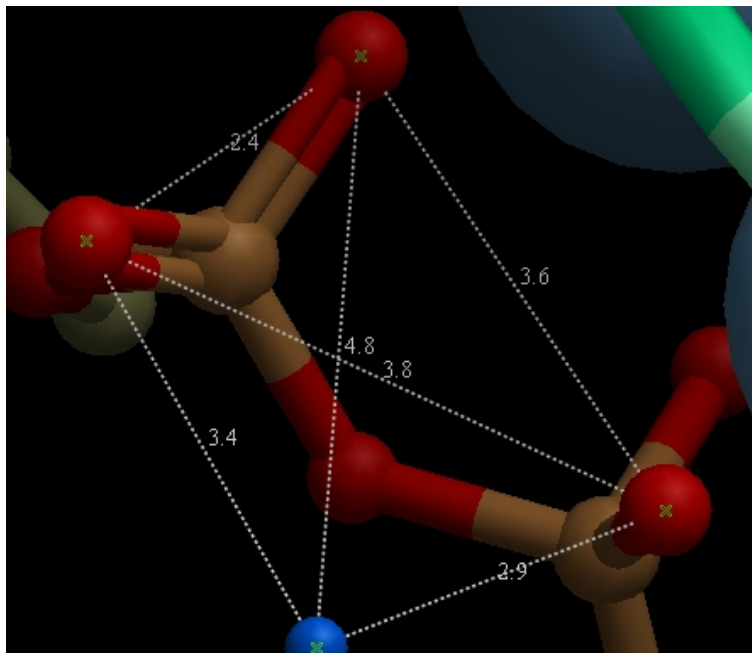
- Select the atoms between which you would like to find the distance. (See `selection toolbar`)
- Tools/Analysis/Distance
- Select all to all

To display **intermolecular** distances

- Select the atoms between which you would like to find the distance. (See `selection toolbar`)
- Tools/Analysis/Distance
- Select **intermolecular**

To display the distances between the same atoms in two objects.

- Select the atoms between which you would like to find the distance. (See `selection toolbar`)
- Tools/Analysis/Distance
- Select **same atoms in two objects**

NOTE: Distances can be displayed and undisplayed in the 3D labels section of the ICM Worskapce. You can change the color of a distance label by right clicking on it in the ICM Workspace. You can alse export the distance to a table.

4.5.7 Planar Angle

If you wish to find the planar angle between three atoms:

- Select **Tools/Analysis/PlanarAngle**

- Right click on the each of the three atoms which you wish to use, and select their name. The spaces next to **First atom**, **Second atom**, and **Third atom** should now contain the name of your atoms.
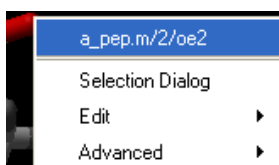
- Click **Apply** to display the angle measure in the terminal window.

```
Angle ( a_pep.m/6/hh21 a_pep.m/2/oe2 a_pep.m/3/o ) = 74.72 deg.
```

4.5.8 Dihedral Angle

In order to find the angle dihedral angle between two sets of atoms:

- Select **Tools/Analysis/Dihedral Angles.**
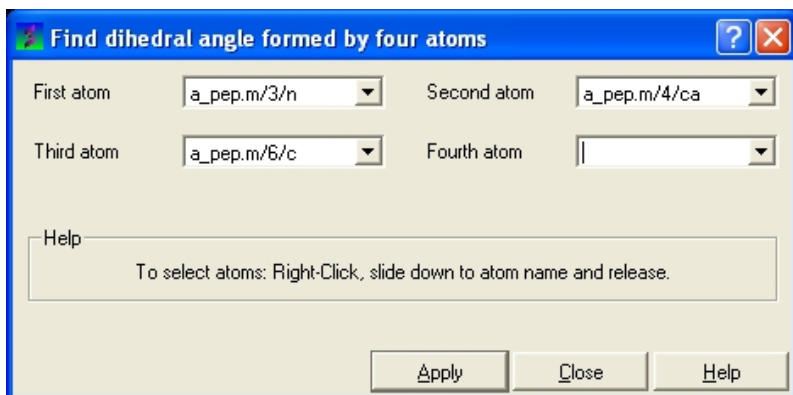
- Right click on each of the four atoms which you wish to use, and select the name of the atoms. The spaces next to **Atom 1**, **Atom 2**, **Atom 3**, and **Atom 4** should now contain the names of your atoms.
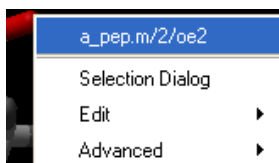
- To find the correct angle, select your atoms according to the following diagram:
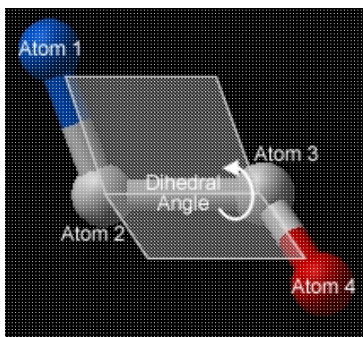
- Click **Apply** to display your dihedral angle measure in the terminal window.

4.5.9 Ramachandran Plot Interactive

To make an interactive ramachandran plot:

- Read in a protein structure (File/Open or PDB Search).
- Select the structure you wish to build the plot for. You can do this by double clicking on the name of the structure in the ICM Workspace (a selection is highlighted blue in the ICM Workspace and green crosses in the graphical display) or you can use the right-click button and drag it over the whole structure in the graphical display.
- Tools/Analysis/Ramachandran Plot Interactive
- The interactive ramachandran plot will be displayed in table called **RAMA.**
- You can view the **Omega**, **Phi/Psi** (Gly) or Phi/Psi angles by clicking on the tabs at the top of the plot. Each point is linked to the data in the table **RAMA** and also to the graphical display. Clicking on a point in the plot will highlight the corresponding angles in the table and also center on this region in the 3D display.

4.5.10 Export Ramachandran Plot

- Read in a protein structure (File/Open or PDB Search).
- Select the structure you wish to build the plot for. You can do this by double clicking on the name of the structure in the ICM Workspace (a selection is highlighted blue in the ICM Workspace and green crosses in the graphical display) or you can use the right-click button anddrag it over the whole structure in the graphical display.
- **Tools/Analysis/Ramachandran Plot Export**

A postscript viewer needs to be downloaded onto your machine in order to view the plot. This can be downloaded from http://www.cs.wisc.edu/~ghost/. Once this software is downloaded you need to tell ICM where it is located by typing the pathname into File/Preferences.

> **NOTE:** You can always export the plot as an image directly in ICM without exporting. You can do this by right clicking on the plot and select **save as image**. Another approach could be to export the RAMA table to Excel and use the plotting tools there. You can do this by right clicking on the table name tab and selecting "Export to Excel" or save as ".csv".

4.5.11 Protein Health

Theory

The protein health option calculates the energy strain of a structure in ICM. It is generally a good idea to investigate the energy strain of any protein structure before undertaking such processes as docking. It is also essential to use this tool after making a model (see Molecular Modeling) to identify strained regions within your model and then some optimization procedure can be undertaken to rectify the problems.

The protein health option calculates the relative energy of each residue for a selection and colors the selected residues by strain. This macro uses statistics obtained in the following paper Maiorov, V.N. and Abagyan, R.A. (1998) Energy strain in three-dimensional protein structures Folding and Design, 3 , 259-269.

To use the Protein Health option:

* Read in a protein structure (`File/Open` or `PDB Search`).
* Convert your PDB structure into an `ICM object`.
* Make a `selection` of the residues you wish to analyze.
* Tools/3D Predict/Protein Health and a window as shown below will be displayed.
* The scale of the coloring can be changed by altering the value within the trimEnergy data entry box.
* Click OK and the structure will be colored according to energy strain (red - high) and a table of residue energy will be displayed in a table.
* A table and plot of Normalized energies for each amino acid in the selection will be displayed. The table is ranked and colored by residues with poor normalized energies. Click on a row in the table or plot to center in on the residue.

4.5.12 Local Flexibility

This option systematically samples rotamers for each residue side-chain in the input selection and uses resulting conformational ensembles to evaluate energy-weighted RMSDs for every side-chain atom. These are stored in the 'field' values on atoms and can be used for example to color the structure by side-chain flexibility. Conformational entropy for each residue side-chain is also calculated and stored in a table. If l_entropyBfactor flag is on, the atom rmsds are normalized within the residue to reflect its total conformational entropy. If l_bfactor flag is set, the bfactors are reset to the same values that are placed in the atom 'field', and occupancy is set to be inversely proportional to it ($O=1/(1+2*rmsd)$)

* Read pdb file (File/Open or PDB Search Tab).
* `Convert` to an ICM Object.
* Tools/3D Predict/Local Flexibility

4.5.13 Protein-Protein Interface Prediction

The ICM Optimal Docking Area method is a useful way of predicting likely protein-protein interaction interfaces. If you do not have mutational data or other experimental data which indicates the likely protein-protein docking site this method will be useful. This procedure can save you time during the docking procedure by focusing your docking only on areas on the receptor and ligand most likely to interact.

Theory

ODA (Optimal Docking Areas) is a new method to predict protein-protein interaction sites on protein surfaces. It identifies optimal surface patches with the lowest docking desolvation energy values as calculated by atomic solvation parameters (ASP) derived from octanol/water transfer experiments and adjusted for protein-protein docking. The predictor has been benchmarked on 66 non-homologous unbound structures, and the identified interactions points (top 10 ODA hot-spots) are correctly located in 70% of the cases (80% if we disregard NMR structures). For a description of the method see `Fernandez-Recio et al Proteins (2005) 127: 9632`.
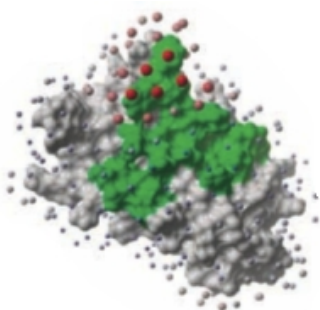
To display the optimal docking area.

- Convert the PDB file to an ICM object.
- Tools/3D Predict/Protein Interface by ODA
- If you select the **Residue Table** option the average ODA score for each residue will be displayed in a table. The lower the number the higher the chance the residue will be involved in protein-protein interactions. Regions colored red represent low ODA score and blue represents a high score.

4.5.14 Identify Ligand Binding Pockets

Theory

The ICM Pocket Finder method (1-2) uses only the protein structure for the prediction of cavities and clefts. No prior knowledge of the substrate is required. The position and size of the ligand-binding pocket are determined based on a transformation of the Lennard-Jones potential by convolution with a Gaussian kernel of a certain size, a grid map of a binding potential and construction of equipotential surfaces along the maps. The pockets are displayed graphically as a surface and the dimensions of each pocket are presented in an interactive table and plot.

Factors that can influence ligand binding to a pocket include the pocket volume and area, buriedness, hydrophobicity, and how compact the pocket is. All these properties are calculated using ICMPocketFinder and tabulated. Scientists at Merck used MolSoft.s ICMPocketFinder algorithm to define a way for quantifying "drugability" of a protein target (3). The metric they used is called Drug-Like-Density (DLID), and this score is also provided in the results table. A good example of the use of the icmPocketFinder method is in the database called Pocketome. The Pocketome (www.pocketome.org) is an encyclopedia of conformational ensembles of all druggable binding sites that can be identified experimentally from co-crystal structures in the Protein Data Bank (4).

1. An, J., Totrov, M. & Abagyan, R. Pocketome via comprehensive identification and classification of ligand binding envelopes. Mol. Cell. Proteomics 4, 752 (2005).

2. Abagyan, R. & Kufareva, I. The flexible pocketome engine for structural chemogenomics. Methods Mol. Biol. Clifton NJ 575, 249.279

(2009).

3. Sheridan, R. P., Maiorov, V. N., Holloway, M. K., Cornell, W. D. &
Gao, Y.-D. Drug-like density: a method of quantifying the .bindability.
of a protein target based on a very large set of pockets and drug-like
ligands from the Protein Data Bank. J. Chem. Inf. Model. 50, 2029.2040
(2010).

4. Kufareva, I., Ilatovskiy, A. V. & Abagyan, R. Pocketome: an
encyclopedia of small-molecule binding sites in 4D. Nucleic Acids Res.
40, D535.540 (2012).

To predict pockets:
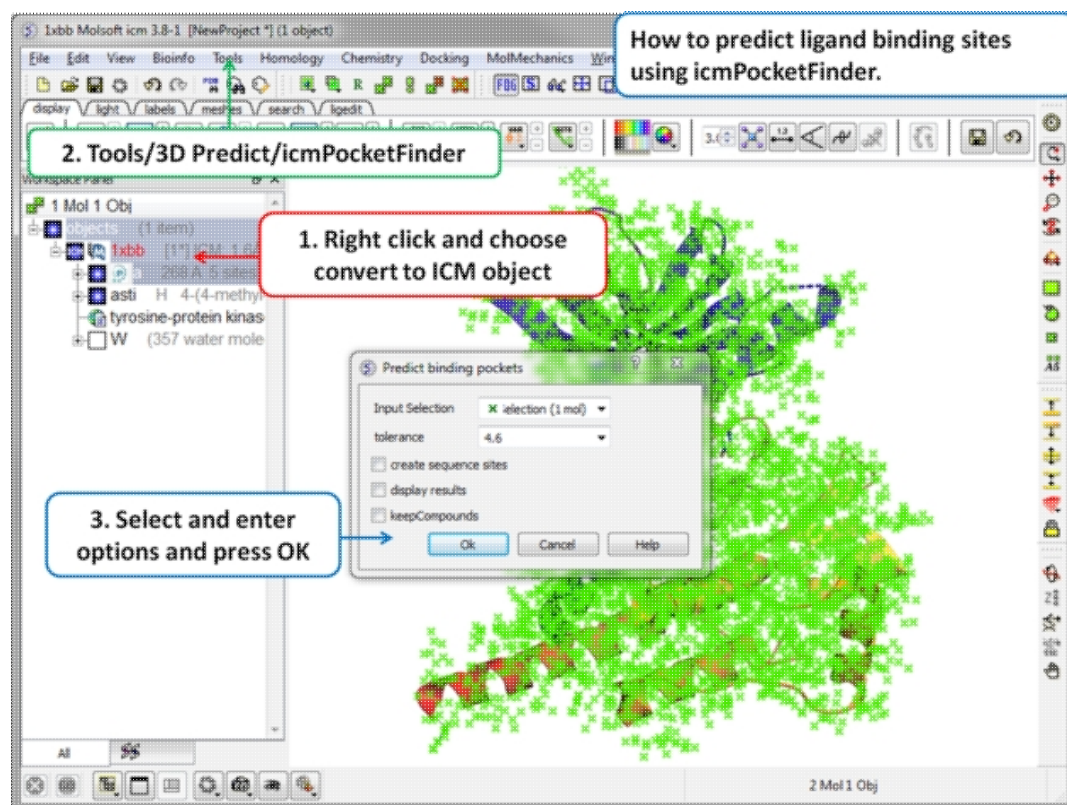
- Read in a protein structure (`File/Open` or `PDB Search`).
- Convert the protein structure to an `ICM object`.
- Tools/3D Predict/icmPocketFinder
- Enter a tolerance level (4.6 is the default value and we
  recommended you to use this). The lower the tolerance value the
  more pockets predicted and the higher the tolerance the less
  pockets predicted.
- Check the box **create sequence sites** if you wish the site to be
  labeled.
- Check the box **display results** to see the predicted pockets as
  grobs in the display panel.
- Check the box **keep compounds** if you wish the compounds (ligands)
  in the receptor to be included in the prediction. If you dont
  check this box the pockets will be calculated based on the
  receptor without ligands.
- Click OK to run icmPocketFinder and the results will be displayed
  in a table.

icmPocketFinder results table.

1. Toggle cavity display on/off here

2. Double click here to select the residues surrounding the cavity

Interactive Pocket Volume vs Area Plot

Results

- Click on a row of the results table to select the residues surrounding the pocket.
- The pocket view can be toggled on or off in the meshes section of the ICM Workspace. Right click on the name of the mesh in the ICM workspace to change properties such as color.
- A fully interactive plot (Area vs Volume) is also provided, pockets that fall within the blue shaded region have a "drug-like" volume and size.

About the icmPocketFinder Results Table

Factors that can influence ligand binding to a pocket include the pocket volume and area, buriedness, hydrophobicity, and how compact the pocket is. These values are reported in the results table:

- **Volume** of the pocket in ˆ .
- **Area** of the pocket in ˆ .
- **Hyrdophobicity** - represents the percentage of the pocket surface s in contact with hydrophobic protein residues (values can range from 0-1)
- **Buriedness** - The buriedness parameter is calculated as follows: One measures the solvent accessible surface area of the pocket (probe radius, 1.4) in isolation. Then one measures the solvent accessible surface area of the pocket covered by its shell. The ratio of the second number to the first is the fraction buried. The lowest possible value is 0.5; i.e. the pocket is completely open and the surface flat. The highest is 1.0, i.e., completely buried.
- **DLID** - Merck's Drug-like density score (see Sheridan et al JCIM 2010). >0.5 is considered "druggable".
- **Radius** gives an indication of how sprherical a pocket is. (3/4 Volume / Pi)^1/3 , i.e. radius of an ideal spherical cavity of the same volume as a pocket blob.
- **Nonsphericity** give an indication of how spherical the pocket is. Area / (area of ideal spherical cavity) . It is 1.0 if the cavity is spehrical

- **Conservation** Average conservation (%identity) of residues in contact with the pocket. Calculation is performed if multiple alignment is present and linked to the protein chain being analyzed.
- **RelCons** Above conservation, relative to the average over the entire chain.
- **Type** - returns the ICM selection for the residues forming the pocket.

5 Molecular Graphics

In this chapter we describe how to make beautiful graphical representations of molecules and manipulate them in the 3D graphics window. This includes how to change color, light, represenations, clipping planes, and how to use built in graphics effects. We also teach how to label and annotate molecules displayed in the graphical user interface.
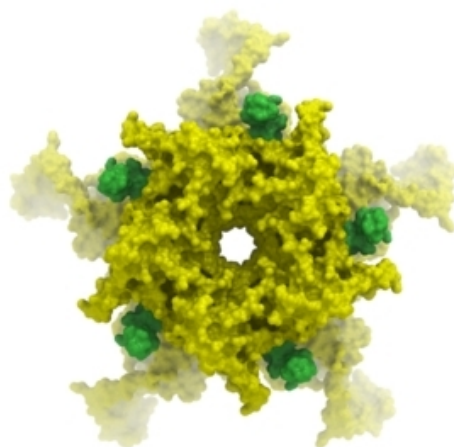
Chapter Contents:

- Molecule Representation
- Meshes-Surface-Grobs
- Coloring
- Lighting
- Labeling and Annotation
- Display Distances and Angles
- Graphics Effects
- Graphics Shortcuts
- Molecule Move Buttons
- Clipping Tools
- Graphic Layers
- High Quality Publication Images
- Movies

5.1 Molecule Representation

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro

To change the molecule display representation:

- Select the atoms, residues, molecules, or objects you wish to change in the graphical display or in the ICM Workspace.
- Then use the molecule representation (e.g. wire, ribbon) options in the Display Tab.

The display tab contains tools for a variety of functions including - structural representations, coloring, labeling and superposition.

There are six main types of structural representation in ICM. They are wire, ball and stick (Xstick), ribbon, skin, CPK and dot envelope (surface).

To display one of these representations:

- Click on the representation button you desire in the **display** tab.

wire · cpk · skin · ribbon · xstick · dot envelope
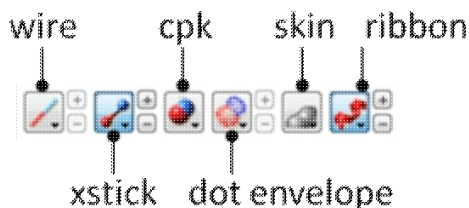
To remove a displayed representation or to toggle between display and undisplay:

- Click on the corresponding representation button in the **display** tab.

Identify which representations are displayed: The button display will change appearance (shaded blue) when selected. This makes it easier to identify which representations are currently being displayed.

ribbon displayed

xstick displayed

Change the display and size of the representations Many characteristics of the graphical representation such as color can be changed by clicking and **holding** on the button. The size can be changed by clicking the plus(+) and minus(-) buttons next to them.

click and hold for more options

+/- to change size
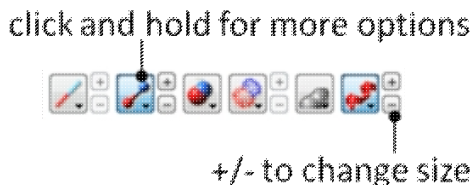
5.1.1 Wire Representation

Click and hold on the **wire representation** button. A menu will be displayed as shown below.
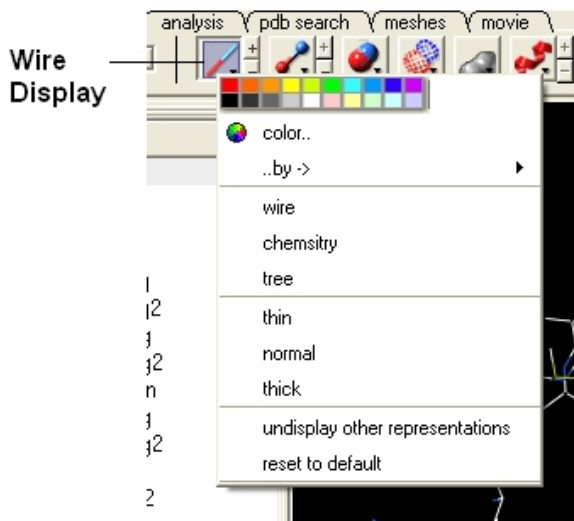
To change the wire style:

- Click and hold on the **wire representation** button and then click on **wire**, **chemistry** or **tree.**

To change the size of the wire representation:

- Click and hold on the **wire representation** button and then click on **thin**, **normal** or **thick.**

NOTE: Clicking on the +/- next to the **wire representation** button also changes the thickness of the wire representation.

To undisplay representations other than wire:

- Click and hold on the **wire representation** button and then click on undisplay other representations.

If you make a mistake or you are not happy with the way your structure is displayed with the wire representation:

- Click and hold on the **wire representation** button and then click on reset to default.

5.1.2 Stick and Ball (Xstick) Representation

Click and hold on the **stick and ball representation** button. A menu will be displayed as shown below.
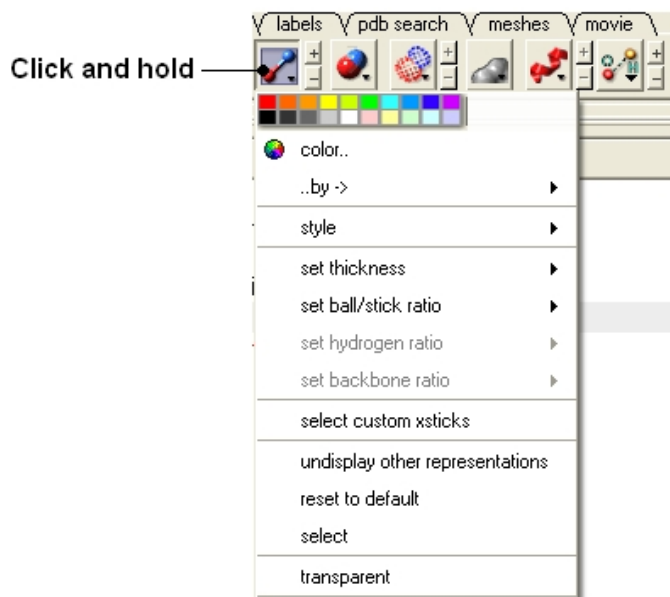
To change the style of the Xstick representation:

- Click and hold on the **stick and ball representation** button and then click on style and choose either **plain**, **chemistry**, **Chem Plus**, or **Chem Plus Aromatic**.

To change the size of the Xstick representation:

- Click and hold on the **stick and ball representation** button and then click on **set thickness** , **set ball/stick ratio**, **set hydrogen ratio**, and **set backbone ratio**.

NOTE: Clicking on the +/- next to the **xstick representation** button also changes the thickness of the xstick representation.

In order to make some parts of your picture clearer, the xstick representation can be set to transparent:

- Click and hold on the **stick and ball representation** button and then click on transparent.

To undisplay representations other than xstick:

- Click and hold on the **stick and ball representation button** and then click on undisplay other representations.

If you make a mistake or you are not happy with the way your structure is displayed with the xstick representation:

- Click and hold on the **stick and ball representation** button and then click on reset to default.

5.1.3 Ribbon Representation

Click and hold on the ribbon representation button and a menu will be displayed.

To change the style of the Ribbon representation:

- Click and hold on the **ribbon representation button** and then click on a style option: **smooth**, **wide**, **wide smooth**, **cylinders**, **protein worm** .

To assign secondary structure:

To accurately represent the secondary structure of the molecule in ribbon representation you may wish to assign secondary structure:

- Click and hold on the **ribbon representation** button and then click on **assign sec. structure**.

To make some parts of your picture clearer, the ribbon representation can be set to transparent:

- Click and hold on the **ribbon representation button** and then click on **transparent.**

To undisplay representations other than ribbon:

- Click and hold on the **ribbon representation** button and then click on undisplay other representations.

If you make a mistake or you are not happy with the way your structure is displayed with the ribbon representation:

- Click and hold on the **ribbon representation button** and then click on **reset to default**.

NOTE: Always use the **ICM** assign sec.** structure tool in the ribbon right click menu to get accurate secondary structure assignment. This is particularly important when studying helices which may have non-cannonical elements within them such as 3/10 or pi. To view non-cannonical helix segments use the segment option in the ribbon right click menu.

To change the display of chain breaks (dotted lines):

- Click and hold on the ribbon represenation button.
- Select the options Display Chain Breaks or Display Chain Break label.

5.1.4 Skin Representation

Click and hold on the **skin representation button**. A menu will be displayed as shown below.
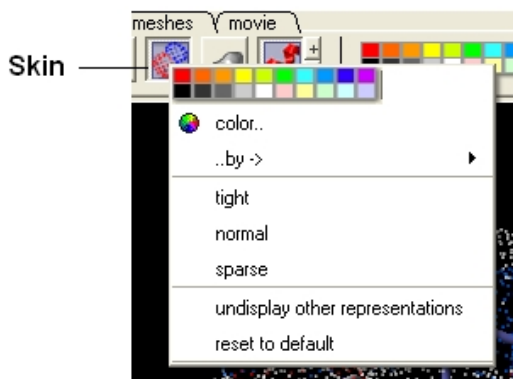
To make some parts of your picture clearer, the skin representation can be set to tight, normal or sparse:

- Click and hold on the **skin representation button** and then click on either tight, normal or sparse.

To undisplay representations other than skin:

- Click and hold on the **skin representation button** and then click on **undisplay other representations**.

If you make a mistake or you are not happy with the way your structure is displayed with the skin representation:

- Click and hold on the **skin representation button** and then click on ** reset to default**.

NOTE: Sometimes due to singularity problems holes may appear within the skin surface. To cure this infliction select atoms nearby and right click select Advanced->RandomizeAtoms

5.1.5 CPK Representation

Click and hold on the **CPK representation button**. A menu will be displayed as shown below.

To undisplay representations other than CPK:

- Click and hold on the **CPK representation button** and then click on **undisplay other representations**.

If you make a mistake or you are not happy with the way your structure is displayed with the cpk representation.

- Click and hold on the **CPK representation button** and then click on **reset to default**.

5.1.6 Surface Representation

Click and hold on the surface representation button. A menu will be displayed as shown below.
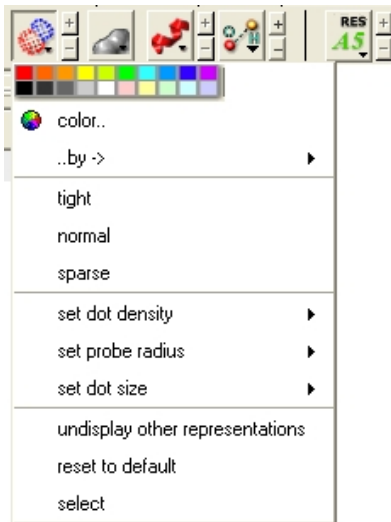
To change the style of the surface representation:

- Click and hold on the **surface representation button** and then click on **tight**, **normal**, or **surface.**

To undisplay representations other than surface:

- Click and hold on the **surface representation button** and then click on undisplay other representations.

If you make a mistake or you are not happy with the way your structure is displayed with the surface representation:

- Click and hold on the **surface representation button** and then click on **reset to default**.

5.1.7 Display and Undisplay Hydrogens

To display and undisplay hydrogens. Click and hold on the **"Change Hydrogen Display"** button shown below. Multiple single clicks will toggle through the hyrogen display options.

- Display Tab
- Click and hold on the **"Change Hydrogen Display"** button shown below.
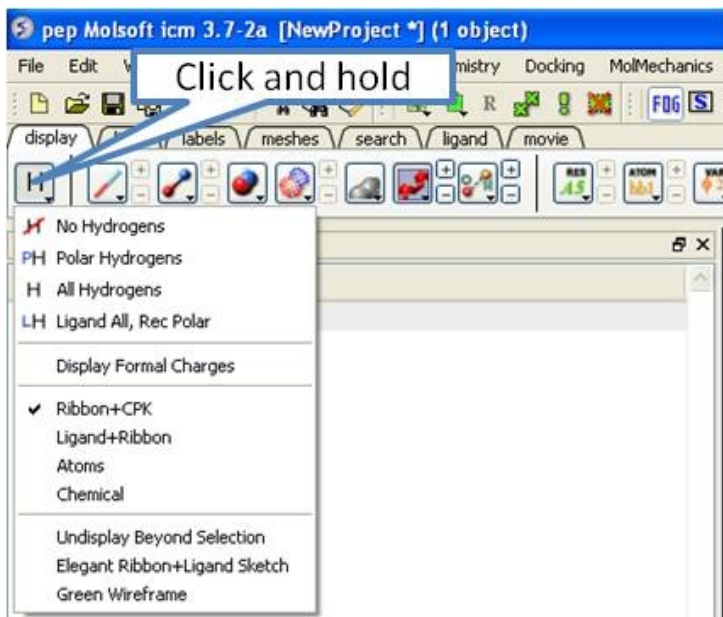
5.1.8 Display Hydrogen Bond

> **NOTE:** The method by which hydrogen bonds are calculated is described here in the command line manual. The GRAPHICS.hbondMinStrength parameter determines the hbond strength threshold for hbond display. The strength value is between 0. and 2. By changing 1. to 0.2 you will see more weak hydrogen bonds.

In order to display potential hydrogen bonds in your structure:

- Convert to an ICM Object
- Make a selection - if you are trying to display the H-bonds between a ligand and the receptor make sure the ligand is part of the selection.
- Click the Display Tab.
- Click on the **Toggle H-bonds** icon in the **display** tab.
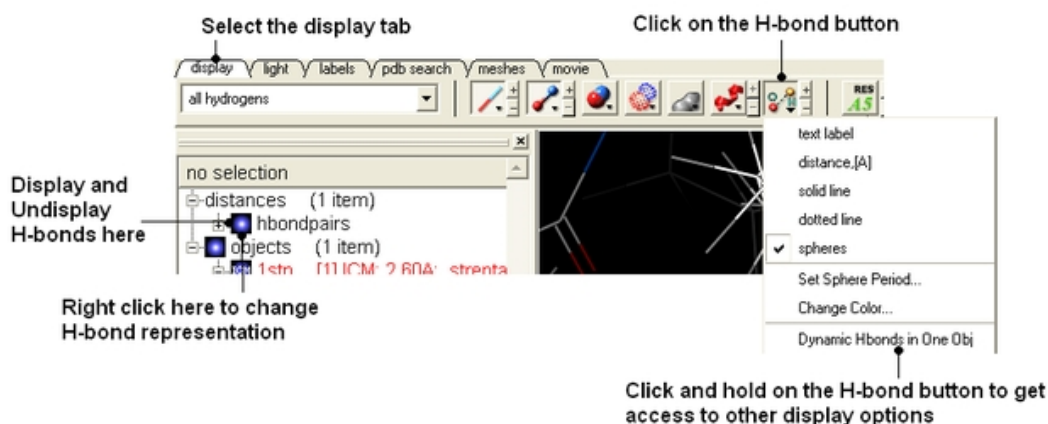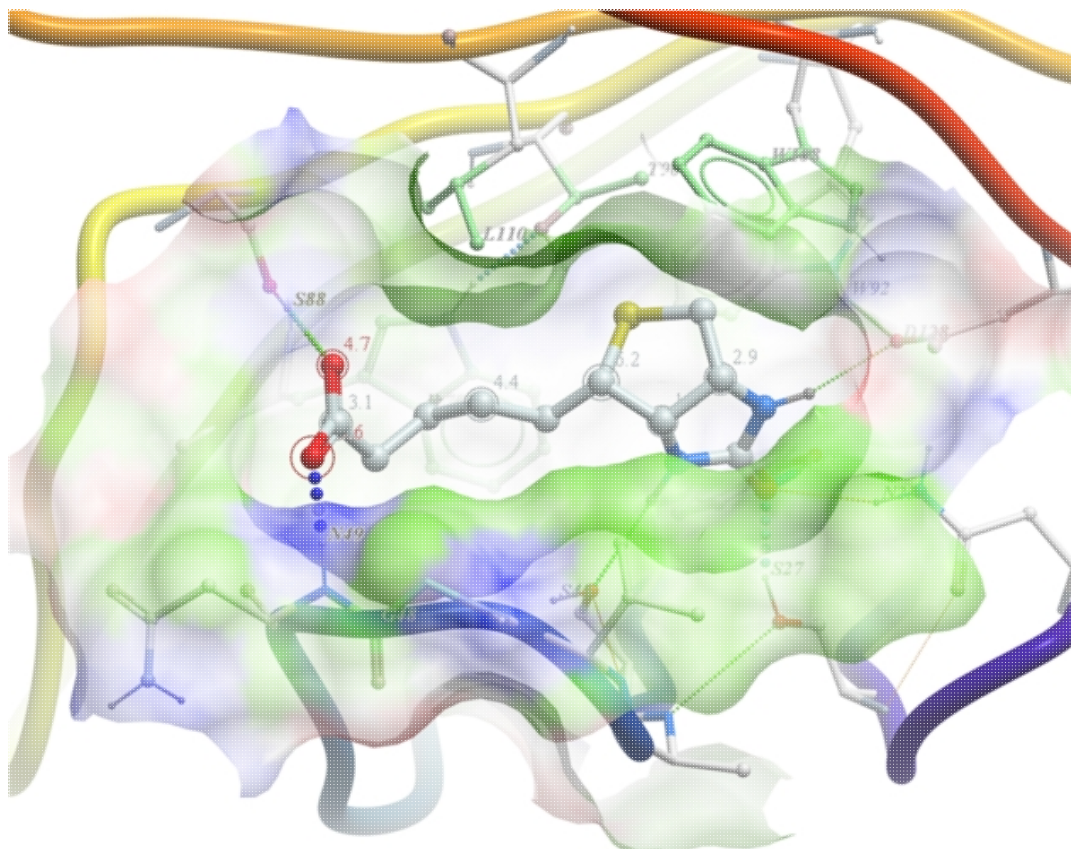
- Click the +/- on the right of the H-Bond button to change thickness of H-bond representation.
- Click and hold the button to change representation or use the **hbondpairs** option in the ICM Workspace.
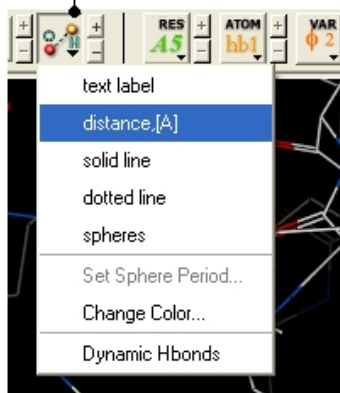
What do the default coloring of the H-bond represent?

Longer and shorter H-X distances in the hydrogen bond are color-coded, from red to blue, respectively.

> **NOTE** Dynamic hydrogen bonds can be set by clicking and holding on the **H-bond toggle** button in the **Display** tab. Hydrogen bonds will then respond to any changes made to the ligand.

5.1.9 Display Formal Charges

You can display formal charges by clicking and holding on the **"Change Hydrogen Display"** button in the **Display tab**.

5.1.10 Ca (carbon alpha) Trace

To generate a carbon alpha (Ca) trace:

- Right click on the object in the ICM workspace and select Tools/Make Ca-Trace object.
- The Ca-Trace object will be displayed in the ICM workspace.
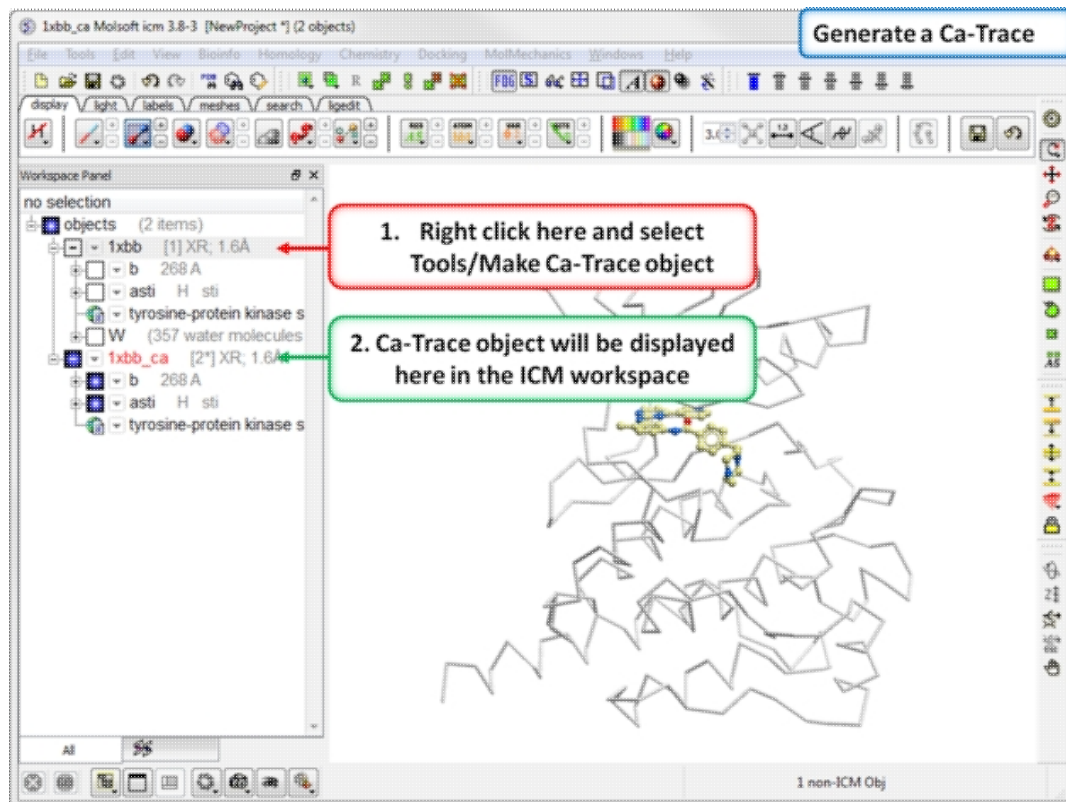
5.2 Multi-Windows

To display more than one window click on the buttons shown below.
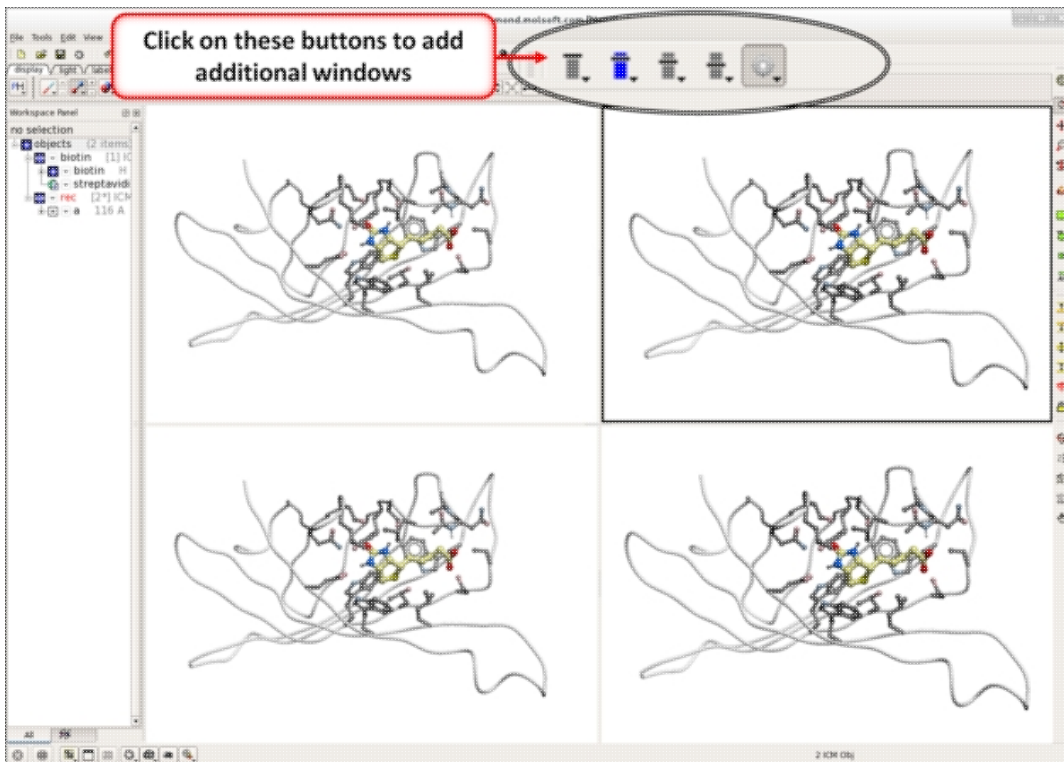
To control the layout and mouse sync mode click on the button shown below.
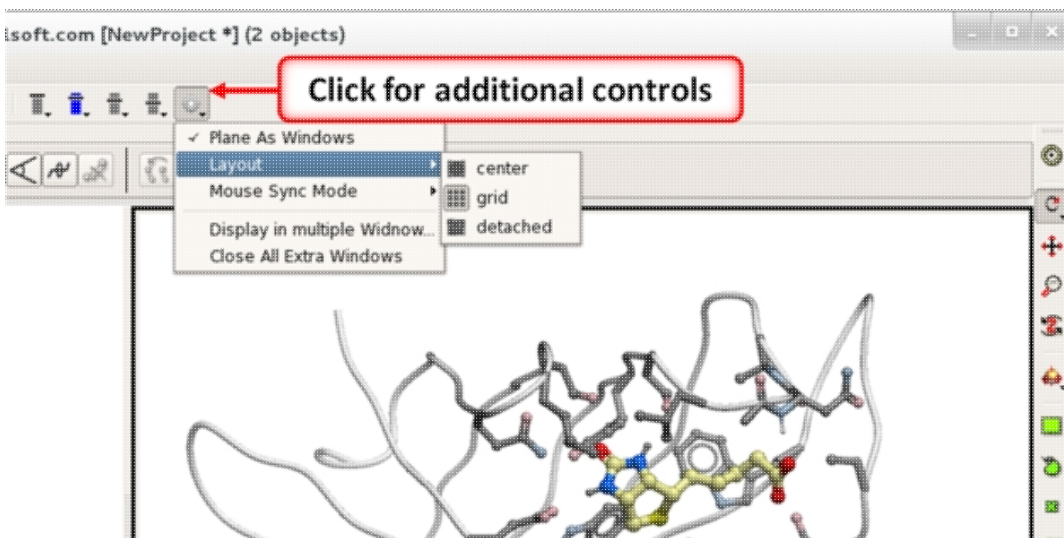
5.3 Meshes - Surface - Grobs
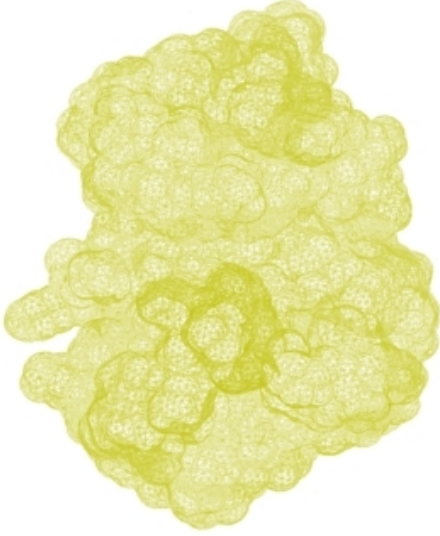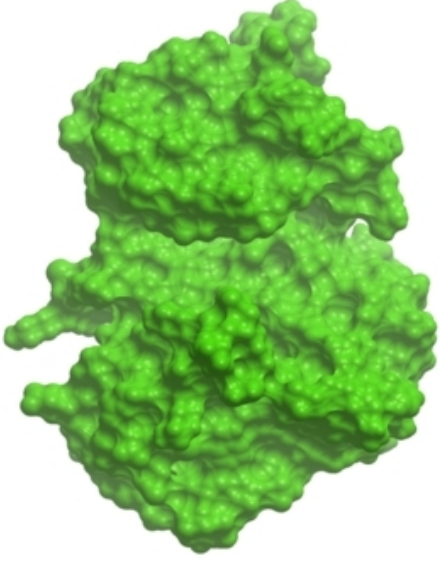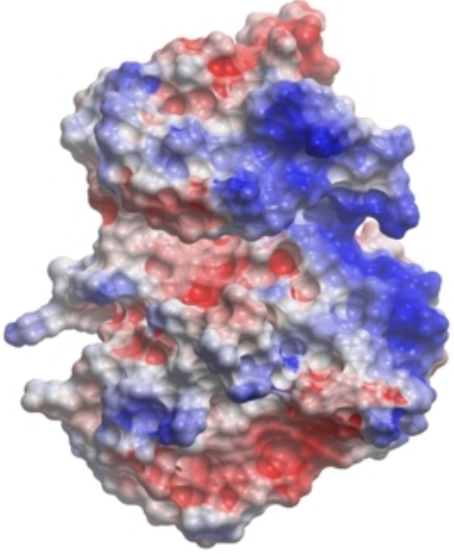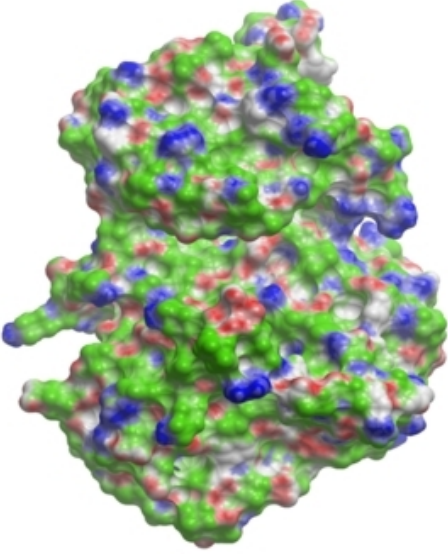
Available in the following product(s): ICM-Browser-Pro | ICM-Pro

Click on the tab button entitled **'meshes'** and more graphics tools for surfaces are available. In ICM surfaces are sometimes referred to as meshes or graphical objects (Grobs).

 Surface	 Plain Solid
 Electrostatic Surface	 Binding Property Surface

| Macroshape Solid | Macroshape Transparent |
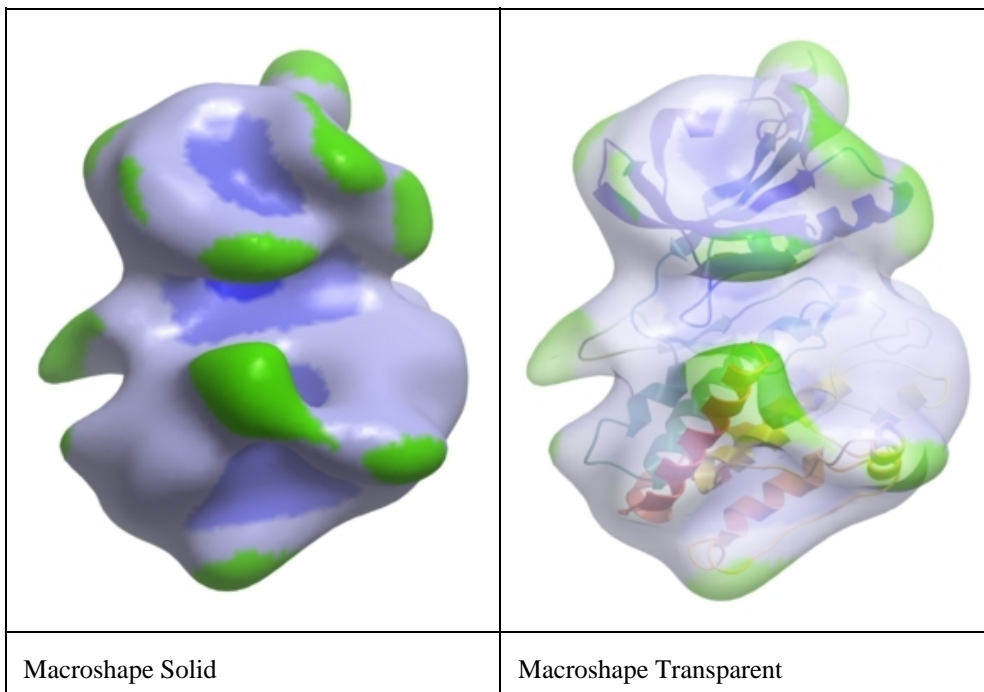
5.3.1 Surfaces

The surface of your structure can be displayed and colored by
electrostatics or **binding** properties. To do this:

- Load a structure into ICM `File/Open` or tab-pdb{PDB Search}
- `Convert` the structure into an ICM object.
- Select the 'meshes' tab button.
- Click on the drop down arrow menu shown below and select which
  surface you wish to generate.
- Click on the generate surface button next to the drop down arrow.

5.3.2 MolSkin
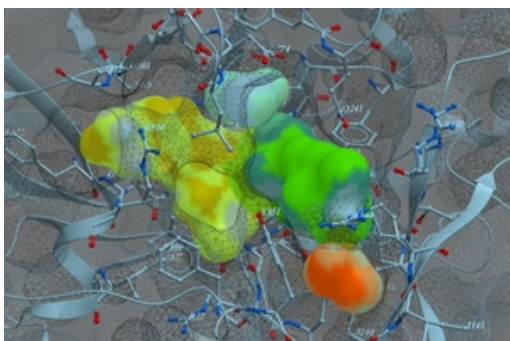
MolSkin is a new macro that instantly makes publication quality graphics
and it gives each molecule a color and colors contact patches. It

undertakes the following:

- Molecular surfaces are generated with those assigned colors and occlusion shaded.
- Carbons of each molecule are colored by its own consistent color.
- Labels, ribbons, are colored accordingly.
- Surfaces are gradually cross-colored by each other to mark the contact patches.
- The labels are brought to the surfaces.

To use MolSkin:

- Right click on the object in the ICM workspace and choose Shapes/Make/Molecular Shapes and Contacts.
- Once the surfaces are made they will be listed in the ICM workspace where they can be toggled on or off.

5.3.3 Color Surface by Proximity

To color a surface by proximity to neighboring molecules:

- `Make` a surface.
- Right click on the molecule you want to color by proximity and choose Shapes/Make/Color by Proximity.

5.3.4 Color Surface by Selection

To color a surface by a selection:

- `Make` a surface.
- `Make` a selection.
- Right click on the molecule you want to color by selection and choose Shapes/Make/Color by Selection.

5.3.5 Meshes

A variety of shapes can be constructed automatically using ICM. These shapes are referred to as meshes. The types of shapes you can build are shown in the drop down option in the meshesh tab
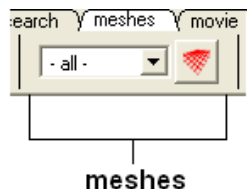
To make a shape select it from the menu by clicking on the down arrow and then click the button next to the menu. The shape will then be displayed in the 3D graphics window.
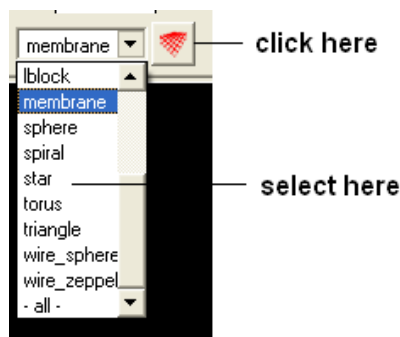
5.3.6 Macroshape

A macroshape can be constructed and allows easy viewing and manipulation of the structural representation. A macroshape representation is ideal for large structures which allows the user to easily identify important regions of the structure and facilitate the return to the 'standard' view of a particular molecule. All the buttons needed to display a macroshape structure are shown below in the 'meshes' tab.
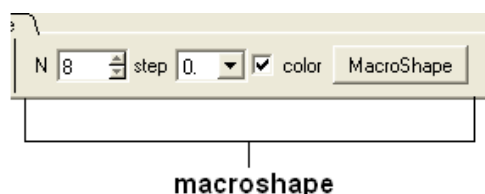
To construct a macroshape:

- Load a molecule into ICM `File/Open` or tab-pdb{PDB Search}
- Select the amount of detail required in the shape by increasing the values in 'N' or 'step' data entry box (note the default values are usually sufficient).
- Check the 'color' if you wish your molecule to be colored.
- Click the button labeled 'MacroShape'.

Macroshape can also be used from the View menu: View/Macro Shape

5.3.7 Google 3D Objects (Sketchup)

To read in a 3D Mesh from Google in KMZ or COLLADA format:

- File/Load/ 3D Mesh in KMZ or COLLADA Format from Google
- Search for the object you would like to view and download it.
- To read the file go to File/Open

5.3.8 Display or Undisplay Meshes or Surfaces

To display or undisplay the surface click in the box in the ICM
workspace as shown below:
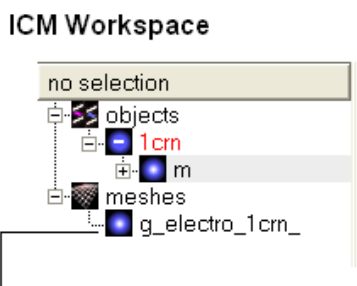
NOTE: All surfaces, meshes and macroshapes come under the one heading
of **meshes** in the workspace panel.

5.3.9 Mesh Options.

A number of options relating to meshes can be found by right clicking on
the mesh in the ICM Workspace. This section describes some of these
options.
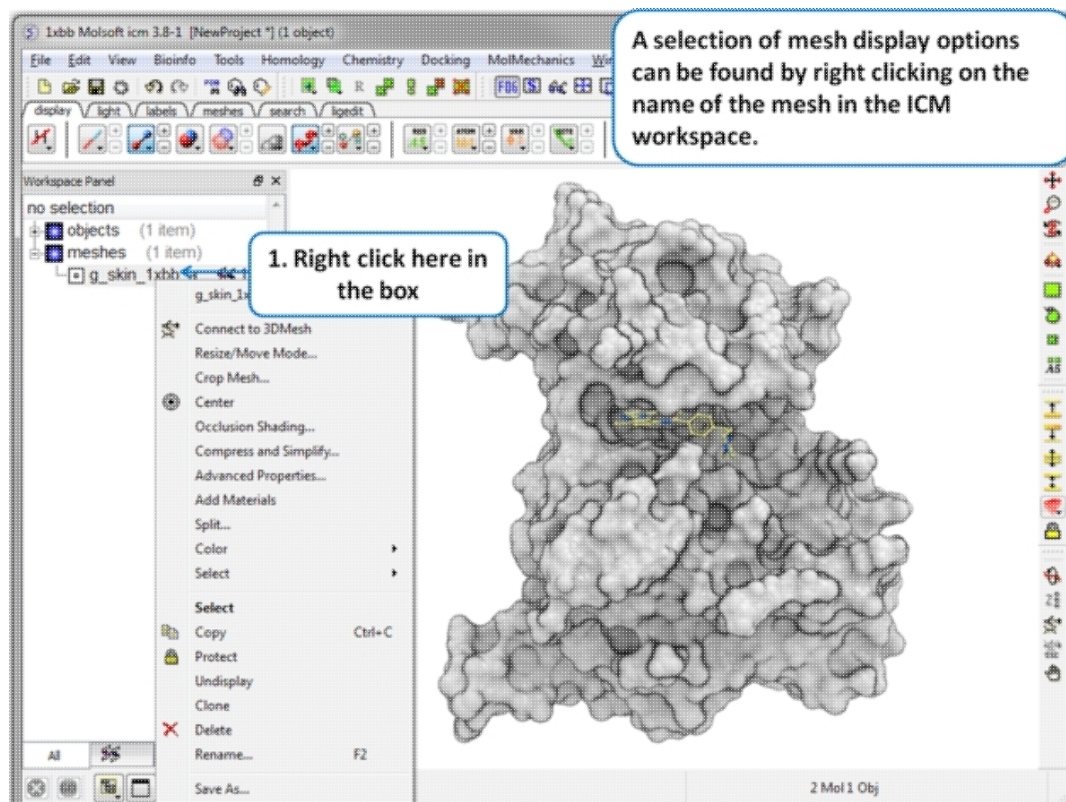
5.3.9.1 Resize Mesh

Once a mesh has been created you can move it and resize it. To do this:

- Locate the mesh in the ICM Workspace and right click on it
- Select the **Resize/Move Mode** in the menu.
- A purple box as shown below will surround the molecule.
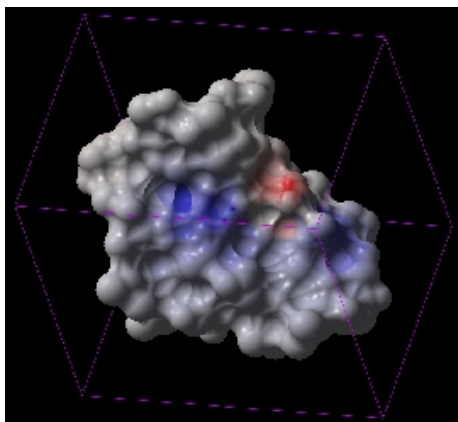
To resize the mesh click on one of the corners of the box and drag to the required size. The number displayed on the edges of the box represent the dimensions.
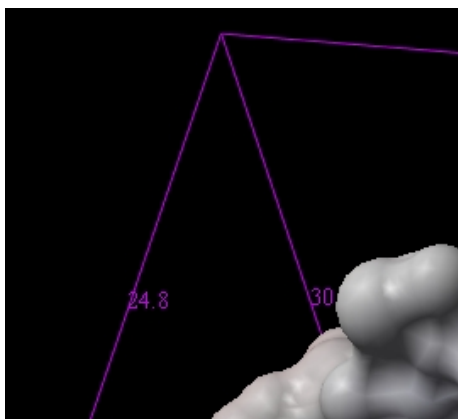
5.3.9.2 Move Mesh

To move a mesh:

- Locate the mesh in the ICM Workspace and right click on it
- Select the **Resize/Move Mode** in the menu.
- Click on the mesh in the graphical display with the **middle** mouse button.

