Table of Contents

Table of Contents

Table of Contents

Table of Contents

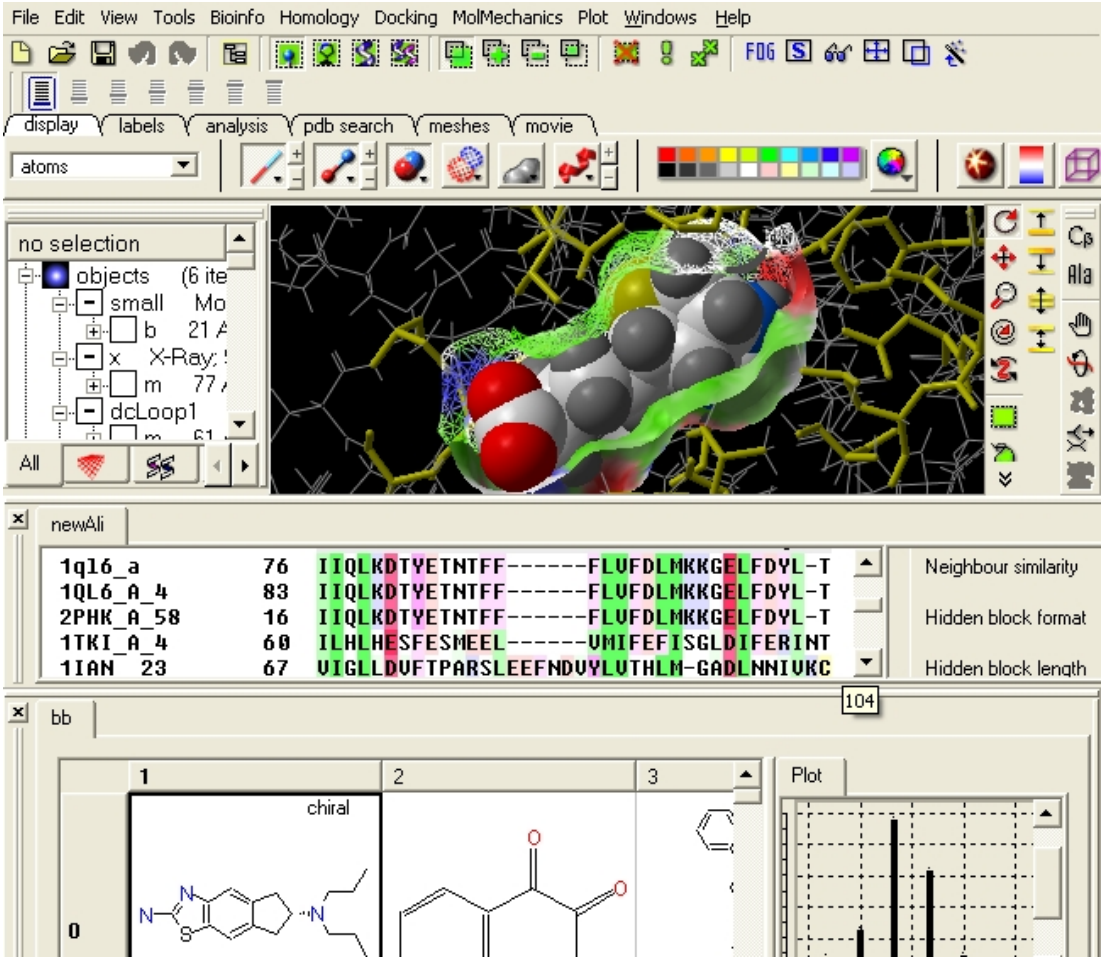
Table of Contents

Table of Contents

Table of Contents

1. Introduction

ICM provides an easy to use general environment for a biologist or chemist who is curious about structure. In just a few seconds you can browse hundreds of structures of interest, analyze and visualize sequences, alignments and binding sites.... Also you can perform molecular modeling, fully−flexible ligand and receptor docking, virtual ligand screening, chemical similarity searching, chemical clustering and much more.... This book describes how to use the program via the **GRAPHICAL USER INTERFACE (GUI)** without the knowledge of the commands and functions running through your terminal window. After reading this book you may read the full ICM language reference for the dying breed of command line users and occasional programmers. This is a separate document which is provided with a full ICM distribution or can be obtained from Molsoft in a printed form.



1.1. Release Notes

NOTE Additions to the manual describing new updates and improvements to ICM are available online at `www.molsoft.com` or in PDF format from our FTP site.

Maintenance Release 3.0.28d. Mar 8, 2004

- A problem with table column popup menus is fixed
- superimpose/Rmsd with **align** option has been extended to graphical alignment from the selection
- the linked alignment is shown more clearly
- the "save as sdf/mol file" now supports the names/formats of columns and ICM−actions for this table.
- "color by alignment conservation" schemes added
- ICM−GUI manual improved

Maintenance Release 3.0.28c. Mar 1, 2004

About 1% of PDB entries had problems with the **convert PDB** operation. Now this problem is fixed.

The swissprot references for the PDB search tool have been updated.

The loop database for homology modeling was updated (it is now 2 times larger than before)

(28b) A major problem which caused crashes and slowdowns with some graphics cards (e.g. `Intel(R) 82855/82865`) has been identified and fixed in 28b.

ICM−Browser(Pro) and ICM: Release 3.0.28. Feb 20, 2004

The following functionality is added to the this version compared to the previous 3.025j/m version released in January:

Graphical Interface:

- a detailed illustrated Graphics User Interface User's Manual (botn on−line and as a book).
- direct control of light to adjust brightness, shininess and other properties of the image (see the "display" tab).
- control over the default display of hydrogen atoms, (see the "display" tab)
- new cylinder representation of the helices in addition to the ICM ribbons (right click+hold on the Ribbon button and choose cylinders)
- nicer display of hydrogen bonds between space atoms shown as "balls−and−stricks" (need to convert an object)
- showing distances and non−linearity angles for hydrogen bonds (try + and − next to the hbond button)
- new drag and drop: to link a molecule to an alignment drag the alignment to the molecule
- ability to name graphical planes (left click on a plane button) and save the names with a project

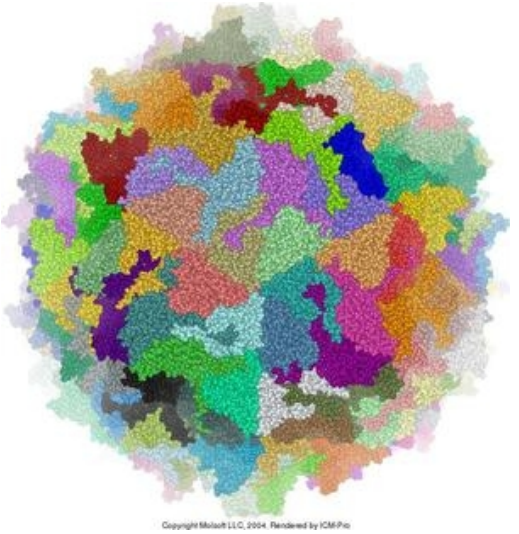
- the quick image button (a photo−camera icon in the top toolbar) for high quality images: set up your preferred resolution (the File/Preferences/Image IMAGE.writeScale preference) and save a png image of high resolution with one click.

Ligand Docking

- if multiple ligand conformations are retained (so called "stacks"), each low energy conformation will be displayed in the hit list table.
- docking in the batch mode is possible under Windows now
- chemical table with properties of the original sdf/mol−file (including catalogue IDs etc.) can be displayed side−by−side with the hit list table.

Tools:

- Easy "on−the−fly" generation of biologically relevant complexes from a PDB file (see the viral structures built with the "Tools.Biomolecules Generator")



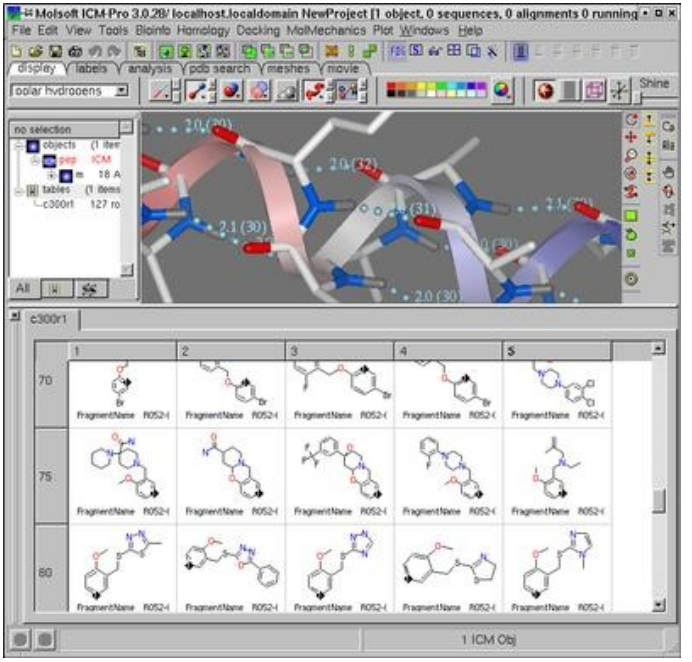
- improved generation of crystallographic neighbors from a selected patch
- improved icmMacroShape (see the "meshes" tab)
- saving movie frames under Linux.
- limits for the number of arrays in tables/chemical tables trippled. (now they are controlled by a separate parameter MnArrays in the icm.cfg file)
- PDB search tools: swissprot references added to the table, the list of ligands in each pdb entry expanded and updated (try the "pdb search" tab and enter an asterisk)
- Direct downloading of swissprot sequences from the File/Load menu or from a molecule popup menu (in this case direct alignment of the sequence to reveal omissions in the structure).

Alignment builder, viewer and editor

- Multiple alignments: building several types of evolutionary trees and rearranging sequences accordingly
- reading and writing the secondary structure display information in multiple alignments
- a bug in writing alignments in the msf format is now fixed
- showing Pi and 3/10 helices in alignments with different colors (try 1c3w, then "Assign Sstructure")

Chem−Informatics:

- Built−in Chemical Editor
- Improved chemical views, convenient shortcuts (Ctrl−3,4,5,6, Ctrl−T).



- Chemical clustering, selecting one representative per cluster
- Printing/exporting chemical tables

Table Data manipulation and analysis

- selecting columns or column ranges, hiding columns, changing column order
- merging several numerical columns into in−line plots
- improve table editing

1.2. Who Should Read This Book

Most of the things you will read here are sort of natural or can be figured out by common sense and trial−and−error. However, if you like to read a "structured" description of the material, go ahead and read this.

Since this book is intended for basically anyone who is even remotely interested in molecules, some basic knowledge of biology and chemistry is implied. Do not try to find definitions of "atom" or "sequence" here, but most terms beyond that will be explained. A keyword search of this manual is availble on the online version at `www.molsoft.com`.

This book covers the graphical user interface only. For detailed information regarding ICM programming please consult the seperate ICM language reference guide.

1.2.1. Basic Prerequisites and Assumptions

Before you can begin you need to download and install ICM−Browser, ICM−Browser−Pro, or ICM−Pro. See the section entitled `ICM Installation` for instructions on how to do this.

1.2.2. Notations

Sometimes we will use **Unix** as a collective term for all non−Windows operating systems

(RH Linux, SUSE Linux, Irix, Dec OSF, etc.).

After all it is only fair to refer to the original.

GUI = Graphical User Interface

1.3. ICM Installation

Here we describe how you can download and begin using the ICM program.

1.3.1. Installing ICM

In order to install ICM you need to register with Molsoft LLC (http://www.molsoft.com). Your E−mail address will become your username and you will obtain a password. This can be achieved by either sending an E−mail to support@molsoft.com or clicking on any download button on the site, which will take you to a **New Registration** window.

Once registered and the necessary payments have been made you are able to download ICM by going to the Molsoft LLC Website (http://www.molsoft.com) by once again clicking on the download button.

- Enter your username (E−mail address) and password.

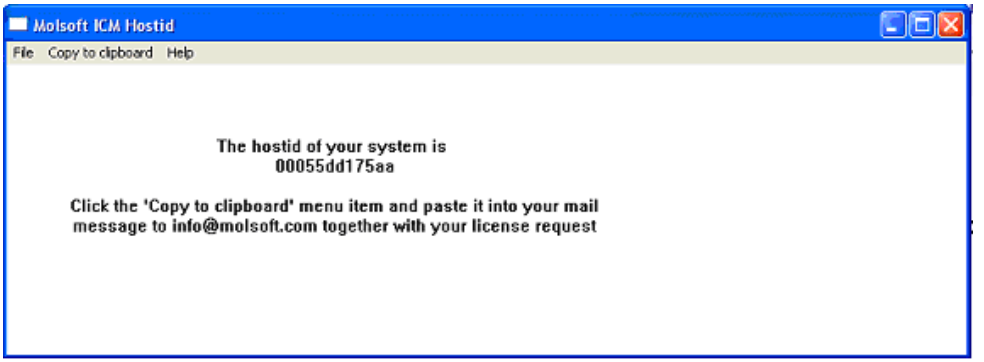
NOTE: Instructions are provided on this page for registering with Molsoft or how to find out your password if you have forgotten it.

- Click on the appropriate operating system for the machine on which you wish to run ICM. ICM will run on the following platforms: Windows98/NT/2000/XP, MacOSX, RedHatLinux and SGI.
- Choose the appropriate ICM product that you wish to download.

You can either follow the instructions on the web page OR carry on reading this section of the manual

- Click on the word **hostid**
- Click the **open** button to obtain your unique hostid number.

If you see this window displayed on your screen then you are well on your way to downloading ICM.



- Click the **copy to clipboard** button.
- Write an E−mail to info@molsoft.com and paste the id number into this E−mail to request a license for the product.
- Download the ICM distribution package by clicking on `ICM−3.0.021.exe`

1.3.2. Windows Platform

There are several ways to start ICM under Windows, including the following:

1. Click the **Start** button on the taskbar, then select **Programs/Molsoft/ICM**
2. Double−click the file with one of the ICM extensions, including pdb files (`*.pdb`) icm projects and binaries (`*.icb`), and other ICM file types.
3. Create a shortcut to the ICM binary and click the

4. Start a command prompt window and type the path the ICM binary, usually it is `C:\Program Files\Molsoft LLC\ICM\icm.exe -g` . In this case you can start ICM with different options.

1.3.3. Unix Platform

Under a **UNIX** platform your executables will reside in the `$ICMHOME` directory.

`$ICMHOME` is an environmental variable of your UNIX shell and it needs to be set to the actual location of the icm files. The installation procedure does tell you to what value the ICMHOME variable needs to be set.

Examples:

```
/usr/icm/icm -X   # returns
/usr/icm/icm -g   # -g means
/usr/icm/icm -h   # help
/usr/icm/icm -bio -g # starts ICM-bio program with gui
```

Once you are in ICM you can spawn another window by choosing **File/New** ICM Window .

In this case if you close the main ICM window, all the children will be closed too.

1.3.4. Activate the Graphical User Interface.

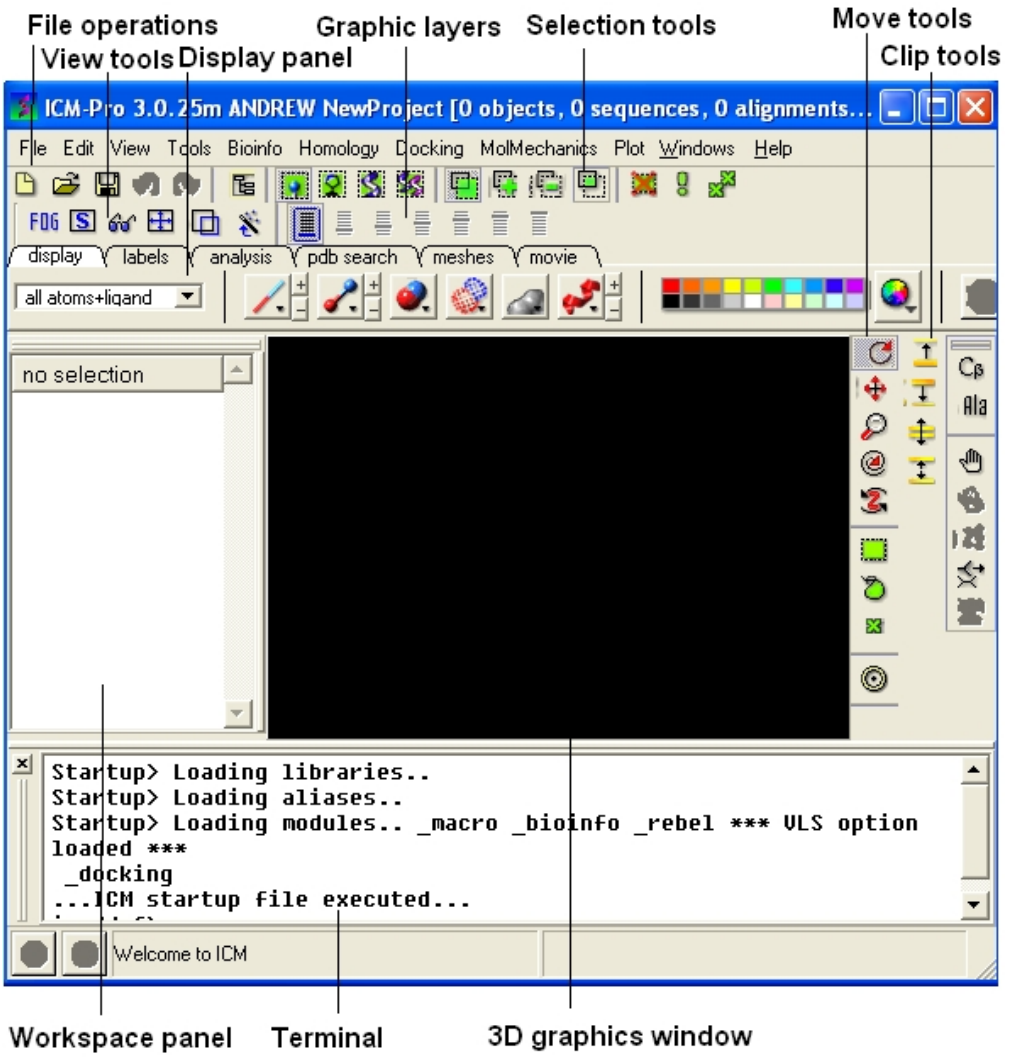
If you are running ICM in Windows then the graphical user interface will be displayed automatically.

However in Unix the GUI version of ICM can be activated by typing icm -g or icm -G and hitting RETURN. Or, to start the graphical user interface from the ICM command line, simply type gui.

2. Graphical User Interface Overview

The graphical user interface (GUI) has many components and is designed to make the life of an ICM user easier. Rather than constantly typing commands in the ICM command line you can now point and click on buttons and menus.

2.1. Default GUI Layout

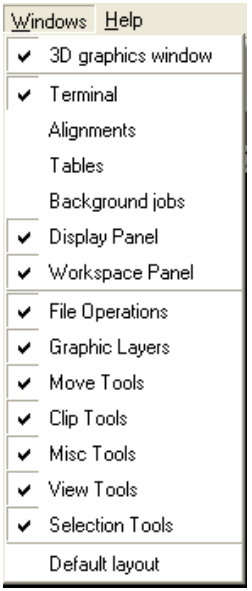


When you first use the GUI the default window layout is displayed as shown above.

2.2. Window Menu

This menu allows you to choose which windows you wish to display. The windows which open automatically when you first open GUI are shown in the `Default GUI` 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.

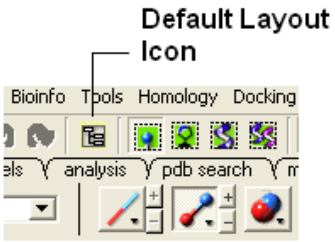
To add or remove windows from the GUI display select the 'window menu'. Other windows not included in the default display such as tables and alignments can be added.



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

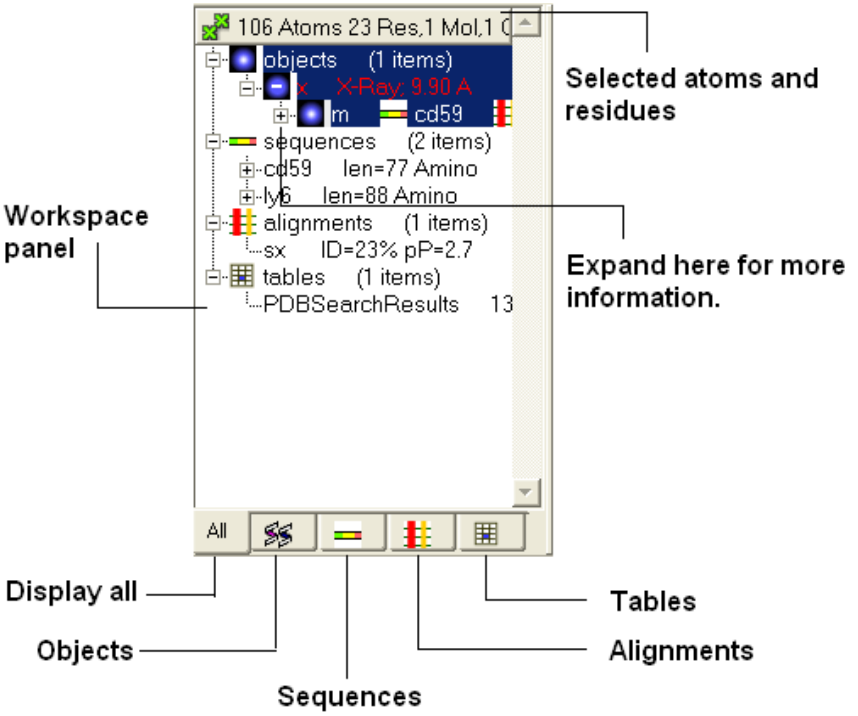
OR

Click the default layout icon.



2.3. Workspace Panel

The workspace panel (see `GUI Overview` for location in 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. Also, from this panel you can make graphical selections and drag and drop objects and sequences to other locations within the GUI.



2.4. GUI Helpful Tips

This section describes some useful tips and suggestions to make life using the graphical user interface easier and more time efficient.

2.4.1. 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.

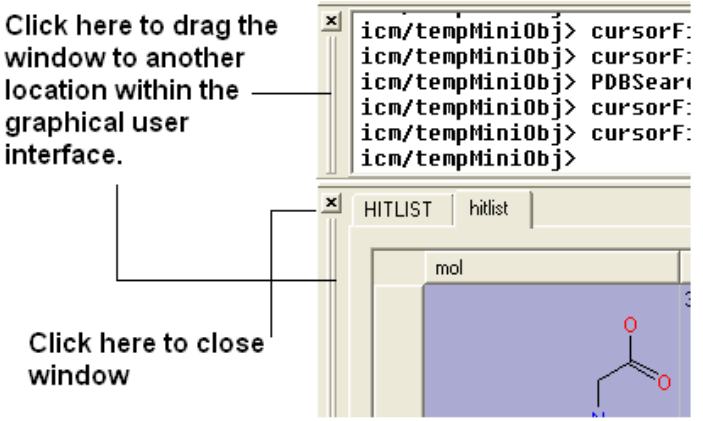
2.4.2. Right Click

NOTE: Use the Right Click Mouse Option

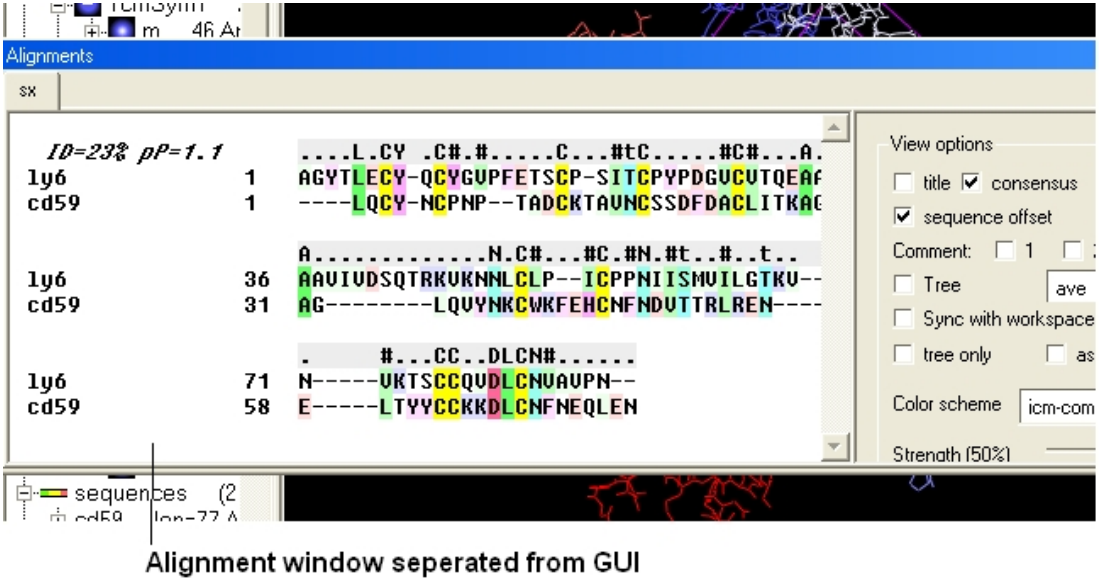
The right click mouse option can be used throughout the graphical user interface. It is a very useful means of opening up a whole new world of menus and options. Most of these options are described in this book. However, when using the graphical user interface it is always a good idea to try right clicking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

2.4.3. Moving Windows

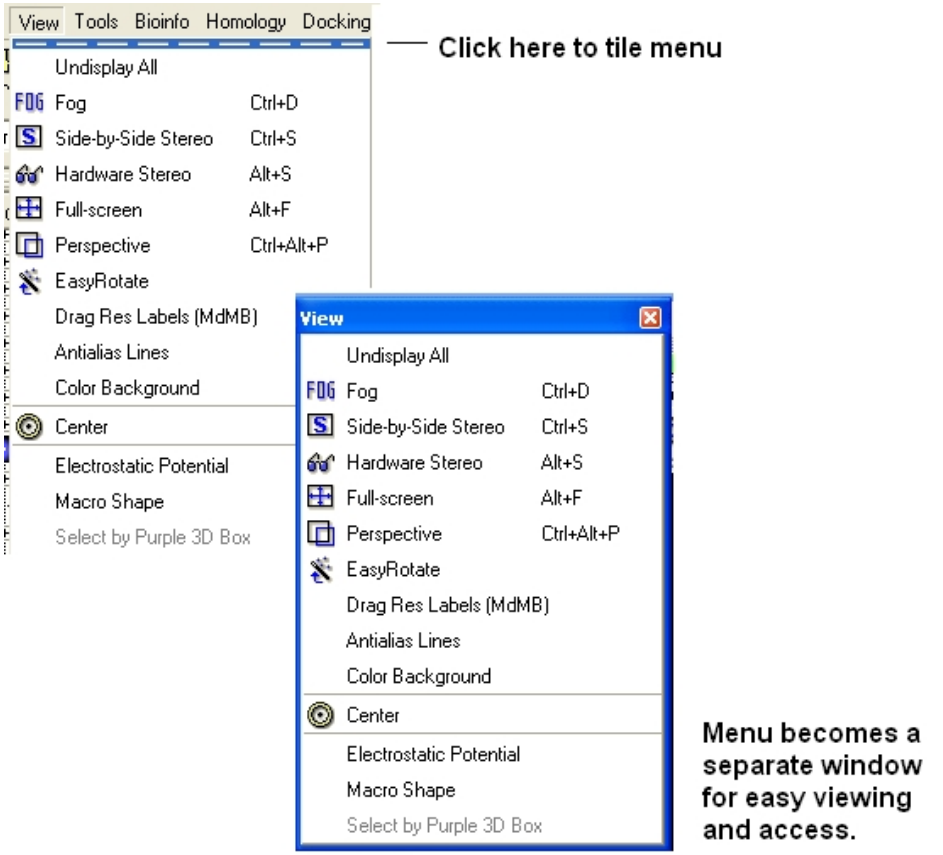
It is possible to move some windows around the graphical user interface to make viewing easier.



For example, you may wish to edit an alignment by moving the alignment display into a more accessible place.



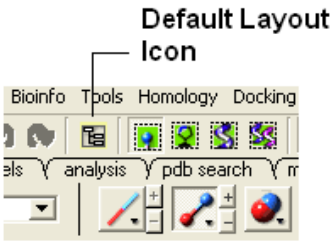
It is also possible to seperate menus from the GUI.



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

OR

Click the default layout icon.



OR

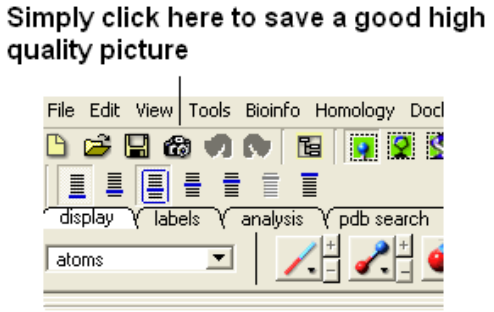
Double click on the window header.

2.4.4. 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.

2.4.5. Making 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 camera button in the view tools panel.



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. Graphical User Interface Basics

In this section you will learn the basics of ICM−GUI including, opening ICM files, saving them and constructing new ICM objects.

3.1. Reading and Saving Your ICM Data

There are two ways to save your work and data in ICM 1) save project and 2) save object. Both will be discussed in this section of the manual. If you finish working with ICM and you know you will want to return to the project at a later date then the easiest thing to do is use the save `project option`. This option is useful for sharing your project with other members of your team.

Any file that ICM can understand can be loaded into ICM by selecting `File/Open`.

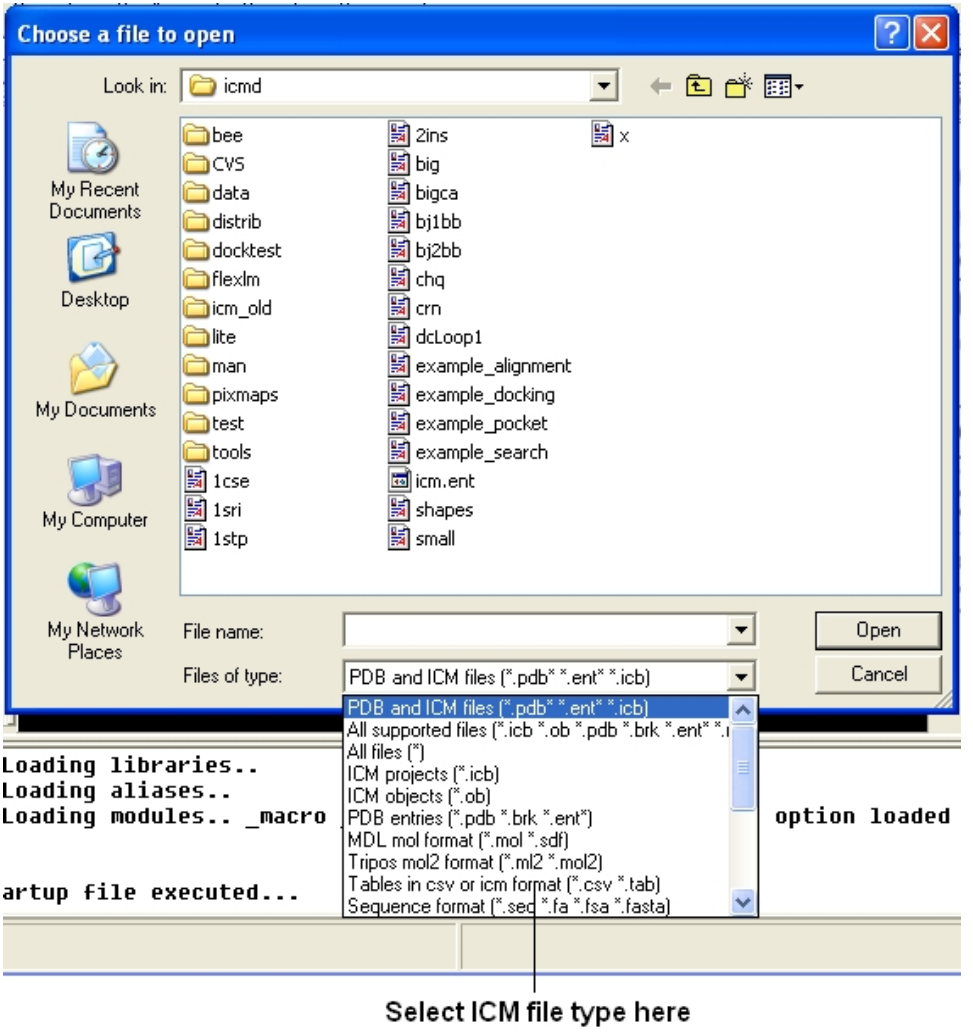
3.1.1. Open an ICM File

Any file that ICM can understand can be opened by:

- Selecting File/Open.

The file types ICM can read and the File/Open screenshot is shown below:

PDB and ICM files (*.pdb* *.ent* *.icb)
All supported files (*.icb *.ob *.pdb *.brk *.e
All files (*)
ICM projects (*.icb)
ICM objects (*.ob)
PDB entries (*.pdb *.brk *.ent*)
MDL mol format (*.mol *.sdf)
Tripos mol2 format (*.ml2 *.mol2)
Tables in csv or icm format (*.csv *.tab)
Sequence format (*.seq *.fa *.fsa *.fasta)
Alignment in ICM format (*.ali)
Alignment in fasta format (*.fa *.fsa *.fasta)
Swissprot format (*.swi *.dat)
ICM movies (*.mov)
conf stack (*.cnf)
3D mesh (*.obj)
ICM maps (*.map)
Amino acid comparison matrix (*.cmp)
Sequence profile (*.prf)



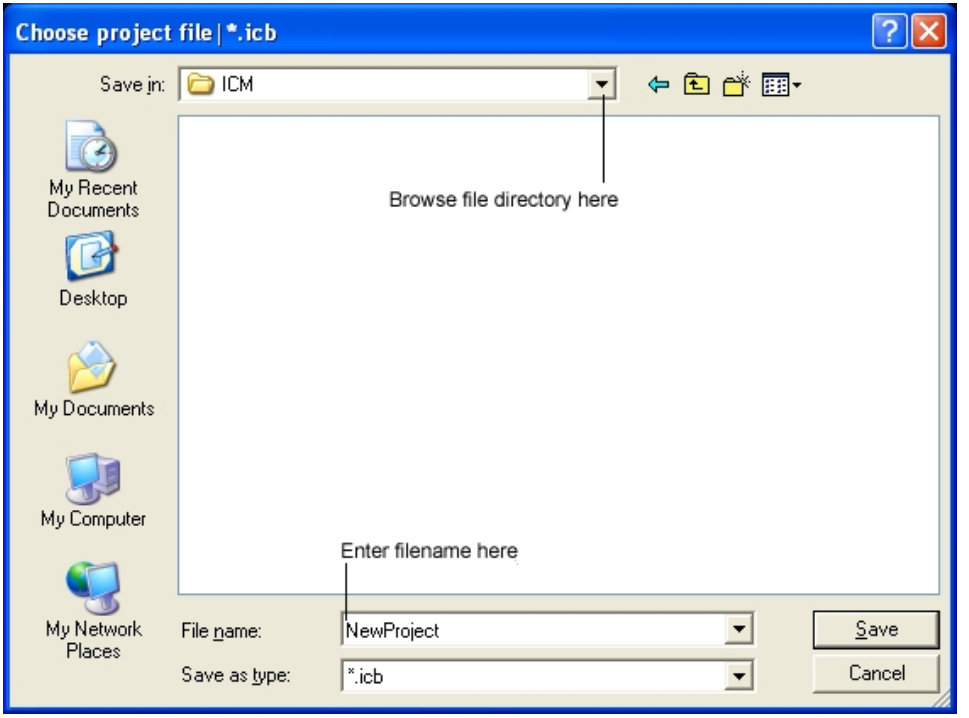
3.1.2. Saving an ICM Project

Saving a project will allow you to quit from ICM and then return to the exact set−up at which you left off at a later date. A complete history of your ICM actions will be saved so that you can pick up exactly where you finished on your previous ICM session.

To save a project:

- Select **File/Save Project** and a data entry window will be displayed. This window will only appear if this is the first time you have saved a project.

- Enter the unique name you wish to call your project in the box labeled **File name:**



- Choose which folder or directory you wish to save your project in by scrolling down in the box labeled **Save** in:
- Once the appropriate information has been entered click on the **Save** button in the bottom right hand section of the window.
- The project is now saved as yourfilename.icb.

NOTE: An alternative way to save a project is to click on the save icon on the toolbar.

3.1.3. Renaming a Saved Project.

If you wish to re−name the project or save different versions of the same project use the **Save Project As** option.

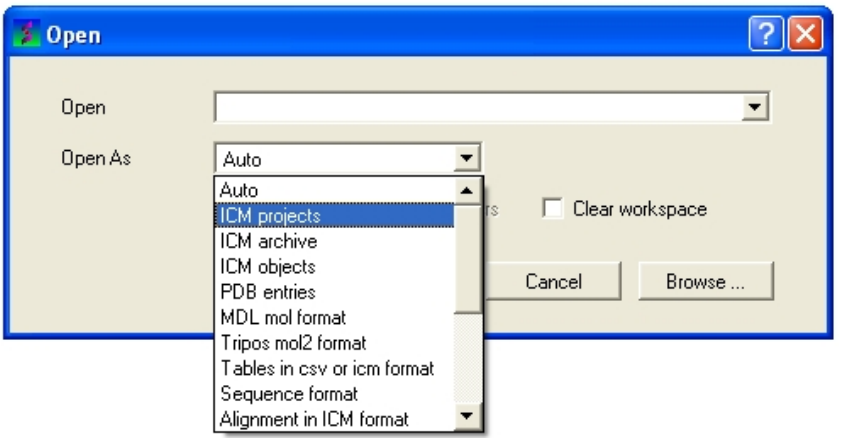
To rename a project:

- Click on the **File/Save Project** option and a data entry window will be displayed. This window will only appear if this is the first time you have saved a project.
- Enter the unique name you wish to call your project in the box labeled **File name:**
- Choose which folder or directory you wish to save your project by scrolling down in the box labeled **Save in:**
- Once the appropriate information has been entered click on the **Save** button in the bottom right hand section of the window.
- The project is now saved as yourfilename.icb.

3.1.4. Reloading a Saved Project When ICM is Running

To reload a saved project when ICM is running:

- Select **File/Open** and a data entry window will be displayed.



- Locate your saved project by clicking on the Browse button in the bottom right hand section of the window.

NOTE: All ICM project files are labeled yourfilename.icb . To make your search easier you can limit the number of files you search through by scrolling down in the "Open as" section and selecting ICM projects.

- Click on **OK** button when the file has been located and your saved project will load.

NOTE: If the file you wish to load has been viewed recently then it will be in the drop down menu in the Open box.

3.1.5. Reloading a Saved Project in Windows when ICM is not Running

To reload a saved project in Windows simply find the file in My Computer and double−click on the icon.

3.1.6. Recent Files

Recently viewed saved projects 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.

3.1.7. Saving an 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.

3.1.8. Reloading a Saved ICM Object when ICM is Running

Once an ICM object has been saved you can re−read it by:

- Click on **File/Open** and a data entry window will be displayed (below).
- Locate your saved ICM Object by clicking on the **Browse** button in the bottom right hand section of the window.

NOTE: To make your search easier you can limit the number of files you search through by scrolling down in the Open as section and selecting the appropriate file ending.

- Click on the **OK** button when the file has been located and your saved ICM Object will load.

NOTE: If the file you wish to load has been viewed recently then it will be in the drop down menu in the Open box.

3.1.9. Reloading a Saved ICM Object in Windows when ICM is not Running

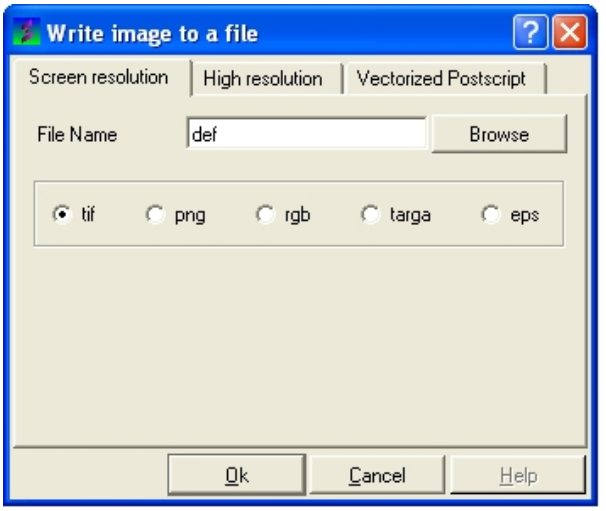
To reload a saved project in Windows simply find the file in the "My Computer" file store and double−click on the icon.

3.1.10. Writing an Image

Once a beautiful ICM image has been constructed you have the option of saving it as an image.

To save and write an image:

- Select **File/Write Image** and the following window will be displayed:



- Enter the name for the picture in the **File name** data entry box.
- Select which file format you would like to save the picture in by clicking in the circular selection button next to the file types. The options are .tif; .png; .rgb; .targa .eps.
- To specify which resolution you wish the picture to be saved click on the **High resolution** button at the top of the panel.
- Click the drop down arrow in the **Resolution Increase** data entry box and select which resolution you require the picture to be. Alternatively you can type the resolution you require into this box.

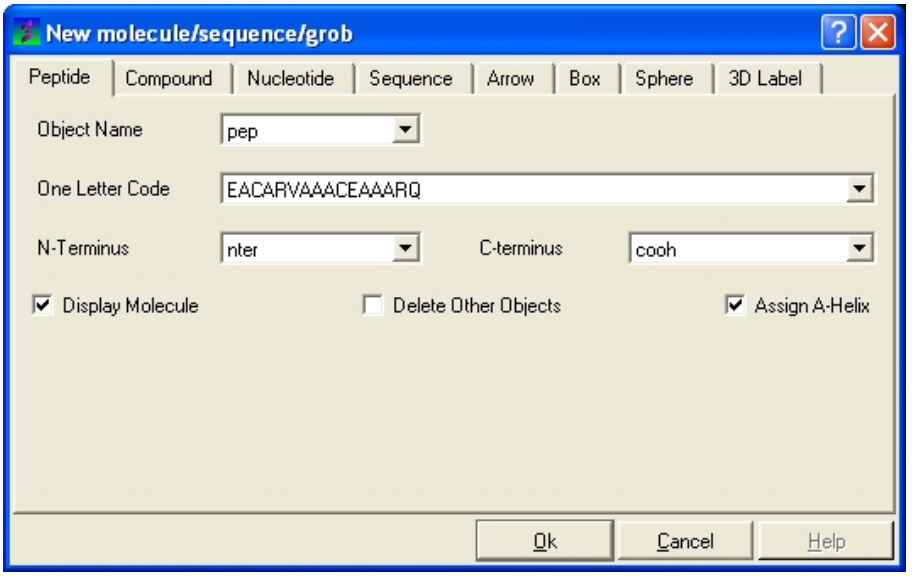
3.2. Exiting ICM

No problem. You do one of the following:

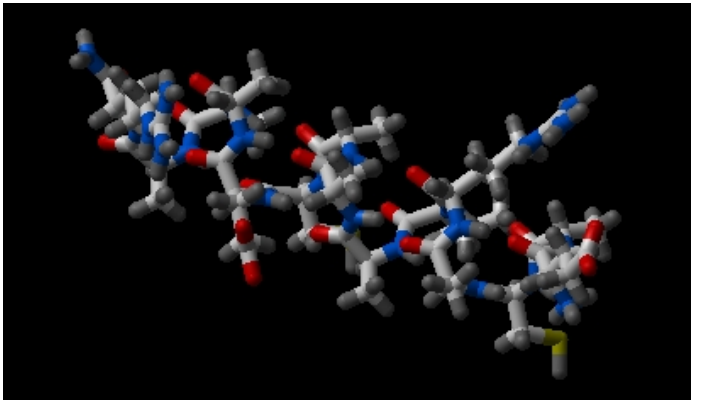
1. Select **File/Quit.** ICM will quit without saving files.
2. Choose the **File/Save** As menu item to save the project for the first time and then select **File/Quit**
3. Click the **Save** icon to save the project and then click **File/Quit**
4. Save and Click **X** at the upper right corner of the ICM window.
5. Type `quit` in the terminal window.

3.3. Constructing New Objects

All the processes in this section can be found under **File/New,** in the **New molecule/sequence/grob window**



3.3.1. Constructing a New Peptide



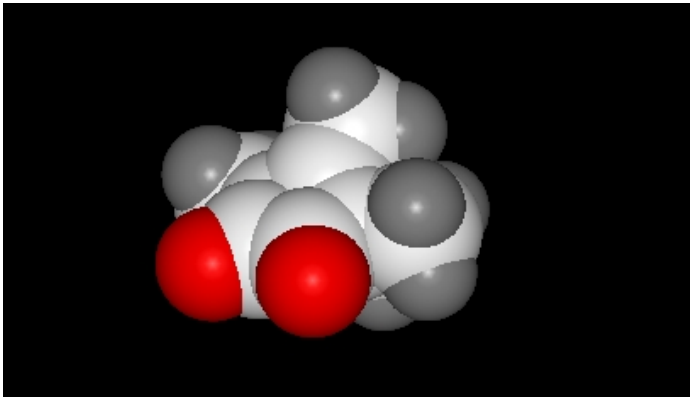
To construct a new peptide:

- Select **File/New** and the **New molecule/sequence/grob window** will appear.
- Type the peptide sequence into the One letter code data entry box. Remember to delete the previous entry if it is in the box.

NOTE: If the peptide you wish to make has been made previously then it will be in the drop down menu in the One letter code box.

- Select the appropriate N−terminal and C−terminal from the drop down menu.
- Check the boxes Display Molecule Delete Other Objects Assign A−Helix (Alpha Helix) according to your particular preference.
- Click the **OK** button.

3.3.2. Constructing a New Compound



To construct a new compound:

- Select **File/New** and the New molecule/sequence/grob window will appear.
- Click the **Compound** tab at the top of the window.