5.3.9.3 Mesh Representation

There are five different display modes:

- Dot
- Wire
- Solid Rugged
- Solid Smooth
- Smooth Transparent.
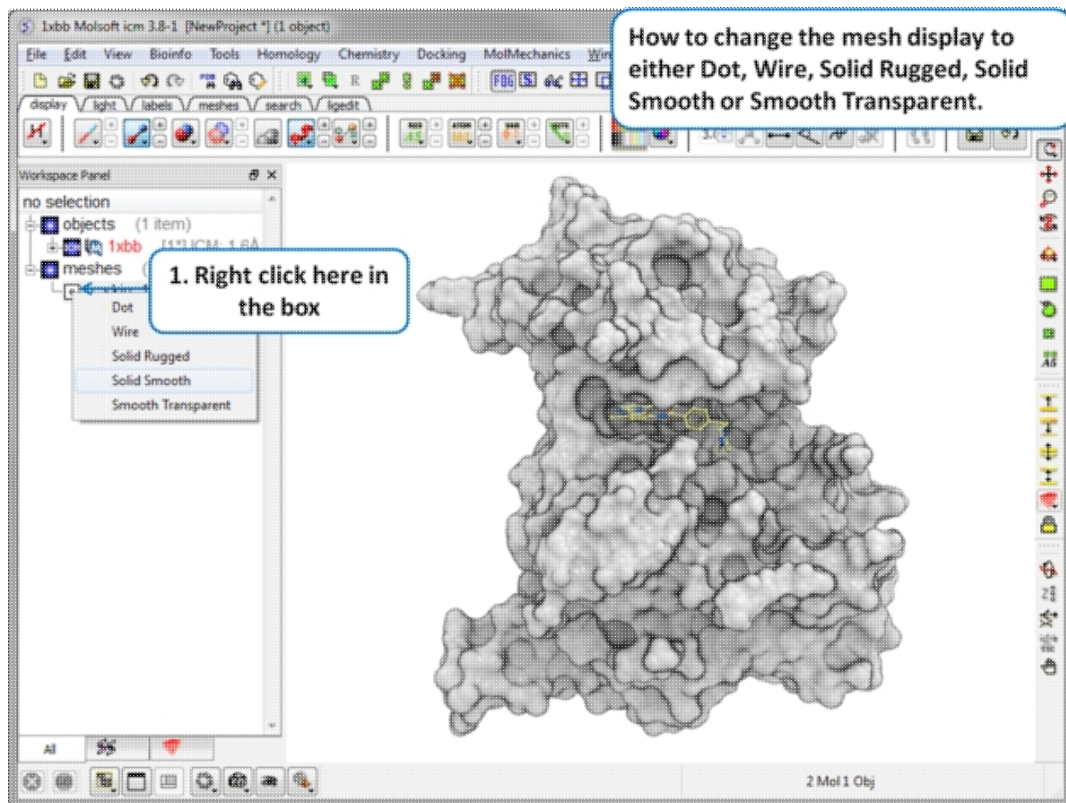
5.3.9.4 Mesh Color and Lighting

There are a number of options to color and change the display of the mesh. These options can be accessed simply by right clicking on the mesh name in the ICM Workspace as shown below.

5.3.10 Mesh Clipping

Clipping tools can be used to adjust the frames of the mesh independently of other objects.

The buttons shown below can be used for this purpose.
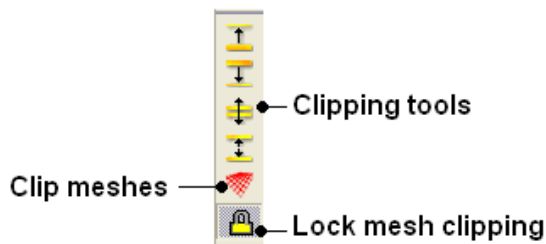
The buttons used for clipping are described in the section entitled Clipping Tools.

To clip the skin independently of the object as shown in the image above:

- Display object and mesh.
- Click and hold on clip meshes button(red button) and select Reset Clipping to Default and check the option Clipping enabled
- Click on the front clipping plane button.

- (Optional) To lock the mesh clipping click on the yellow lock mesh clipping button.

5.3.11 Save Mesh

You can save a mesh as a wavefront object by right clicking on the mesh in the ICM Workspace and selecting **SaveAs.**

5.3.12 Occlusion Shading

The occulusion shading option provides better representation of depth within a cavity. The color of each surface element of a grob (mesh) is changed by mixing its own color with the background depending on the burial of the surface element.

To add occlusion shading:

- Right click on the mesh in the ICM Workspace and select **Occlusion Shading**. The occlusion shading value can also be changed before generating the mesh in the meshes tab.
- Enter a depth value - default is 0.8. Higher values will generate a more dramatic shading.

See example `here`.

5.4 Coloring

Available in the following product(s): `ICM-Browser` | `ICM-Browser-Pro` | `ICM-Pro`

To change the coloring of molecules:

- `Select` the atoms, residues, molecules, or objects you wish to color in the graphical display or in the ICM Workspace.
- Then use the color options in the `Display Tab`.
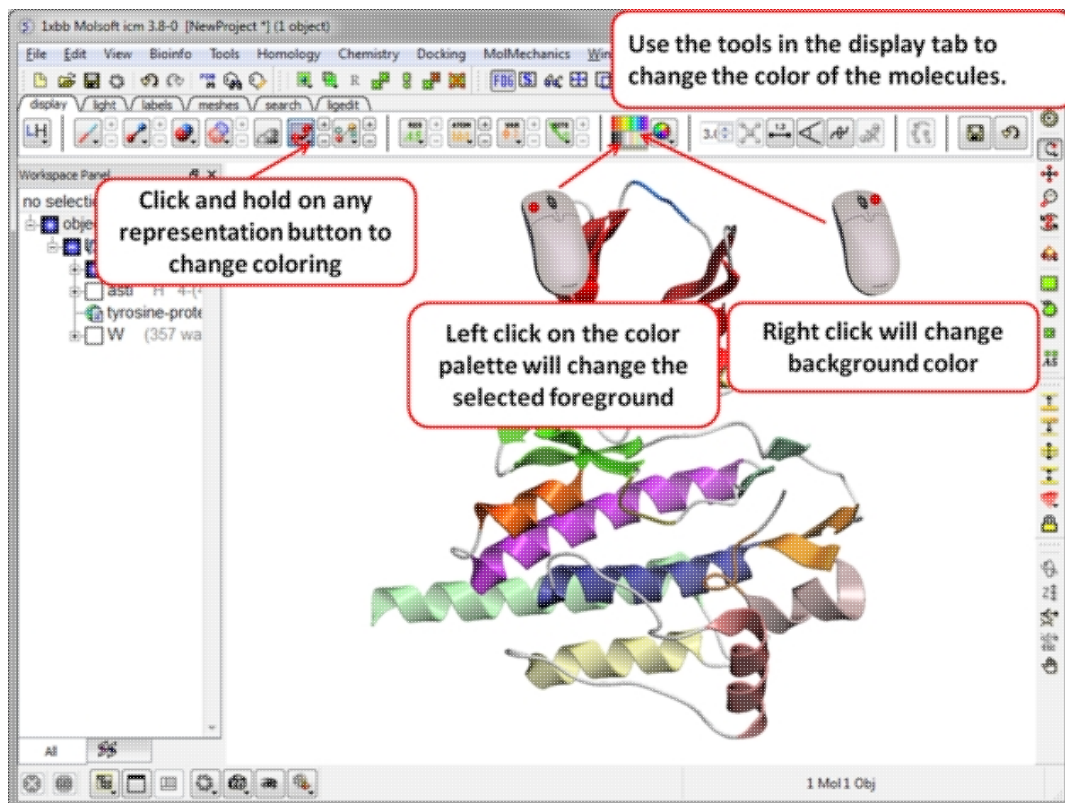
5.4.1 Coloring

To change the color of a structural representation such as CPK, Xstick, wire or ribbon.

- `Select` the atoms, residues, molecules, or objects you wish to color.
- Click and hold on the structural representation button for the representation you wish to color (e.g. wire, ribbon etc...) in the **Display** tab.
- Select a color by clicking color.

To color by a particular parameter such as atom type, b-factor, secondary structure etc...

- Click and hold on the structural representation button for the representation you wish to color (e.g. wire, ribbon etc...) in the **Display** tab.
- Select ..by-> option

To change the color of everything displayed :

Click on the color palette in the `Display Tab`.

5.4.2 Color Background

To change the color of the background:

- Select **View/Color** background.
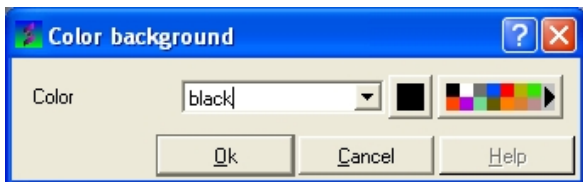
- Click on the square of your desired color. If you are not satisfied with the color palate, click on the arrow next to the colors to customize a color.

OR

- Right click on a color in the colors panel in the display tab.

Right click here in the display panel to set the background color.

5.4.3 Background Image

A background image can be added to the graphical display. This can be useful for making cool images or for comparing structures (e.g.compare displayed object with background image of object).

To add a background image from an image file (png or jpeg):

- File/Open Image
- Right click on the image in the ICM Workspace and select "Set as Background in Graphics."
- Choose one of the following: (1) Original Resoution, (2) Original Resolution Centered, or (3) Scale to Fit
- Enter the Scale (%)
- Enter whether you want to keep existing background.

To move and resize a background image: Version 3.7-2b and higher.

- File/Open Image
- Right click on the image in the ICM Workspace and select "Set as Background in Graphics."
- Choose the option **Original Resoution**.
- Click on the drag atom button (looks like a hand).
- Click and drag image.
- Remember to deselect drag atom button.

Background images

1. File/Open Image file

2. Right click on image in ICM Workspace and select "Set as Background in Graphics"

4. If you select the option "Original Resolution" you can drag and resize the image around the window. Click on the "hand" button to do this.

3. Background Image You can drag around window see point #4.
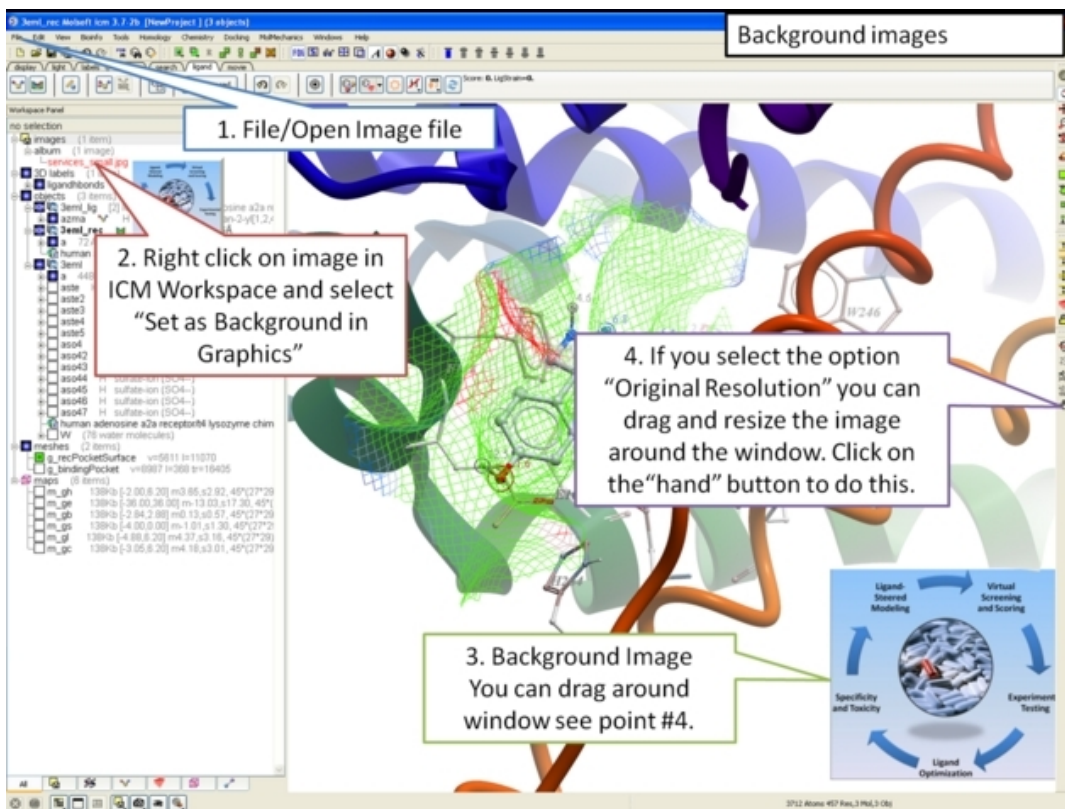
To set current display as background image:

- Click and hold on the "Copy Image to Clipboard" button at the bottom of the gui and select the "Set as Background" option.

To remove a background image:

- Select the **display** tab and then click and hold on the color sphere button and select "Remove Background Image".
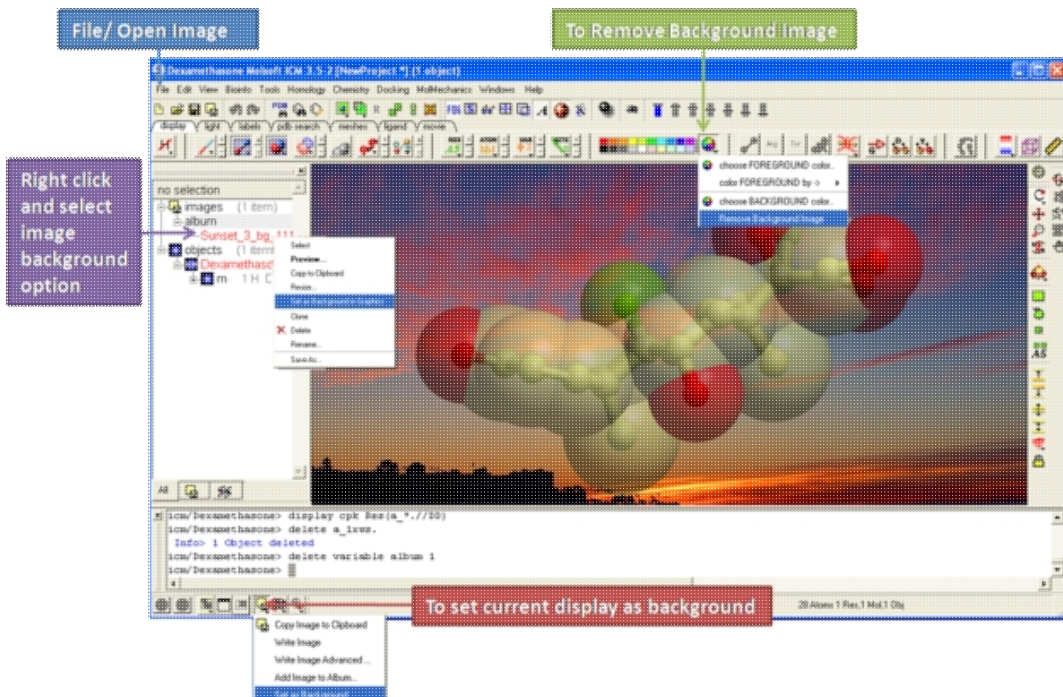
File/ Open Image

To Remove Background Image

Right click and select image background option

To set current display as background

OR

- Right click on the image in the ICM Workspace and select "Set as Background in Graphics."
- Choose the option **Remove From Background**

5.5 Lighting

Available in the following product(s): `ICM-Browser` | `ICM-Browser-Pro` | `ICM-Pro`

These options are in the `light tab`
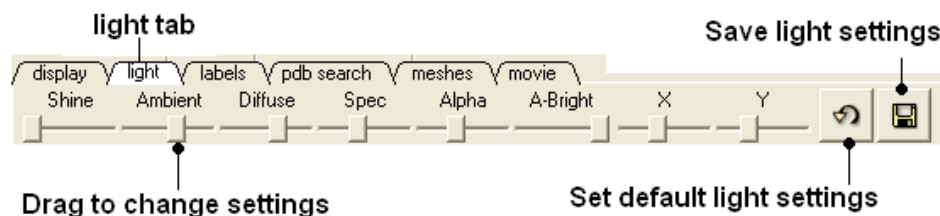
CLick and drag the sliders to change the lighting. You can also save your preferred lighting settings and return to default.

Shine - shininess property of the solid material

Ambient - ambient light intensity of RGB for ambient light

Diffuse - diffuse light intensity of RGB for diffuse light

Spec - specular light intensity of RGB for specular light

Alpha - transparency setting for grob

A-Bright - light intensity shinning on grob

X and Y - Change the position of the light source in the graphics window

5.6 3D Stereo

Available in the following product(s): `ICM-Browser` | `ICM-Browser-Pro` | `ICM-Pro`

Click and hold on the stereo hardware button (see image below).
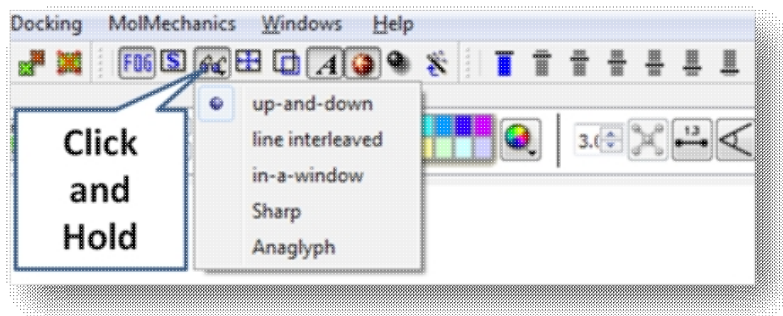
There are 5 different options available which are described here
`http://molsoft.com/man/icm-table.html#GRAPHICS.stereoMode`.

1. up-and-down
2. line interleaved
3. in-a-window
4. Sharp
5. Anaglyph

The Anaglyph option is the easiest to used with inexpensive 3D glasses
and and without any expensive 3D compatible hardware or monitors. The 3D
effect is better with a lighter background.
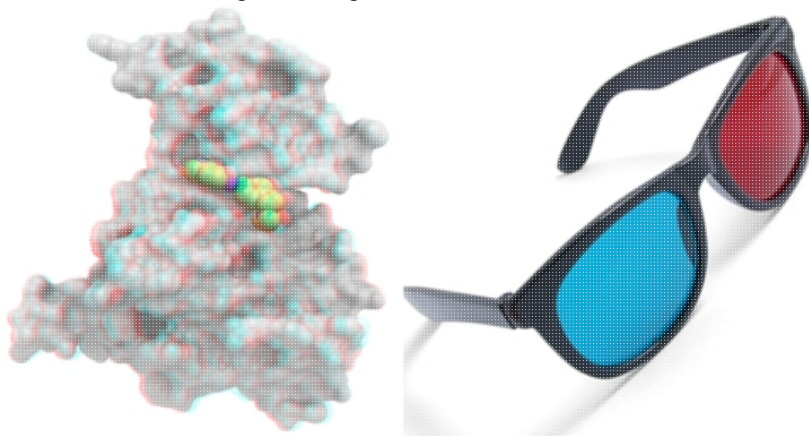
5.7 3D Printing

You can save surfaces in the STL format for 3D printing. To do this:

- Make a mesh e.g. go to the meshes tab - plain solid.
- The mesh (surface) will then be displayed in the ICM workspace
  (left hand side).
- Right click on the mesh and choose Save as.. and choose stl from
  the format option list

5.8 Labeling and Annotation

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro

To add labels or display or undisplay pre-defined annotation:

- Select the atoms, residues, molecules, or objects you wish to
  label in the graphical display or in the ICM Workspace.
- Then use the label options in the Display Tab.

To add new user-defined annotation:

- Select the atoms, residues, molecules, or objects you wish to
  label in the graphical display or in the ICM Workspace.
- Right click on the selection and choose "Annotate Selection".

5.8.1 Labeling

Labeling options are contained within the Labels or Display Tab. In many
cases clicking and holding a label button will allow you to view more
options.

5.8.2 Labeling Atoms

Select the atoms you wish to label (see `display structure` or `selection toolbar`).

- Select the **display** tab.
- Click the **label ATOM** button.
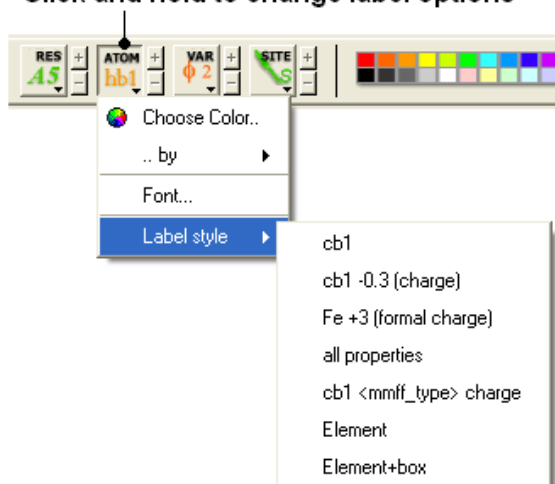
To change the level of label detail:

- Click and hold the **label ATOM** button and select the desired level of label detail, color or style.

5.8.3 Labeling Residues

To label residues:

- Select the **display** tab.
- Select the residues you wish to label (see `display structure` or `selection toolbar`).
- Click the **label RES** button.

Click and hold for more options
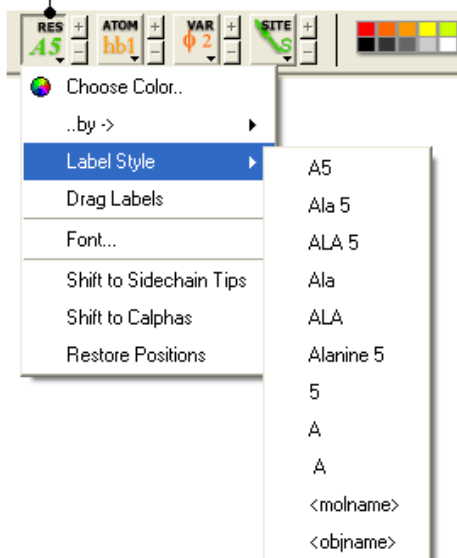
To change the level of label detail:

- Click and hold the **label RES** button and select the desired level of label detail or style.

5.8.4 Move Residue Label

To change the location of your residue label:

- Select View/Drag res labels.
- If your mouse has a middle mouse button, then click on handle (as shown) of the label you wish to move, and drag it to your desired area.

Click on this area to drag your label.

- If your mouse has no middle mouse button, then click on the Translation icon on the toolbar, and click on the handle (as shown) of the label you wish to move, and drag it to your desired area.

The +/- buttons on the side of the Residue and Atom buttons will shift the label. There are also other **residue label move** options available when you click and hold the residue label button. These options include **Shift to Sidechain Tips**, **Shift to Calphas**, and **Restore Positions**

5.8.5 Label Variables

To label variable angles (dihedral-torsion, planar and phase angle) the molecule needs to be converted into an `ICM object`.

- Convert the molecle to an `ICM object`.
- Select the atoms for which you would like to display the variables (see `display structure` or `selection toolbar`).

- Click on the **toggle variable label** button shown above located in the **display** tab.
- Change the font size by using the +/- buttons.
- Change the font and color by clicking and holding on the variable atom label button.

To change the variable label style click and hold the variable atom label button as shown below.
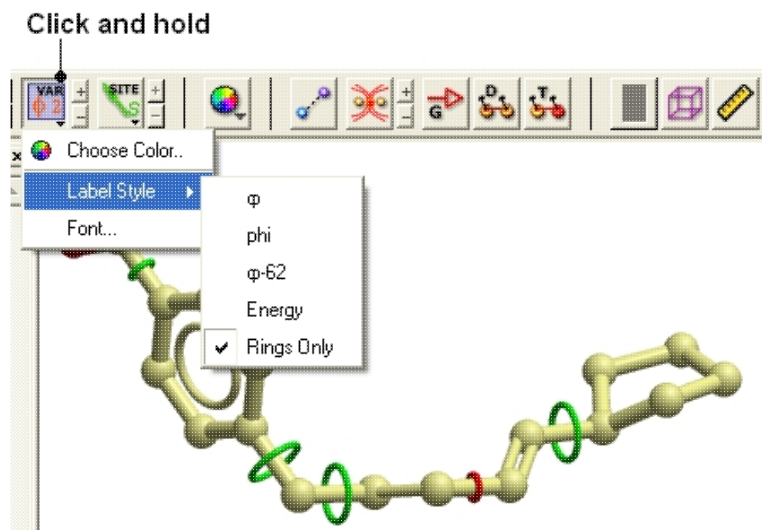
Rings of varying diameter and color are superimiposed on rotatable bonds. Green rings with large diameter are considered less constrained than rings with small green rings. Red rings are highly constrained. When the **Label Style/Energy** option is selected the first number displayed represents the bond angle, the second the energy and the third the worst energy that could be achieved by rotating the bond.

5.8.6 Sites and Annotation
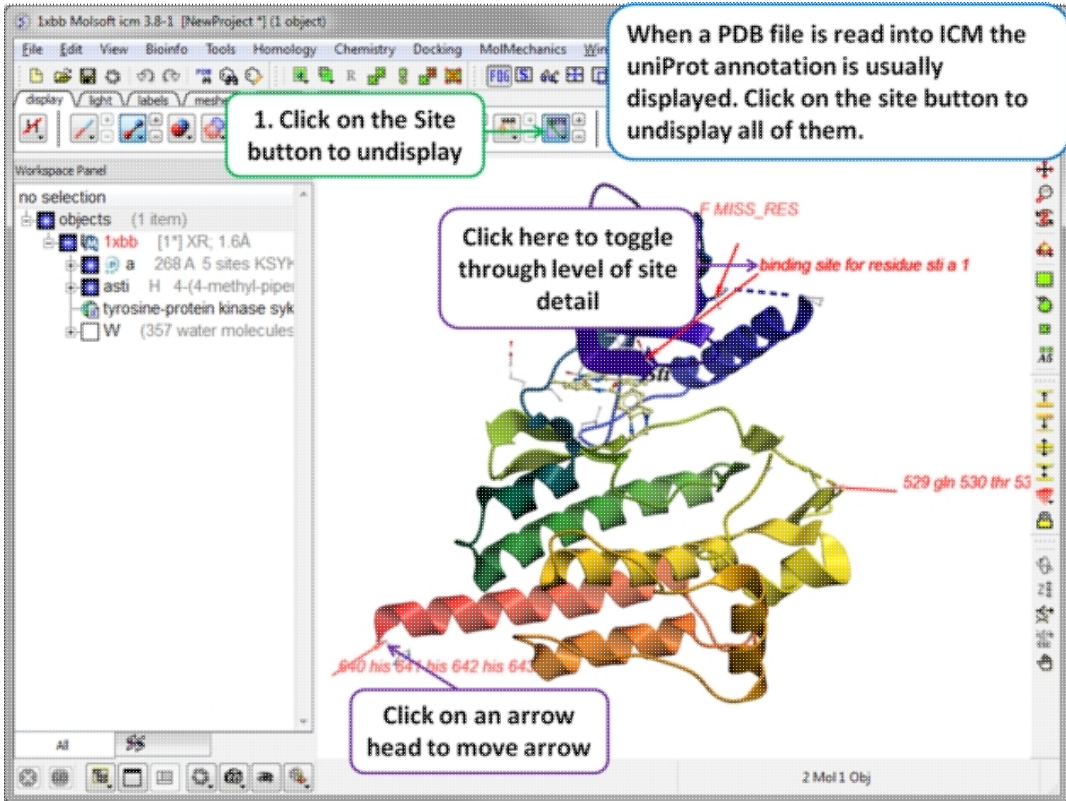
When a PDB file is read into ICM the sequence functional/mutation sites listed in Uniprot are automatically displayed. To undisplay all displayed sites click on the **Sites** button in the `display tab`. Click in the bottom left hand corner of the annotated site to change the level of detail or click on an arrow head to move the location.
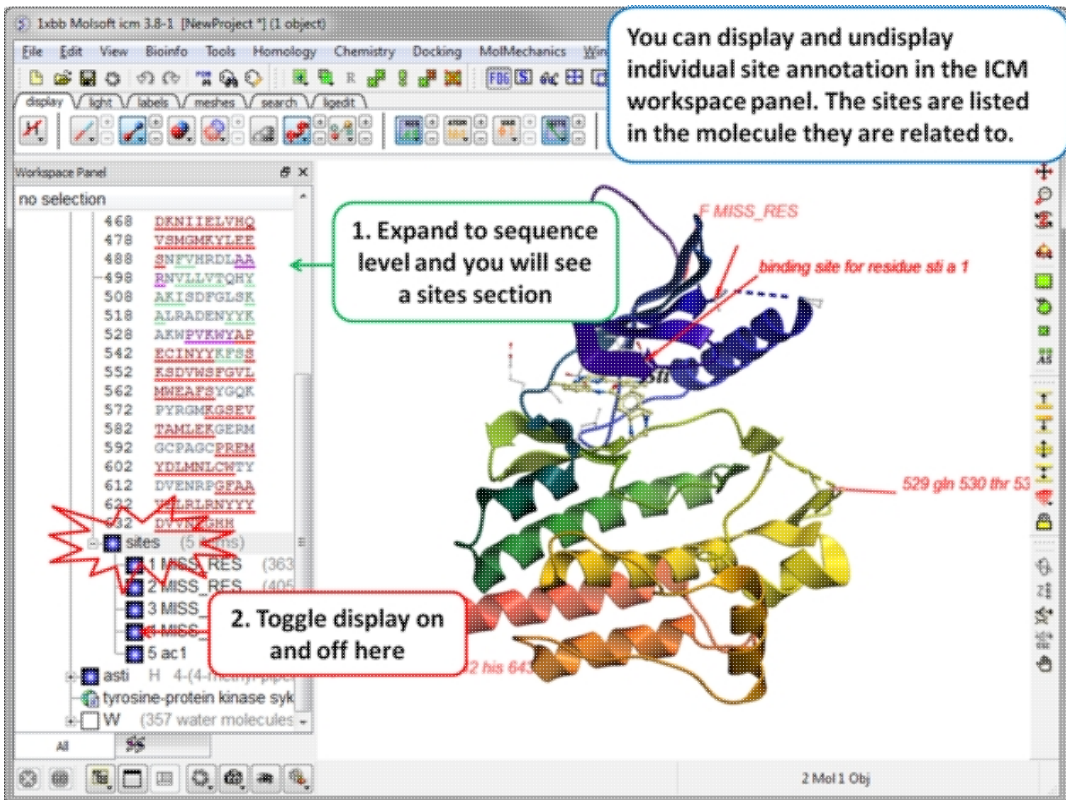
Individual sites can be displayed or undisplayed in the ICM workspace.
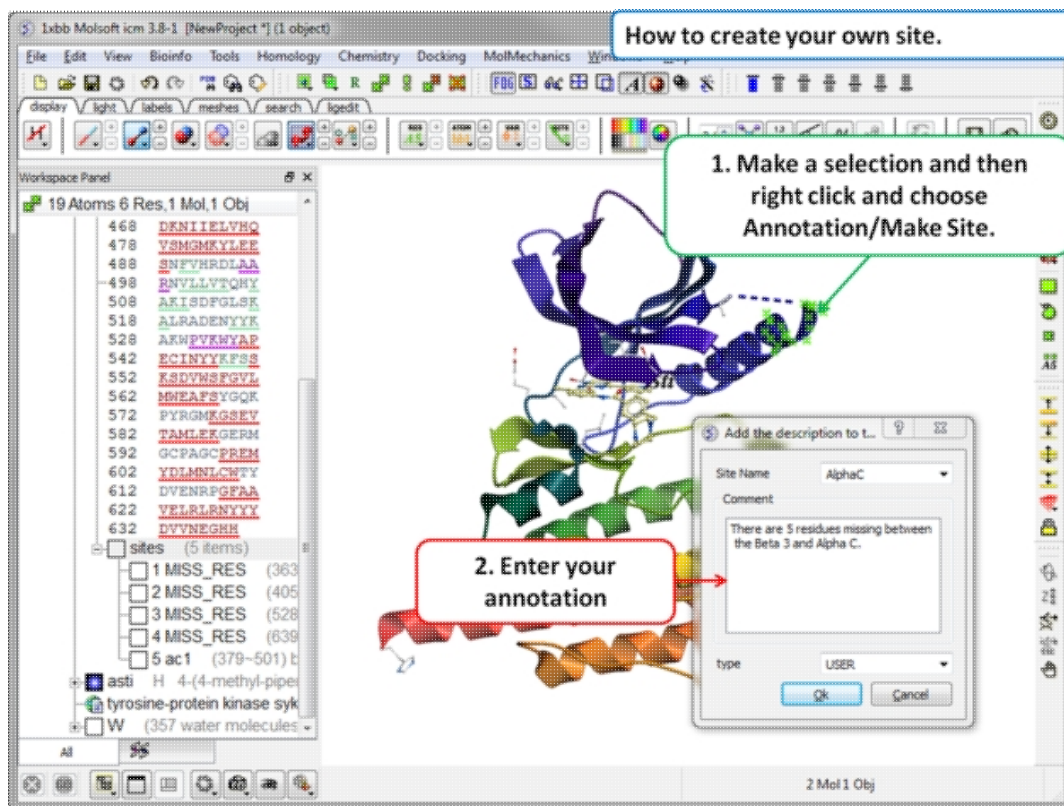
To create your own annotated site:

- Select the region you wish to annotate as a Site.
- Right click on the selection.
- Choose the option Annotate/Make Site
- Enter the annotation into the text box and select ok
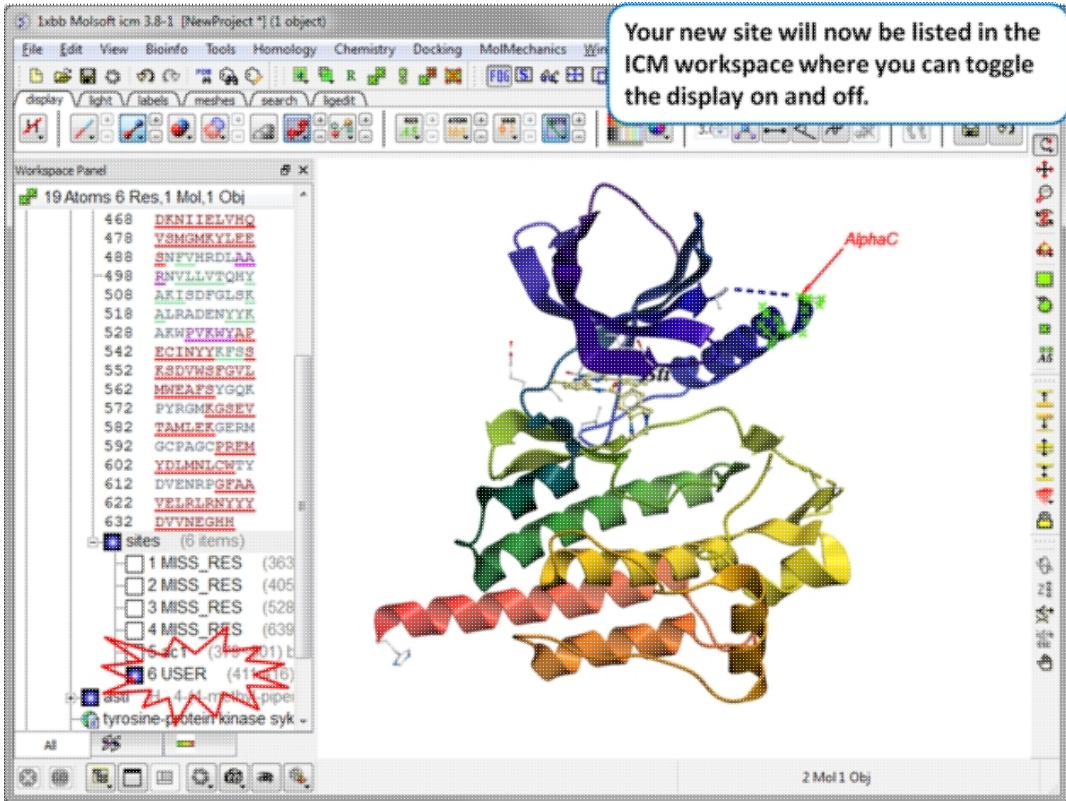
Your new site will now be listed in the ICM Workspace.
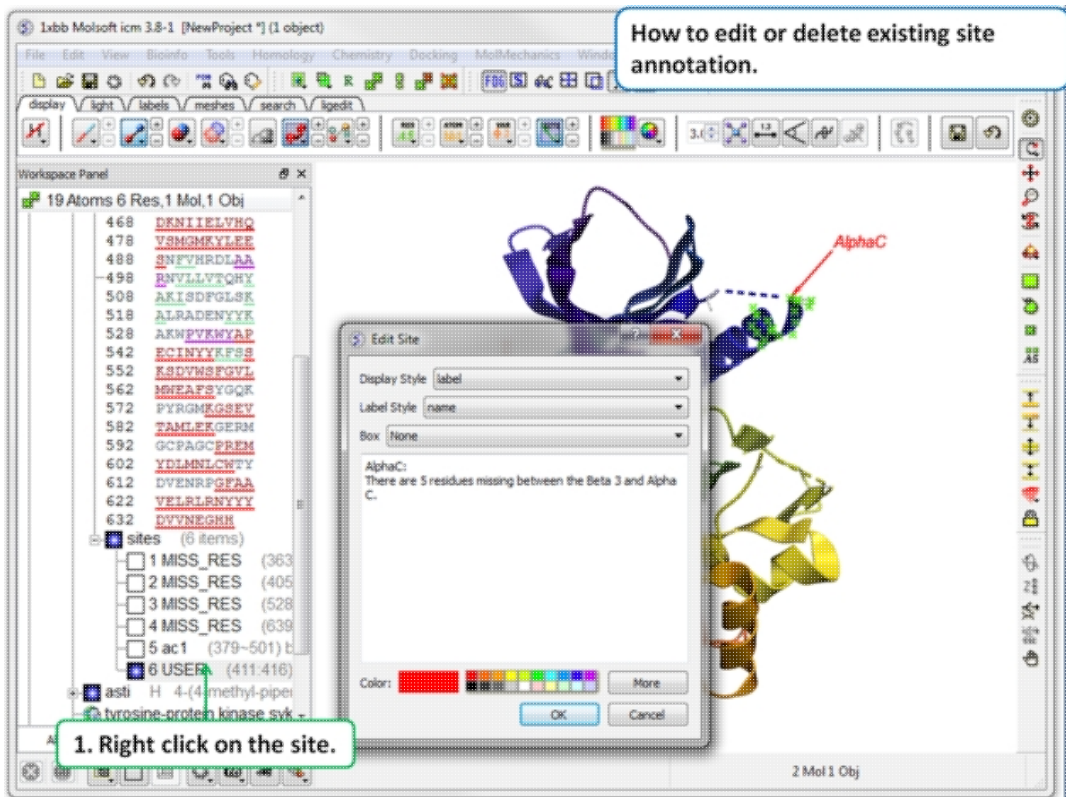
To edit or delete a site:

- Right click on the site in the ICM workspace.

5.8.7 Changing Label Colors

To change the color of any label:

- Click and hold down the required label button and a menu as shown below will be displayed.

- Select **color.**

5.8.8 Customized Label 2D or 3D

To generate a customized a label:

- Select the **labels** tab.
- Select either 2D or 3D button.
- Enter your label and select the desired color, font and size.
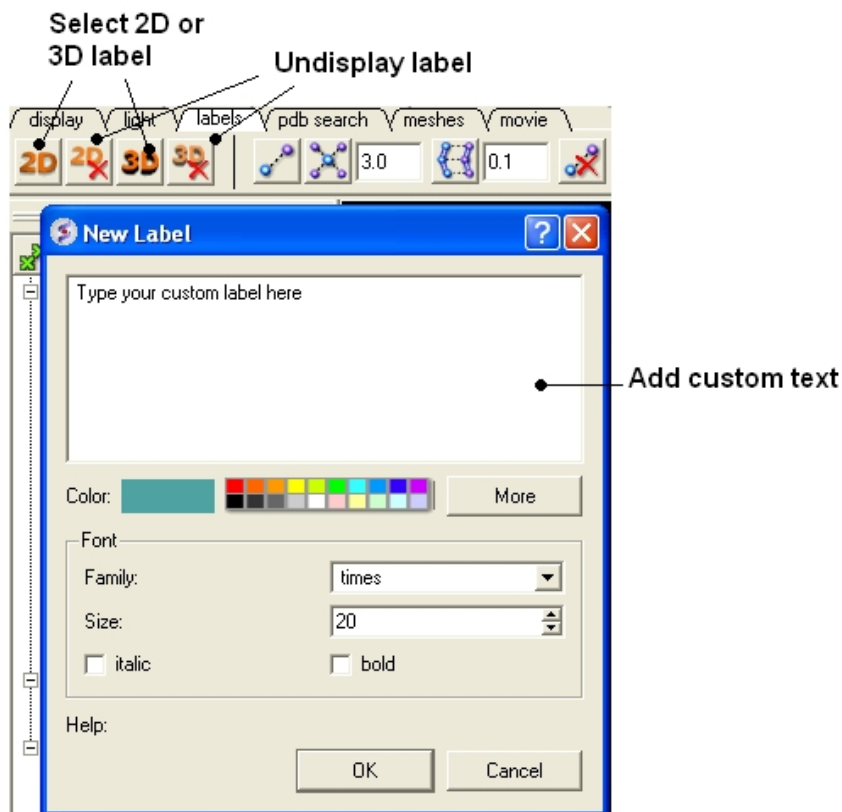
To **edit** or **delete** a label - right click on the label in the graphical display as shown below.

Right click here to Edit or Delete label
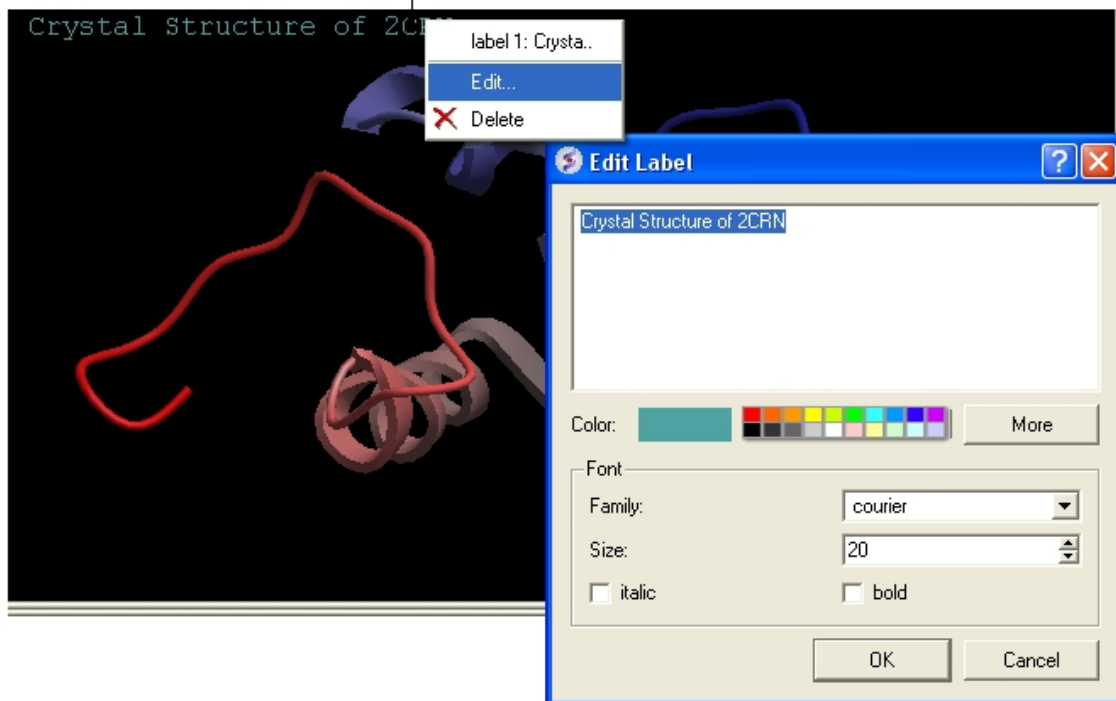
To move a custom 3D label you need to right click on it in the ICM
workspace (under meshes) and choose Connect/Move independently. Press
the Esc key to disconnect when you have finished moving it.

5.8.9 Undisplay Customized Label

Undisplay Residue, Atom, and Variable Label Any label that is displayed
can be undisplayed by selecting the region of the molecule related to
the label and clicking on the corresponding label button in the labels
tab. For example if you wish to undisplay an atom label - click the atom
label button. If a label is displayed the coresponding button in the
display tab will be shaded blue. When you delete the button will return
to grey. 2D and 3D labels have an undisplay button (red cross on the
button see customized label section).

Undisplay 2D or 3D label Click onthe undisplay label button in **labels**
tab.

NOTE: A label can also be deleted by right clicking on the label in
the graphical display and selecting **delete.**

5.8.10 Labeling Distances

Within the **labels** tab there are tools for calculating and displaying
distances. These tools can also be found in the Tools/Analysis menu.
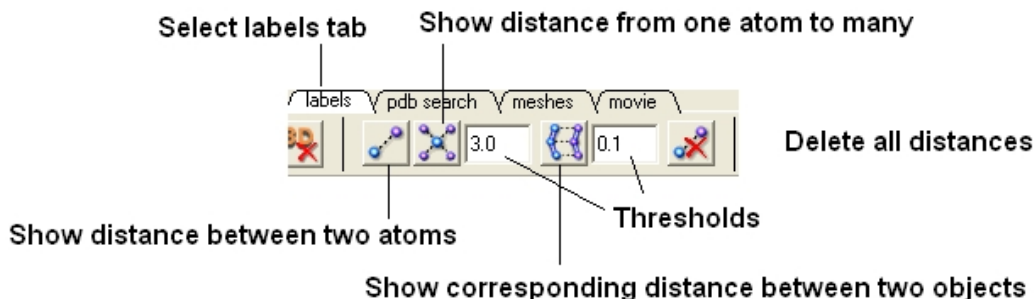
To display distance between two atoms:

- Click on the labels tab (previously called advanced tab).
- Select the atoms between which you would like to find the distance. (See `selection toolbar`)
- Click on the 'Show Distances Between Two Atoms' Button
- The distance will be displayed in angstroms, in green.
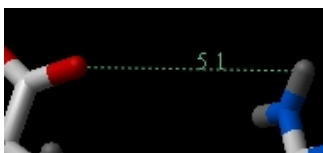
To find the distance from one atom to many:

- Click on the labels tab (previously called advanced tab).
- Select the atom from which you wish to measure the distance from (See `selection toolbar`)
- Click on the 'Show Distances From One Atom To Many' button.
- The distances will be displayed in green.

The maximal and minimal distances can be selected by entering values in the boxes shown here (below) in the labels tab (previously called Advanced tab).
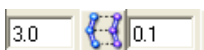
NOTE: Distances can be displayed and undisplayed in the 3D labels section of the ICM Workspace. See image below.
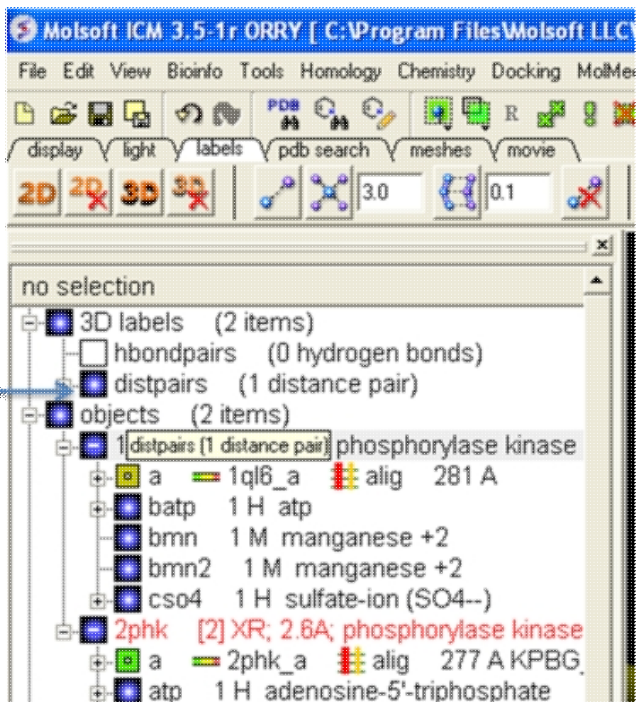
To change the color of the distance label

- Right click on the **distpairs** under the **3D labels** section of the ICM workspace and select **Change Color**.

5.8.11 (Un)display Origin

To display and undisplay the axis of the coordinate frame (origin):

- Select the **labels** tab and select the **toggle origin** button.

Display or undisplay origin button - located in the labels tab
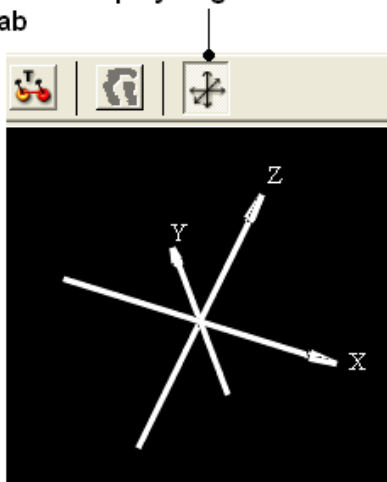
5.8.12 Displaying Tethers

Theory

A tether is a harmonic restraint pulling an atom in the current object to a static point in space. This point is represented by an atom in

another object. Typically, it is used to relate the geometry of an ICM molecular object with that of, say, an X-ray structure whose geometry is considered as a target. Tethers can be imposed between atoms of an ICM-object and atoms belonging to another object, which is static and may be a non-ICM-object. You cannot create tethers in ICM-Browser, however, if the project that you have loaded contains tethers between two objects, then they can be displayed:

- Click on the **display tab** (previously called advanced tab).
- Click on the 'Toggle Tethers' button.

5.8.13 Displaying Distance Restraints

Theory

A distance restraint imposes a penalty function on the distance between two atoms in the same object. You cannot create distance restraints in ICM-Browser, however, if the project that you have loaded contains distance restraints, then they can be displayed:

- Click on the **display tab** (previously called advanced tab).
- Click on the 'Toggle distance restraints' button.

5.8.14 Display Clash

To display a clash the file needs to be an ICM Object.

- Select the region around which you would like to identify clashes.
- Select the display tabs and the "toggle clashes" button shown below.

Toggle clashes button

5.8.15 Display Rainbow, Box, Ruler

To (un)display a rainbow scale, box or ruler use the buttons shown below located in the Labels Tab.
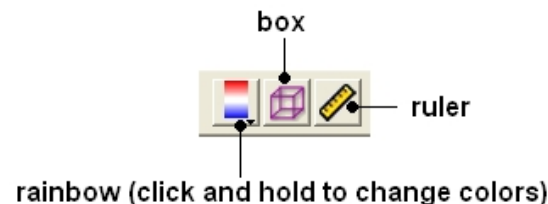
5.8.16 Display Gradient

This button is located in the **display** tab.

Toggle energy gradient button

This option is described in detail in the language manual
http://www.molsoft.com/man/icm-commands.html#display-gradient

5.9 Display Distances and Angles

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro

5.9.1 Display Distance Between Two Atoms - the quick way

- Click on the **Display** tab
- Click on the **Distance between two atoms** button shown below.
- Click on the atoms you wish to measure.
- Distance will be displayed in the graphical display. You can turn this on and off in the ICM Workspace panel under the heading **3D labels**.

Display distance

5.9.2 Display Planar Angle

- Select the **display** tab.
- Select three atoms.
- Select the button shown below.
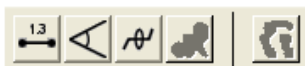
Display planar angle

5.9.3 Display Dihedral Angle

- Select the **display** tab.
- Select four atoms.
- Select the button shown below.
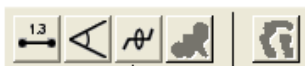
Dihedral angle

5.9.4 Delete Label

To delete distance or angle labels

- Select the **display** tab.
- Select the delete distance or angle label button shown below.

Delete distance or angle labels button in display tab
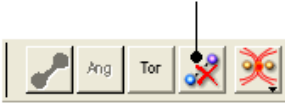
5.10 Graphics Effects

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro

All the visual effects tools can be accesed by the View Menu or click on
the corresponding button in the View Tools panel shown below.

View Tools

5.10.1 Fog

Fog Toggle(Ctrl + D) : this feature creates a fog-like environment for
your object, so that the part of your structure that is closer appears
clear and the distant parts are faded as if they are in fog. The
clipping planes control the point at which the fog begins.

- View/Fog

5.10.2 Shadows

- View/Shadows

OR

select the shadow button shown below.

Toggle shadow
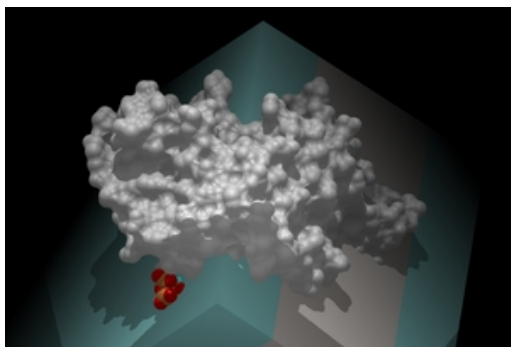
5.10.3 Sketch Accents

To make images as shown below use:

- View/Sketch Accents
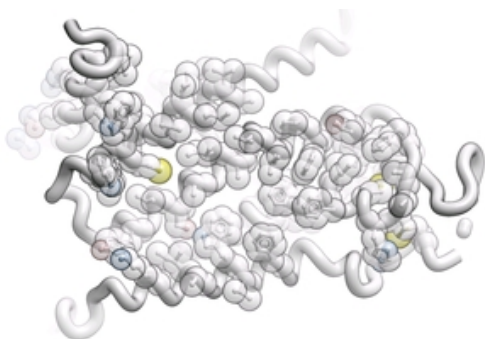
5.10.4 Elegant Ribbon & Ligand Sketch

- Display Tab
- Click and hold Hydrogen button
- Select Elegant Ribbon+Sketch
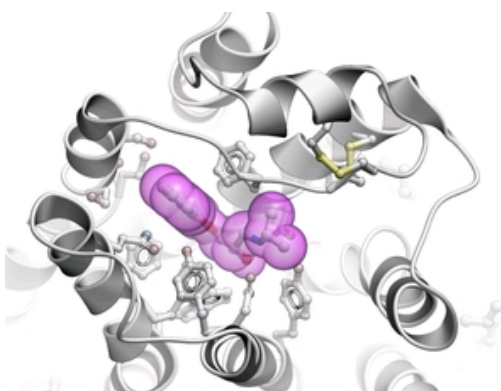
5.10.5 Occlusion Shading

Occlusion shading is for surfaces and meshes to give a perception of depth.

To add occlusion shading:

- Right click on the mesh in the ICM Workspace and select Occlusion Shading. The occlusion shading value can also be changed before

generating the mesh in the meshes tab.

- Enter a depth value - default is 0.8. Higher values will generate a more dramatic shading.
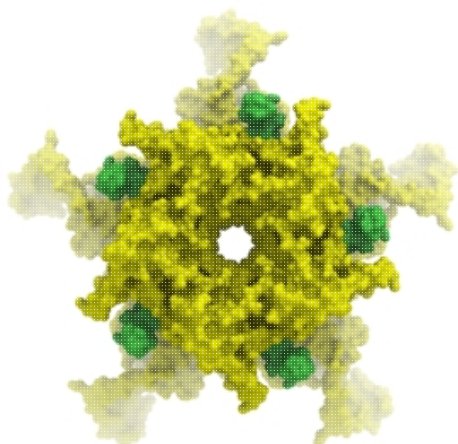
5.10.6 Perspective

Toggle perspective Ctrl_P this will add perspective to your structure, enhancing depth in the graphical display.

- View/Perspective

5.10.7 Animate View

Learn how to build fully interactive and interruptable animations.
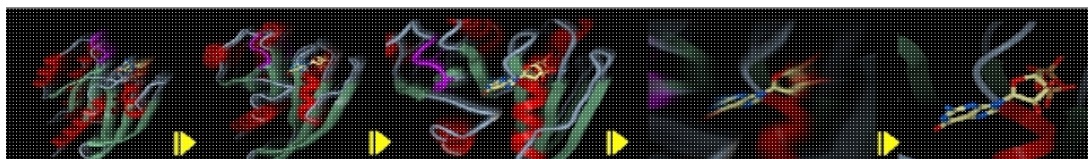
Smooth Animated Transitions

5.10.7.1 Make Animation

To quickly produce an ICM Molecular Animation:

- Click and hold down the "Begin rocking/rotation" button shown in the picture below.
- Choose from the following options - X-Rock, Y-Rock, Xy-Rock, xY-Rock, X-Rotate, and Y-Rotate.
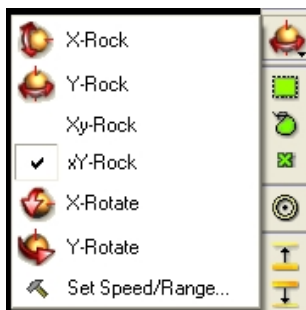
> **NOTE:** Default rocking representation can be changed in the
> `File/Preferences/Gui` menu.

5.10.7.2 Change Speed, Range and Cycle Length of Animation

To change the speed, range and cycle length of the animation:

- Click and hold down the "Begin rocking/rotation" button shown in the picture above.
- Choose the set speed range option and change the speed and range using the drag bars. Any change will appear in the graphical display behind this box.
- If desired you can change the number of cycles of the animation. This is an ideal tool for `screen-shot movie making`.
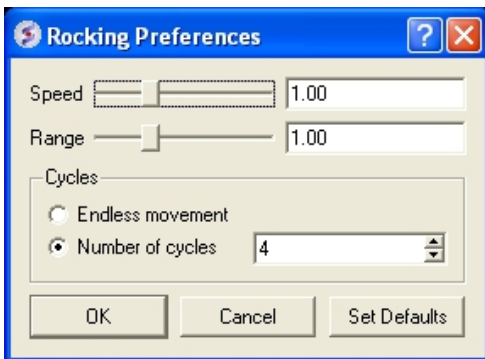
> **NOTE:** There is a return to default button in the Rocking Preferences dialog box shown above and defaut values can be changed in `File/Preferences/Gui`.

> **NOTE:** Default rocking speed can be changed in the `File/Preferences/Gui` menu.

5.10.7.3 Interrupt Animation

An ICM Animation or Transition is fully interactive and is interrupted by a single click of the mouse.

To stop or change an animation or transition:

- Click the "Begin rocking/rotation" button shown in the picture below.

To temporarily halt an animation or transition:

- Click in the graphical display. Once you release the mouse button the animation will start again.

> **NOTE:** If you click on the graphical display during an animation the animation will be interrupted. Whilst clicking and holding the mouse button other operations can be performed such as zooming and selections.
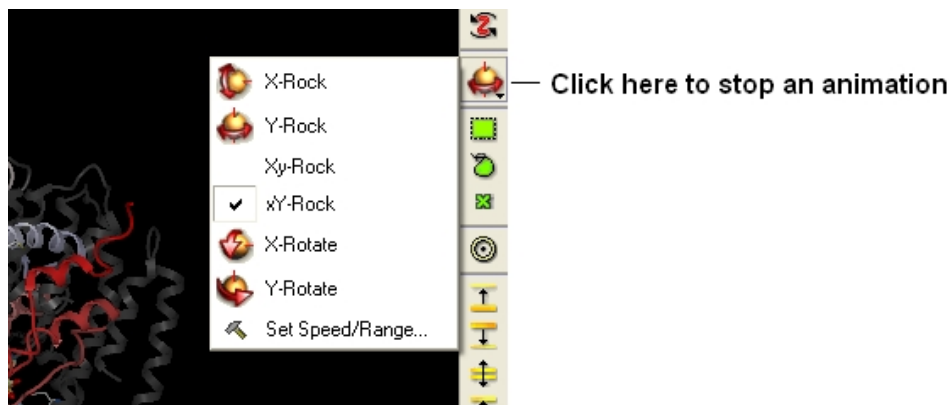
Click in the graphical display window to temporarily interrupt an animation

5.10.7.4 Saving an Animation

An animation can be saved in an ICM project:

```
File/Save Project
```

Or

as a `slide`.

5.11 Graphics Shortcuts

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro

The left mouse button can be mapped onto different graphics tools which can be selected from the right hand tool bar.

Note: (1) You can access many non-rotation modes directly from the rotation mode by using Middle and Right-mouse buttons, as well as by using the right, top and left margins of the graphics window. (2) You can access the rotation mode from non-rotation modes by pressing Ctrl.

- rotation (**the default** , press Ctrl if you in the non-rotation modes)
- translation (the middle mouse button in the rotation mode)
- zooming in and out by dragging the mouse up and down (the left margin in the rotation mode, or use the mouse **wheel)**
- Z-rotation (the top margin in the rotation mode)
- selecting by box (the right mouse click in the rotation mode)
- selecting by lasso (Ctrl-draw lasso in the rotation mode)
- picking out atoms (a toggle)
- picking out and labeling residues (a toggle)
- moving the front clipping plane (the top section of the right margin in the rotation mode)
- moving the rear clipping plane (the bottom section of the right margin in the rotation mode)
- moving the slab (the middle section of right margin in the rotation mode)
- unclipping (Ctrl-U)
- rotating torsions (Ctrl-left-mouse-click in the rotation mode)

- connect and unconnect separate molecules to movement controls

Many useful graphics tips are summarized here.

> **NOTE:** Key mouse controls are summarized in the command line manual
> here http://www.molsoft.com/man/graphics-controls.html

5.12 Molecule Move Buttons

> Available in the following product(s): ICM-Browser | ICM-Browser-Pro
> | ICM-Pro

To move your structure it must first be displayed in the graphics window
(for instructions on how to display a structure see the Display Tab).
All of the following options are displayed in the Move Tools toolbar
(shown below).
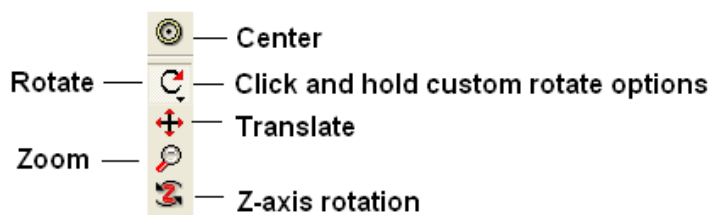
5.12.1 Rotation

**In order to achieve the best pose for a picture or to enable the study
of a certain region of your structure in more detail you may need to
rotate the structure:**

- Click on the **rotation** icon on the toolbar.
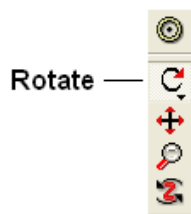
- Click and drag on your structure in the display window until it is
  in the desired position.

5.12.2 Custom Rotation

An option is provided to customize the rotation of the molecule. This
allows exact rotation by a specified number of degrees.
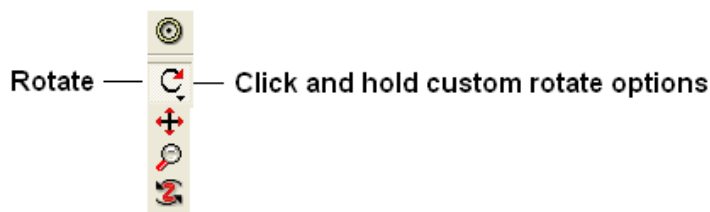
- Click and hold down the rotation button and a data entry box as shown below will be displayed.
- Enter the number of degrees of rotation you require and in which X, Y or Z coordinate.
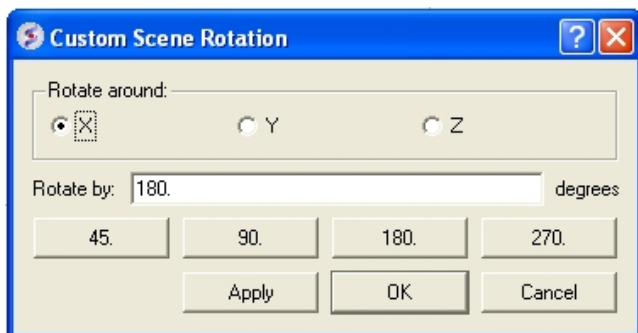
To continuously rotate the picture:

- Click on the **continuous rotation** icon on the toolbar.
- Click, hold, and slightly move your mouse anywhere on the graphical display window. The point at which you hold your mouse, is the direction to which the object will turn.
- Positioning the mouse towards the center of the display will move the object slower than if the mouse is positioned towards the edge of the graphical display.

In order to rotate your picture around the Z-axis:

- Click on the **Z-axis rotation** icon on the toolbar.

- Click and drag your object around the Z-axis until it is in the desired position.

5.12.3 Translation

To translate your structure up, down, left, or right:

- Click on the **translation** icon on the toolbar.
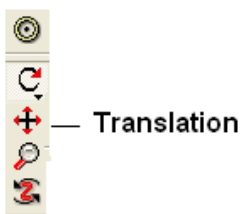
- Click and drag on your structure in the display window until it is in the desired position.

When you are displaying more than one object and you wish to translate one object in relation to the other on the Z-axis:

- Right click on the name of the object you wish to move in the ICM workspace and select connect to object. This object is now independent from the other object and can now be manipulated separately.
- Click on the **Z translate** icon on the toolbar.
- Click and drag your structure along the Z-axis, moving it closer or further from your unconnected structure.
- Once you are finished, right click on the name of the object which is connected, and click on disconnect.

5.12.4 Zoom

To zoom in or out of your structure:

- Click on the **zoom** icon on the toolbar.

- Click and drag your mouse up to zoom in and down to zoom out.

You can also zoom in and out directly with the right-mouse-button *without* explicitly switching to the zoom tool, if you use the **left 5%-margin** of the graphics window.

5.12.5 Center

To restore your picture to the center of the graphical display window or to center on a selection:

- Make a `selection` of the region you wish to zoom into - if no selection is made the whole structure will be centered.
- Click on the **center** icon on the toolbar.

5.12.6 Torsion Angles

To alter the torsion angle of certain residues of your structure manually:

- `Convert` your pdb structure into an ICM object.
- Click on the **change torsion angles** icon on the toolbar (see image below).
- Click on the bond until you see the torsion angle displayed in yellow (see image below). Click and hold on the bond around which you wish to rotate a residue. The changing torsion angle will be displayed in orange.
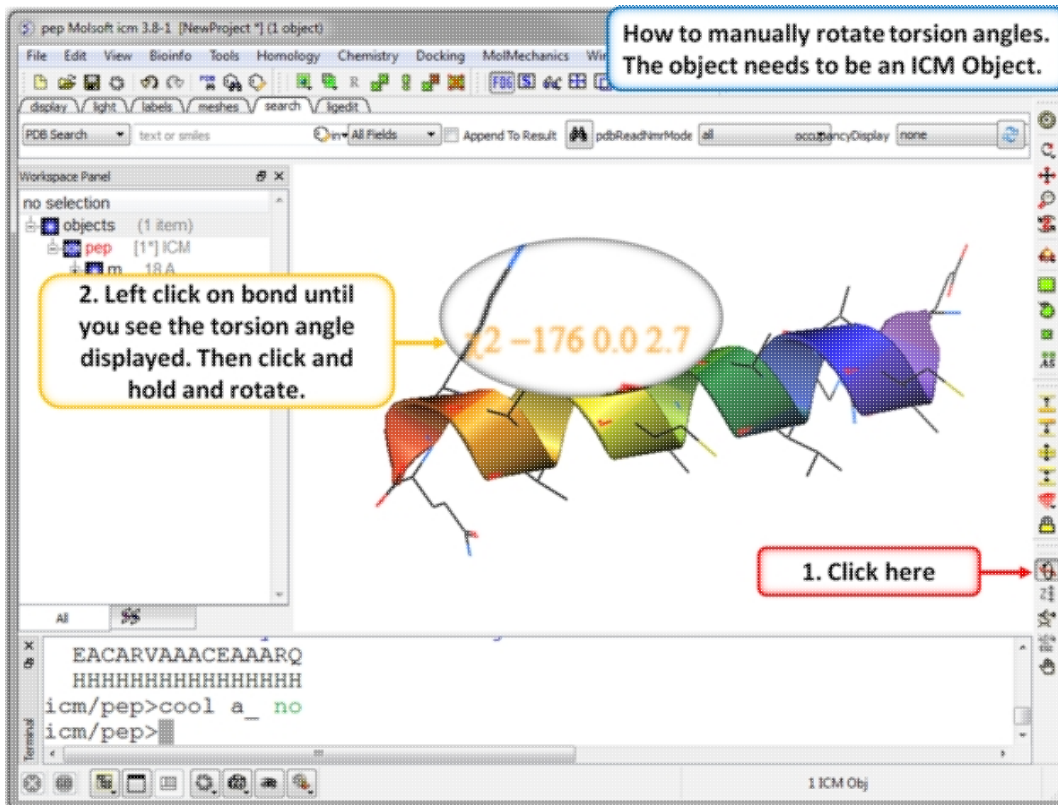
NOTE: This option can be used more effectively in conjunction with the `variable label` option.

To alter torsions by entering specific angle values:

- You can edit torsions by specifically defining the exact angle as described here.

5.12.7 Connect (Move)

When there is more than one object displayed in the graphical display window the objects are connected to one another. If you wish to move or manipulate one object independently from the others you need to **connect** to it

To do this from the ICM Workspace:

- Right click on the name of the object you wish to move in the ICM workspace and select **Connect to Object**. The object will now be colored yellow.
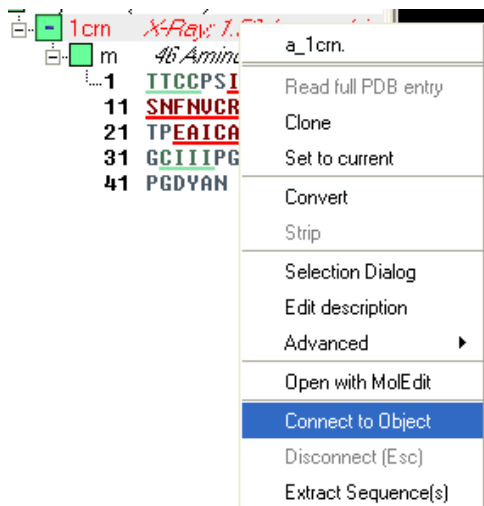
- The object is now controlled separately from the rest of your objects by your mouse.
- Disconnect your object by once again right clicking on the name of the object in the ICM Workspace and selecting disconnect in the drop down menu or **Press the ESCAPE key**.

Note: you can temporarily switch to the global rotation in the connected state if you press Shift

Note: use the Escape button to disconnect

5.13 Clipping Tools

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro

The clipping tools allow you to adjust the frames of the ICM window, changing the clipping planes.

Clipping planes can also be moved *without* switching to the clipping tool, if you click the right hand margin of the graphics window:

- The top section of the right 5% margin of the graphics window: moves the back clipping plane
- The middle section of the right 5% margin of the graphics window: moves the slab (both clipping planes)
- The bottom section of the right 5% margin of the graphics window: moves the front clipping plane

In order to move the front or rear clipping planes of your screen:

- Click on the **Move front clipping plane** or **Move rear clipping plane** icons on the toolbar.
- Click and drag the respective plane frontward or backward, depending on how you wish to clip it.

You can also move the **slab** of viewing window, keeping the distance between the front and back clipping planes. In order to adjust the area

of the structure where your viewing window is located:

- Click on the **Slab** icon on the toolbar.
- Click and drag the slab frontward or backward, depending on the desired area of the structure you wish to see.

If you have made changes to the clipping planes which you do not wish to keep or you wish to automatically fit your entire structure within the clipping planes:

- Click on the **Unclip** icon on the toolbar. This will automatically set the clipping planes to fit your object.

5.13.1 Mesh Clipping

Clipping tools can be used to adjust the frames of the mesh independently of other objects. This is described here.

5.14 Graphic Layers

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro

To display and undisplay layers of a structure you can use the buttons shown below. Seven layers can be created and within each layer different structural representations can be displayed.

Right click on one of the layer buttons and a number of options can be chosen as shown below.
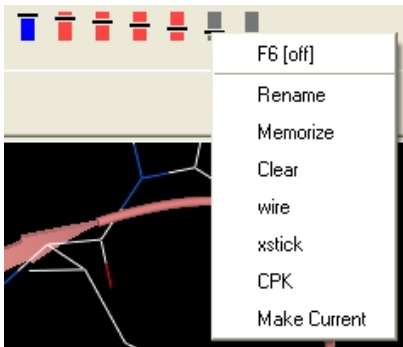
To change the display in one of the layers:

- Right click on one of the layer buttons.
- Select a representation wire, xstick or CPK.
- You can do this for each of the seven layer buttons.
- Click on the layer button to display and undisplay. If the layer button is shaded red then the layer is not displayed. If the layer button is shaded light blue then it is displayed. You can switch between layers by clicking on the button or using the. You can use the **memorize** button to store a particular representation and **clear** to remove a memorized representation.

5.15 High Quality Publication Images

5.15.1 High Quality Image

To make high quality publication images:

The first step is to improve the quality of the image using the **High
Quality Image** and **Antialias** buttons shown below.

To save and write an image:

- Select **File/Write Image** and the following window will be
  displayed:
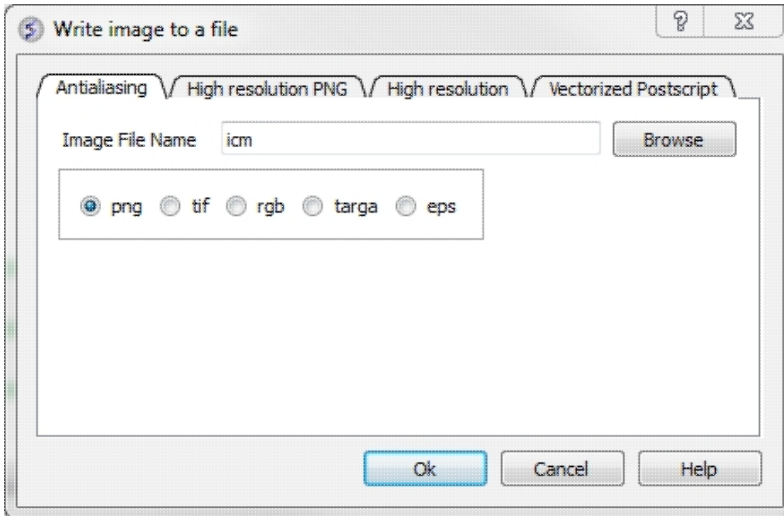
Choose one of the tabs depending on the image type you require:

- **Antialiasing** allows you to make png, tif, targa, eps or rgb file
  types.
- **High Resolution PNG** Allows you to specify a resolution increase
  and transparent backgroun.
- **High Resolution** Allows you to specify a resolution increase for
  tif, targa and rgb files.
- **Vectorized Postscript** This creates a vectorized postscript model
  of the screen image. Instead of the bitmap snapshot this command
  generates lines, solid triangles and text strings corresponding to
  the displayed objects. Since the postscript language is directly
  interpreted by high-end printers, the printed image may be even
  higher quality than the displayed image. The final resolution is
  limited only by the printer since the original image is not
  pixelized. Warning: there may be inevitable side-effects for some
  types of solid images at the intersection lines of solid surfaces
  (i.e. large scale cpk representation, hint: use display skin
  instead).

5.15.2 Quick Image

A quick image can be saved using this option. The image will be saved as icm1.png in the current directory in which you are working. Each subsequent image produced will be incrementally numbered.

This option is also available via a button as shown below:

Quick Image Button

5.15.3 Image Options

Click and hold on the **image** button at the bottom of the GUI to:

- Copy image to clipboard (single click on the button also does this).
- Resize image (Write Image Advanced)
- Transparent Background Write Image Advanced)
- Store an image in ICM (Write Image Advanced)
- If you are making an `ICM document` you may want to store images inside ICM (Add Image to Album).
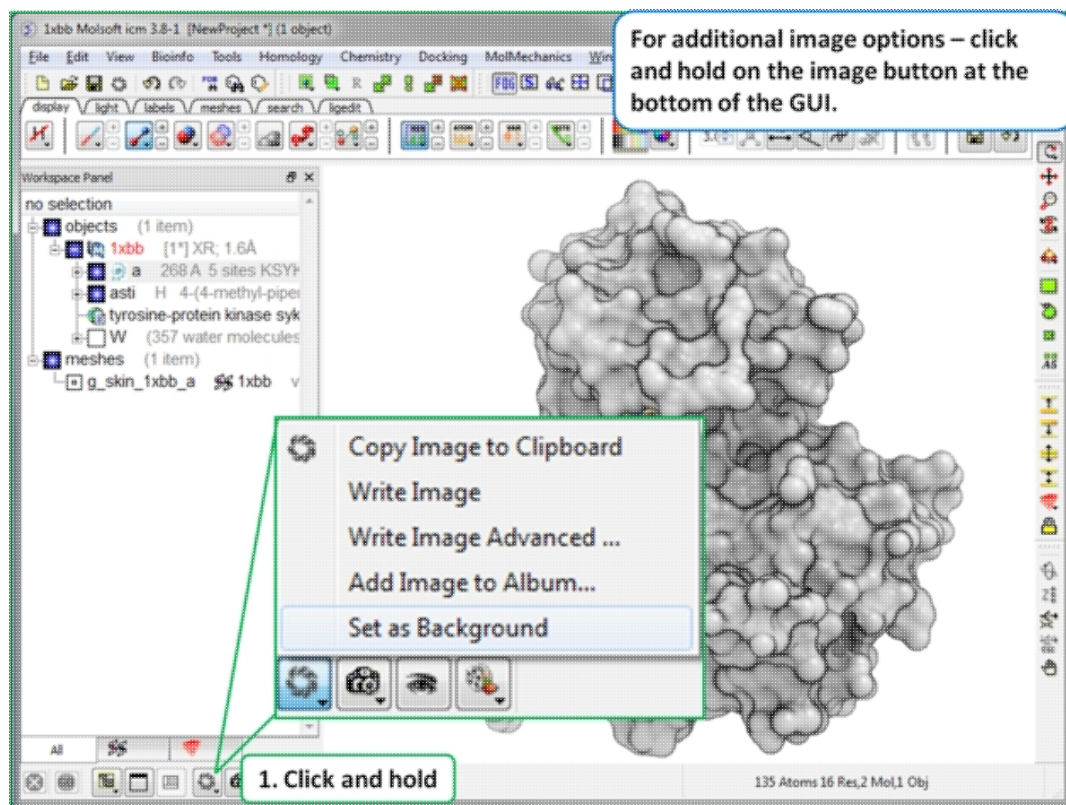
5.16 Movie Making
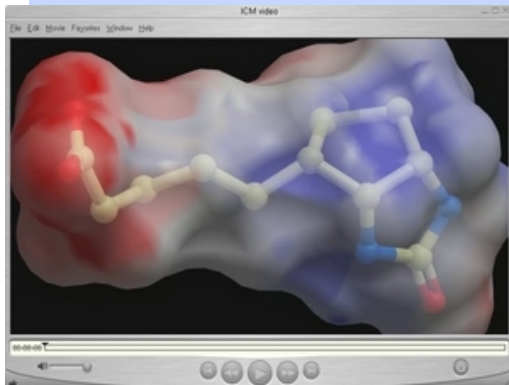
A movie is an excellent way of communicating 3D structural data. The resulting movie can easily be transfered into other applications such as Microsoft Powerpoint.

There are two ways to make a movie:

- Make a movie directly from a series of `slides`.
- Make a movie directly from `screenshots`.

An alternative to a movie is a fully interactive 3D document embedded in Windows PowerPoint or a Web Browser, this can be undertaken using slides and the `ActiveICM` plugin.

5.16.1 Making a Movie from a Set of Slides

To make a movie from a set of slides:

Make a series of `slides` using the camera button.
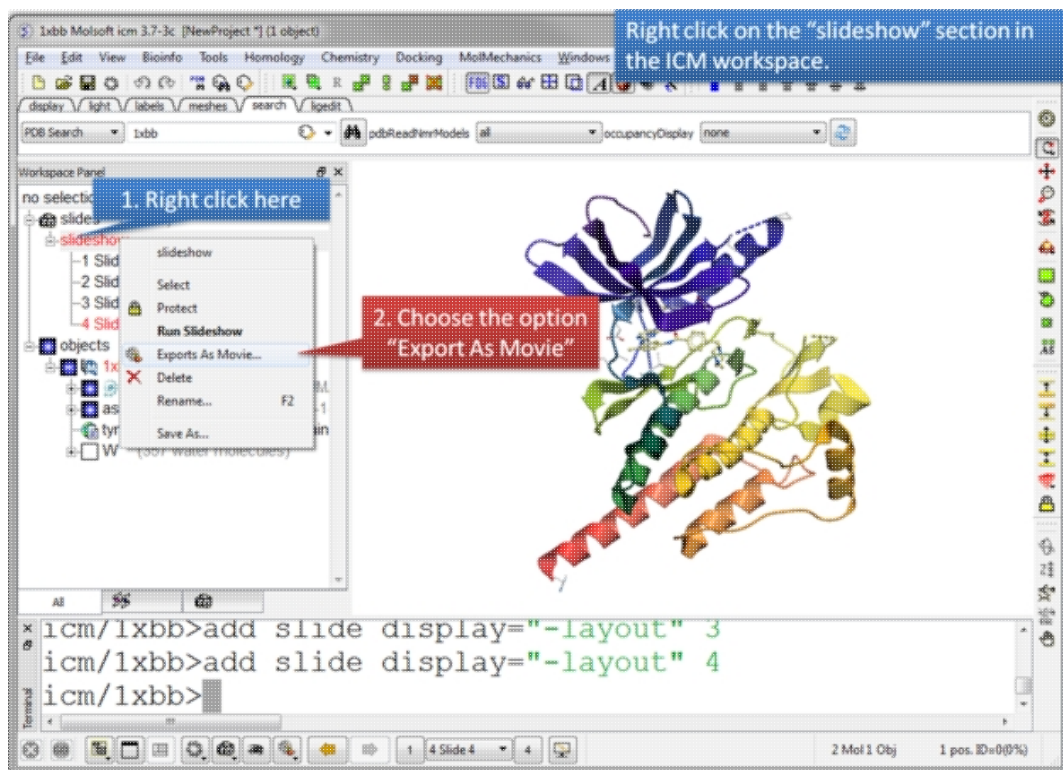
Right click on the slideshow in the ICM Workspace and choose the option
"Export as Movie".
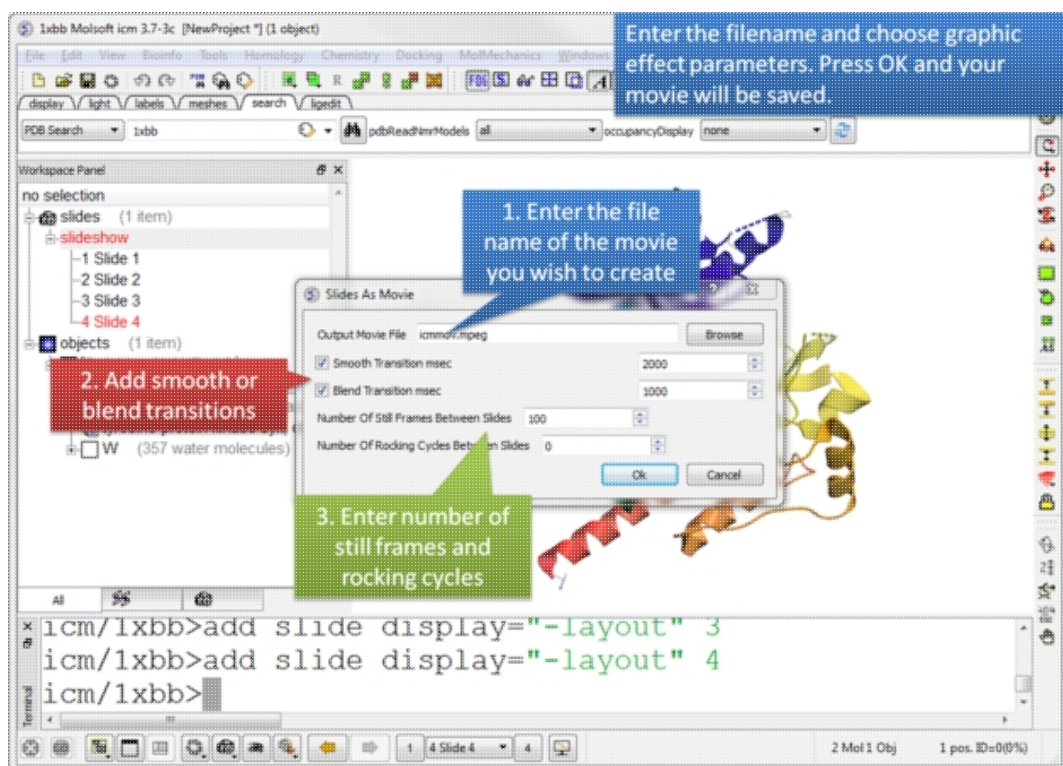
Enter the filename for your movie and set the movie effect parameters.
Press OK and the movie will be written to a file.

5.16.2 Screenshot Movie

Screenshot movie making

To make a **Screen-grabbing Movie** follow these steps:

- Resize the graphical display to the screen size/resolution you
  need. You may also want to select the `high quality` image button
  and antialiasing to improve the quality of the movie or add visual
  effects such as `shadows`.
- To begin making a movie click on the movie making button at the
  bottom of the graphical user interface (as shown below).
- Enter a file name for your movie and select the movie format
  (.mov, .avi, mpeg).
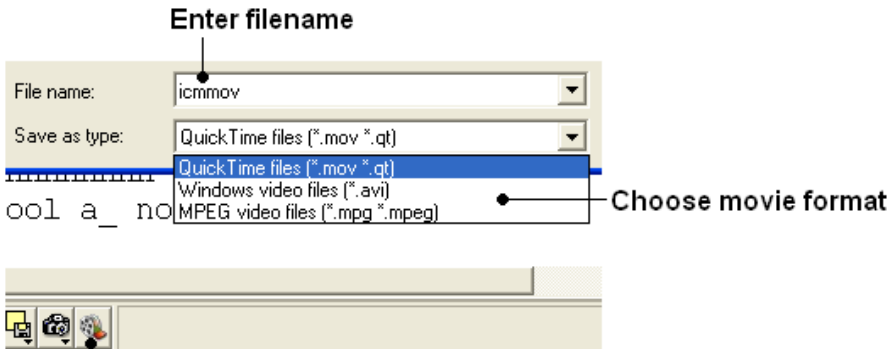
Screenshot movie making button

> **NOTE:** If you want to make a movie to include in a PowerPoint
> presentation you need to save the movie in AVI format.

- To begin recording the screenshot movie click on the red **Record
  video** button. Anything displayed in the graphical display will be
  recorded, for example you can record `animations and transitions`.
  Specifying the `number of cycles` in the animation (rocking,
  rotation) is an ideal tool for screen-shot movie making. If you
  have a fast computer you can use **Realtime screen grabbing** which
  can be selected by clicking and holding the **Record video** button.
  The real time option can also be set in `File/Preferences/Gui` menu.

Record video button

- The length of the movie in minutes, seconds and milliseconds is
  displayed in the top right hand corner of the graphical display.

- You can pause the movie and fade out by clicking on the button shown below. The number of frames for the fading out option can be controled using the option in `File/Preferences/Gui`

Pause and fade out
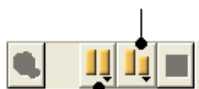
Pause recording

- You can record a smooth transition from a previous frame by clicking on the button shown below.

Smooth transition from previous frame

NOTE: Anything you do in the graphical display will be recorded in the movie. For example you can change representations, lighting, add new molecules etc. This can be achieved in a more controled manner using the **pause** and **record smooth transition** button.

- Once you have paused the recording the viewpoint and representation of the molecules can be changed and a smooth transition from the previous frame can be generated by selecting the **Record smooth transition from previous frame** button.

- To stop recording a video press the button shown below.

Record smooth transition from previous frame

Stop recording

6 Slides & ActiveICM
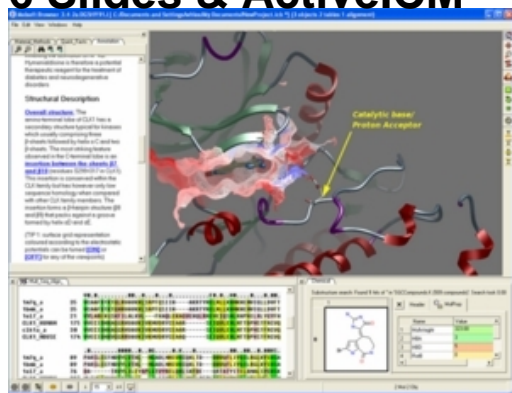
In this chapter you will find a description of the tools available to create files (.icb) containing fully-interactive three-dimensional (3D) molecules and two-dimensional (2D) data. These files can contain multiple interactive views and animations of molecular structures and objects in conjunction with related hyperlinked text, chemical, biological sequence, alignment and data views. The files are small and easily transferable and downloadable. The files can be used for Molecular Presentation and Documents inside the ICM browser or displayed on the web and in PowerPoint using the `ActiveICM` plugin. For examples of ICM Molecular Documents please see MolSoft's ActiveICM product page at www.molsoft.com/activeicm.html

Chapter Contents:

- Making Molecular Slides
- How to View and Navigate Slides
- How to Edit Slides
- How to Add Smooth Blending and Transition Effects Between Slides
- How to Make Molecular Documents - Link HTML Text to Slides
- `ActiveICM` - ActiveICM enables you to view and display ICM graphical slides and animations interactively inside Windows Microsoft PowerPoint and web browsers such as Internet Exporler and Mozilla Firefox.
- `IcmJs` is a JavaScript/HTML5 version of the ActiveICM plugin.

6.1 Making Slides

> Available in the following product(s): `ICM-Browser` | `ICM-Browser-Pro` | `ICM-Pro`

A slide enables you you to store a large number of different 3D visualization along with text and window layout.The following information can be stored in a slide:

- Viewpoint (e.g. rotation, translation, zoom, lighting and depth effects)
- A set of atom-specific graphical representations such as surfaces, which can be represented as smooth, transparent or wireframe, ball-and-stick models, CPK (space-filling) models.
- A set of atom, residue or distance labels on any of the atomic items
- A set of arbitrary 3D textual annotations assigned to a point in space
- A set of arbitrary 2D annotations assigned to specific 2D coordinates on a screen
- Parameters of the parametric `animation`
- Window layout - for when the slides are viewed in the browser.
- Current table(s)
- Sequences and Alignments
- HTML text with hyperlinks
- 2D images
- For each grob (mesh): representation and colors.

A slide can be made by clicking on the Camera button at the bottom of the GUI.

Make a slide by clicking on the camera button.

1. Click on the camera button to make a slide.
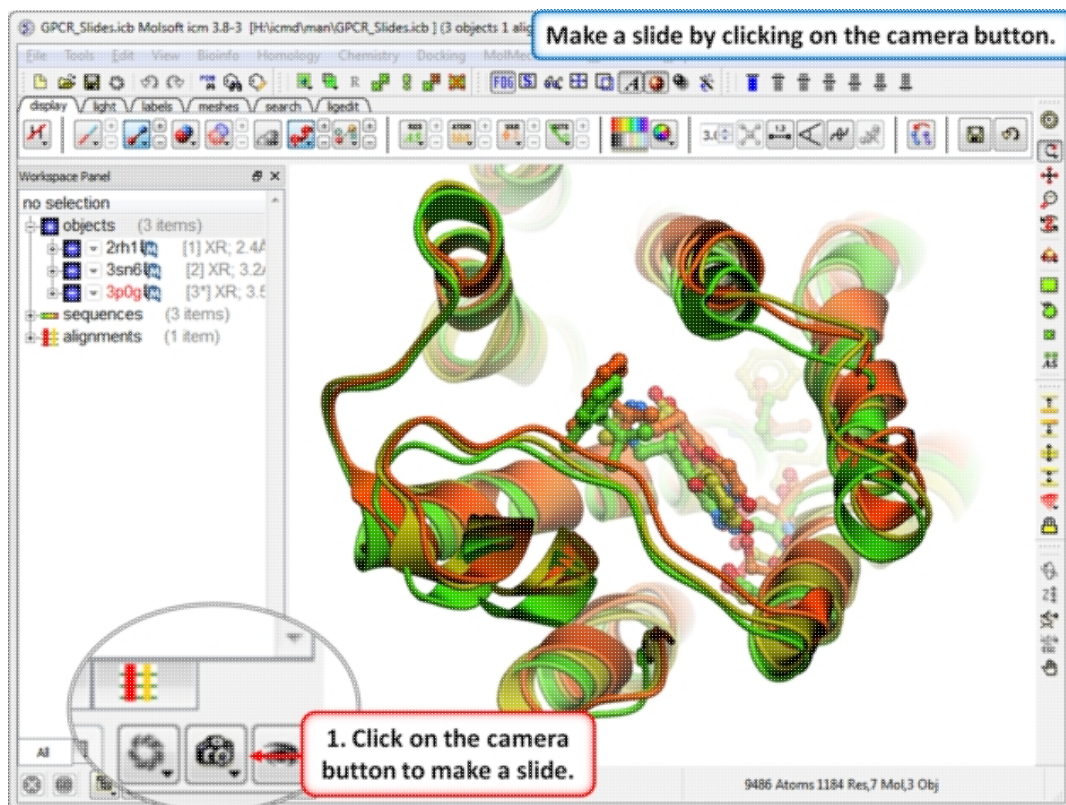
Slides can be viewed in the GUI, can be edited and graphical effects can be added to them. Slides can also be embedded into the web, or PowerPoint using ActiveICM or viewed in ICM-Browser (or ICM-Pro).

This tutorial takes you through the steps to create a series of fully interactive 3D slides. Another tutorial can be found here. **To begin making ICM Molecular Slides:**

- First load the structure or structures you wish to display in your first slide. Additional structures, labels etc and text can be added at any point during the slide making process. In this example we will load the PDB file 1XWS a PIM1 kinase.

Load a kinase structure from the PDB.

1. Click on the "Search" tab

2. Enter search string "1xws"

3. Click on the search button and a table of hits will be displayed

4. PDB stucture is automatically listed in the ICM Workspace and displayed
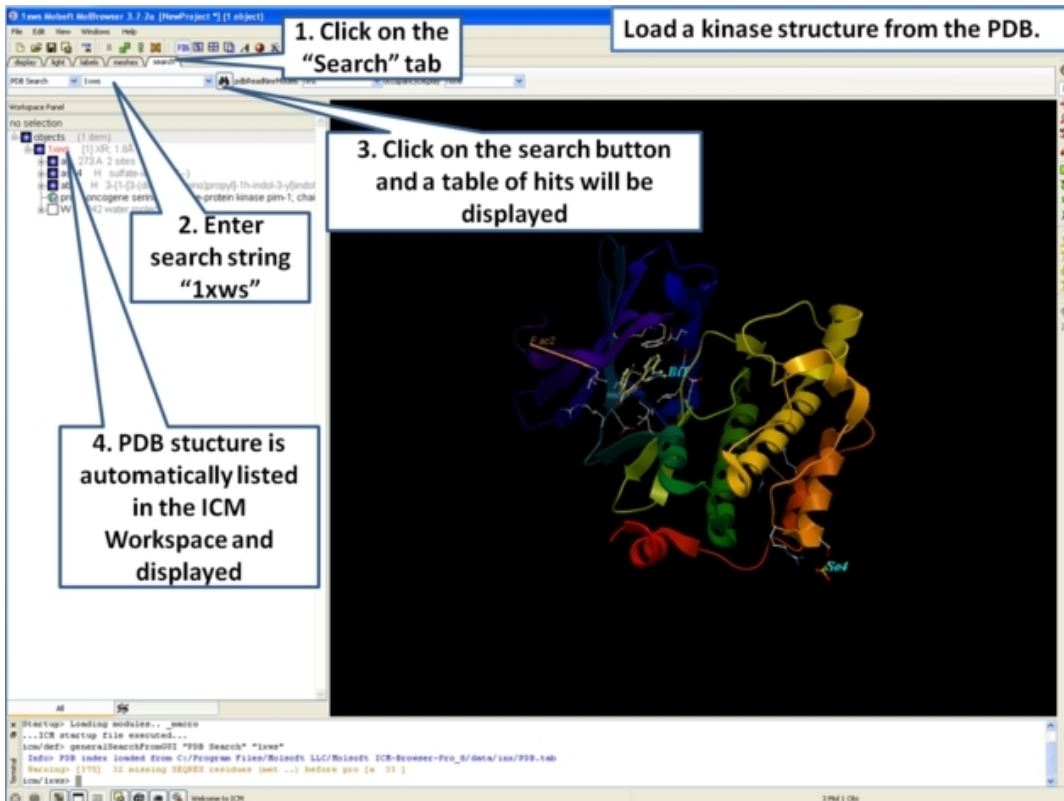
- Next, we will convert the PDB file to an ICM object so we can make slides of the ligand-receptor hydrogen bonds and binding pocket surface.

Convert the structure from PDB format into an ICM object.

1. Right click here and select "Convert PDB"

2. Enter the options as shown and press OK.
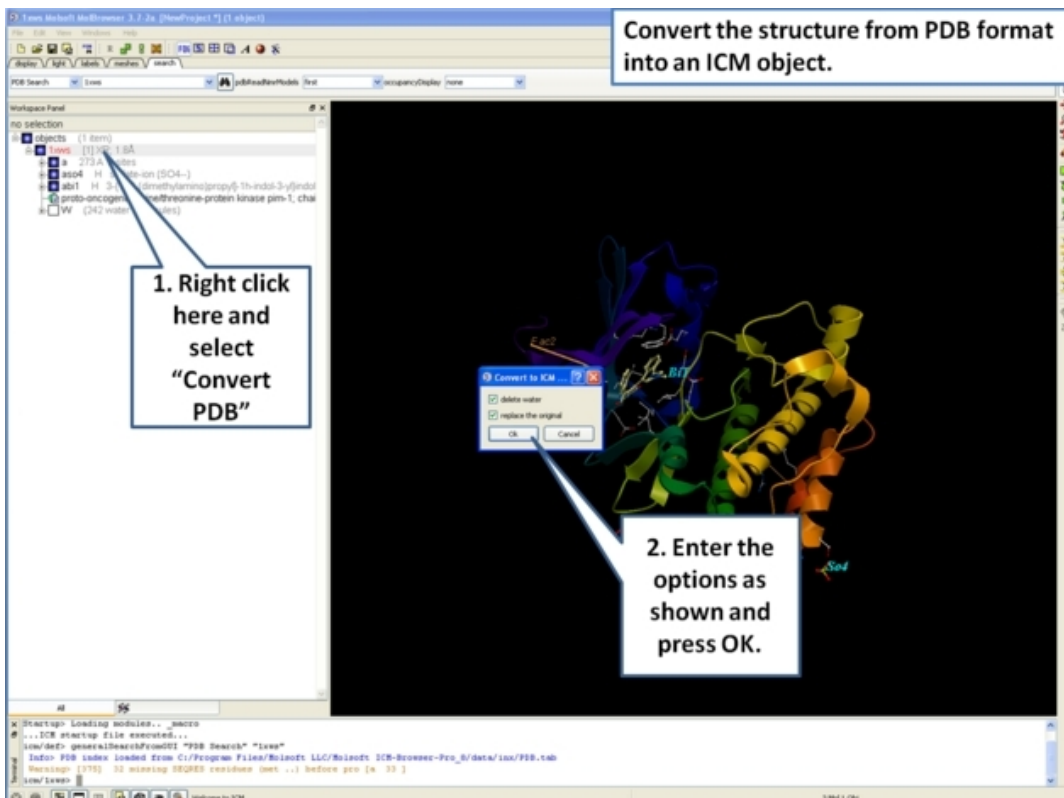
- Now we are going to prepare the first slide by rotating the protein structure to an orientation which allows the viewer to see

the key features of the kinase. For example the bulge in the hinge region (between the N- and C- lobes) which is unique to PIM proteins.
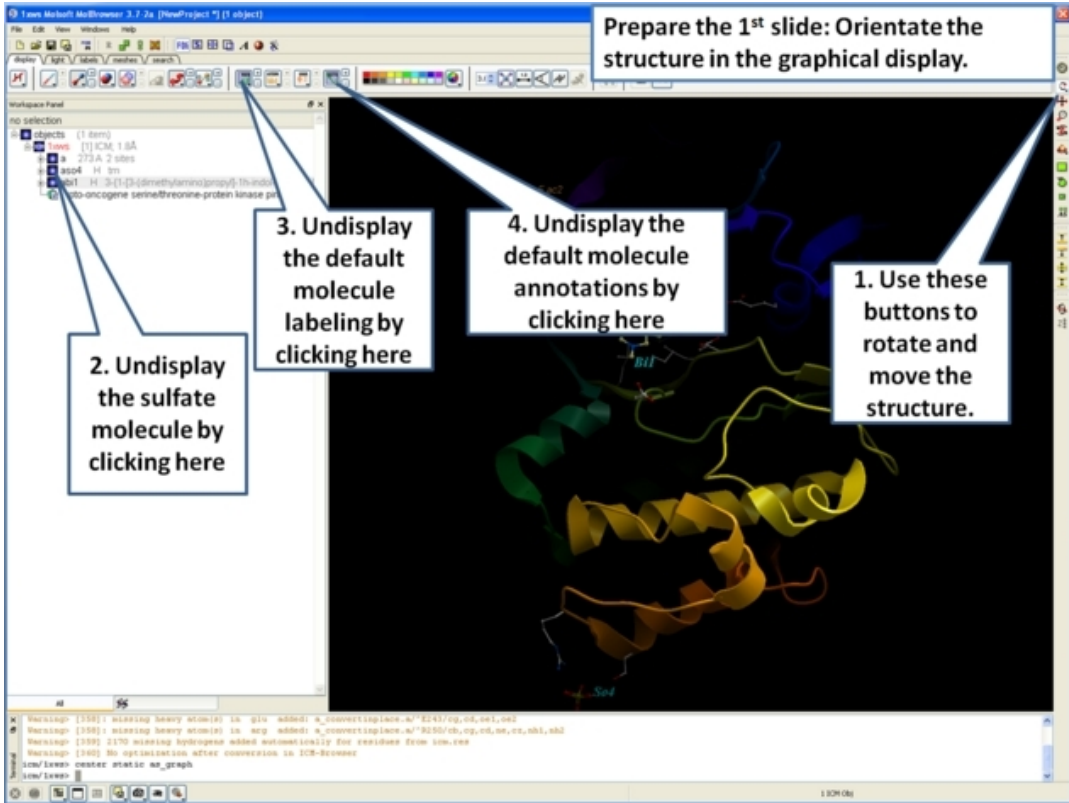
- Next, make the first slide by clicking on the camera button at the bottom of the graphical user interface.

Make the 1st slide: A view of the first subunit with ligand displayed.

2. Note: Slide is listed in the ICM Workspace

1. Click here to make a slide
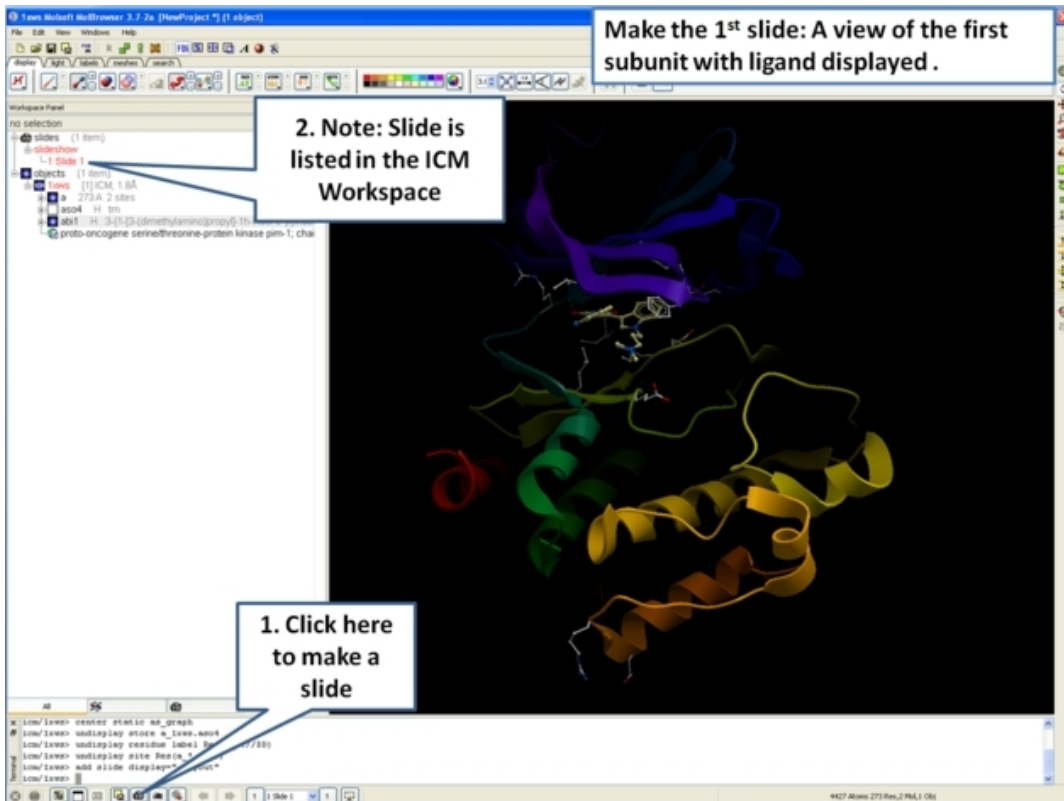
Click to add slide

- Once you have clicked on the camera button you will see that the first slide has been generated. The first slide is shown in the ICM Workspace window as shown below.
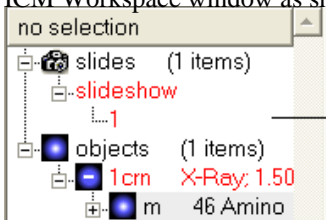
The number and name of the first slide is displayed in the ICM Workspace

- Slides can consist of Static views or Transitions and Animations. Here we will zoom into the flexible glycine rich region of the kinase which lays across the roof of the ATP-binding pocket. Click on the camera button and make the second slide
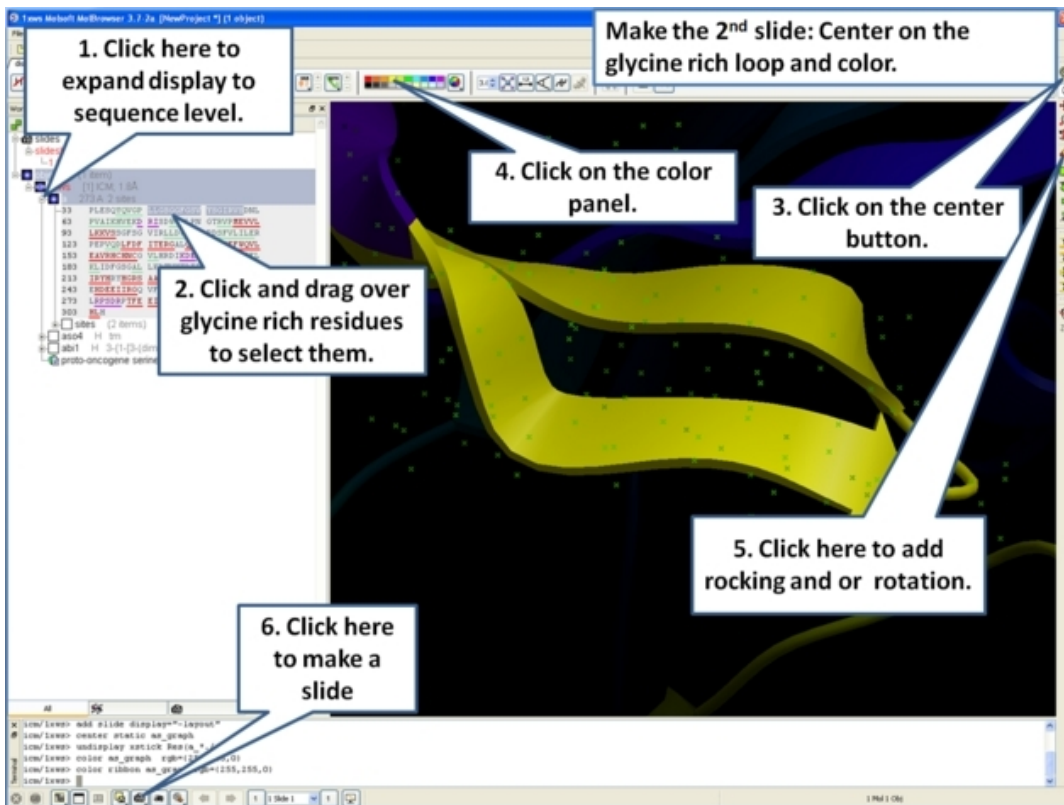
- Next, we will make a slide of the surface of the ligand binding pocket colored by binding property.
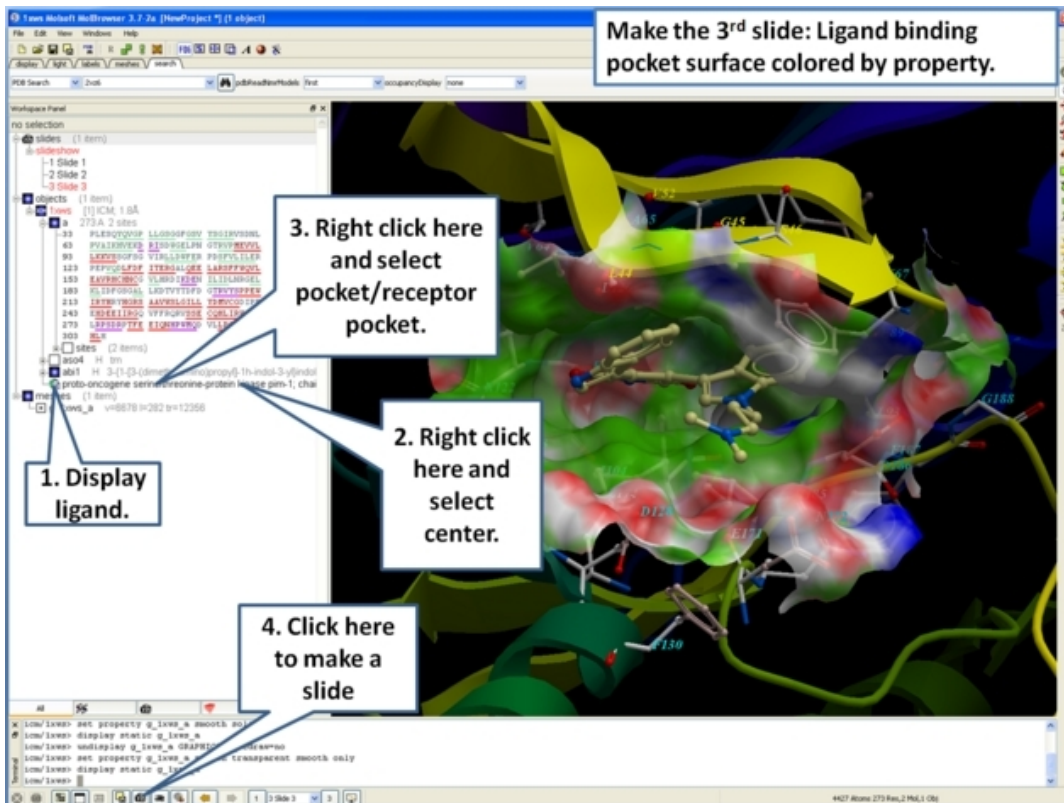
- Now save the document as an icb file. Go to File/Save as...

6.2 Make a Movie from a Set of Slides

Please see the `movie` chapter on how to make a movie from a set of slides.

6.3 How to View and Navigate Slides

6.3.1 View Slide Show

To view a slide show select the buttons shown below:
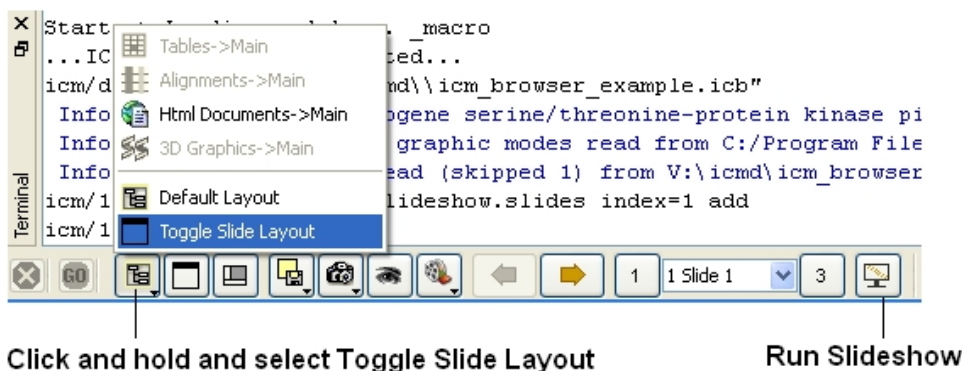
Click and hold and select Toggle Slide Layout   Run Slideshow

NOTE: Slides are associated with the objects currently loaded into ICM. Therefore if you delete an object then the slides will not work. However if you delete an object and then re-read the same object with the same name and structure the slides will be ok.

To save a slide show

* File/Save Project

6.3.2 Slide Navigation

You can make as many slides as you wish as described in the `Making Molecular Slides section`.

To navigate through the slides you can use the buttons shown below, the cursor keys for some operations or the right click options in the ICM Workspace.

Previous Slide — Jump to Selected Slide

Next Slide

Add a Slide — Final Slide
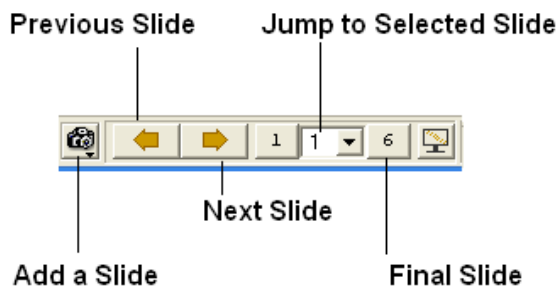
The slide currently displayed is highlighted in red in the ICM
Workspace.

Currently displayed slide
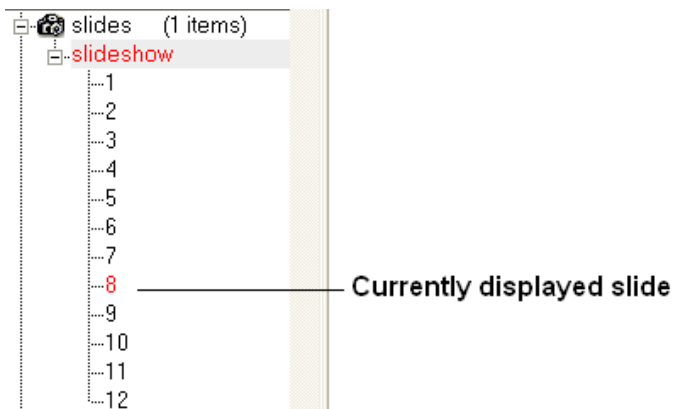
To jump to another slide right click and select "Set Slide".

All slides are displayed in the ICM Workspace. You can hover the mouse
over a slide name in the ICM Workspace and a thumbnail sketch of the
slide is displayed as shown below. This can be used for slide navigation
purposes.
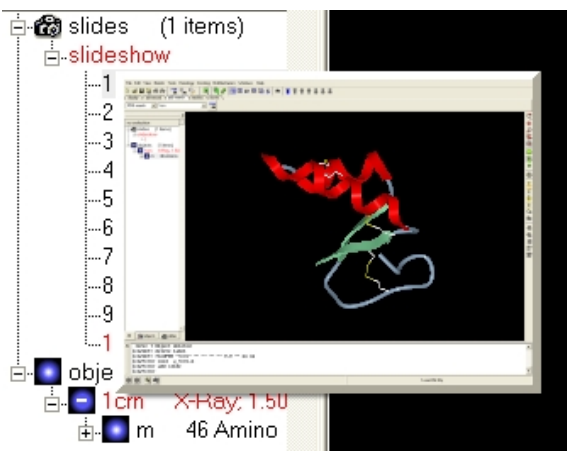
Hover mouse over slide name in the
ICM Workspace and a thumbnail
sketch of that slide will be displayed.

Or you can right click on the name of the slide in the ICM Workspace and
select the option "Preview".
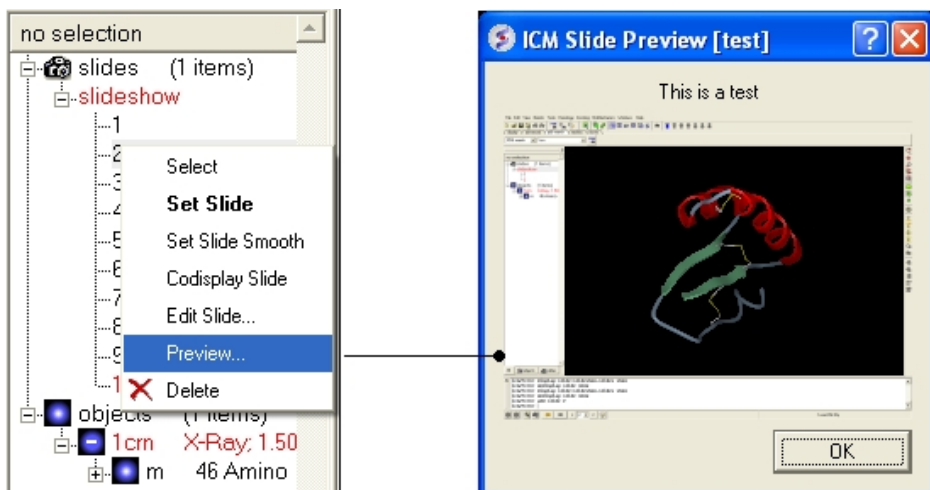
6.4 How to Edit Slides

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro

You can jump to the slide you wish to edit by following the slide
navigation instructions.

6.4.1 Edit Slide

Edit slide contents: To edit the content of a slide the procedure is to
add a new slide and then delete the old one or use the "overwrite
current slide" option as shown below:

- Click and hold down on the camera button.
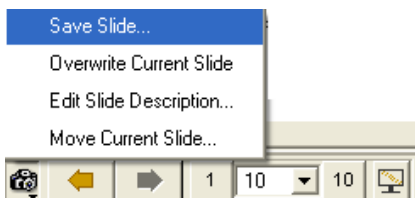
To edit a slide description.

- Click and hold down on the camera button and select the option
  "Edit Slide Description".
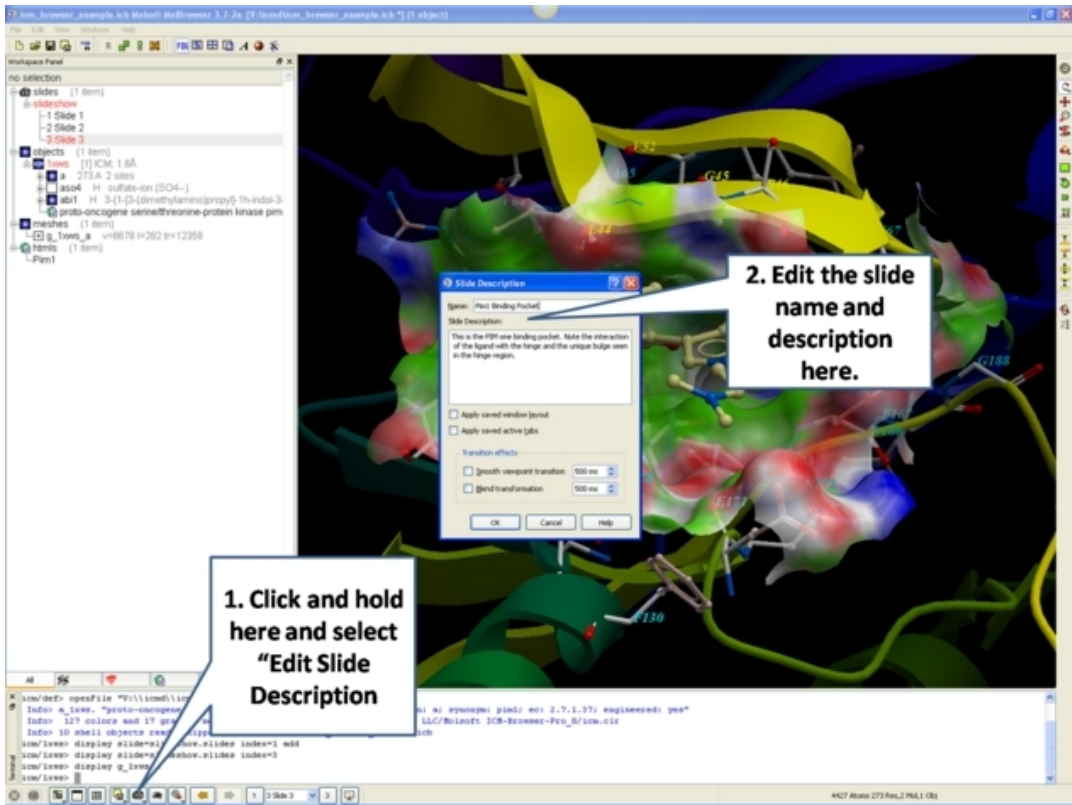
- Enter the name of the slide
- Enter a description of the slide.
- If you wish to keep the current window layout or active tabs check the boxes provided

To delete a slide:

- Right click on the name of the slide in the ICM Workspace and select Delete.

To change the name of a slide

- Right click on the name of the slide in the ICM Workspace and select Edit Slide.

6.4.2 Move Slide

To change the slide's position in the slideshow use the Move Current Slide option and select the new position from the list.