OPTION 1:

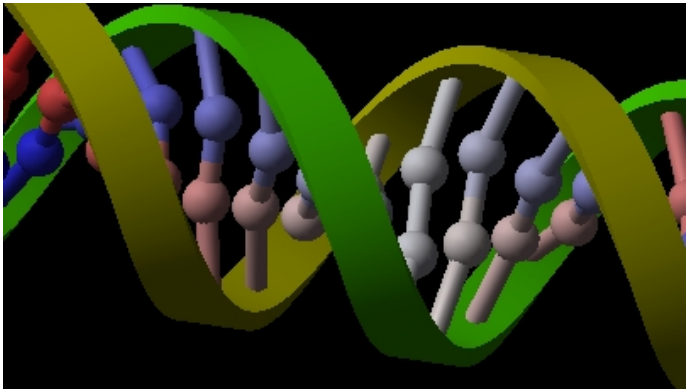
- Type in the Smiles String in the Smiles String data entry box. Remember to delete the previous string. If a string has been entered previously it will be available by clicking on the drop−down button.
- Check the boxes Display Molecule Delete Other Objects according to your preference.
- Click the **OK** button.

OPTION 2:

- Click the Launch Molecule Editor button.

Please refer to the Launch Molecule Editor section of this manual for instructions.

3.3.3. Constructing New DNA or RNA



To construct new strand of DNA or RNA:

- Select **File/New** and the **New molecule/sequence/grob** window will appear.
- Click the **Nucleotide** tab at the top of the window.
- Check the appropriate box for the nucleotide you are constructing, either DNA RNA or DNA Duplex
- Enter the nucleotide sequence into the **One Letter Code** data entry box. Remember to delete the previous nucleotide sequence. If a sequence has been entered previously it will be available by clicking on the drop−down button.
- Check the boxes **Display Molecule** or **Delete Other Objects** according to your preference.
- Click the **OK** button.

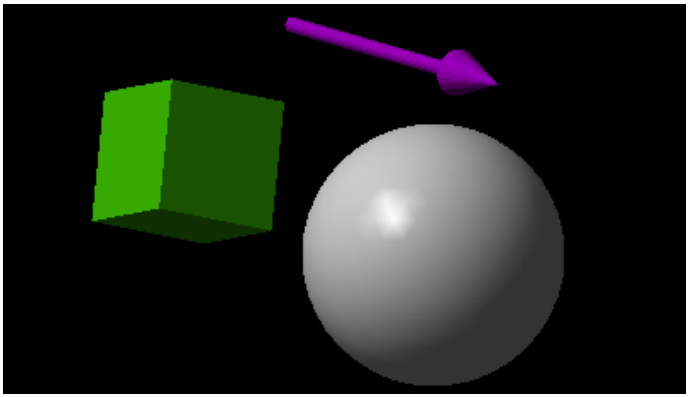
3.3.4. Constructing New Protein and Nucleic Acid Sequences

To construct a new protein and nucleic acid sequence:

- Select **File/New** and the **New molecule/sequence/grob** window will appear.
- Click the **Sequence** tab at the top of the window.
- Copy and paste a Fasta−format sequence into the Sequence data entry box.

- ICM will automatically determine what kind of sequence you have constructed but if you wish to specify then you can check either the protein or nucleic acid box.
- Click the **OK** button.

3.3.5. New Graphical Objects



To construct a new graphical object, such as a arrow, box, sphere, or text:

- Select **File/New** and the **New molecule/sequence/grob** window will be displayed.
- Click the Arrow, Box, Sphere, or Text tab at the top of the window, according to your desired object.
- Adjust the color and size of your graphical object.
- Click the **OK** button.

4. Working with the PDB

Protein structures solved by X−ray crystallography, NMR or other experimental methods are stored in the Protein Data Bank (PDB). These structures can be easily accessed, displayed and analyzed using ICM.

4.1. Finding a PDB Structure

There are four ways to find a structure from the PDB database and load it into ICM:

1. Query by keyword or PDB code.
2. Query by sequence pattern.
3. Query by sensitive similarity search.
4. Load the PDB file directly from FTP, http, local drive.

4.1.1. Querying PDB by Keyword or PDB Code

There are four ways of querying the PDB using ICM and keywords. OPTION 3 allows for a much more refined search.

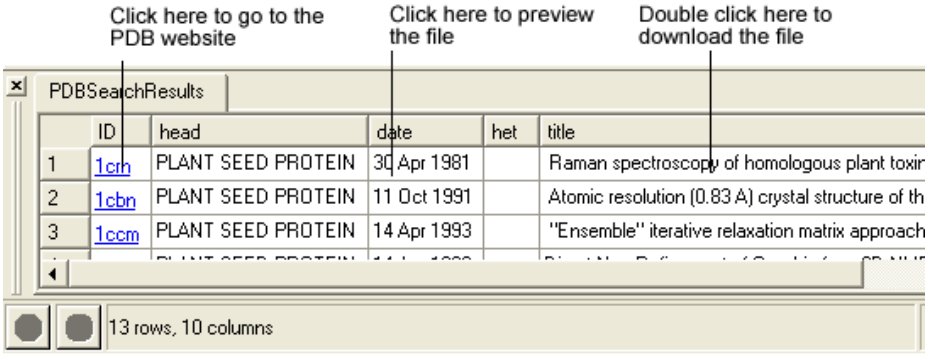
OPTION 1:

- Select **Edit/PDB** search and the "Find PDB Entries by Keyword" data entry window will be displayed.
- Enter a keyword or PDB code into the Keywords data entry field.

 :

NOTE: If a keyword has been entered previously it will be available by clicking on the drop−down button.

- Click the **OK** button and a list of related PDB entries will be displayed in the PDBSearchResults section of the graphical user interface.



OPTION 2:

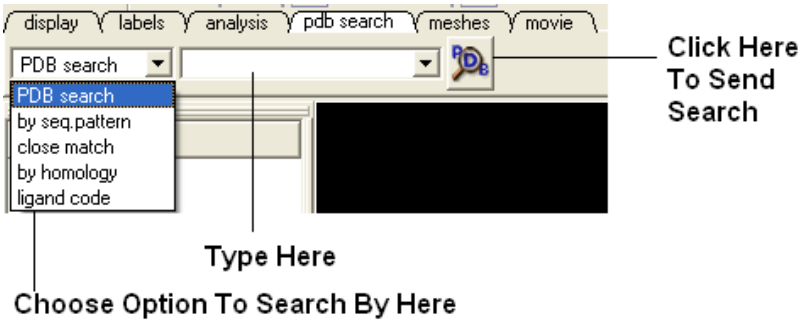
Use the pdb search tab on the tool bar. Select which parameter you wish to search by. Enter some text and this will be searched against the PDB.

Seq Pattern− Enter a protein sequence and this option will tell you whether a protein structure exists in the PDB for that sequence.

Close Match − Enter a protein sequence and this option will tell you which sequences are similar to your entered sequence.

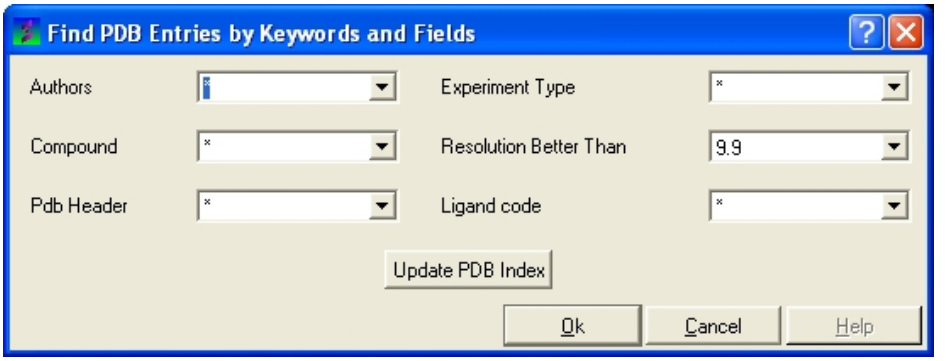
Homology − Enter a protein sequence and homolopgous proteins in the PDB will be displayed in a table.

Ligand Code − Enter the PDB ligand code.(e.g. 1crn)



OPTION 3:

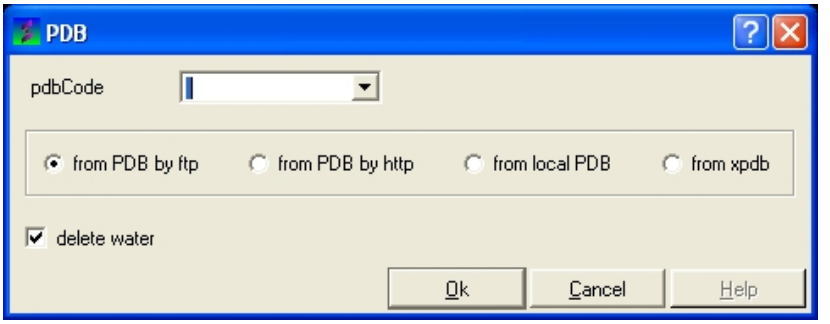
- Select **Edit/PDB** search by field and the ** Find PDB Entries by Keywords and Fields ** data entry window will be displayed.



- Enter your search words into the appropriate fields: **Author; Compound; PDB Header; Experiment Type; Resolution Better Than**
- Click the **OK** button.

OPTION 4:

- Select **File/Load** and the PDB data entry window will be displayed.
- Enter the PDB code and select the source of your PDB file.



4.1.2. Querying PDB by Sequence Pattern

There are two ways to query the PDB by sequence pattern.

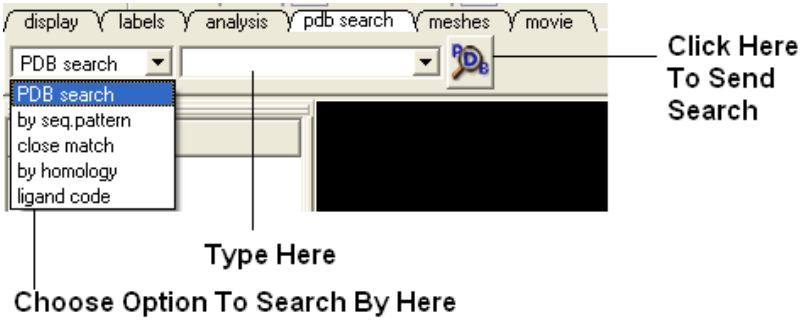
OPTION 1:

- Select **Edit/PDB Search by sequence pattern**
- Cut and paste or type your sequence into the Sequence data entry field.

- Choose whether you wish to display All entries or Entries with unique sequence by checking the appropriate button.
- Click the **OK** button.

OPTION 2:

Use the word search option on the tool bar



4.1.3. Sensitive PDB Similarity Searches

There are two ways to search a sequence against the PDB database.

OPTION 1:

If your sequence is already loaded into ICM:

- Select **Edit/PDB Search by sensitive similarity**
- Type the sequence name into the **Sequence name** field. Sequences which are already loaded into ICM can be seen by clicking the drop−down button
- Select the number of hits you wish to see by typing the number into the Limit field. A number can also be selected by clicking on the up and down arrows. (Default is 50)
- Select the sensitivity of your search by typing a value into the Expect field. This value is a database−size error estimate and the default value is 0.01.
- Choose whether you wish to display All entries or Entries with unique sequence by checking the appropriate button.
- If you wish to load the sequences leave the Load Sequences box checked.
- If you merely want to see the PDB codes which are similar to your sequence then un−check the Load Sequences box.

- Click the **OK** button.

OPTION 2:

If your PDB sequence is not loaded into ICM:

- Select **Edit/Search with external sequence**
- Cut and paste or type (shown below) your sequence into the **Sequence** data entry field.
- Select the number of hits you wish to see by typing the number into the **Limit** field. A number can also be selected by clicking on the up and down arrows. (Default is 50)
- Select the sensitivity of your search by typing a value into the **Expect** field. This value is a database−size error estimate and the default value is 0.01.
- Choose whether you wish to display **All entries** or ** Entries with unique sequence ** by checking the appropriate button.
- If you wish to load the sequences leave the **Load Sequences** box checked.

NOTE: If you merely want to see the PDB codes which are similar to your sequence then un−check the Load Sequences box.

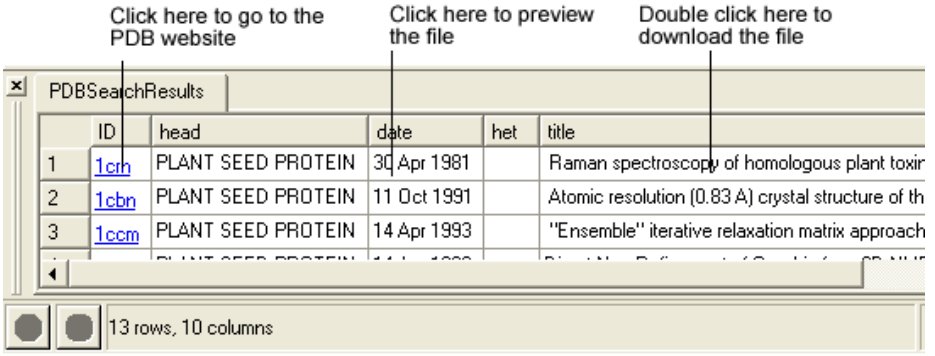
- Click the **OK** button.

NOTE: You can also use the toolbar search option by homology if you wish.

4.1.4. Working with PDB Search Results

Once you have searched for a PDB structure, a table with the search results will be displayed on the bottom of the ICM window. See the `Tables` section for more information on how to use ICM tables. See the next section `loading your PDB file` for information how to view the PDB file. More information about working with tables can be found in the `Tables Section` of this manual.

4.1.5. Loading Your PDB File



More information about working with tables can be found in the `Tables Section` of this manual.

4.2. Converting PDB Files Into ICM Objects

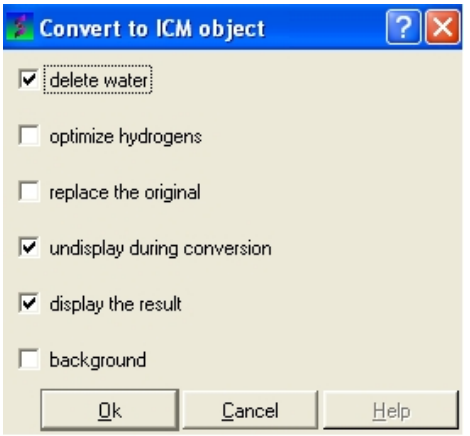
Sometimes it is necessary to have a PDB file in the form of a true ICM−object for which you may calculate energy, build a molecular surface and perform all operations. There are two ways of converting a PDB file into an ICM object.

OPTION 1:

- Right click on the name of the protein, displayed in red, in the ICM workspace panel and a menu will be displayed.
- Select **ConvertPDB** in the menu and the following data entry box will be displayed as shown below.

OPTION 2:

- Select **MolMechanics/Convert/Protein** and the following data entry box will be displayed as shown below.

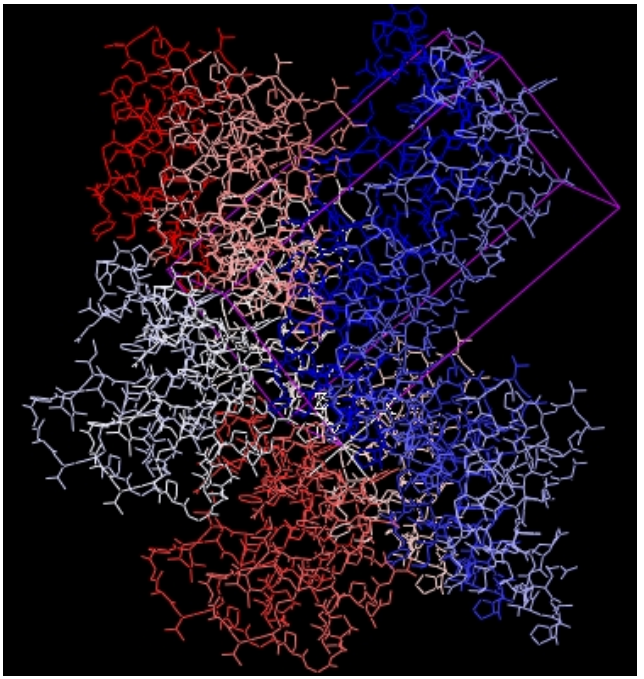


Six options can be selected:

1. Delete water molecules if you are not interested in them.
2. Optimize hydrogens if you wish but this takes time.
3. Delete the original PDB file and replace it with the converted ICM object.
4. Undisplay during conversion will speed up the conversion procedure.
5. You can display the final converted object if you wish.
6. Running the conversion in the background will speed the procedure up.

4.3. Creating Symmetry Related Molecules and Crystallographic Cells

Here we will describe how you can generate symmetry related molecules and display the crystallographic cell of a PDB structure.



4.3.1. Creating Symmetry Related Molecules

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.

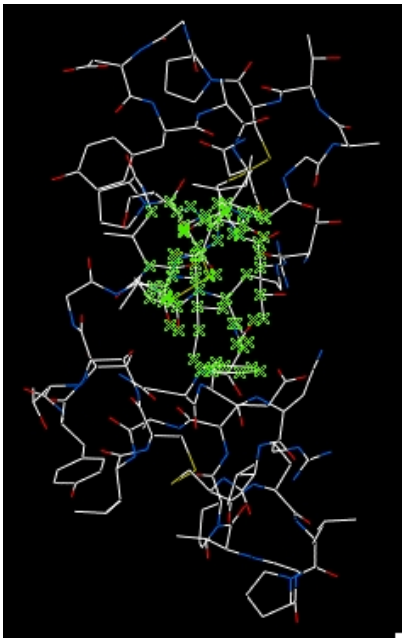
ICM has a function which generates crystallographic neighbors for the selected atoms. For large proteins it is impractical to generate neighbors for the whole molecule due to the high number of atoms in all neighboring molecules.

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

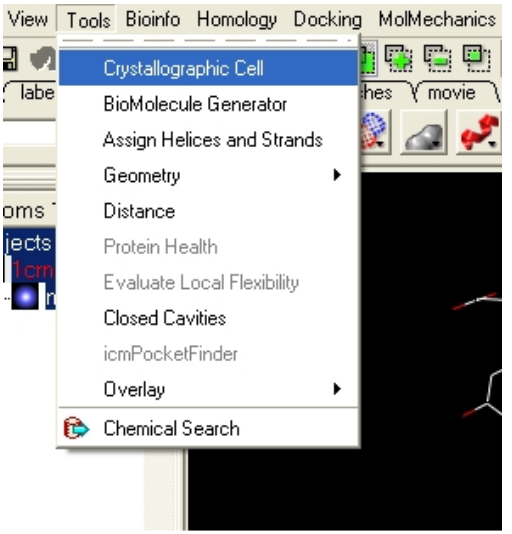
To generate symmetry related molecules around a selection of atoms:

- Read a PDB file into ICM. For instruction see the section entitled `Finding a PDB Structure`.

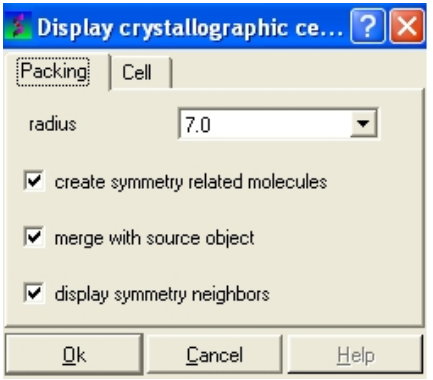
- Display the structure and select the residues around which the symmetry will be generated. For information on how to select residues see the `Making Graphical Selections` section.



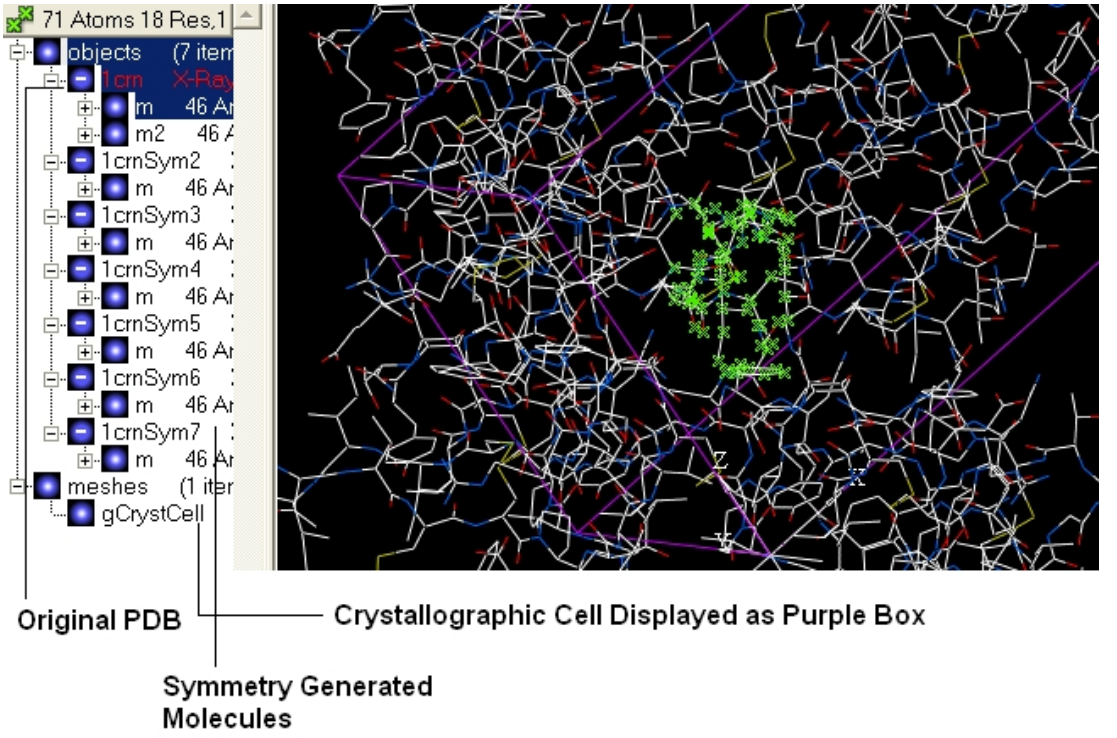
- Select the menu Tools/Crystallograhic Cell.



A data entry box as shown below will be displayed.

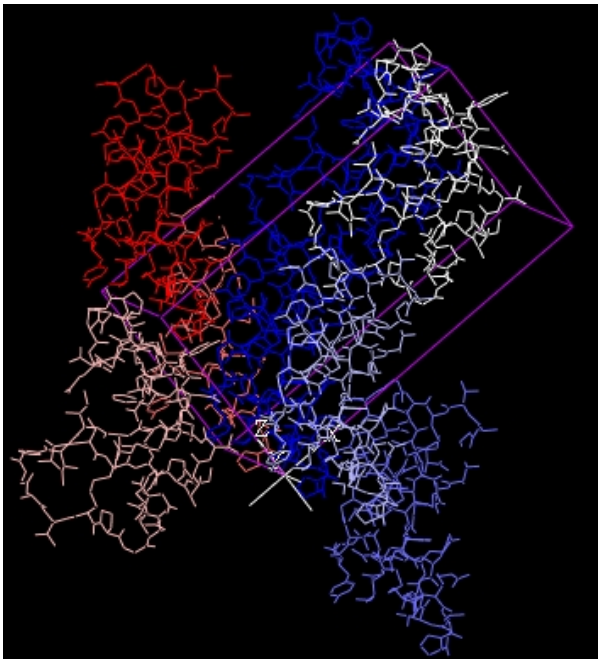


- Select the radius around your selction from which you wish to construct the symmetry related molecules.
- Tick the create symmetry related molecules box.
- Select whether you want the symmentry generated molecules to be merged into the original PDB object.
- Select whether you wish for the symmetry related generated molecules to be displayed.
- Click OK.



The crystallographic symmetry neighbors will be displayed in the Workspace Panel along with the crystallographic cell.

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.

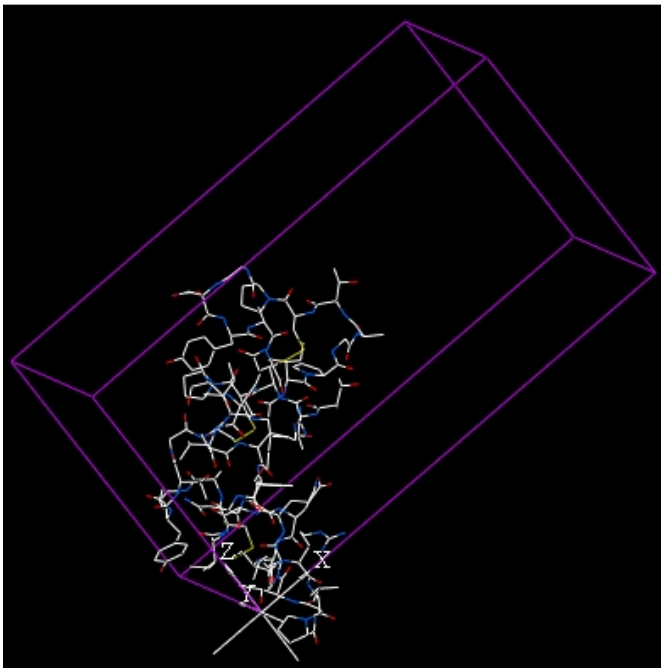


4.3.2. Displaying the Crystal Cell

To display the crystal cell of a PDB structure:

- Read a PDB file into ICM. For instruction see the section entitled `Finding a PDB Structure`.
- Select the whole object.
- Select the menu Tools/Crystallograhic Cell and a data entry box will be displayed.
- Click the tab labeled Cell.
- Click OK

The crystallographic cell will be displayed as a purple box as shown below.

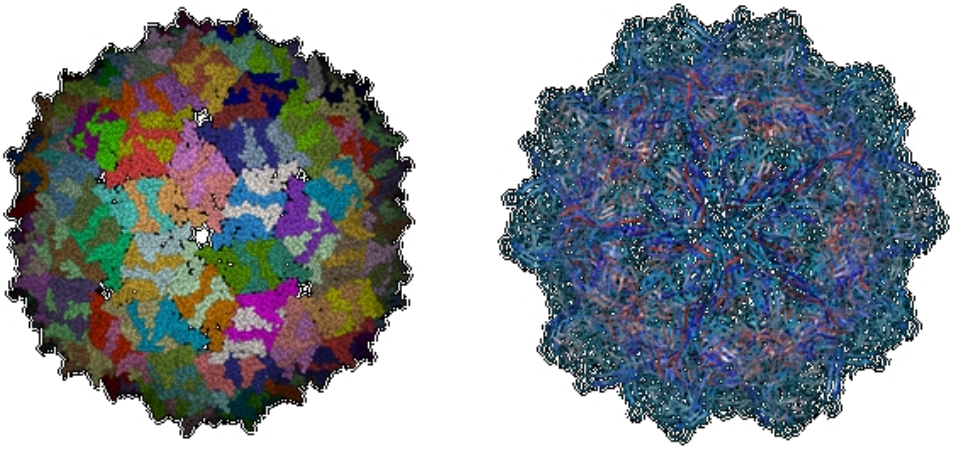


4.4. Generating Biologically Meaningful Structures from a PDB File

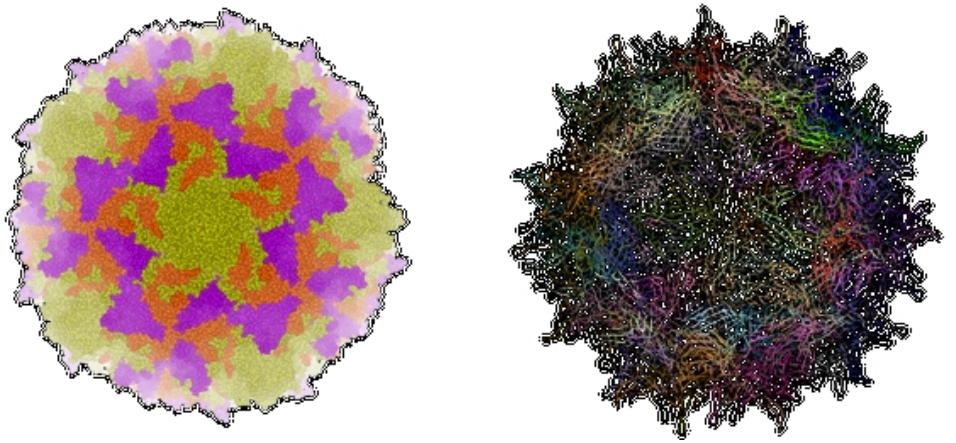
It is very useful to know how a protein from the PDB may look in a biological environment. The PDB entries solved by X−ray crystallography and deposited in the PDB contain the information about the crystal structure rather than the biologically relevant structure. For example, for a viral capsid only 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

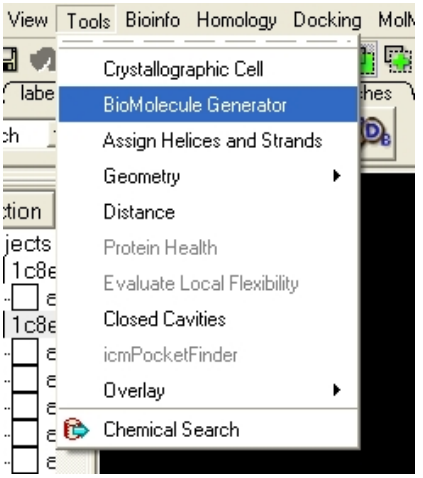


Left: PDB: 1AL2 P1/Mahoney Poliovirus, Single Site Mutant V1160I At 2.9 A Resolution **Right:** PDB: 1LP3 Adeno−Associated Virus (Aav−2), A Vector For Human Gene Therapy At 3.0 A Resolution

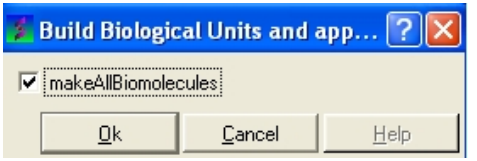
NOTE: Right click on a PDB structure in the ICM workspace to determine whether a structure from the PDB has biological unit information. If it does have this information then there will be an option in the menu entitiled "Generate Biomolecules" if not the option will be blanked out.

To generate a biological unit with ICM:

- Select the menu Tools/Biomolecule Generator.



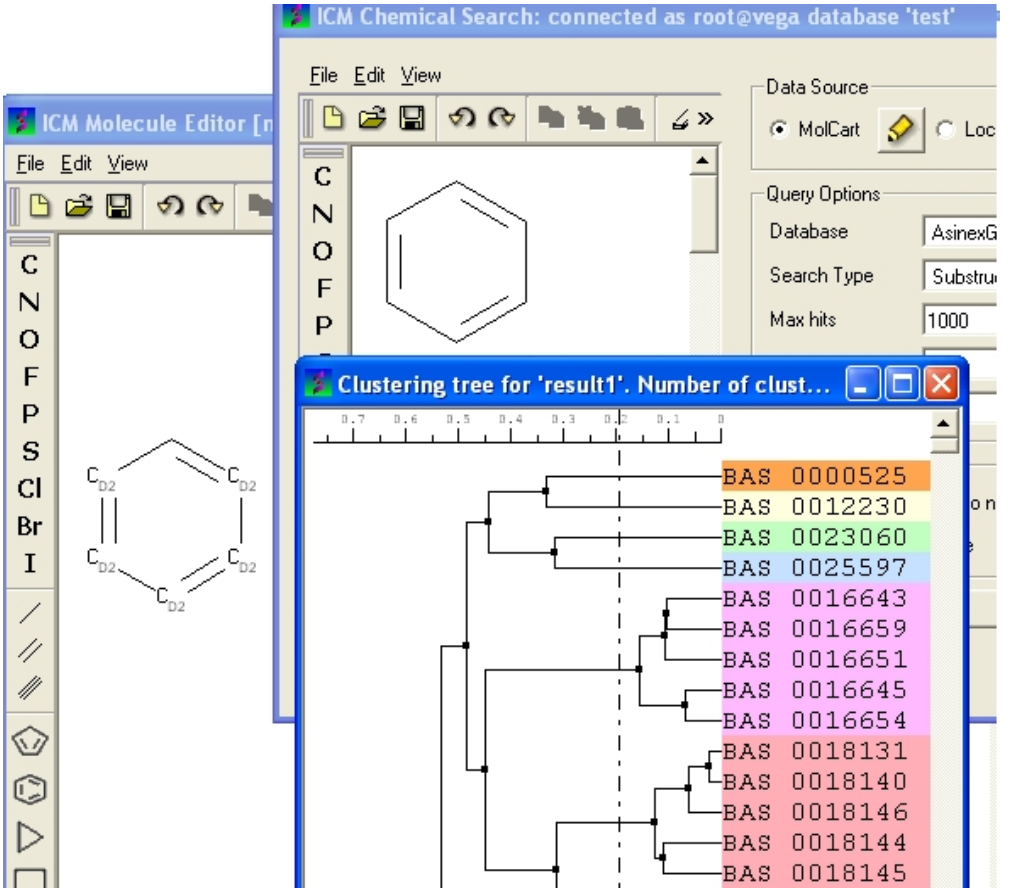
- Tick the makeAllBiomolecules box
- Click OK



5. Working with Chemical Structures

ICM provides an environment under which many chemical structure analysis functions can be undertaken. These functions are critical for enhancing any drug discovery project. They include:

1. Chemical structure drawing.
2. Chemical structure similarity searching.
3. Chemical structure clustering.



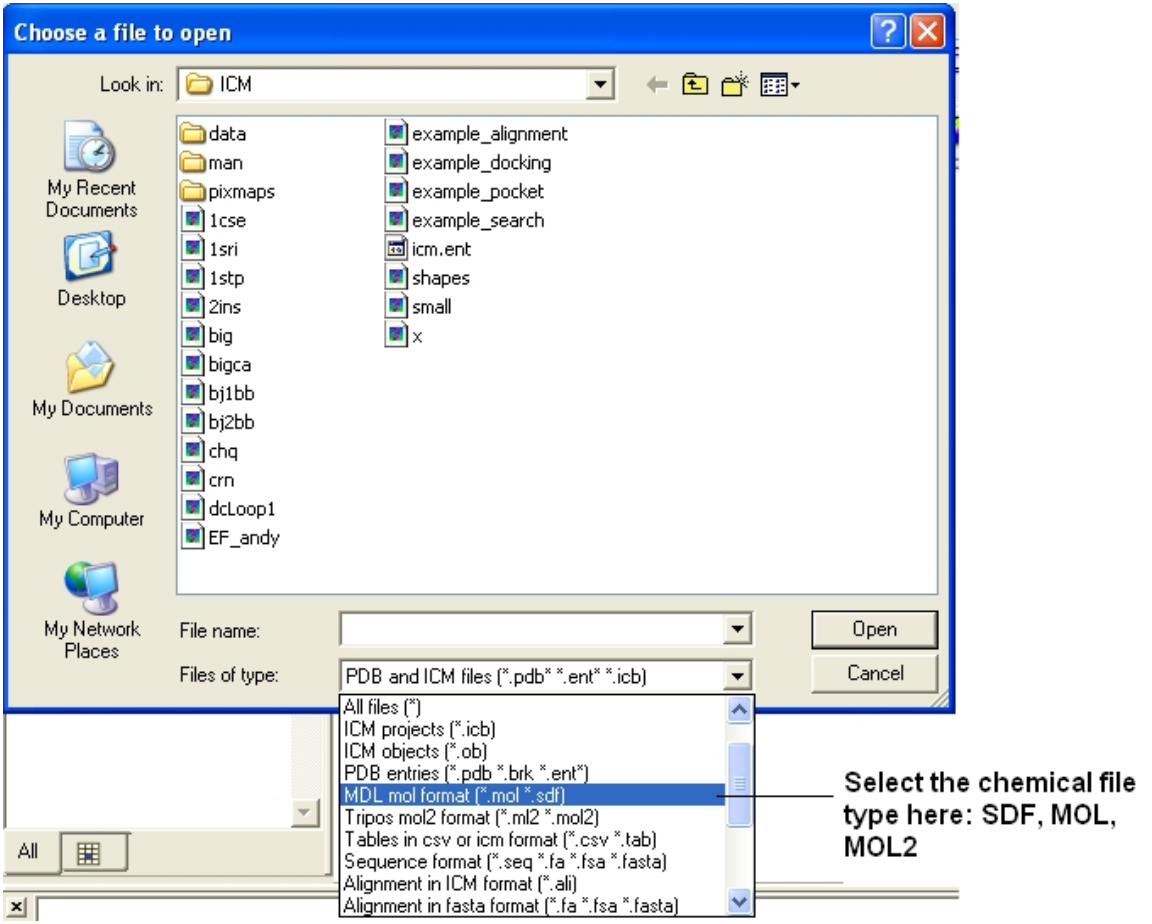
5.1. Reading Chemical Structures

Chemical structures can be read into ICM from MOL/MOL2 and SDF files OR you can construct your own structures by drawing them in the ICM molecular editor.

5.1.1. Loading Chemical Structures

Chemical structures from pre−existing molecular files such as MOL, MOL2 or SDF can be read into ICM by:

- Select File/Open and the window as shown below will be displayed.
- Select the chemical structure file you wish to open: MOL, MOL2 or SDF
- Once selected the file will be displayed as a chemical table (See `ICM molecular tables` section).



5.1.2. Chemical Smiles

If you know the chemical smiles string for the compound you wish to construct:

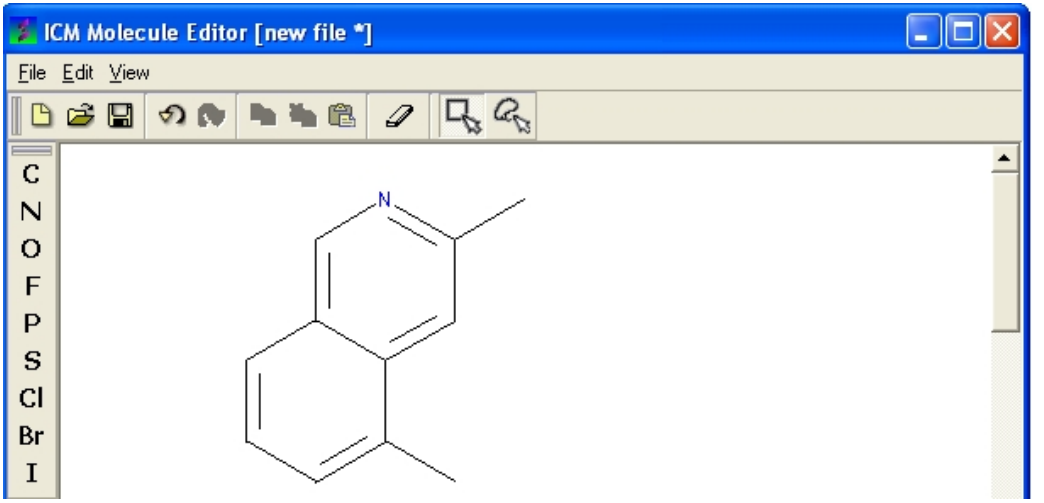
- Select File/New and the New molecule/sequence/grob window will appear.
- Click the Compound tab at the top of the window.
- Type in the Smiles String in the Smiles String data entry box. Remember to delete the previous string. If a string has been entered previously it will be a
- Check the boxes Display Molecule Delete Other Objects according to your preference.
- Click the OK button.

5.2. Molecular Editor

In ICM you can draw compounds by using the ICM Molecular Editor.

The molecular editor can be activated by:

- Select Edit/Molecular Editor and the editor as shown below will be displayed.



5.2.1. Drawing a New Chemical Structure

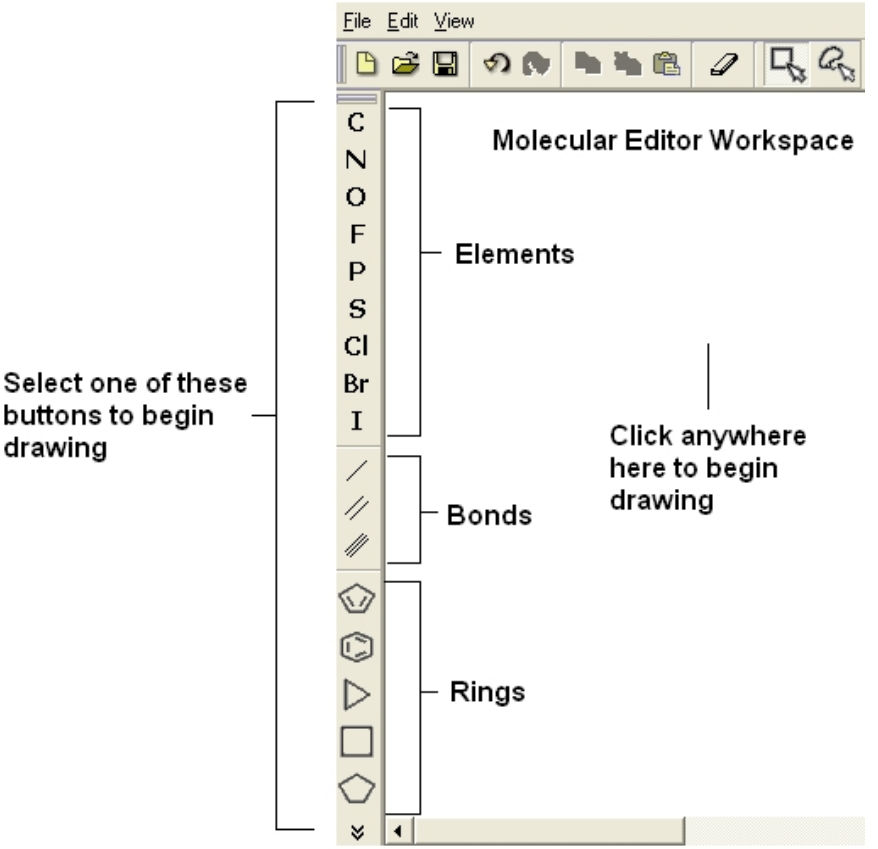
To draw a new chemical structure the ICM Molecular Editor should be loaded.

To do this:

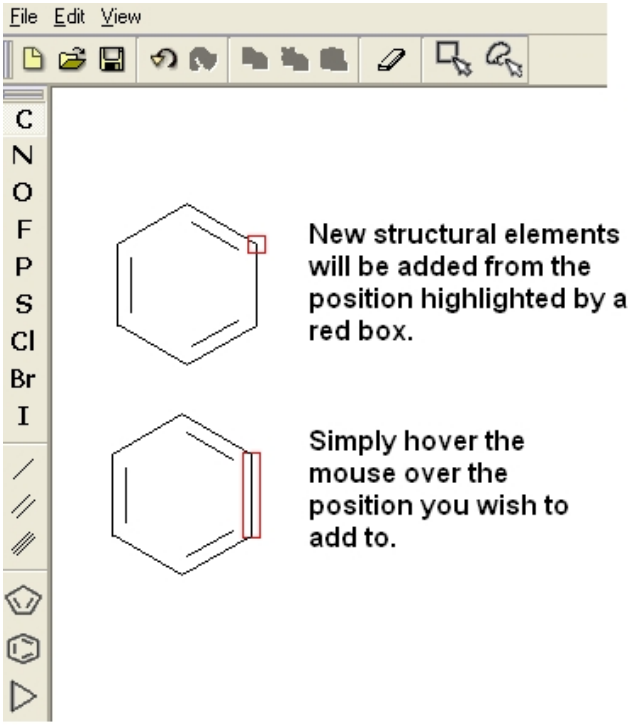
- Select Edit/Molecular Editor

Now you can start drawing your structure.

- First select one of the appropriate buttons on the left hand side of the molecular editor.
- Then click in the white Molecular Editor Workspace and your element, ring or bond will be displayed.

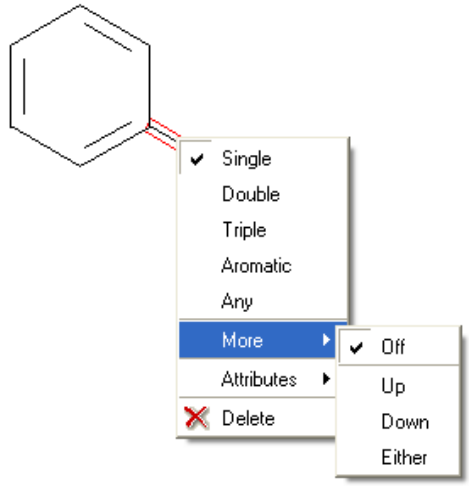


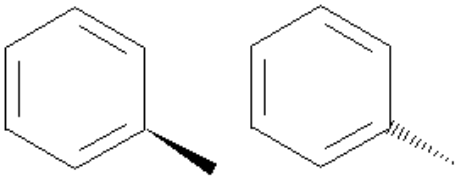
- You can extend your structure by selecting another button from the left hand−side as before.
- Select where on the structure you would like to add the new group by hovering the mouse over the desired position. The position you will add to will be highlighted in a red box.

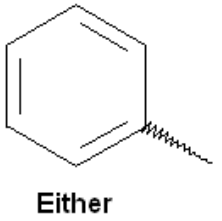


To change the direction of a bond:

- Right click on the bond and a menu as shown below will be displayed.
- Select which bond direction you desire from up, down or either.



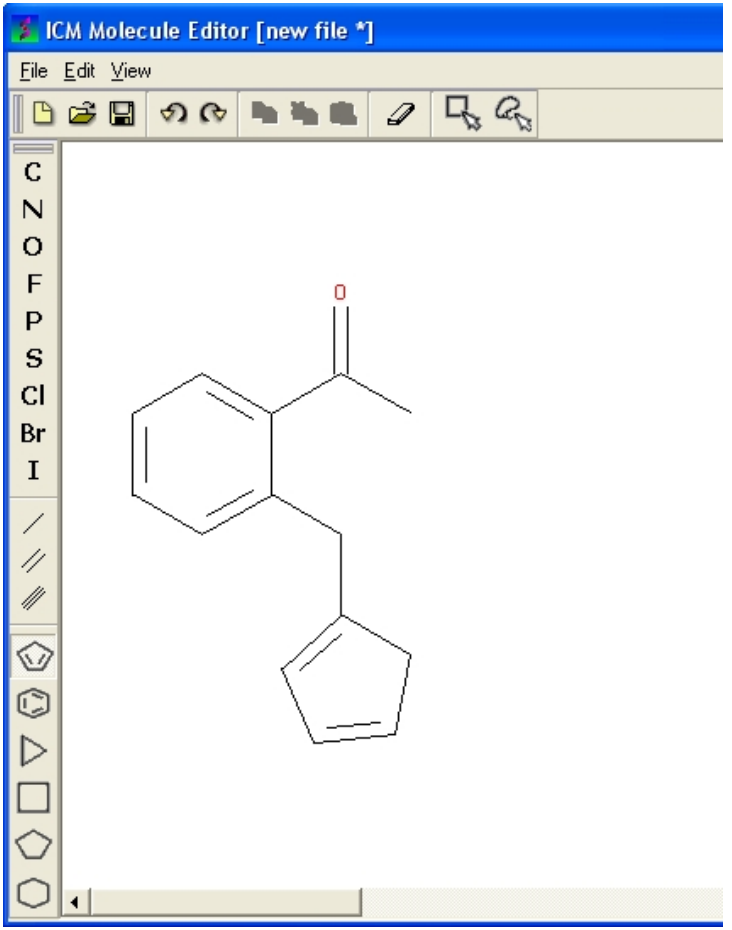




Carry on drawing until your structure is complete! See the other sections in this chapter.

1. To save your structure read the `save and append chemical structure` section.
2. To append your structure to an existing SDF file read the `save and append chemical structure` section.
3. To save your structure to an ICM table read the `save and append chemical structure` section.

4. To edit your structure read the `edit your structure` section.



5.2.2. Save and Append Chemical Structures

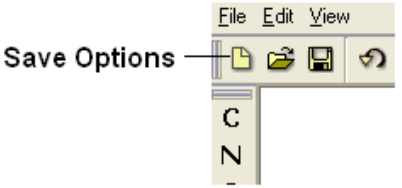
Once you have drawn a chemical structure `(see Drawing a New Chemical Structure)` then you can save the structure in the following ways:

1. By saving the structure as a MOL file or SDF file on your machine or fileserver.
2. Appending the structure to an already created SDF file.
3. Appending the structure to an ICM Molecular table.

To save the structure as a MOL or SDF file on your machine or fileserver.

- In the Molecular Editor window select File/Save
- Enter a filename and select where you wish to save the file.

NOTE: Other save options can be found on the ICM Molecular Editor Toolbar.

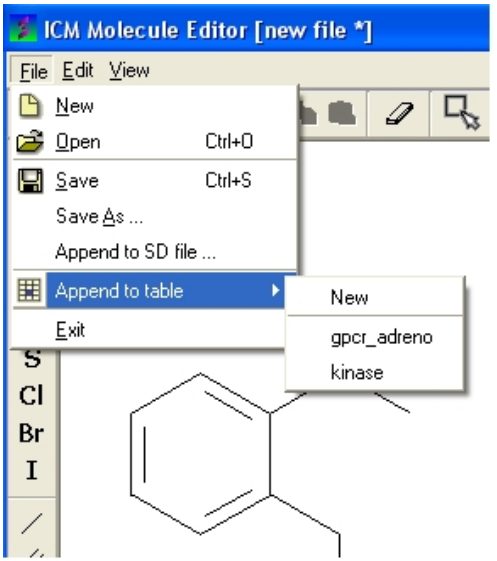


To append the structure to an already created sdf file.

- In the Molecular Editor window select File/Append to SD file...
- Search for the SDF file you wish to append to and select OK. This SDF file can be read into ICM as described in `Read Chemical Structure` section of this manual.

To append your structure to an ICM table:

- In the Molecular Editor window select File/Append to table
- A list of loaded ICM tables will be displayed as shown below. If you dont have any ICM table loaded or you wish to add the structure to a new table select the "new" option.
- The structure will be automatically added to an ICM table and displayed in the GUI.



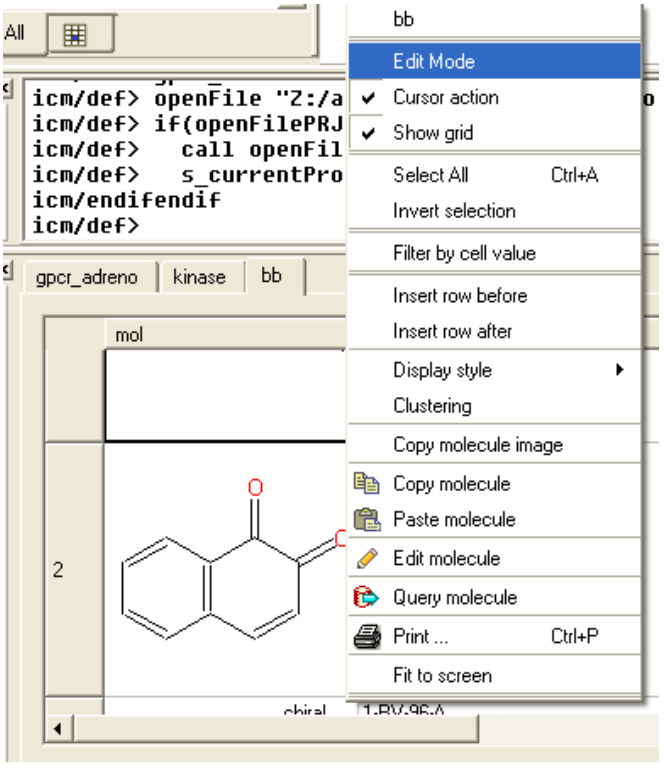
NOTE: For more information on how to manipulate standard ICM Tables and Molecular Tables see the `Table` section of this manual.

5.2.3. Editing a Chemical Structure

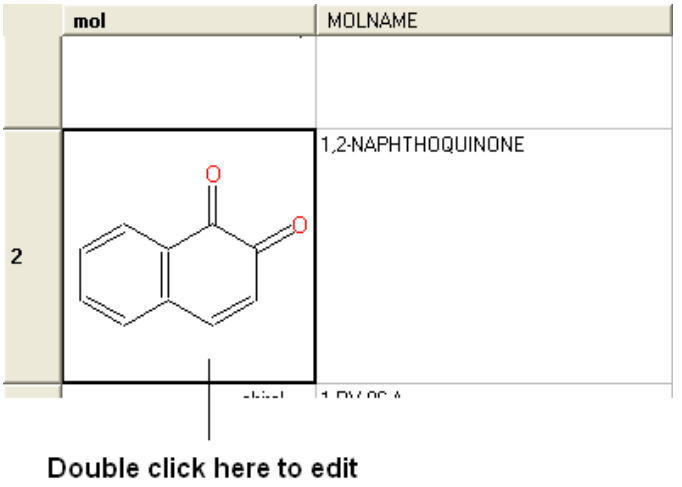
If you make a mistake whilst drawing a chemical structure or if you wish to change an already saved and loaded structure there are a number of editing tools and techniques which can be used.

To edit a structure which is in a loaded ICM molecular table:

- Right click on the structure in the table and a menu as shown below will be displayed.

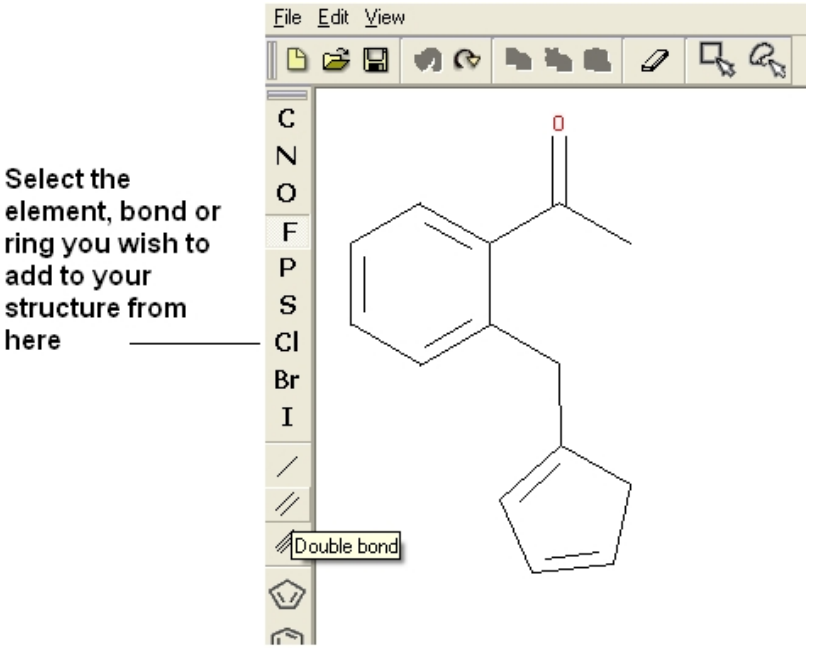


- Select the Edit Mode and a black square will be displayed around the chemical you wish to edit.
- Double click on the structure and the ICM Molecular Editor will be activated.

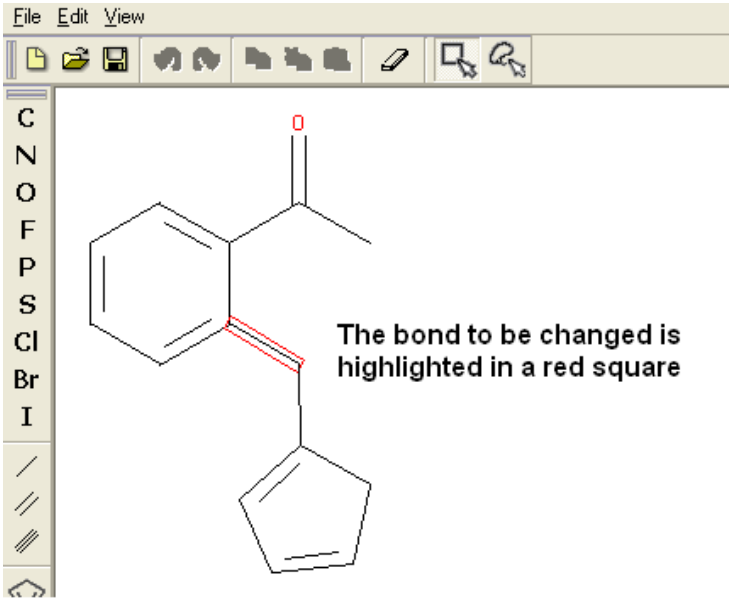


To edit a bond or atom in the structure:

- First select the new bond, atom or ring from the buttons on the left of the ICM Molecular Editor.



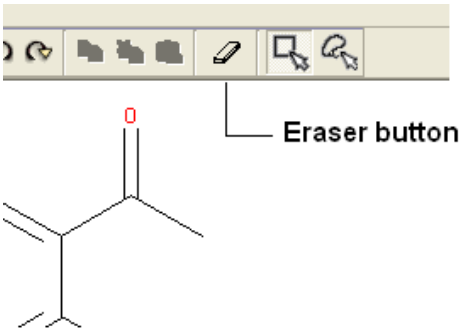
- Hover over the element or bond you wish to change in the ICM Molecular Editor workspace. A red square will be displayed over the bond or element you select as shown below.



- Click on the bond or element and it will automatically change to your selection.

To delete parts of a structure:

- Select the eraser button on the ICM Molecular Editor Toolbar.

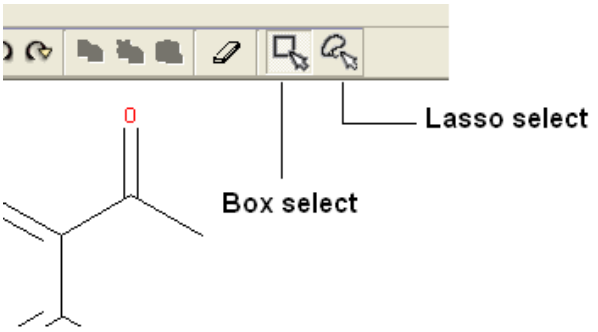


- Click on the regions you wish to delete.

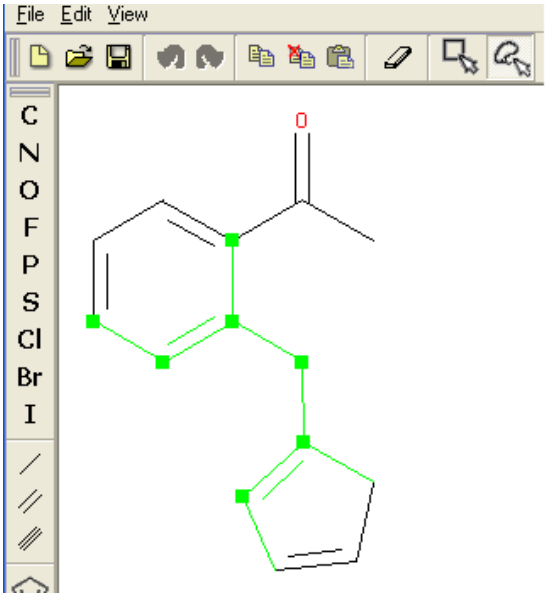
Alternatively you can select the delete option in the ICM Molecular Editor "Edit" menu.

5.2.4. Molecular Editor Selections

Selections can be made in the ICM Molecular Editor using the two buttons shown below.



Selections are displayed in green.



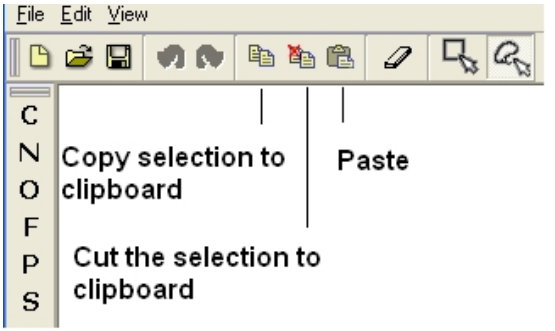
5.2.5. Copy, Cut and Paste

To copy, cut and paste part or all of your structure.

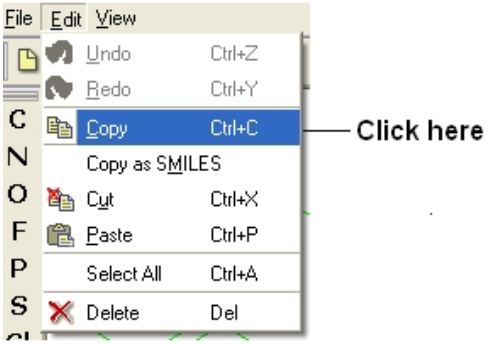
- First select the parts you wish to copy or cut by reading the instructions in the `Molecular Editor Selections` section of this manual.

- Select copy or cut from the ICM Molecular Editor "Edit" menu.
- The selected regions will then be placed on the copy clipboard and can be pasted into the ICM Molecular Editor or any other program.

The copy and paste buttons and menus are shown below:



OR



To copy your structure as a smiles string:

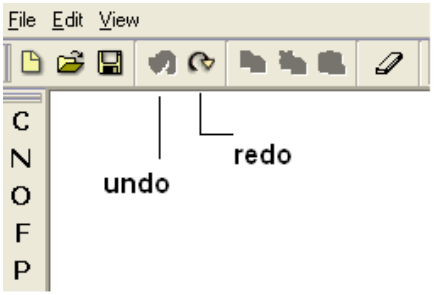
C1C=CC=C(C=1C(C)=O)CC(CC=C1)=C1

- Select the "Copy as SMILES" option in the ICM Molecular Editor "Edit" menu.

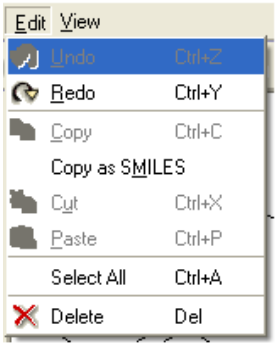
This will place the SMILES string on the clipboard which can then be pasted into any application.

5.2.6. Undo and Redo

The undo and redo options for the ICM Molecular Editor are located in the Edit menu and on the toolbar as shown below.



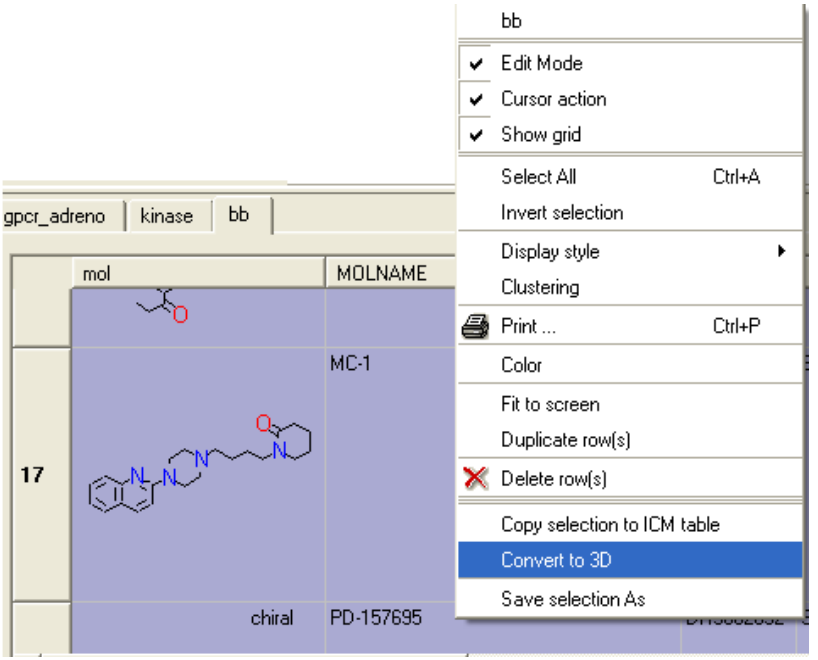
OR



5.3. Converting Chemical Structures from 2D to 3D

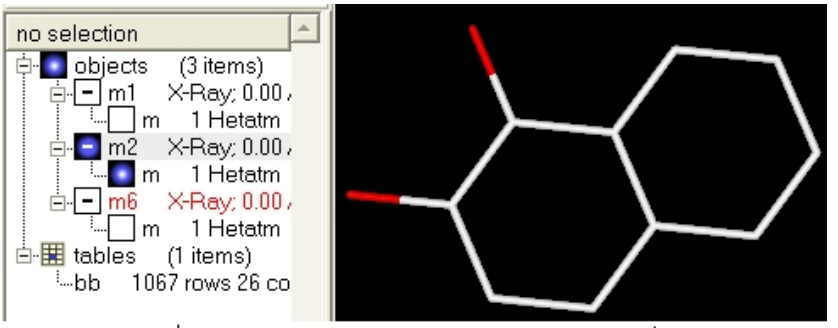
To convert a chemical structure from 2D to 3D:

- Select which structures you wish to convert in the molecular table. For instructions on making selections within tables see the `Making Table Selections` part of this manual.
- Right click on one of the selections you have made and a menu as shown below will be displayed.



- Select the Convert to 3D option and you will see the compounds being converted and minimized in the 3D graphical display window.

Once converted the compounds will be displayed in the 3D graphical display window and also in the ICM workspace.



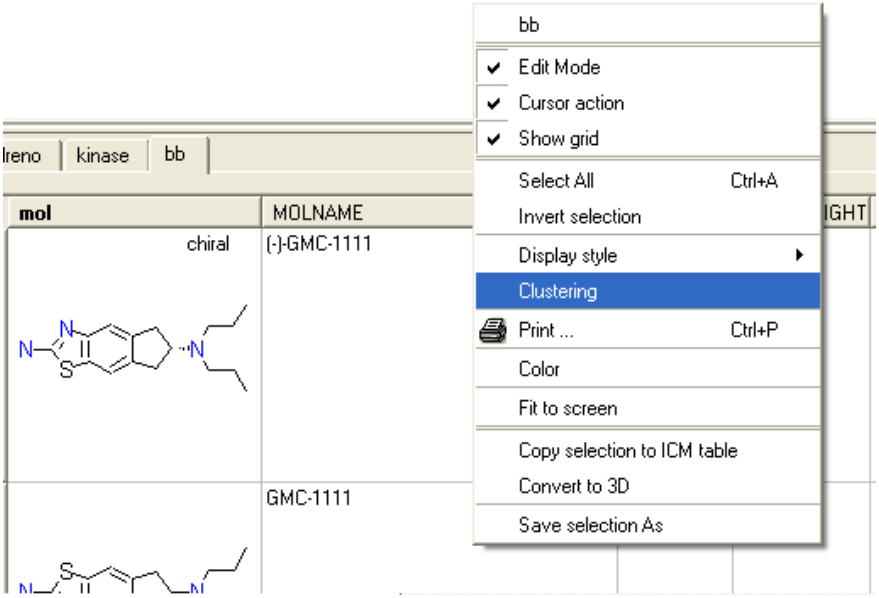
Converted chemical stuctures are displayed in the 3D graphical window and the ICM workspace

5.4. Chemical Clustering

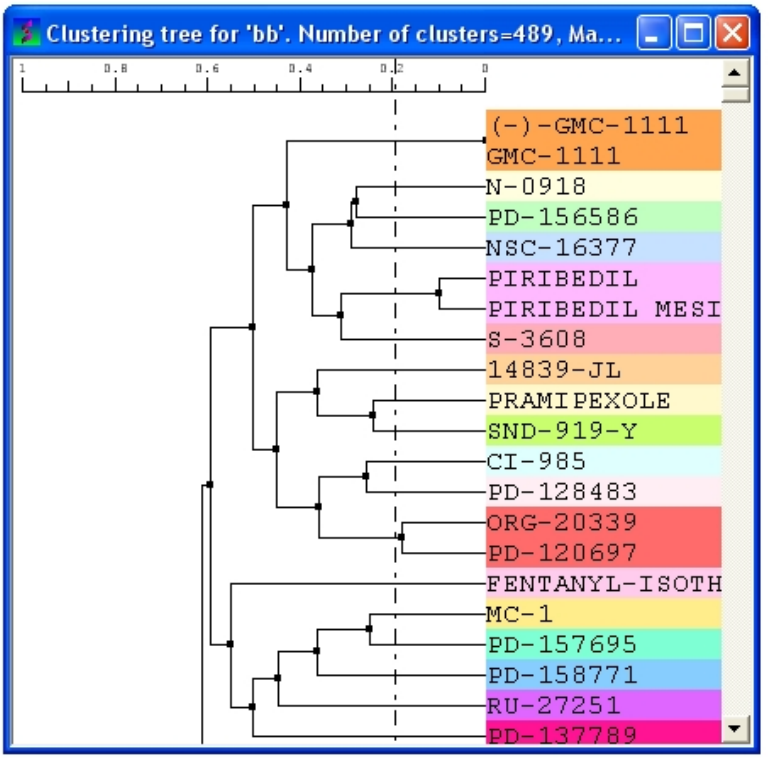
To perform clustering based on chemical similarity first you must have an SDF file or a molecular table loaded into ICM.

- Right click on the table and a menu will be displayed as shown below.
- Select the clustering option either tree or k−means.

The 'tree' method is a vigorous clustering apporach and computes all to all distances and might be slow for more than 1000 compounds. The k−means approach is less vigorous and therefore quicker.



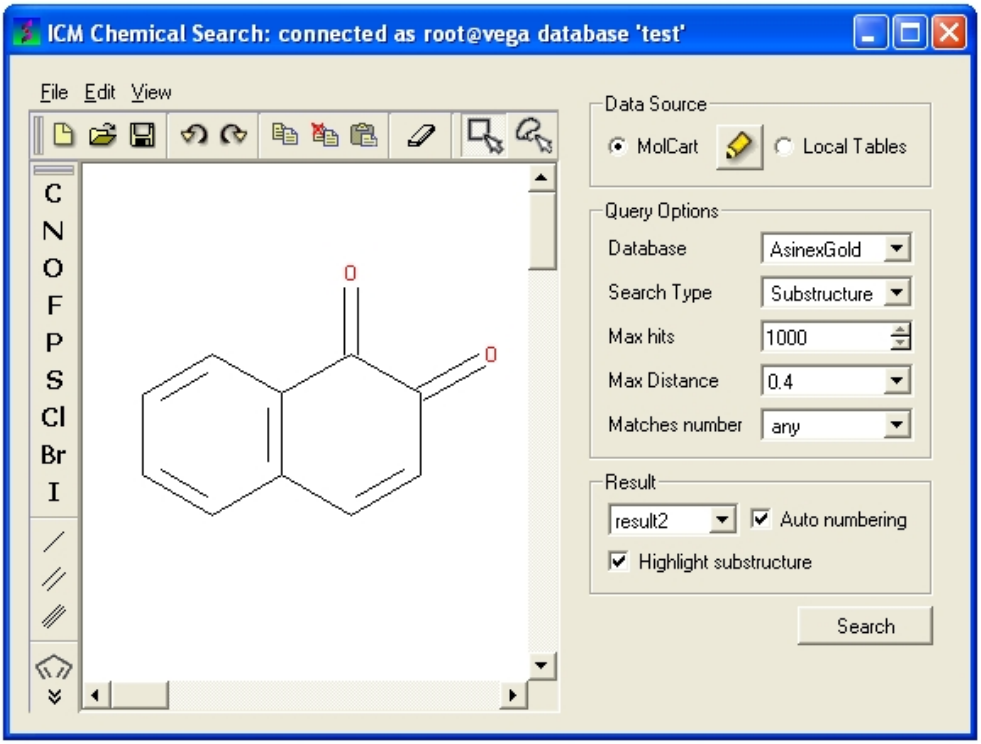
The clustering may take a few sections to complete. Once completed a tree of the clustered compound names will be displayed.



The color coding within the tree refers to the color coding in the table.

5.5. Chemical Similarity Searching

Chemical similarity searching can be used to screen a database of compounds for structural similarity to a query chemical structure. The chemical similarity search window is shown below.

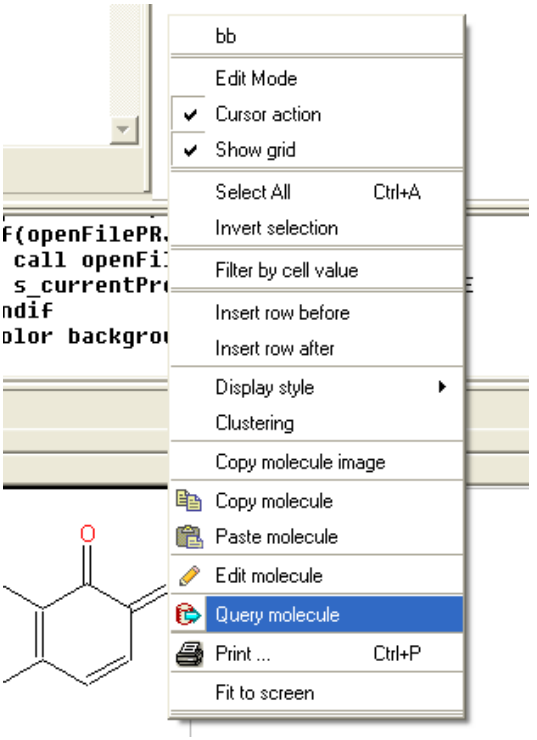


5.5.1. Query Setup

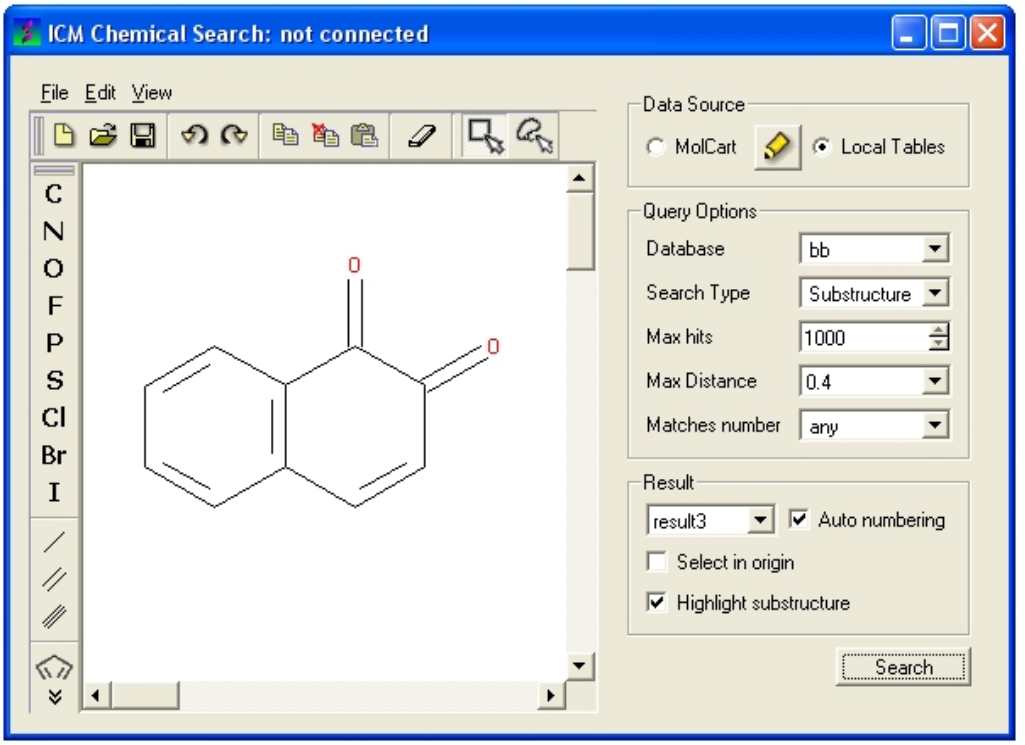
To set up a query first you must have either drawn or loaded a chemical structure into ICM. Instructions for this are described in the `Reading Chemical Structures` and `Molecular Editor` sections of this manual.

To perform chemical similarity searching:

- Right click on the compound and a menu as shown below will be displayed.



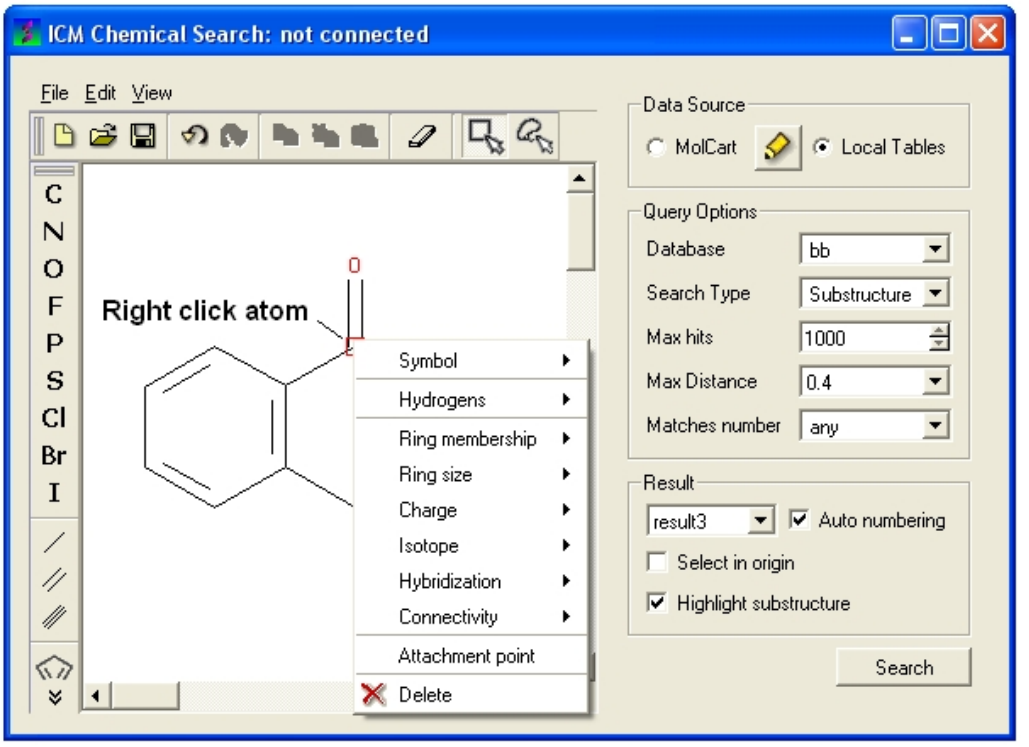
- Select the "Query molecule" option and the ICM Chemical Search window with the query structure will be displayed as shown below.



At this point your query can be modified as described in the `Molecular Editor` sections of this manual. **However,** there are a number of ways to specifically modify your query and filter your search. The way to accomplish this is to right click on a bond or atom and a menu as displayed below will be displayed. The menus differ depending on whether you right click on a bond or atom.

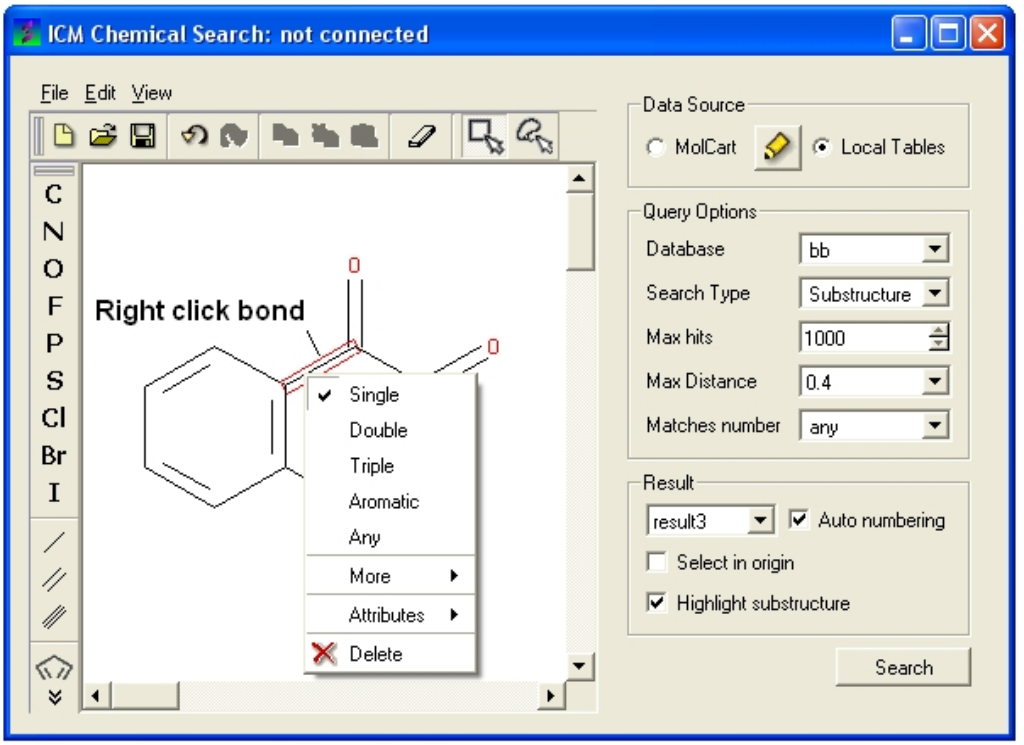
If you wish to specify a filter at an atom.

- Right click on the atom and the menu shown below will be displayed.



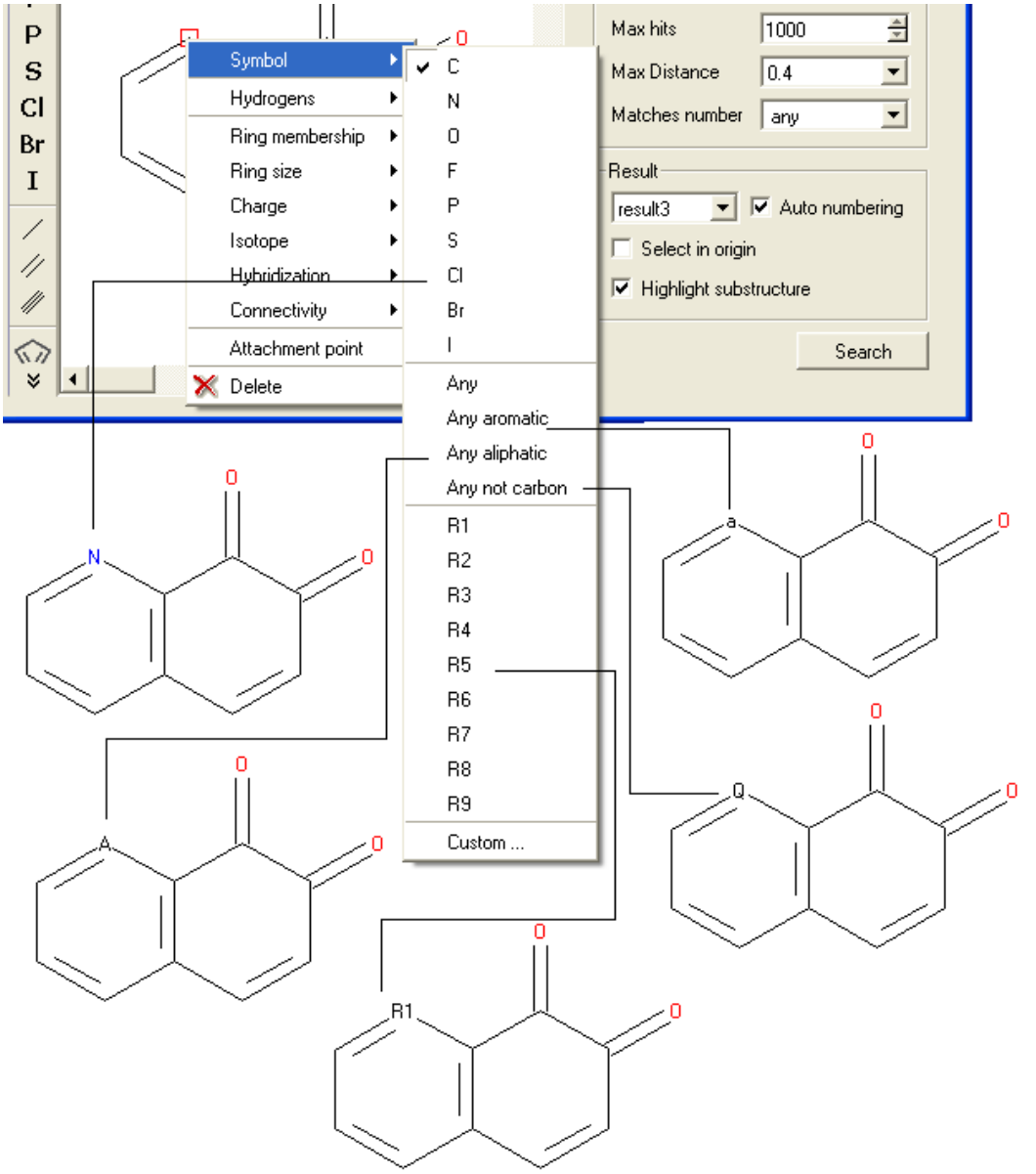
If you wish to specify a filter at a bond.

- Right click on the bond and the menu shown below will be displayed.



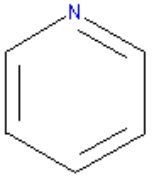
To specify a particular atom type, aromatic, aliphatic or R−group at a particular atom site.

- Right click on the atom and select the "symbol" option as shown below.
- Select the desired atom type, aromatic, aliphatic or R−group and a symbol will be displayed as shown below.


P
S
Cl
Br
I
Symbol
Hydrogens
Ring membership
Ring size
Charge
Isotope
Hybridization
Connectivity
Attachment point
Delete
C
N
O
F
P
S
Cl
Br
I
Any
Any aromatic
Any aliphatic
Any not carbon
R1
R2
R3
R4
R5
R6
R7
R8
R9
Custom ...
Max hits
1000
Max Distance
0.4
Matches number
any
Result
result3
Auto numbering
Select in origin
Highlight substructure
Search
N
A
R1

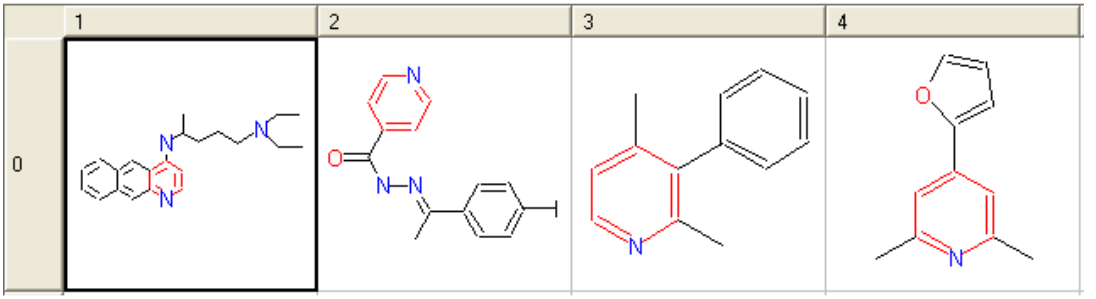
For example:

Query:



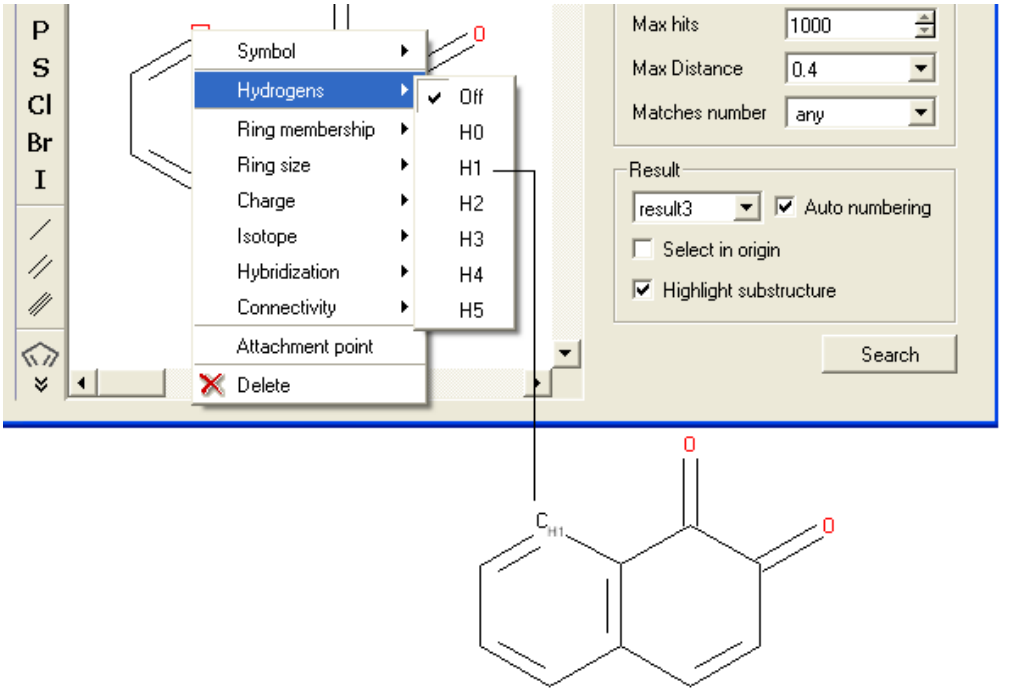
Right click atom - Symbol - N

Selection of chemical substructure search results



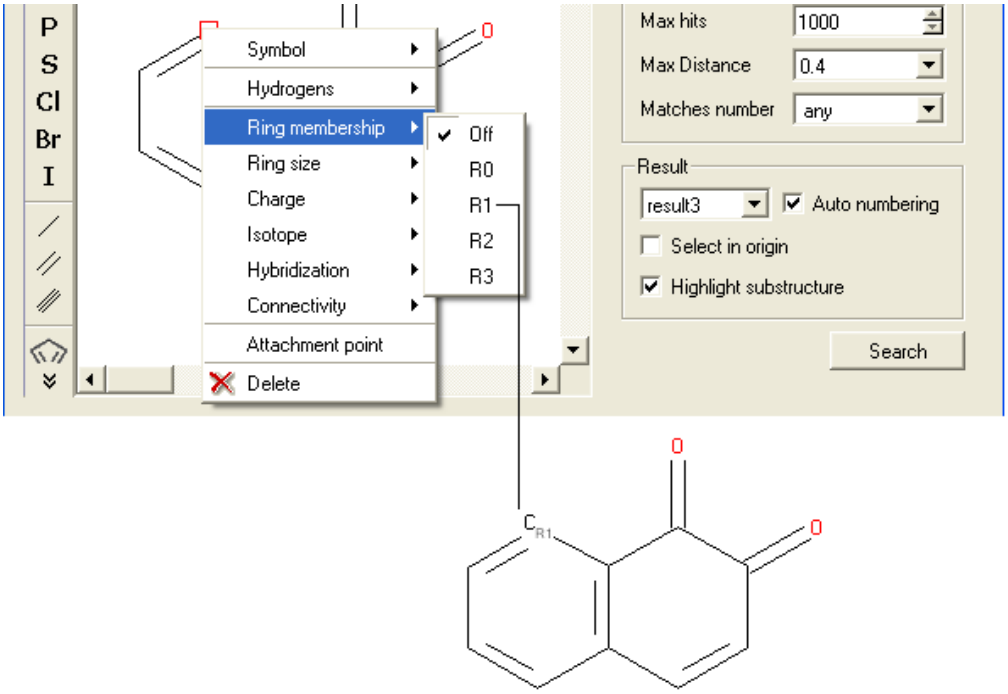
To specify a particular number of hydrogen atoms at a particular site:

- Right click on the atom and select the "Hydrogens" option as shown below.
- Select how many hydrogens you wish to specify and a symbol will be displayed as shown below.



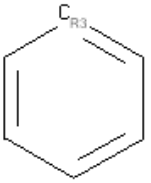
To specify the number of rings a particular atom will be a member of:

- Right click on the atom and select the "Ring membership" option as shown below.
- Select whether the atom should be part of 1, 2 or 3 rings.