- Click and hold on the "make slide button".
- Select Move Current Slide.
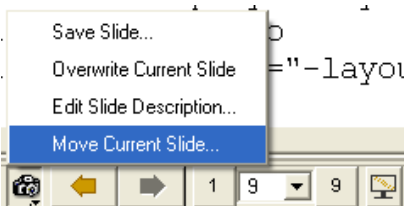
Click and hold

- Select the position in the slide show where you want to move the slide to.
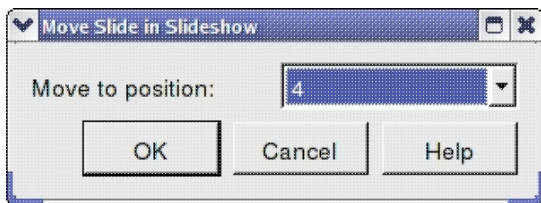
Co-display more than one slide

- Right click on the name of the slide in the ICM Workspace you wish to co-display with the curently displayed slide.
- Select the option co-display slide.

6.5 How to Add Smooth Blending and Transition Effects Between Slides

Available in the following product(s): ICM-Browser | ICM-Browser-Pro | ICM-Pro

How to add smooth and blend transitions to a slide.

- Right click on the name of the slide in the ICM Workspace.
- Select **Edit Slide**.
- Select the desired transition effect - **smooth** or **blend** as shown below.
- Select the length of the transition in milli seconds.
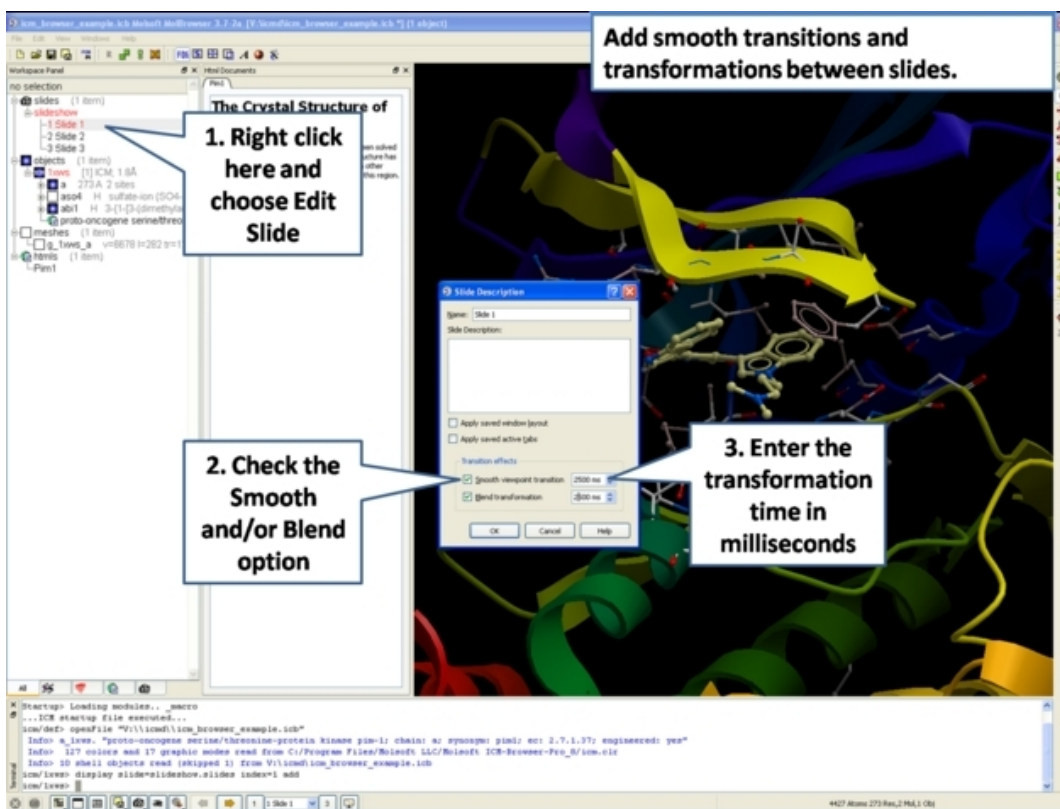
6.6 How to Make Molecular Documents - Link HTML Text to Slides

Available in the following product(s): ICM-Browser | ICM-Browser-Pro
| ICM-Pro

An ICM Molecular Document contains text and images which can be
hyperlinked to the graphical display. Click on the hyperlinked text and
then a fully-interactive 3D slide will be displayed. The hyperlinks are
usually linked to a set of slides but can also be linked to a series of
commands in a script, a web page, a table or alignment. Once a molecular
document has been made you can view it in the ICM-Browser (File/Save
Project .icb file) or download ActiveICM and view it in a web page or
Powerpoint.

To begin creating an ICM document

- File/New/ and click on the HTML tab.
- Enter some text. E.g the Name of the HTML document. Formatting can
  be changed as described in the edit section below.
- Click OK
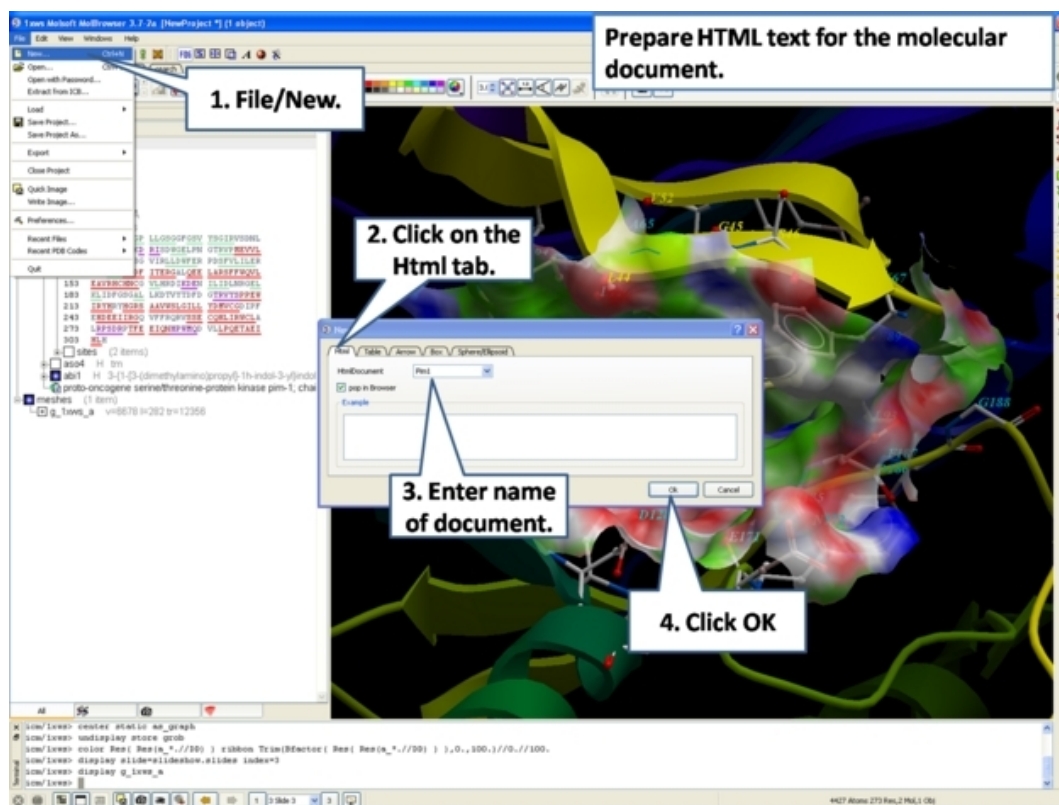
- A HTML text panel will be displayed in the graphical user
  interface.

NOTE: You can add multiple documents into a single file. The
documents will be accessible via tabs at the top of the HTML panel.

6.6.1 How to Add Text or Edit a Molecular Document

To edit the HTML text in the graphical display

- First `create` an HTML document and the text panel will be displayed in the graphical user interface.
- Right click in the body of the text display panel and select **Edit.**
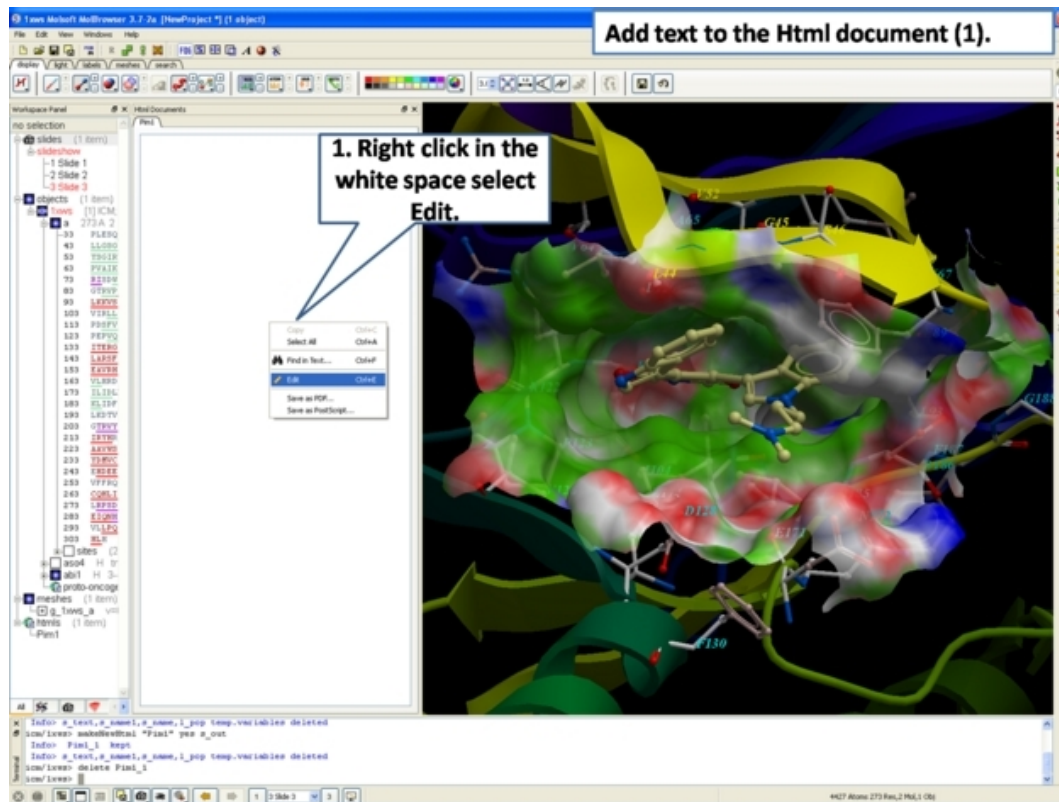
- Enter text and use the formatting tools provided in the panel above the text editor. Make sure you have selected the **Edit** tab in the HTML editor. You can see your page in the **View** tab or write directly in HTML in the **Source** tab.
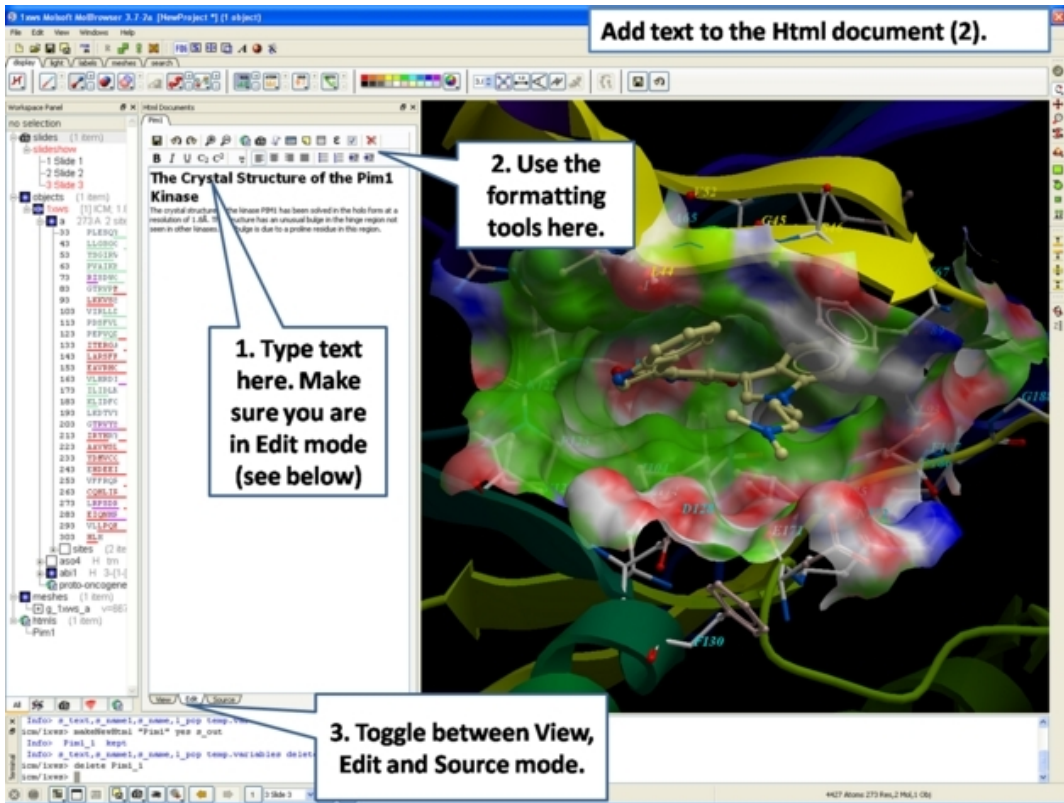
The key formatting tools in the HTML editor are shown below.
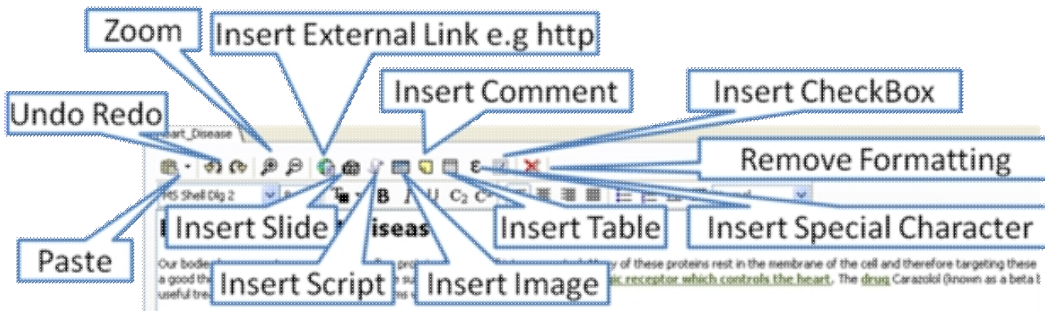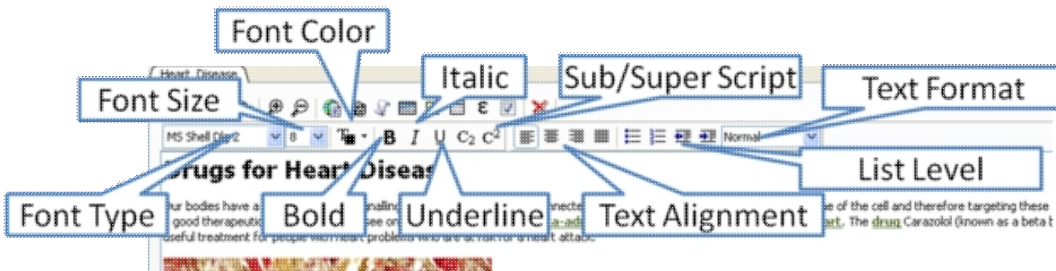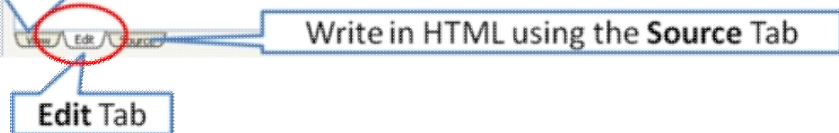
6.6.2 How to Make a Hyperlink Between Text and a Slide

To make a hyperlink between the text and the graphical display (slide)

Make a slide or set of slides of the graphical display you wish to link
to. See `Making Molecular Slides` for help on this.Once slides have been
created:

- File/New/Html
- Right click in the body of the text display panel.
- Select Edit.
- Highlight the text you wish to link to a graphical display - you
  can do this by left clicking and dragging over the text (selected
  text will be highlighted in blue).
- Click on the "Camera button" in the HTML editor formatting tool
  panel.
- Select the **Slide** tab.
- Select which number slide you wish the text to be linked to from
  the drop down menu.
- There is an option to display the slide as a thumbnail image in
  the text document panel. Check if appropriate.
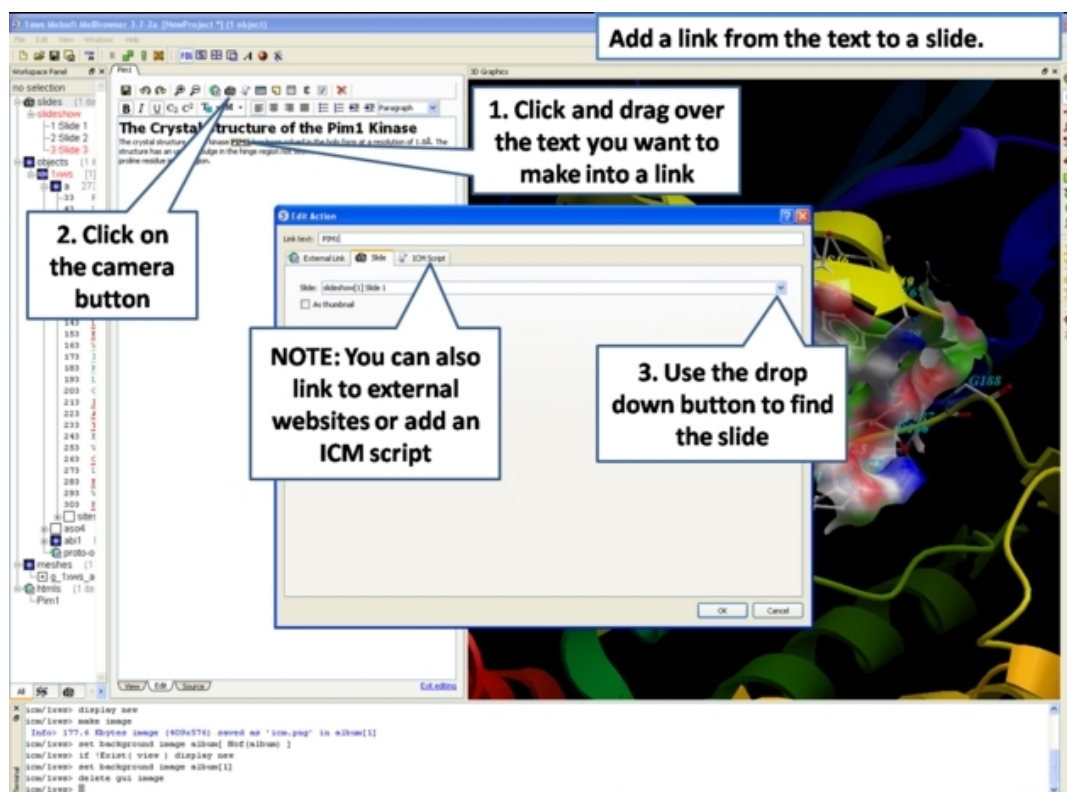
6.6.3 Insert Image

NOTE: The easiest way to add images (PNG or JPEG) into an ICM
Document is to use drag and drop. You can drag and drop the image
into the ICM Workspace or go to File/Open. Once the image is in the
album in the ICM Workspace you can then drag it from the ICM
workspace into tthe HTML editor.
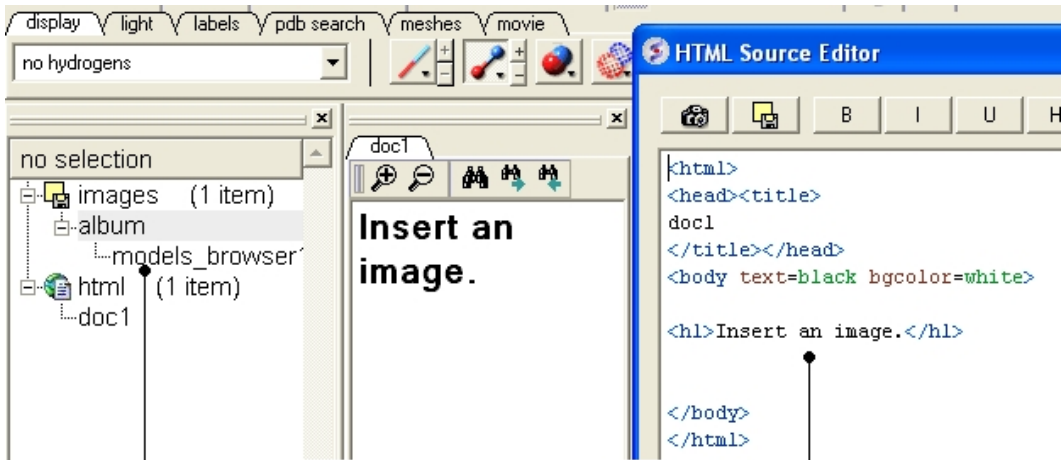
Drag and Drop from the ICM Workspace to the HTML source editor

Another way to insert a picture into the HTML text panel

- First read the image into the ICM photo album File/Open OR Drag and Drop from directory into the ICM Workspace.

The image name and preview will then be displayed in the ICM Workspace.
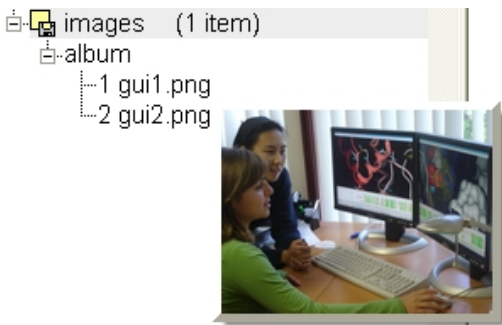
- Create HTML text - File/New/HTML. Add text.
- Right click in the HTML window and select 'Edit Source'.
- Right click on the position in the ICM Script Editor where you would like to insert the image.
- Select 'Insert Image'
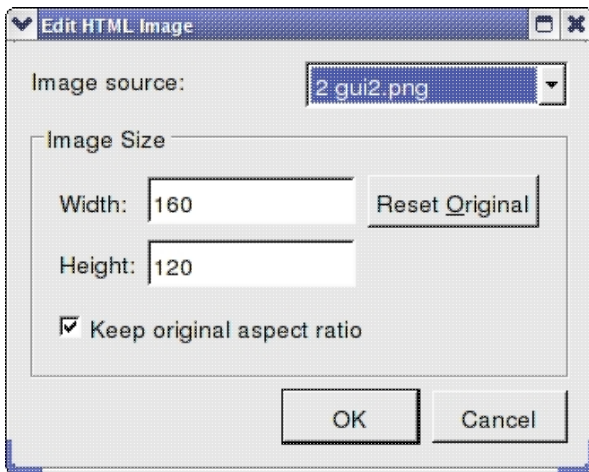
- Select the image name source.
- Choose the desired Width and Height.

- Click OK.
- Click Save in the ICM Script Editor.

6.6.4 Insert Script

How to insert a script to the text panel

There are 3 ways to add a script - described in more detail below

1. Drag and drop script from ICM Workspace

2. In the HTML Source Editor - right click and select Insert Slide or Action

3. Create an "inline" script

These methods are described below:

Drag and Drop Method

- Create a script File/New/Script
- The script will be displayed in the ICM Workspace.
- Right click in the HTML Text Panel (for instructions on how to create this panel see `create molecular document`) and select edit source and the HTML Source Editor will be displayed.
- Click-Drag and Drop the script into the HTML Source Editor

A line as shown below will be added.

```
<a href="#icm/script/script1">text placed here will be displayed as a link in the document</a>
```

Another way to add a script to the document is to Insert Action:

- Right click in the body of the text display panel.
- Select Edit Source
- Highlight the text you wish to link to a graphical display - you can do this by left clicking and dragging over the text (selected text will be highlighted in blue).
- Right click and select 'Insert Slide or Action' or select the button in the HTML Source Editor and a window as shown below will be displayed.
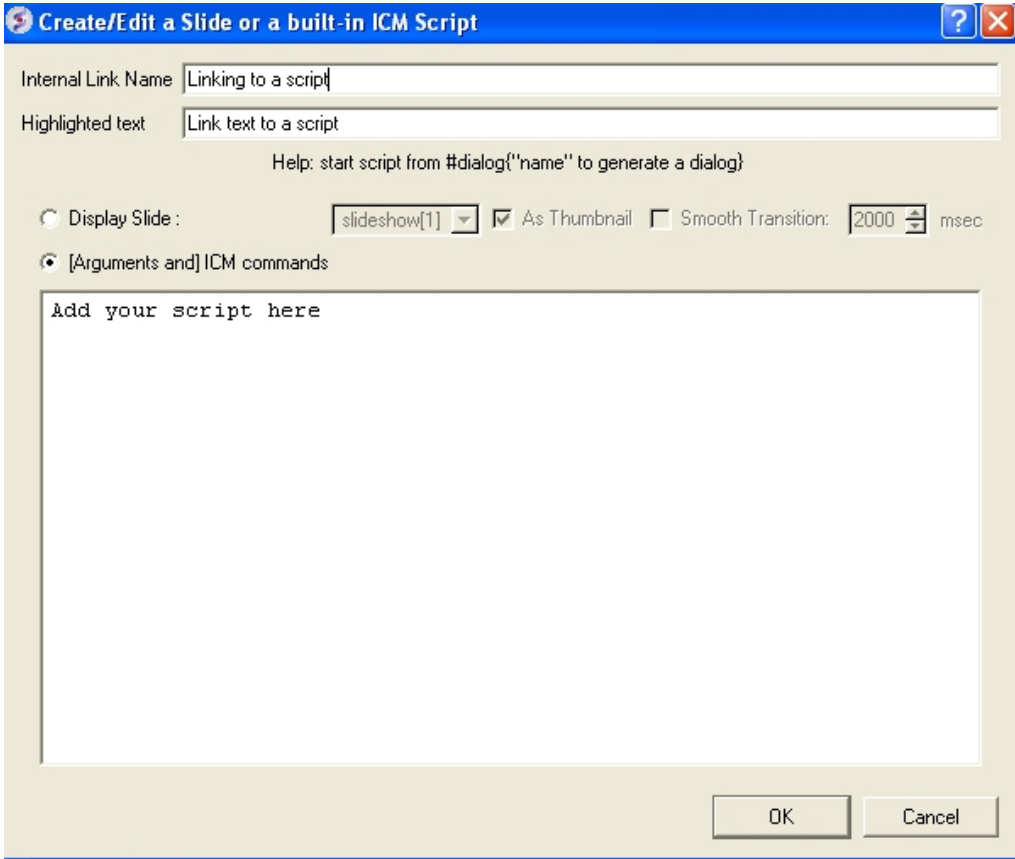
- Select the option [Arguments and] ICM commands
- Add script in the editor provided
- Select ok

Inline Script

A script can be added to the HTML text in the following way

- Right click in the body of the text display panel.
- Select Edit Source
- Enter script in the format as shown below.

```
<!--icmscript name="script2"
#dialog{"Test"}
# i_number1 (2)
# i_number2 (3)
print $1 + $2
--><a name="script2" href="#_">script2</a>
```

6.6.5 Insert a Dialog Box

Dialog boxes are provided to enable a viewer to interact with a
presentation or document file. The dialog box will be a gui data entry
box. For an example here is a script to prompt the user of the file to
enter a pdb code:

```
#dialog{"Read PDB File"}
# s_pdbcode (1crn)
read pdb $1
ds a_1.
```
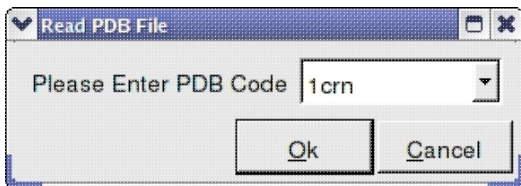
The code above can be saved as a script or inside the html text. To do this:

1. Right click on the HTML text display and select "Edit Source".
2. Highlight the text you wish to link to a dialog box and then select the right click and select 'Insert Slide or Action' or select the button in the HTML Source Editor and a window as shown below will be displayed.
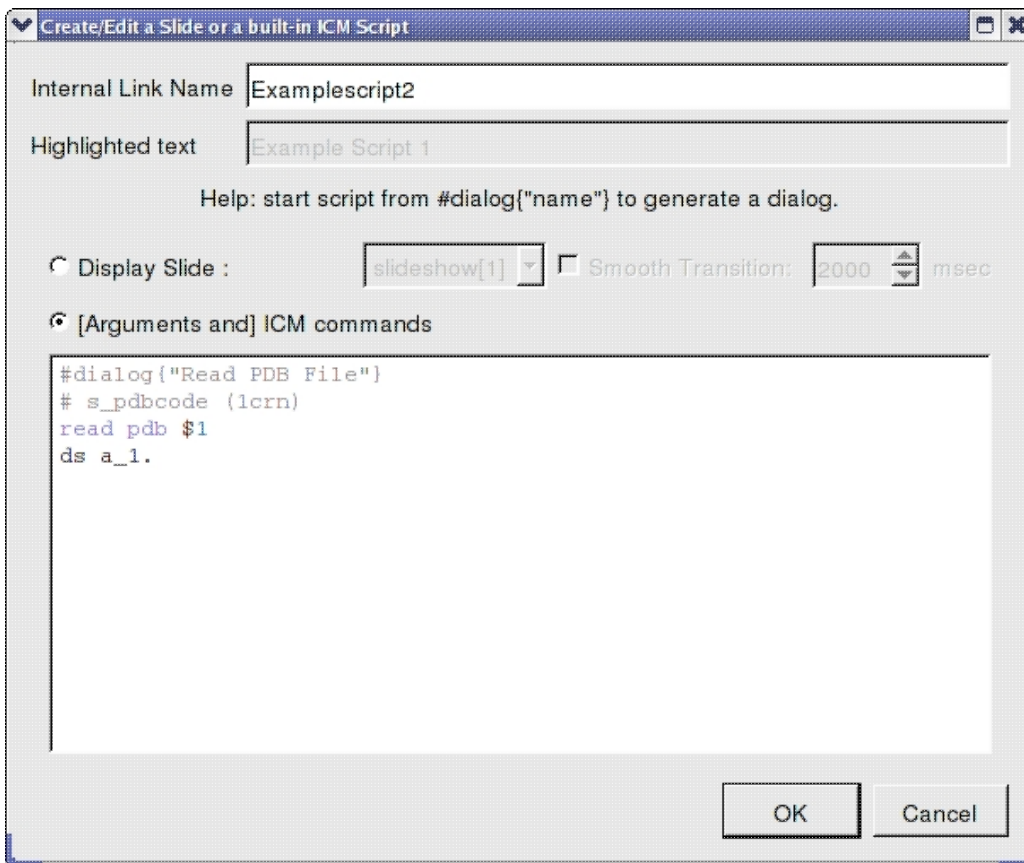
OR.

1. Right click on the HTML text display and select "Edit Source".
2. Add a link to a script as shown below.

```
<a href="#icm/script/script1">Example Script 2</a>
```

6.6.6 How to add a check box.

To add a check box:

- Click on the check box button in the HTML editor tools panel (see below).
- You can choose the check box to toggle an object or molecule on or off. Or you can be more specific about the toggle actions using the ICM command line. You can choose different actions for On and

Off.
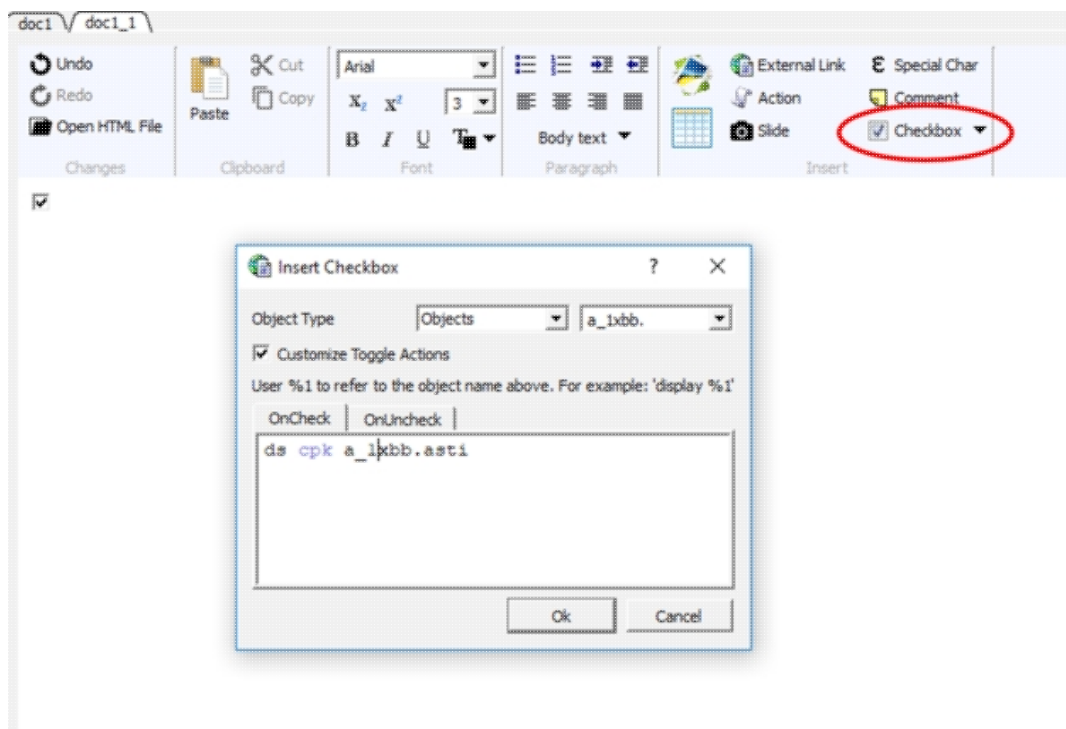
6.6.7 Document Navigation

The following buttons shown below aid document navigation. Also remember
that more than one document can be stored and the header of each
document file will be displayed in multiple tabs in the text panel
window.
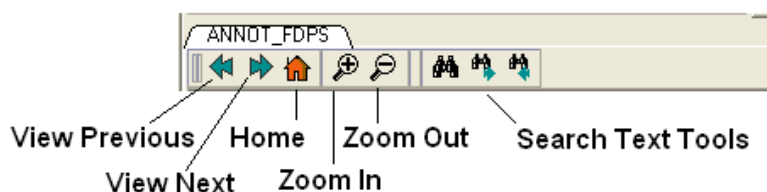
6.6.8 Protect Shell Objects From Deletion

When making a molecular document you can protect objects from deletion
by the person who reads your document by:

- Right click on the object in the ICM Workspace.
- Select the **Protect** option.

6.7 ActiveICM
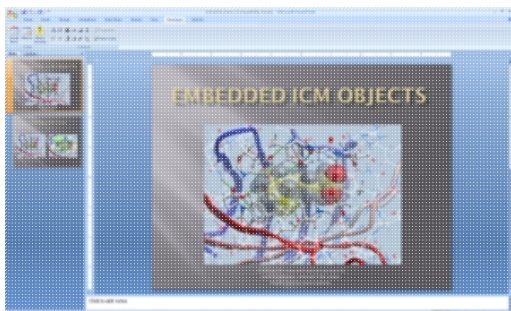
`ActiveICM` enables you to view and display ICM graphical `slides` and `animations` interactively inside Windows Microsoft PowerPoint and web browsers such as Internet Exporler and Mozilla Firefox.

Chapter Contents:

- `How to Embed in Microsoft PowerPoint 2003`
- `How to Embed in Microsoft PowerPoint 2007`
- `How to Embed in Microsoft PowerPoint 2010`
- `How to Embed in a Web Browser`
- `How to Use ActiveICM in PowerPoint`
- `How to Change ActiveICM Component Properties`
- `Advanced use of activeICM: Macros to direct visualisation changes`
- `Background Images`

6.8 How to Embed in Microsoft PowerPoint 2003

Setup

- Download ActiveICM from www.molsoft.com/support
- Save an ICM file (.icb) containing slides. Click `here` to see how to make slides.

Embed icb file

- Open the Insert menu from the top bar of PowerPoint and select **Object**
- This opens up the Object dialogue. Select **ActiveIcmCtlClass:**
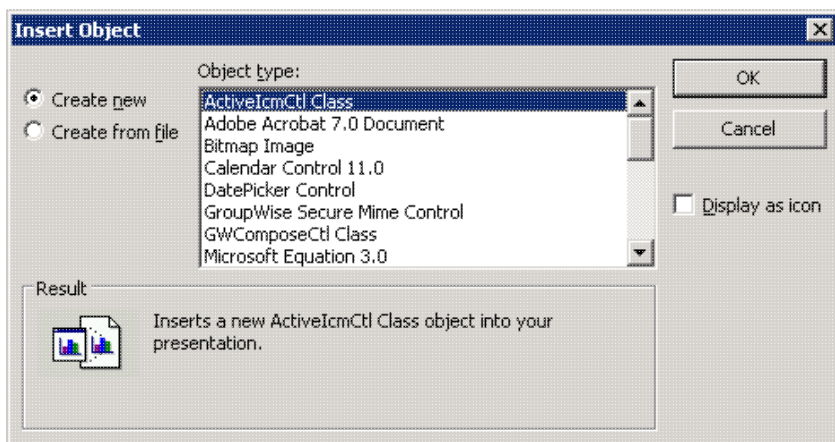
- Click on OK. A file dialogue will then be opened. Open the ICB file you wish to use via this dialogue. IMPORTANT: To avoid later problems, make sure the ICB file is in the same folder as the PowerPoint file.
- A low-resolution snapshot of the first slide in the ICB file will be shown in the activeICM control you created. You can change the shape of the control by dragging the corners of the control with

the mouse, once selected.

- Right-click on the activeICM control and select the Properties menu item
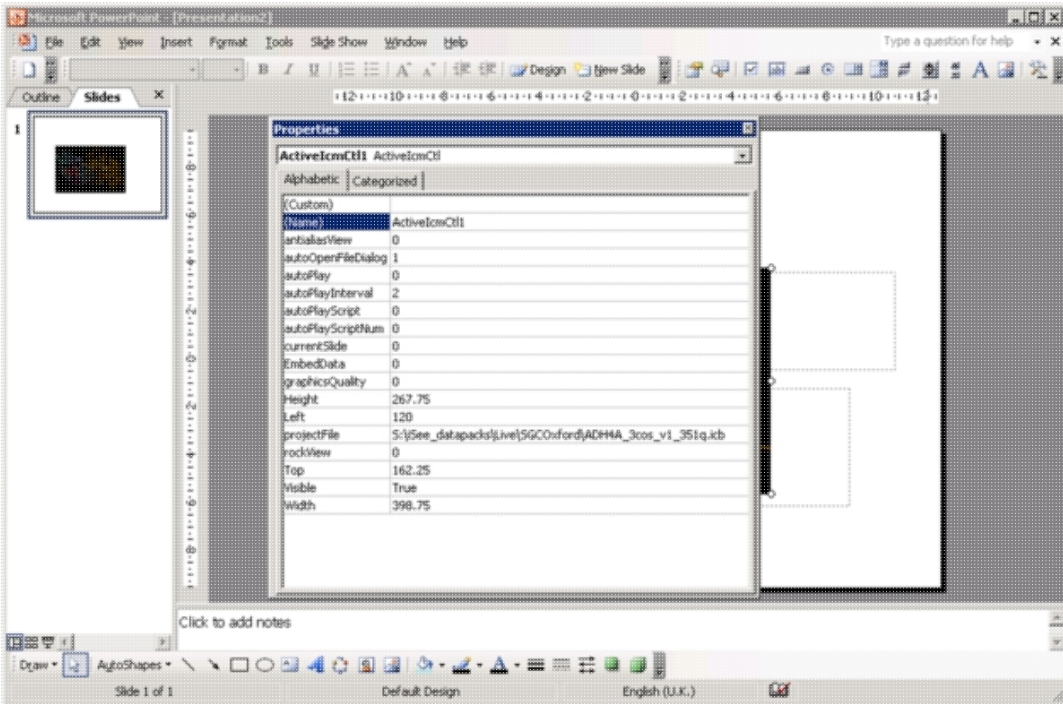
- Save the PowerPoint presentation

6.9 How to Embed in Microsoft PowerPoint 2007

Setup

- Download ActiveICM from www.molsoft.com/support
- Save an ICM file (.icb) containing slides. Click here to see how to make slides.

NOTE: Here are the instructions for ActiveICM in Microsoft Office 2007, for older versions of PowerPoint see here.

Enable the Developer Menu:

- Click the **Microsoft Office Button** (button top left), and then click **PowerPoint Options**.
- In the **PowerPoint Options** dialog box, click Popular.
- Under **Top options for working with PowerPoint**, selet the **Show Developer tab in the Ribbon** check box, and then click **OK.**
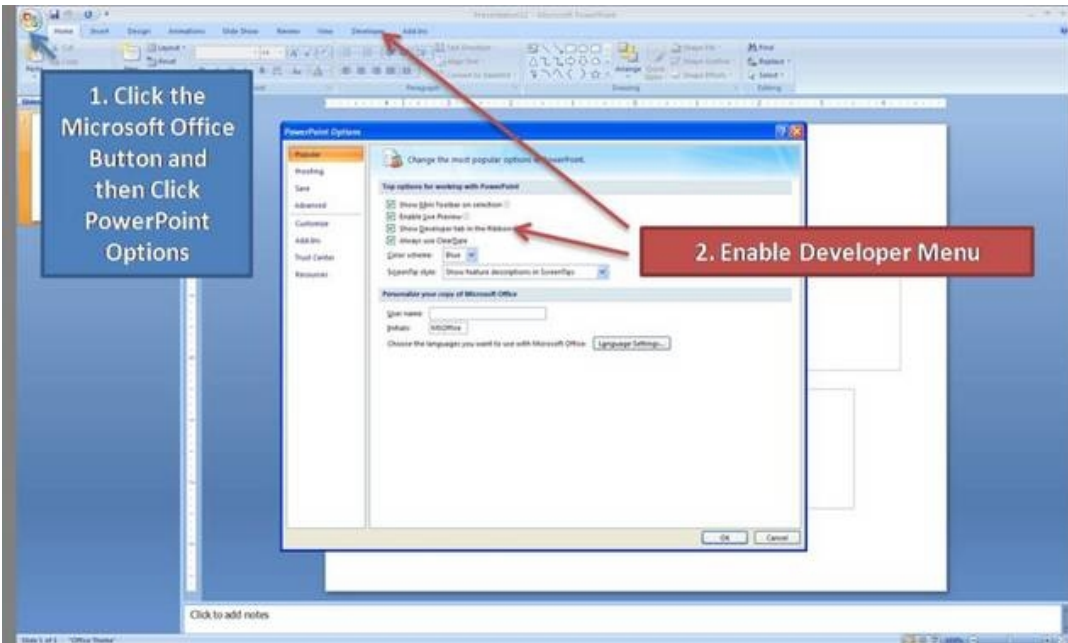
Insert ActiveICM into PowerPoint:

- Select the **Developer** menu.
- Select the **More Controls** button in the **Controls** field.
- Select **ActiveICMCtl Class** from the list of controls and click **OK.**
- Click the mouse anywhere in the white PowerPoint space and a dialog box will be displayed asking you to select your ICM (.icb) file.
- Click and drag at the corners of the image to resize the normal way you would resize an object in PowerPoint.

6.10 How to Embed in MicroSoft PowerPoint 2010

Setup

- Download ActiveICM from www.molsoft.com/support . Please always check you are using the latest version.
- Save an ICM file (.icb) containing slides. Click here to see how to make slides.

Enable the Developer Menu:

- Click on the File tab, and then click Options menu.
- Select the **Customize Ribbon** option and then check the **Developer** option in the right hand side panel (see image below).
- Click on the **Developer Menu**.
- **IMPORTANT!** Click OK and then you will see the Developer Menu in the Ribbon at the top of PowerPoint.
- Select the Developer menu.
- Select the **More Controls** button in the **Controls** field.
- Select **ActiveICMCtl Class** from the list of controls and click **OK.**
- Click the mouse anywhere in the white PowerPoint space and a dialog box will be displayed asking you to select your ICM (.icb) file.
- Click and drag at the corners of the image to resize the normal way you would resize an object in PowerPoint.
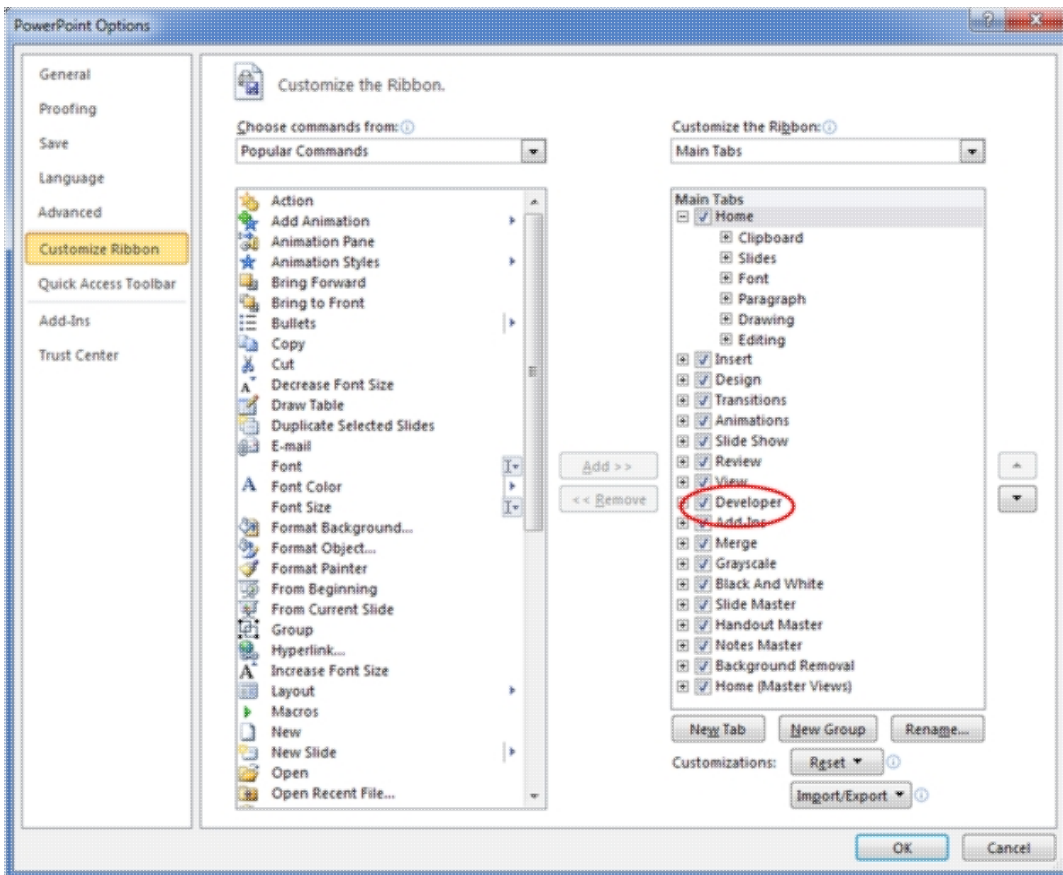
6.11 Embed in Web Browser

NOTE: There is now a plugin-free version using JavaScript/HTML5 - click here for more information.

To embed in a web browser.

1. Download ActiveICM from here
   http://www.molsoft.com/getbrowser.cgi?product=activeicm&act=list
   (it is free!).

2. `Create` an HTML page in ICM (File/New/Html).
3. Add a series of `slides`.
4. File/Export As ActiveICM Html..

NOTE. There is an issue with FireFox21 because it disables the ActiveICM plugin. The workaround at the moment is:

- type about:config in the location (address) bar and press the "Enter" key to open the about:config page, just like you open a website by typing the URL in the location bar.
- if you see a warning message then you can confirm that you want to access the about:config page.
- in the Search bar at the top of the about:config page type 'load_appdir_plugins'
- double click on the preference to set value to true
- restart FF

6.12 How to Use ActiveICM in PowerPoint

**IMPORTANT There are two ways to open a presentation:

- Double click on the ppt file in windows folder. (in this case PowerPoint will set the current directory to the one which contains the file and there should be no problems with both relative and absolute paths)
- Open ppt through the "File-Open" or recent files. (in this case PowerPoint DOES NOT SET the current directory to the one which contains the file -> relative path might not work and user will be prompted to locate the ICB file unless file is found in absolute location)

To view the slides you must be in Slide Show mode

- Press the **F5** button to start the **Slide Show**. In edit mode (i.e. not presentation mode), the control is shown as a static image ï¿‰ it is not possible to interact with the ICB file. Therefore, to prepare the presentation so that the control shows the correct initial visualisations it is necessary to run the PowerPoint slide(s) in presentation mode

Change Slides

- Use the left and right cursor keys to change slides.

A number of other options can be accessed by right clicking on the slide. These options include:

- Select Slide
- Auto Play
- Set on/off rocking
- Center
- Load a new ICM File
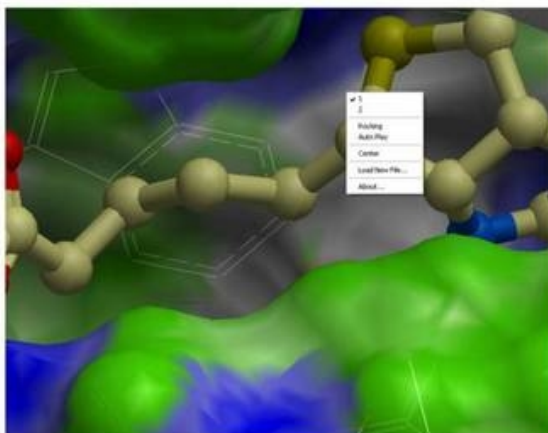
You can also add multiple ActiveICM 3D displays in one slide:

- To display mutliple ActiveICM 3D displays in one slide just copy the original display or repeat the steps described above. All powerpoint slides should point to the same ICM file (.icb) but they can point to different slides.
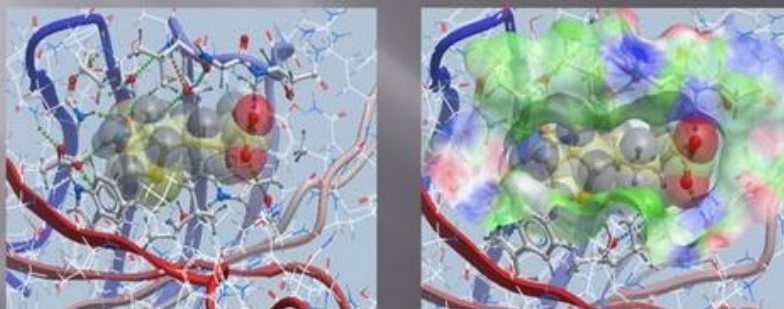
6.13 How to Change ActiveICM Component Properties

A number of properties of ActiveICM can be changed once embedded in powerpoint. The options include:

- Select the first slide to be displayed.
- Set slide auto play.
- Set auto play of a script.
- Embed the powerpoint file and the icb file all into one file.

To change these options:

- Right click on your embedded activeICM in Powerpoint.
- Select **Properties** and click on the button shown below.
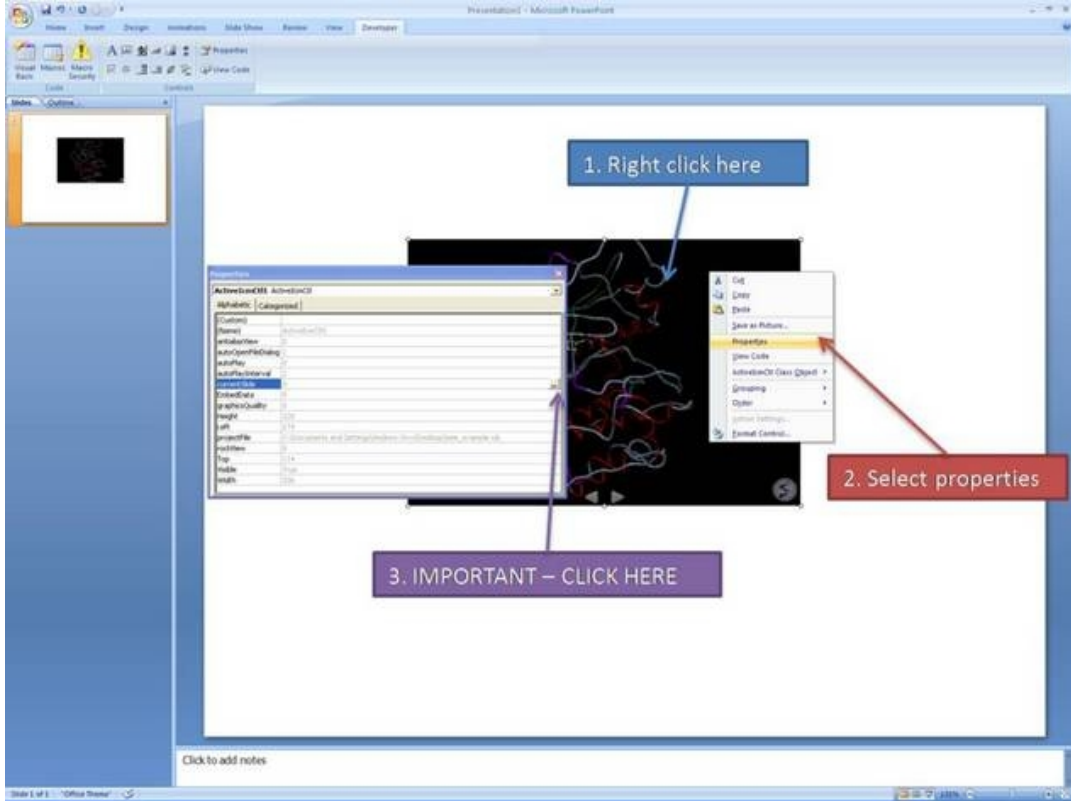
- A **Property Pages** window will then be displayed as shown below.
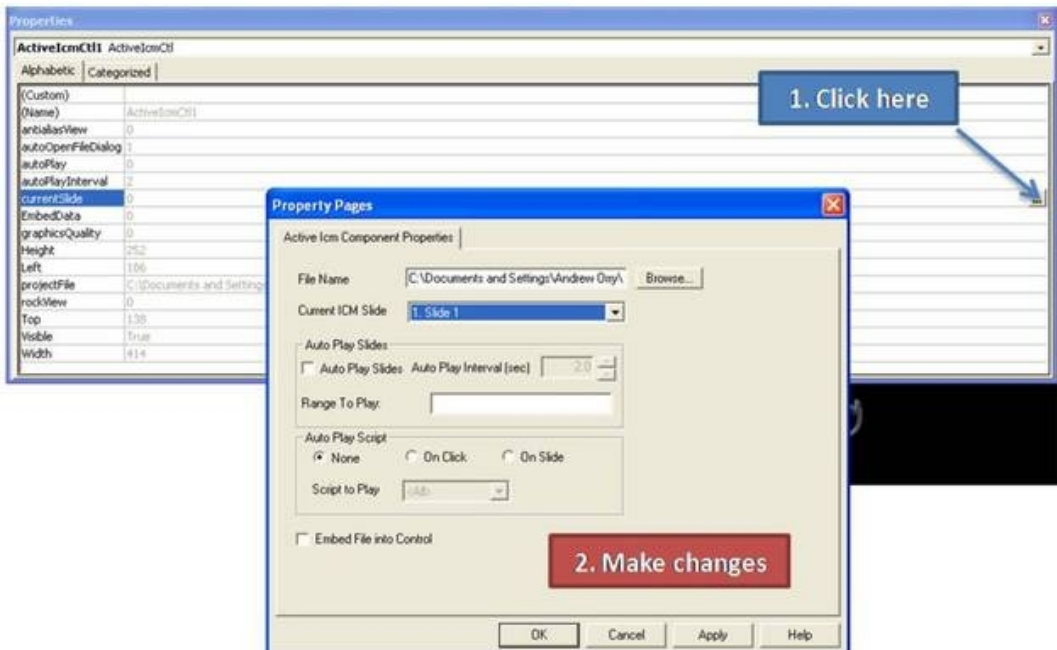
To change the file name of the icb file linked to activeICM: Simply type in the path to the file or use the browse option.

To change the current ICM slide: Use the drop down button next to **Current ICM Slide** to select the slide you wish to display first in your presentation.

To auto play slides: Check the **Auto Play Slides** box and select the interval between slides option. A range of slides can be played by entering the number of the slides separated by a comma.

To auto play a script: Select whether you want the script to run **On Click** or **On Slide** then select the script from the **script to play** drop down button. You should first save your script in the icb file.

To embed the icb file in the ppt file Click the **Embed File into Control** option. **Important** - Please save your PowerPoint file in the t 1997-2003 ppt format not pptx.

6.14 Advanced use of activeICM: Macros to direct visualisation changes

Documentation kindly provided by Dr. Brian Marsden (SGC Oxford
`http://www.sgc.ox.ac.uk/people/brian/`)

It is possible to write simple VisualBasic scripts to avoid having to use the right-click menu approach to changing activeICM control slides within the control itself. This allows one to place buttons outside of the activeICM control, but in the same PowerPoint slide, which controls the control's behaviour. Below are a couple of useful examples of this approach.

Creating a button to set the control's active slide:

Insert a button Office 2003

- In edit mode, make sure the control toolbox toolbar is shown by right-clicking the blank area at the top of the top bar and ensuring **Control Toolbox** is ticked.
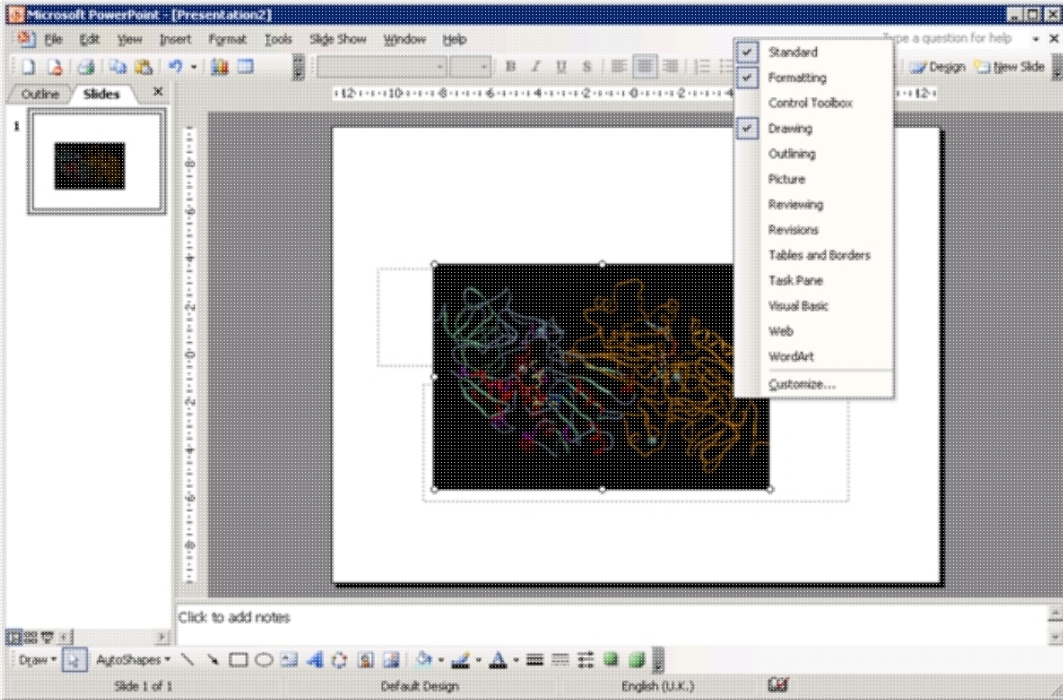
- Click on an icon in the Control Toolbox which corresponds to the sort of button you wish to use. Then click and drag in the PowerPoint slide to generate the button.

Insert a button Office 2007:

- In edit mode, click on an icon in the Developer menu or ribbon which corresponds to the sort of button you wish to use. Then click and drag in the PowerPoint slide to generate the button.
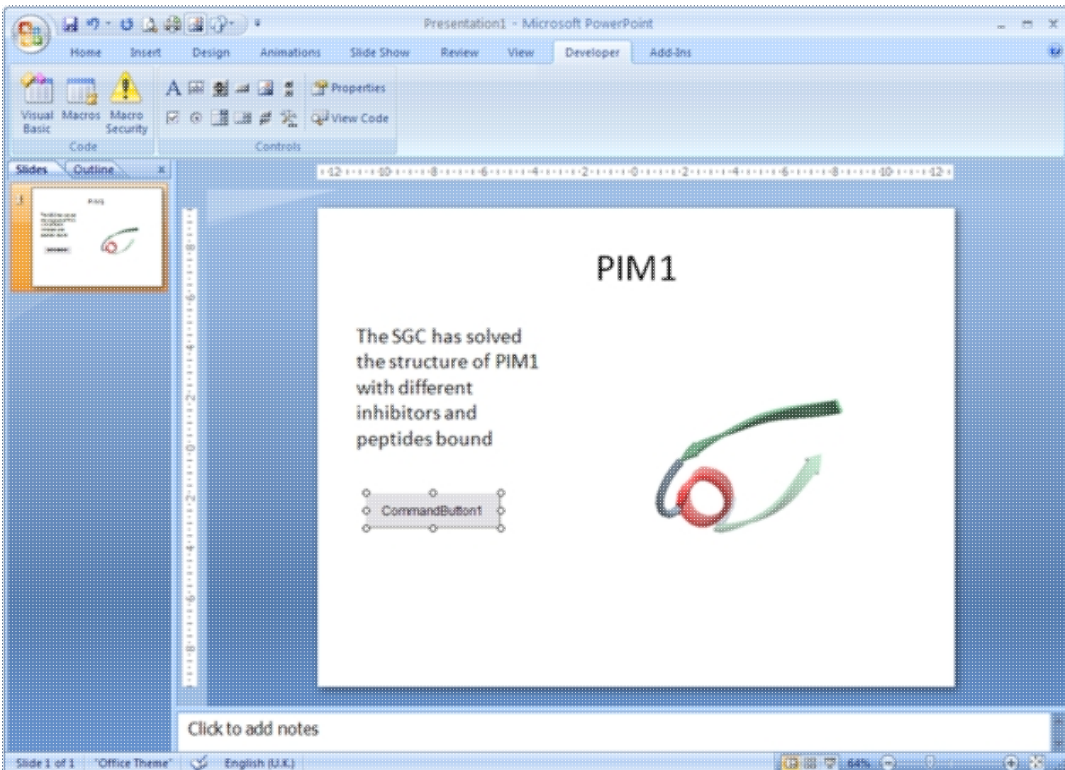
- Double-click on the new button to open the VisualBasic editor with two empty functions pre-defined. The first one pertains to the control itself and can be ignored in this context *For the second function (which is for the newly-created button), copy the following into the editor, between the two lines of function code:

```
ActiveIcmCtl1.currentSlide = 2
```

- This sets the current activeICM control's slide to be number 3 **note** that the value placed in this code needs to be 1 less than the actual slide number (confusing, no?). Obviously, use a value here that makes sense in the context of your ICB file.
- This should leave the editor looking like this:
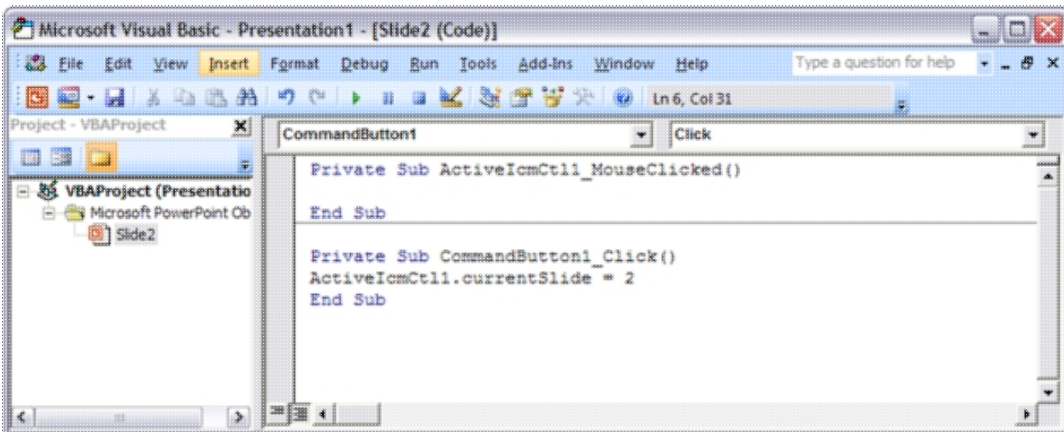
- Close the Visual Basic editor
- To change the physical properties of the button e.g. text, colour e.t.c.right-click on the button and select the Properties menu option. This opens up a dialogue as below, where many properties of the button can be changed:
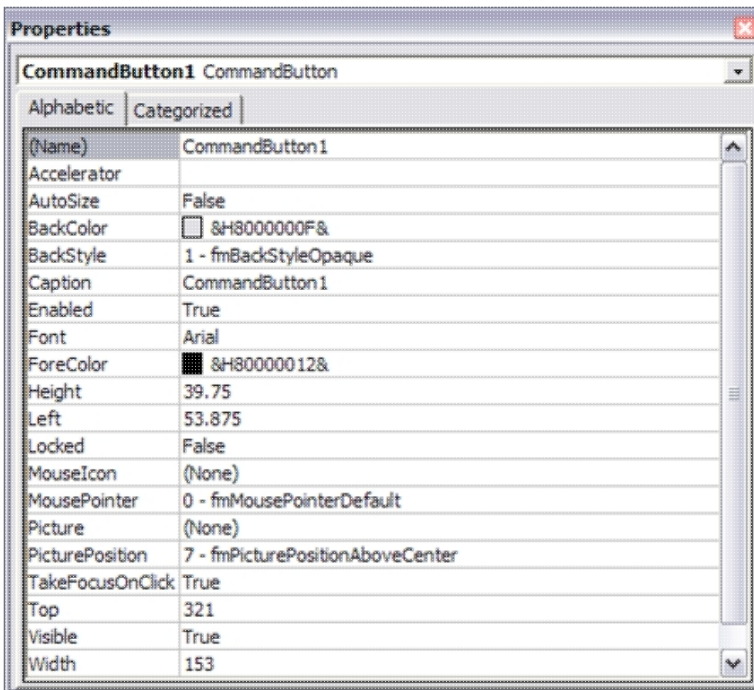
- Using this dialogue, it should be possible to disguise the button to look like normal text (for example) which can be clicked on during the presentation to change the visualisation of the

6.14 Advanced use of activeICM: Macros to direct visualisation changes

control, apparently magically. Note that the button will only work in presentation mode.
- IMPORTANT: In Office 2007, remember to save the PowerPoint presentation now as a pptm file that is, a macro-enabled PowerPoint file otherwise the macros will not work next time you load the presentation.

Other code examples: Just copy and paste the example of interest inside the function for the button in the Visual Basic editor. Code that enables a button to cycle through the ICB files slides in order (including wrap-around)

```
currentSlide = ActiveIcmCtl1.currentSlide
numSlides = ActiveIcmCtl1.nofSlides
If currentSlide = numSlides - 1 Then
ActiveIcmCtl1.currentSlide = 0
Else
ActiveIcmCtl1.currentSlide = currentSlide + 1
End If
```

6.14.1 PowerPoint Cache Errors

PowerPoint caches some information about active controls. Sometimes after an ActiveICM upgrade you may get an error when trying to access some property or method: "Wrong number of arguments or invalid property assignment" or something similar.

In this case you need to close PowerPoint and remove all files from the location below:

C:\Documents and Settings\seva\Local Settings\Temp\PPT11.0

6.15 Background Images

In ActiveICM version 1.1-5 and higher you can add background images to your icb file and display in PowerPoint and the web using ActiveICM. The documentation on how to insert background images can be found `here`.

6.16 ICM JavaScript (IcmJS)

IcmJS (formerly known as ActiveIcmJS) is a JavaScript/HTML5 version of the ActiveICM plugin. You can read more about IcmJS `here`.

To make a fully interactive 3D molecular web page from ICM and embed using IcmJS:

- Use ICM vesion 3.8-5 or higher. If you do not have access to this please contact MolSoft to request it.
- Make a set of `slides`.
- Go to File/Export as ActiveICM Html and then select the IcmJS check box.
- The IcmJS file will then be saved to your computer which you can open in any modern browser e.g. Chrome, FireFox or Safari.

7 Working with Sequences and Alignments

Many powerful sequence manipulation tools are contained within ICM. In this chapter we will describe some of the bioinformatic manipulations you can perform with sequences and `alignments`.

Chapter Contents:

- Read and Save Sequences
- Sequence Secondary Structure
- Bioinfo Menu Options
- BLAST - Sequence Search and Align
- Sequence Alignments
- Alignment Editor
- Color Structure and Surfaces by Alignment

7.1 Read and Save Sequences

> Available in the following product(s): `ICM-Pro`

There are a number of different ways to load a sequence into ICM via the Graphical User Interface.

7.1.1 Read a Sequence from UniProt

Uniprot is a curated protein sequence database which provides a high level of annotation (such as the description of the function of a protein, its domains structure, post-translational modifications, variants, etc.).

To read a sequence from UniProt:

- File/Load/UniProt
- Enter UniProt ID

or use the option in the `Search Tab`.

7.1.2 Cut and Paste a Sequence

Paste your own sequence into ICM

- File/New/Sequence
- Paste the Sequence into the box provided.
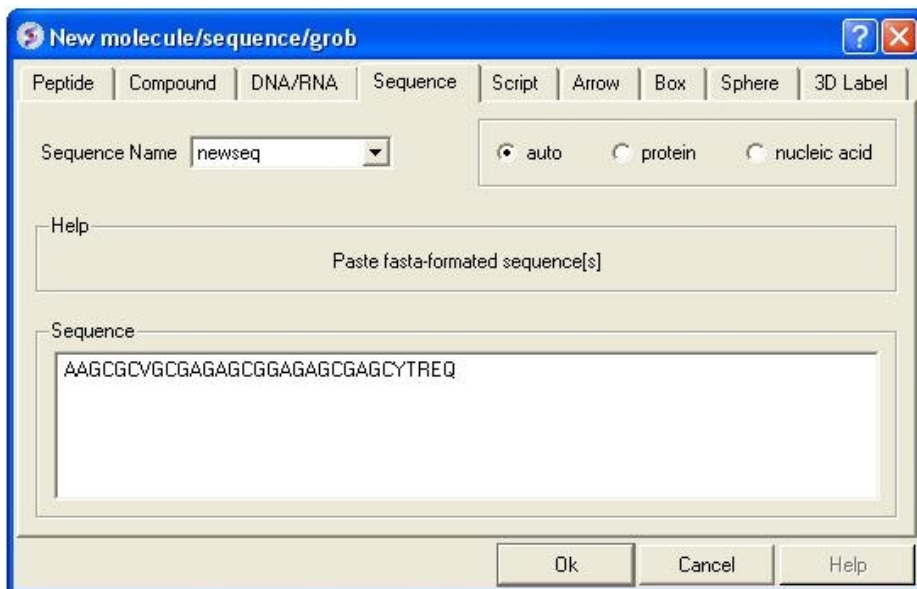
7.1.3 Extract a Sequence from a PDB File

Extract a Sequence from a PDB file

- Right click on a molecule in a loaded PDB file in the ICM Workspace.
- Select Extract Sequence(s) and the sequence will be laoded into the Sequences section of the ICM Workspace.

7.1.4 Read directly from a Sequence File

To read a sequence from a file:

If you have a sequence file saved in FASTA format it can be read into ICM by:

- File/Open

7.1.5 Save Sequence

To save a sequence in FASTA format:

- Right click on the sequence name in the ICM worksapce.
- Select the option Save As.
- Choose FASTA format from the Windows "Save as type:" drop down menu option.

7.2 Sequence Secondary Structure

If a sequence has assigned secondary structure then colored lines will appear under the sequence in the ICM Workspace. Regions underlined in red = alpha helix, green = beta sheet, blue = pi helix, magenta = 3/10 helix.

The colored lines under the sequence in the ICM Workspace refer to secondary structure.
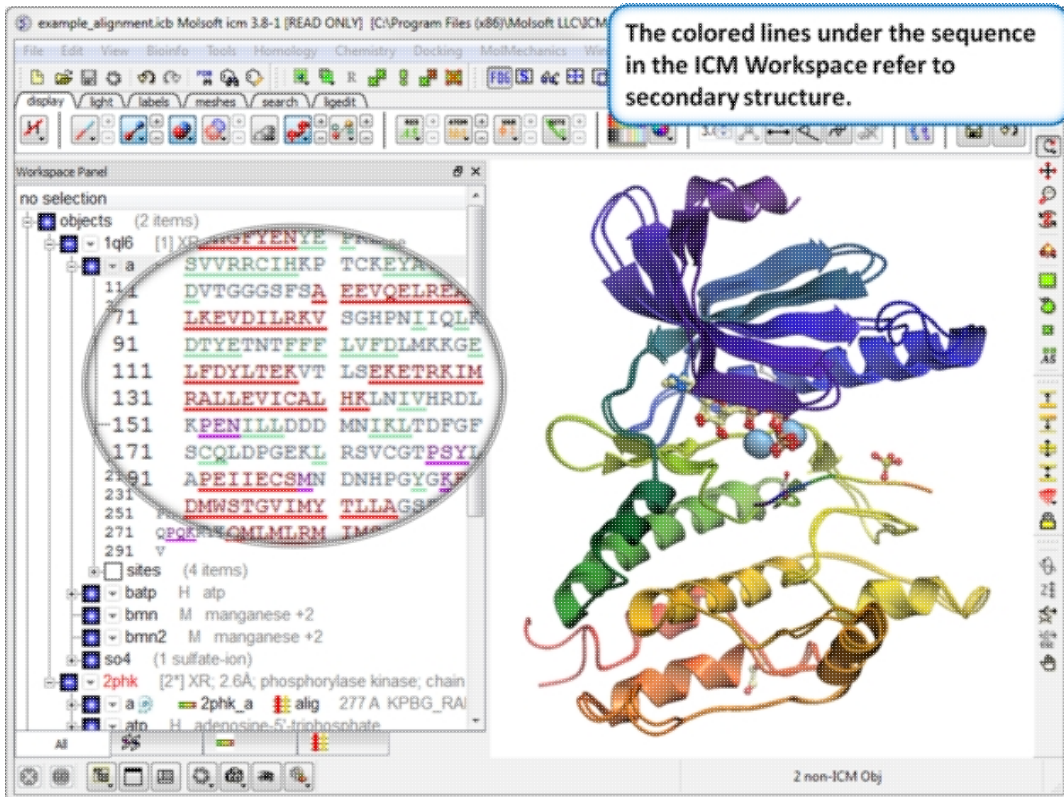
7.3 Bioinfo Menu

Available in the following product(s): ICM-Pro

Note: Click **Next** (top right hand corner) to navigate through this chapter. Headings are listed on the left hand side (web version) or by clicking the **Contents** button on the left-hand-side of the help window in the graphical user interface.

Bioinformatics Menu
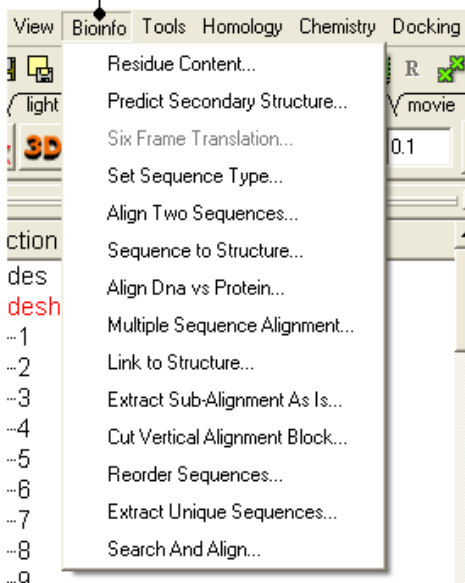
7.3.1 Residue Content

To determine the residue content of a sequence.

- Bioinfo/Residue Content and a data entry box as shown below will be displayed.
- Enter the sequence name. See the `Load Sequence` section for more information on how to load a sequence into ICM using the Graphical User Interface.
- A table and graph of residue frequencies will be displayed.

7.3.2 Predict Secondary Structure

To predict the secondary structure of a sequence:

- Bioinfo/Predict Secondary Structure
- Enter the sequence name. See the `Load Sequence` section for more information on how to load a sequence into ICM using the Graphical User Interface.
- An option is provided to ignore currently assigned secondary structure.

To view the secondary structure prediction click on and expand the sequence in the ICM workspace. Regions underlined in red = alpha helix, green = beta sheet, blue = pi helix, magenta = 3/10 helix.

7.3.3 Six Frame Translation

This options returns the translated DNA or RNA sequence ('-' for a Stop codon, 'X' for an ambiguous codon) using the standard genetic code.

- Read into ICM a DNA sequence from a file (eg File/Open FASTA) or use the File/New option and cut and paste a DNA sequence.
- Bioinfo/Six Frame Translation
- Translate all frames or use start codon.

7.3.4 Set Sequence Type

This option allows you to define whether a sequence that is read into ICM is a protein or nucleotide sequence.

- Read into ICM a sequence (eg File/New and cut and paste sequence or File/Open FASTA)
- Bioinfo/Set Sequence Type
- Select the sequence name using the drop down button

- Select sequence type protein or DNA.

7.3.5 Align Two Sequences

Please see the `Alignment Chapter`.

7.3.6 Sequence to Structure alignment

Please see the `Alignment Chapter`.

7.3.7 Multiple Sequence Alignment

Please see the `Alignment Chapter`.

7.3.8 Alignment Strength Profile

To view a fully interactive alignment strength profile:

- `Create` an alignment.
- Bioinfo/Alignment Strength Profile
- A fully interactive table and plot will be displayed.
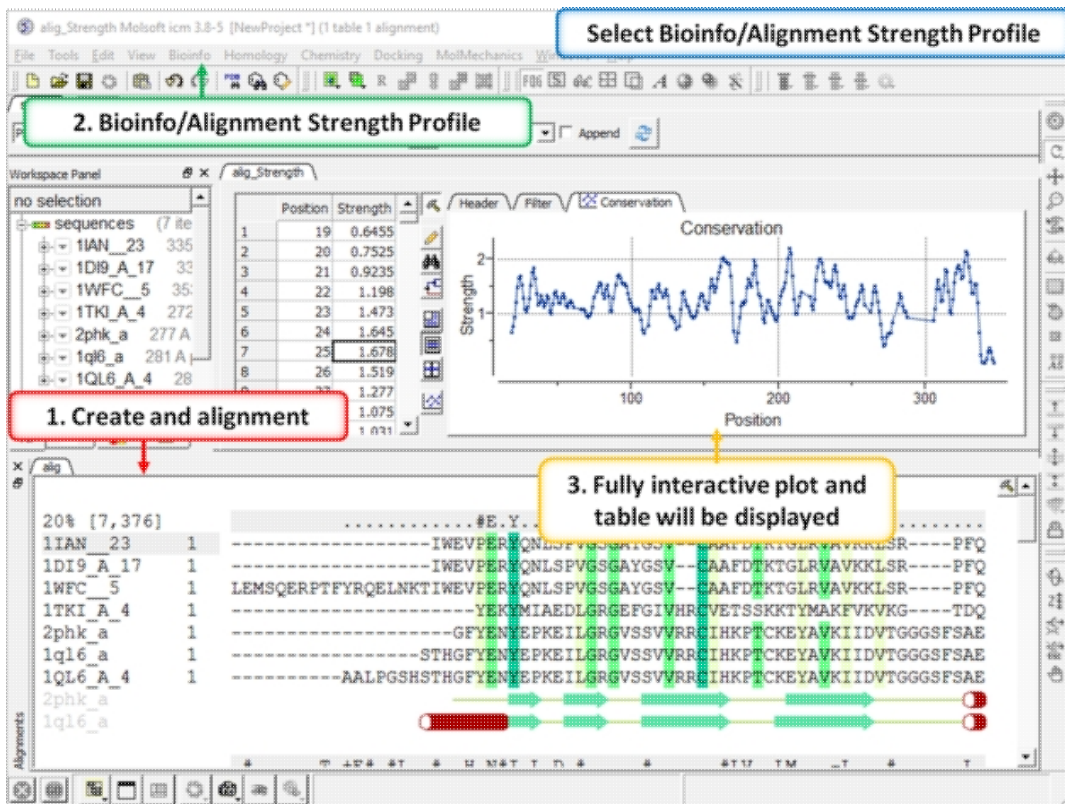
The profile can be plotted onto the alignment:

- Open the alignment editor panel.
- Select the plot checkbox and choose either bars or line plot.
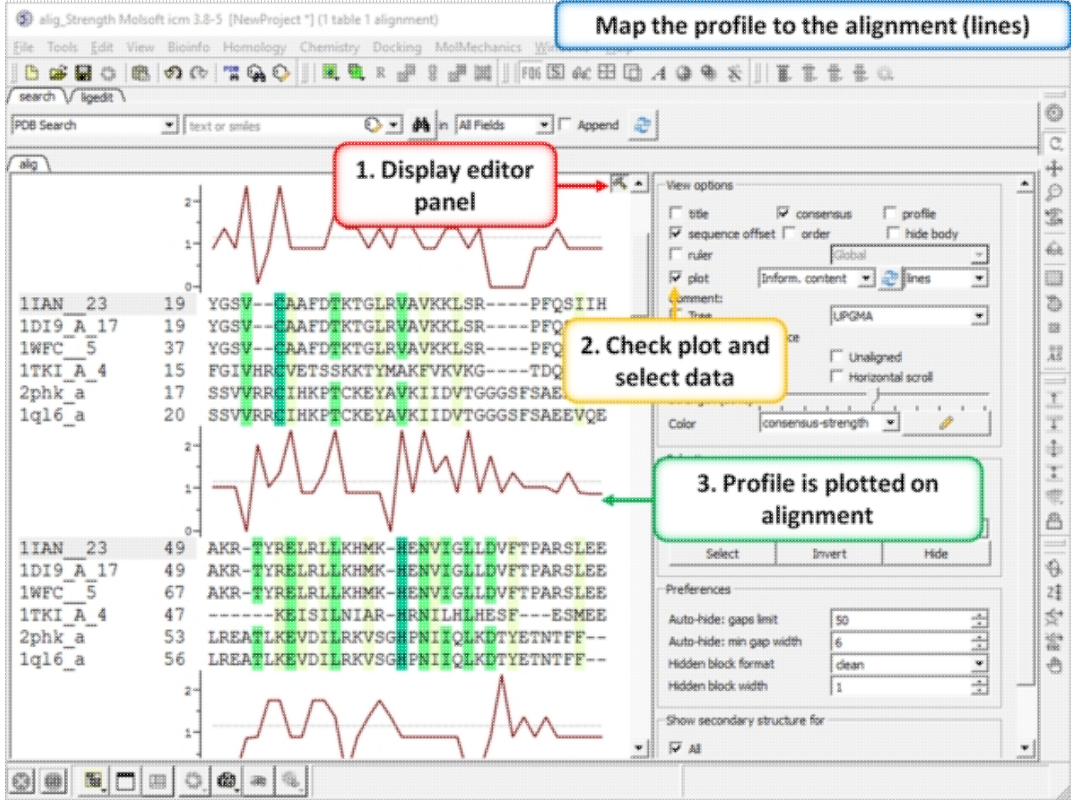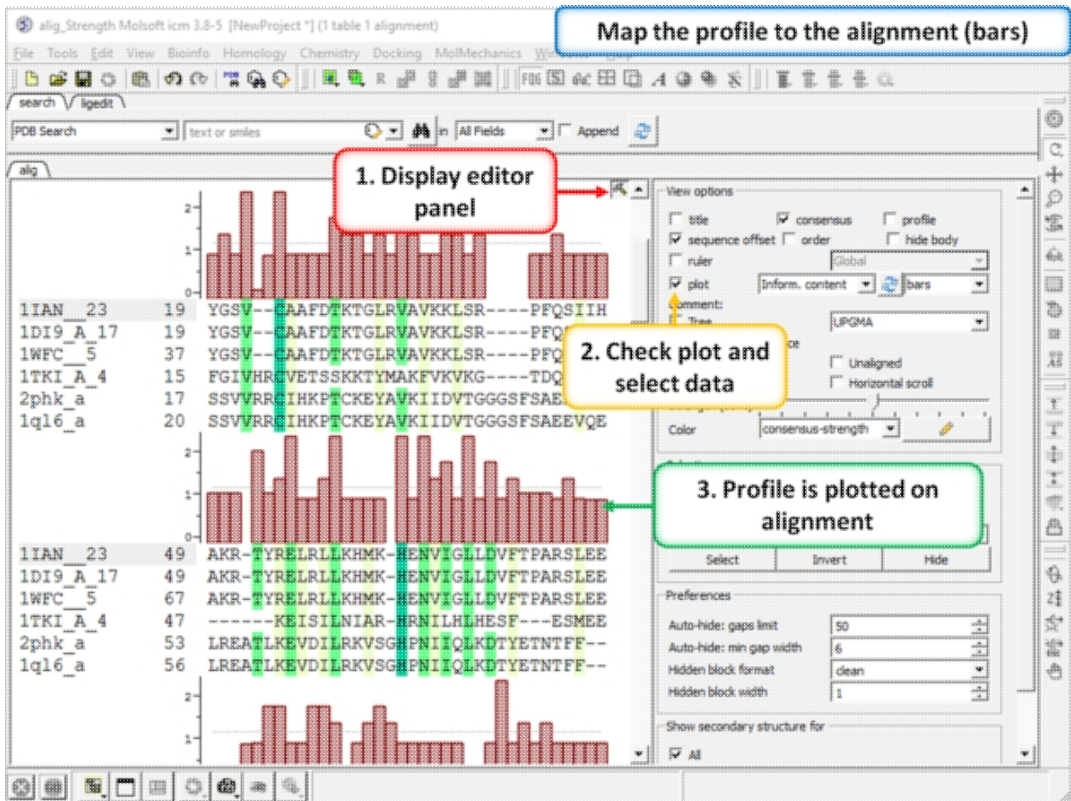
7.3.9 Link to Structure

To link a structure to an alignment:

- Double click on the structure in the ICM workspace to select it.

- Bioinfo/Link to Structure

> **NOTE** Links are described in more depth in the `Making Links Section` of the manual.

7.3.10 Extract Sub-Alignment As Is

On occasion you may want to extract a sub alignment from a bigger alignment. For example you wmay only wanto extract the alignment for the sequences linked to a structure.

To extract a sub-alignment:

- An initial `multiple sequence alignment` must first be displayed in the graphical user interface.
- Bioinfo/Extract Sub-Alignment As Is
- Enter the name of the algienment from which you wish to extract a sub-alignment from.
- Specify the sequence order numbers you wish to extract - enter each number separated by a space.You can see the sequence order alignment number by selecting the **order** option in the alignment view options panel. See image below below.
- Click OK and the extracted sequence alignment will be displayed in a separate alignment tab.
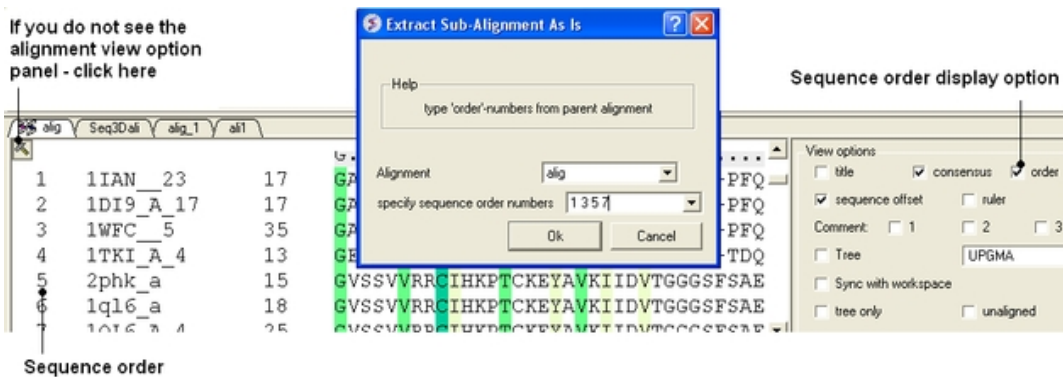
7.3.11 Cut Vertical Alignment Block

To cut a vertical alignment block:

- An initial `alignment` must first be displayed in the graphical user interface.
- Bioinfo/Cut Vertical Alignment Block
- Enter the alignment from which you wish to cut from.
- Enter the region of the alignment you wish to cut (from: to:). The easiest way to determine the region to cut is to display the ruler in the alignment. This is an option in the alignemtn view panel - see image below.
- Click OK and the cut section will be displayed in a new alignment.
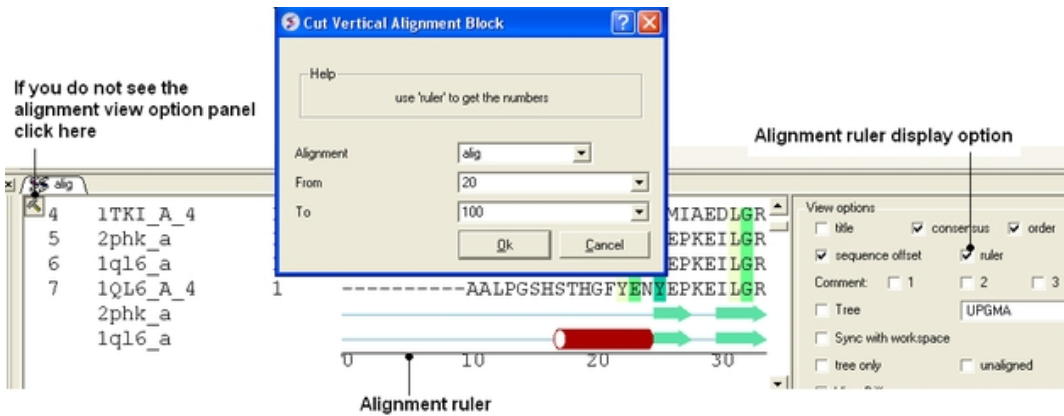
7.3.12 Reorder Sequences

To reorder sequences in an alignement

- An initial `multiple sequence alignment` must first be displayed in the graphical user interface.
- Bioinfo/Reorder Sequences
- Enter the alignment name
- Enter the new sequence order. You can see the sequence order alignment number by selecting the **order** option in the alignment view options panel. See image below below.
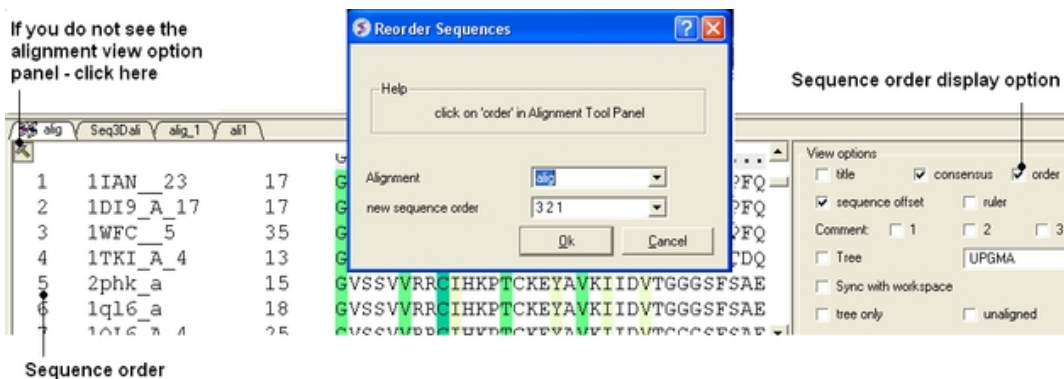
7.3.13 Extract Unique Sequences

To extract unique sequences from a group of sequences:

- `Read` into ICM the sequences you wish to make unique.
- Select the sequences. A sequence can be selected by double clicking (highlighted blue in ICM workspace) - a range of sequences in the ICM Worskpace can be selected by holding down the SHIFT button and double clicking. A non-contiguous selection can be made by holding down the CTRL button and double clicking.
- Right click on the sequence selection in the ICM Workspace and select Group sequences
- Bioinfo/Extract Unique Sequences
- Enter the name of the sequence group.
- Enter the number of residue mismatches necesary to determine that a sequence is unique or not.
- Select whether you want to keep the redundant sequences or delete them from ICM.
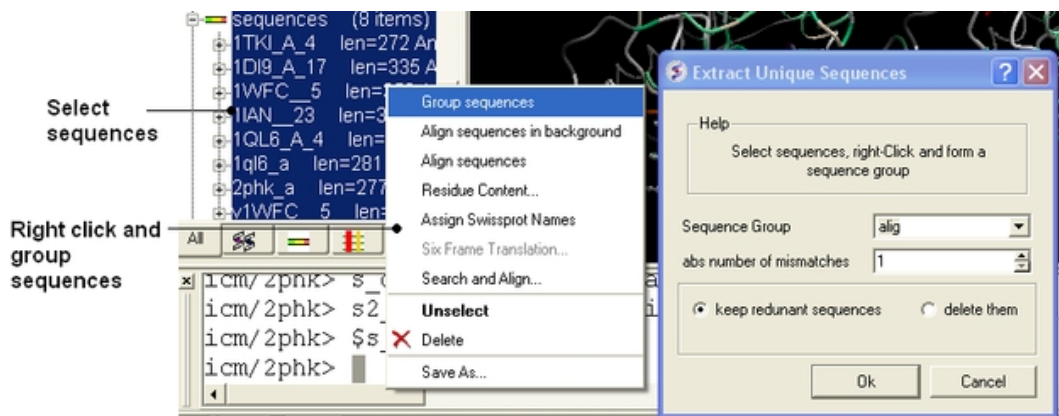
7.3.14 Load Example Alignment

To see an example of an alignment select:

- Bioinfo/Load Example Alignment

7.4 BLAST Search

Available in the following product(s): `ICM-Pro`

To BLAST the NCBI sequence database (including PDB sequences):

- Go to the Search tab
- Choose the drop down option for BLAST and follow the instructions
  `here`.

To BLAST search a local BLAST formatted file:

ICM provides a fast tool to search any Blast-formatted database with a
query sequence and generate an alignment on the fly. For example you can
use this option to find template structures for homology modeling.

1. Select Bioinfo/Search and Align (or right click on a sequence)
2. Enter the sequence name you wish to search against the database
   with.
3. Locate the blast-formatted database to search. You can download
   Blast formatted databases from here
   `ftp://ftp.ncbi.nih.gov/blast/db/` eg. pdbaa - PDB sequence
   database.
4. Specify the speed parameter (0 is the slowest, but the most
   detailed, 100 is the fastest search for nearly identical
   sequences).
5. Enter the number of hits you would like to view
6. Enter an identity threshold filter to narrow down the number of
   hits.
7. The top hits will be displayed in a table.

7.5 Sequence Editor

The sequence editor allows you to easily display and create sequence
sites. To open the editor:

- `Read` in a sequence into ICM.
- Right click on the sequence in the ICM workspace and choose
  "Sequence Editor".

To toggle site display on and off and change view options:
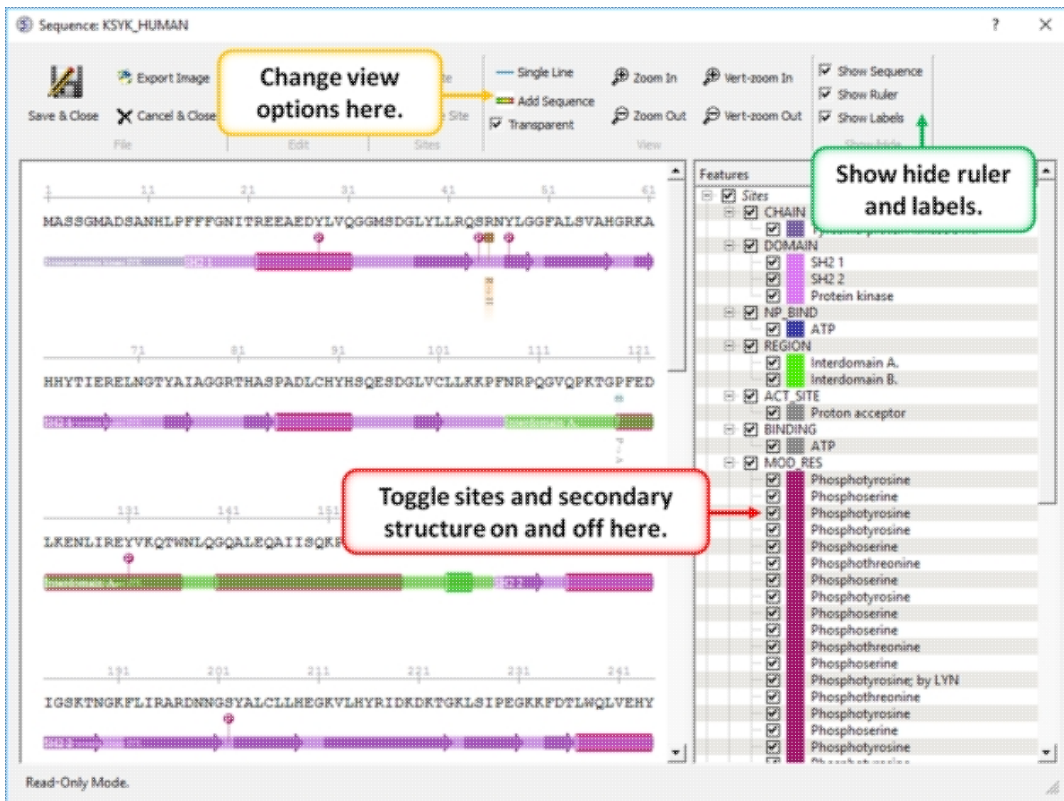
To edit a sequence or site:

To save your changes or export an image:

7.6 Sequence Alignments

Available in the following product(s): ICM-Pro

7.6.1 Default Alignment

To make an alignment using the default parameters:

How to make a sequence alignment.

1. Select the sequences you wish to align. Hold CTRL key and click.

2. Right click on the selection and choose "Align Sequences".
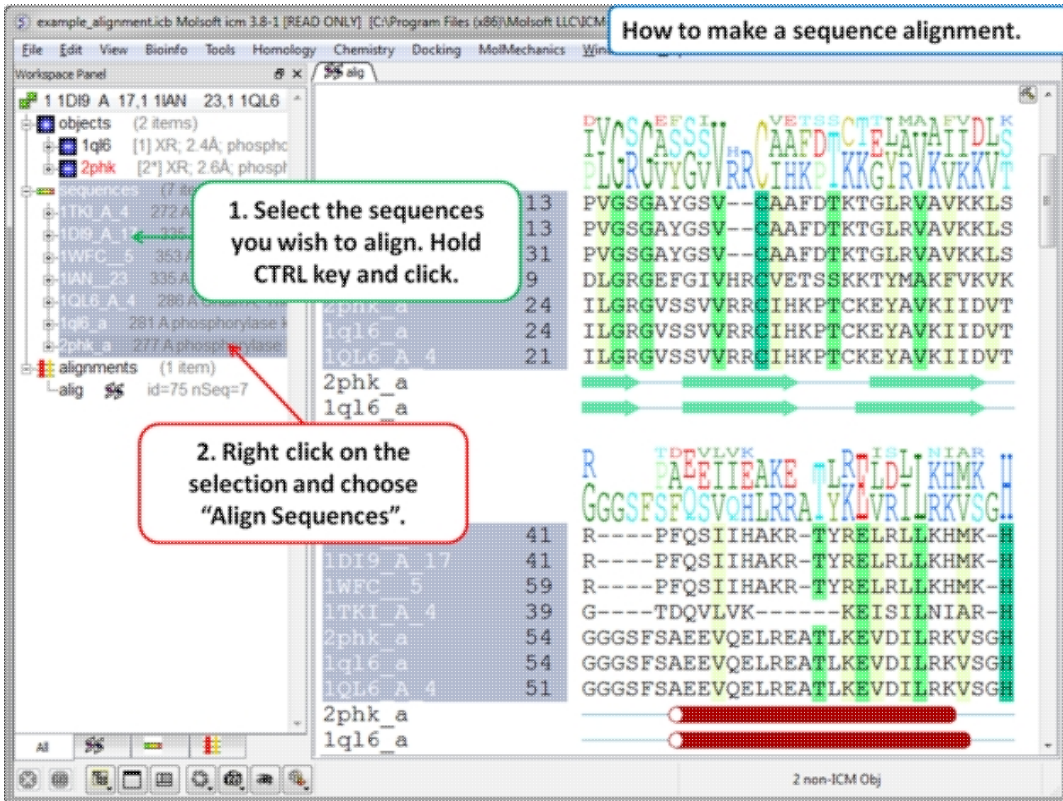
- `Read` in the sequences you wish to align.
- Select the sequences in the ICM workspace.
- Right click and choose "Align Sequences".

.

Alignment parameters (such as alignment method) can be changed using the options in the Bioinfo menu (e.g. `Align Two Sequences` and `Multiple Sequences`).

7.6.2 Align Two Sequences

To align two sequences:

- `Read` into ICM two or more sequences.
- Bioinfo/Align Two Sequences
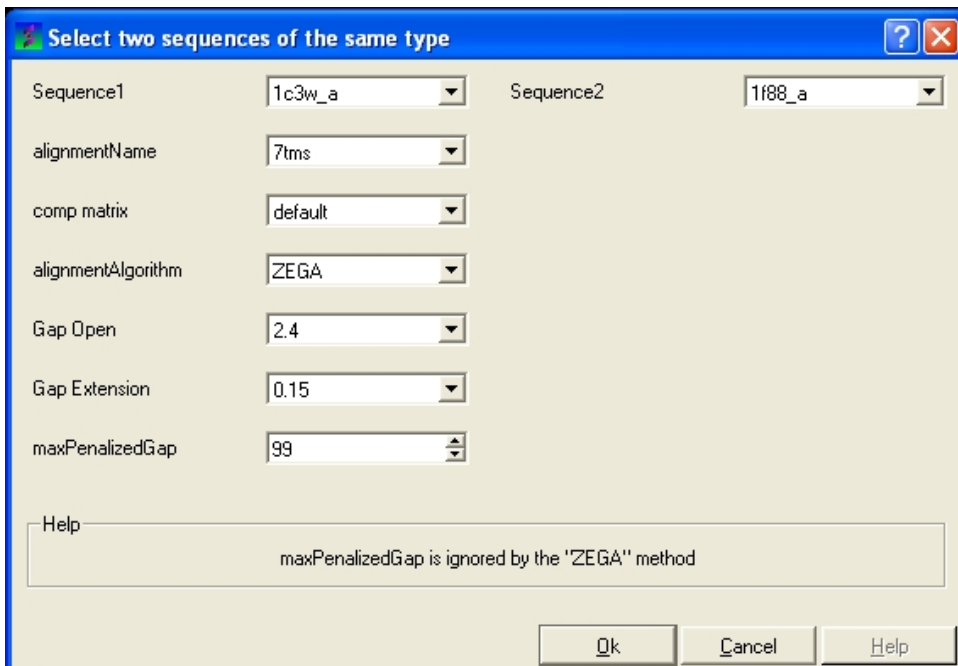
- Enter the name of your first sequence in the 'Sequence 1' data entry box.
- Enter the name of your second sequence in the 'Sequence 2' data entry box.

NOTE: Any sequences already loaded into ICM can be seen by clicking on the down arrow next to the 'Sequence 1 and 2' data entry boxes. This can save typing and trying to remember what you called your sequence.

- Enter a unique alignment name in the 'alignmentName' data entry box.
- Select a comparison matrix from the list shown below by clicking on the arrow next to the 'comp matrix' data entry box.
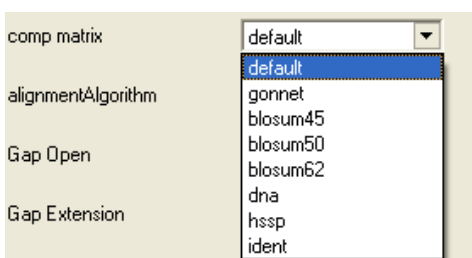
- Select the alignment algorithm you wish to use from the list shown below by clicking on the arrow next to the 'alignmentAlgorithm' data aentry box.
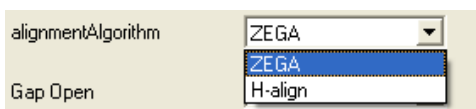
ZEGA - a Zero End-gap Global Alignment, that is a pairwise alignment method based on the Needleman and Wunsch algorithm modified to use zero gap end penalties. This type of alignment was first described by Michael Waterman, who called it the "fit" alignment. The paper of Abagyan and Batalov, 1997 describes the statistics of the structural significance of

the alignment score and optimization of the alignment parameters for the best recognition of structurally related proteins.

H-Align - alignment method used in the Align and Score functions and find database command (as described in Batalov and Abagyan, 1999)

- Enter the values you wish to use for Gap Open, Gap Extension and the maximum penalized gap penalty.

Gap Open The absolute gap penalty is calculated as a product of gapOpen and the average diagonal element of the residue comparison table You may vary gapOpen between 1.8 and 2.8 to analyze dependence of your alignment on this parameter. Lower pairwise similarity may require somewhat lower gapOpen parameter. A value of 2.4 (gapExtension=0.15) was shown to be optimal for structural similarity recognition with the Gonnet et. al.) matrix, while a value of 2.0 was optimal for the Blosum50) matrix (Abagyan and Batalov, 1997).

Gap Extension The absolute gap penalty is calculated as a product of gapExtension and the average diagonal element of the residue comparison table.

maxPenalizedGap The maximum penalized gap which is used for Gap Open and Extension

- Click OK and the alignment will be displayed in the alignment editor window at the bottom of the graphical user interface.
- Remember to save the project or write the alignment if you wish to keep the alignment for use at another time.
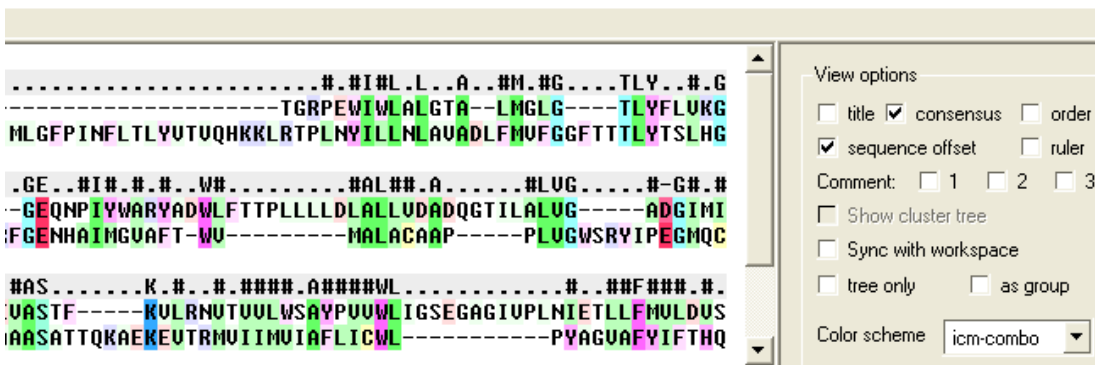
7.6.3 Sequence to Structure alignment

This option allows you to align a sequence to a template structure sequence using secondary structure weighting.

- `Read` into ICM the sequence (ModelSeq) you wish to align to the template sequence.
- Read in the template (TemplateSeq) structure and `extract the sequence` from this structure
- Bioinfo/Sequence to Structure Alignment
- Enter the ModelSeq and the TemplateSeq name
- Enter the name you wish to call the alignment.
- Enter the weights you wish to use for apha and beta secondary structure. The default values have been very well tested.
- This function uses a dynamic algorithm to find the alignment of the locally structurally similar backbone conformations. The RMSD is calculated within a certain residue window. The default is 3.
- Press OK and the alignement will be displayed in the bottom of the gui interface.

7.6.4 Align DNA vs Protein

To align DNA to protein:

- Select the 'Bioinfo' menu.
- Select the option Align DNA vs Protein
- Follow the data entry instructions shown in `the previous section entitled "align two sequences"` but enter one DNA sequence and one protein sequence.

7.6.5 Multiple Sequence Alignment

To align more than 2 sequences:

- `Read` into ICM the sequences you wish to align.
- Select the sequences you wish to align in the ICM workspace. A sequence can be selected by double clicking (highlighted blue in ICM workspace) - a range of sequences in the ICM Worskpace can be selected by holding down the SHIFT button and double clicking. A non-contiguous selection can be made by holding down the CTRL button and double clicking.
- Bioinfo/Multiple Sequence Alignment
- Enter the name of the sequence group. If you selected the sequences as described above then the name of the group is **selection.** Other named groups of sequences can be made by right clicking on the sequence selection.
- Select the comparison matrix you would like to use.
- Enter Gap open and extension values.

Gap Open The absolute gap penalty is calculated as a product of gapOpen and the average diagonal element of the residue comparison table You may vary gapOpen between 1.8 and 2.8 to analyze dependence of your alignment on this parameter. Lower pairwise similarity may require somewhat lower gapOpen parameter. A value of 2.4 (gapExtension=0.15) was shown to be optimal for structural similarity recognition with the Gonnet et. al.) matrix, while a value of 2.0 was optimal for the Blosum50) matrix (Abagyan and Batalov, 1997).

Gap Extension The absolute gap penalty is calculated as a product of gapExtension and the average diagonal element of the residue comparison table.
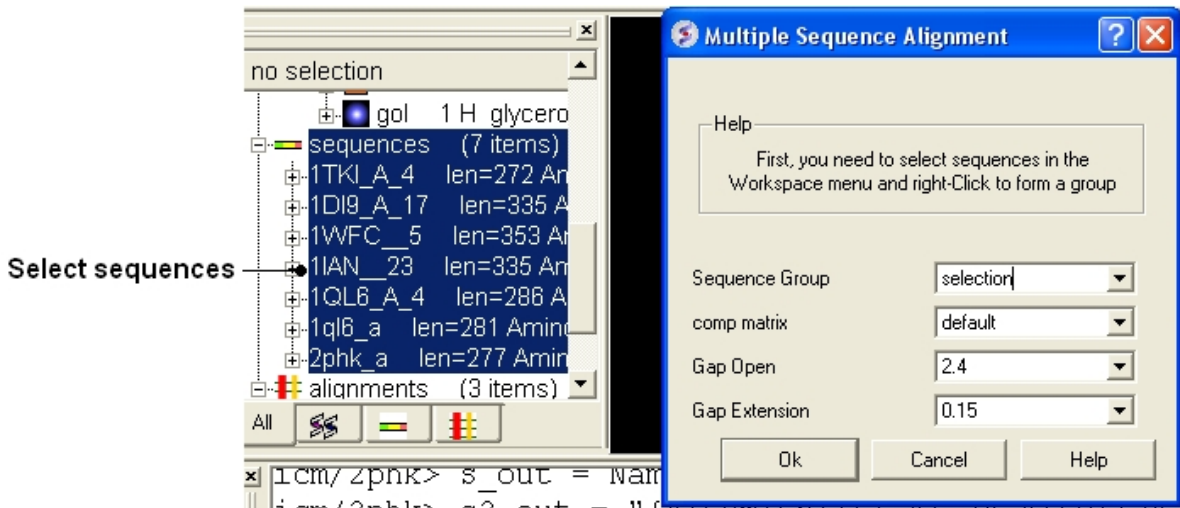
7.6.6 Drag and Drop

An easy way to add another sequence to an alignment is to drag and drop
a loaded sequence from the ICM workspace panel to the alignment window.
The sequence automatically becomes part of the alignment.
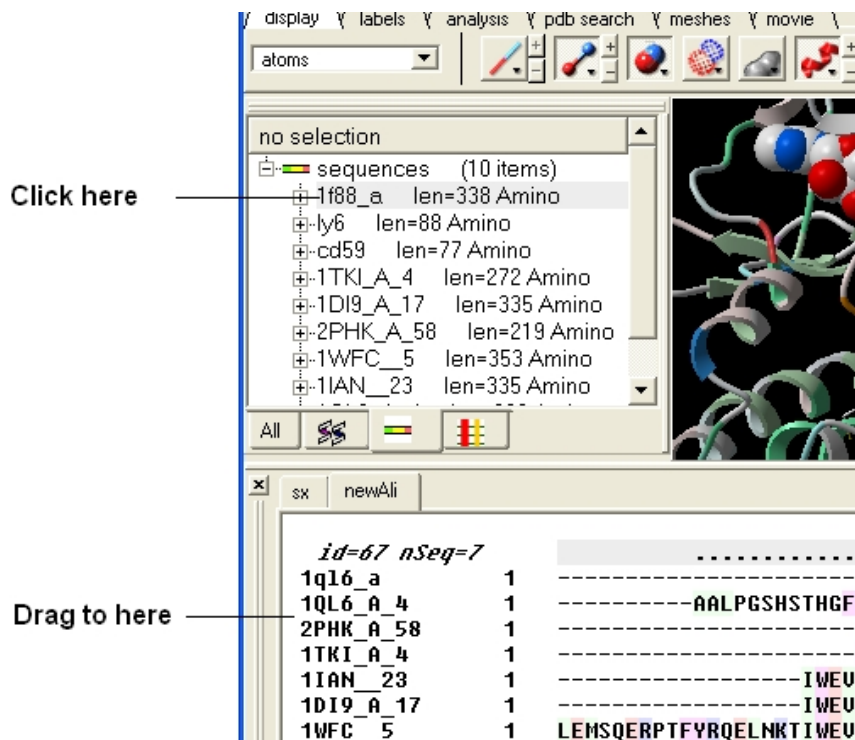
7.7 Alignment Editor

Available in the following product(s): ICM-Pro

The default position for the alignment editor is at the bottom of the
graphical user interface. If you have made an alignment and you cannot
see the alignment you can select Window/Alignments (See Window Menu
section of this manual) and it will be displayed.

ICM has an easy to use editor for pairwise and multiple alignments. ICM alignment editor is robust and always protects the integrity of your alignment by protecting you from making unintended changes in the alignment.

> **NOTE:** To increase or decrease the size of the font in the Alignment Editor press the CTRL key and the '+' or '-' keys.
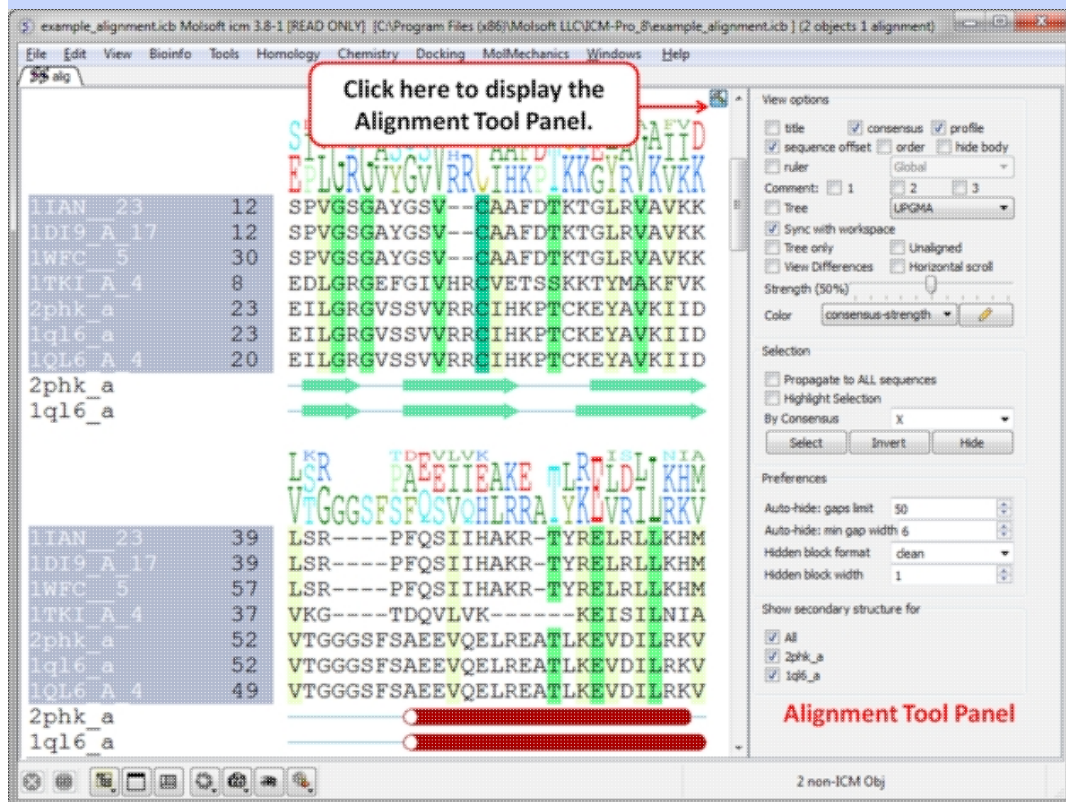
7.7.1 Edit an Alignment

To edit an alignment one only needs four types of operations:

- select a block with one or several sequences to be moved (press Ctrl to add blocks). **Important:** since you can only move the selection **to the gapped space** , the moving front of the selection must be next to the gaps.
- (optional) create space on both sides around a vertical section of the alignment
- use the keyboard *arrows* to move the selected block with respect to the other sequences
- squeeze out the excessive gaps (an item in the alignment popup menu)

OPERATION	KEYS
set a vertical selection for ALL sequences in the alignment	**Double-Click**
add white space by hitting the Space bar	**SpaceBar**
remove white space	**Backspace**

| select a sub-block for shifting | **Drag**
Left-Mouse-Button |
| shift the selected block next to a gapped area | **Right and Left Arrows** |

7.7.2 Save Alignment Image

To save your alignment as an image file:

- Right click on the alignment and select the Save/Export image option.
- Choose the option save as image.
- Enter the filename you wish to call your alignment. We advice you to keep the .png file extensions.
- Select the style you desire from full-width, as is, or 60.
- Select the resolution for the image. We recomend 3.0.
- Select the browse button if you wish to save the picture in a directory other than the one you are running ICM in. If you decide to change directories you will have to reenter the desired file name and click ok. The path of the file will then be entered in the save options data entry box.
- Click OK.

To save your alignment to the clipboard:

- Right click on the alignment and select the Save/Export image option.
- Choose the Save to clipboard option.

7.7.3 Save Alignment

To save an alignment or tree:

- Right click on the alignment or tree and a menu will be displayed.
- Select the save as option.
- In the windows "Save as Type" dialog box you can choose icb (ICM format), ali or FASTA.

7.7.4 Print Alignment

To print an alignment or tree:

- Right click on the alignment or tree and a menu will be displayed.
- Select the print option.

7.7.5 Delete Alignmnet

To delete an alignment or tree:

- Right click on the alignment or tree and a menu will be displayed.
- Select the delete option.

7.7.6 Annotate Alignment

To annotate an alignment:
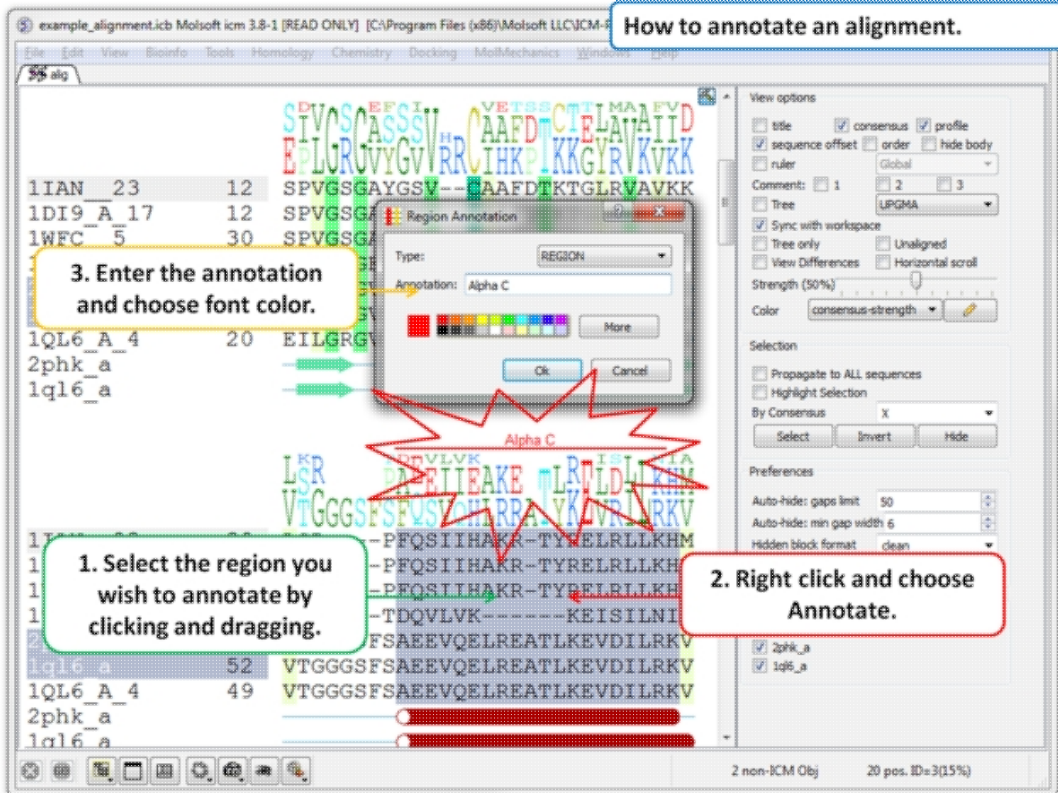
- Click and drag over the region of the alignment you wish to annotate. The selected region will be highlighted in blue.
- Right click on the blue selected region and select 'Annotate'.
- Enter your comment text and color of font.
- Unselect by clicking away from the selection.