P
S
Cl
Br
I
Symbol
Hydrogens
Ring membership
Ring size
Charge
Isotope
Hybridization
Connectivity
Attachment point
Delete
Off
R0
R1
R2
R3
Max hits
1000
Max Distance
0.4
Matches number
any
Result
result3
Auto numbering
Select in origin
Highlight substructure
Search
O
O
O
C
R1

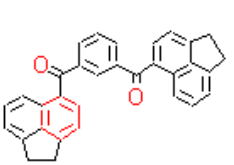
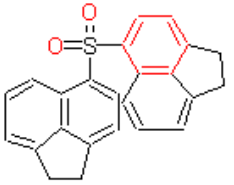
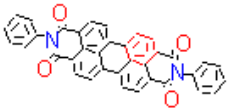
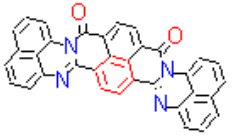
For example:

Query:



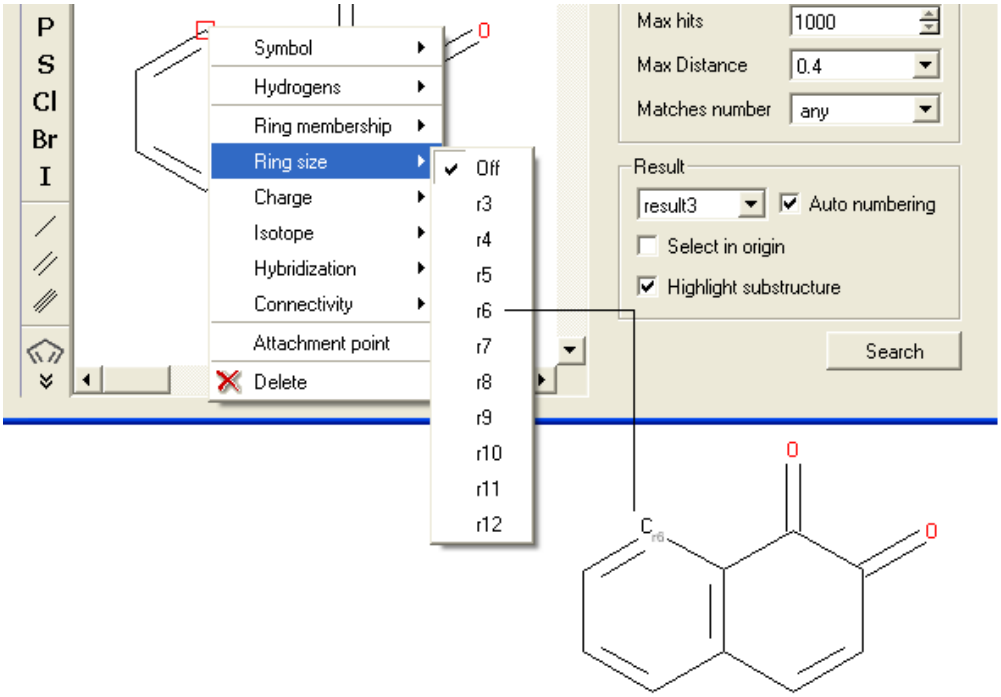
Right click atom - Ring Membership - R3

Selection of chemical substructure search results

	1	2	3	4
24				

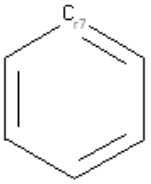
To specify the ring size connected to an atom:

- Right click on the atom and select the "Ring size" option as shown below.
- Select the size of the ring the atom should be connected to and a symbol will be displayed as shown below.


P
S
Cl
Br
I
Symbol
Hydrogens
Ring membership
Ring size
Charge
Isotope
Hybridization
Connectivity
Attachment point
Delete
Off
r3
r4
r5
r6
r7
r8
r9
r10
r11
r12
Max hits
1000
Max Distance
0.4
Matches number
any
Result
result3
Auto numbering
Select in origin
Highlight substructure
Search
O
O
O
C

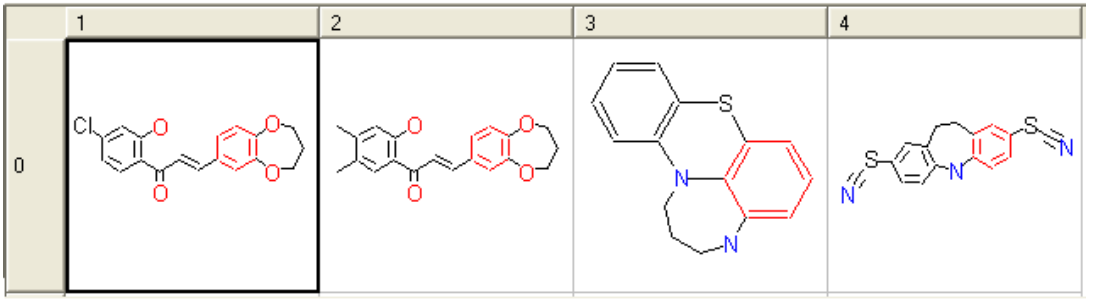
For example:

Query:



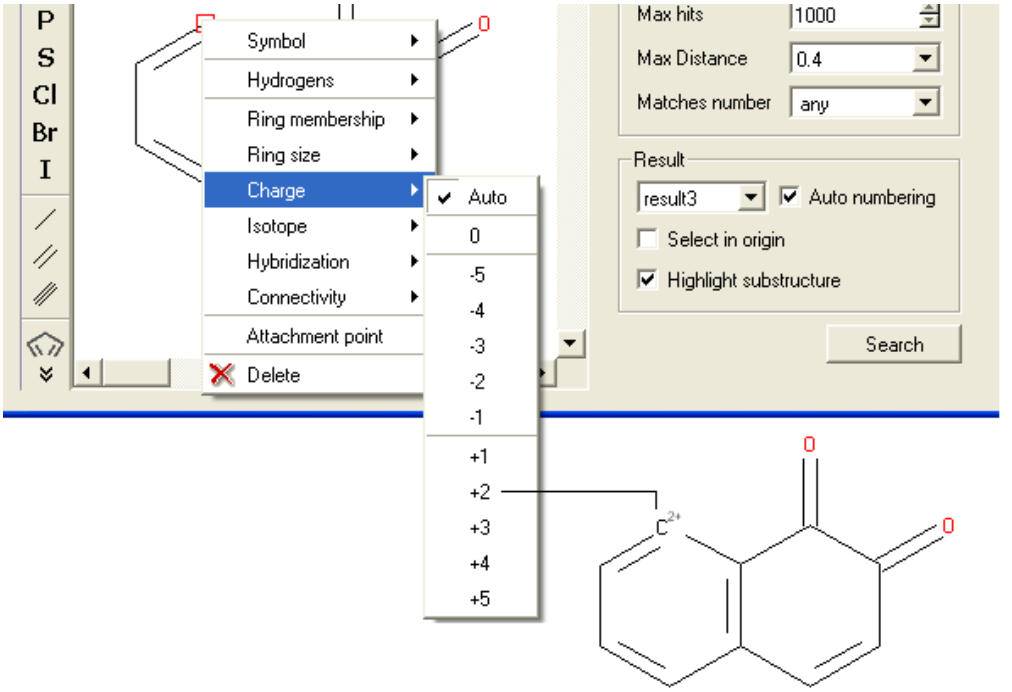
Right click atom - Ring size - R7

Selection of chemical substructure search results



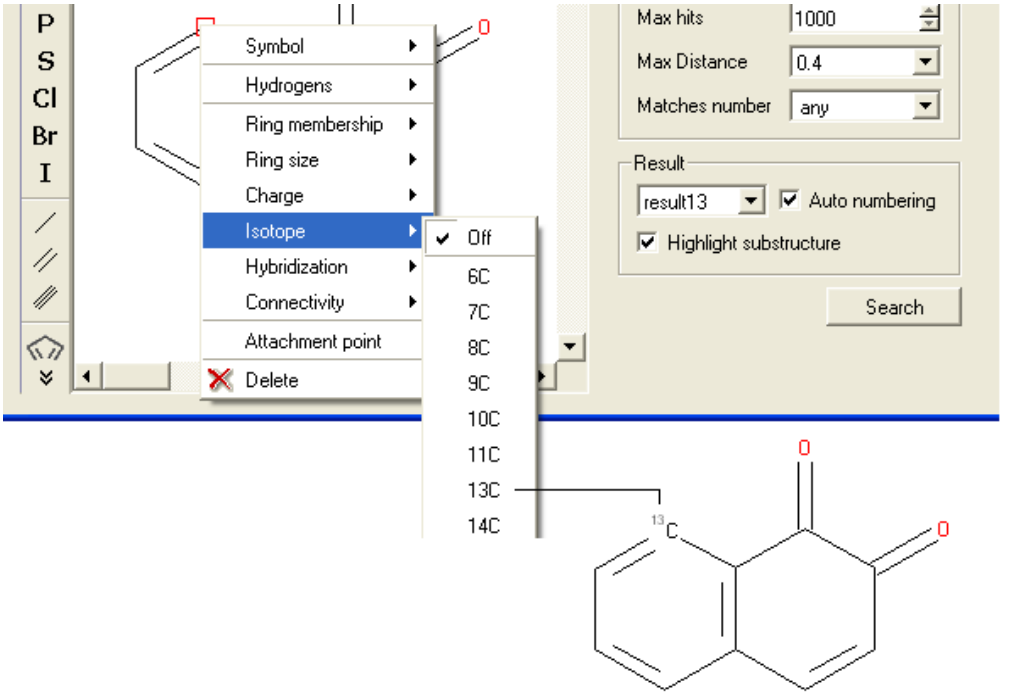
To specify the charge at a particular point:

- Right click on the atom and select the "Charge" option as shown below.
- Select the desired charge and a symbol will be displayed as shown below.



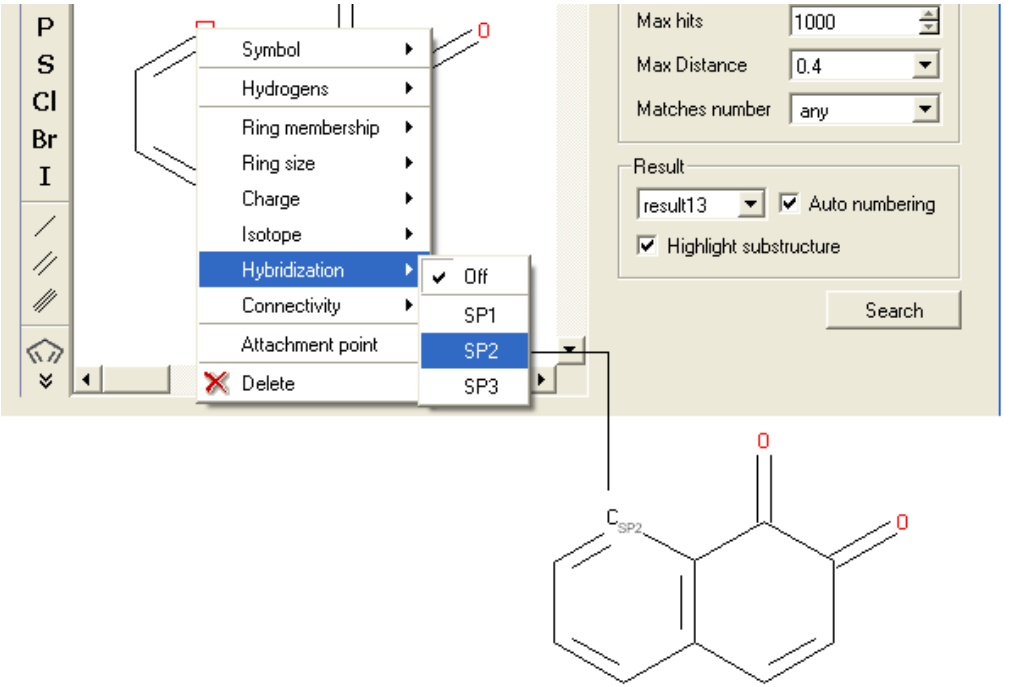
To specify an isotope at a particular atom

- Right click on the atom and select the "Isotope" option as shown below.
- Select the desired isotope from the list and a symbol as shown below will be displayed.



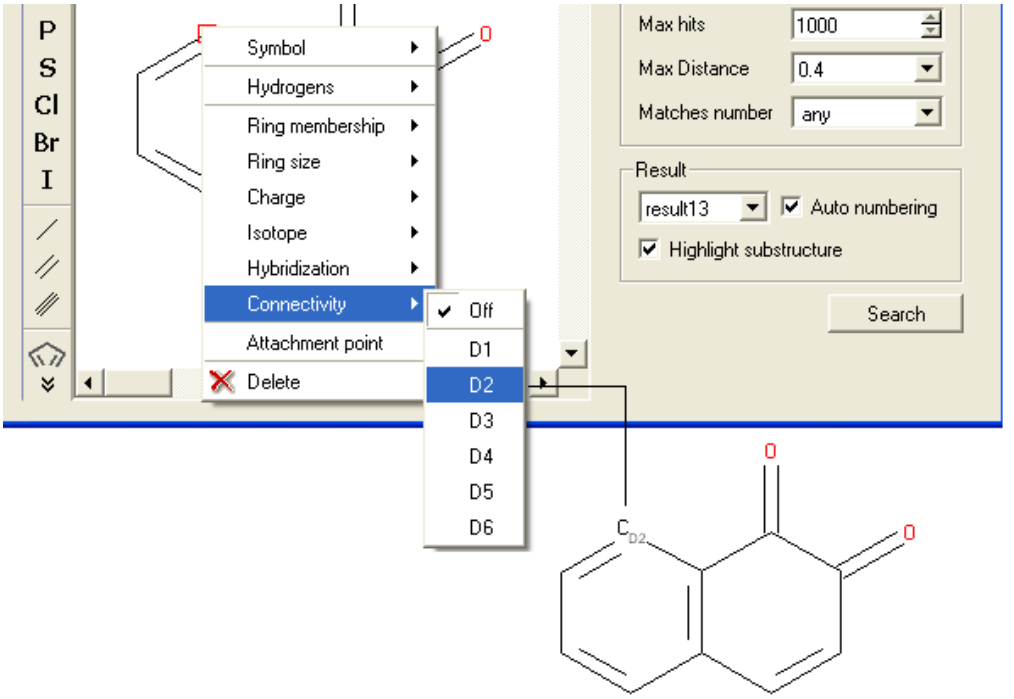
To specify the hybridization state of the atom:

- Right click on the atom and select the "Hybridization" option as shown below.
- Select the desired hybrization state and a symbol will be displayed as shown below.



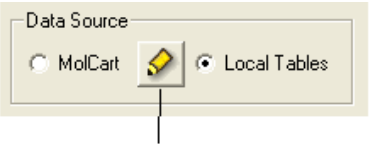
To specify the number of atoms you wish an atom to be connected to:

- Right click on the atom and select the "Connectivity" option as shown below.
- Select the number of atoms you wish the atom to be connected to and a symbol will be displayed as shown below.



5.5.2. Query Processing

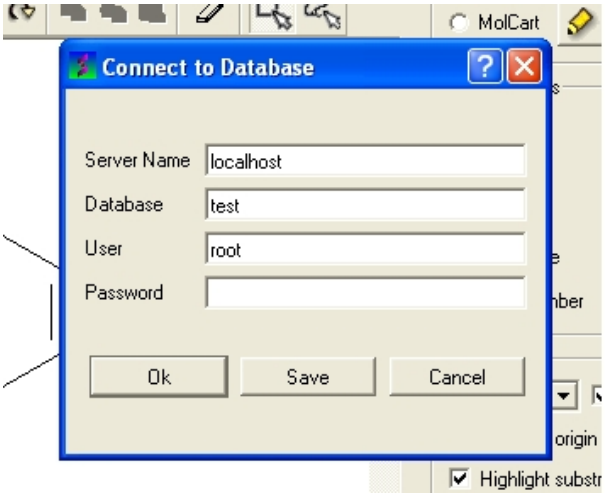
To begin processing your query first you need to decide which database to search. The options are listed in the "Data Source" section of the ICM chemical search window.



To connect to MolCart database for the first time click here.

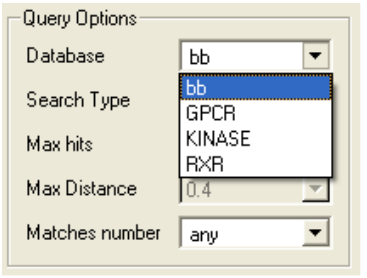
You can either search a local table (molecular table) or you can search MolCart.

If you select MolCart you first need to setup the link to the correct database. Click on the button shown above (yellow pencil) and the Connect to Molcart window will be displayed as shown below.



- Enter the Server Name in which the database is stored.
- Enter the database name.
- Enter your username and password for the server.
- You can save these details so you dont have to re−enter this information each time you use the chemical similarity search.

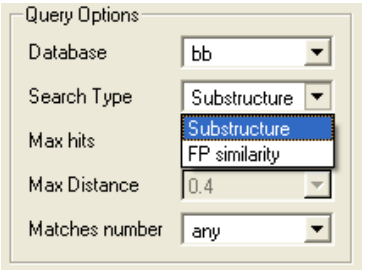
If you are searching a local table, the names of your currently loaded tables are in the drop down menu shown below.



Now select a search type:

- Click on the drop down arrow next to the "Search Type" option in the Query Options panel.
- Select either substructure or FP similarity as shown below.

A substructure search is a search whereby only the defined molecule in the query will be searched against the database. Whereas, a FP search which stands for fingerprint search enables any fingerprint within a structure to be searched for in the database. The "Max distance" option is available for use with the FP search and the "Matches number" option is for use with the substructure search. The option you do not require based on your search method will be blanked out. A "Max distance" value of 0 means that the search will only identify matches exactly the same as the fingerprint − the default is 0.4. The "Matches number" option allows you to stipulate how many times within a structure in the database your query can be found.



Enter how many hits you wish to be returned to you.

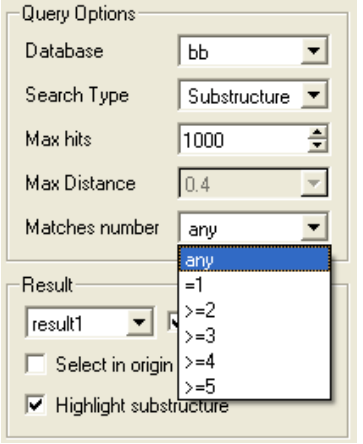
- Click on the up or down arrows or type into the "Max hits" data entry box.

Select the "Max Distance". Only for use with fingerprint (FP) similarity search.

- Click on the drop down arrow next to the "Max Distance" data entry box.

Select the number of matches. Only for use with the substructure similarity search.

- Select the number of matches you require from the drop down box as shown below.

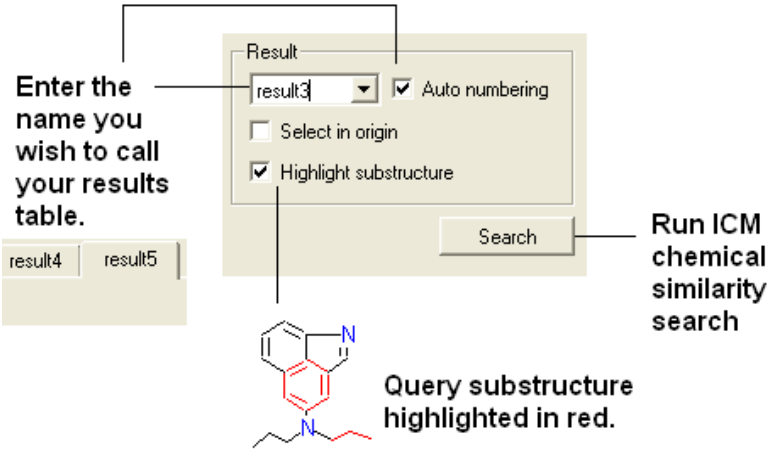


Before processing the query see the next section entitled `query results`

5.5.3. Query Results

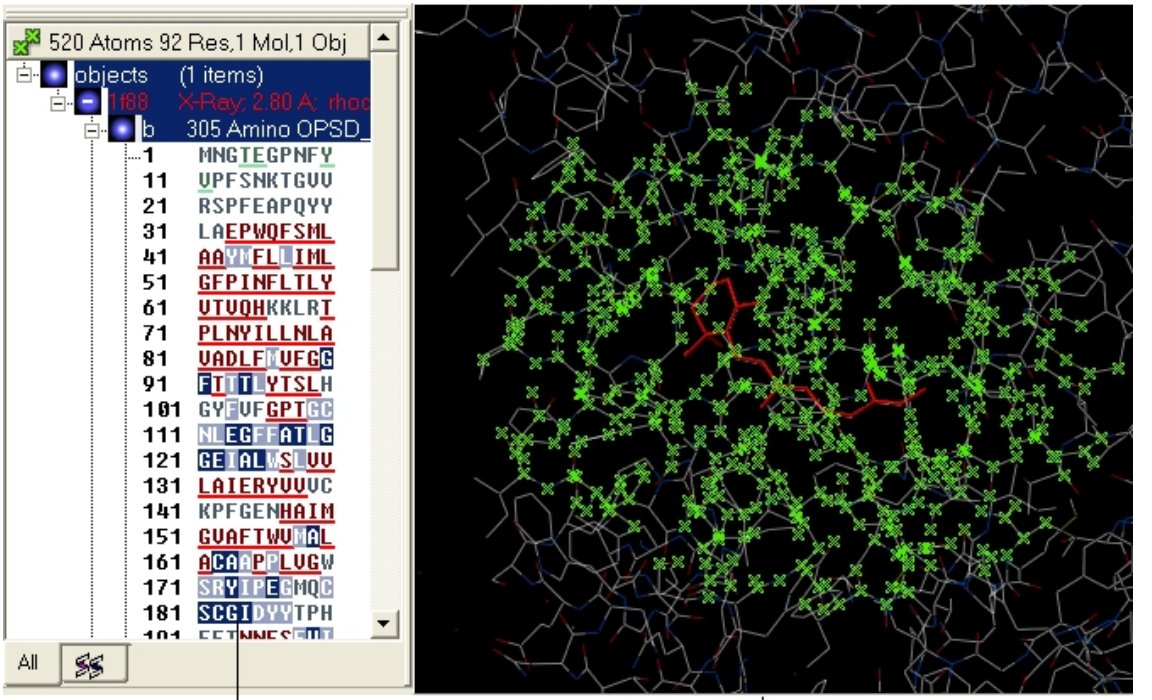
The query results will be displayed in a table. You can select the title of the table, and if you are doing a large number of queries then this name can be incremented by 1. (See diagram below)

To run the query click on the "search" button.



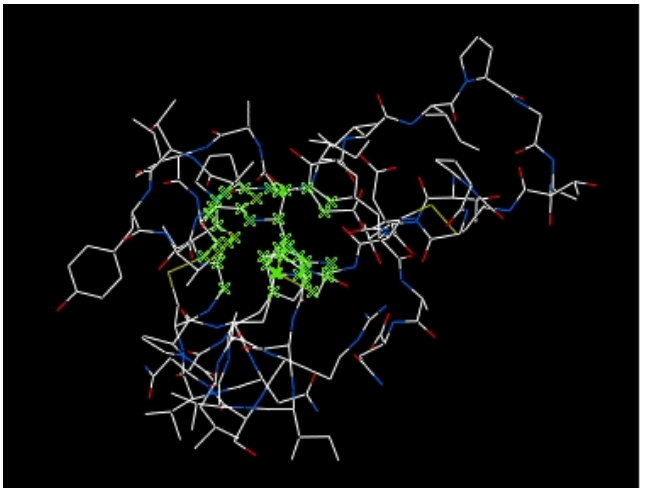
6. Making Selections

When using ICM there will be many occasions when you will have to make selections. For example, if you only wish part of your structure to be displayed 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.



6.1. Graphical Selections

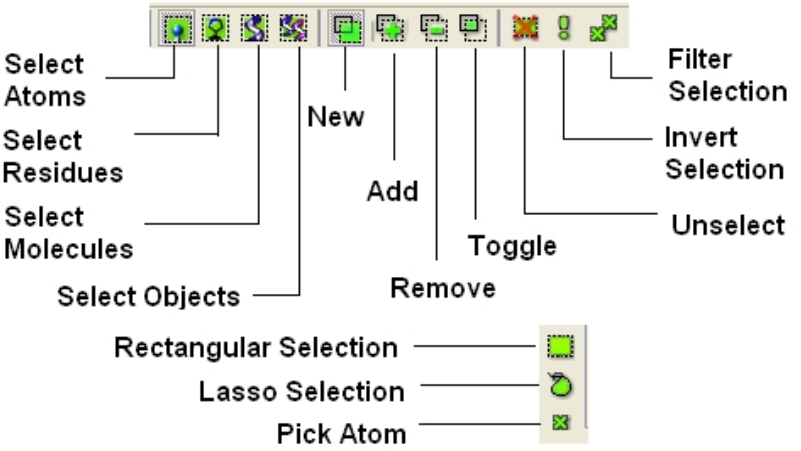
In this section you will learn how to select parts and certain regions of molecules from the 3D graphical display. Graphical and molecule selections are required in many operations within ICM. For example, if you wanted to display graphically part of a molecule or if you wanted to perform a minimization of residues within a sphere of an imporant atom.



Selection shown in green.

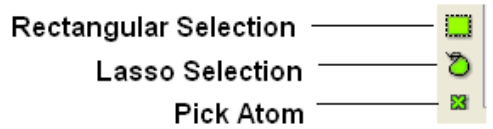
6.1.1. Selection Tools

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



6.1.2. Basic Selections

To make a basic selection (ie nothing too complicated!) the following buttons can be used.



To unselect everything you have previously selected:

- Simply click on the **Unselect** icon on the selection toolbar.

OR

- Right click and drag away from the displayed structure.

To select parts of your structure:

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

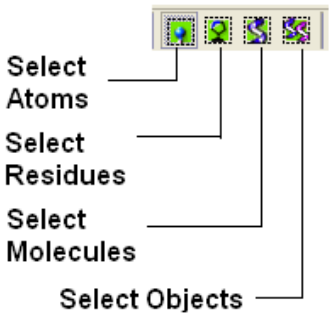
OR

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

To pick individual atoms:

- Click on the 'pick atom' button

You may want to specify which part of your structure you will be selecting. To select by a specific part of the structure:

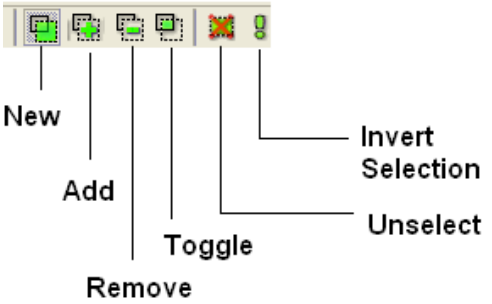


- Click on the **Select** objects **, Select molecules**, **Select residues**, or **Select atoms** icon, depending on which part of the structure you wish to be highlighted.

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 stored in the as_graph variable.

6.1.3. Altering 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.



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 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

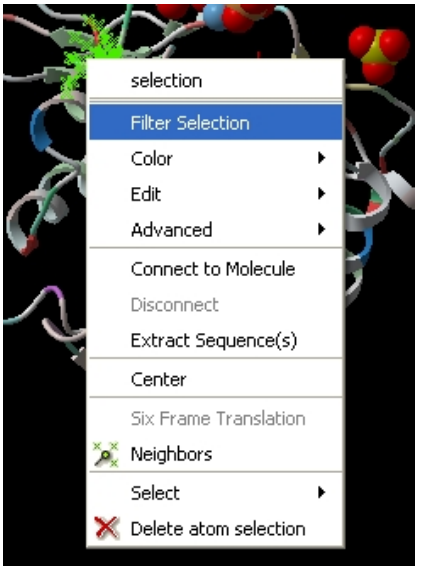
6.1.4. Filter Selection

You may want to be very specific about a selection you want to make. For example you may only wish to select protein backbone atoms.

The button shown below enables you to filter your selection:

Or

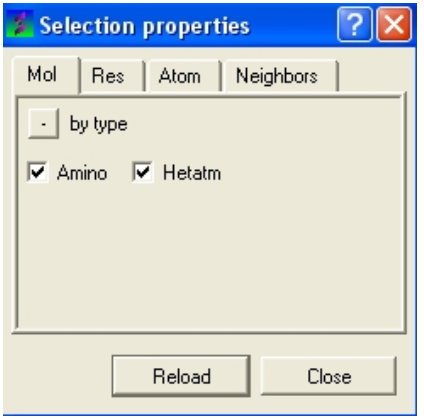
Right click on a selection and a menu as shown below will be displayed.



- Select the Filter Selection option.

If you wish to filter and select by residue or atom type:

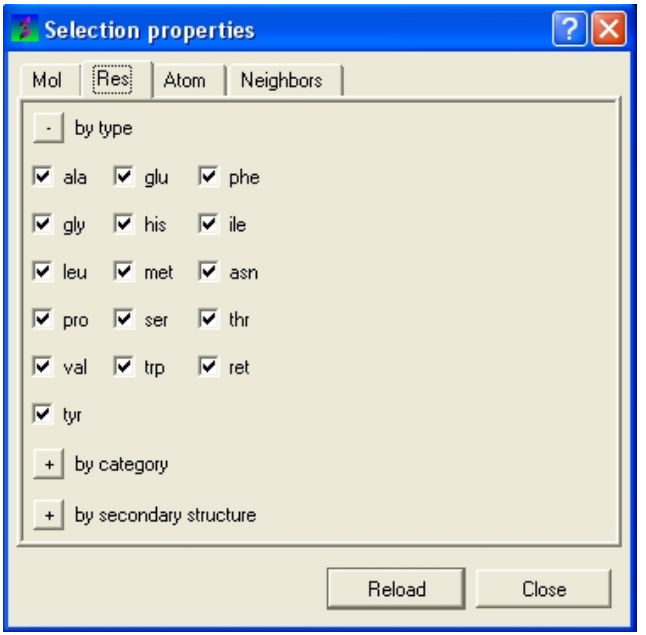
- Click on the Filter graphical selection icon on the toolbar and a data entry box as shown below will be displayed.



To select just the protein or just the hetatoms as well:

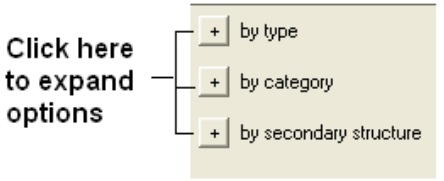
- Click on the Mol tab.
- Check the appropriate boxes depending on your desired selection.

To filter by residue type or secondary structure:

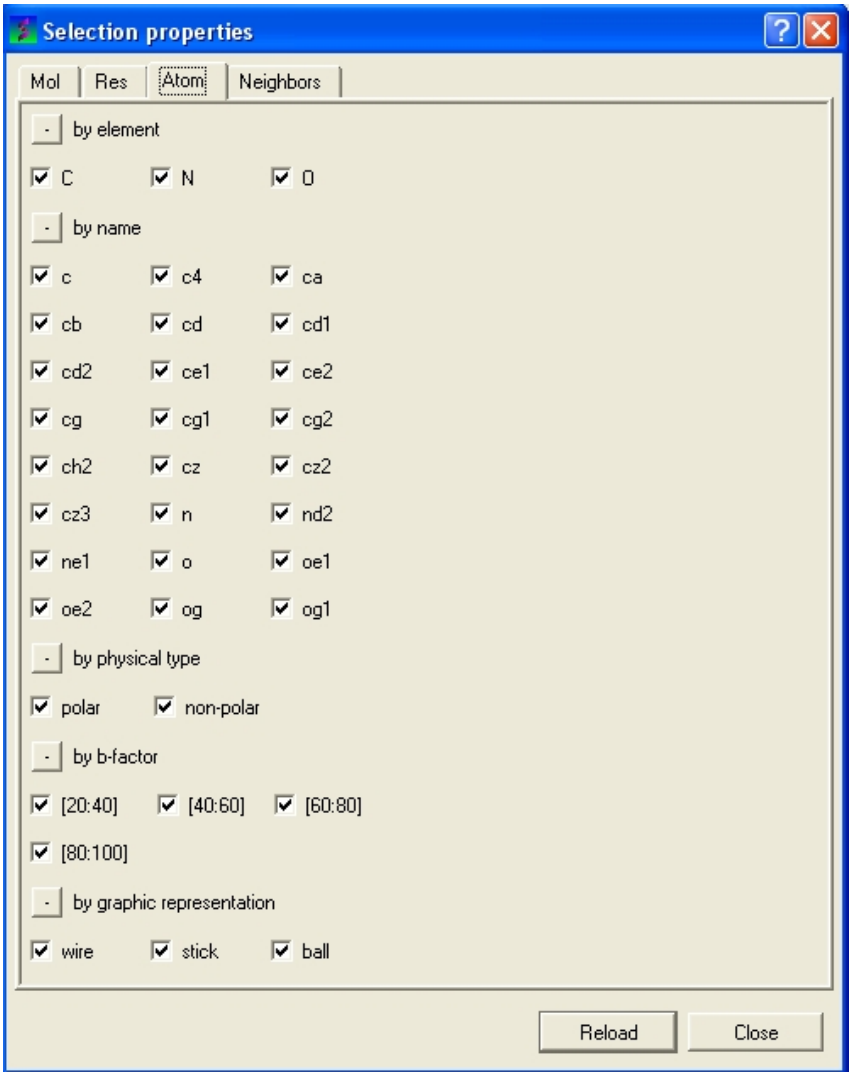


- Click on the Res tab.
- Check the appropriate boxes.

NOTE: You may need to click on the button marked with a '+' symbol to expand the options.

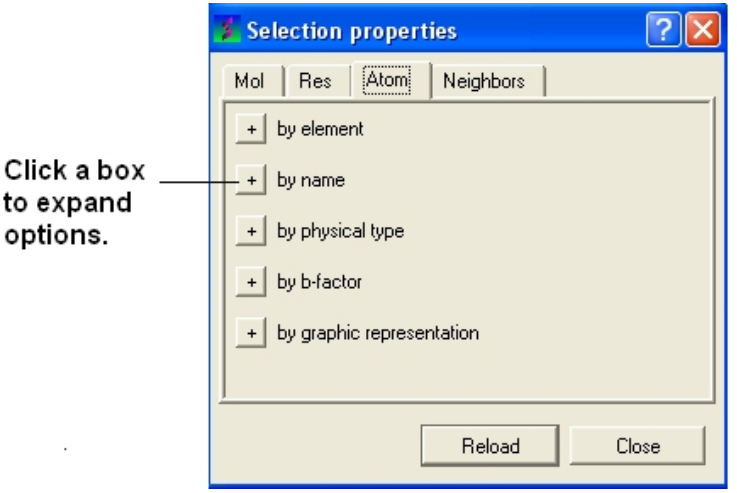


To filter by atom type.



- Click on the Atom tab.
- Check the appropriate boxes.

NOTE: You may need to click on the button marked with a '+' symbol to expand the options.



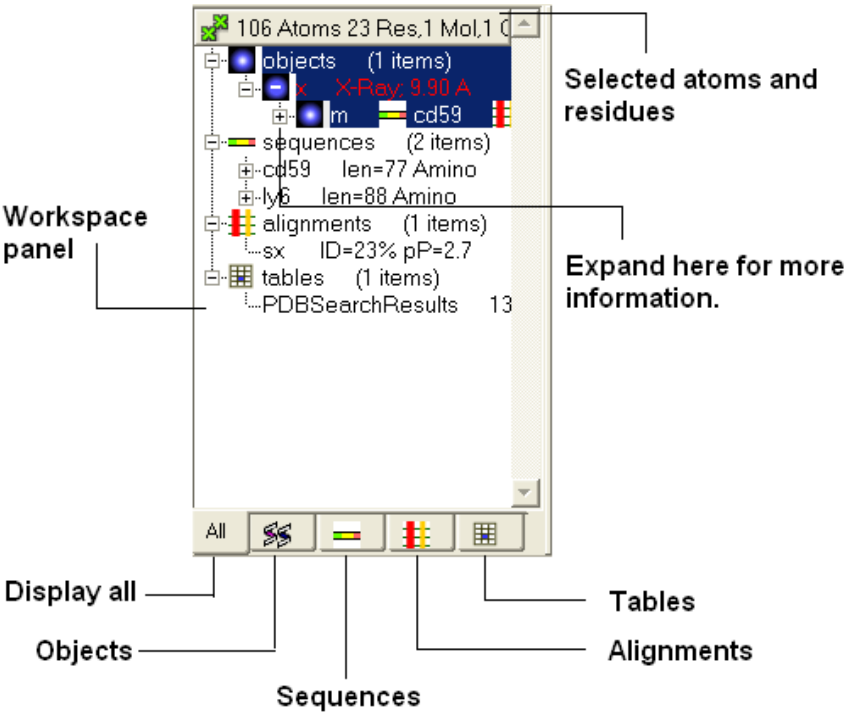
To select neighbors to a particular selection.

- See the `select neighbours` section for detailed instructions.

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

6.2. 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.



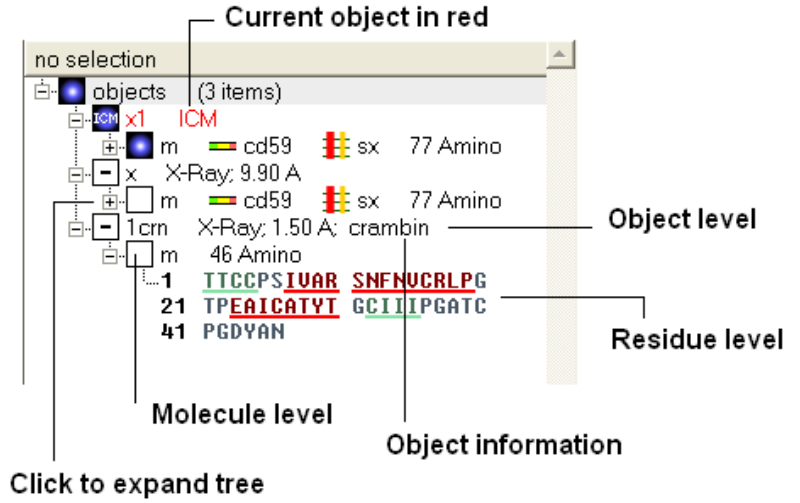
6.2.1. Workspace Navigation

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.

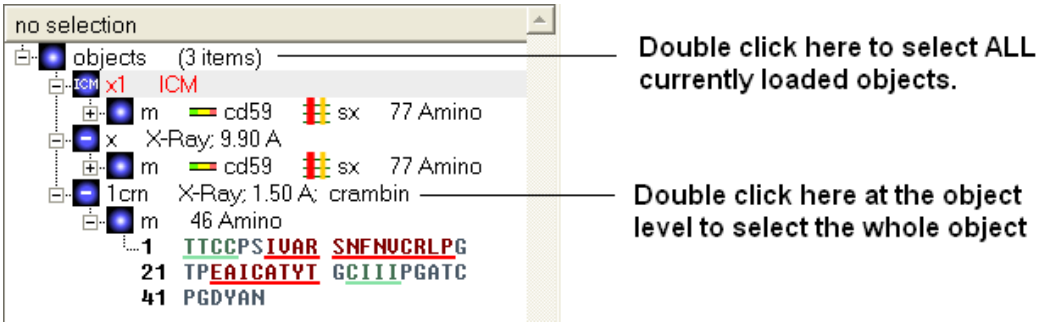


NOTE: You can expand each level of the ICM workspace by clicking the "+" button as shown above.

6.2.2. Selecting the Whole Object

To select the whole object:

- Double click on the object level.

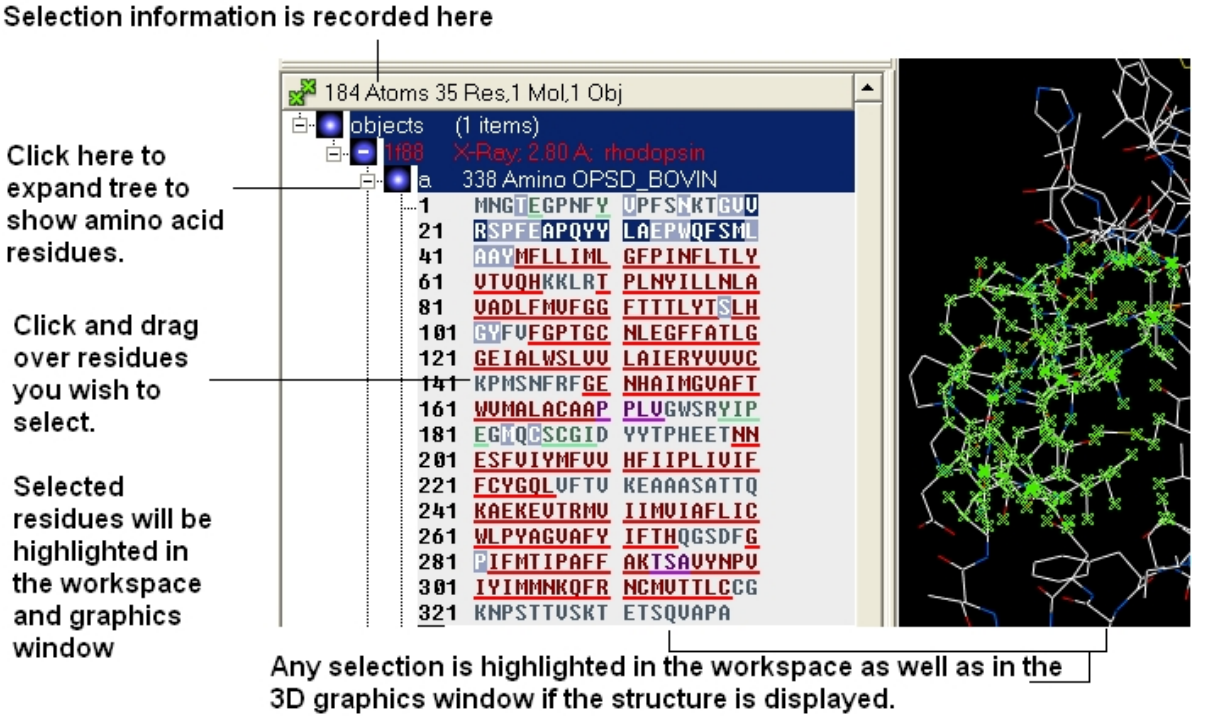


6.2.3. Selecting Amino Acids

There are three options to select individual amino acid 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.

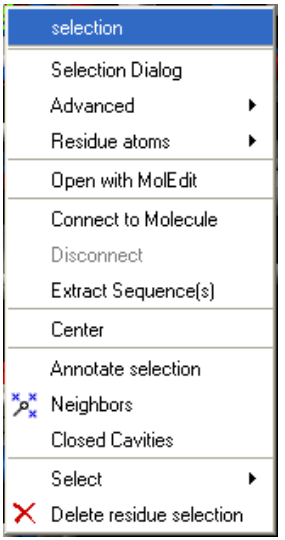


OPTION 2:

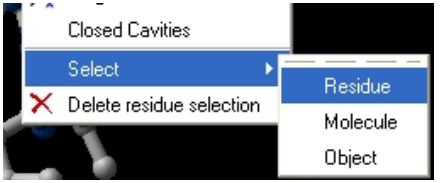
- Click on the **rectangular selection** icon or **lasso selection** icon on the toolbar.
- Click and drag around the residues you wish to select. Selected residues will be displayed by green crosses on the graphical display and blue in the ICM workspace.
- Click on the Pick Atom button.

OPTION 3:

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



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



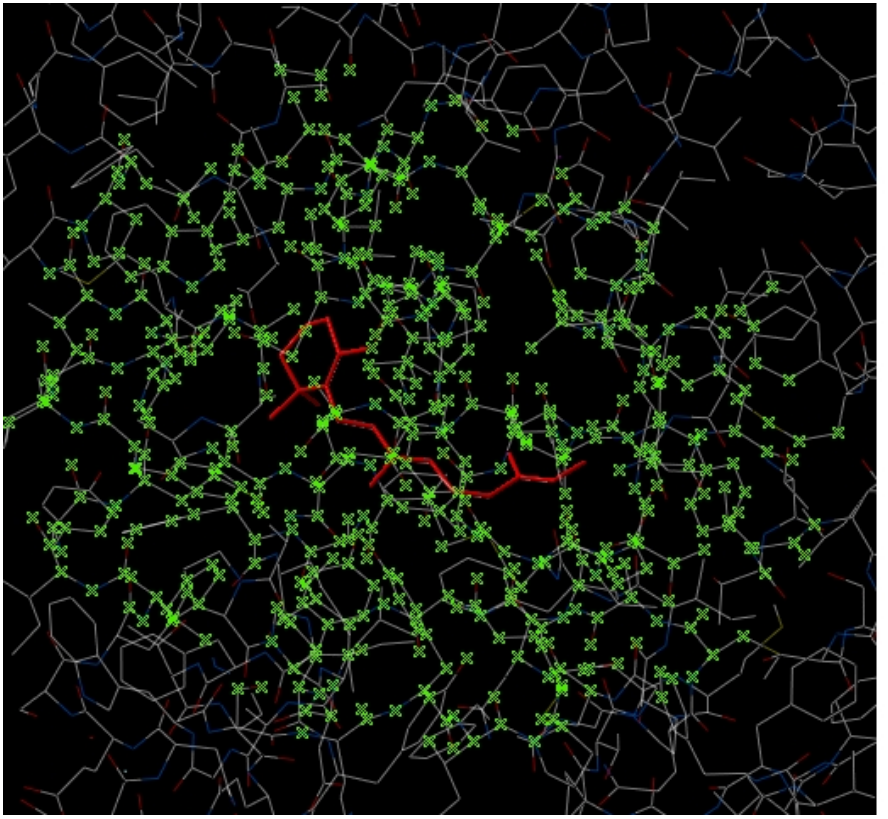
NOTE: Ctrl + A will select everything in the ICM workspace, and Ctrl + Shift + A will unselect your objects.

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

6.3. 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.

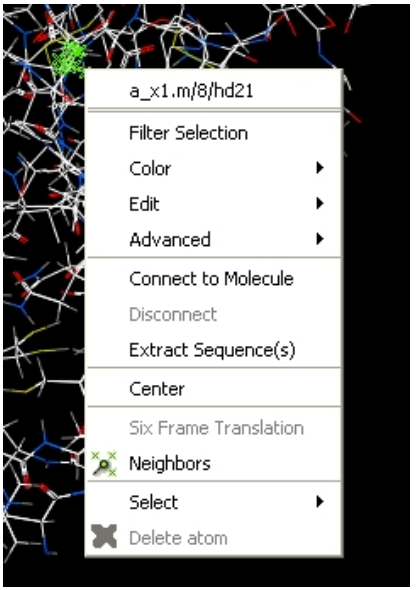
There are two ways of selecting neighbours to a particular atom or residue in ICM. Either by right clicking in the graphical display or by right clicking in the ICM workspace.