To Edit an annotation:

- Right click on the annotation and choose **Edit**}

To Delete an annotation:

- Right click on the annotation and choose **Delete**}

7.7.7 Box, Shade, Change Font

To draw a box, shade or change the font around a segment of the alignment:
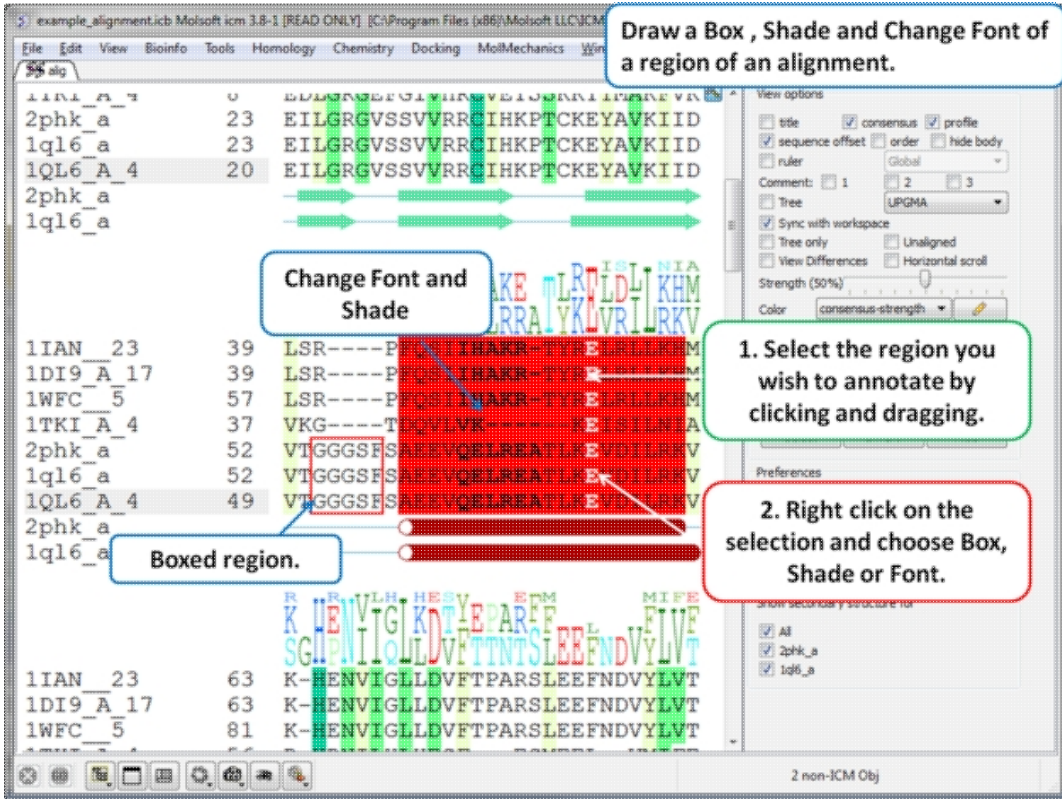
- Click and drag over the region of the alignment you wish to box or shade. The selected region will be highlighted in blue.
- Right click on the blue selected region and select either font, shade or box.
- Unselect by clicking away from the selection.

7.7.7.1 Undisplay box, font or shading

To undisplay a box, font or shading:

- Click and drag over the boxed or shaded region of the alignment.
- Right click and choose the option "Clear Font/Color/Border".

7.7.8 Phylogenetic Trees

NOTE: Before constructing a phylogenetic tree you need to align the sequences as described in the `alignment section`

To view a tree:

- Check the 'Tree' option in the alignment editor.

The tree will be displayed in the editor as shown below:
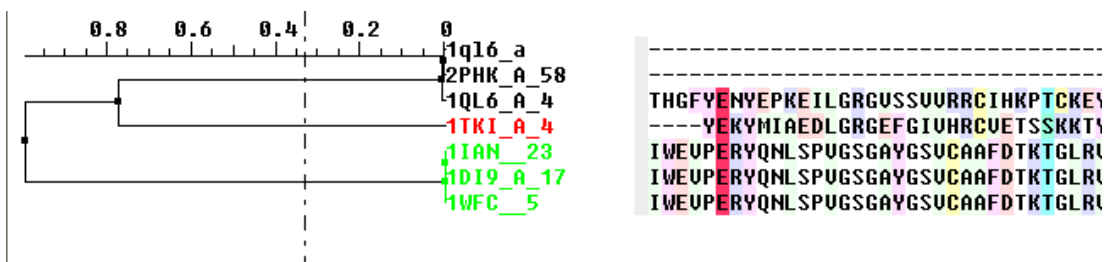
To display the tree alone without the alignment:

- Check the 'tree only' option in the alignment editor.

Tree functionality:

- the tree-section in the alignment is **resizeable**, just grab the rightmost end of the top ruler and drag it
- **branch swapping** : the sequences can be reordered by swapping the tree branches. Just do the following:
  - ♦ **Right-click** on a tree-node to get a popup-menu
  - ♦ **swap** the branches
- **selecting a branch**: double click on a tree-node to select the sequences belonging to it.

7.7.9 Coloring an Alignment.

How to color an alignment:

There are a wide range of default coloring schemes.

- Click on the drop down arrow beside the "Color" data entry box and a number of color schemes will be displayed.
- Select the color scheme.

NOTE: The colors are shaded from pale to bright where the brighter color represents higher conservation at that point in a multiple alignment.

To view the alignment color key:

- Click the pencil icon next to the color scheme selection tools and a table will be displayed.

To edit the alignment color key:

- Click the pencil icon next to the color scheme selection tools and a table will be displayed.
- Click on the "Edit Cells by Double Click" button on the right hand side of the table.
- Double click on a color panel in the table to change the color.

To color by strength of consensus:

To color your multiple alignment by the strength of consensus at each point in an alignment:

- Choose the Color option "Consensus Strength"
- Click and drag on the consensus strength button shown below:
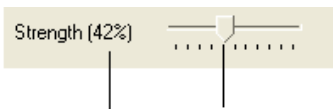
7.7.10 Alignment View Options

The alignment view options are located on the right hand side of the alignment editor.
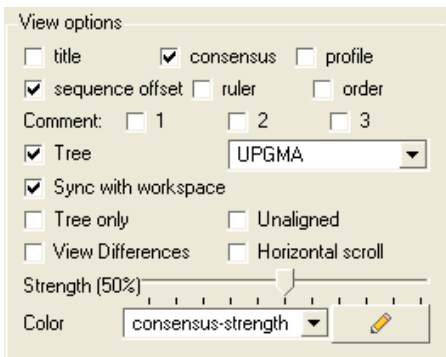
To add or remove the alignment title:

- Check the title box in the view options.

To rename an alignment:

- Right click anywhere in the alignment or on the alignment tab and a menu will be displayed.
- Select the 'Rename' option.
- Type the new name for your alignment in the data entry box which becomes activated in the ICM workspace (See below).
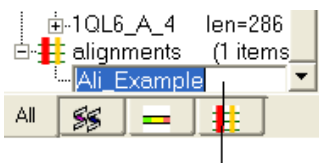
To add or remove the alignment consensus display.

- Check the box labeled 'consensus' in the view options.

`id=67 nSeq=7        ....%-.%.....%g.g....%..%%...t...%%%+.%.......%.%.. . %+E#.#L+`

The alignment consensus line

If you have a large alignment it may be convenient to show the number of each sequence

To number your alignment:

- Check the 'order' box in the view options.

```
 id=67 nSeq=7
1    1q16_a
2    2PHK_A_58
3    1QL6_A_4
4    1TKI_A_4
5    1IAN__23
6    1DI9_A_17
7    1WFC__5
```

Alignment order number
displayed here

Horizontal Scroll

To view the alignment in **Horizontal scroll** click on the "Horizontal scroll" button in the **View Options** panel in the Alignment tool bar.

To view the sequence offset number for each of your sequences in an alignment:

- Check the 'offset' box in the view options.

```
                     ..K.
1q16_a          106  TEKU
2PHK_A_58        46  TEKU
1QL6_A_4        113  TEKU
1TKI_A_4         91  TSAF
1IAN__23        103  CQK-
1DI9_A_17       103  CQK-
1WFC__5         121  CQK-
```

Sequence offset number

To view the sequence ruler:

- Check the 'ruler' box in the view options.

```
------------------IWEUPERYQNLSPUGSGAYGSUCAAF
------------------IWEUPERYQNLSPUGSGAYGSUCAAF
LEMSQERPTFYRQELNKTIWEUPERYQNLSPUGSGAYGSUCAAF
0         10        20        30        40
```

Alignment ruler

To view the sequence consensus.

- Check the consensus option in the view options.
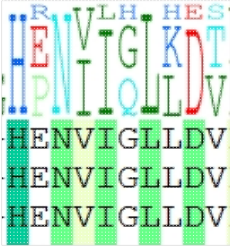
To view secondary structure.

If one of the sequences of the alignment is linked to a structure then you can display the secondary structure by:

- Check the "show secondary structure for" box.

The secondary structure will be displayed at the bottom of the alignment. The color code is: red cones = alpha helix, green arrows = beta sheet, blue cones = pi helix, magenta cones = 3/10 helix.
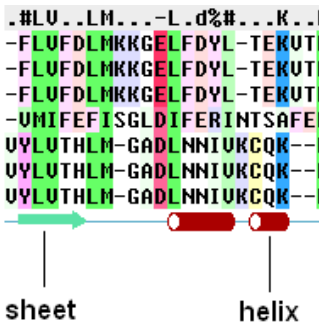
7.7.11 Alignment Gaps

To make an alignment clearer you may wish to HIDE gap regions.

To hide all gap regions:

- Right click on the alignment and a menu as shown below will be displayed.
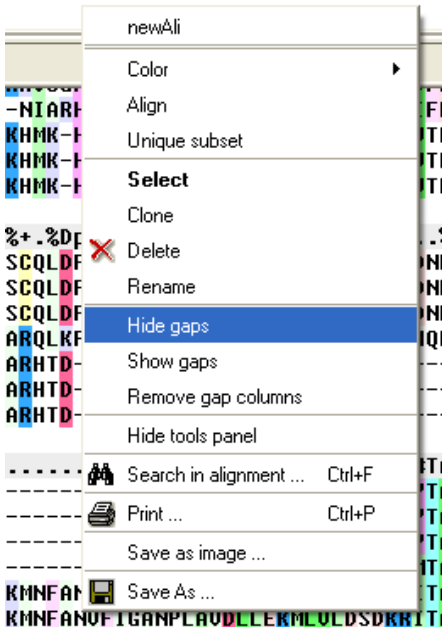
- Select the "Hide gaps" option.

The gaps in your alignment will be hidden according to the preference made in the alignment tools panel shown below. Click on the drop down arrow in the "Hidden block format" data entry box.

Two parameters can be specified directly from the Tools Panel in the alignment window:

1. the "Hidden Block Format can use the following special symbols:
   - ♦ %l number of hidden chars
   - ♦ %L length of the hidden block
   - ♦ %f hidden from
   - ♦ %t hidden to
   e.g. " %f .. %t " or " %L "
2. the "Hidden block width" which defines the total length of the hidden section.
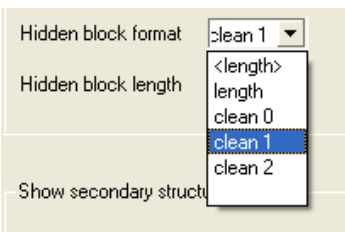
Some predefined hidden block formats are shown here:

length: displays the length of the gap

length: displays the length of the gap in .

clean0: displays no indication of a gap

clean1: displays grey panel in the gap position.

clean2: displays a wider grey panel in the gap position

> **NOTE:** The width of the hidden panel can be changed as shown below.

Enter length of hidden block here

If you have hidden all the gaps individual gaps (or blocks) can be displayed by:

- Right clicking on the gap and select "Show hidden block" option.

To show all gaps:

- Right click on the alignment away from a gap region and a menu will be displayed.

- Select the "Show gaps option.

7.7.12 Searching an Alignment

If you have a large alignment and you wish to find a specific group of amino acids within that residue the you can use the Alignment search tool.

- Right click on the alignment away from any hidden gaps and a menu will be displayed.
- Select the "Search in alignment option" and a data entry box as shown below will be displayed.
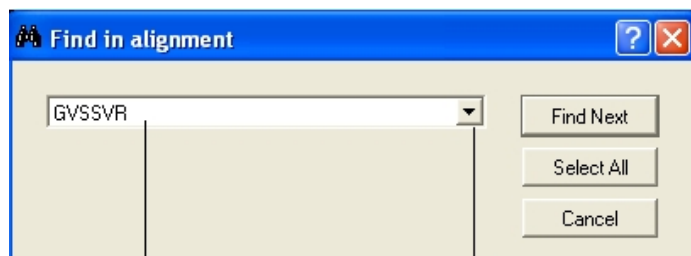
NOTE: Another way of searching an alignment is to use the alignment selection tools which are linked to the ICM workspace and 3D graphical window. This is described in the section entitled `Making Selections in Alignments`.

7.7.13 Making Alignment Selections

ICM has a very powerful alignment selection tool which enables sequences and structures to be interlinked with the 3D graphical window, the alignment window and the ICM workspace.

Selection is displayed in the ICM workspace (blue) the alignment window (blue) and the 3D graphics display (green cross).

To select a single column of an alignment:

- Double click.

One column selected by double clicking

To select parts of an alignment:

- Click and drag over the region you wish to select.

Click and drag over to select

To select multiple discontinuous parts of an alignment:

- Click and drag whilst holding down the control key.

Click and drag whilst holding down the
control key.

To enable the easy selection of all sequences in an alignment:

- Check the box labeled "Select by mouse in all sequences".
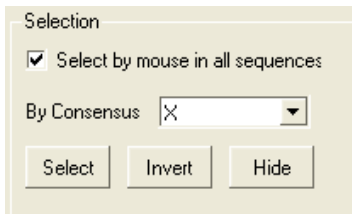
All sequences are selected
using the mouse

> **NOTE:** All selections made in the alignment window are linked to the
> 3D graphics window and the ICM workspace if a structure is in the
> alignment.

7.7.14 Select by Consensus

This is a very useful tool, for example, you may want to color the
consrved regions of your structure in the 3D display windowa different
color to the rest of the structure. This tool allows you to select the
conserved regions in the sequence alignment. Once the selection has been
made it can be used for a number of different ICM operations such as
`coloring` and `displaying secondary structure`.

A selection can be made based on the alignment consensus. The buttons relating to this are in the alignment tool panel.
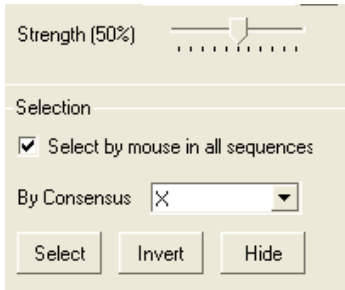
Before selecting by consensus you first need to define a consensus strength:

- Click and drag on the bar labelled "Strength" and select your desired percentage.
- Enter which elements of the consensus you wish to select separated by comma. Refer to the language manual for definition of each consensus symbol.

Enter which elements of the consensus you wish to select seperated by a comma.

- Click the Select button and your selection will be highlighted in blue in the alignment windown and ICM workspace and as green crosses in the 3D graphical display window.

Once the selection has been made it can be used for a number of different ICM operations such as `coloring` and `displaying secondary structure`.

To invert a selection:

- Click on the invert button.

To hide a selection.

- Click on the Hide button

> **NOTE:** All selections made in the alignment window are linked to the 3D graphics window and the ICM workspace if a structure is in the alignment.

7.7.15 Calculate Sequence Identitiy and Similarity

To calculate the sequence identity and similarity between one sequence and other sequences in an alignment:

How to calculate sequence identity and similarity.
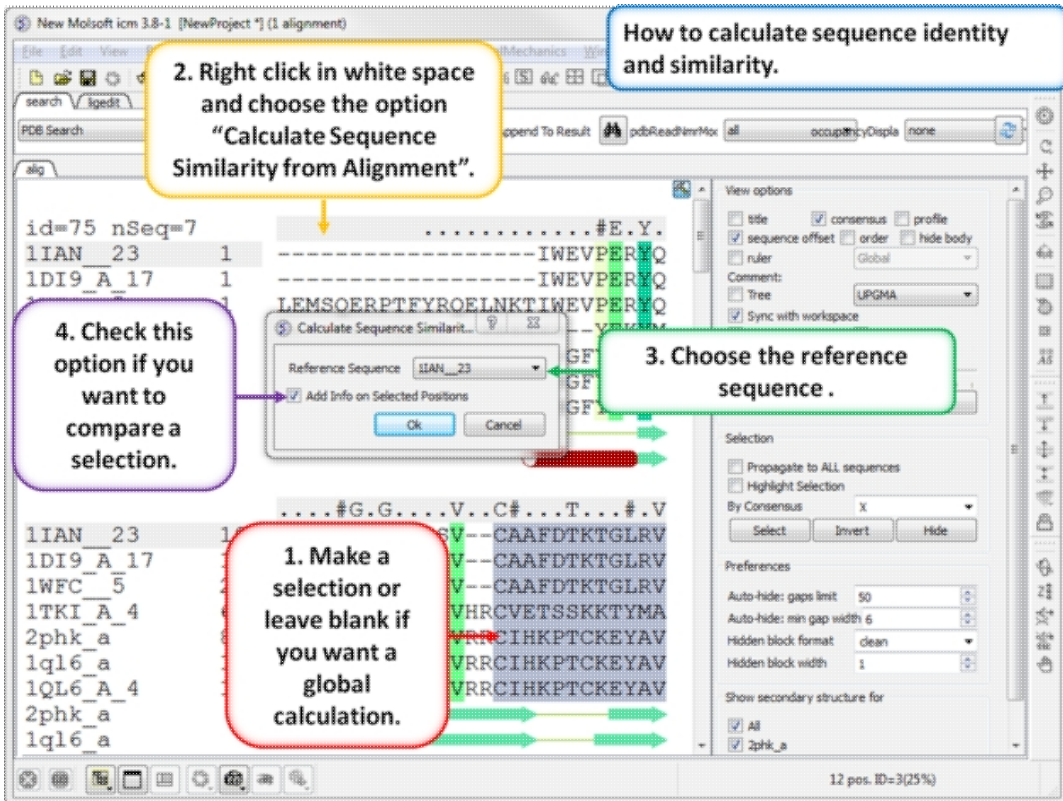
- Make an alignment.
- If you wish to calculate local sequence identity you need to select the region of interest.
- Right click in white space in the alignment and choose the option "Calculate Sequence Similarity from Alignment".
- Choose the reference sequence from the dialog box.
- If you wish to calculate local sequence identity you need to select the option "Add info on Selected Positions".

A table of sequence similarity and identity will be displayed. A guide
to the columns in the table:

- **Ref** = name of reference sequence.
- **Seq** = name of comparison sequence.
- **Ident** = identity percentage
- **Sim** = similarity percentage
- **i** = order
- **IDSel** = identiy percentage for selection
- **SimSel** = similarity percentage for selection
- **p..** = residues in selection.

7.7.16 Alignment Secondary Structure

If the one or more of the sequences contain secondary structure
annotation then you will see colored cylinders and arrows under the
alignment. Red Cylinder = alpha helix, green arrow = beta sheet, blue
cylinder = pi helix, magenta cylinder = 3/10 helix.

The colored cylinders and arrows under the sequence alignment refer to secondary structure.
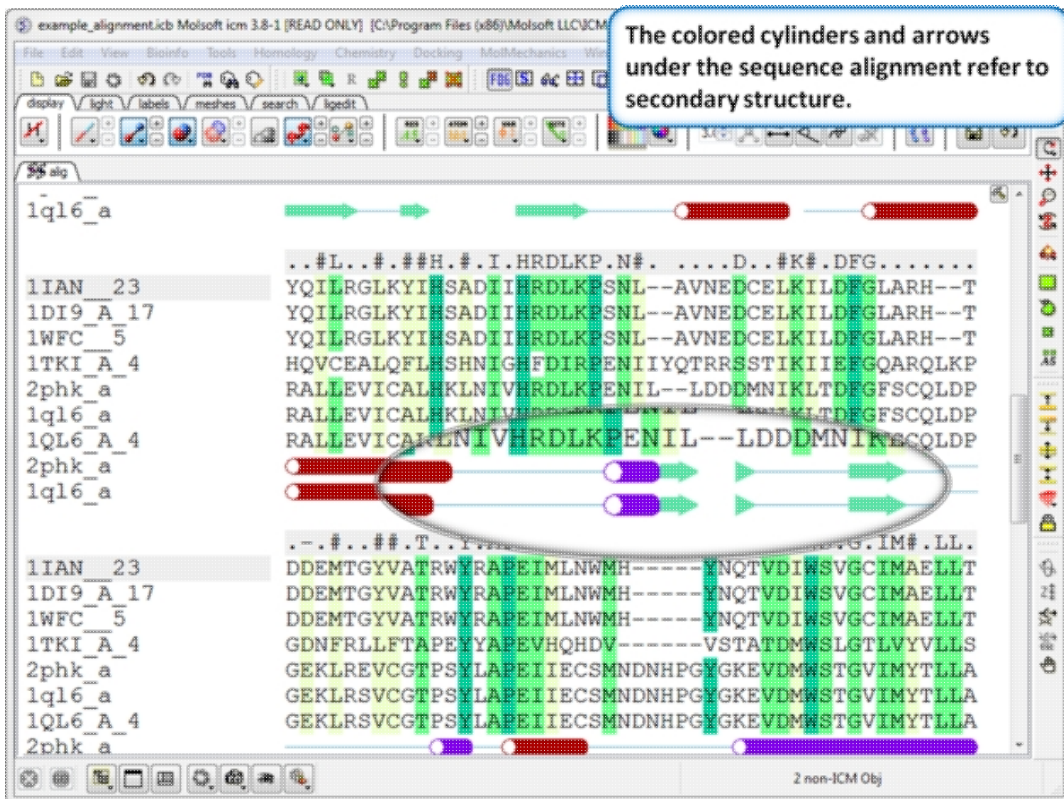
Use the alignment tool panel to toggle the secondary structure display on and off.

To toggle secondary structure display on and off use the options in the alignment tools panel.

1. Click here to see the tools panel.

2. Toggle on and off
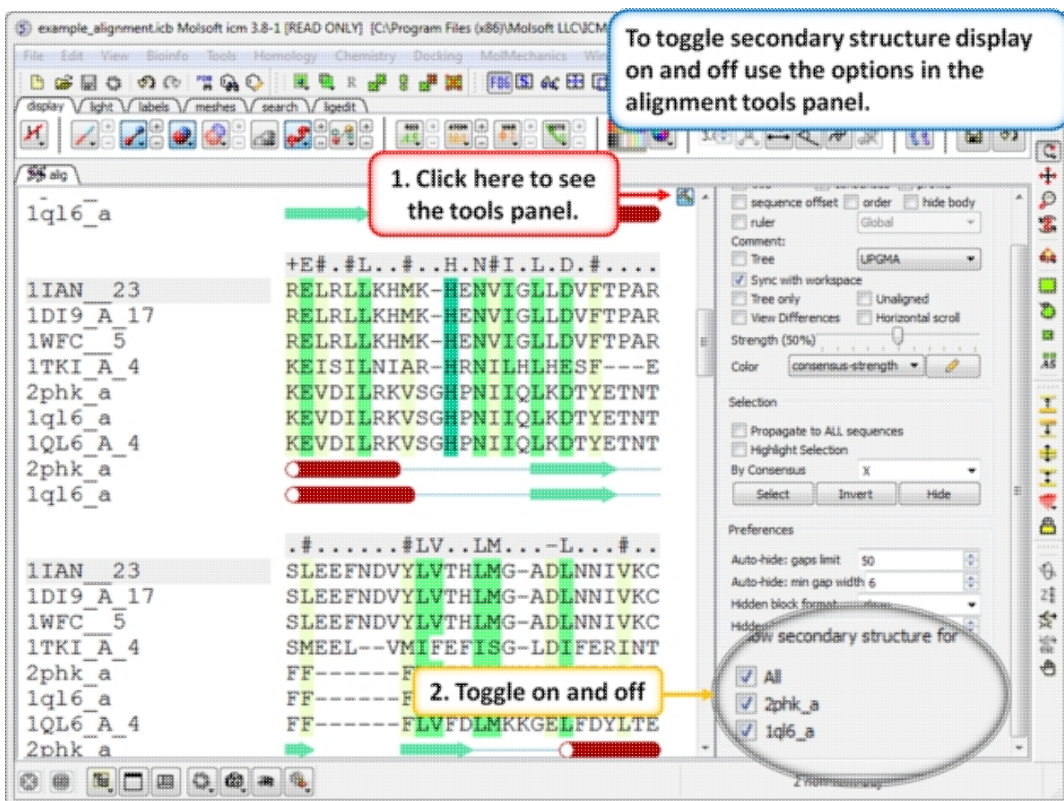
7.8 Color Structure and Surface by Alignment

To color your protein molecule by the color of the alignment e.g. color by conservation.

- `Extract` the sequence from the PDB structure.
- `Make` the alignment.
- `Color` the alignment.
- Propagate the alignment coloring to the 3D structure by clicking and holding on the representation button (e.g. ribbon) in the display tab. Select Color by Alignment.
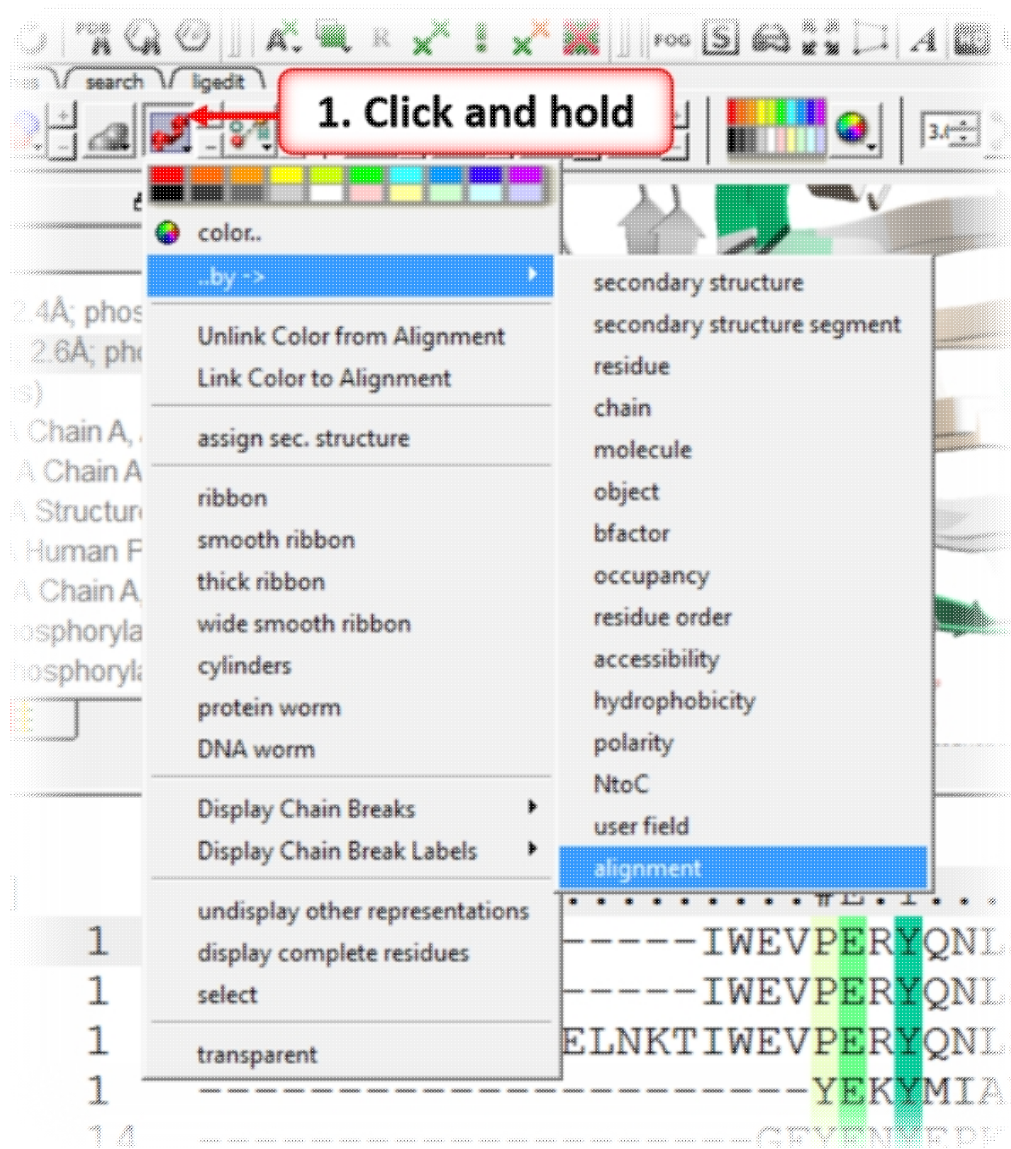
To color a surface by the alignment conservation.

- Make a `plain solid surface` using the meshes tab.
- Make a selection in the alignment by using the options in the Alignment Editor panel and select `by consensus`.

- Right click on the surface in the ICM workspace and choose Color
  by Selection.

8 Tutorials

Graphics Tutorials		
Molecule Representation, Coloring, Labeling and Annotation	HTML	
Creating Fully Interactive Slides for PowerPoint and the Web	HTML	`Reference Guide| Webinar`
Sequence and Alignment Tutorials		
Read Sequences and Align	HTML	`Video`
Link Sequence to Structure	HTML	`Video`
Alignment Annotation	HTML	
Protein Structure Tutorials		
PDB Search	HTML	
Convert PDB to ICM Object	HTML	
Superimpose Proteins	HTML	
Structure Analysis	HTML	
Calculate contacts	HTML	
Display hydrogen bonds	HTML	
Identify ligand binding pockets	HTML	
PDB Preparation: Symmetry	HTML	
PDB Preparation: Occupancy & B-Factors	HTML	
PDB Preparation: Alternative Orientation	HTML	
PDB Preparation: Biomolecule	HTML	
Protein Modeling Tutorials		
Building an Homology Model	HTML	
GPCR Modeling Example	HTML	
Loop Modeling	HTML	
Predicting the Effect of a Mutation on Binding	HTML	
Predicting the Effect of a Mutation on Stability	HTML	
Cheminformatics Tutorials		
Chemical Sketching using the Molecular Editor	HTML	`Reference Guide`
Chemical Search	HTML,	`Refrence Guide`
Chemical Clustering	HTML,	
Working with Large Chemical Space	HTML,	
3D Pharmacophore Search	HTML	`Video`
2D Pharmacophore Search	HTML	`Video`
How to Create a Markush Structure	HTML	`Video`
How to Enumerate a Markush Library	HTML	`Video`
How to Decompose a library based on a Markush Library	HTML	`Video`
How to Enumerate a library by reaction	HTML	`Video`
Detecting Activity Cliffs	HTML	
Atomic Property Fields (APF)		
APF Superposition, Score, Consensus, Screening and Clustering.	HTML	
Ligand Editor Tutorials		
Working with the ICM Interactive 3D Ligand Editor	HTML	Reference Guide.
Ligand Docking Tutorials		
Dock Biotin to the Streptavidin Receptor	HTML	`Video`
Re-Dock an Inhibitor to Ricin Crystal Structure	HTML	`Video`

Covalent Docking	HTML	`Video`
Protein-Protein Docking Tutorial		
Protein-Protein Docking using FFT Method	HTML	
Virtual Screening Tutorials		
Virtual Ligand Screening to Ricin Receptor	HTML	
Virtual Ligand Screening to Cyclooxygenase	HTML	`Video-1`| V`ideo-2`
Docking a Markush Library	HTML	
Induced Fit Docking Tutorials		
Multiple Receptor Conformation Ensemble Docking Example	HTML	
Explicit Group Docking	HTML	
QSAR Tutorials		
2D FingerPrint QSAR Model	HTML	
2D 3D Pharmacophore QSAR Model	HTML	

8.1 Graphics Tutorials

Introduction

The following examples are focused on the structure of the kinase catalytic domain. The catalytic domain is nestled between the N- and C-terminal and has high sequence conservation between kinase families. The adenine moiety of ATP interacts with the hinge region which links the N- and C-terminals of the catalytic domain. The ATP sugar binding region is on the "floor" of the pocket where ribose makes a hydrogen bond with a polar residue. A conserved lysine residue on the beta3 strand is part of the "roof" of the pocket where the triphosphate group of ATP binds. A flexible glycine-rich loop moves in and out of the pocket depending on the ligand bound state of the PK and is regulated to some extent by the movement in and out of the pocket by the AlphaC helix. A buried region at the "back" of the pocket is protected by a "gatekeeper residue" forming a variable hydrophobic cavity. The hydrophobic cavity along with the `DFG motif` region are of interest for drug design because it opens up regions of the pocket which are not conserved and do not bind ATP.

To learn the basics of the graphical user interface we will annotate the key structural regions of a protein kinase.
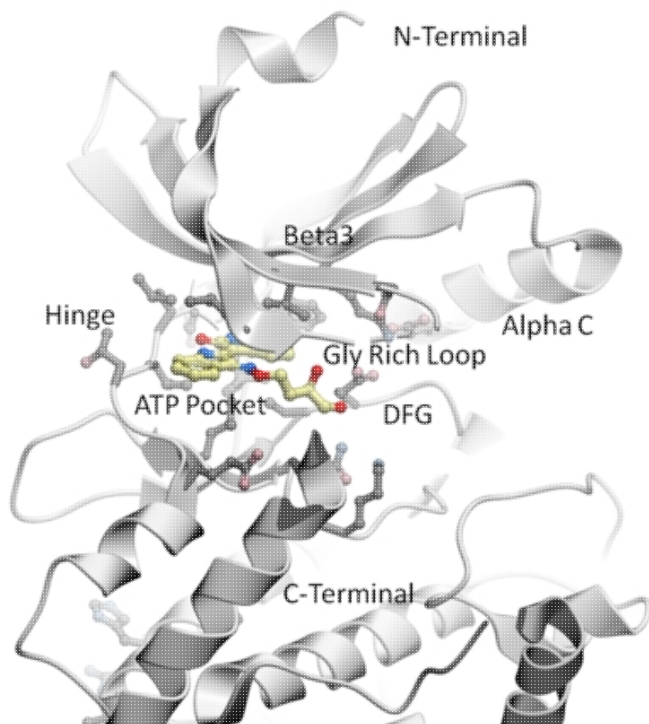
8.1.1 Change Molecule Representation and Color

This tutorial shows you how to:

- How to load a PDB structure into ICM.
- How to change the background color.
- How to display and undisplay a molecule.
- How to color ribbon representation.
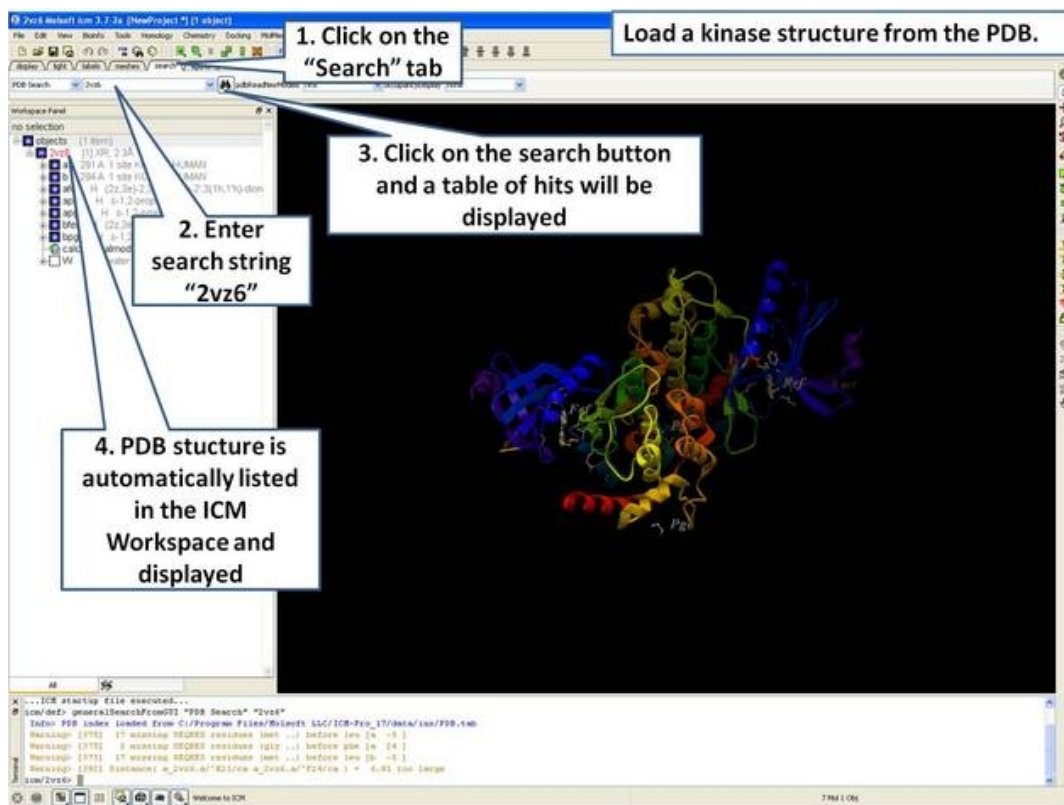
Step 1: Click on the search tab to load and display the PDB structure 2vz6
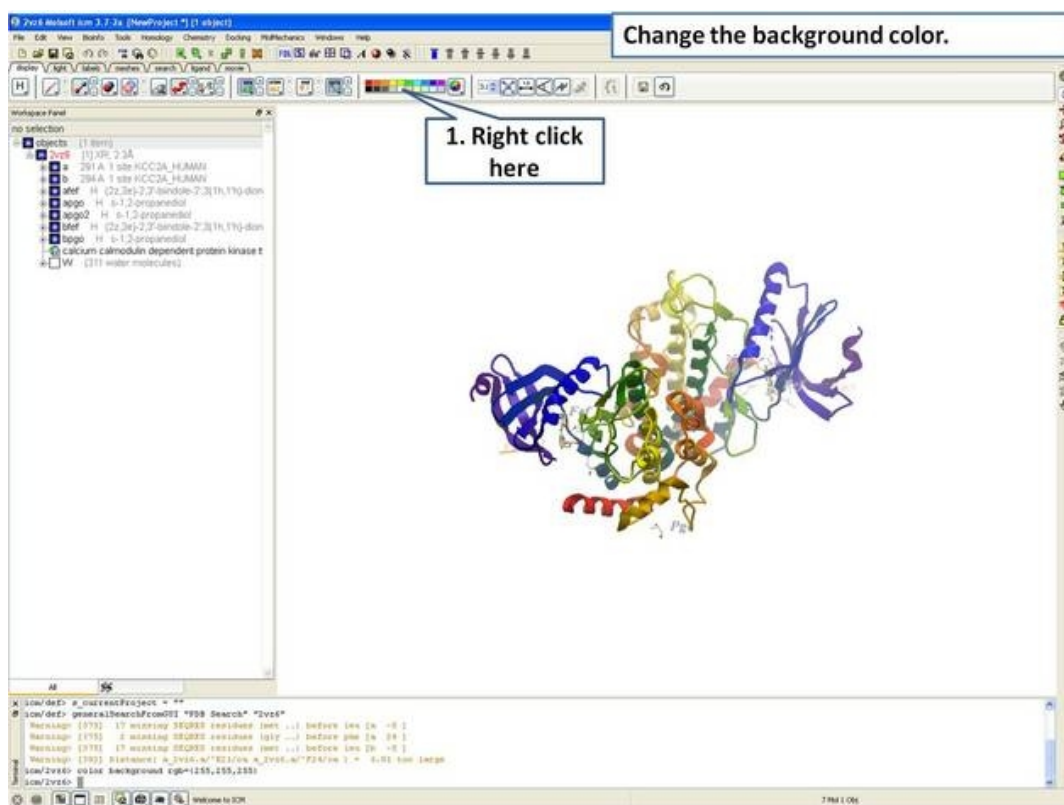
Step 2: Change the background color by right clicking on the color palette.
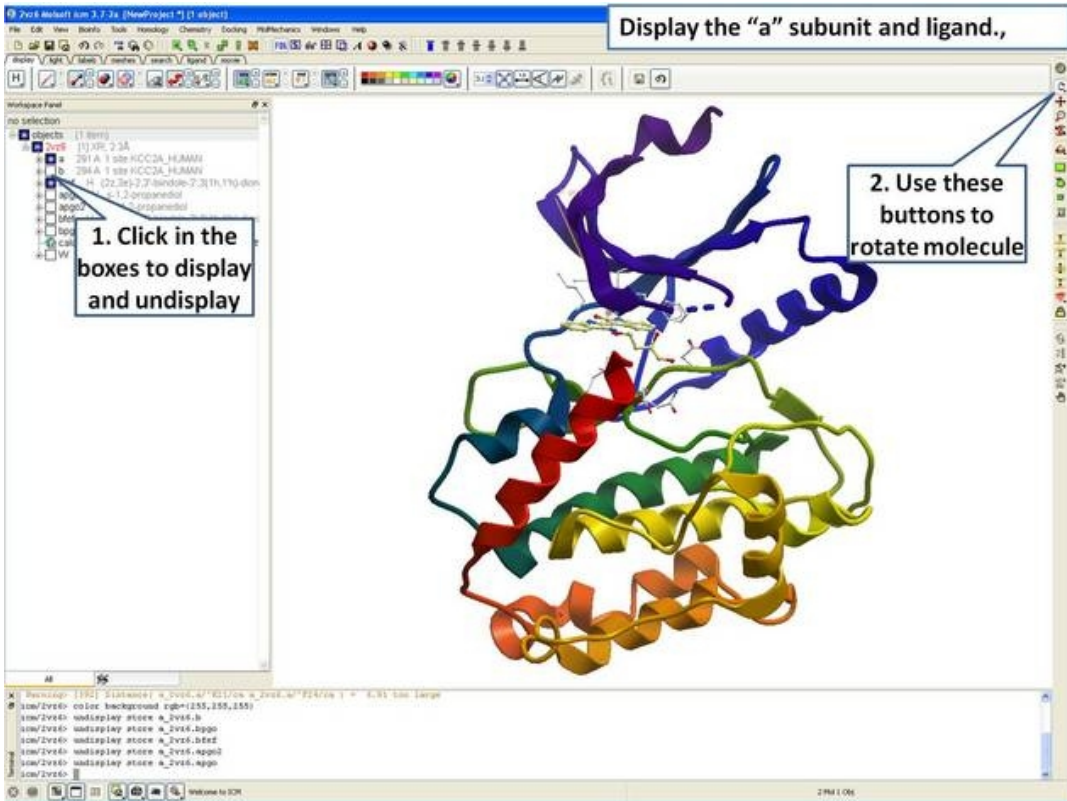
Step 3: Display the "a" subunit of the kinase.
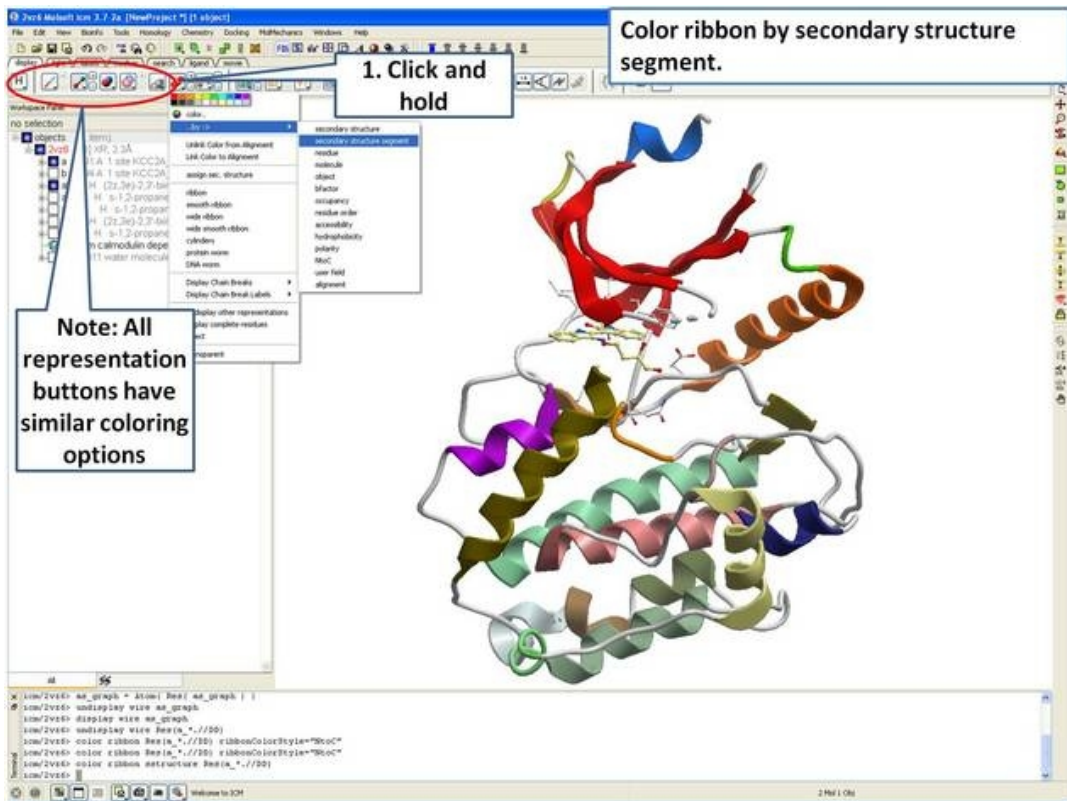
Step 4: Use the options in the "Display Tab" to color the ribbon.

Color Beta 3 strand yellow.

Step 5: Select the beta 3 strand (residues 37:43) and color yellow.

8.1.2 Annotation

This tutorial shows you how to add user defined annotation to a particular region of a protein structure.

Annotate the Alpha C helix.

4. Use middle mouse to move

5. Left click to change level of detail

2. Right click and select "Annotate Selection"

1. Use the lasso to select the helix

3. Enter annotation
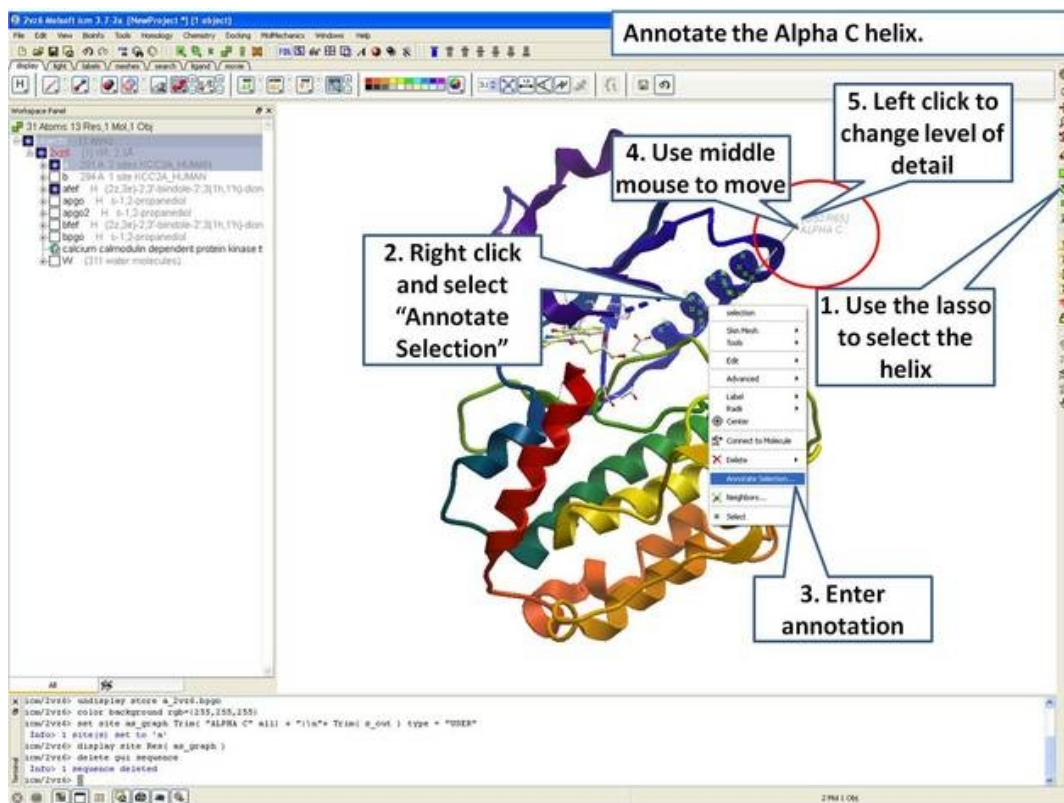
Step 6: Select the region you wish to annotate and right click on the selection for options.

8.1.3 Labels

This tutorial is a continuation from the previous tutorials in this section and shows you how to:

- How to select individual residue types.
- How to propagate a selection from one atom to all.
- How to use the residue label button.
- How to make a spherical selection.
- How to label a residue selection.
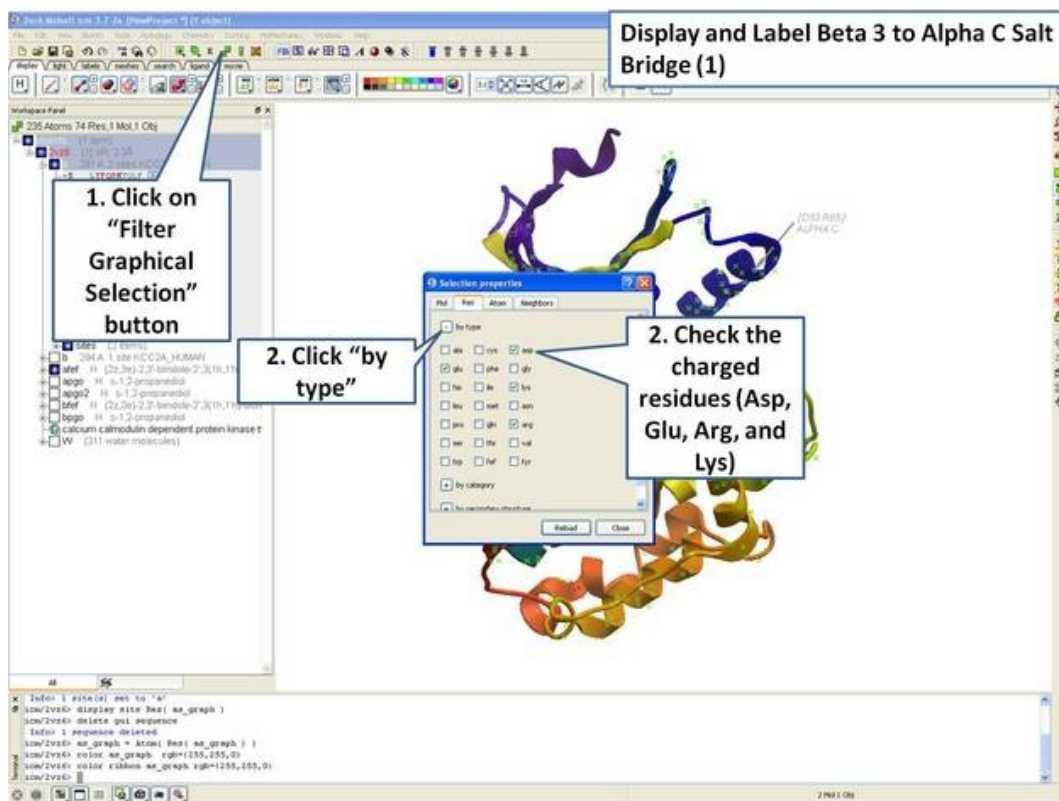
Step 7: Use the "Filter Graphical Selection" button to select charged residues.
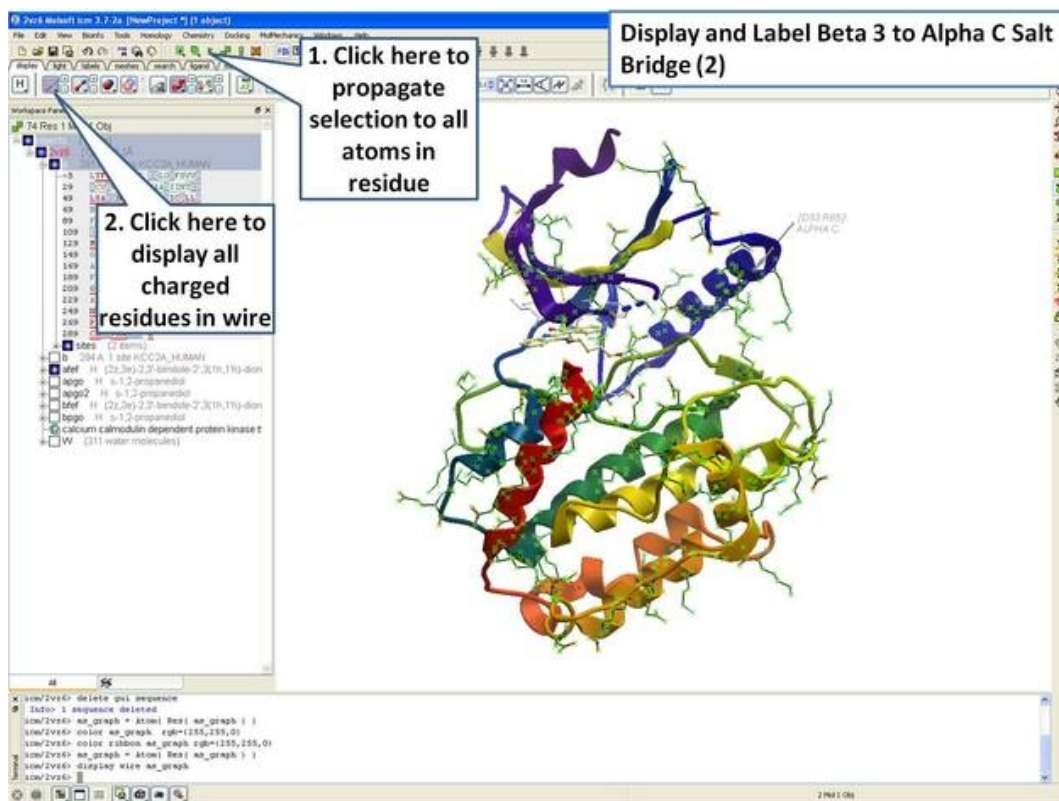
Step 8: Propagate selection to all taoms to display all atoms of the side chain.

Display and Label Beta 3 to Alpha C Salt Bridge (3)

1. Undisplay all xstick representations for clarity

2. Use the zoom in button to locate alpha C to beta 3 salt bridge

5. Click and hold on the residue label button for label coloring and style options

3. Click on the residue label button

4. Click on each residue to label
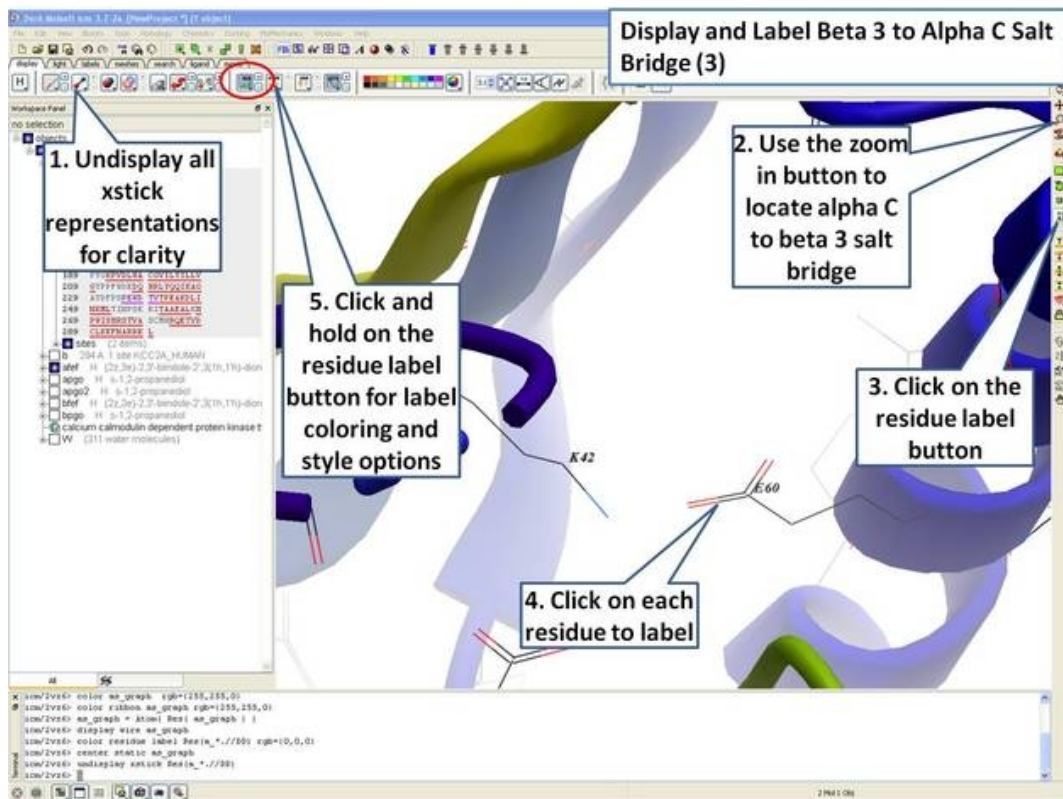
Step 9: Locate the B3 to AlphaC salt bridge (K42- E60) and label using residue label button. **Note:** Once you have selected the two residues you can invert selection (click on invert selection button) and then undisplay all the other charged residues.

Display and label the residues near the ligand binding pocket (1).