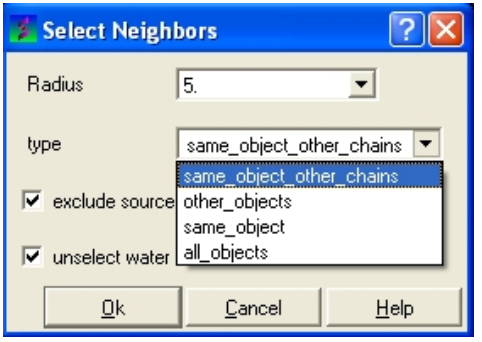
6.3.1. Selecting Neighbors: Graphical

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.



- Select the Neigbors option and a data entry box as shown below will be displayed.



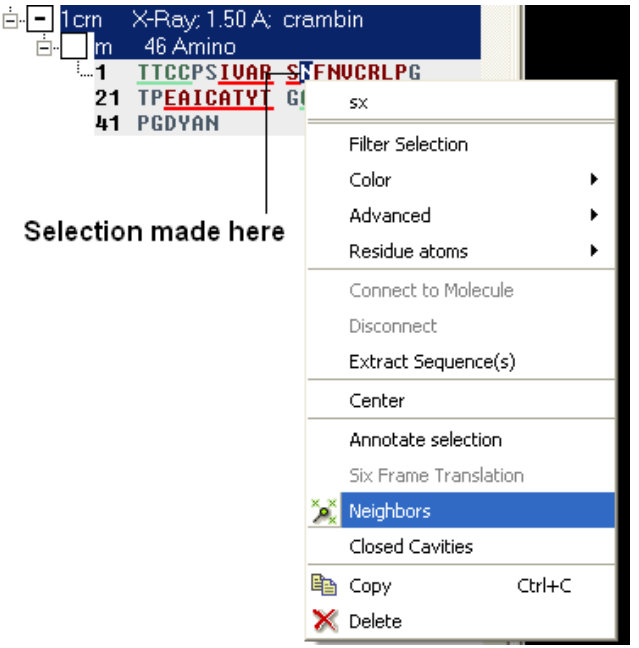
- Enter the selection radius around which you wish to select.
- Select from which object you wish to make the selection from the drop down list in the "type" data entry box.
- Select whether you wish your original selection to be selected by checking the "exclude source" box.
- Select whether you wish water molecules to be selected by checking the "unselect water" box.
- Click OK and your selection will be displayed as green crosses.

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

6.3.2. 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`.

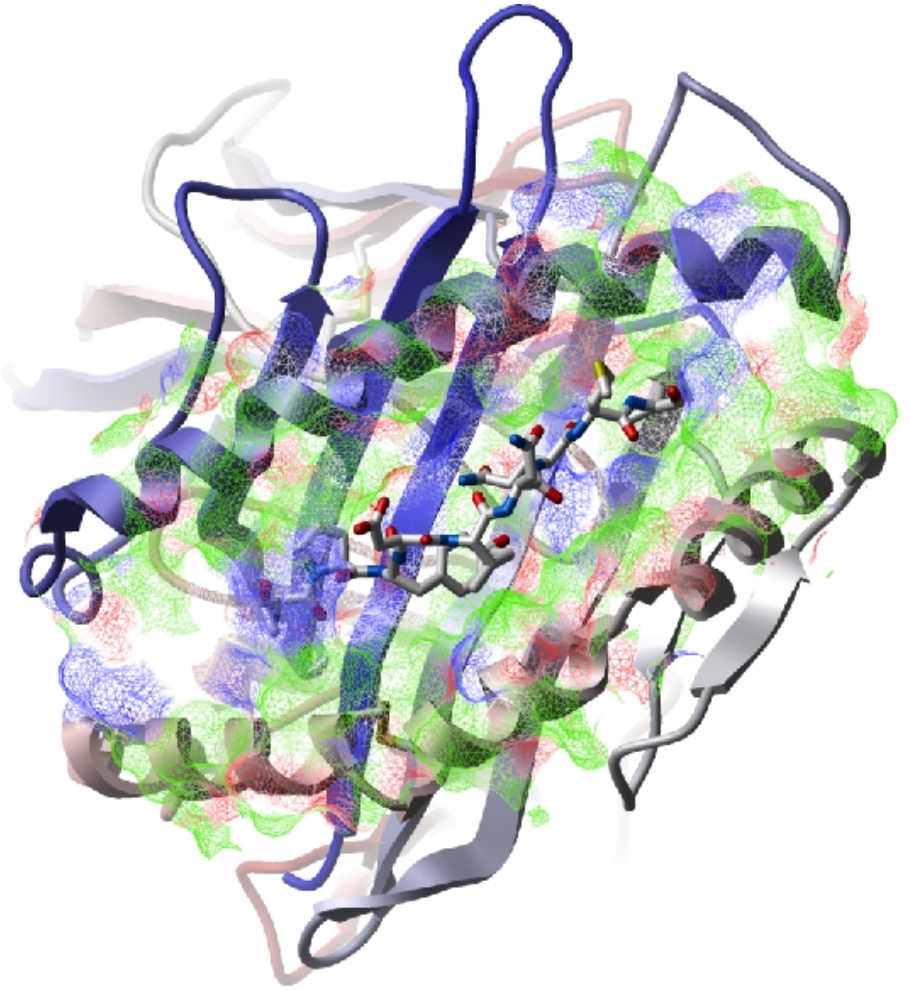
NOTE: The selection you have made is always recorded at the top of the ICM workplace. If you are familiar with using the ICM terminal (See language manual) the atoms, residues, molecules or objects selected interactively in the graphics window are automatically s

6.4. Other Selections

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

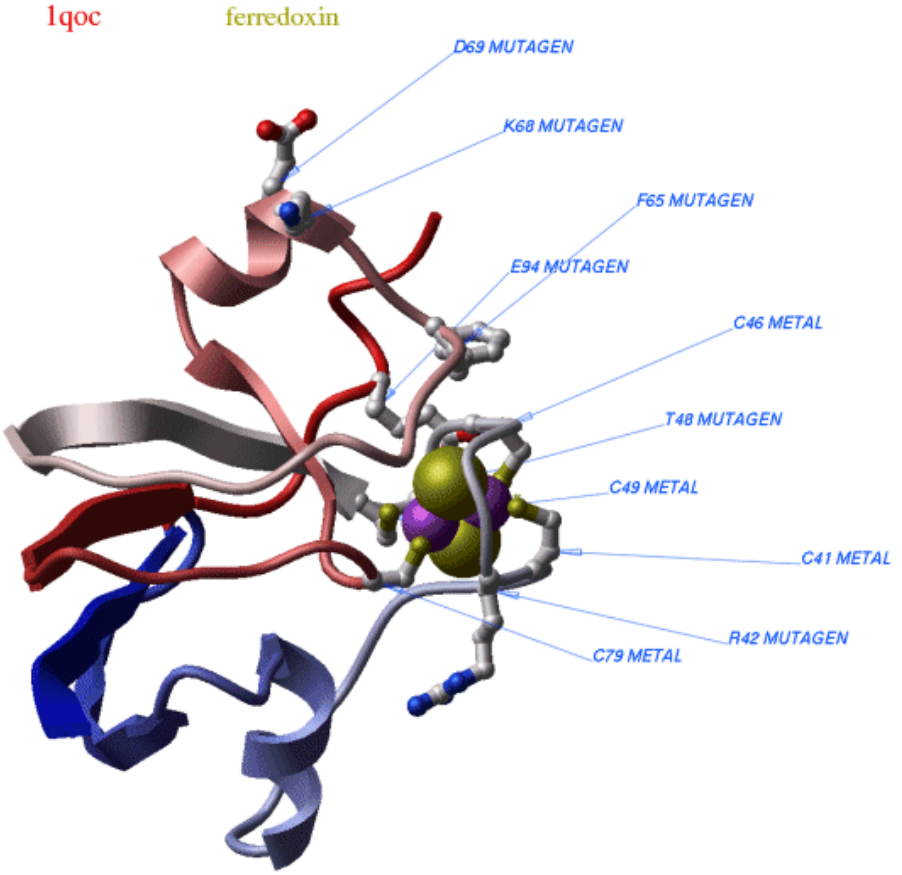
7. Molecule Viewing and Graphical Representation

Once a structure has been loaded into ICM (see `GUI Basics`) then you may want to display the structure in a way that allows for a more detailed inspection or the construction of a beautiful image. This section will show you how.



7.1. Displaying a Structure

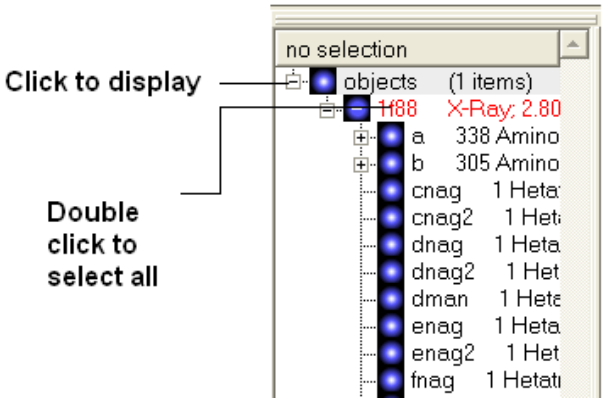
Once a structure has been loaded into ICM the individual components of that structure (i.e. amino acids, metal ions, binding sites etc) are listed in the ICM workspace.



7.1.1. Displaying and undisplaying the entire structure

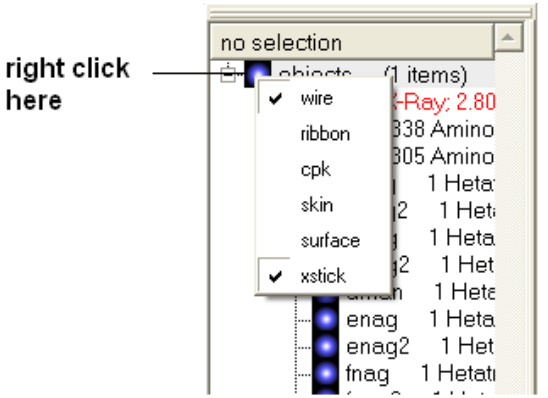
To display every component of the object except for binding sites and water atoms:

- 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 green 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:

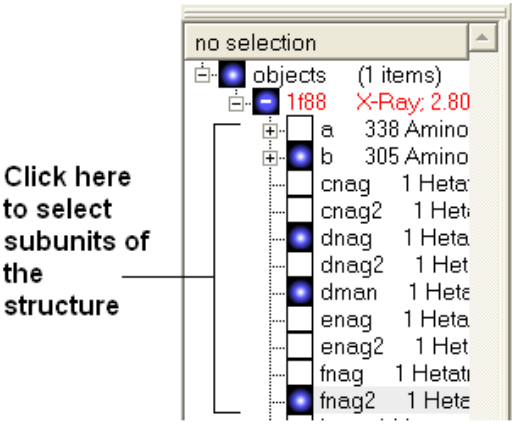
- Select **View/Undisplay** All

7.1.2. How to display structural subunits

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

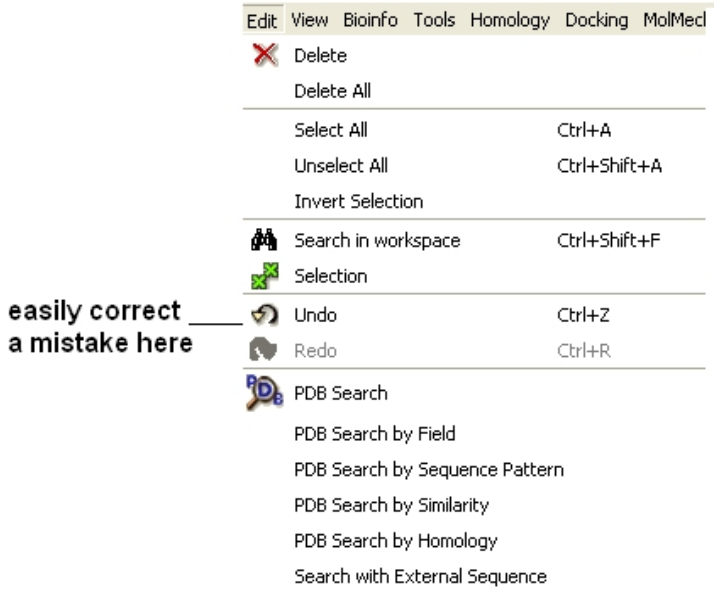
To display the selected regions of the structure in wire, ribbon, cpk, skin, surface and xstick representations:

- Right click on the appropriate box in the **ICM** workspace. 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.



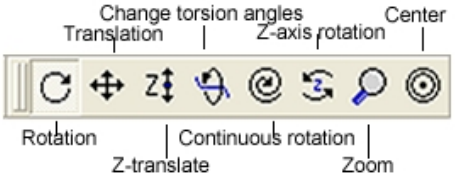
7.1.3. Undo and Redo

Most of the viewing and graphical representation functions descibed in this section can be undone or restored using the 'undo' and 'redo' option located in the edit menu. It is an easy tool for correcting a mistake. However all actions in ICM can be rectified by a variety of other ways. Such as, if you accidently label the wrong reside, this label can be removed by selecting the residue and pressing the label button again.



7.2. Moving Your Structure

To move your structure it must first be displayed in the graphics window (for instructions on how to display a structure see `Molecule Viewing and Graphical Representation section`). All of the following options are displayed in the Move Tools toolbar (shown below).



7.2.1. 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.

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.

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.

7.2.2. 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.

7.2.3. 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.

7.2.4. Center

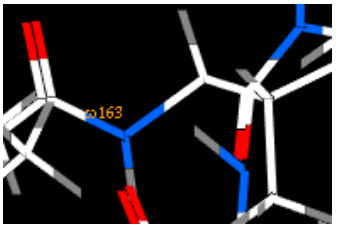
To restore your picture to the center of the graphical display window:

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

7.2.5. Torsion Angles

To alter the torsion angle of certain residues of your structure:

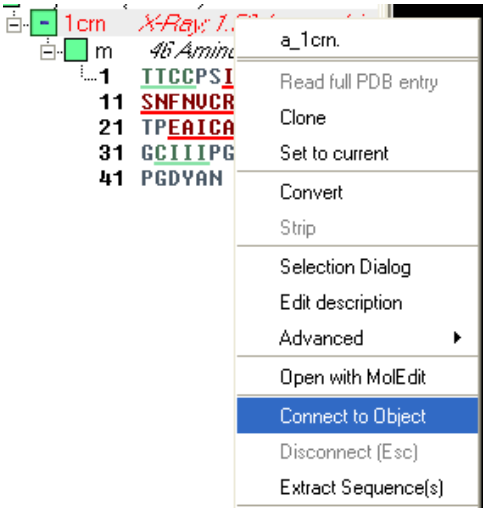
- Click on the **change torsion angles** icon on the toolbar.
- Click and drag on the atom around which you wish to rotate a residue. The changing angle will be displayed in orange.



7.2.6. 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 separately from the other you can use the connect option.

- Right click on the name of the object you wish to move in the ICM workspace and select **Connect to Object**

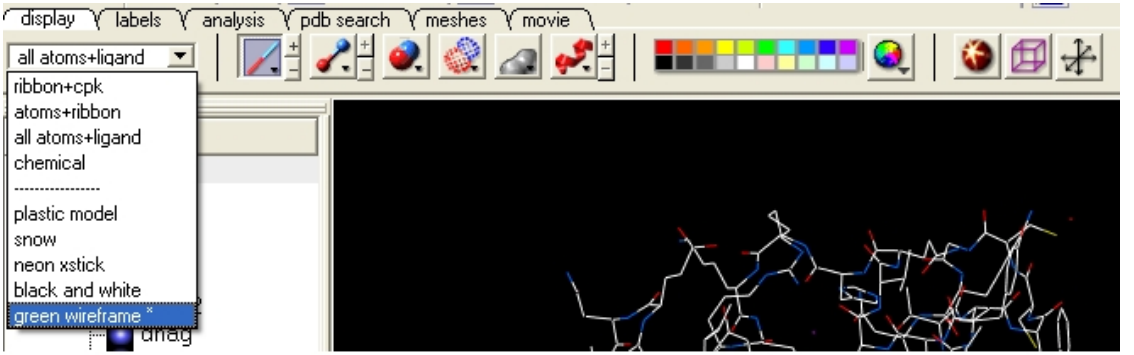


- The object is now controlled separately from the rest of your objects by your mouse.

- Disconnect your object by once again right clicking on the name of the object and selecting disconnect in the drop down menu.

7.3. Structural Representations

All structural representations can be controled from the display tab at the top of the screen. This tab is shown below.



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

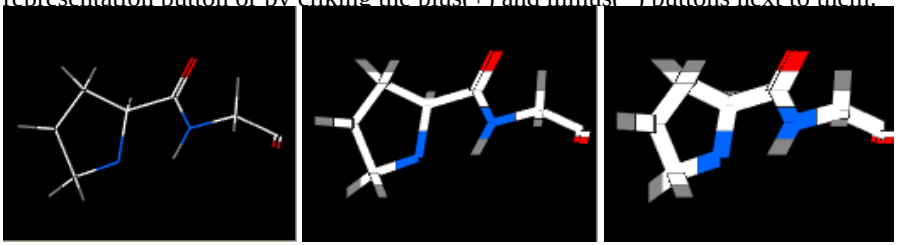
To display one of these representations:

- Click on the representation button you desire in the display tab (shown above).

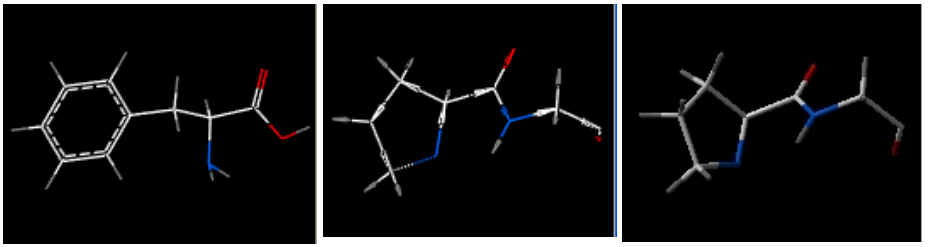
To remove a displayed representation:

- Click on the corresponding representation button at the bottom of the graphical user interface window.

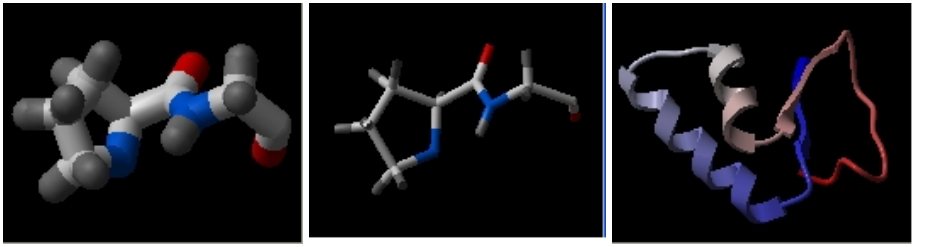
Many characteristics of the graphical representation such as color can be changed by right clicking on the representation button or by cliking the plus(+) and minus(−) buttons next to them.



Wire : Thin Wire : Normal Wire : Thick

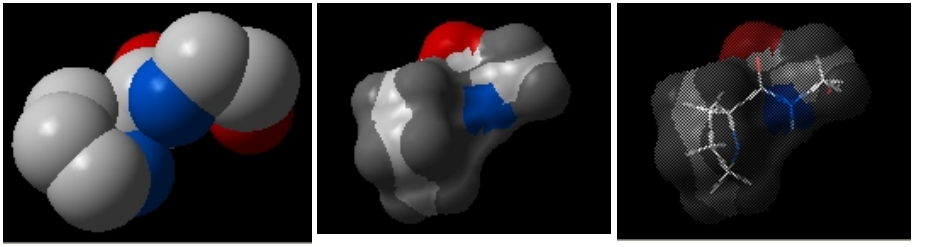


Wire : Chemistry Wire : Tree Xstick− Thin

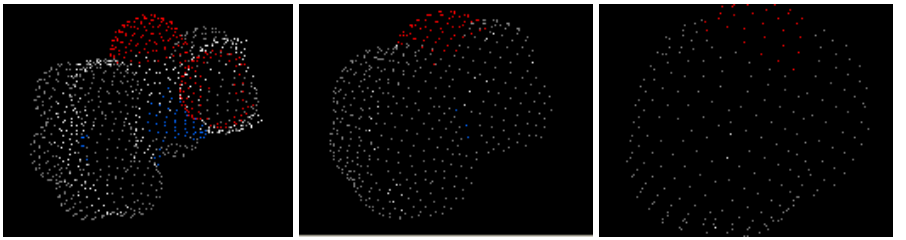


Xstick : Thick Xstick : Stick / Ball Ribbon : Ribbon

Ribbon : Segment Ribbon : Protein Worm Ribbon : Transparent



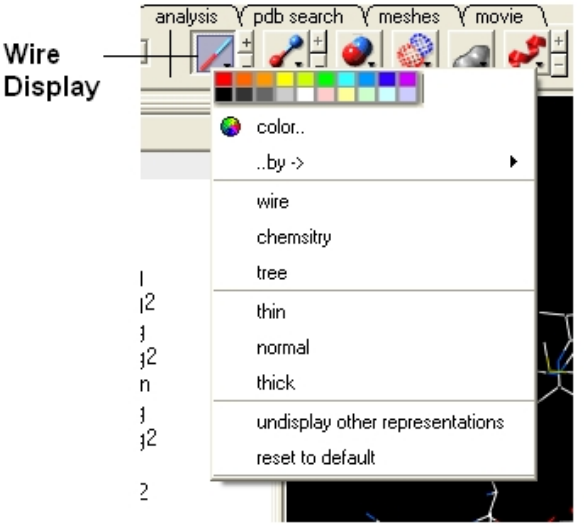
CPK : Default Skin : Default Skin : Transparent



Surface : Tight Surface : Normal Surface − Sparse

7.3.1. 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 arrow next to the **wire representation** button also changes the thickness of the wire representation.

To undisplay representations other than wire:

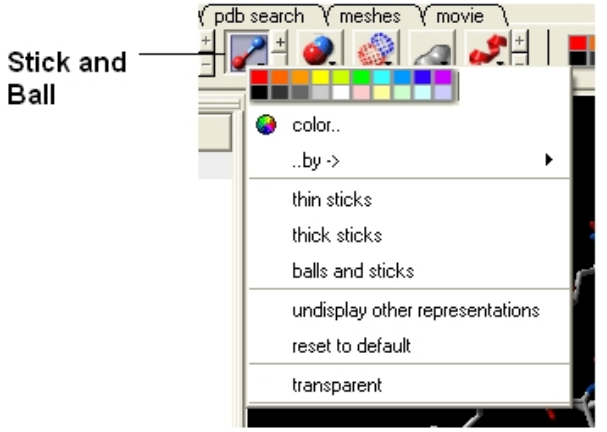
- Click and hold on the **wire representation** button and then click on undisplay other representations.

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.

7.3.2. 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 size and style of the Xstick representation:

- Click and hold on the **stick and ball representation** button and then click on **thin sticks, thick sticks** or **balls** and sticks**.

NOTE: Clicking on the arrow next to the **Stick and ball representation** button can also change 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.

7.3.3. Ribbon Representation

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

To change the style of the Ribbon representation:

- Click and hold on the **ribbon representation button** and then click on **ribbon, segment, protein worm** or **DNA worm.**

In order 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 .**

In order 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:

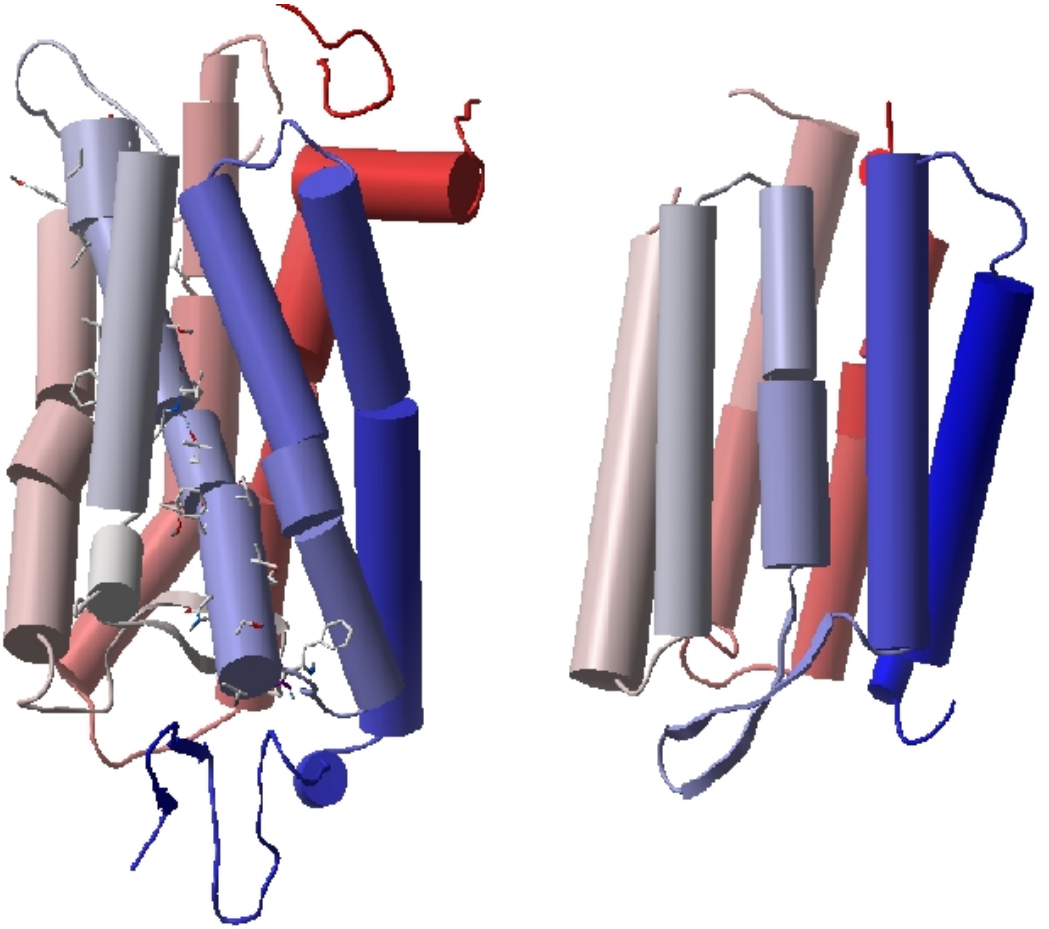
- Click and hold on the **ribbon representation** button and then click on undisplay other representations.

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 .**

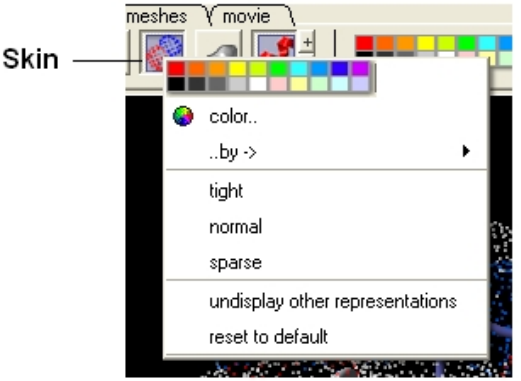
NOTE: Always use the **ICM** assign sec.** structure tool in the ribbon right click menu to get accurate secondary structure assignment. This is particularly important when studying helices which may have non−cannonical elements within them such as 3/10 or pi. To view non−cannonical helix segments use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

The helices in rhodopsin and bacteriorhodopsin are shown below in segment representation. Non−cannonical regions are represented as breaks in the helix segment.



7.3.4. Skin Representation

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



In order 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 .**

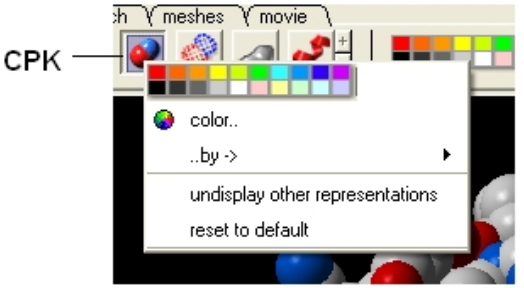
If you make a mistake or you are not happy with the way your structure is displayed with the skin representation:

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

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

7.3.5. CPK Representation

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



To undisplay representations other than CPK:

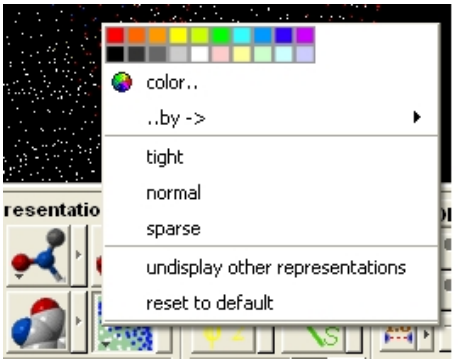
- Click and hold on the **CPK representation button** and then click on **undisplay other representations .**

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 .**

7.3.6. 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.

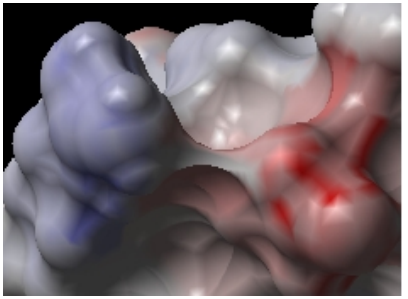
If you make a mistake or you are not happy with the way your structure is displayed with the surface representation:

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

7.3.7. Electrostatic potential

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

- Select **View/Electrostatic** potential.
- Areas colored blue represent positive areas and red represents negative areas.



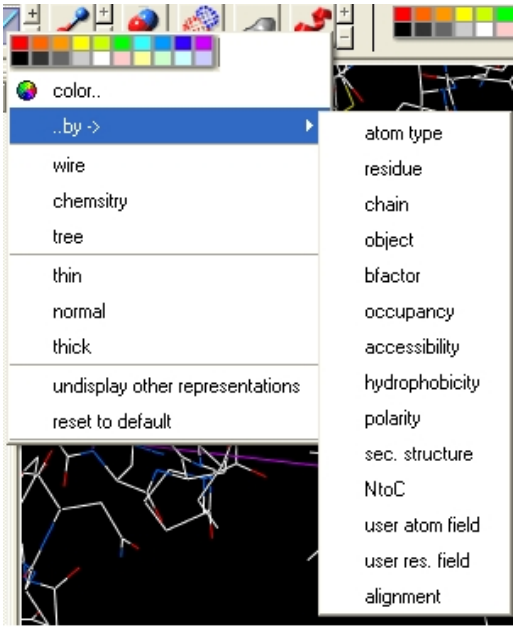
7.3.8. Coloring

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

- Click and hold on the structural representation buttons in the Display tab.
- Select a color by clicking color.

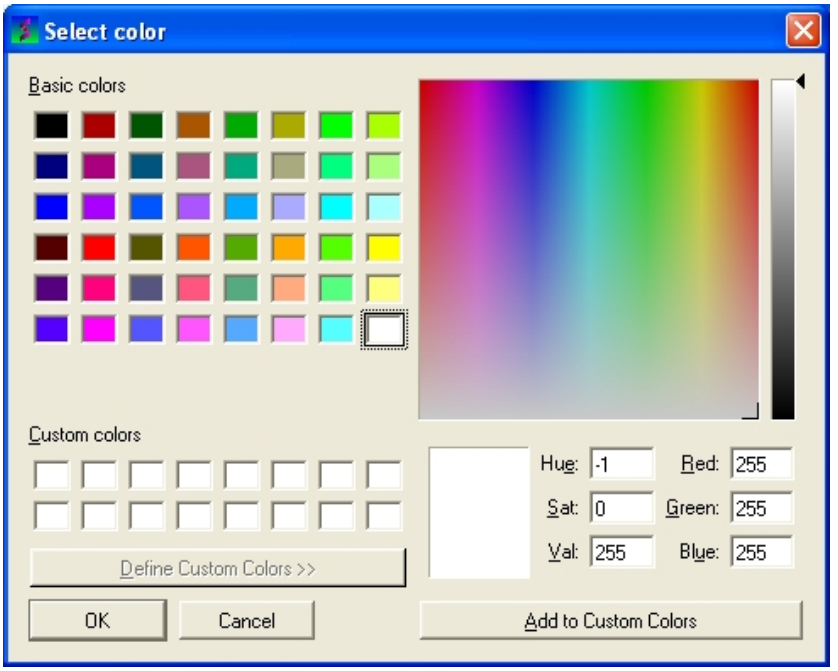
OR, if you wish to color by a particular parameter such as atom type, b−factor etc...

- Right click on the structural representation buttons in the Display tab.
- Select ..by−> option



To change the color of the whole of your displayed structure:

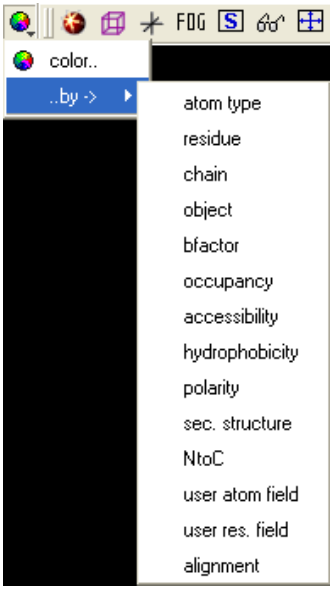
- Click on the color palate displayed on the toolbar.
- If you are not satisfied with these colors, click on the color wheel on the toolbar. A window as shown below will be displayed. Select the desired color by either clicking on one of the basic colors or by selecting the desired color on the right hand side of the window.



- Once the desired color has been selected it can be added to custom colors for future use by clicking on the Add to Custom Colors button.
- Click the **OK** button and the color will be applied to the structure.

To change the color of the desired representation by a defined structural characteristic:

- Click and hold on the **wire representation button** and then click **..by−>.** A menu as shown below will be displayed.

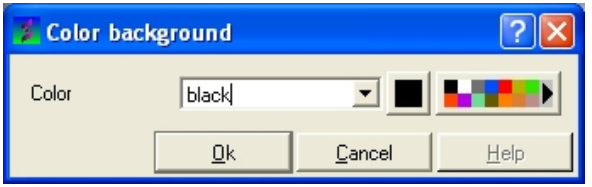


- Select the characteristic that you wish to color by and click on it.

7.3.9. Coloring Background

In order to change the color of your background:

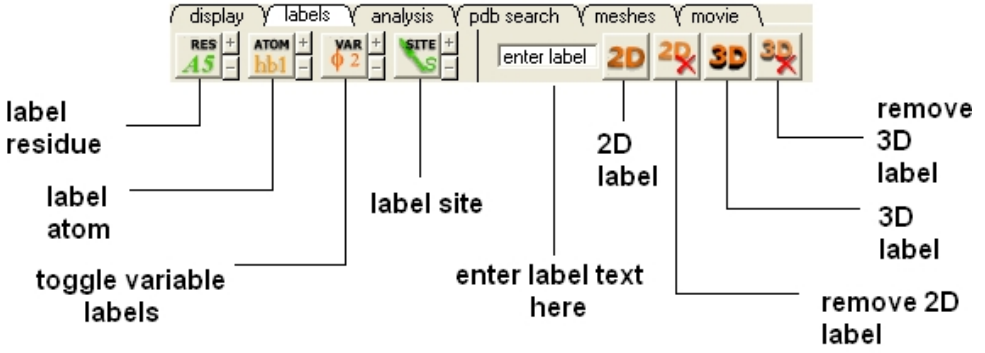
- Select **View/Color** background.



- 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.

7.4. Labeling

Click on the label tab shown below to enable access to the labelling options.



7.4.1. Labeling Atoms

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

- Click the **label ATOM** button.

To change the level of label detail:

- Click the +/− options on the **label ATOM** button until the desired level of label detail is displayed.

7.4.2. Labeling Residues

To label residues:

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

To change the level of label detail:

- Click the +/− options on the **label RES** button until the required level of detail is displayed.

7.4.3. 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.

- 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.

7.4.4. Labeling Sites

Select the sites you wish to label (see `Displaying structure` or `Selection Toolbar`):

- Click the **label SITE** button.

To change the level of label detail:

- Click the arrow +/− option on the label SITE button until the required level of detail is displayed.

7.4.5. 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.**

7.4.6. Customized Label

To add your own text to a label you must first select the atom or residue you wish to label (see `selecting structure`).

- Type your label in the **enter label** text box which is shown below.

* Click the 2D button.

* To remove the label click the 2D button with the red cross on the side shown above.

NOTE: If you wish your label to be represented in 3D click the 3D button.

7.4.7. Delete Label

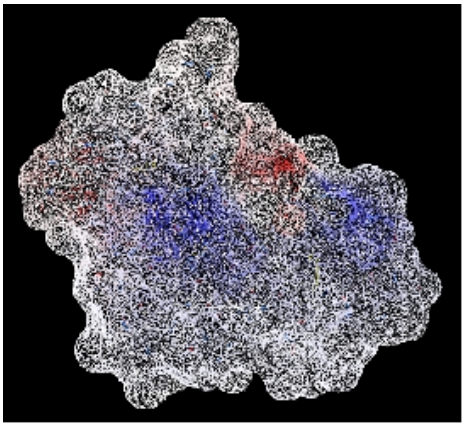
Any displayed label can be deleted by clicking on the corresponding label button in the labels tab. For example if you wish to delete an atom label click the atom label button. If a label is displayed this button will be shaded blue. When you delete the the button will return to grey.

7.5. Surfaces Meshes and Macroshape

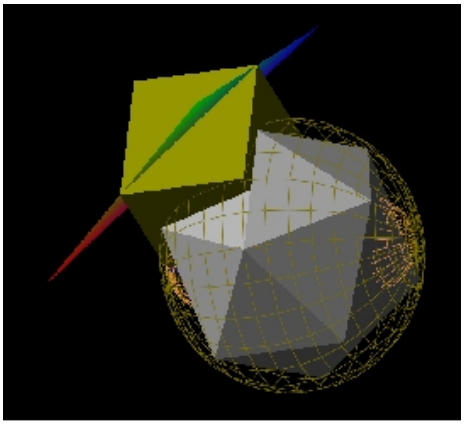
If you click the tab button entitled '**meshes**' three different graphical display tools are available for you to use. The three displays are surface, meshes and macroshape and are collectively referred to as meshes.

The benefits and applications of each display are described in this section.

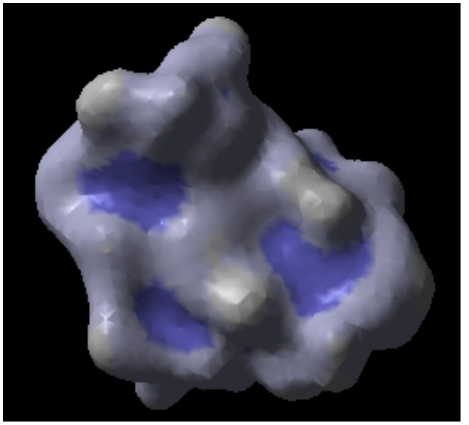
Surface:



Meshes:



Macroshape:

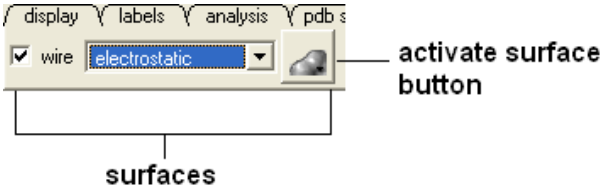


7.5.1. Surfaces

The surface of your structure can be displayed and colored. To undertake this

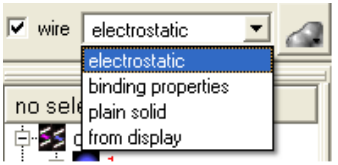
- Load a structure into ICM `(see Molecule Viewing and Graphical Representation section)`.
- Select the 'meshes' tab button.

The buttons and options you need to use are shown below:



First you need to decide by what parameter you wish to color the surface:

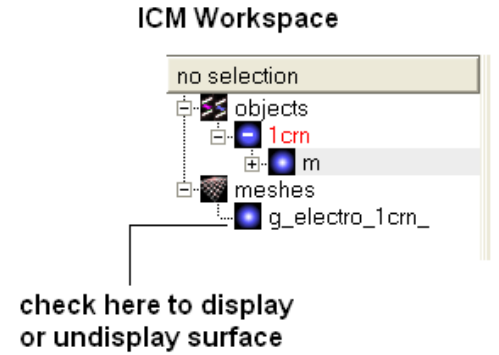
- Click on the drop down arrow menu shown below and select which parameter you wish to color the surface by.



- Click the button next to the menu to color the surface of your molecule.

If you wish to have your surface displayed in wire check the 'wire' box next to the menu.

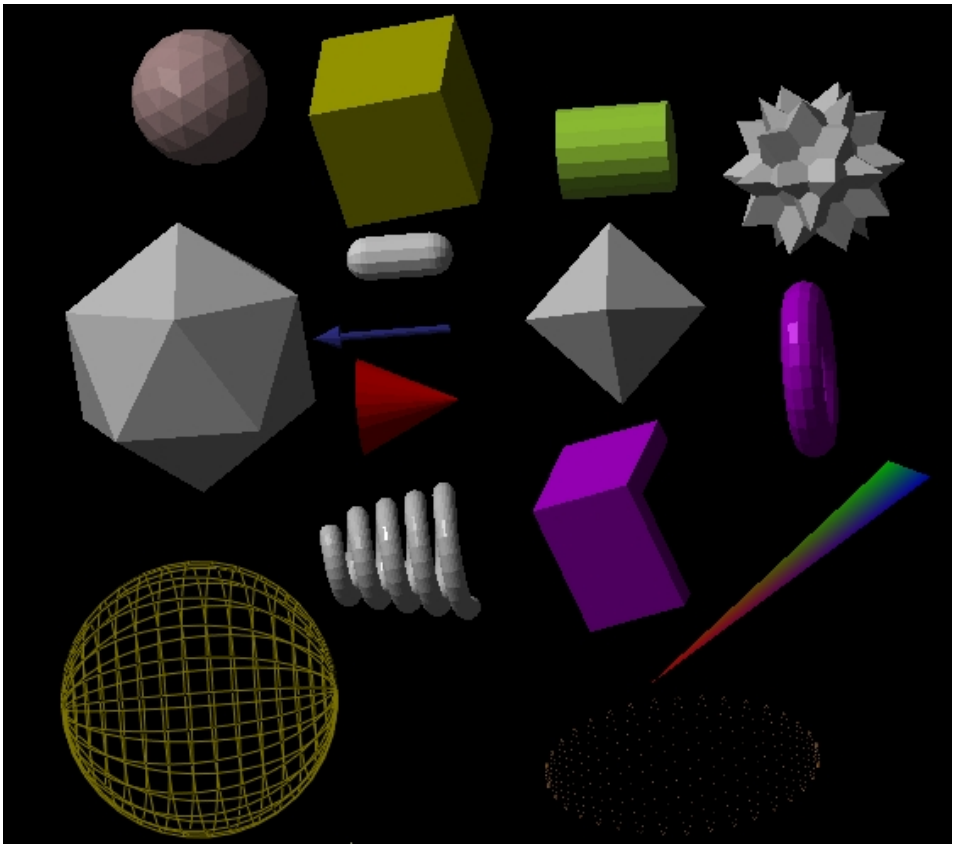
To display or undisplay the surface click in the box in the ICM workspace as shown below:



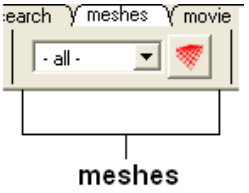
NOTE: All surfaces, meshes and macroshapes come under the one heading of **meshes** in the workspace panel.

7.5.2. Meshes

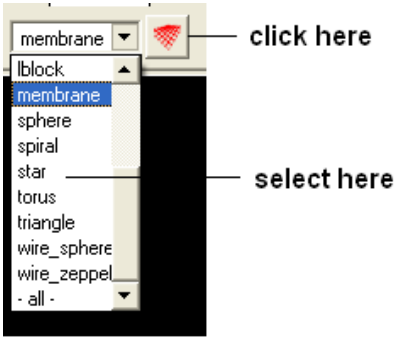
A variety of shapes can be constructed using ICM. These shapes are referred to as meshes. The types of shapes you can build are shown below:



All the buttons for creating these shapes are shown here:

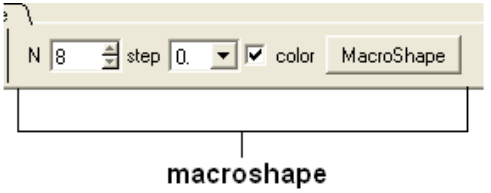


To make a shape select it from the menu by clicking on the down arrow and then click the button next to the menu. The shape will then be displayed in the 3D graphics window.



7.5.3. Macroshape

A macroshape can be constructed which 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:

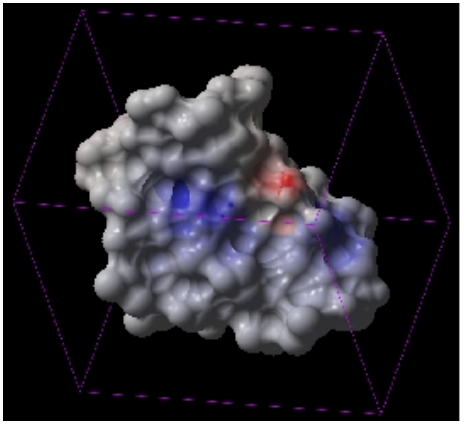
- Load a molecule into ICM (`see Molecule Viewing and Graphical Representation section`).
- Select the amount of detail required in the shape by increasing the values in 'N' or 'step' data entry box (note the default values are usually sufficient).
- Check the 'color' if you wish your molecule to be colored.
- Click the button labeled 'MacroShape'.

7.5.4. Move and Resize Mesh

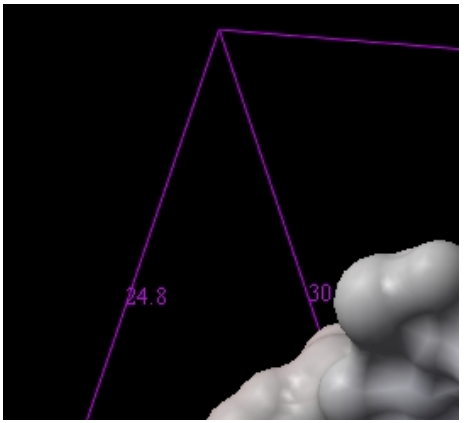
Once a mesh has been created you can move it and resize it. To do this, locate the mesh you wish to either move or resize in the ICM Workspace and right click on it as shown below.

- Select the **Resize/Move Mode** in the menu.

A purple box as shown below will surround the molecule.



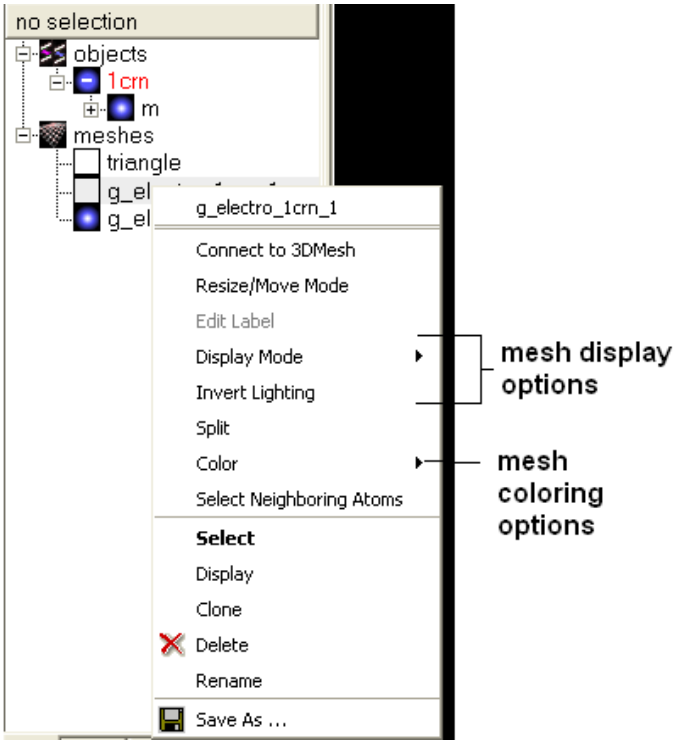
To resize the mesh click on one of the corners of the box and drag to the required size. The number displayed on the edges of the box represent the dimensions.



To move the mesh click on it with the center mouse button or selct the connect option.

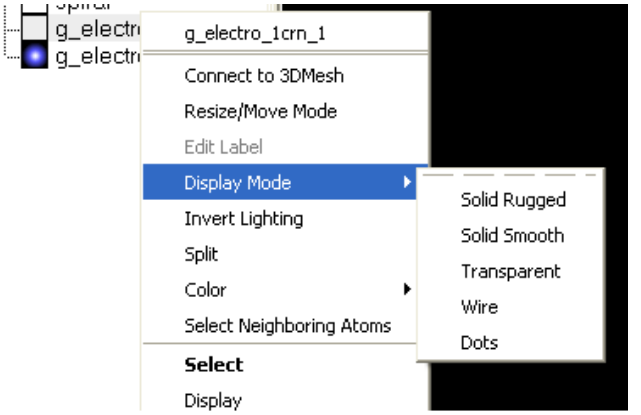
7.5.5. Color and Mesh Display

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.



The lighting and display can be changed by selecting the options 'Display Mode' or 'Invert Lighting'.

There are five different display modes as shown below:



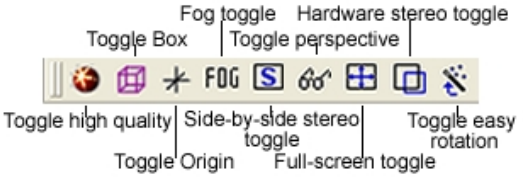
To change the lighting effects select 'Invert Lighting'.

The mesh colors can be changed by using the 'Color' option in the menu.

7.6. Advanced Image Processing

Here we describe some more ways to improve your images.

7.6.1. View Tools



Toggle High Quality : this will make your ICM object to have 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.

Toggle Box around Selection − this creates a 3D box around your structure, allowing you to make 3D selections. Click and drag the vertices of the box to obtain your desired selection, and then go to View/Select by purple 3D box.

Toggle Origin : this shows the origin and the coordinate axes of your graphical display.

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.

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

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

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

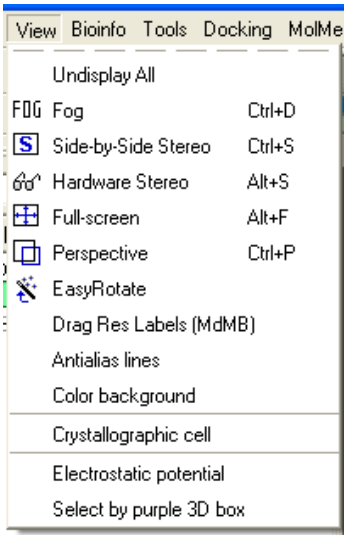
Hardware stereo toggle (Alt + S) − if you have 3D goggles and you wish to view your structure in 3D form, this feature will allow you to do so.

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.

NOTE: Most of these options can be found in the **View** menu.

7.6.2. View Menu

The following options can be found in the **View** menu shown below.



In order to smooth your image by creating a more gradual transition between the color of a line and the background color:

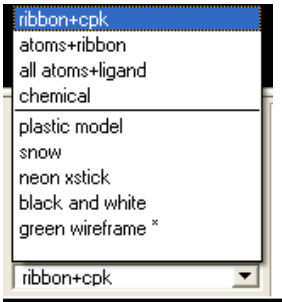
- Select **View/Antialias lines.**

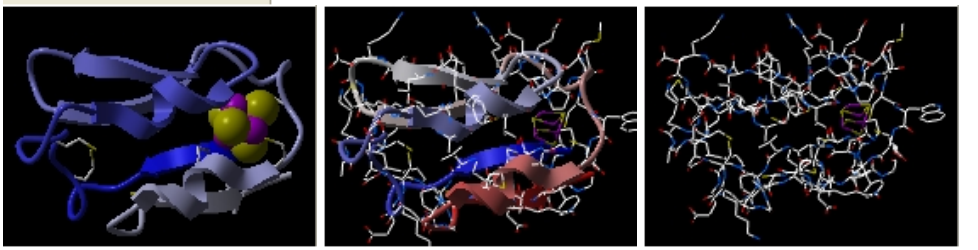
In order to view the crystallographic cell (more information in the `Displaying Crystal Cell section`) of your loaded PDB structure:

- Select **View/Crystallographic cell.**

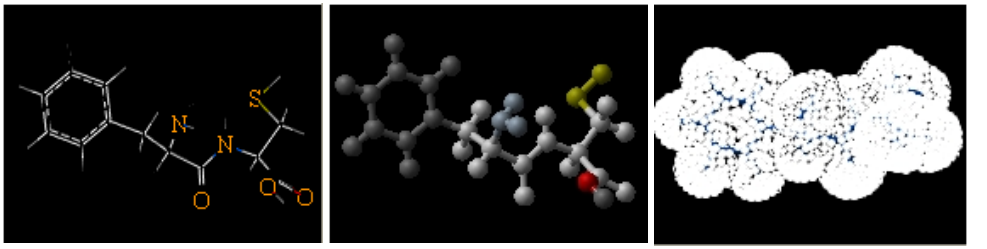
7.6.3. Advanced Representations

There are several convenient representations that are preset in a drop down menu below the other representations.

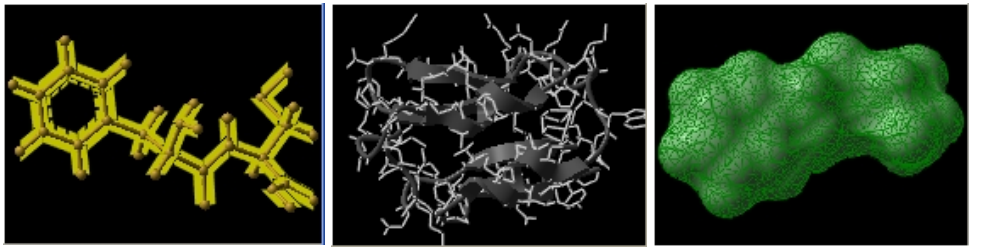




Ribbon + CPK Atoms + Ribbon All atoms + Ligand

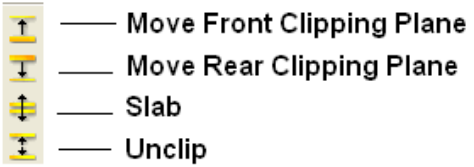


Chemical Plastic Model Snow



Neon Xstick Black and White Green Wireframe

7.7. Clipping Tools



The clipping tools allow you to adjust the frames of the ICM window, changing the clipping planes.

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 undesirable changes to the clipping planes 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.

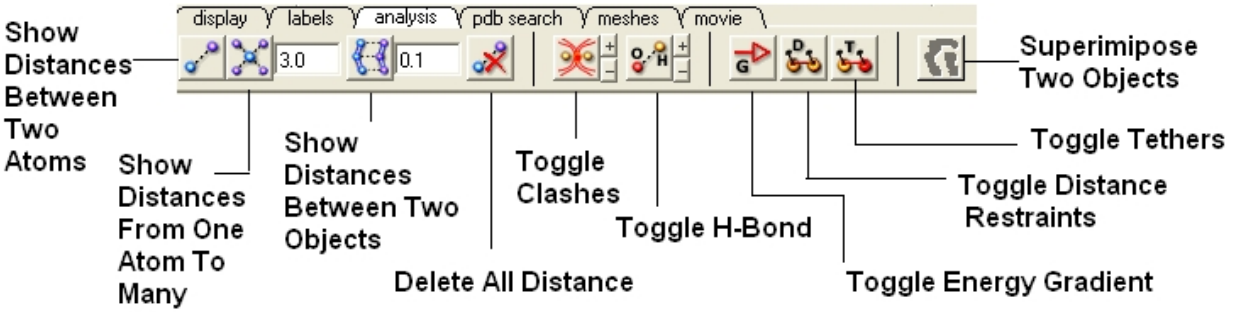
8. Structure Analysis

Once you have loaded your structure or made a model there are a number of functions that allow you to display and analyze specific regions.

8.1. The Structure Analysis Tab

All of these options can only be performed on ICM objects. (To convert your structure to an ICM object see `Convert PDB`)

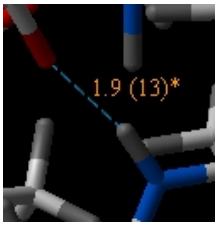
To perform any of the functions described in this section click on the analysis tab shown below:



8.1.1. Hydrogen Bonding

In order to display potential hydrogen bonds in your structure:

- Click the analysis tab.
- Click on the **Toggle H−bonds** icon in the **analysis** tab.



- Click the arrow to the right of the icon once to display the bond, twice to display the bond and the distance between the two bonding atoms, and thrice to display the bond, the distance, and the hydrogen bonding angle.

NOTE: The hydrogen bonds labeled with a star represent the ones whose hydrogen bonding energy is of exceptionally good quality.

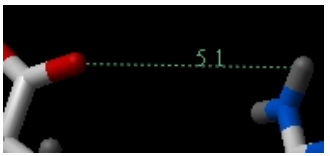
8.1.2. Finding the Distance Between Atoms

In order to display the distance between two atoms:

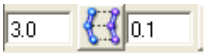
- Click on the analysis tab.
- Click on the 'Show Distances Between Two Atoms' Button
- Select the atoms between which you would like to find the distance. (See `selection toolbar`)
- The distance will be displayed in angstroms, in green.

In order to find the distance from one atom to many:

- Click on the analysis 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.



NOTE: The maxinal and minimal distances can be selected by entering values in the boxes shown here (below) in the analysis tab.



8.1.3. Displaying Tethers

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 analysis tab.
- Click on the 'Toggle Tethers' button.

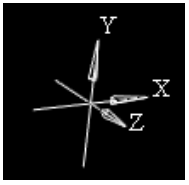
8.1.4. Displaying Distance Restraints

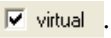
A distance restraint imposes a penalty function on the distance between two atoms in the same object. You cannot create distance restraints in ICM−Browser, however, if the project that you have loaded contains distance restraints, then they can be displayed:

- Click on the analysis tab.
- Click on the 'Toggle distance restraints' button.

8.1.5. Virtual Atoms and Variables

Virtual atoms are additional immaterial geometrical points (referred to as "virtual atoms") attached to each molecule for technical reasons, and internal coordinates ("virtual bonds, angles, torsions and phases") associated with them. These points help to have a standard yet flexible treatment of parameters defining absolute position (translation and rotation) of each molecule with respect to the coordinate frame. Each molecule is connected to the origin via two virtual atoms attached to it. In order to display this part of the ICM−molecular tree:



- Check the box next to **virtual** 

.

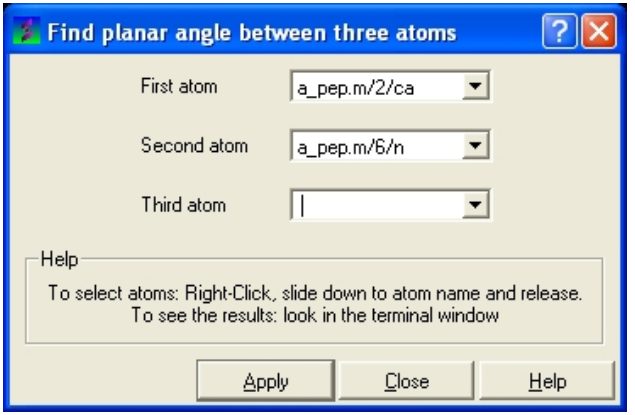
8.2. Calculating and Displaying Angles

All the tools required to calculate and display angles are in the Tools menu. Here we explain how to use them.

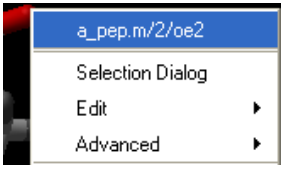
8.2.1. Finding the Planar Angle

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

- Select **Tools/Geometry/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.



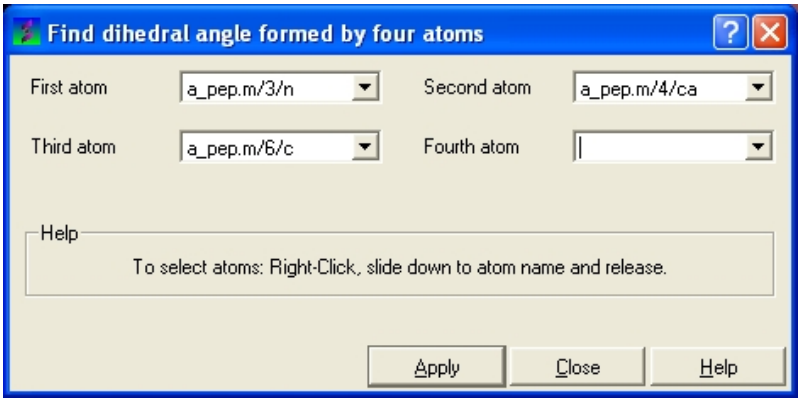
- Click **Apply** to display the angle measure in the terminal window.

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

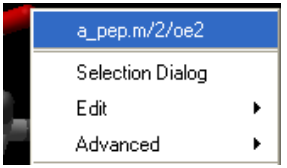
8.2.2. Finding the Dihedral Angle

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

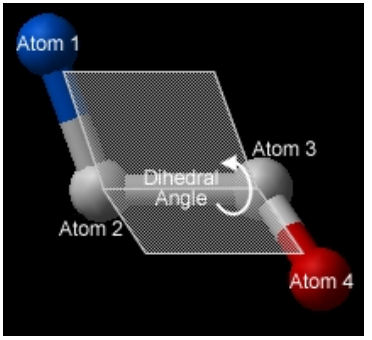
- Select **Tools/Geometry/Dihedral Angles.**



- 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:



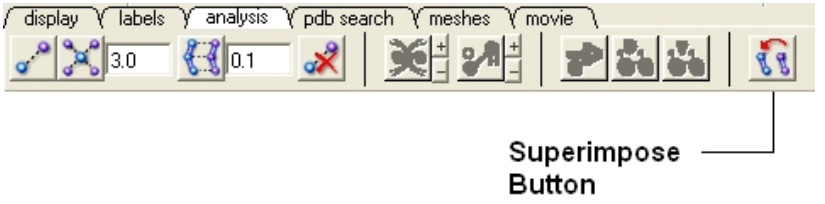
- Click **Apply** to display your dihedral angle measure in the terminal window.

8.3. How to Superimpose Two Structures

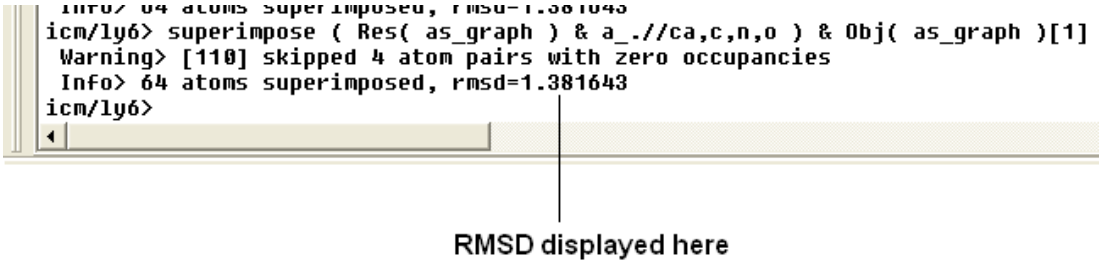
In order to calculate the root mean square deviation (RMSD) between two structures it is necesary to superimpose them. By using the superimpose button in the 'analysis' tab, ICM will calculate the Ca−atom, backbone atom and heavy atom differences between the two structures.

To superimpose two structures which have the same number of residues and atoms:

- First load the two structures into ICM.
- Select which parts or all of the two structure you wish to superimpose (see `selection toolbar`).
- Select the analysis tab at the top of the GUI.
- Select the superimpose button.



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

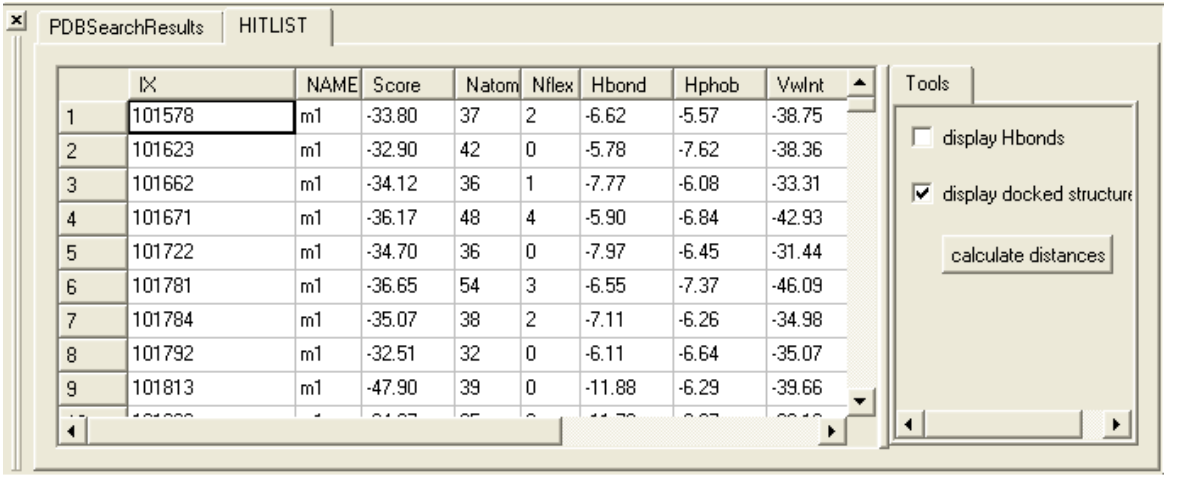


9. Working with Tables

One of the easiest ways to store, sort and display data in ICM is by the use of a table. In most cases tables are automatically created, for example, if you search for a PDB file or when you load a compound database. It is also possible for you to create your own table. Once a table is created, ICM provides easy to use tools to sort, add, edit and plot data.

Here we will concentrate on describing the actions you can perform on a table once it has been read into ICM. We will start by describing a simple table and then describe a molecular table in the next section.

A standard ICM table:



9.1. Standard ICM Tables

9.1.1. Reading a Table

A table can be read and saved as a .csv file or a .tab file. Saving or reading your table as a csv (comma seperated value) file enables the table to be transfered or loaded from other applications such as Microsoft Excel. A compound database such as an .sdf file can also be viewed as a table in ICM, additional details on how to manipulate a molecular table is explained in the next section.

A table can be read into ICM by selecting:

- File/Open and then selecting the table you have saved.

NOTE: If you have loaded a table and it is not displayed it maybe because the table display is not selected. To select the table display, select the window menu and select table see the `Window Menu Section`.

OR

Sometimes data is naturally stored and displayed in a table − eg PDB data. A common use of tables is for compound data. An explanation of how to use compound molecular tables is in the next section entitled `ICM Molecular Tables`.

For an example of a table try the following:

- Select PDB search tab.
- Type * into data entry box.
- Click on the button next to the data entry box.

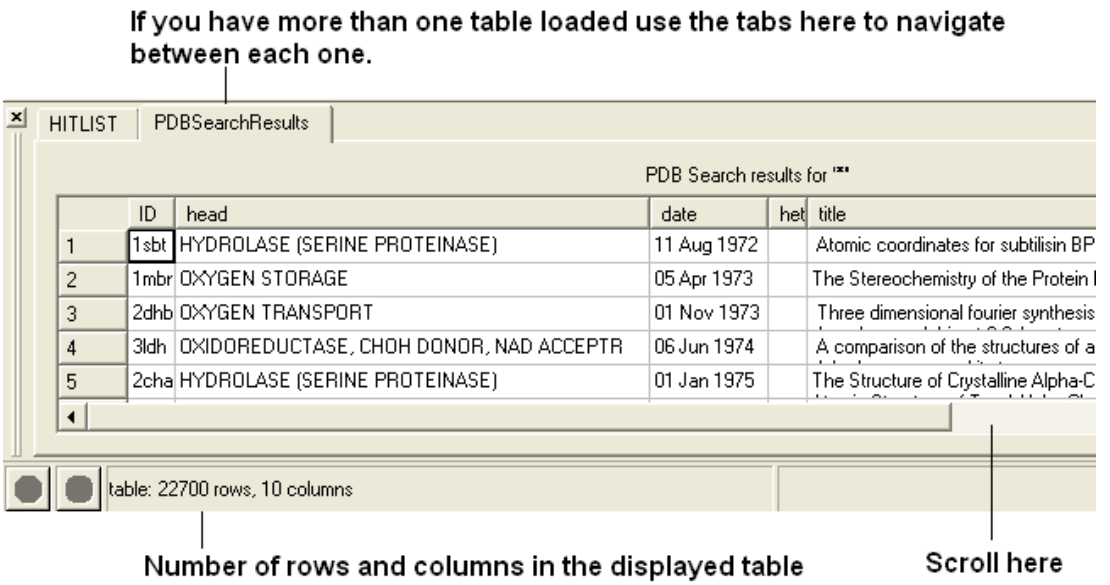
A table of all the PDB structures will be displayed at the bottom of the GUI.

9.1.2. Basic Table Navigation

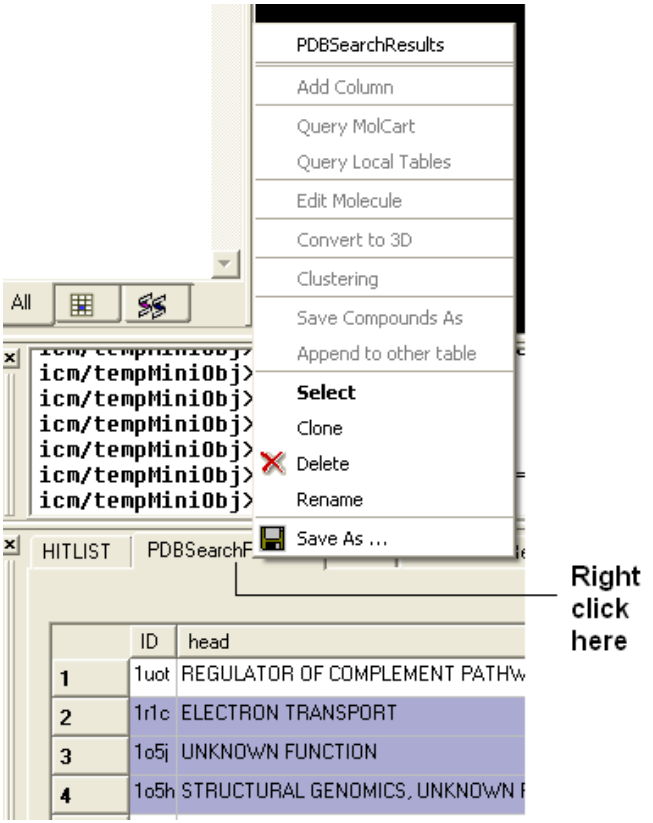
To view the contents of a table you can move the table up and down using the scroll bars on the side and bottom of the display.

NOTE: If you have loaded a table and it isnt displayed it may be because the table display isnt selected. To select the table display, select the window menu and select table (See `Window Menu Section`.

If you have read more than one table into ICM you can select a table by clicking the tab on the top of the table (See Below).



Right clicking on the tab allows you to clone, rename, delete or save a table.



NOTE: Double clicking on the tab allows two tables to be displayed at once. Double cliking again returns to the default table layout.

NOTE: Information regarding the number of rows and columns within a table is displayed at the bottom of the table.

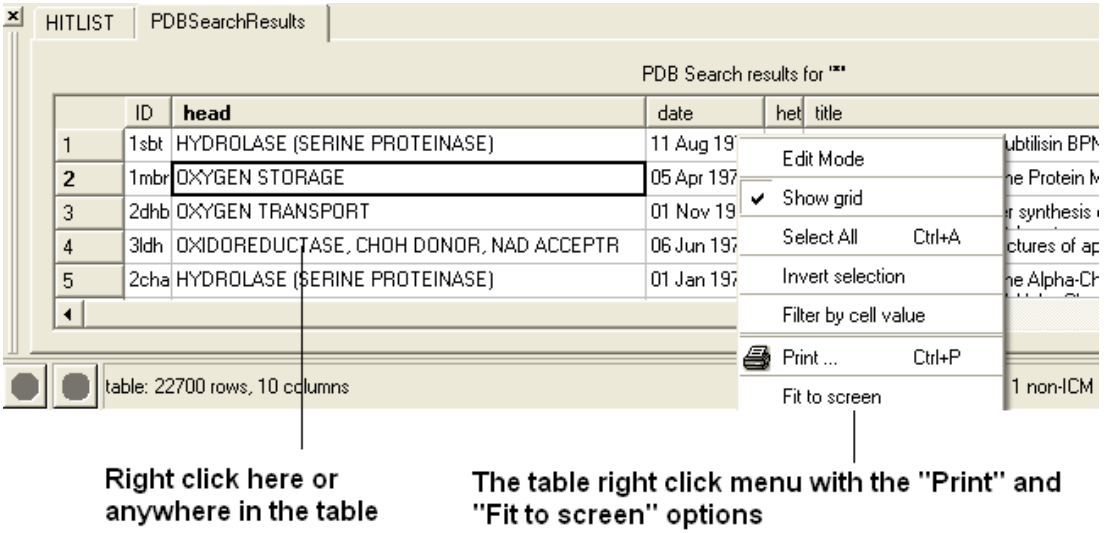
A table can be made to fit the screen by:

- Right clicking on the table and a menu will be displayed.

- Select the "Fit to screen" option.

A table can be printed by:

- Right clicking on the table and a menu will be displayed.
- Select the "Print" option.



The grid lines within the table can be removed from the view by:

- Right clicking on the table and a menu will be displayed.
- Select the "Show grid" option.

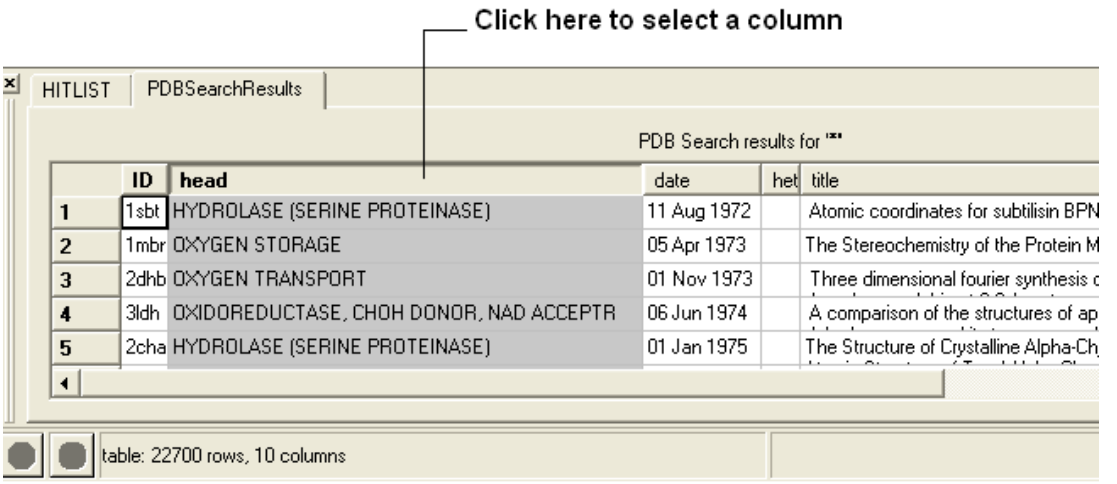
To change the width of column and rows:

You can change the width of a row or column by clicking on the separating line and dragging. You can make each row the same width by holding down the Ctrl key and dragging.

9.1.3. Making Table Selections

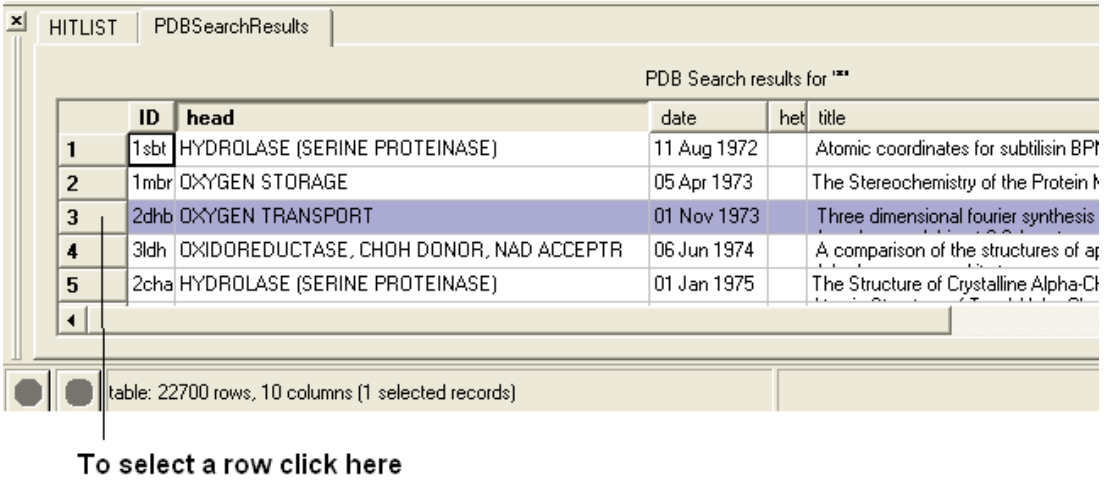
To select one column of a table:

- Click on the column header



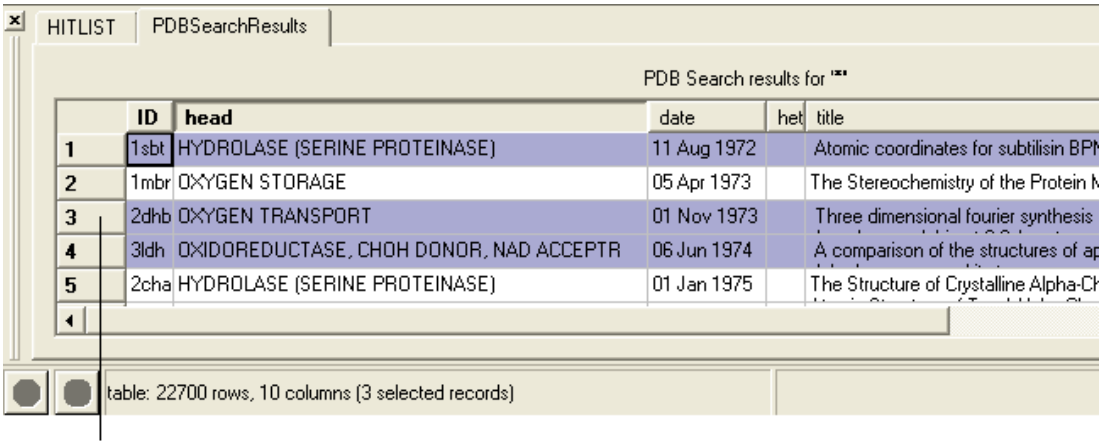
To select one row of a table:

- Click on the row header



To select more than one row or column:

- Click on one row or column whilst pressing the Ctrl key
- Select multiple number of rows or columns whilst still pressing the Ctrl key

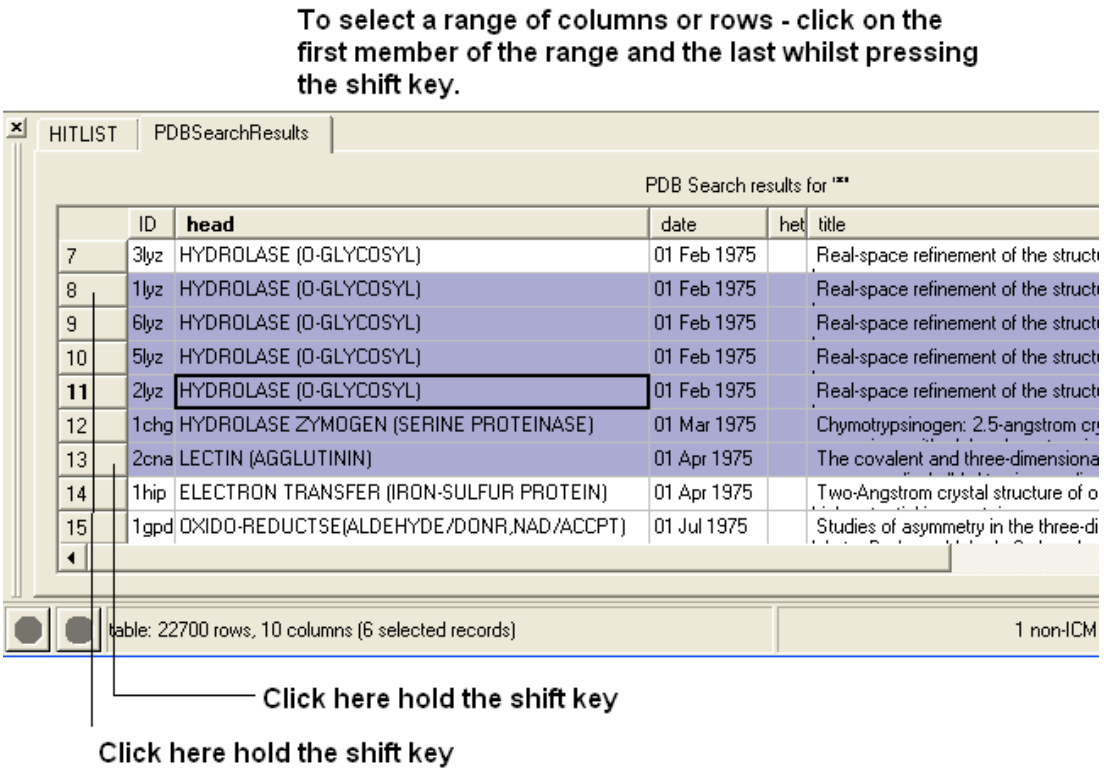


Select multiple rows and columns by clicking and selecting whilst pressing the Ctrl key.

NOTE: The Ctrl key acts as a toggle enabling select and unselect.

To select a range of columns or rows:

- Click on the first row or column in the range whilst pressing the Shift key.
- Click on the last row or column in the range whilst pressing the Shift key.



To invert a selection:

- Right click on the original selection and a menu will be displayed.
- Select the 'Invert selection' option.

NOTE: Invert selection can only be used on rows.

To select the whole table:

- Right click in the table and a menu will be displayed.
- Select the "Select All" option.

To remove a selection:

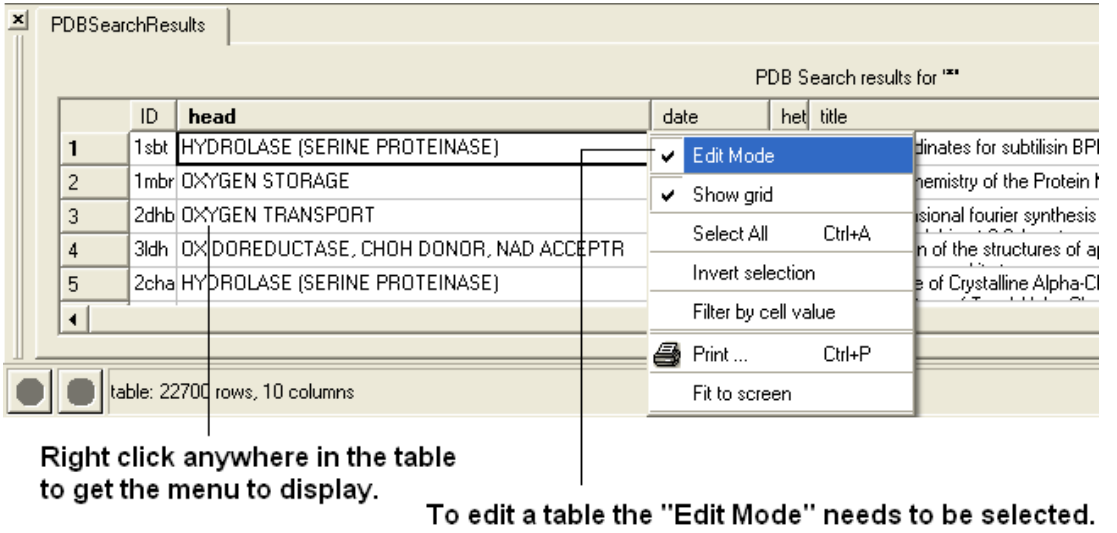
- Click anywhere within the table.

A selection can also be made from a plot select(`See Select plot section`).

9.1.4. Editing a Table

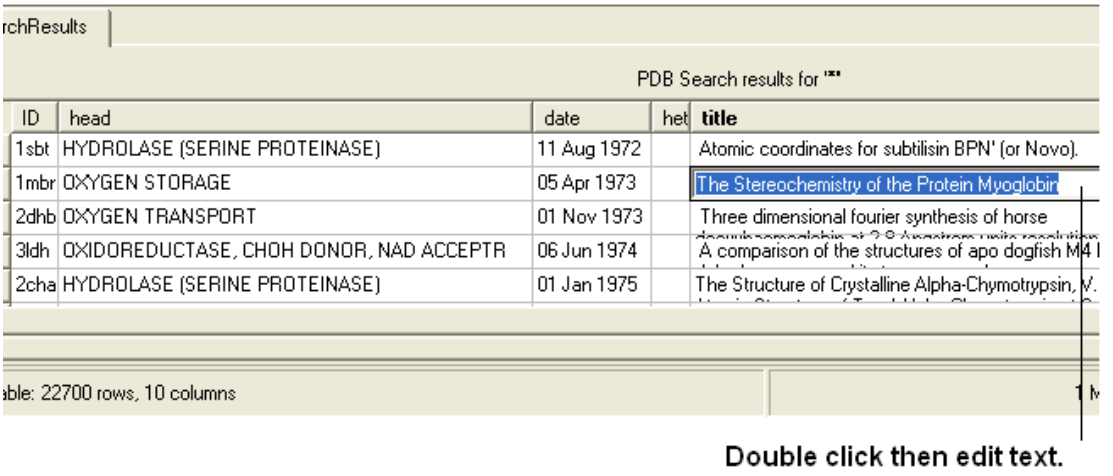
To edit a table:

- Right−click on the table and a menu will be displayed.
- Select the "Edit Mode" option. A tick will be displayed if it is selected.



To edit the text or values within a cell:

- Double click within the field you wish to edit and the text will be highlighted blue.



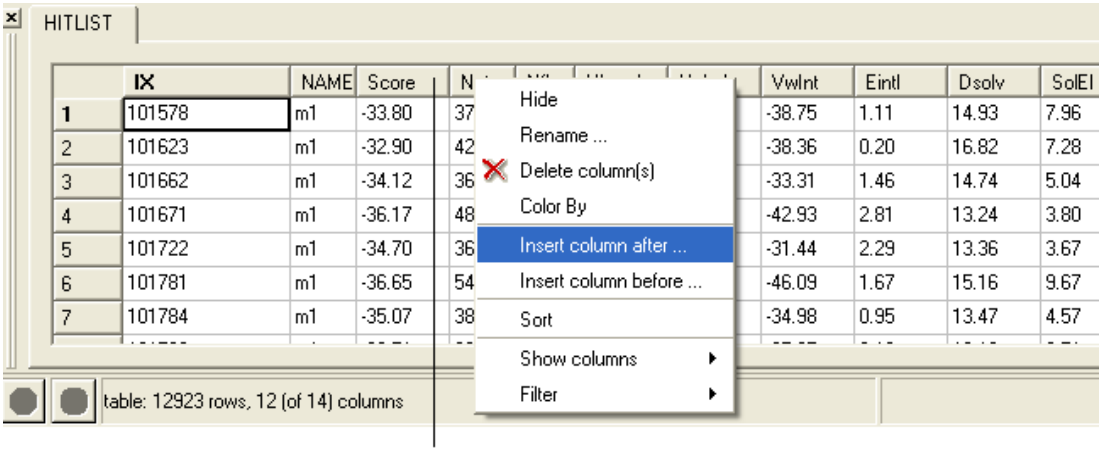
To edit the name of a column:

- Right click on the column header and a menu will be displayed.
- Select the option "Rename" and enter the appropriate new text.

9.1.5. Inserting Columns

To insert a column:

- Identify the position within the table where you wish the column to be inserted.
- Right click on the column header and a menu will be displayed.
- Select "Insert column before" or "Insert column after".



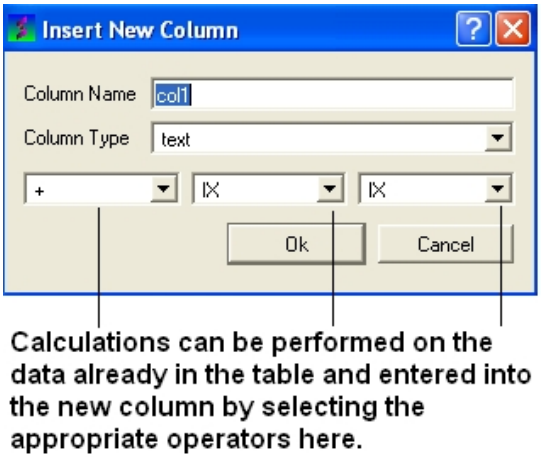
Right click on the column header and select insert column.

A data entry box will then be displayed.

- Enter the name of the column.
- Select which kind of data will be entered into the column text, integer, real or ariphmetrics.

Calculations can be performed on the data within columns and the results displayed in a new column by:

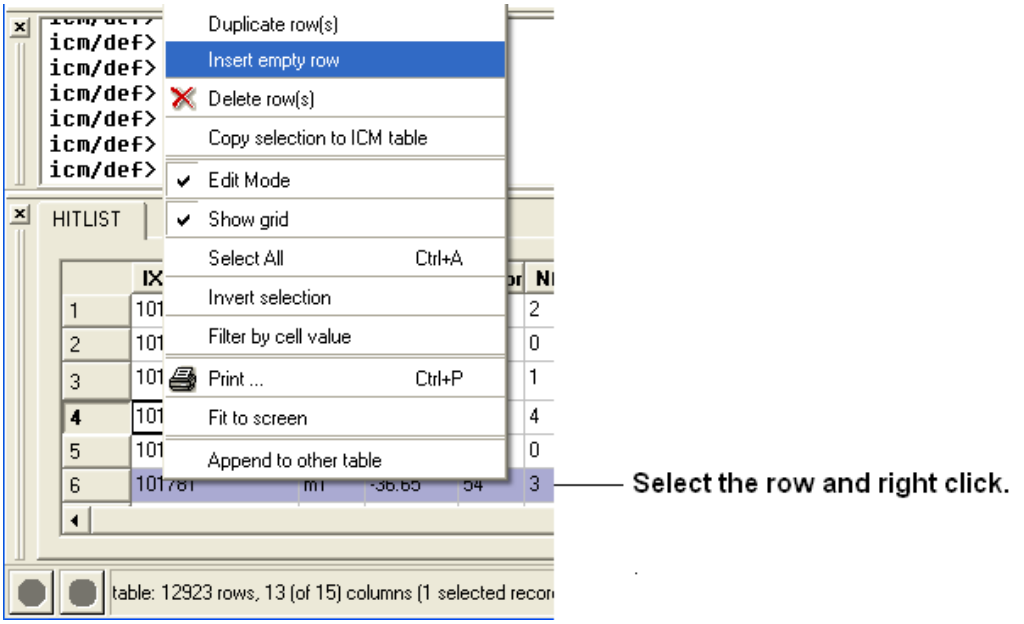
- Selecting ariphmetrics in the column type data entry box.
- Selecting which mathematical operator you wish to use from the drop down menu and which columns you wish to perform the calculation on.



9.1.6. Inserting Rows

To insert a row:

- Identify the position within the table where you wish the row to be inserted.
- Select the row (`See table selection section`).
- Right click and a menu will be displayed.
- Select insert empty row.



A blank row will be inserted. You can add data to this row by following the instructions in the `edit table section`.