4. Propagate selection to all atoms

1. Undisplay wire and other label annotations from previous examples

2. Right click on the "a" subunit ligand and choose "Select Neighbors"

3. Enter the parameters as shown
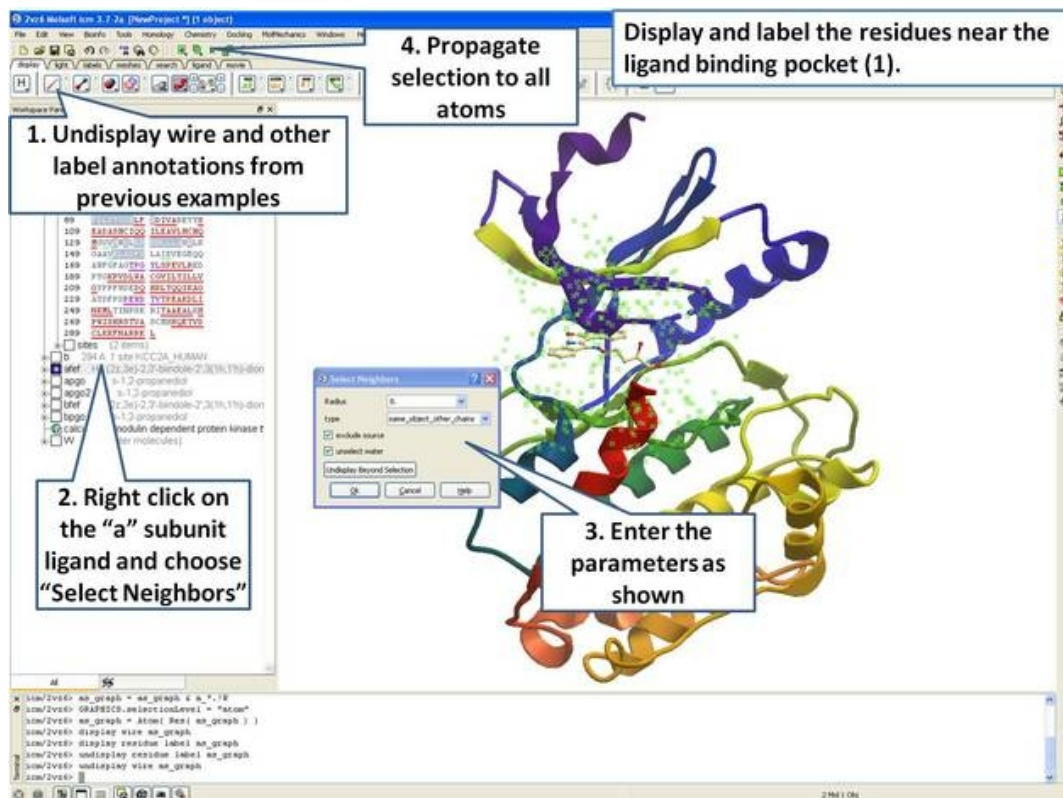
Step 10: Select neighboring residues to the ligand.
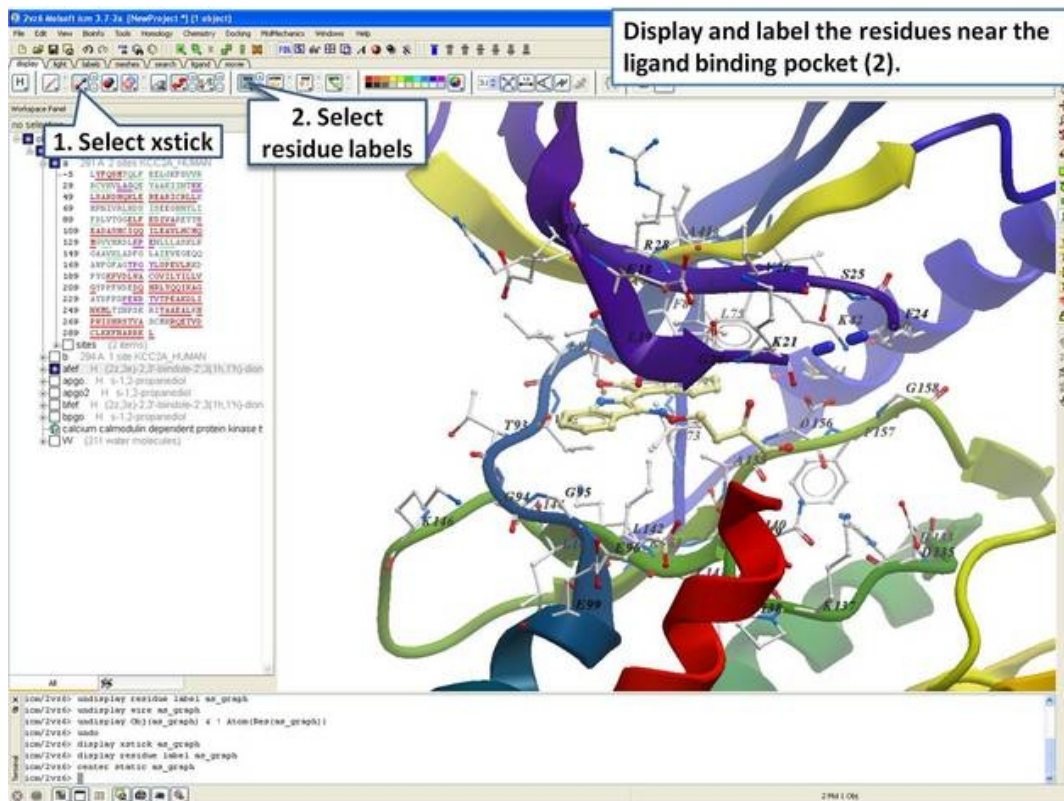
Step 11: Display the residues and label them.

8.1.4 2D and 3D Labels

This tutorial is a continuation from the previous tutorials in this
section and shows you how to:

- Create and display a 2D label.
- Delete and move a 2D label.
- Create and display a 3D label.
- Delete and move a 3D label.
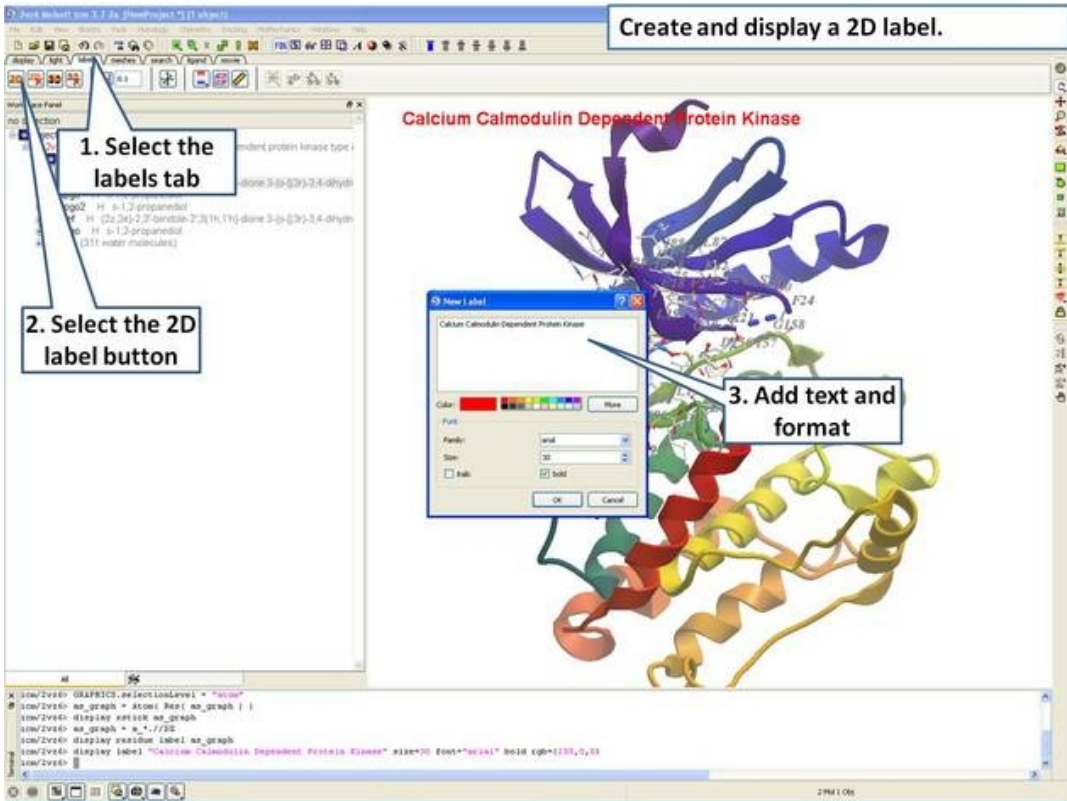
Step 12: Create a 2D label using the options in the "labels" tab.
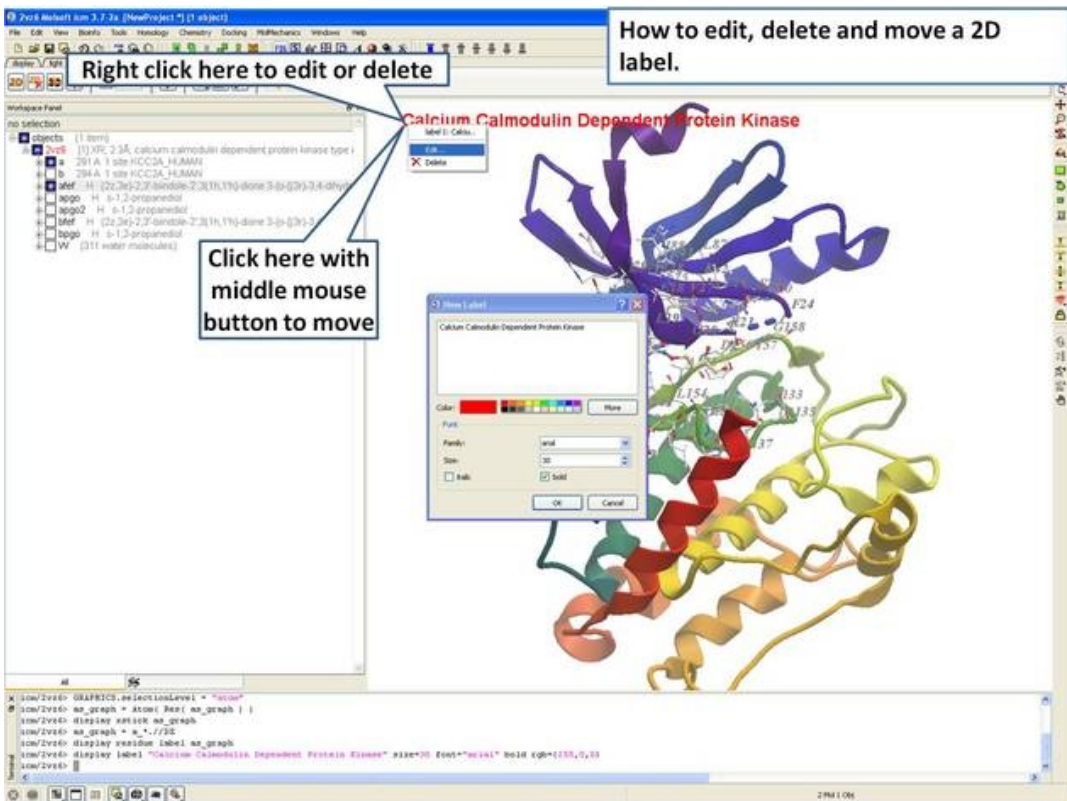
Step 13: Right click on the 2D label to edit or delete it.
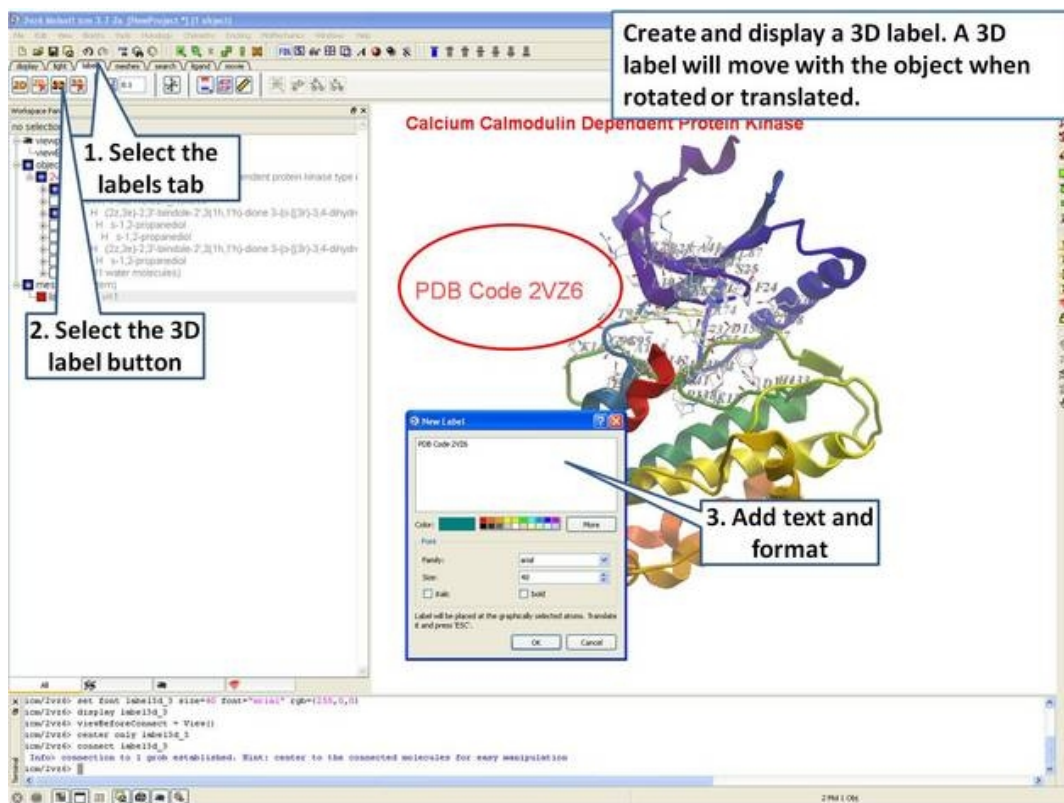
Step 14: Create a 3D label using the options in the "labels" tab.
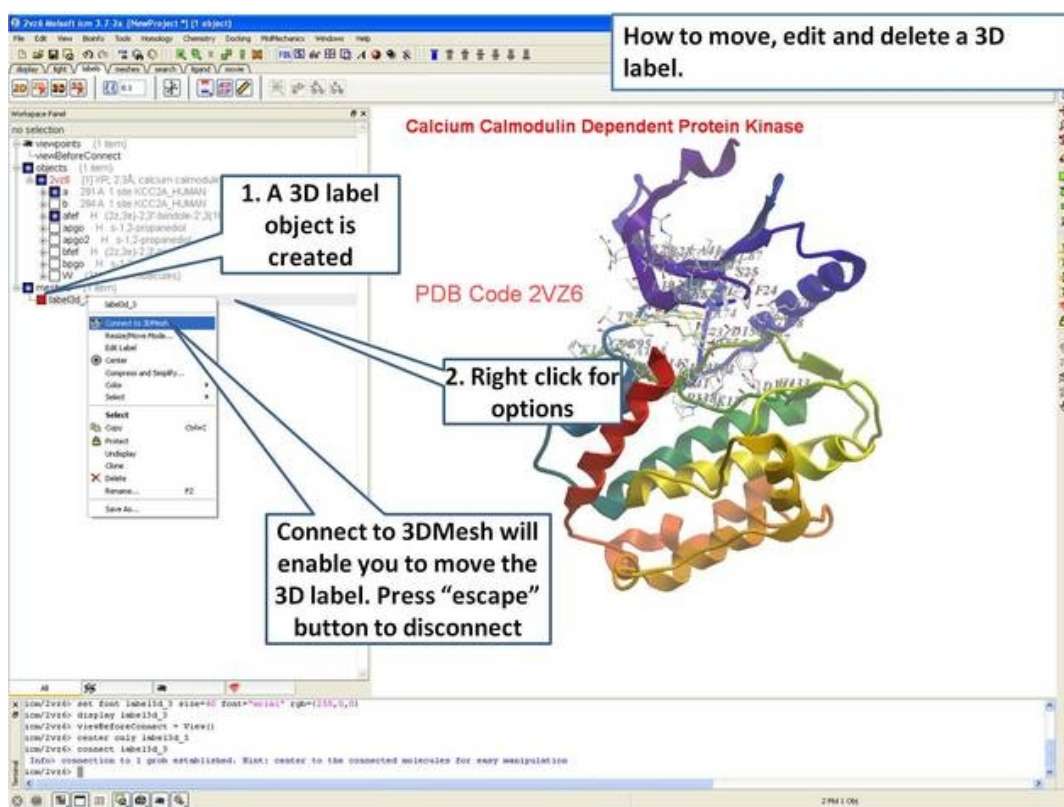
Step 15: Right click on the 3D label to edit or delete it.

8.2 Creating Fully Interactive Slides for PowerPoint and the Web Tutorial

Introduction

A slide enables you you to conveniently store a large number of
different 3D visualization properties (e.g. color, viewpoint,
representation etc...) along with text and window layout. `ActiveICM`
enables you to view and display ICM graphical `slides` and `animations`
interactively inside Windows Microsoft PowerPoint and web browsers such
as Internet Exporler and Mozilla Firefox.

In this example we are going to look at the effect of an agonist binding
to the Beta-2-Adrenergic GPCR (B2AR) and prepare a molecular document. A
prepared ICM file containing three B2AR GPCRs superimposed can be
downloaded here `ftp://ftp.molsoft.com/pub/workshop/` (1. cut and paste
the address into a web browser. 2. Right click on GPCR_slides.icb and
choose "Save Link As". 3. Open the .icb in ICM File/Open). The file
contains pdb files 2RH1 (R inactive state), 3P0G (R* active state, with
G-alpha mimic), and 3SN6 (R*G G-protein signalling state). The agonist
ligand causes Helix 5 to shift inwards and Helix 6 to swing outwards.
This agonist binding poses were predicted using ICM a year before the
crystal structures were solved, you can read about this `here` and a good
review of the structure and function of GPCRs `here`.

Tutorial

In this tutorial we will build a series of slides and then annotate them
using HTML to create hyperlinks to the slides. The resulting molecular
document can be exported in movie format or in fully interactive 3D
files.
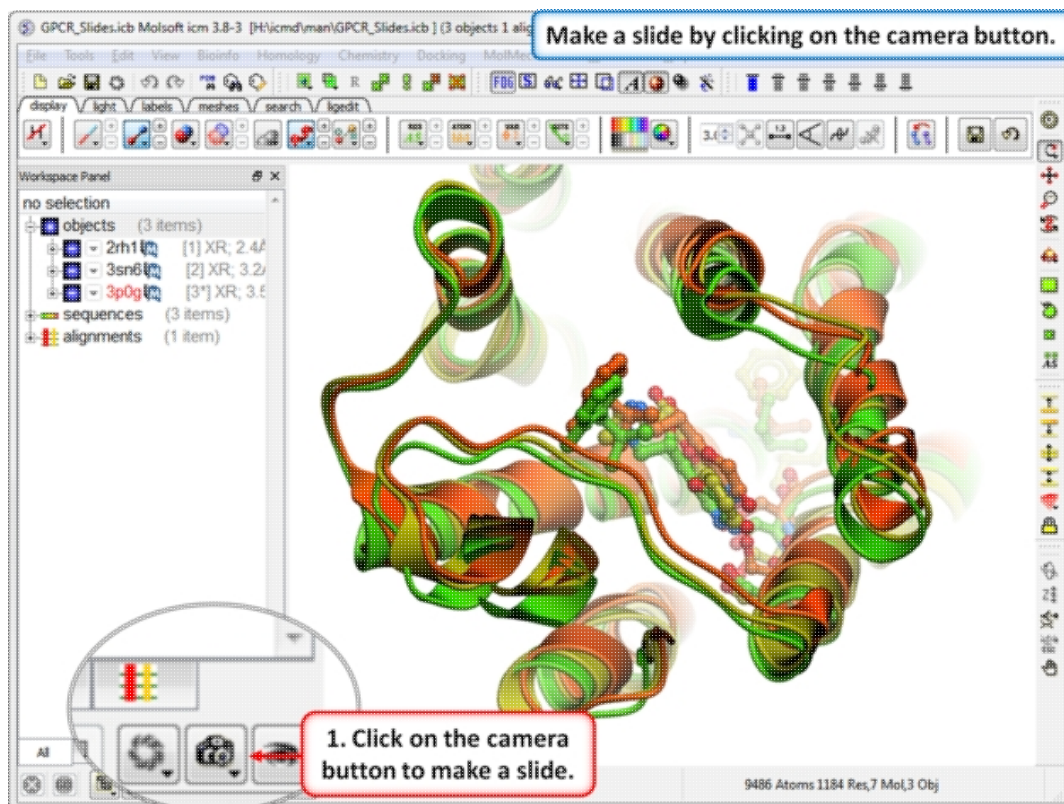
Step 1: Download the example file here
`ftp://ftp.molsoft.com/pub/workshop/` (1. cut and paste the address into

a web browser. 2. Right click on GPCR_slides.icb and choose "Save Link As". 3. Open the .icb in ICM File/Open). Make a slide by clicking on the camera button at the bottom of the GUI.
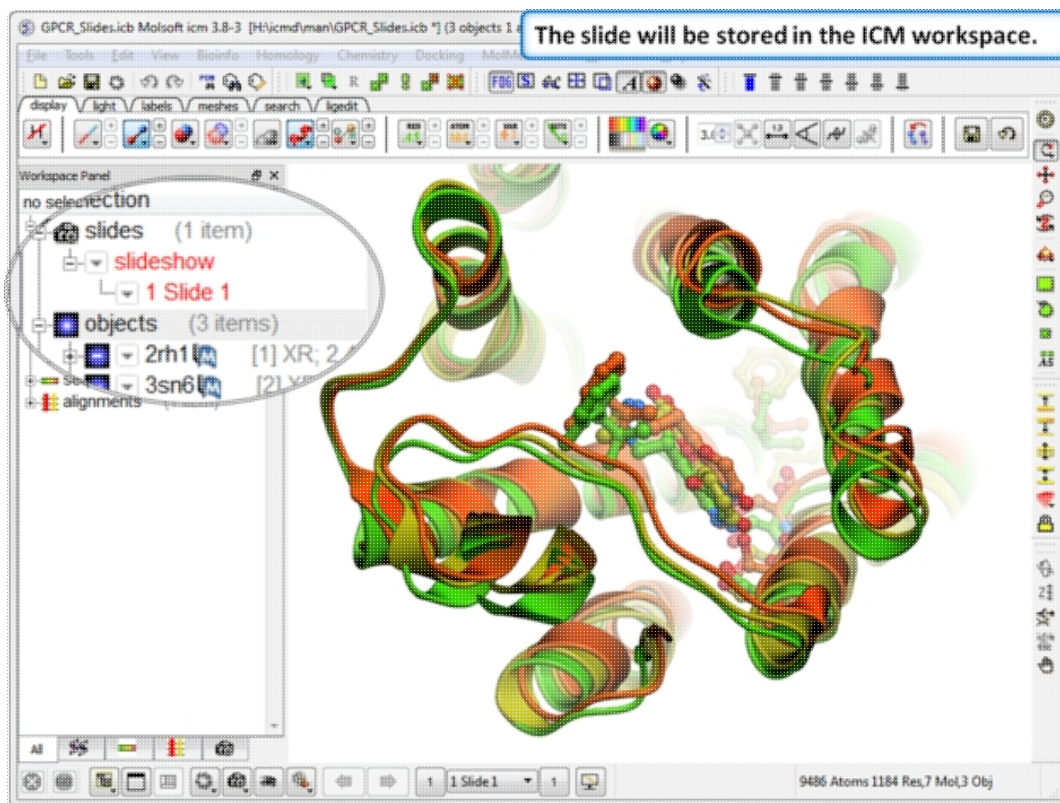
Step 2: The slide is stored in the ICM Workspace panel (left hand side of the GUI). You can make changes to the 3D display and return to the display in the slide by clikcing on the slide in the ICM Workspace.
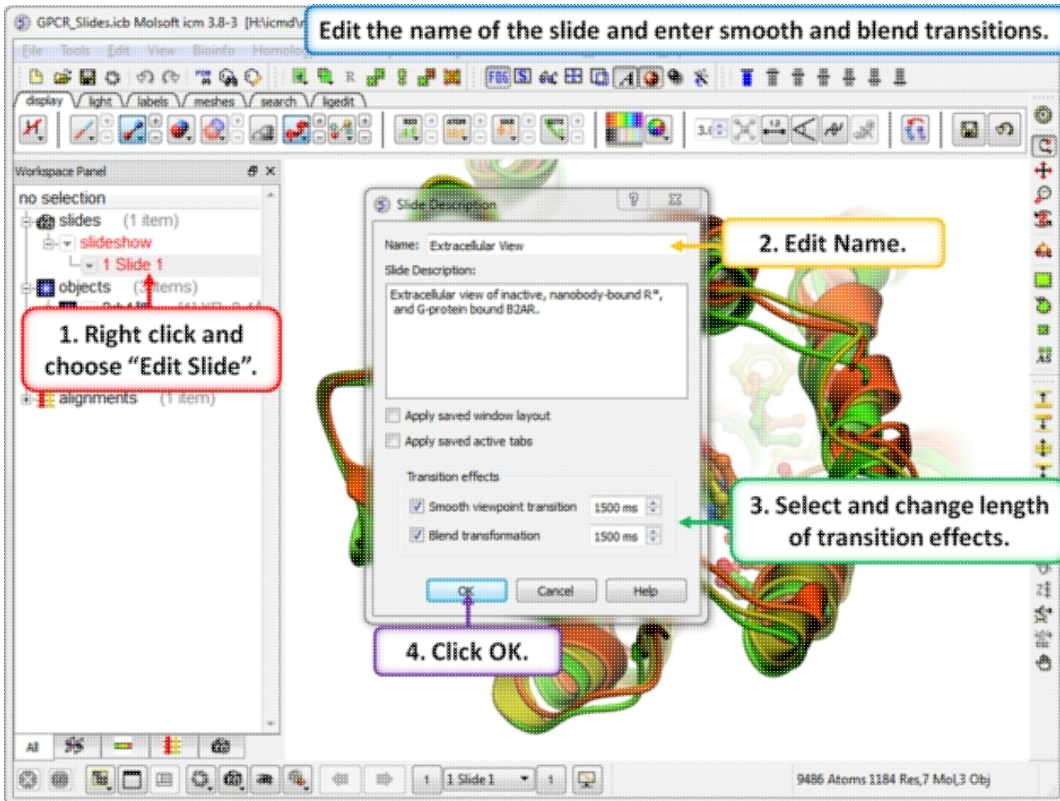
Step 3: Properies of the slide can be edited by right clicking on the slide in the ICM workspace.
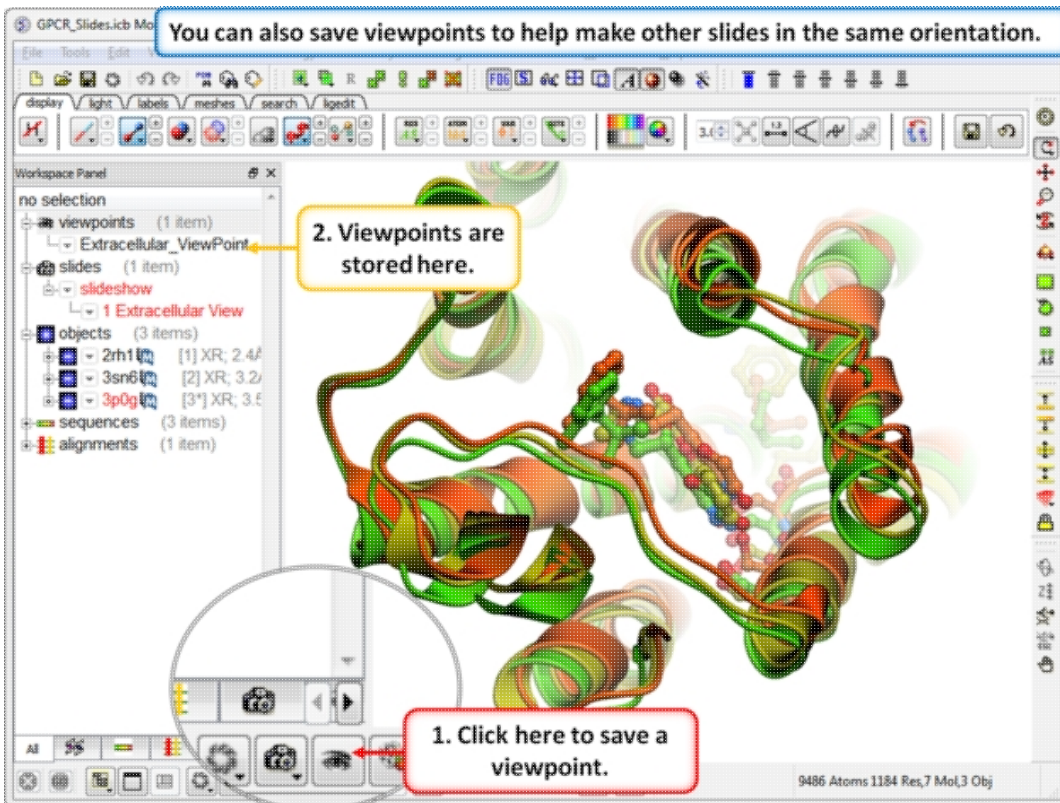
Step 4: Saving a viewpoint is useful if you are making many slides from the same viewing position.
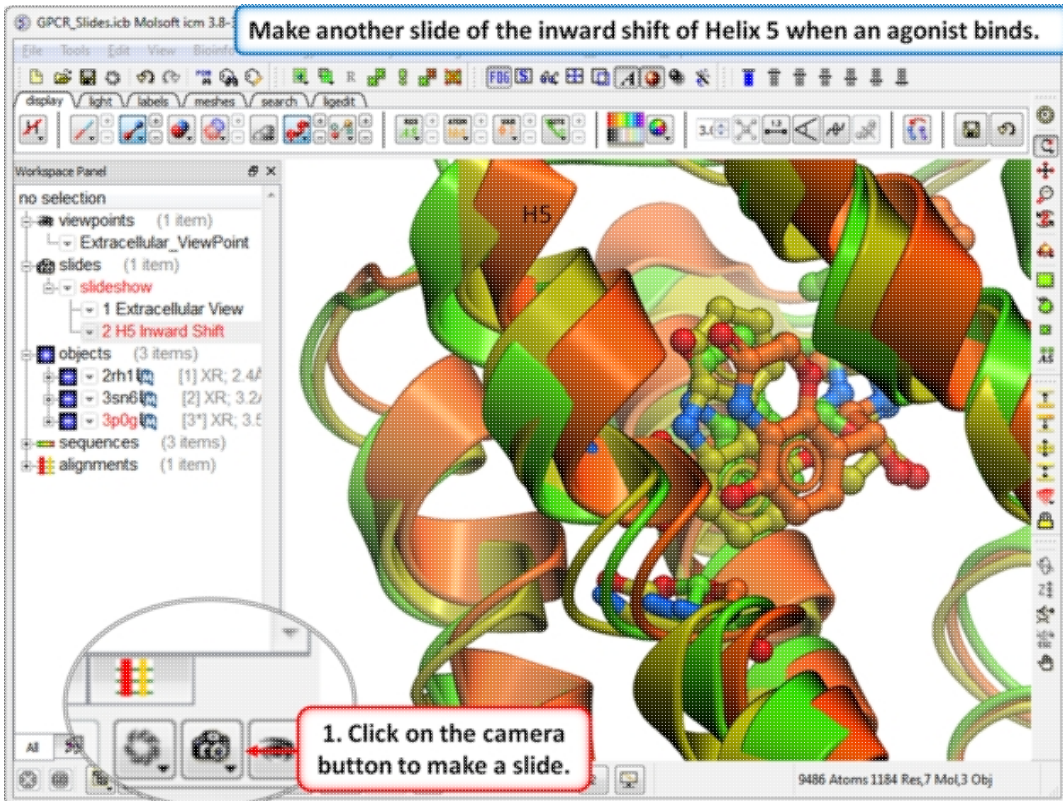
Step 5: In this slide we show that Helix 5 makes an inward shift when an agonist binds.
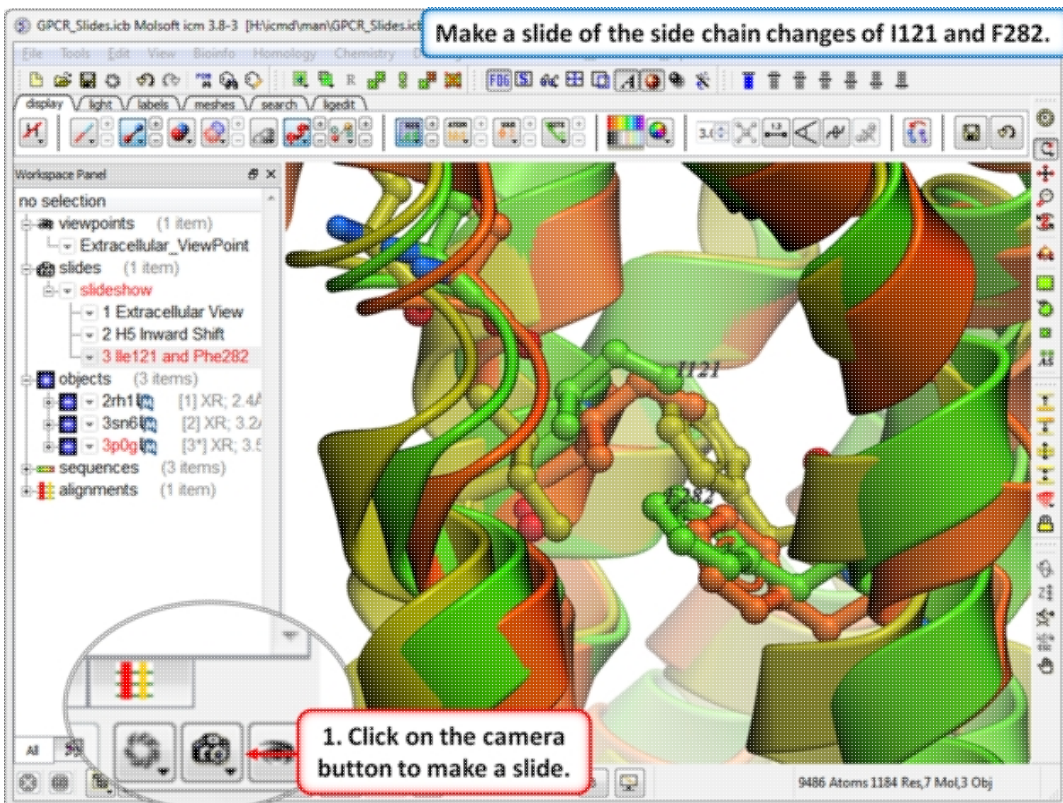
Step 6: In this slide we show that upon binding an agonist Ile121 switches its rotameric state and Phe 282 moves.
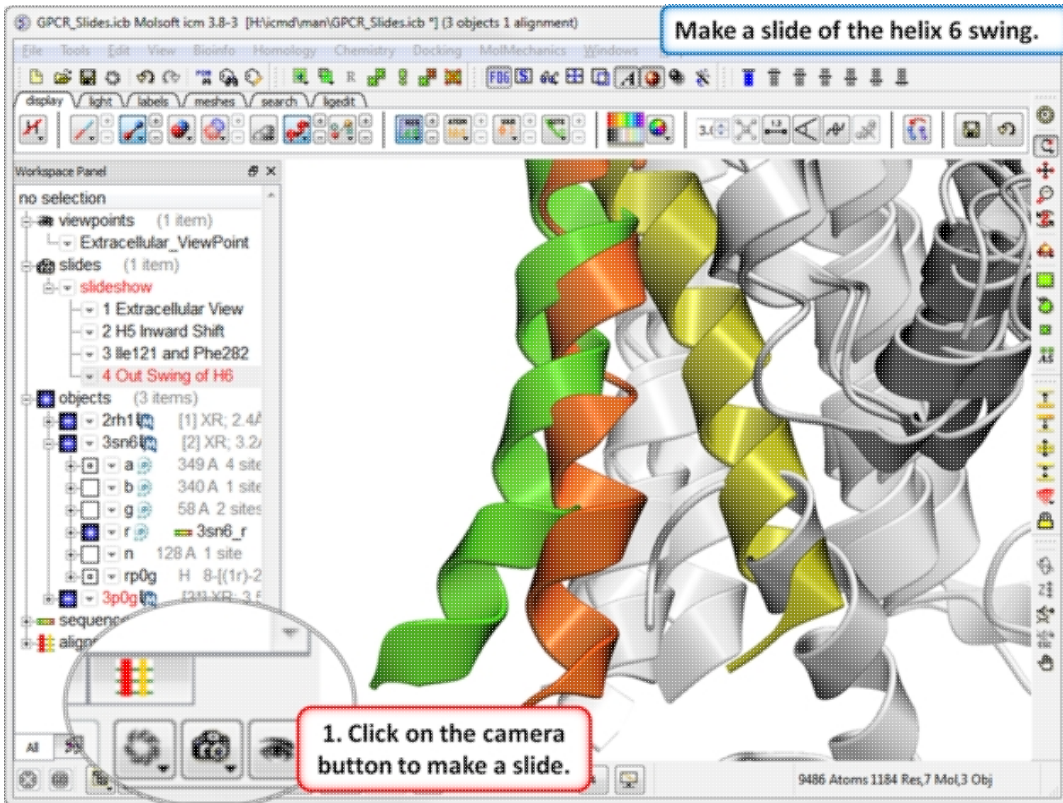
Step 7: In this slide we show that agonist binding causes Helix 6 to swing out.
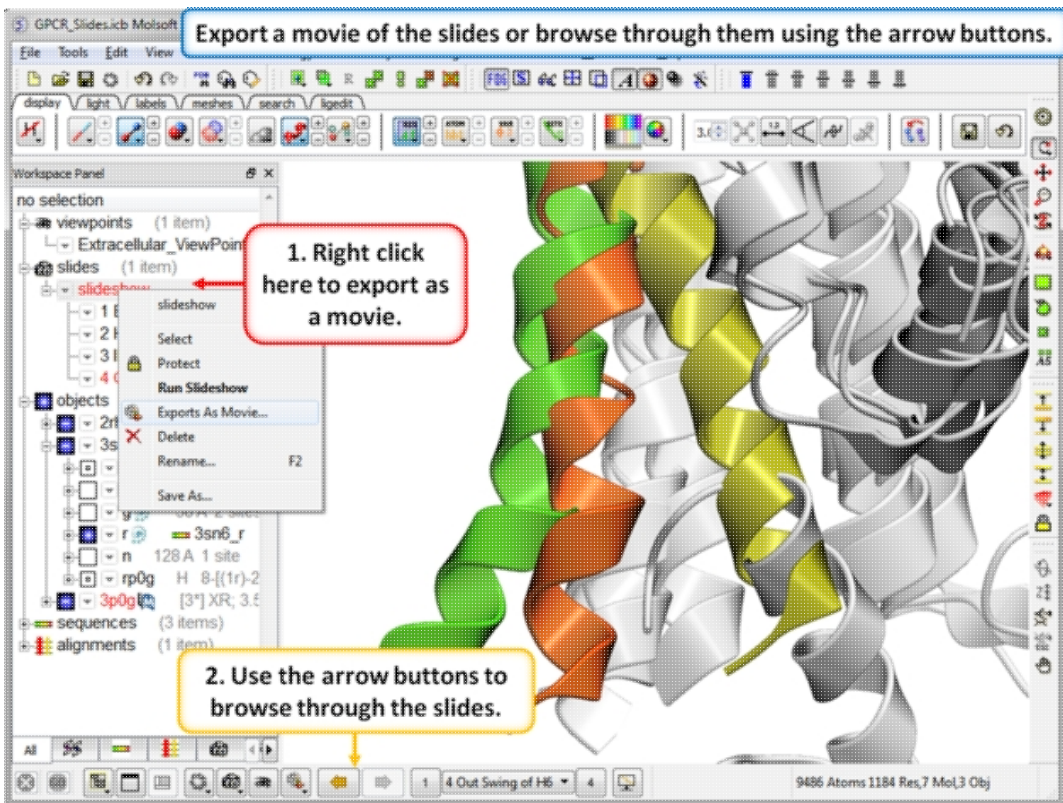
Step 8: You can export the slides as a movie or you can browse through them using the arrow buttons.
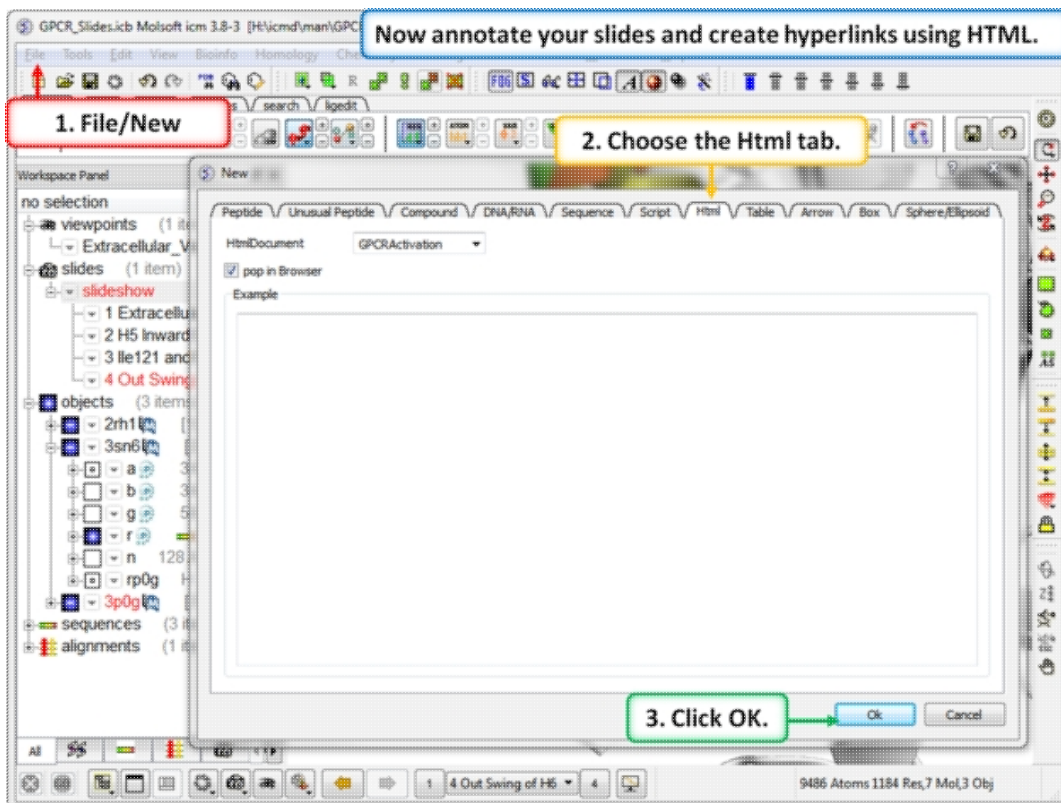
Step 9: To build a molecular document you can annotate the slides using HTML.
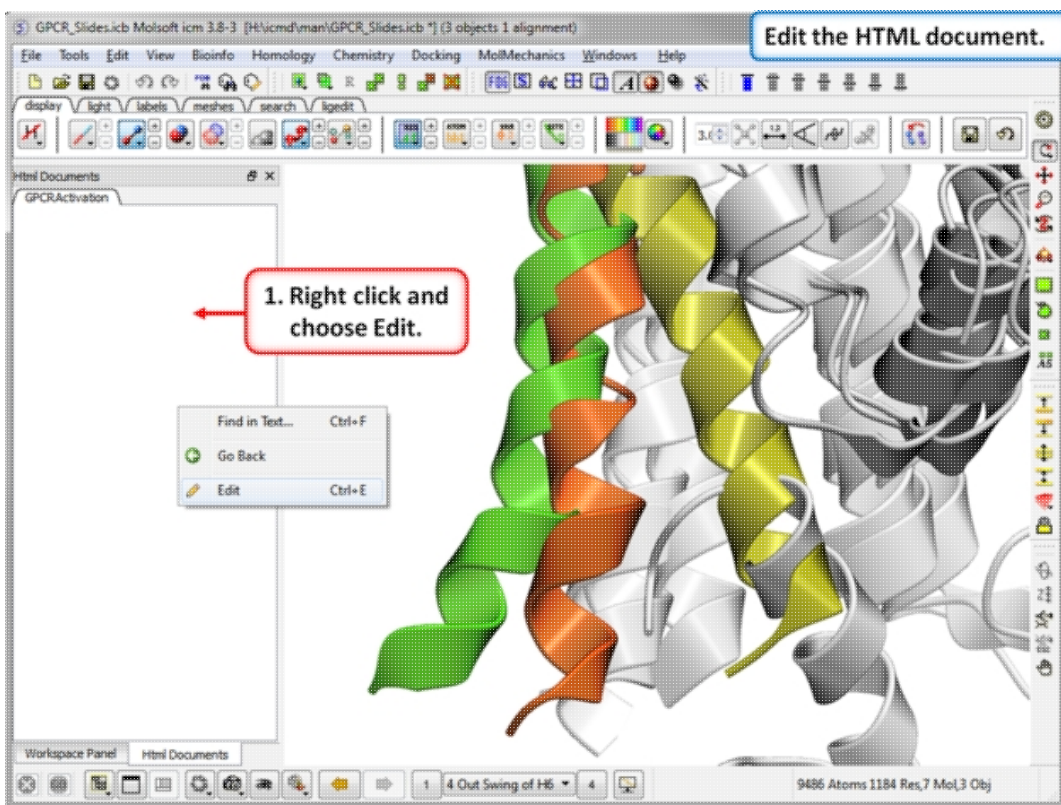
Step 10: Edit the document by right clicking on the HTML panel and choosing **Edit.**
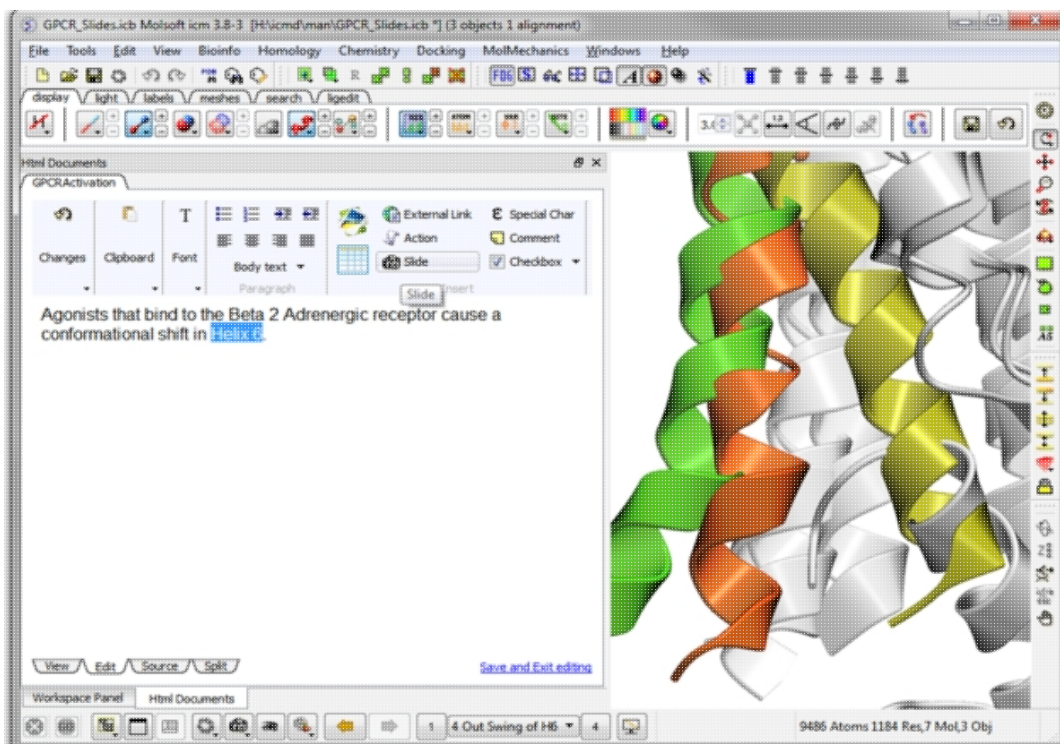
Step 11: Hyperlinks can be made to the slide. Other `functions` such as adding check boxes, ICM scripts and external links are also available.
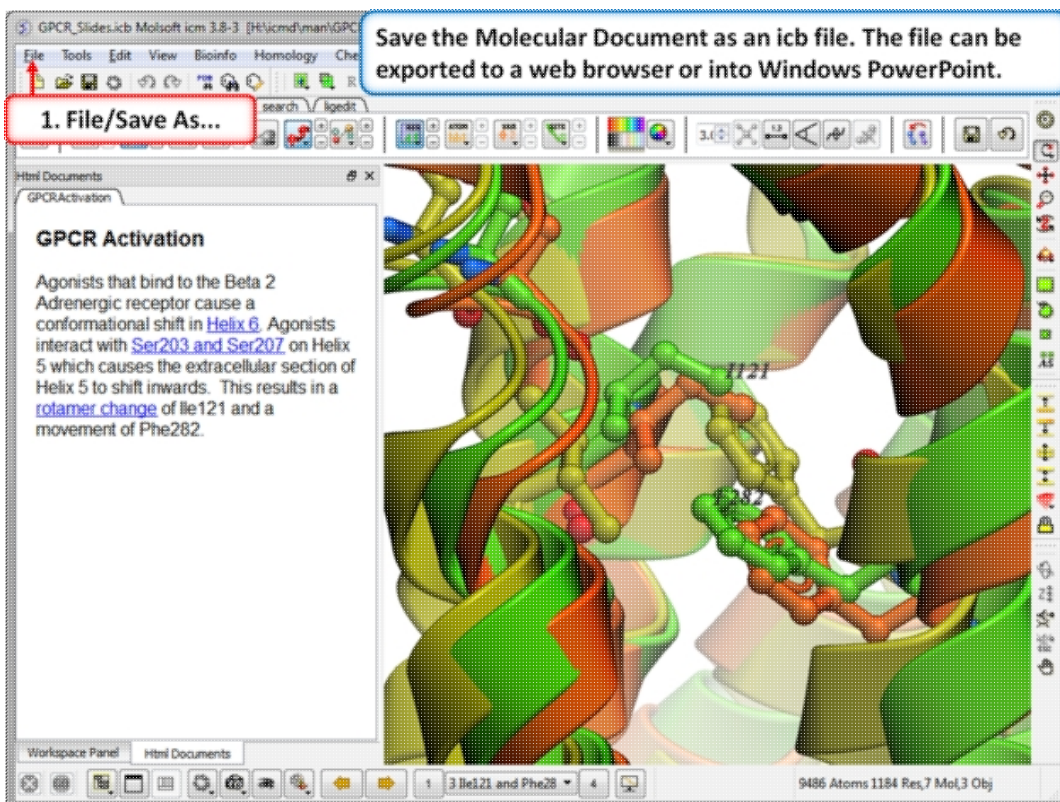
Step 12: Save the molecular document in icb format and view in ICM-Pro or ICM-Browser. You can also export to a `web browser` or view the fully interactive slides in `Windows Powerpoint` using a plugin called `ActiveICM`.

8.3 Protein Structure Tutorials

Introduction

The positioning of the DFG motif in the activation loop of a protein kinase has an effect on the size and properties of the ATP binding pocket. Most kinase inhibitors target the kinase with the DFG inwards to the ATP binding site. Type-II inhibitors target the site where the DFG motif is in the out position which opens up the pocket and provides additional hydrophobic binding sites. Targeting DFG-out conformations can improve inhibitor specificity and slower-off rates.
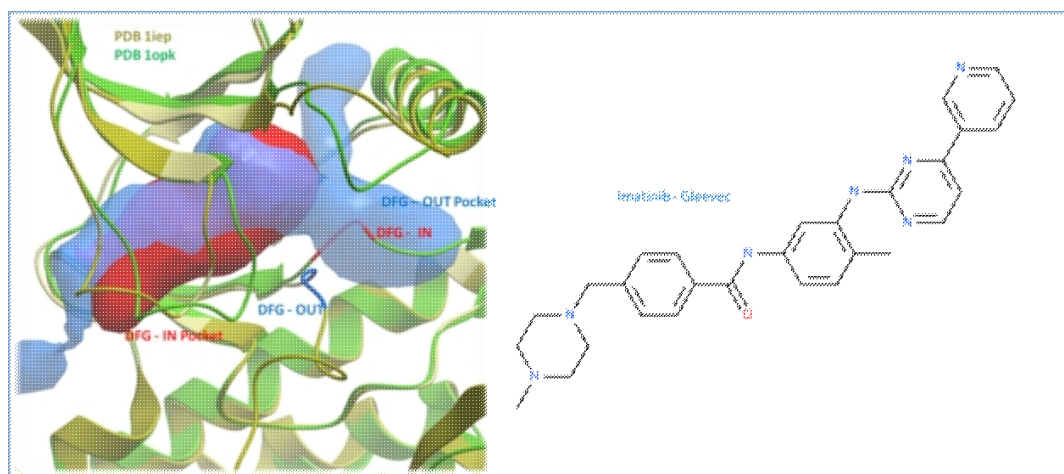
Comparison of the crystal structures of Abl kinase with DFG-IN and DFG-OUT (Left). Imatinib (Gleevec - right) binds to the DFG-OUT conformation.

Tutorial

In this example we will look at two kinase structures and compare their binding pockets. One of the structures is bound to the Type-II inhibitor Imatinib (Gleevec) a drug used to treat Chronic Myelogenous Leukemia. We will use ICM tools to look at the side-chains that interact with the inhibitor and compare the pocket with a DFG-in structure using `ICMPocketFinder`. Please click on the links provided to learn more about the methods used.

8.3.1 PDB Search
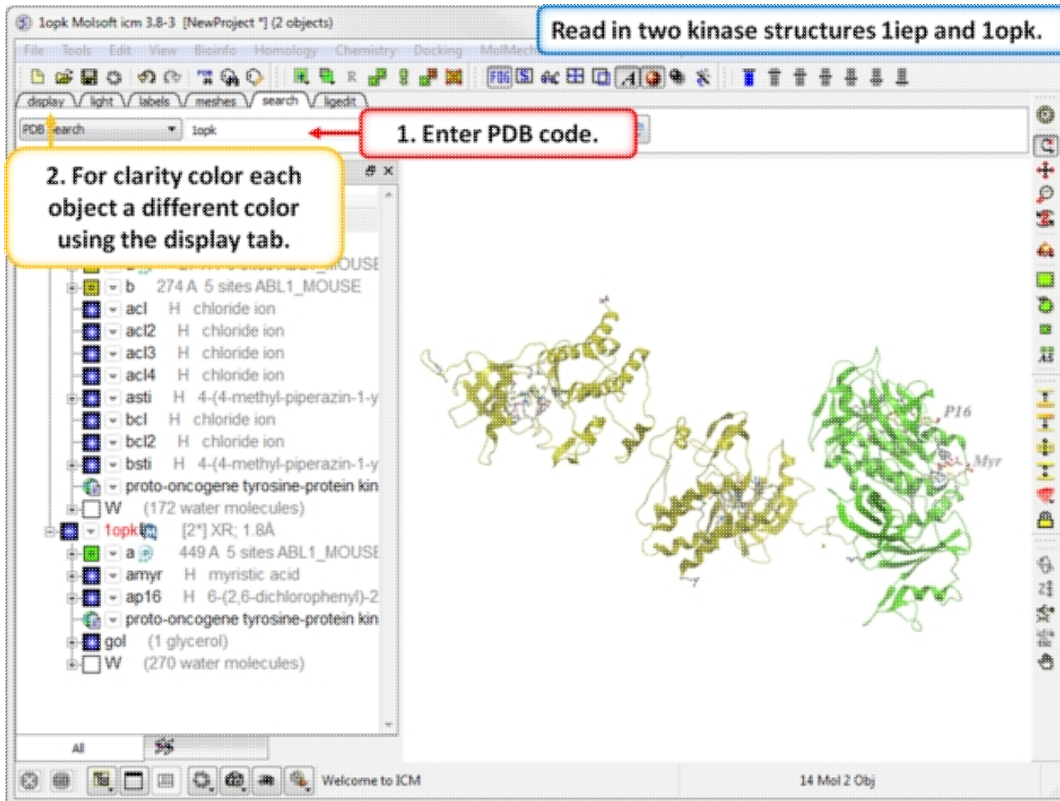
Step 1: Click on the Search Tab and choose "PDB Search" and oad the
PDBs structures 1iep and 1opk. You can color each object a unique color
by clicking on the Display Tab and clicking and holding on the ribbon
button and selecting Color by>Object.

8.3.2 Convert PDB to ICM Object
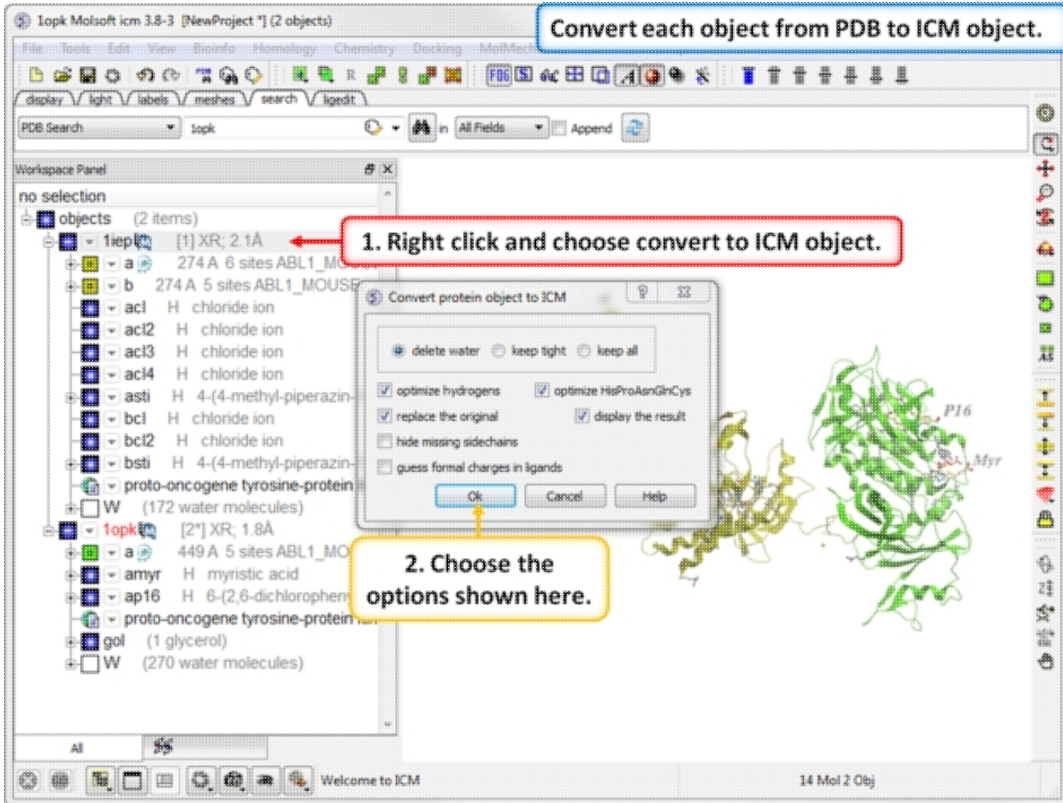
Step 2: Convert each PDB file into an ICM object.