To insert a duplicate row:

- Identify the position within the table where you wish the row to be inserted.
- Select the row (`See table selection section`).
- Right click and a menu will be displayed.
- Select insert duplicate row.

9.1.7. Deleting Columns and Rows

To delete a column or row:

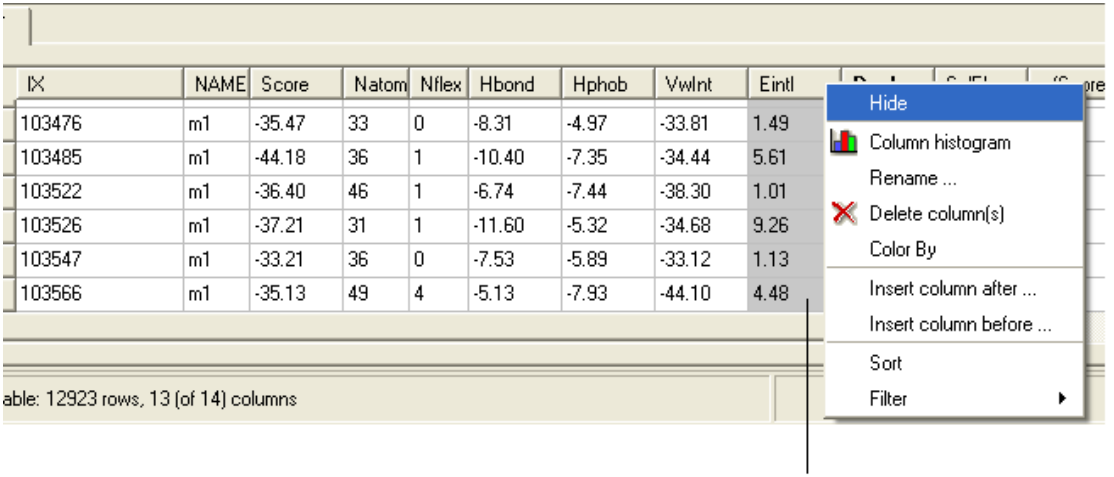
- Select the column(s) or row(s) you wish to delete. See the `select table` section for information on how to make table selections.
- Right click and select the delete option from the menu.

9.1.8. Hide and Show Columns

If you have a large table you may wish to only show and display certain columns and hide others. By default any loaded table will have all the columns displayed.

To select which columns you wish to hide:

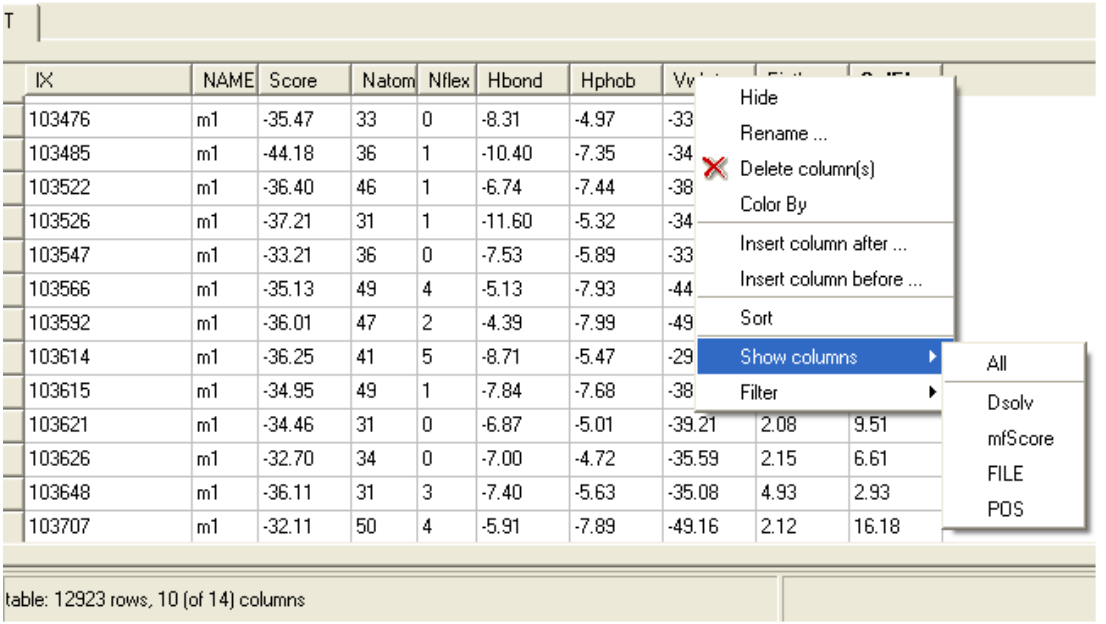
- Select the column(s) you wish to hide. See the `select table` section for information on how to make table selections.
- Right click and select the hide option from the menu.



Select column(s), right click and then select the hide option.

To show hidden columns:

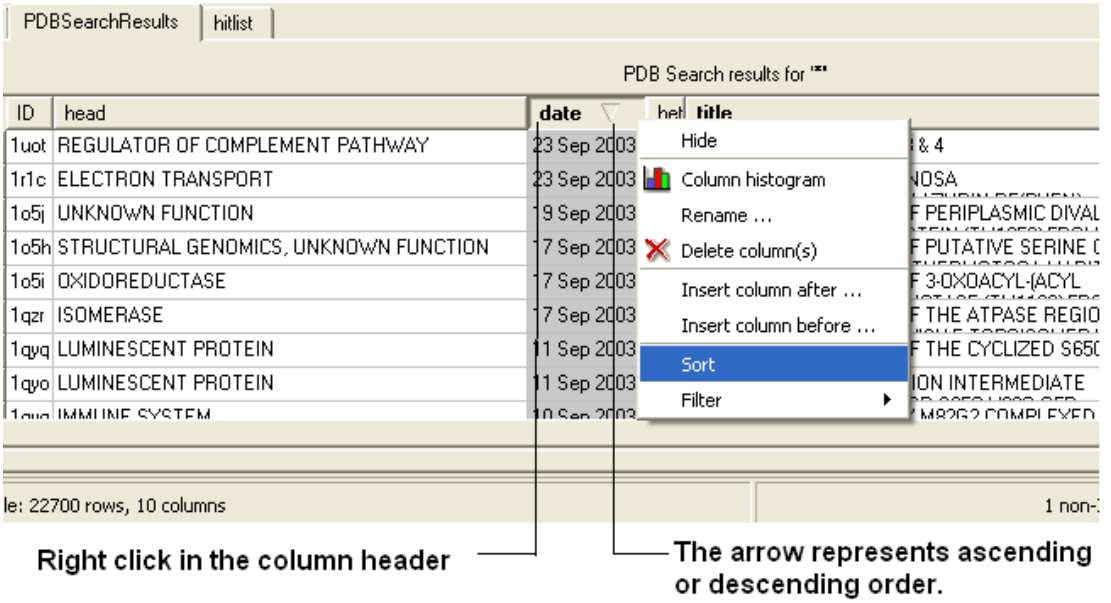
- Right click on the column header and a menu will be displayed.
- Select the show columns options.
- Select which column you wish to show from the drop down list.



9.1.9. Table Sorting

To sort a table by a column value:

- Right click on the column header.
- Select the **sort** option.

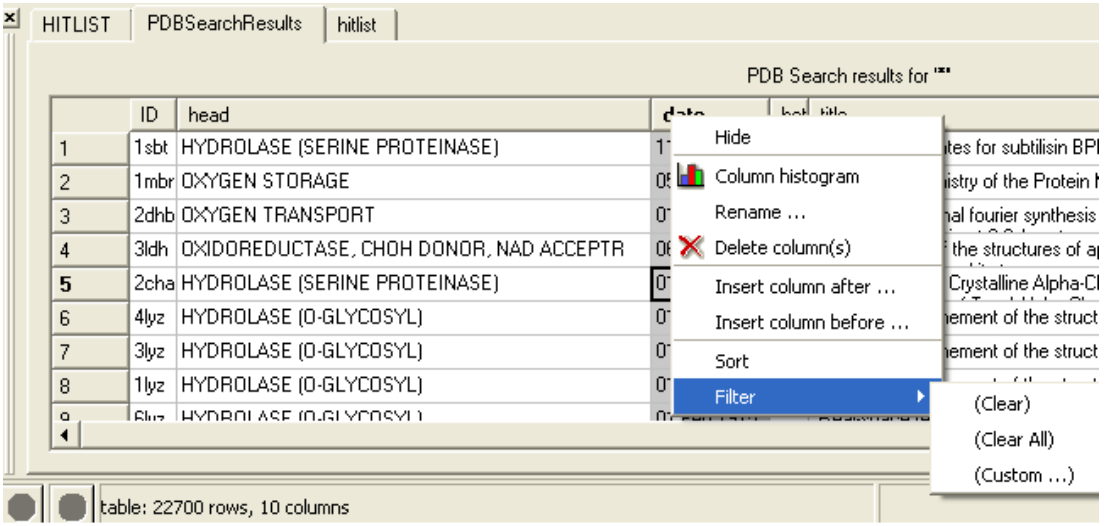


9.1.10. Table Filtering and Appending

Here we will describe how you can filter your table so that you can then append the filtered data to a new table or display only relevant information to your filter query.

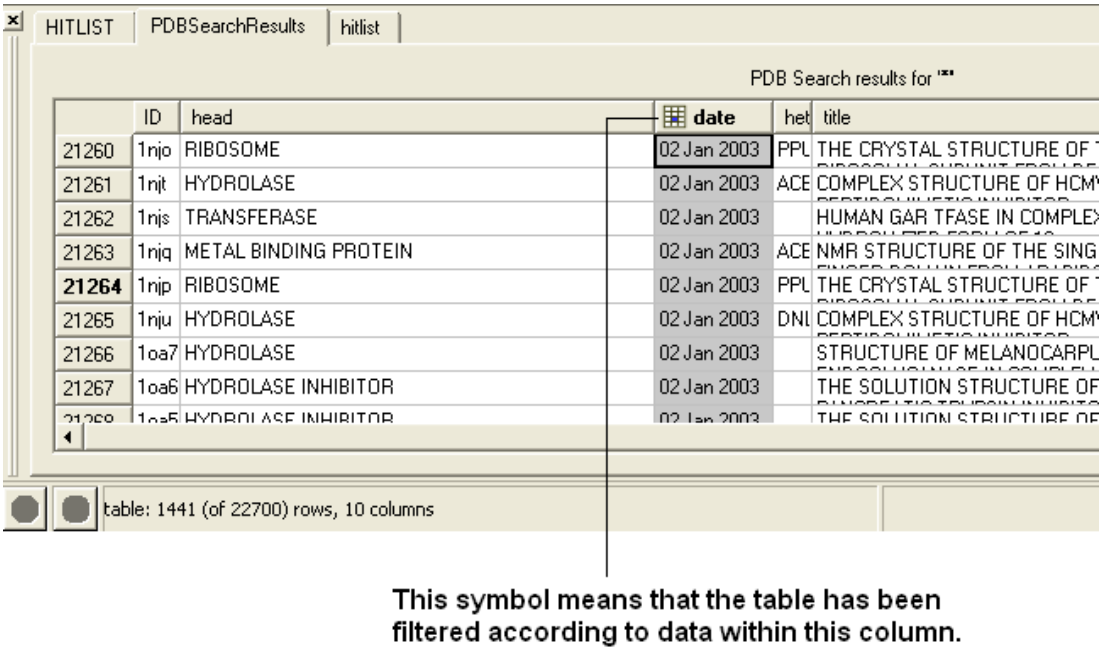
To filter a table:

- Select the column you wish to filter. See the `select table` section for information on how to make table selections.
- Right click on the column header.
- Select the filter option.



- Select the "Custom" option and a data entry box as shown below will be displayed.
- Enter the appropriate operations and filter values for your search.
- Click OK.

NOTE: When a column has been filtered a symbol as shown below will appear in the header of the column.



To append the filtered information into a new table:

- Select the whole table either by right clicking or pressing Ctrl A.
- Right click on the table and select "Append to other table".
- Enter a new name for the table you are appending with your filter results.

OR

Selected rows can be appended to a new table by:

- Right clicking on the selected rows and a menu will be displayed.
- Selecting the "copy selection to ICM table" option.

A table can be filterd by a cell value:

- By clicking once in a cell.
- Right click and a menu will be displayed.
- Select the option "Filter by cell value".

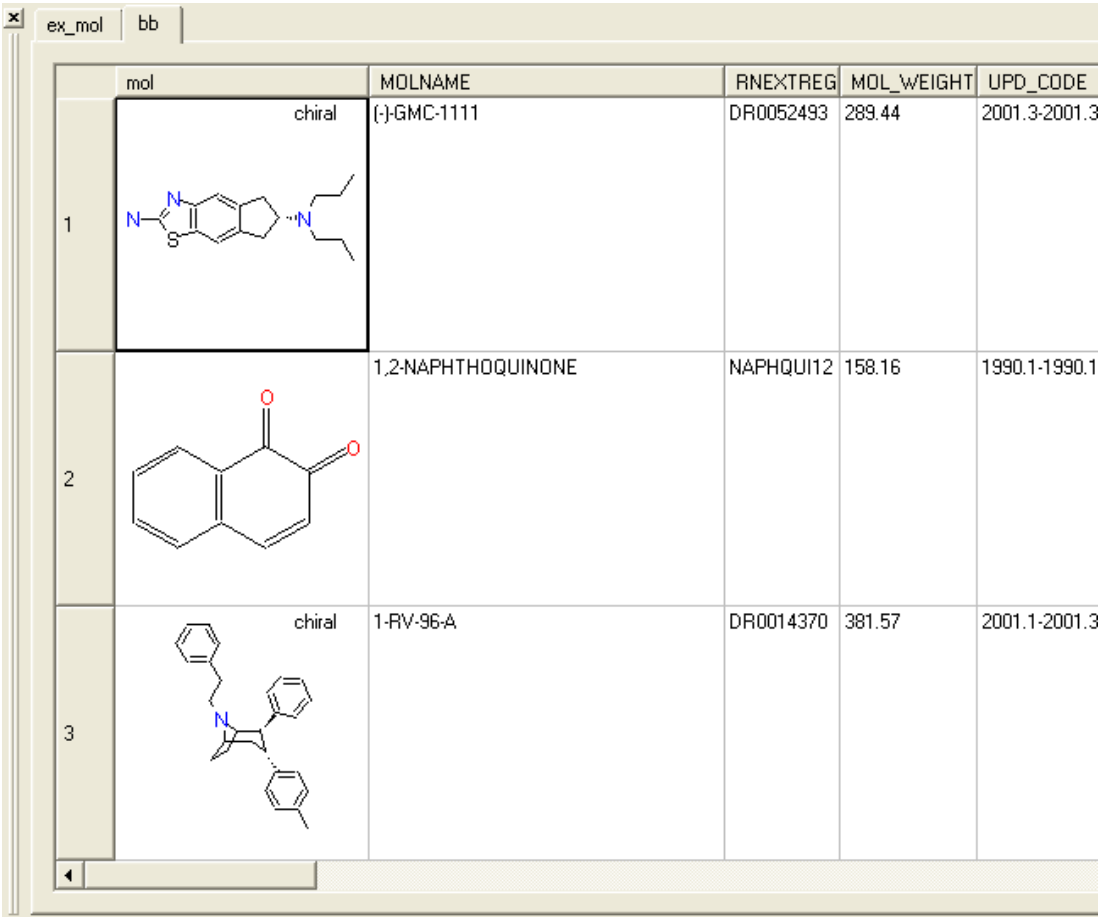
A filter can be cleared by:

- Right clicking on the column selection and selecting Filter/Clear or Filter/Clear All

9.2. ICM Molecular Tables

An ICM molecular table is created when an SDF or Mol file is read into ICM. To read and open a mol or sdf file go to File/Open `(See Open an ICM file section)` All of the table functions described in the previous section `Standard ICM Table` can be applied to molecular tables.

An ICM molecular table:

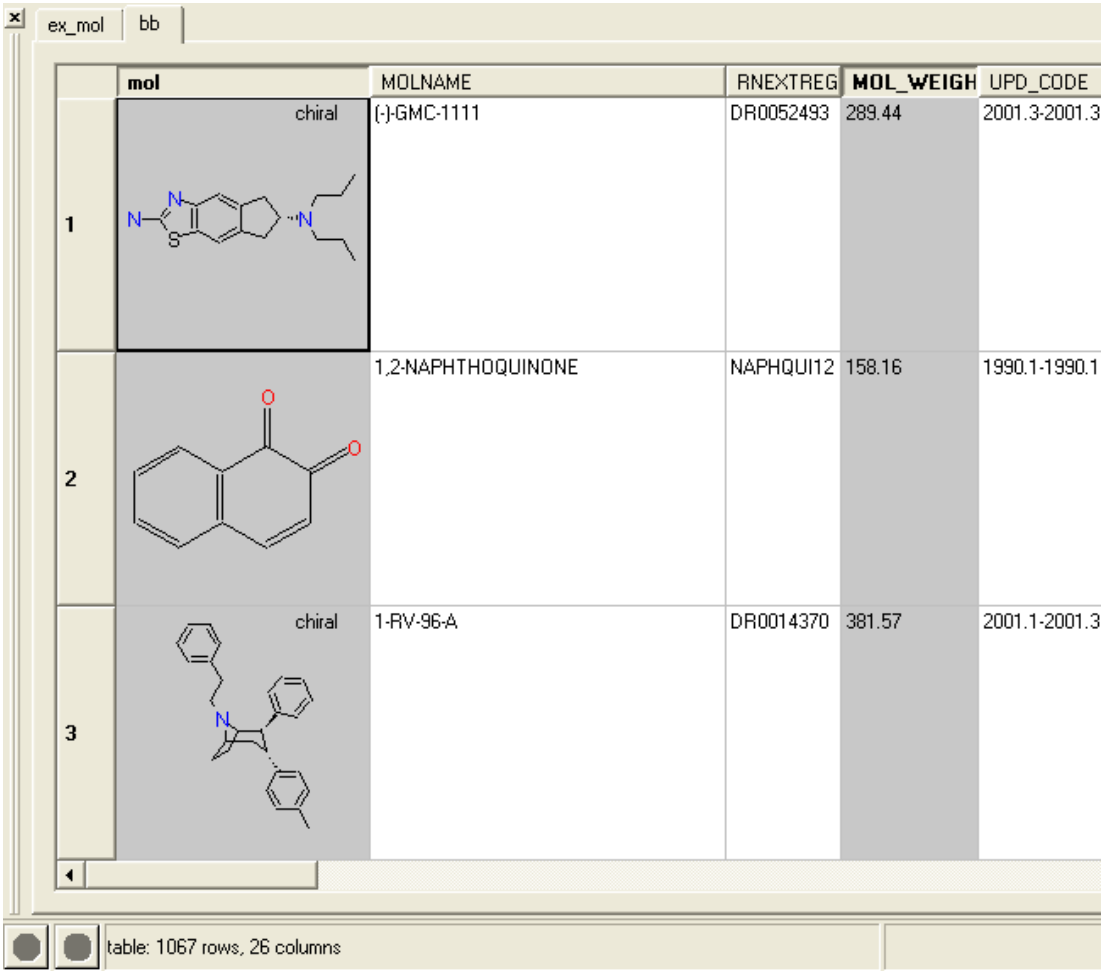


9.2.1. Molecular Table Display

There are many ways in which a molecular table can be displayed. For example you can select whether you want to have just the structure displayed or maybe you want to display the structure with alot of other important information such as molecular weight, docking score, energy etc...

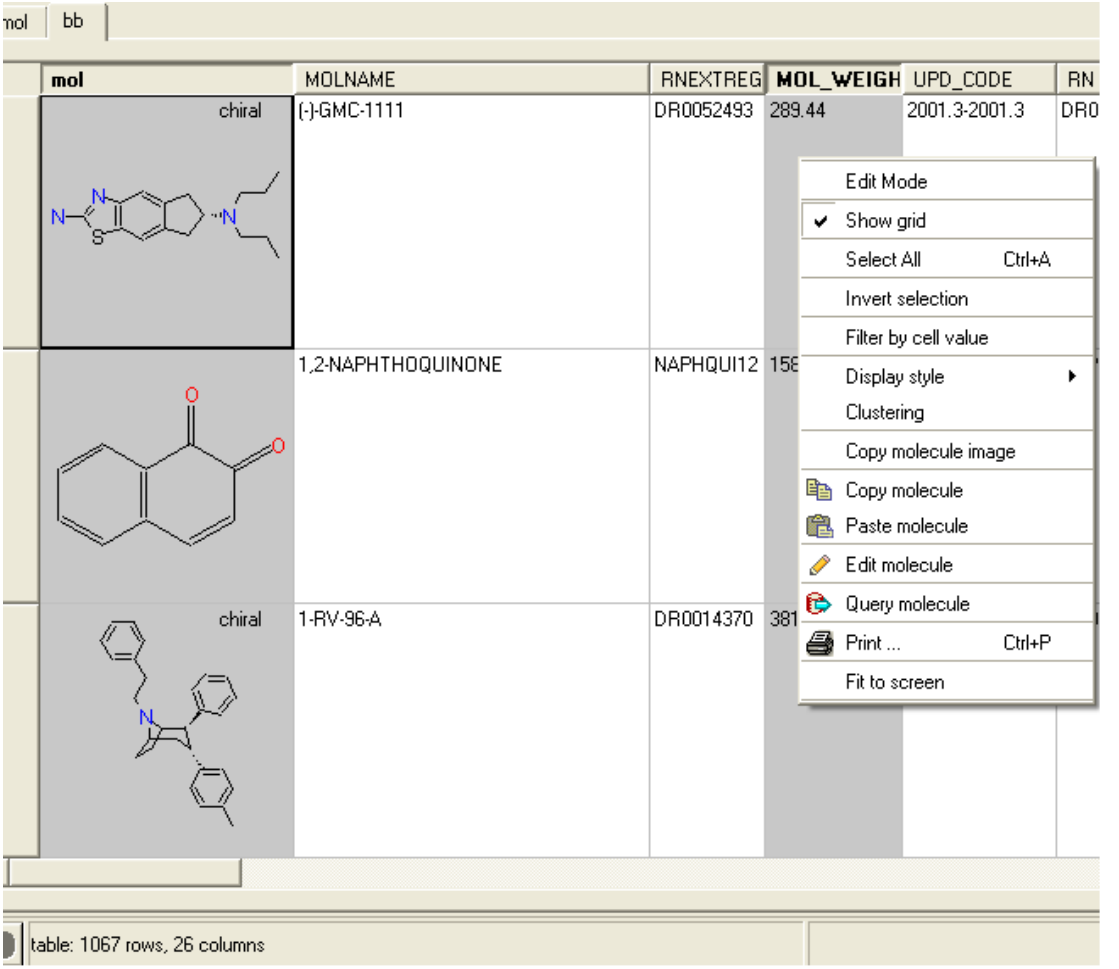
The default layout displays all the columns and tables. However using the table selection tools described in the previous section `Standard ICM Table` you can customize the display.

- First select which columns you wish to display.

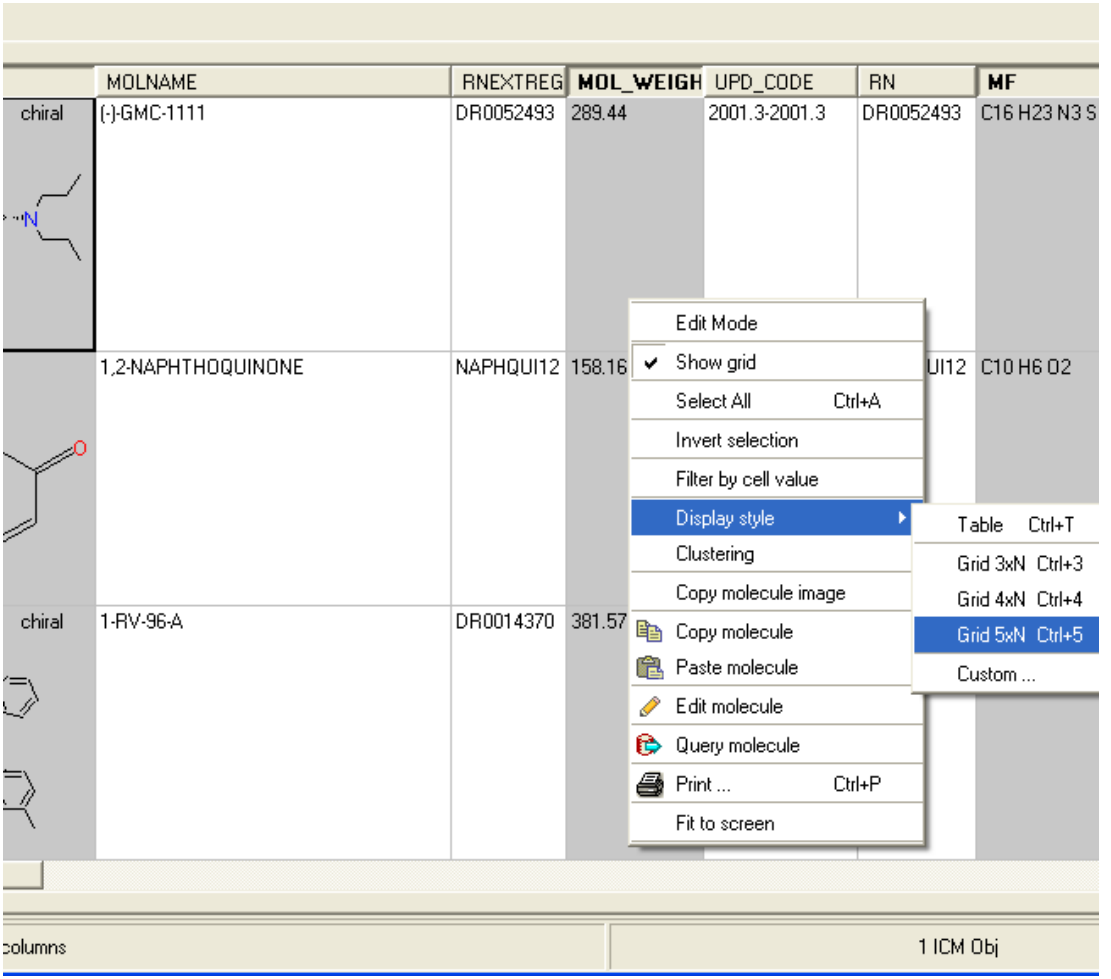


Next,

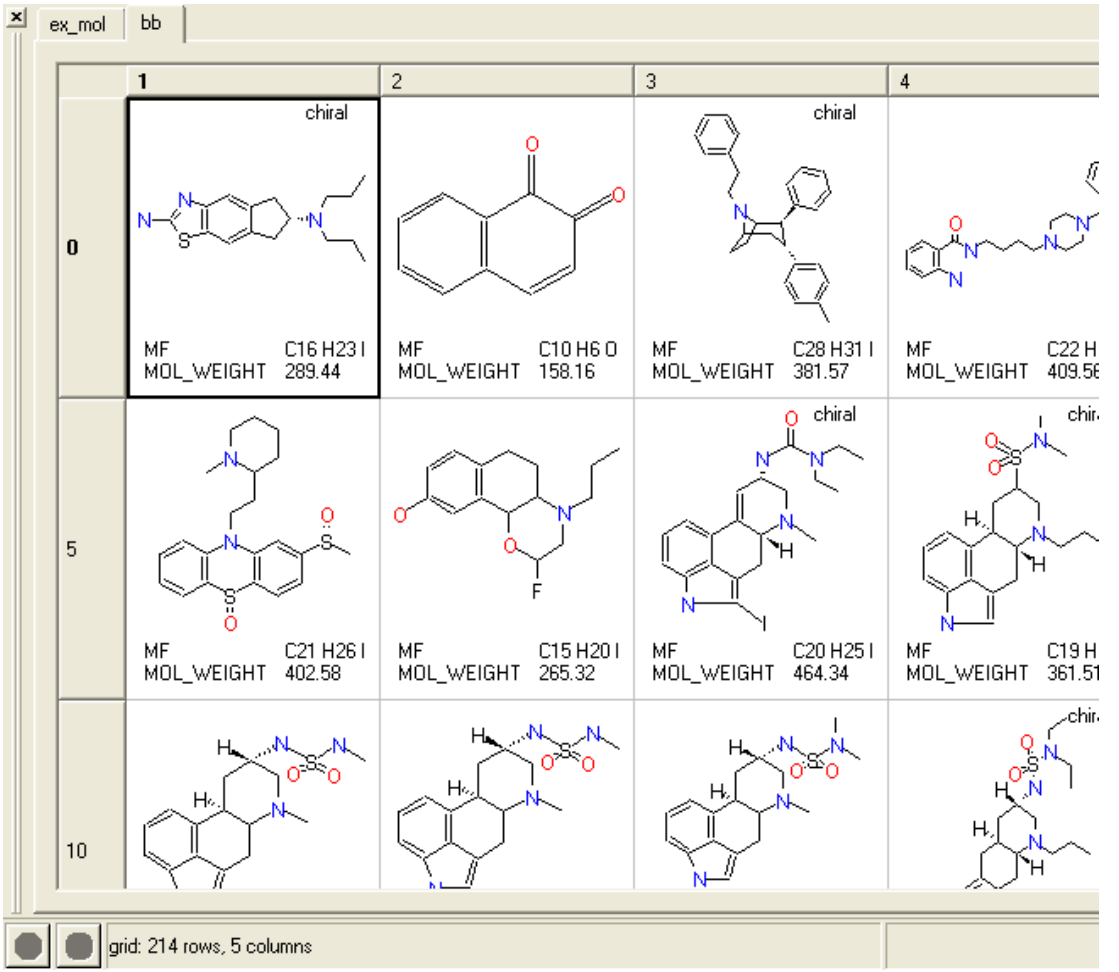
- Right click on the selection and the following menu will be displayed



- Select Display Style
- Select which kind of display style you like − table, grid 3xn, grid 4xn, grid 5xn or you can customize your own size.



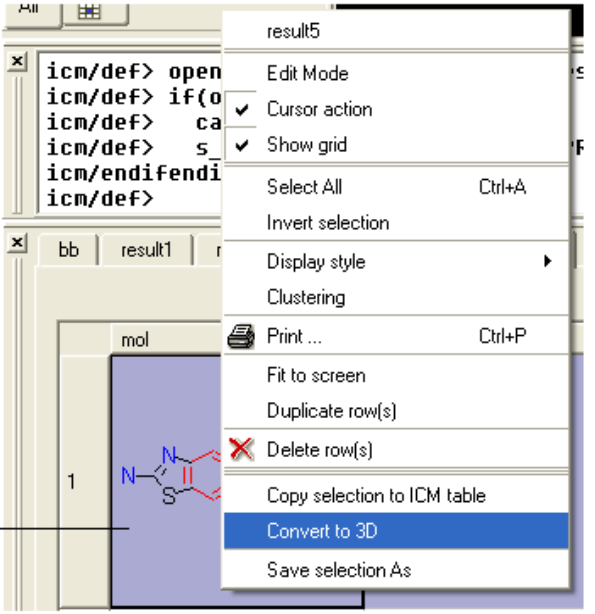
Here is an example of 5xn:



9.2.2. Display and Convert Molecule

To display and convert a molecule from a molecular table in the 3D graphics display window:

- Select the molecule image or images in the molecular table.
- Right click and select the Convert to 3D option.



Select and right click to display and convert to 3D.

9.2.3. Copy Molecule

To copy a molecule to paste into another application such as Microsoft Word or Excel:

- Right click on the molecule and a menu will be displayed.
- Select the option "Copy Molecule Image"

To copy a molecule or image to paste into another row within an ICM table:

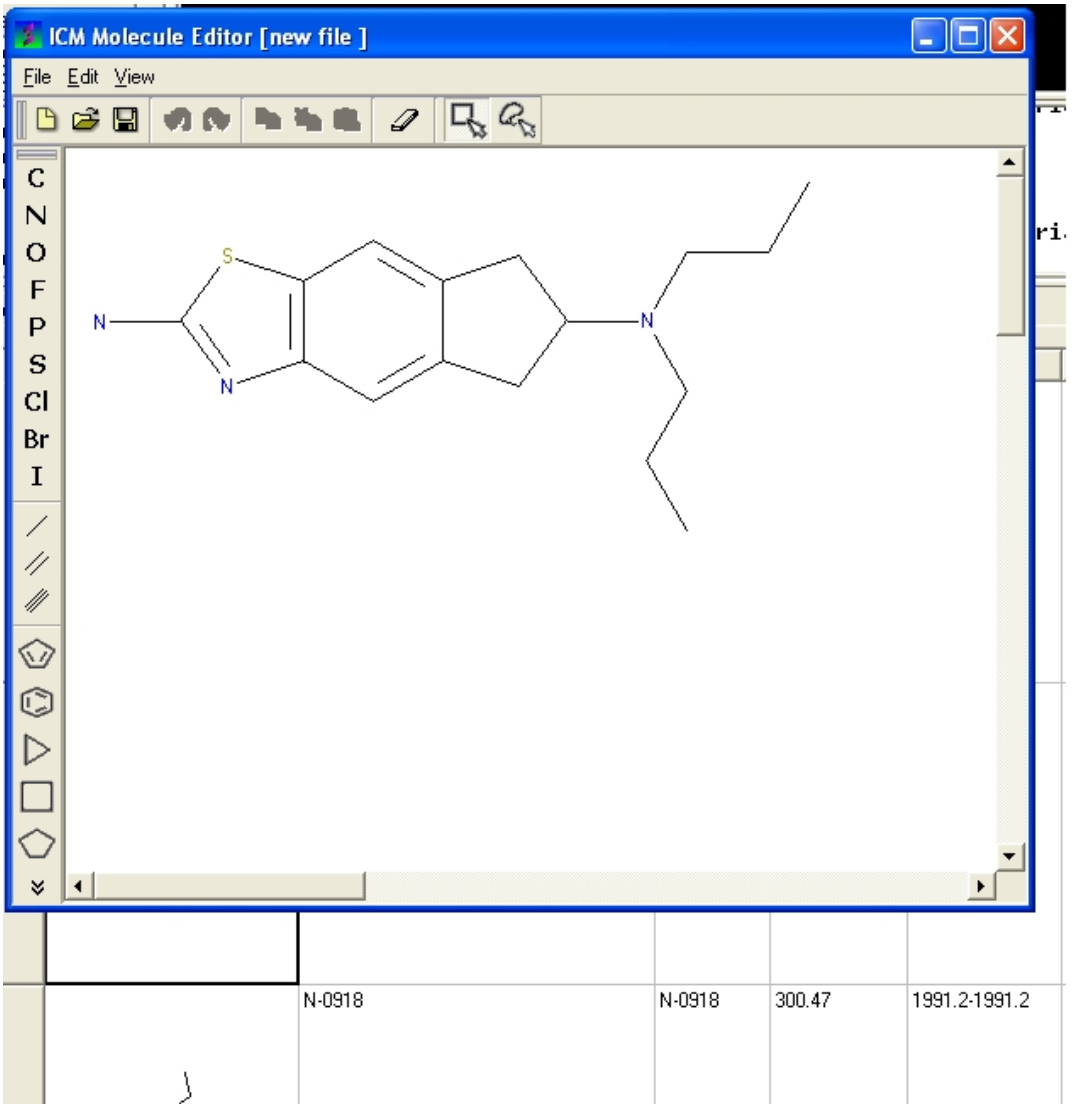
- Right click on the molecule and a menu will be displayed.
- Select the option "Copy Molecule"
- Right click in the cell into which you wish to paste the molecule.
- Select the option "Paste Molecule"

NOTE: To learn how to insert a row read the `insert row` section.

9.2.4. Edit Molecule

To edit a molecule:

- Right click on the molecule and a menu will be displayed.
- Select the option "Edit Molecule" and the ICM molecular editor will be displayed.
- Edit the molecule.
- Click Exit in the ICM molecular editor.

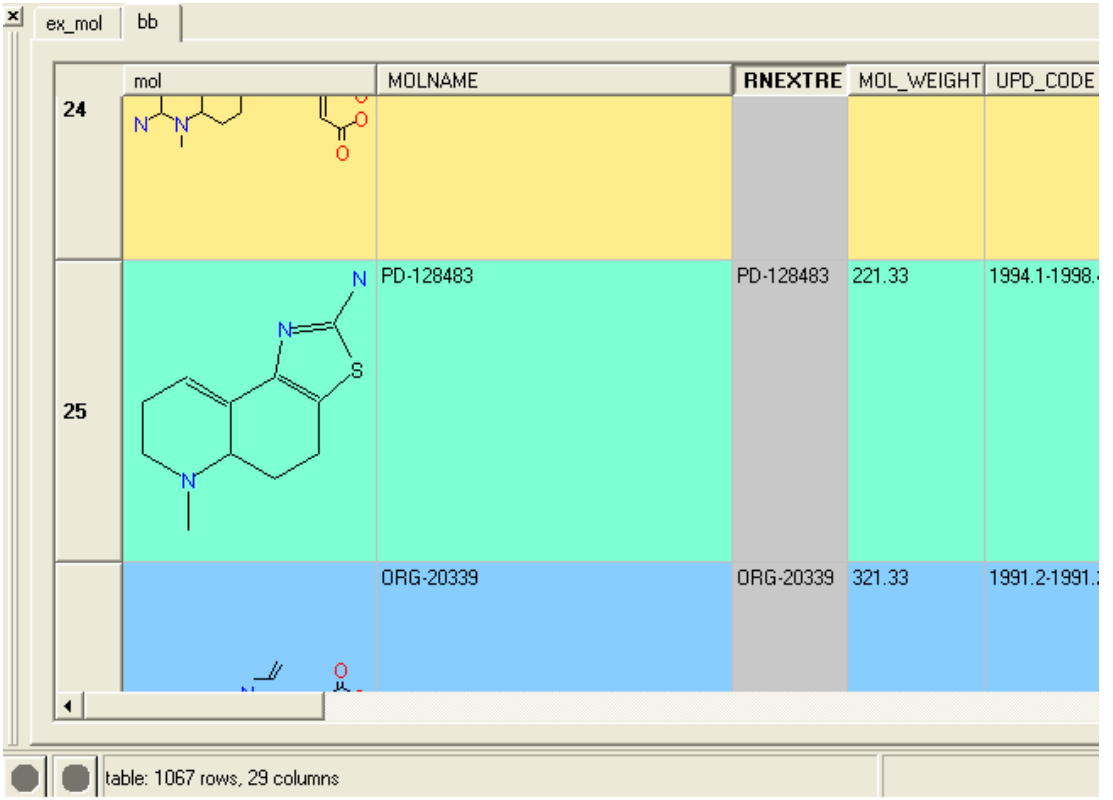


9.2.5. Color Table

You can color your table based on values within a column by:

- Selecting the column.
- Right click on the column header and a menu will be displayed.

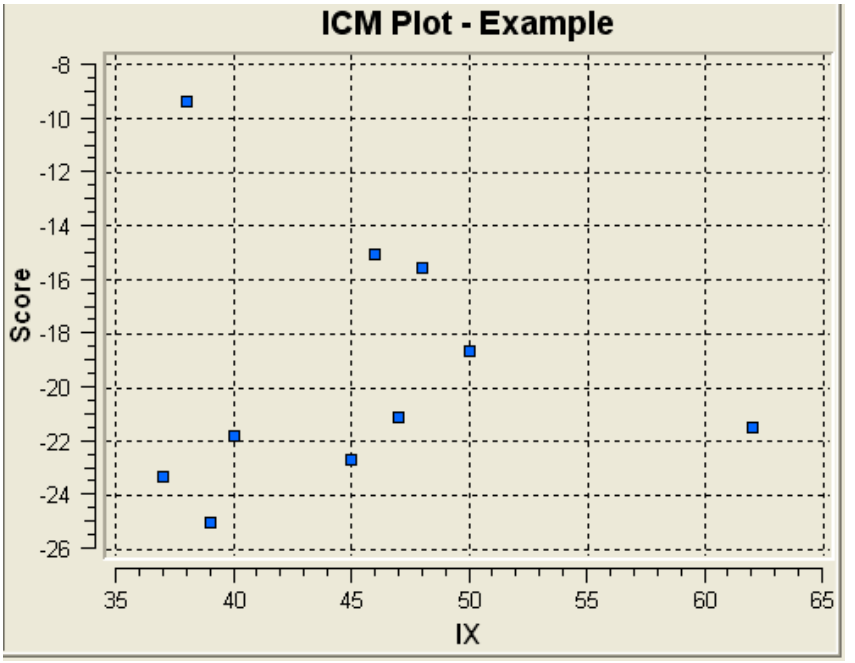
- Select the option "Color by".



NOTE: You can remove the color from the table by clicking the color option in the menu again.

9.3. Plotting Table Data

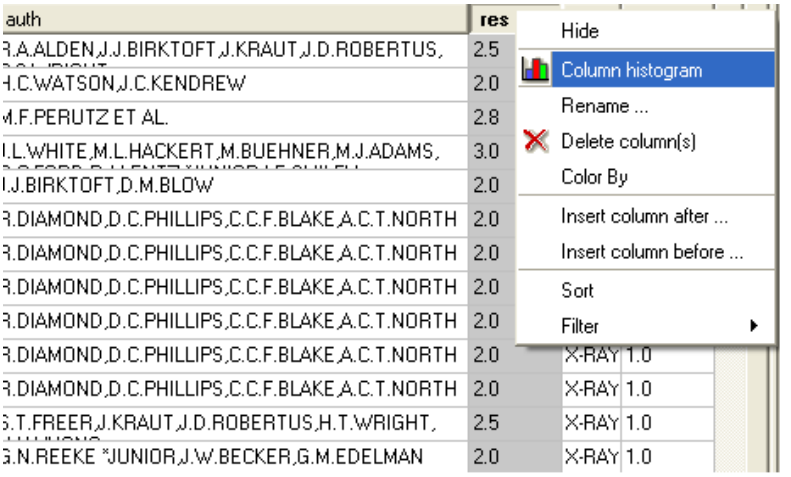
The data within a table can be plotted graphically. A histogram can be made for the data within one column or a plot can be constructed for the data within two columns.



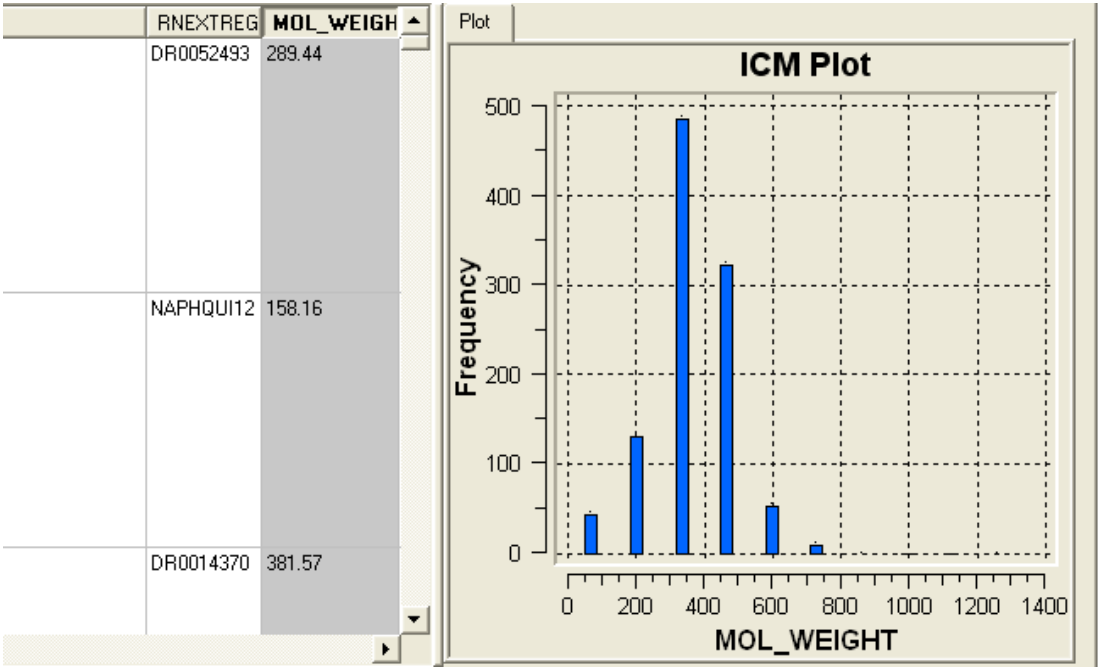
9.3.1. Column Histogram

To plot a histogram of the data within one column:

- Select the column.
- Right click on the column header.
- Select the "Column histogram" option.



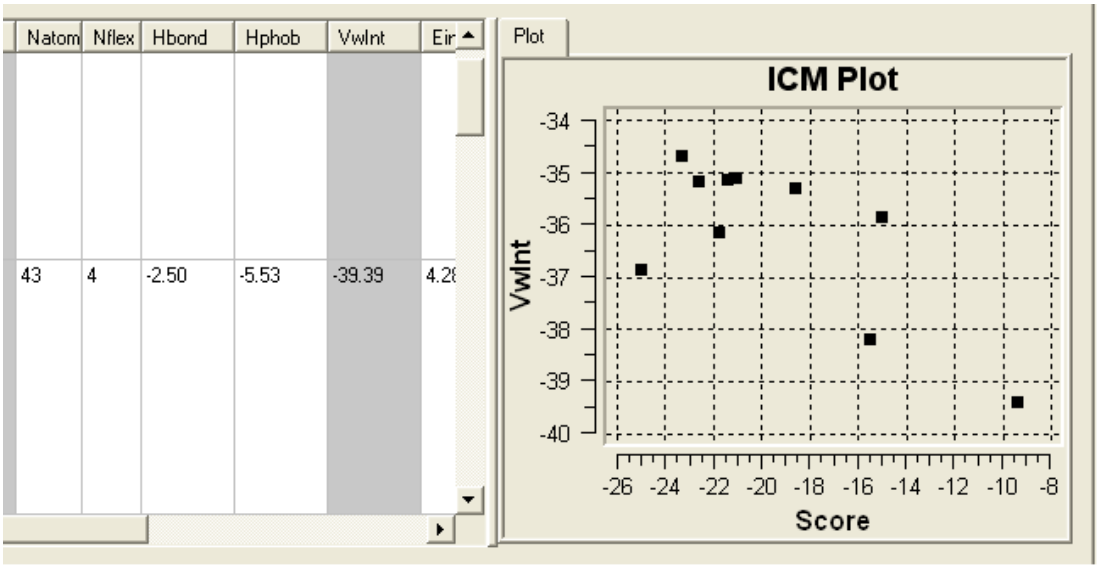
A plot will then be displayed next to the table.