8.3.3 Protein Superposition
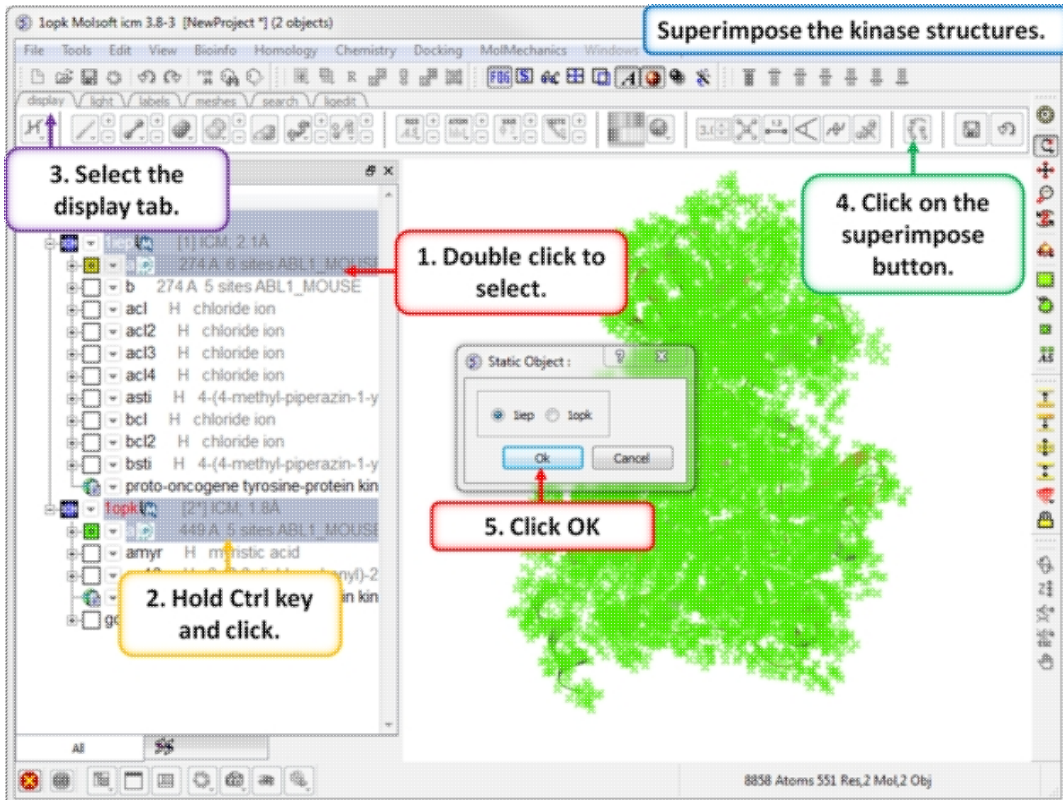
Step 3: Superimpose the two kinase structures.

8.3.4 Protein Structure Analysis
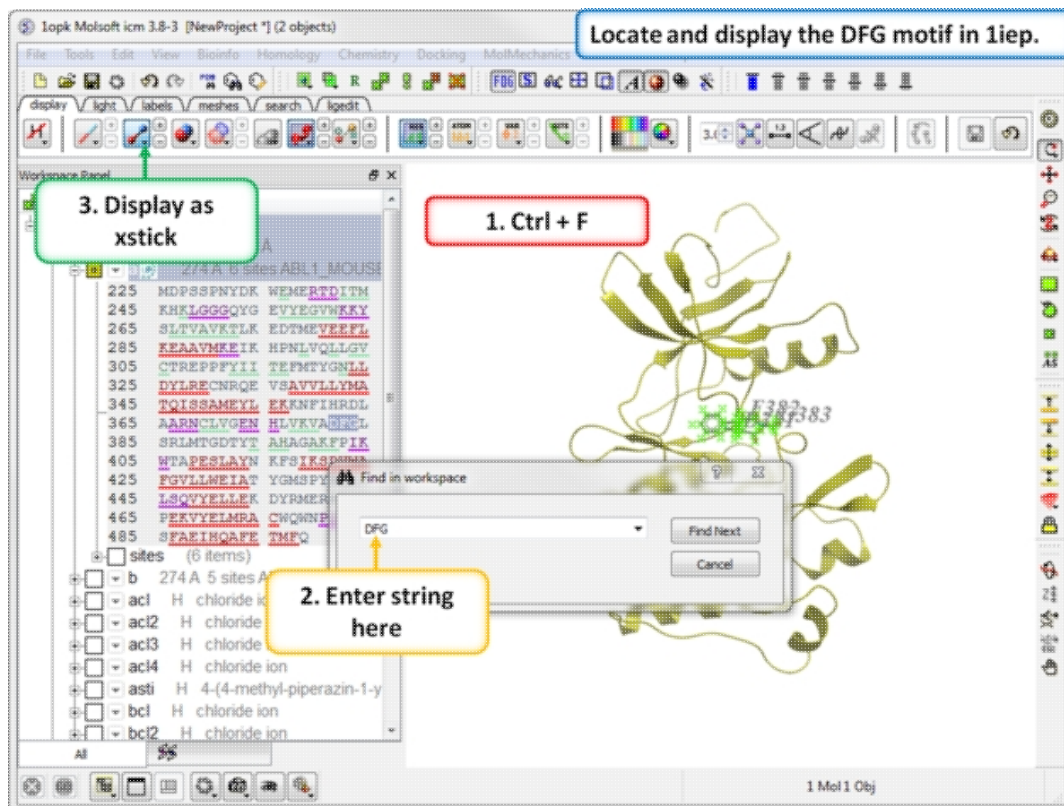
Step 4: Search the "a" subunit of 1iep for the "DFG" motif. You can press Ctrl F and expand the sequence in the ICM workspace. Display the DFG region in xstick.
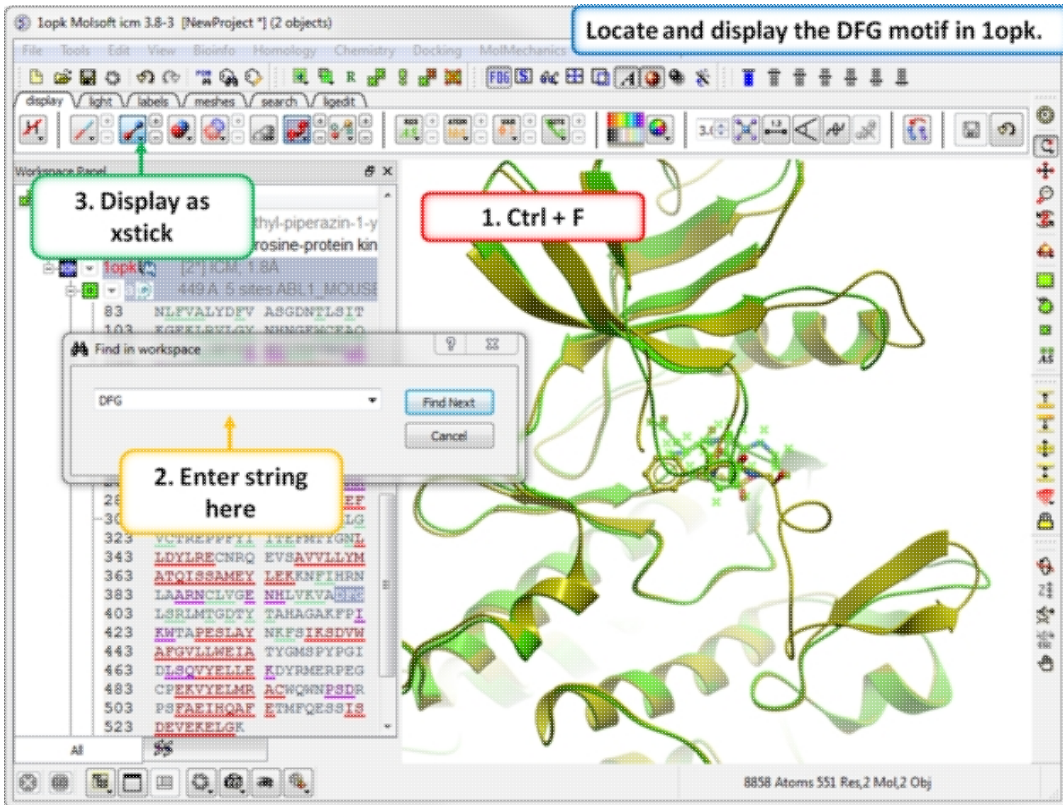
Step 5: Search the "a" subunit of 1opk for the "DFG" motif. You can press Ctrl F and expand the sequence in the ICM workspace. Display the DFG region in xstick.
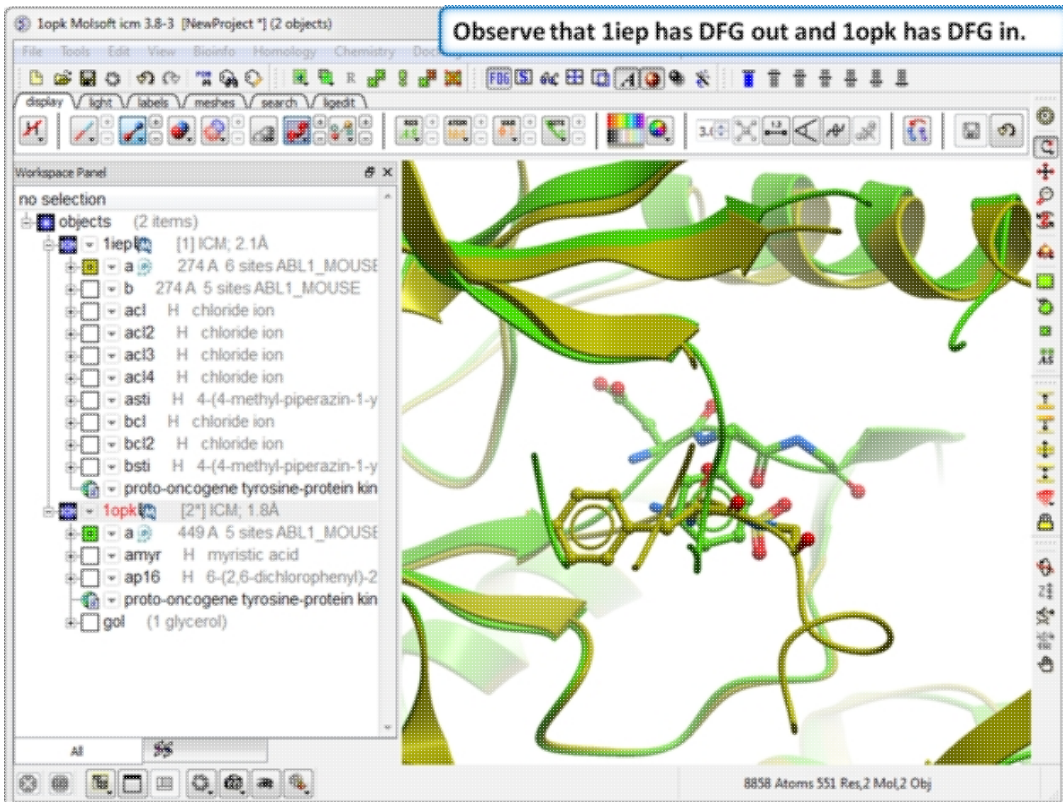
Step 6: Observe the DFG location in both structures.

8.3.5 Contact Areas

Step 7: Use Tools/Analysis/Contact Areas to display the contacts with the Gleevec ligand.

Step 8: Tag the residues surrounding the ligand. Repeat Steps 7 and 8 for the 1opk structure and ligand.

8.3.6 Hydrogen Bond
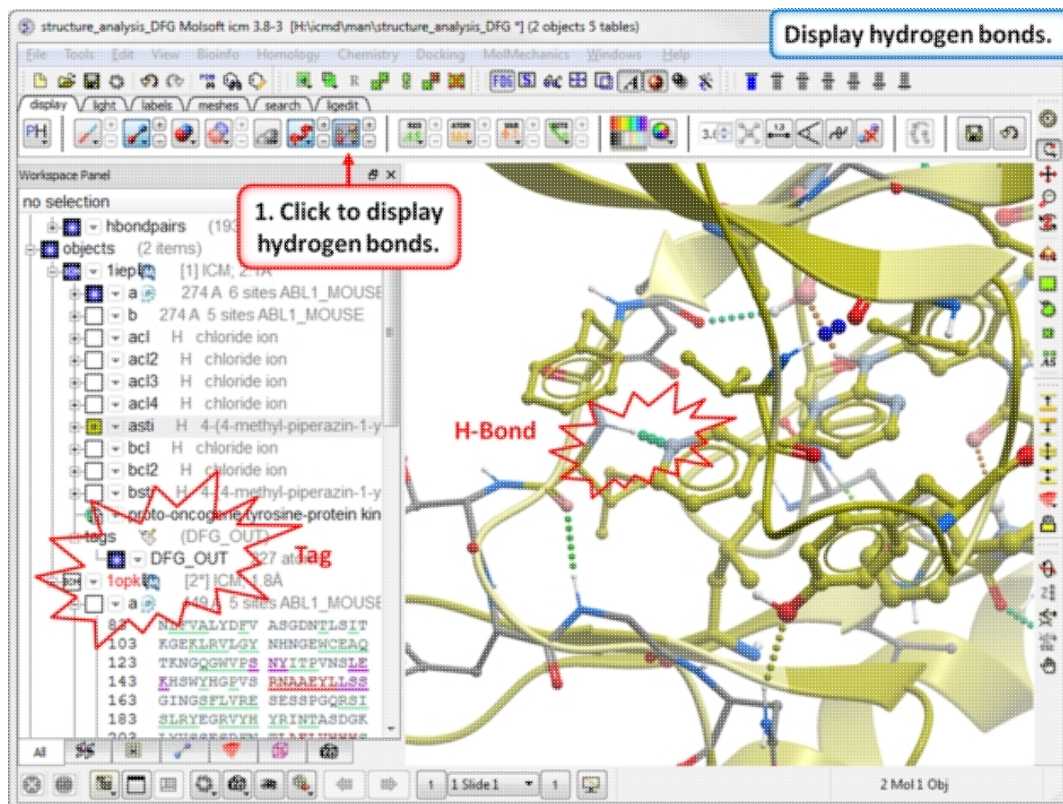
Step 9: Compare the hydrogen bond interaction of the Type I and Type II inhibitors with the hinge region (1iep - residues 317:322) of the kinase.

8.3.7 Pocket Identification

Calculate the binding pocket surface using ICMPocketFinder.

1. Tools/3D Predict ICMPocketFinder.

2. Right click and rename.
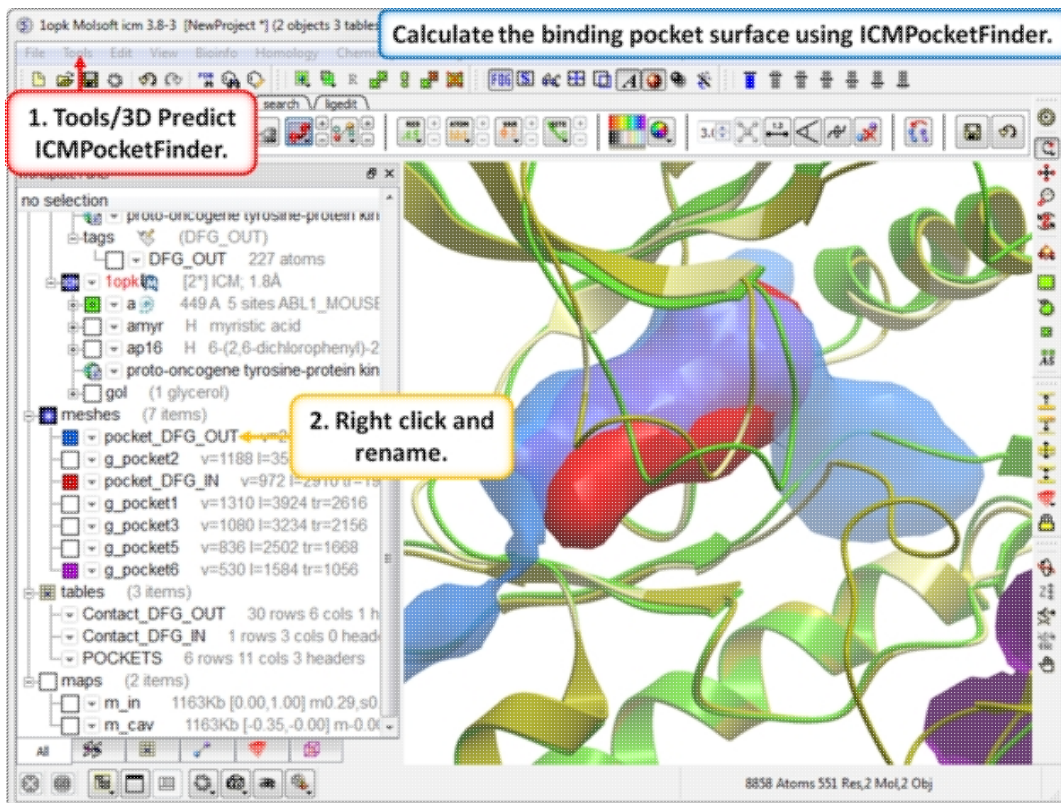
Step 11: Use `ICMPocketFinder` to compare the pockets of DFG-IN and DFG-OUT. Run ICMPocketFinder for each structure - rename the pocket so as not to overwrite it.

8.3.8 Protein Preparation and Crystallographic Analysis Tutorial

8.3.9 PDB Preparation - Symmetry

Background When inspecting a ligand binding pocket it is important to check that the true pocket is formed by chains which are not explicitly present in a PDB entry. Therefore it is necesary to use **Tools/X Ray/Crystallographic Neighbor** to find all molecules/subunits or chains involved in the interaction with the ligand. Molecular objects and 3D density maps may contain information about crystallographic symmetry. It consists of the following parameters:
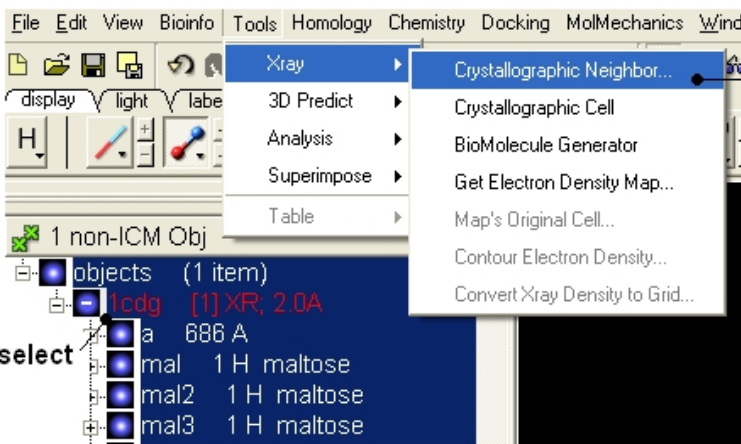
1. Crystallographic group eg. P2121 that determine N (depends on a group) transformations for the atoms in the asymmetric unit.
2. Crystallographic cell parameters A, B, C, Alpha, Beta and Gamma

To generate the coordinates within one cell one needs to apply N transformations and then to generate neigboring cells the content of one cell needs to be translated in space according to the cell position.

Example As an example let us look at Cycloldextrin glycosyltransferase (PDB Code: 1CDG). The problem with docking to this receptor is that the true pocket is formed by chains which are not explicitly present in the PDB entry. Site mb1 includes serine 382. This cannot be predicted just by looking at the structure. Therefore we need to identify symmetry related molecules to this protein.

- Use the PDB search tab to load the crystal structure 1cdg.
- Inspect the ligand binding pocket of **maltose** (mal)

- To identify if there are any other chains involved in the interaction with the ligand select the whole structure in the ICM Workspace.

- Tools/Crystallographic Neighbor
- Select a 7A radius
- Check "create symmetry related molecules" and "display symmetry neighbors".
- Inspect the neighbors surrounding **maltose(mal).** Each symmetry related subunit can be colored by object by clicking and holding the representation button in the display tab and selecting color-by.
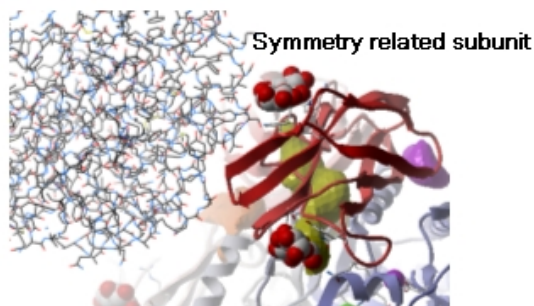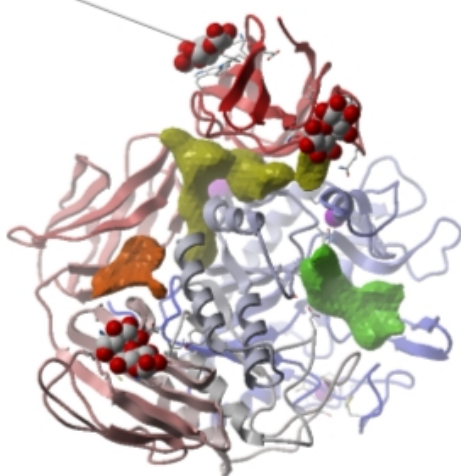
8.3.10 PDB Preparation - Occupancy and B-Factors

Background When preparing a PDB for analysis (eg docking or modeling) it is important to check the reported occupancies and b-factors. The occupancy is a fraction of atimic density at a given center. If there are two eqally occupied conformers both will have an occupancy of 0.5 - the normal value is 1 range 0-1. The *{B-Factor} is the mean-square displacement of atom from its position in the model - the normal range is 5-50.

One way of visualizing the occupancy and b-factor is by coloring the structure by these values. You can do this by clicking and holding on a representation button in the **display** panel and selecting Color-by.

As an example let us look at the crystal structure 1ATP

- Type in the PDB search tab 1atp and the structure will be displayed in the graphical display.
- Use the ICM workspace to undisplay everything except for the "e" subunit. You can do this by clicking in the blue boxes in the ICM Workspace.
- Display the "e" subunit in wire representation using the wire button in the **display** tab.
- Click and hold on the wire button and select Color-by B-Factor. Regions of high B-factor are colored red.
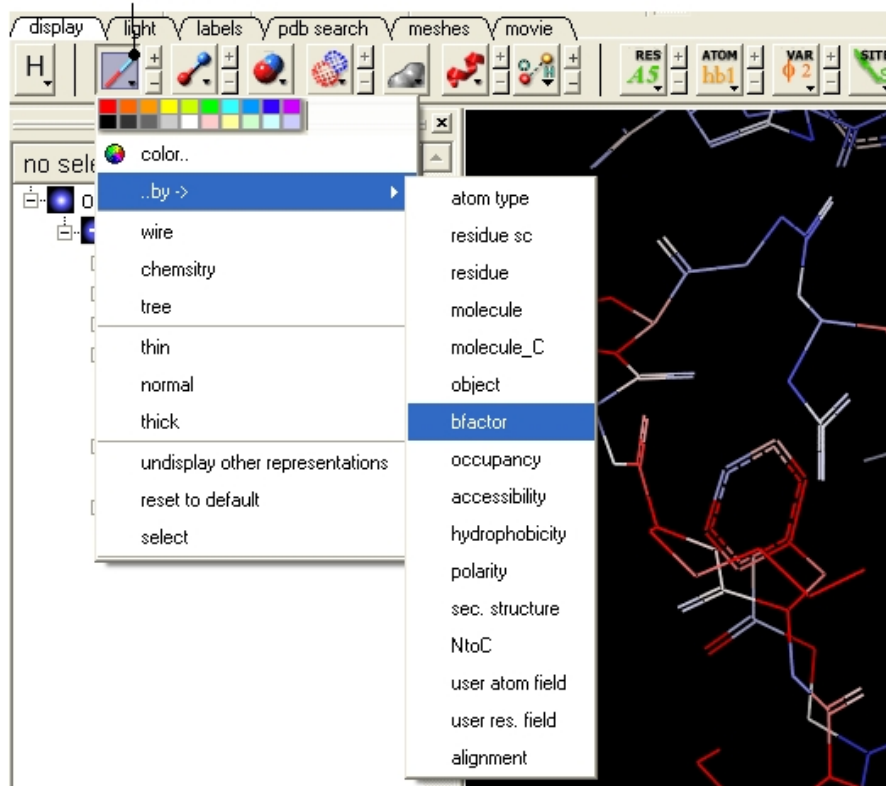
Residues with high B-Factor are colored red

8.3.11 PDB Preparation - Residue Alternative Orientation

For some very high resolution structures two alternative conformations for a residue are provided. Therefore for docking you need to decide to use one conformation of the residue or generate seveal separate docking models. This could be performed using multiple receptor conformation docking.

Here is an example of alternative residue orientations found in a crystal structure of a Fatty Acid Binding protein in complex with stearic acid.

Load and display the PDB structure 1HMT.

1. PDB search 1HMT

2. Undisplay xstick, residue and variable labels

Display alternative conformations for V32 and T36.

3. Click the xstick button

2. Hold the control key and click and drag over V32 and T36

4. Click center button

1. Click here to expand residue level

T36

V32

8.3.12 Biomolecule Generator

Objective

Here we will investigate the biological environment of a virus protein .
PDB code 1DWN.

Background

It is very useful to know how a protein from the PDB may look in a
biological environment. The PDB entries solved by X-ray crystallography
and deposited in the PDB contain the information about the crystal
structure rather than the biologically relevant structure. For example,
for a viral capsid only one instance of capsid protein complex will be
deposited and only one or two molecules of haemoglobin that is a
tetramer in solution maybe deposited. In some other cases the asymmetric
unit may contain more than one copy of a biologically monomeric protein.
ICM reads the biological unit information and has a tool to generate a
biological unit. Not every PDB entry has the biological unit
information.

Instructions

- Read and load the PDB file 1DWN
- Tools/Xray/Biomolecule Generator
- Tick the makeAllBiomolecules box (Warning this may take a few
  minutes to generate)
- The generated molecules will be listed in the ICM Workspace. Each
  one can be selected and displayed. The biomolecule is shown below.
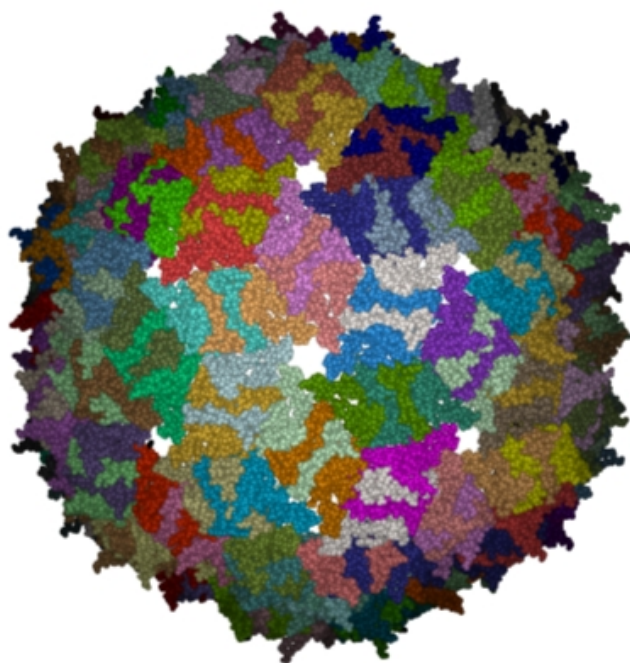
NOTE: Please note that right clicking on a PDB file in the ICM
Workspace will tell you whether there is any **Biomolecule** information
available for the structure. If this information is not present then
the option will be greyed out.

Manual References (Web Links)

```
Biomolecule Generator
```
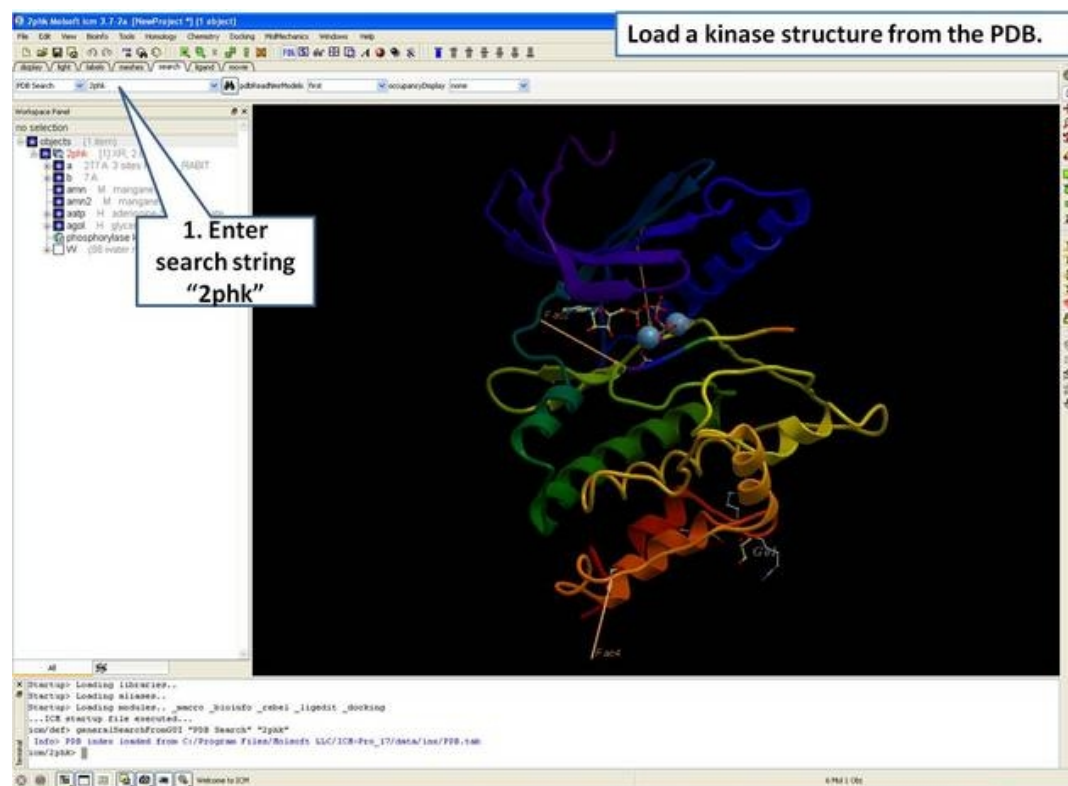
8.4 Sequence and Alignment Tutorials

The key topics in this tutorial are:

- How to extract sequences from PDB structures.
- How to load sequences from UniProt.
- How to make an alignment.
- How to link the alignment to 3D structure.
- How to display sequence conservation in ligand binding pocket.
- How to color 3D structure by alignment.

8.4.1 Display and Superimpose Protein Kinase Structures in Preparation for the Tutorial

In preparation for the tutorial we will read in two PDB file and superimpose them. We will then extract sequences from the PDB structures and read in additional kinase sequences from Uniprot.

Step 1: Load and display the kinase structure 2PHK.

Step 2: Load and display the kinase structure 1ql6.
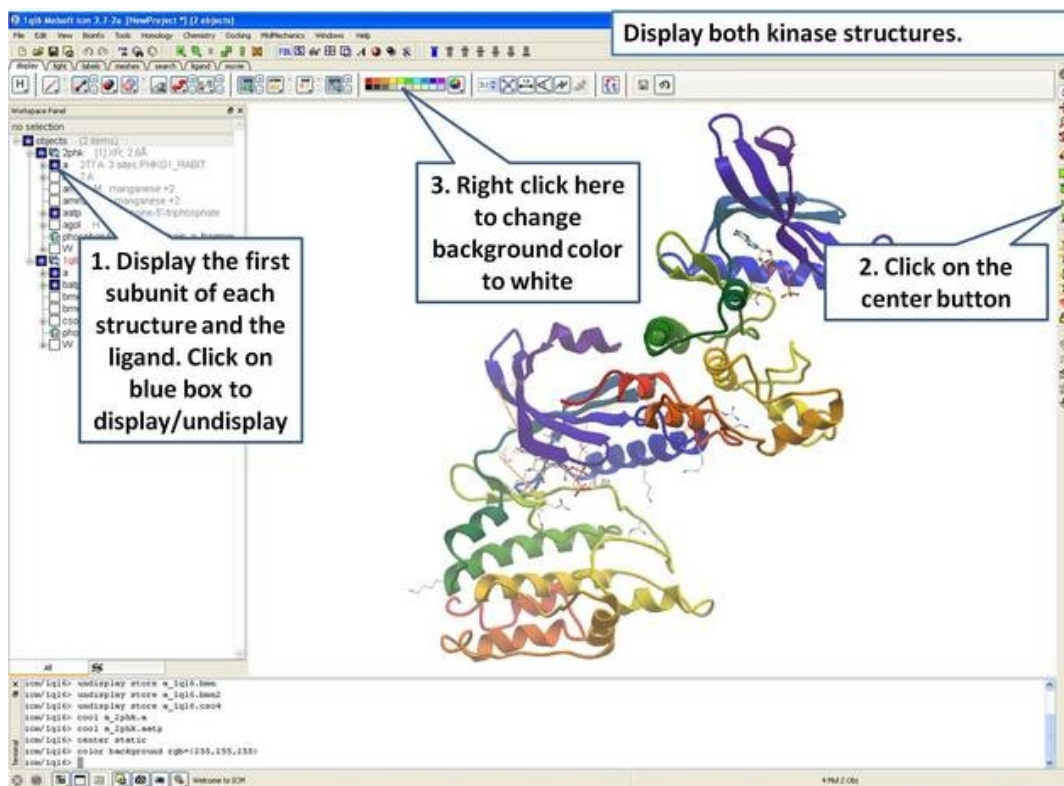
Step 3: Display both receptors.

Step 4: For clarity, color each receptor a different color.

Step 5: Superimpose both kinase structures using the superimpose button
in the display tab.
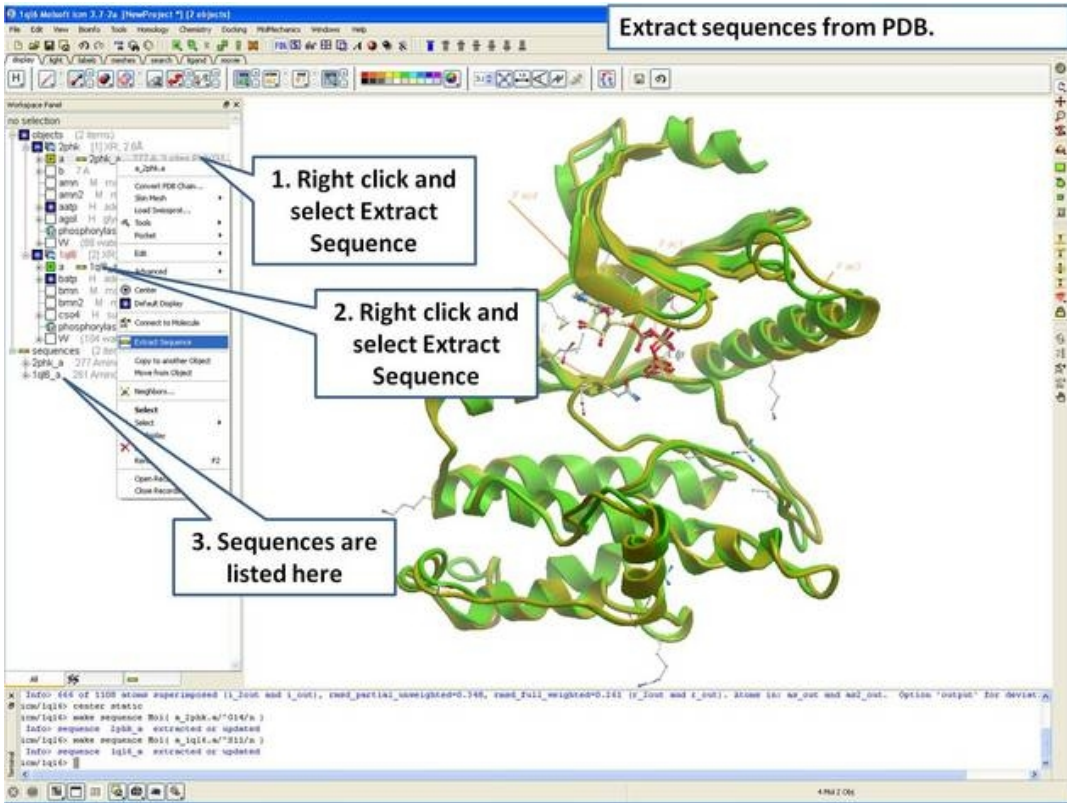
Step 6: Extract the sequences from the PDB structure.
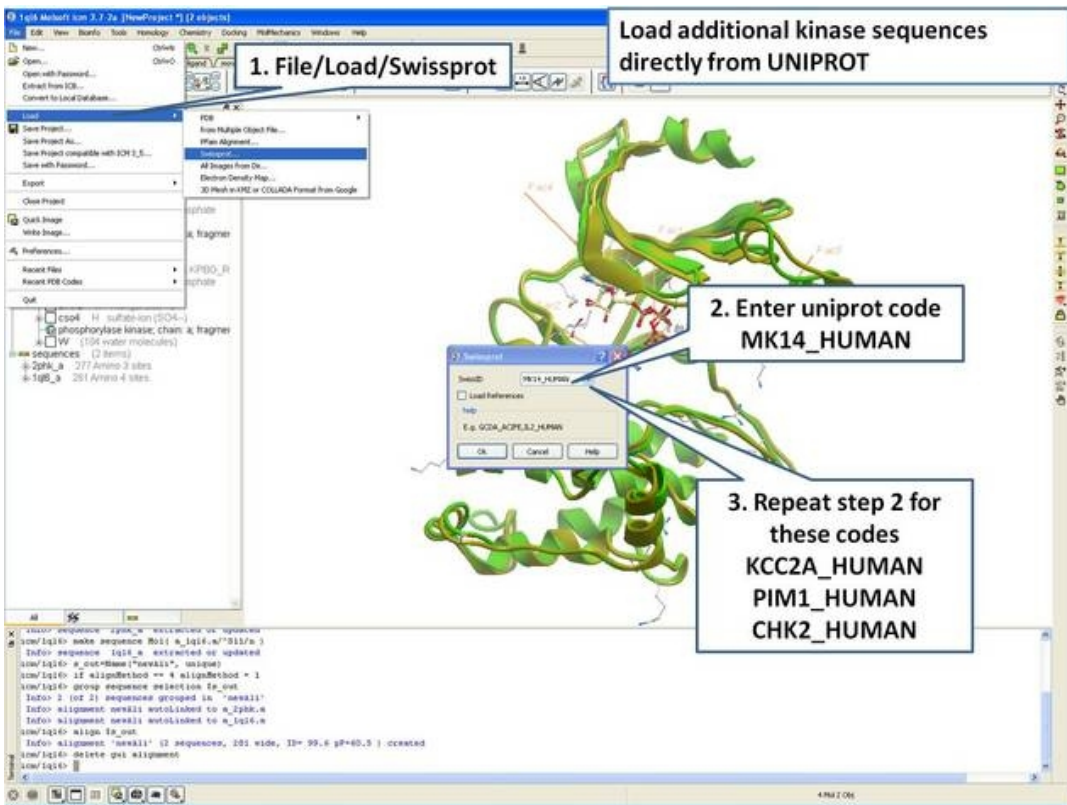
Step 7: Load UniProt sequences.
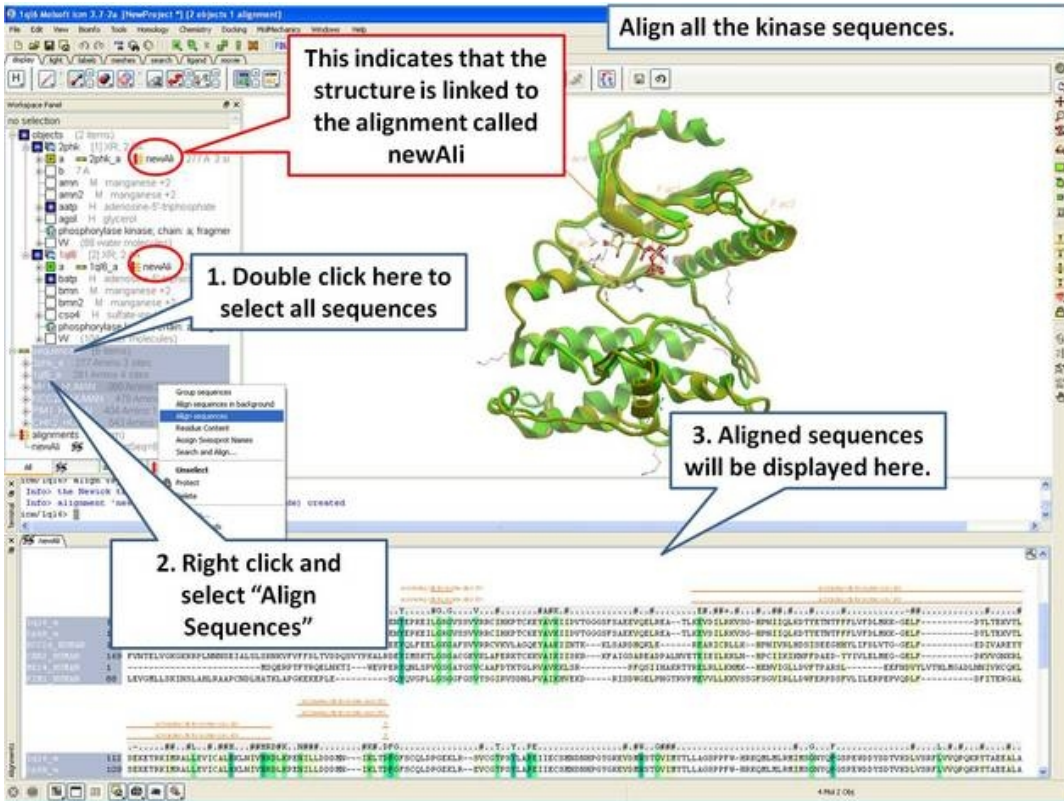
Step 8: Make a sequence alignment.

8.4.2 Linking Sequence Alignment to Structure

An alignment can be directly linked to the 3D structure. If the sequence has been extracted from a displayed PDB file this will be done automatically. If multiple alignments are loaded you can change the link using Bioinfo/Link to Structure... menu. This examples continues from the previous section.

Step 9: Color the alignment by sequence conservation.

Step 10: Color the 3D structure by the alignment color.

8.4.3 Identify Sequence Conservation in Ligand Binding Pocket

In this example we show how to display sequence conservation in the ligand binding pocket. This examples continues from the previous section.
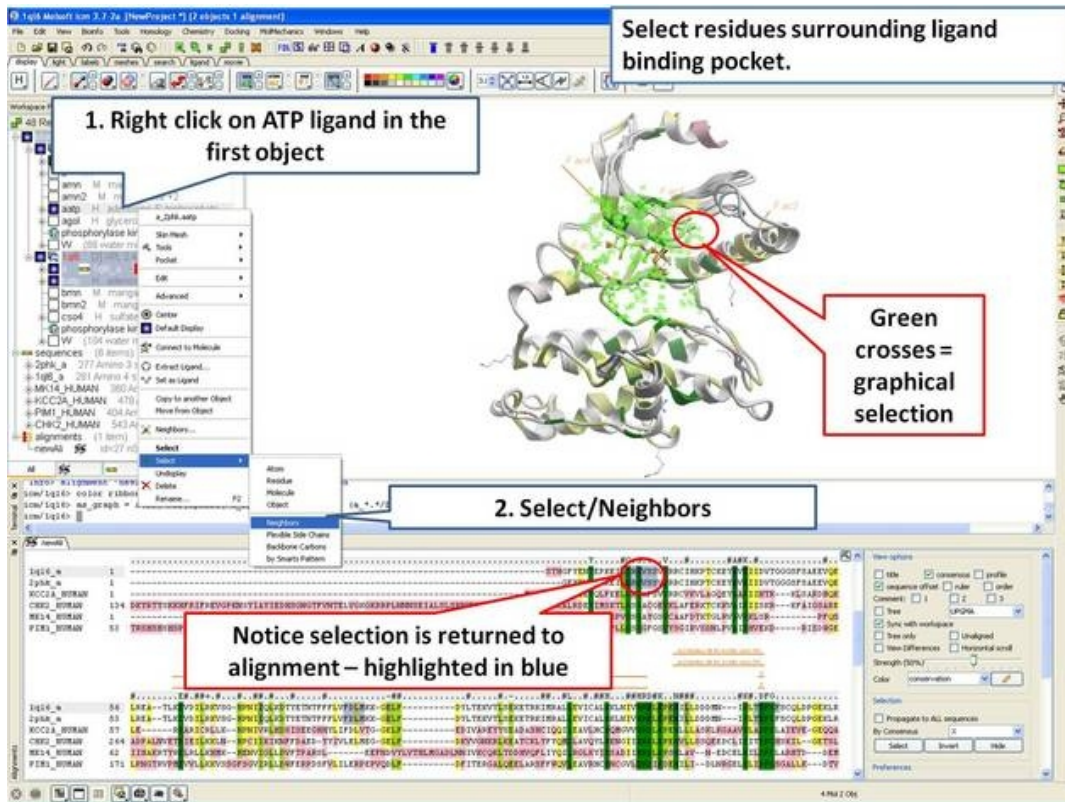
Step 11: Select the residues surrounding the ligand binding pocket (or any other selection of interes).

Propagate selection to all sequences in alignment.

Notice selection is propagated to all sequences in the alignment – highlighted in blue

1. Propagate to all sequences

Step 12: The 3D structure and alignemnt are linked, therefore the selections made in 3D are transferred to the alignment. Only the sequences that have 3D structures will be selected in the alignment, therefore we need to propagate the selection to all sequences in the alignment.

Display only the residues in the ligand binding pocket.

Residue conservation in the ligand binding pocket

3. Click again to restore view

2. Hide selection

1. Invert selection

Step 13: Display only the residues in the pocket in the alignment.

Step 14: Display the residues surrounding the pocket in stick representation.

Step 15: Color stick by the alignment color.

8.4.4 Alignment Annotation

Introduction

G-Protein Coupled Receptors (GPCRs) all share a common structural core
of seven transmembrane helices but they lack significant sequence
homology between subfamilies. When modeling GPCRs it is important to get
a good alignmnent between the query and template structure. Each helix
has one or more conserved motifs:

Helix 1: GX$_3$**N** or G**N**

Helix 2: N(S,H)LX$_3$**DX**$_{7,8,9}$ P

Helix 3: SX$_3$LX$_2$IX$_2$D(E,H)**RY**

Helix 4: **W**X$_8$,9P

Helix 5: FX$_2$**PX**$_7$Y

Helix 6: FX$_2$CW(Y,F)X**P**

Helix 7/Helix 8: LX$_3$NX$_3$N(D)**PX**$_2$YX$_{5,6}$ F

The ProSite class A alignment `http://prosite.expasy.org/PDOC00210` can
be used to guide GPCR alignments. For this example we have prepared an
icb file containing a subset of the class A GPCR sequences from ProSite.
We will use this file to annotate the key conserved motifs this will
help with the GPCR modeliing example. The file can be downloaded here:
`ftp://ftp.molsoft.com/pub/workshop/` (1. cut and paste the address into
a web browser. 2. Right click on gpcr_ClassA and choose "Save Link As".
3. Open the .icb in ICM File/Open).

Step 1: Read in the class A GPCR alignment (gpcr_ClassA.icb - see link
above). Make the alignment the main window (Windows/Alignment Main).

Display the secondary structure of PDB 3p0G in the alignment.

1. Click here to open tools panel.

2. Click here to display secondary structure.
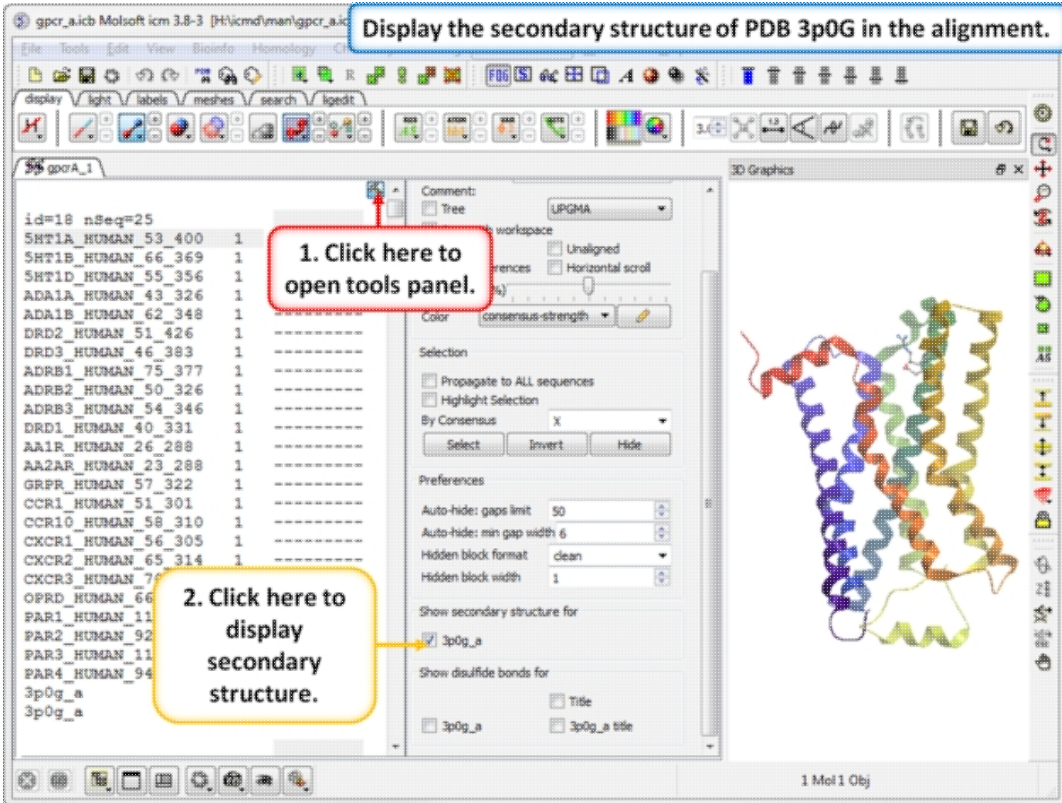
Step 2: The sequence of the beta 2 adrenoceptor is appended to the alignment. This allows us to display the secondary structure of the protein in the alignment.

Secondary structure is displayed colored cylinders and arrows under the alignment. Red Cylinder = alpha helix, green arrow = beta sheet, blue cylinder = pi helix, magenta cylinder = 3/10 helix.

Secondary structure
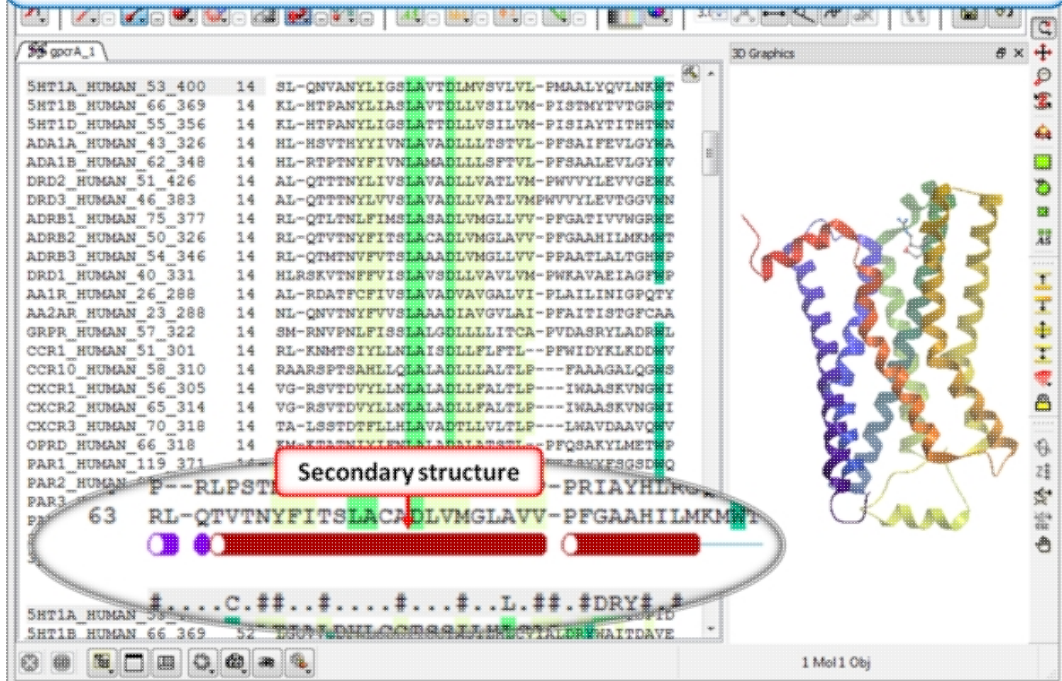
Step 3: Secondary structure is displayed as colored cylinders and arrows under the alignment. Red Cylinder = alpha helix, green arrow =

beta sheet, blue cylinder = pi helix, magenta cylinder = 3/10 helix.
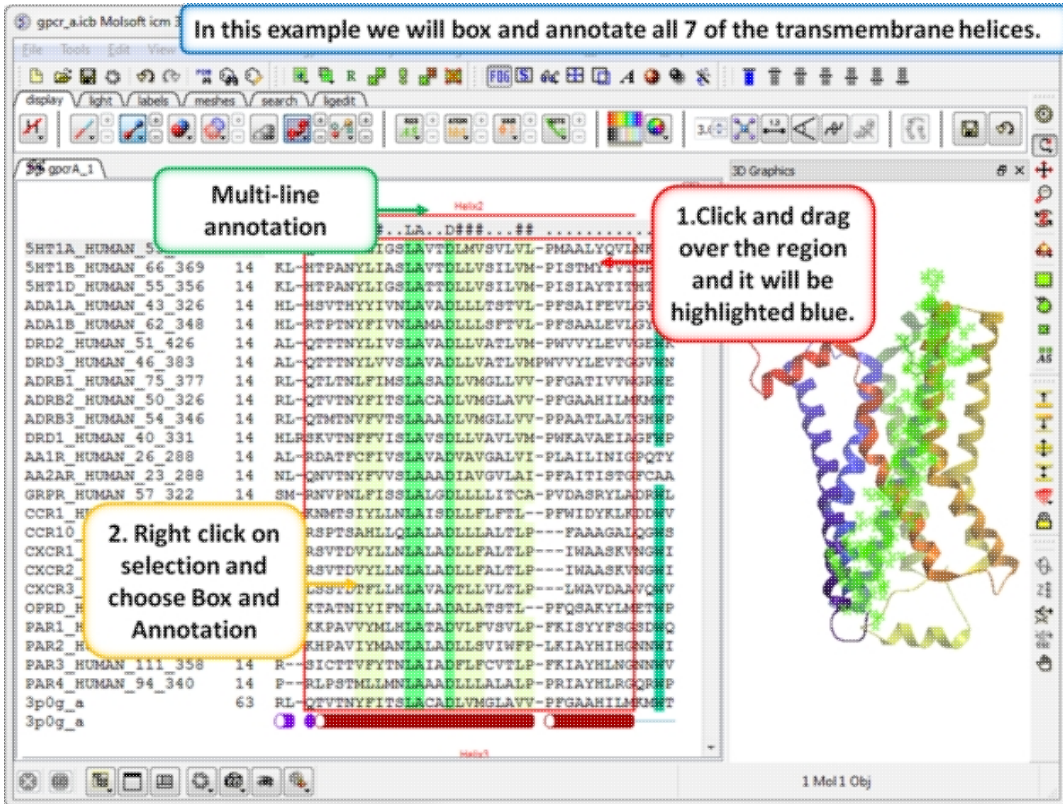
Step 4: Box and annotate the seven transmembrane helices.
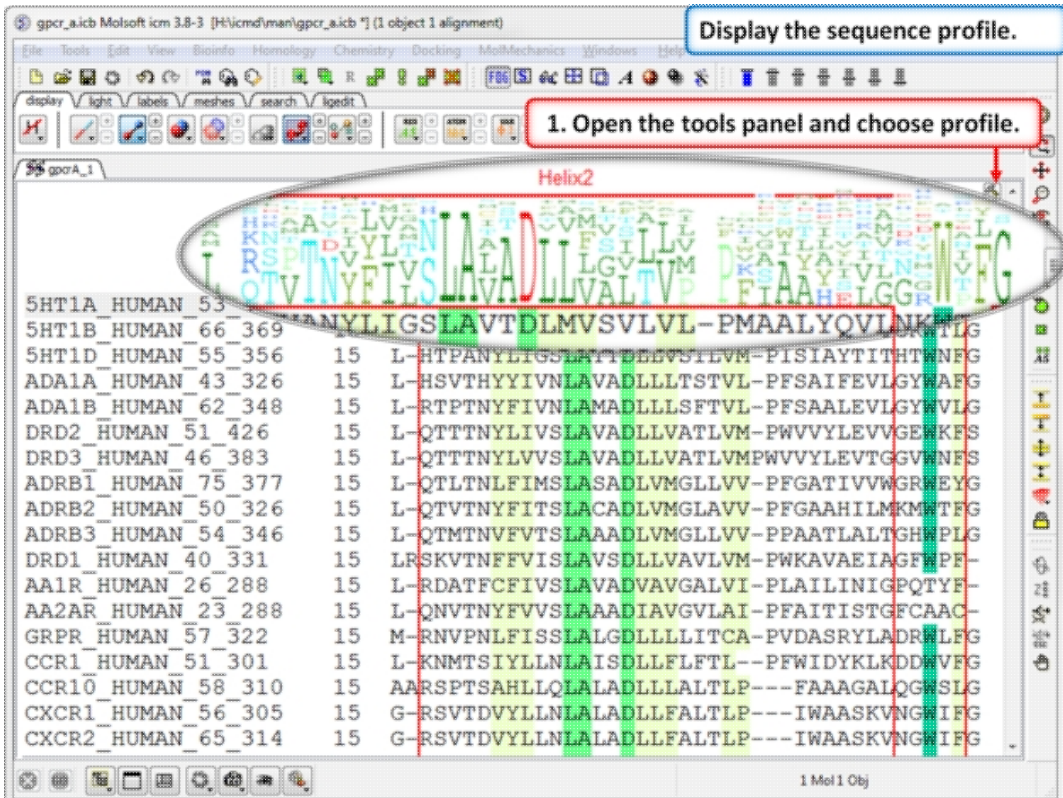
Step 5: A sequence profile helps to get a better understanding of the sequence conservation at each point.

To highlight the conserved motifs shade the residues that are 100% conserved.

1. Drag strength to 100%.

3. Right click on the selection and choose shade color.

2. Choose Select.

5HT1A_HUMAN_53_400	12	ERSL-QNVANYLIGSLAVT
5HT1B_HUMAN_66_369	12	TRKL-HTPANYLIASLAVT
5HT1D_HUMAN_55_356	12	TRKL-HTPANYLIGSLATT
ADA1A_HUMAN_43_326	12	HRHL-HSVTHYYIVNLAVA
ADA1B_HUMAN_62_348	12	NRHL-RTPTNYFIVNLAMA
DRD2_HUMAN_51_426		IVSLAVA
DRD3_HUMAN_46_383		VVSLAVA
ADRB1_HUMAN_75_377		IMSLASA
ADRB2_HUMAN_50_326		ITSLACA
ADRB3_HUMAN_54_346		VTSLAAA
DRD1_HUMAN_40_331		VISLAVS
AA1R_HUMAN_26_288	12	NQAL-RDATFCFIVSLAVA
AA2AR_HUMAN_23_288	12	NSNL-QNVTNYFVVSLAAA
GRPR_HUMAN_57_322	12	VKSM-RNVPNLFISSLALG
CCR1_HUMAN_51_301	12	YKRL-KNMTSIYLLNLAIS
CCR10_HUMAN_58_310	12	ARRAARSPTSAHLLQLALA
CXCR1_HUMAN_56_305	12	SRVG-RSVTDVYLLNLALA
CXCR2_HUMAN_65_314	12	SRVG-RSVTDVYLLNLALA
CXCR3_HUMAN_70_318	12	RRTA-LSSTDTFLLHLAVA
OPRD_HUMAN_66_318	12	YTKM-KTATNIYIFNLALA

View options
- title
- [x] consensus
- [x] profile
- [x] sequence offset
- order
- hide body

View Differences ☐ Horizontal scroll
Strength (100%)
Color   no color

Selection
- Propagate to ALL sequences
- Highlight Selection
By Consensus   X

Select   Invert   Hide

Preferences

Hidden block format   clean
Hidden block width   1

Show secondary structure for
- [x] 3p0g_a

1 Mol 1 Obj

Step 6: To highlight the conserved sequence motifs - set the consensus strength to 100% and then color the fully conserved residues. You may need to lower the conservation strength to pick out all the motifs.

Calculate sequence similarity and display residues at each position.

ADA1A_HUMAN_43_326	54	RVFCNIWAAVDVLCCTASIMGLCIISIDRYIGVSYPLRYPT
ADA1B_HUMAN_62_348	54	RIFCDIWAAVDVLCCTASILSLCAISIDRYIGVRYSLQYPT
DRD2_HUMAN_51_426	54	RIHCDIFVTLDVMMCTASILNLCAISIDRYTAVAMPMLYNT
DRD3_HUMAN_46_383	55	RICDIFVTLDVMMCTASILNLCAISIDRYTAVVMPVHYQH
ADRB1_HUMAN_75_377		YIALDRYLAITSPFRYQS
ADRB2_HUMAN_50_326		YIAVDRYFAITSPFKYQS
ADRB3_HUMAN_54_346		ALAVDRYLAVTNPLRYGA
DRD1_HUMAN_40_331	54	GSFCNIWVAFDIMCSTASILNLCVISVDRYWAISSPFRYER

1. Right click and choose "Calculate sequence similarity".

SeqSimilarity

	p29	p30	p31	p32	p33	p34	p35	p36	p37	p38	p39
1	N2	A3	C4	V5	V6	A7	A8	I9	A10	L11	E12
2	N2	A3	F4	V5	I6	A7	T8	V9	Y10	R11	T12
3	N2	A3	F4	V5	L6	T7	T8	I9	L10	L11	T12
4	N2	I3	L4	V5	I6	L7	S8	V9	A10	C11	H12
5	N2	I3	L4	V5	I6	L7	S8	V9	A10	C11	N12
6	N2	V3	L4						S10	R11	E12
7	N2	G3	L4						L10	K11	E12
8	N2	V3	L4						A10	K11	T12
9	N2	V3	L4						A10	K11	F12
10	N2	L3	L4	V5	I6	W7	A8	I9	A10	W11	T12
11	N2	T3	L4	C6	A7	A8	V9		I10	R11	F12
12	N2	V3	L4	V5	I6	W7	A8	V9	K10	V11	N12
13	N2	V3	L4	V5	C6	W7	A8	V9	W10	L11	N12
14	N2	T3	T4	I5	I6	W7	I8	F9	C10	T11	H12

2. Percentage similarity and residues at each position.

table: 25 rows, 470 columns   1 Mol 1 Obj

Step 7: Calculate the sequence similarity and display the alignment residue profile.

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