9.3.2. Plotting two columns

To construct a plot from data within two columns:

- Select the two columns.
- Right click on the column header.
- Select the "Columns plot" option.



9.3.3. Print Plot

To print a plot:

- Right click on the plot and a menu will be displayed.
- Select the print option.

9.3.4. Saving a Plot Image

To save a plot image:

- Right click on the plot and a menu will be displayed.
- Select the "Save as image" option.

9.3.5. Hide a plot

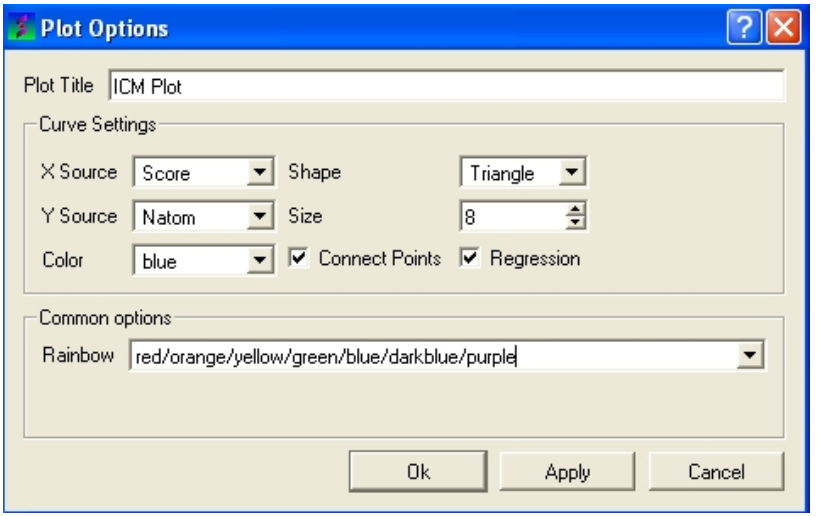
To hide a plot:

- Right click on the plot and a menu will be displayed.
- Select the "Hide" option.

9.3.6. Plot Options

To change plot options such as color, scale etc...

- Right click on the plot and a menu will be displayed.
- Select "Options".



From the plot options data entry box you can

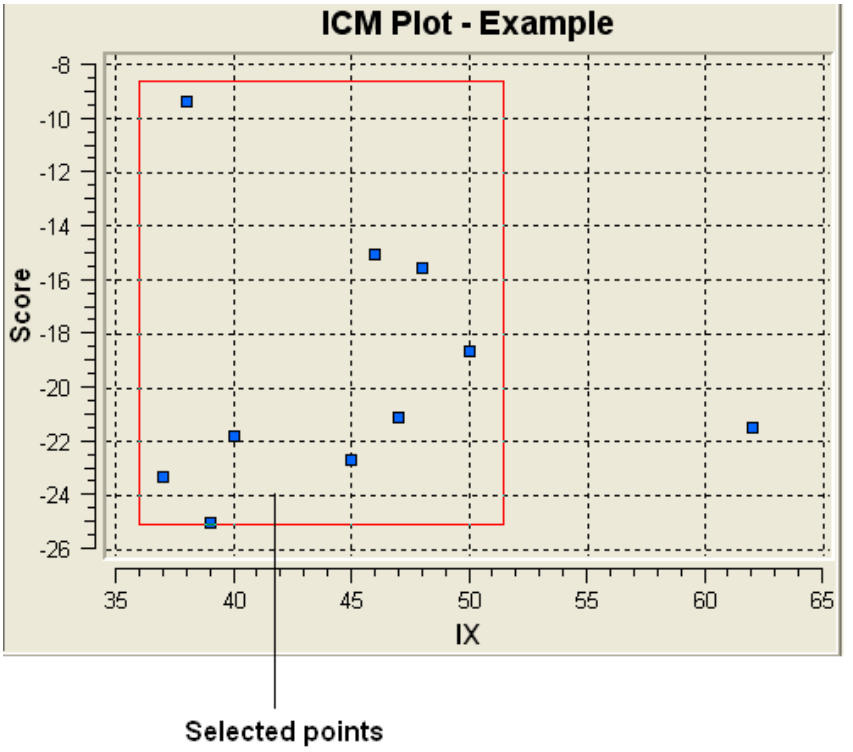
1. Change the title of the plot.
2. Select which columns will be plotted on each axis.
3. Change the shape of the dots in the plot.
4. Color the plot.
5. Connect points on the plot.
6. Perform regression analysis.

9.3.7. Making Selections From a Plot

To make a selection from an ICM plot:

- Right click on the points in the plot you wish to select.

A red box will surround the points you select. The points within the box will be selected in the table.

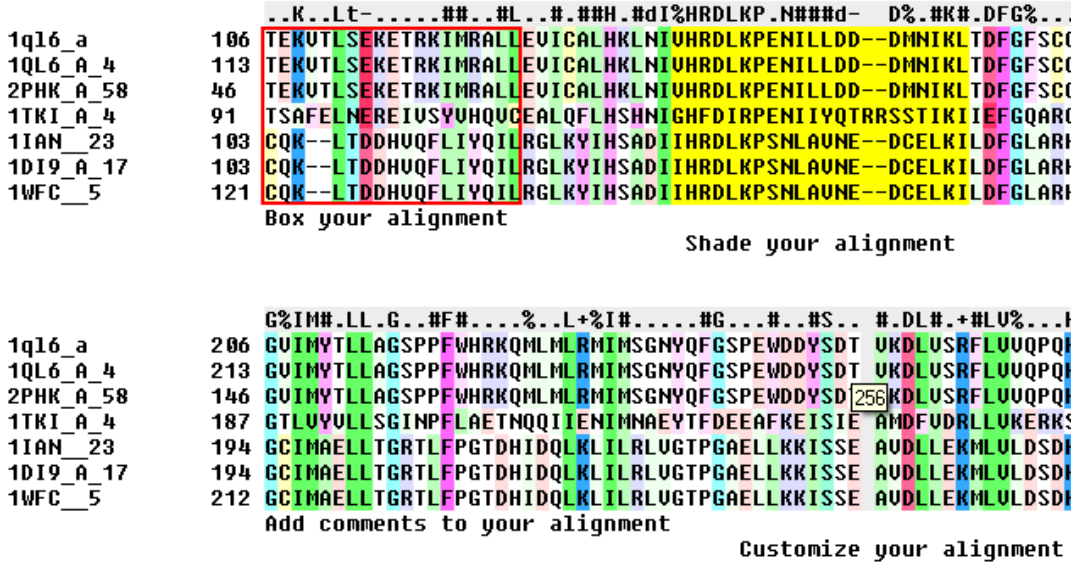


10. Sequence Alignments

ICM provides a powerful sequence alignment editing tool.

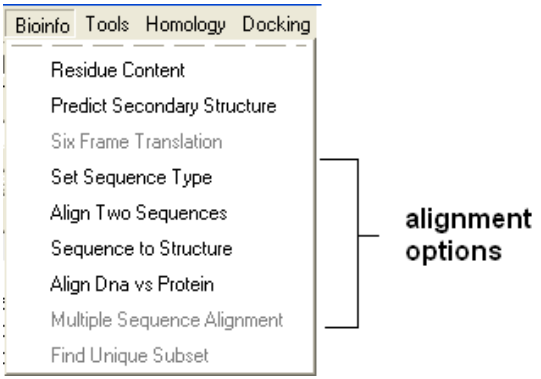
You can customize your sequence alignments in a number of ways:

1. Coloring according to a number of different consensus schemes.
2. Customizing your own consensus tables.
3. Shading areas of interest.
4. Boxing areas of interest.
5. Adding comments to an alignment.
6. Saving an alignment as a high quality image for publication.
7. Displaying and analyzing phylogenetic trees.
8. Direct selection from the alignment to the 3D object.



10.1. Alignment Introduction

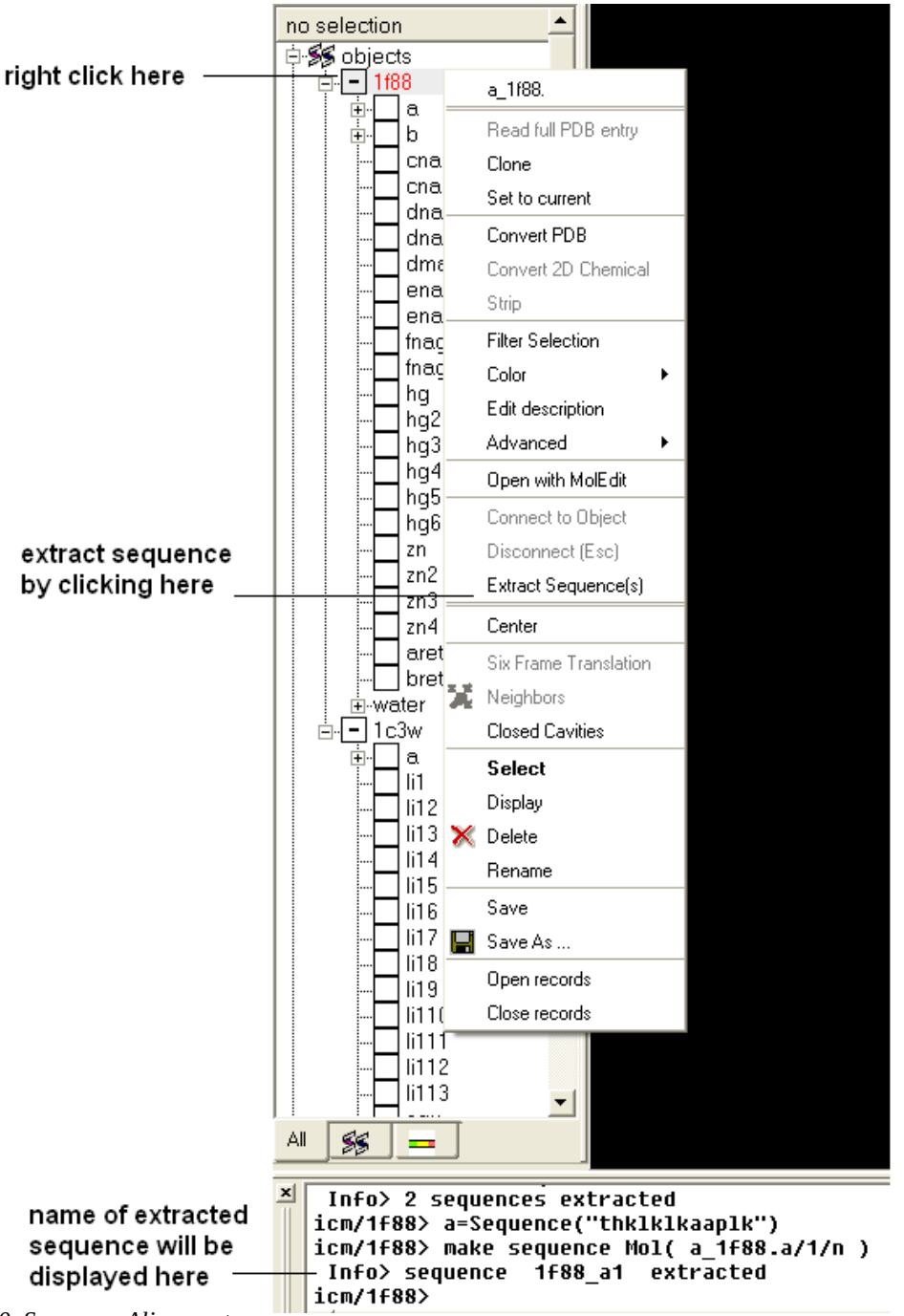
To align two or more sequences you need to use the options in the 'Bioinfo' menu shown below.



To construct an alignment, two or more sequences need to be loaded into ICM. This can be done be done in one of the following ways:

1. Constructing your own sequence see `new sequence section.`
2. Extracting the sequence from a loaded PDB sequence by:

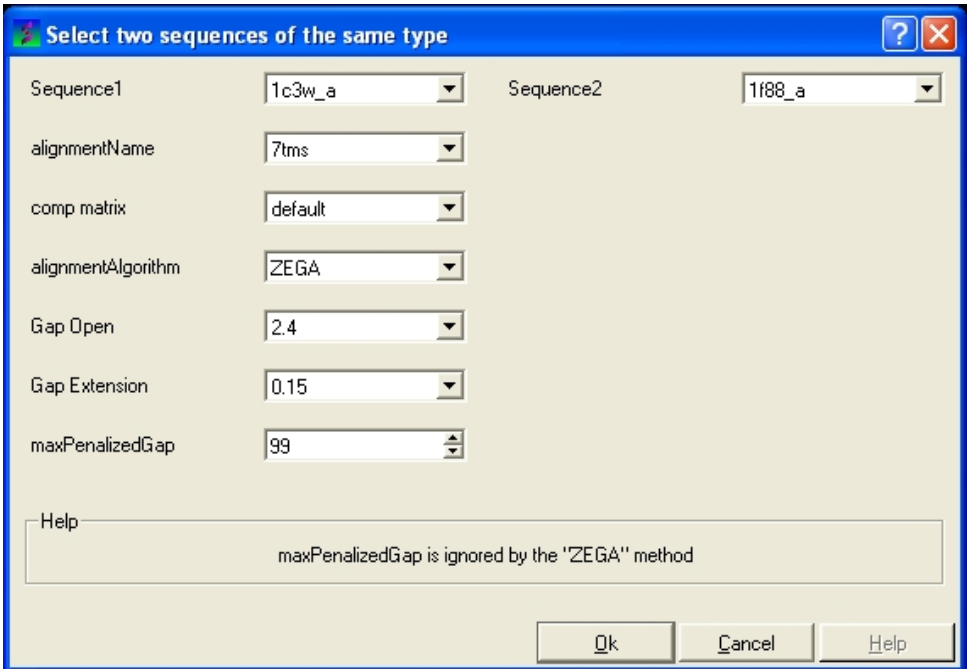
- Right clicking on the object name in the workspace panel
- Select 'extract sequence' and the name of the extracted sequence will be displayed in the terminal window.


right click here
extract sequence by clicking here
name of extracted sequence will be displayed here
no selection
objects
1f88
a
b
cna
cna
dna
dna
dma
ena
ena
fnac
fnac
hg
hg2
hg3
hg4
hg5
hg6
zn
zn2
zn3
zn4
aret
bret
water
1c3w
a
li1
li12
li13
li14
li15
li16
li17
li18
li19
li110
li111
li112
li113
All
a_1f88.
Read full PDB entry
Clone
Set to current
Convert PDB
Convert 2D Chemical
Strip
Filter Selection
Color
Edit description
Advanced
Open with MolEdit
Connect to Object
Disconnect (Esc)
Extract Sequence(s)
Center
Six Frame Translation
Neighbors
Closed Cavities
Select
Display
Delete
Rename
Save
Save As ...
Open records
Close records
Info> 2 sequences extracted
icm/1f88> a=Sequence("thklklkaaplk")
icm/1f88> make sequence Mol(a_1f88.a/1/n)
Info> sequence 1f88_a1 extracted
icm/1f88>

10.2. Align Two Sequences

To align two sequences:

- Select the 'Bioinfo' menu.
- Click on 'Align Two Sequences' and the following data entry box will be displayed.

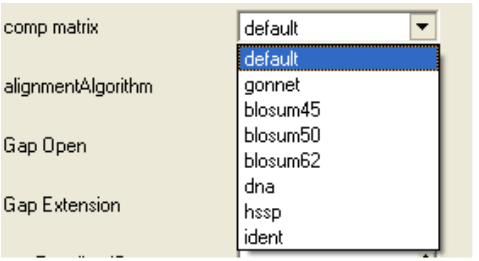


- Enter the name of your first sequence in the 'Sequence 1' data entry box.
- Enter the name of your second sequence in the 'Sequence 2' data entry box.

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.

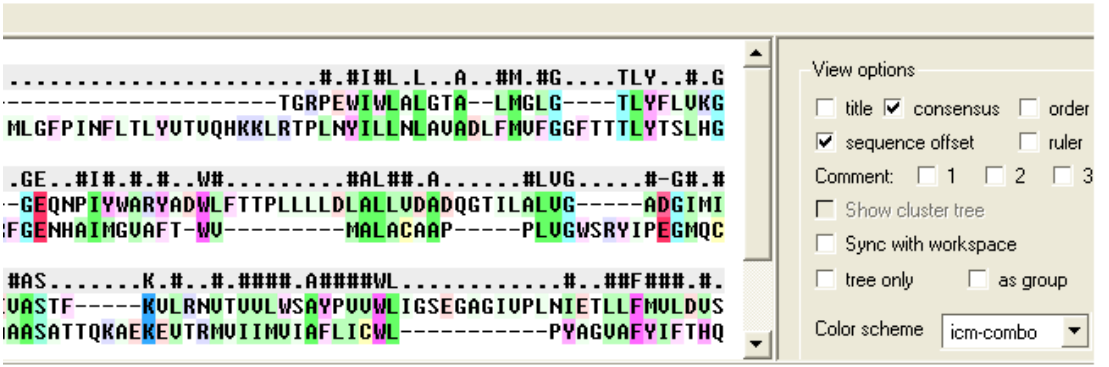
- Enter a unique alignment name in the 'alignmentName' data entry box.

- 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.

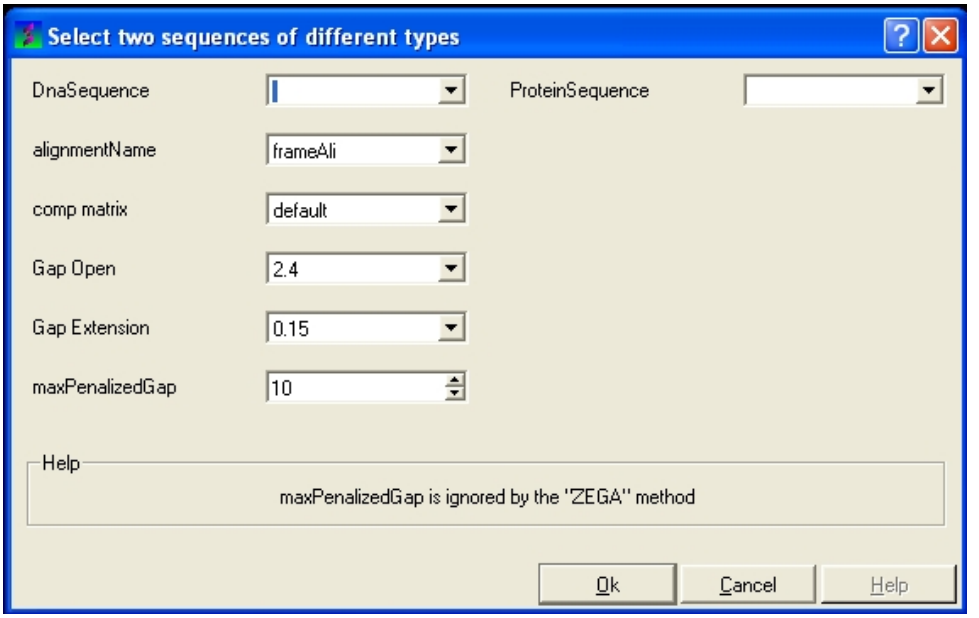
- Enter the values you wish to use for Gap Open, Gap Extension and the maximum penalized gap penalty.
- Click OK and the alignment will be displayed in the alignment editor window at the bottom of the graphical user interface.
- Remember to save the project or write the alignment if you wish to keep the alignment for use at another time.



10.2.1. Align DNA to 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.

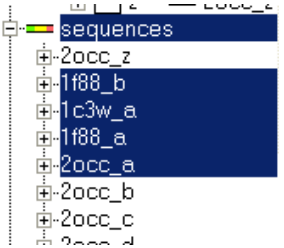


10.3. Align Multiple Sequences

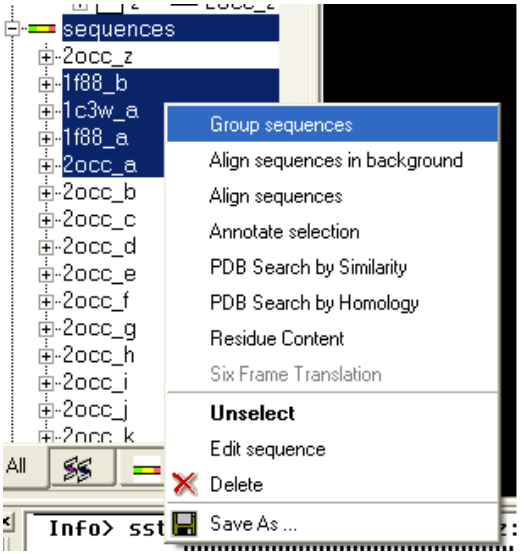
To construct a multiple alignment of more than two sequences the first thing you must do is group the sequences together.

The easiest way to do this is:

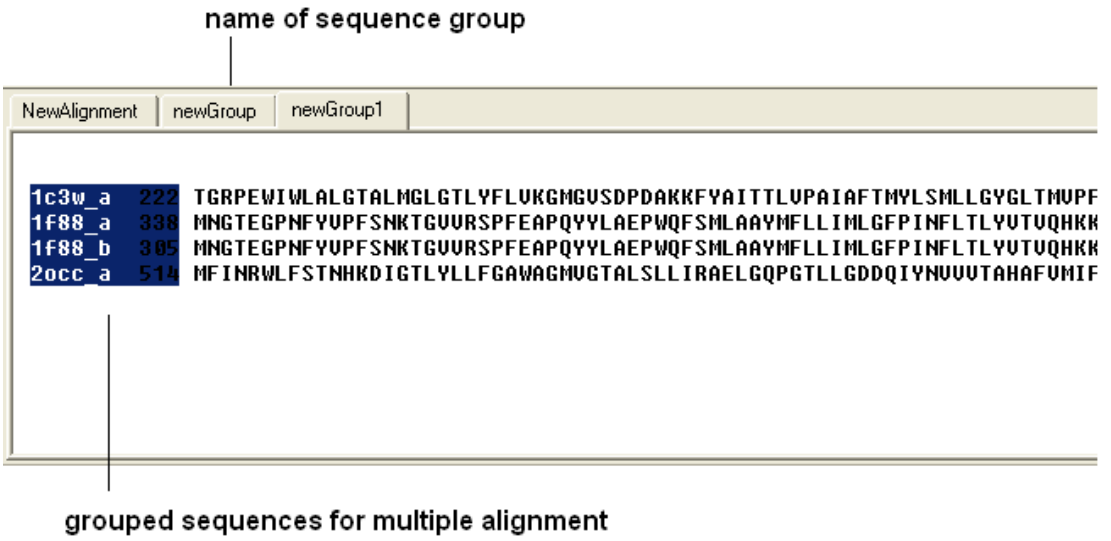
- Locate the sequences in the 'workspace panel' as shown below.
- Then select the sequences you wish to group by holding down the CTRL button and clicking on them. If the sequence is selected it will be highlighted in blue.



- Then right click on the highlighed sequences and the following menu (below) will be displayed.

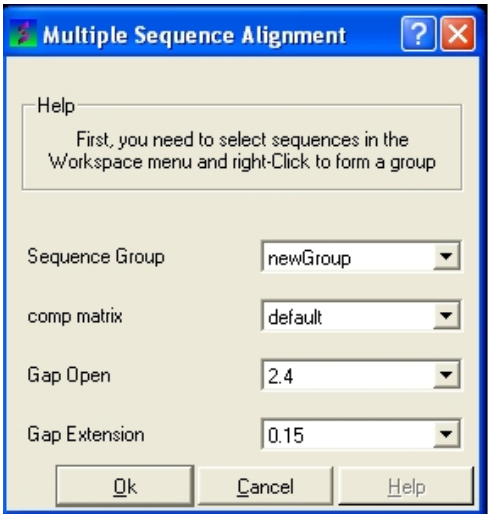


- Select the option 'Group sequences'.
- The sequences will then be displayed in the alignment window at the bottom of the graphical user interface.
- The name of the group is displayed in the tab above the group sequences. Usual name for a group is newGroup1 newGroup2.... etc depending on how many grouped sequences you have.



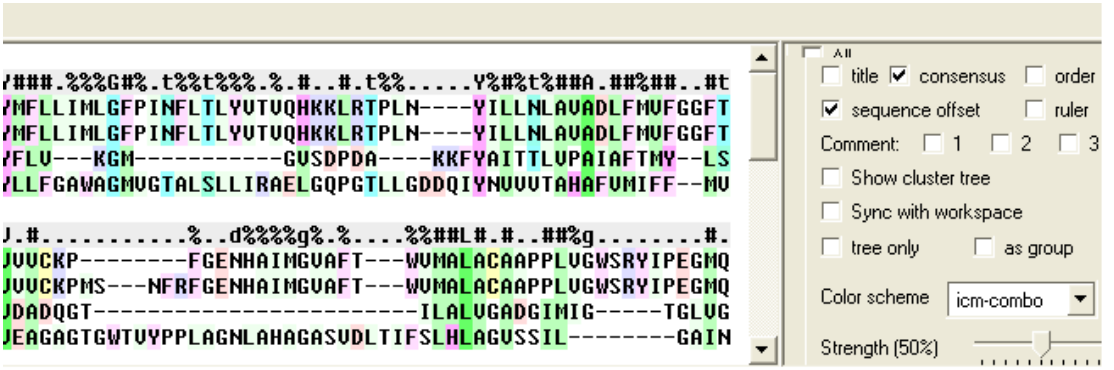
Once the sequences are grouped you need to

- Select the 'Bioinfo' menu.
- Select the option 'Multiple Sequence Alignment' and the data entry window shown below will be displayed.



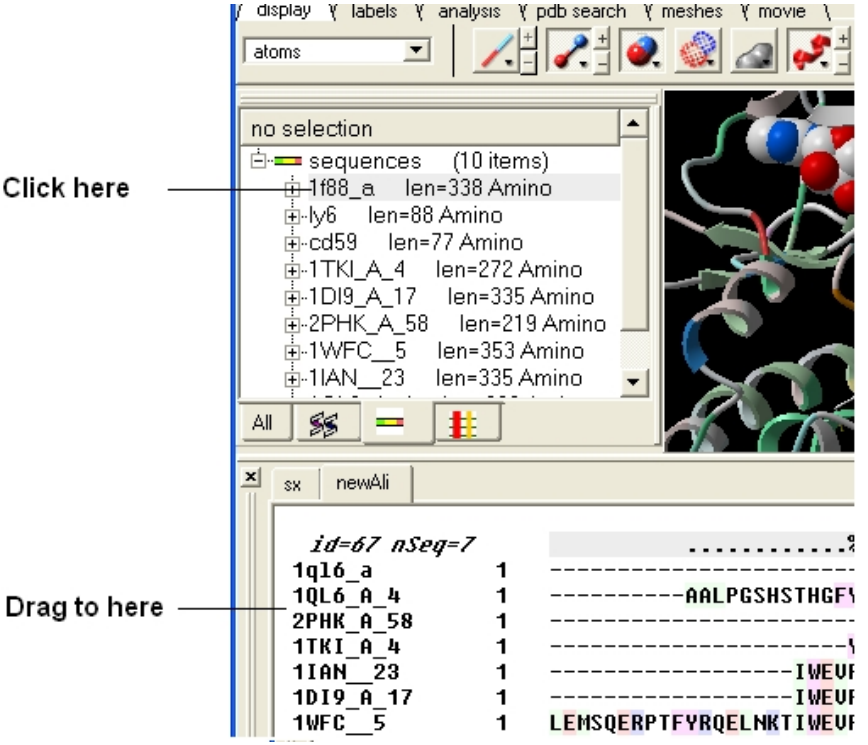
- Enter the name of your group of sequences in the 'Sequence Group' data entry box.

- Select the comparison matrix you wish to use.
- Select the gap opening and gap extension penalties.
- Click OK and the alignment will be displayed in the alignment editor window (below).



10.3.1. 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.



10.4. Alignment Editor

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.

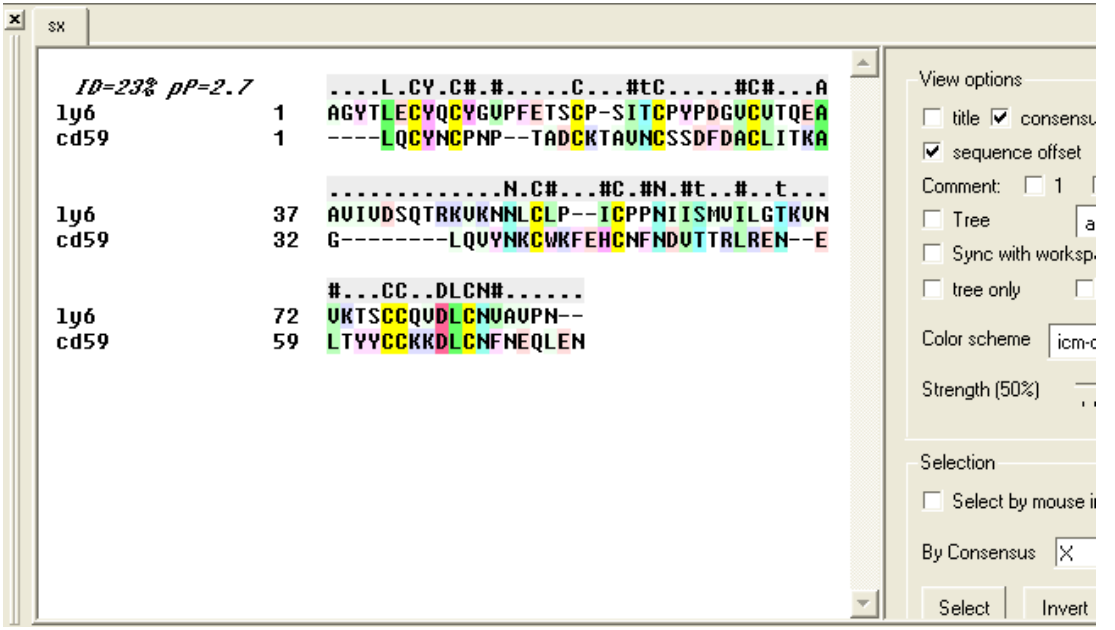
10.4.1. Edit an Alignment

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**

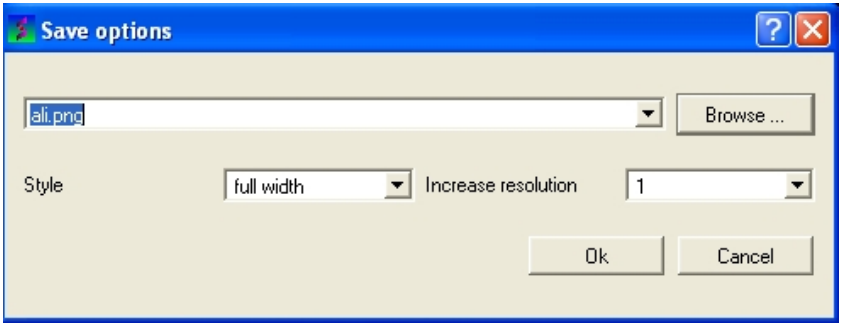


10.4.2. Save, Print and Delete

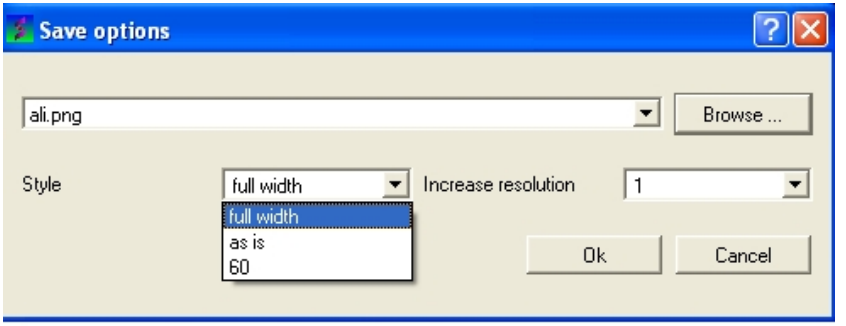
To save your alignment as a picture:

- Right click on the alignment and select the Save as image option.

- A data entry box as shown below will be displayed.

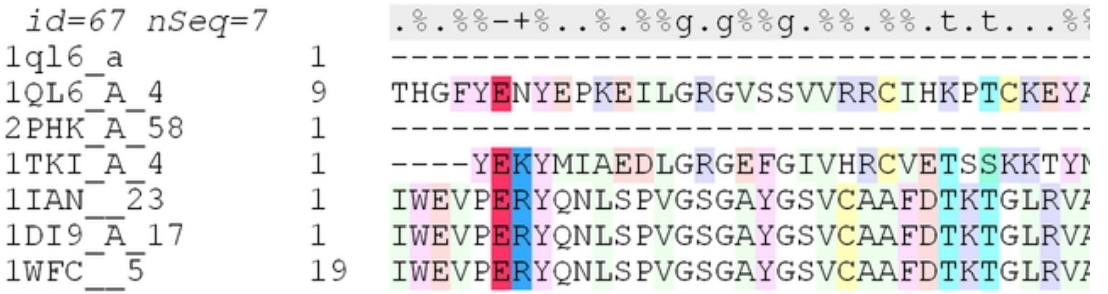


- Enter the filename you wish to call your alignment. We advice you to keep the .png file extensions.
- Select the drop down arrow next to the Style data entry box as shown below.

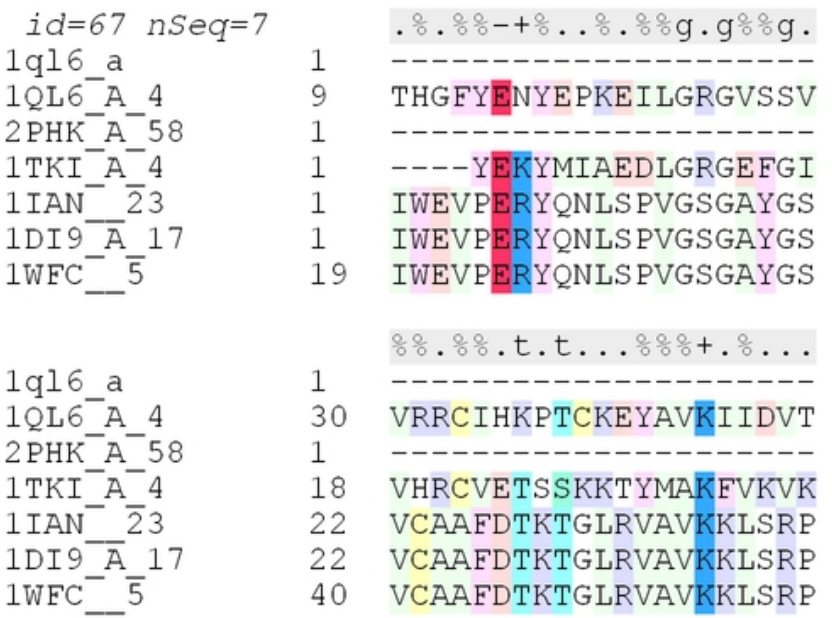


- Select the style you desire from full−width, as is, or 60.

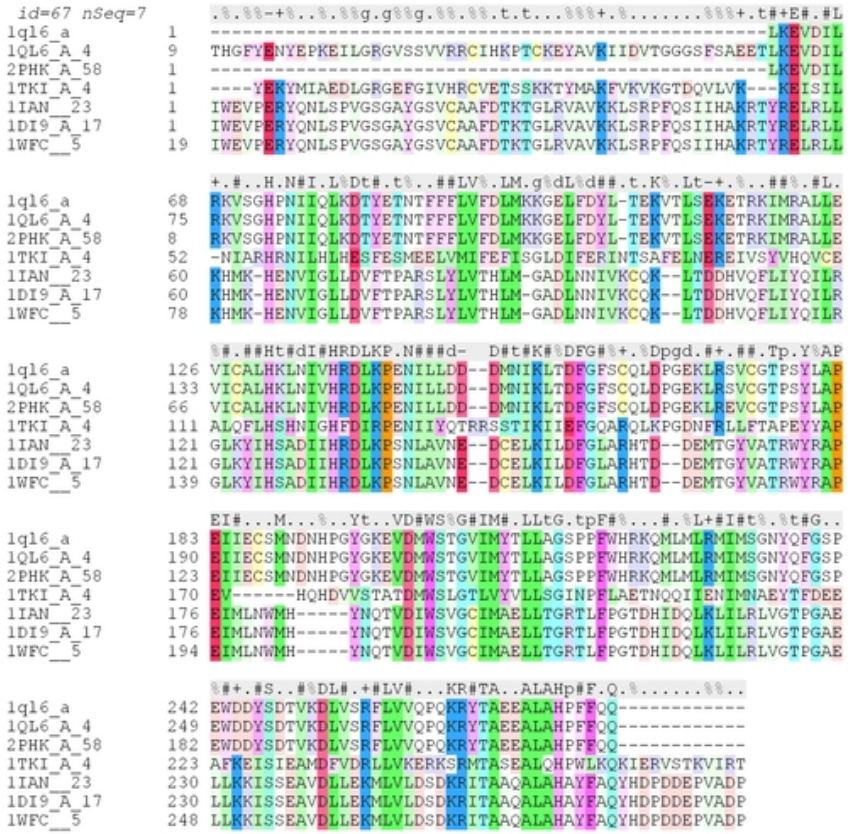
Full−width:



Asis: As displayed in GUI.



60: 60 residues width



- 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 an alignment or tree:

- Right click on the alignment or tree and a menu will be displayed.
- Select the save as option.

To print an alignment or tree:

- Right click on the alignment or tree and a menu will be displayed.
- Select the print option.

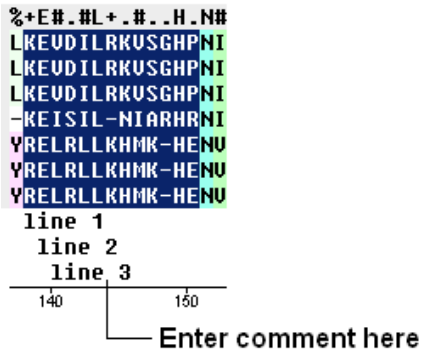
To delete an alignment or tree:

- Right click on the alignment or tree and a menu will be displayed.
- Select the delete option.

10.4.3. Add a Comment

To add a comment to an alignment:

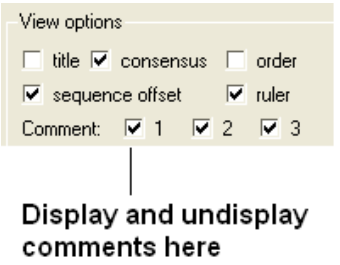
- Click and drag over the region of the alignment under which you wish to add a comment. The selected region will be highlighted in blue.
- Right click on the blue selected region and select 'Edit Comment' and either Line 1 ,2 or 3.
- Enter your comment text.
- Unselect by clicking away from the selection.



NOTE: The length of text added in a comment line can only be as long as the selected region in the alignment. However, there are up to 3 comment lines which you can add.

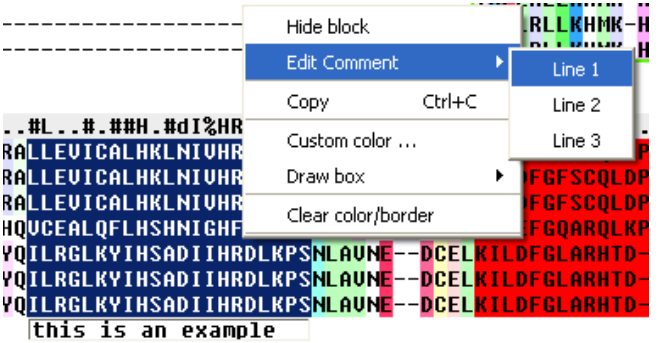
To display and undisplay comments:

- Check and un−check the comment boxes in the view options section of the alignment editor shown below.



To edit a comment:

- Select the comment in the alignment window.
- Right click and a menu will be displayed as shown below.



- Select edit comment and the line number you wish to edit.
- Type the new comment.

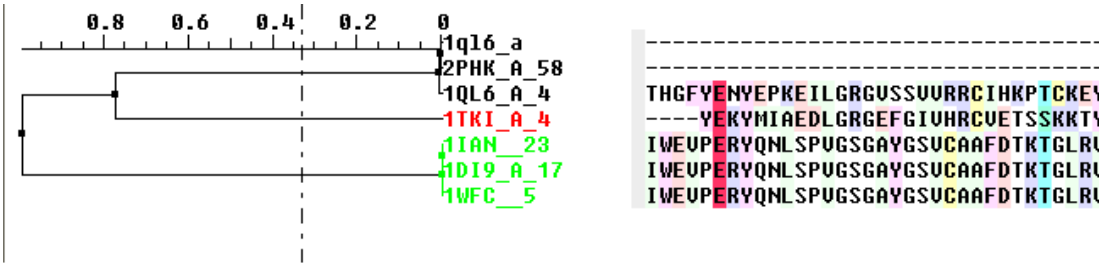
10.4.4. Phylogenetic Trees

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:

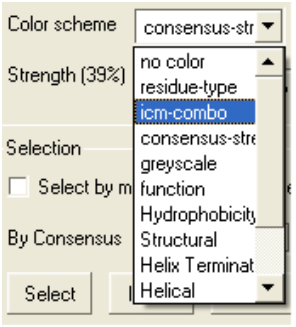
- Check the 'tree only' option in the alignment editor.

10.4.5. Coloring an Alignment.

To color an alignment:

There are a number of ways to color an alignment in ICM. ICM offers a wide range of default coloring options to choose from in the Alignment Editor.

- Click on the drop down arrow beside the "Color scheme" data entry box and a number of color schemes will be displayed.
- Select the color scheme.

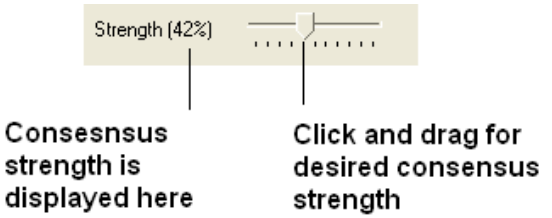


NOTE: You can keep selecting from the list until you find an appropriate color scheme. See the ICM language manual for other ways of coloring, definitions of color schemes and customizing the color.

To color by strength of consensus:

To color your multiple alignment by the strength of consensus at each point in an alignment:

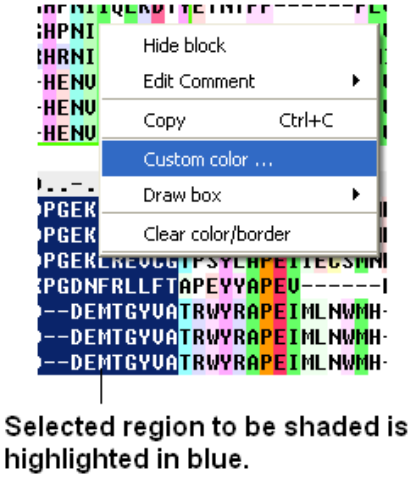
- Click and drag on the consensus strength button shown below:



10.4.6. Shading and Boxing an Alignment

To shade an alignment:

- Click and drag over the region of the alignment you wish to shade. It should be highlighted in blue.
- Right click and a menu will be displayed as shown below.
- Select the Custom color... option

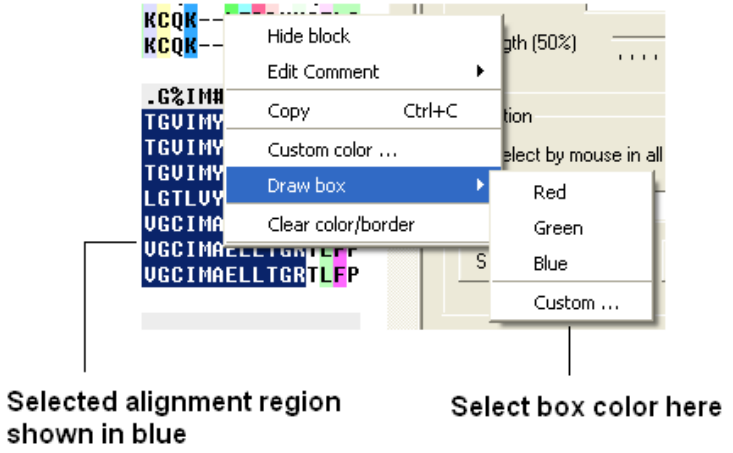


- Select your desired shading color.

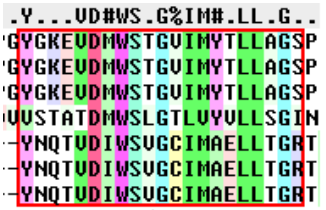
A shaded alignment

To box an alignment

- Click and drag over the region of the alignment you wish to shade. It should be highlighted in blue.
- Right click and a menu will be displayed as shown below.
- Select the Draw box option.



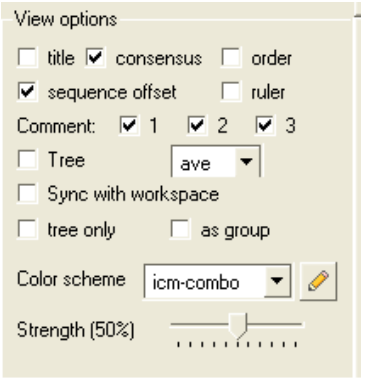
- Select which color you wish to box your alignment in.



A boxed alignment

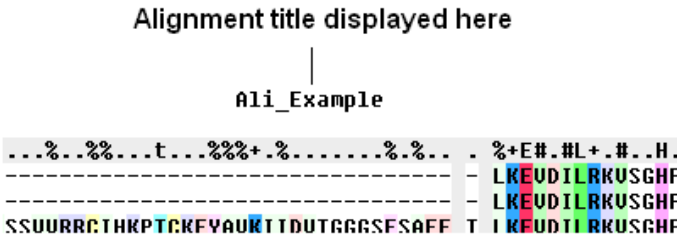
10.4.7. Alignment View Options

The alignment view options are located on the right hand side of the alignment editor.



To add or remove the alignment title:

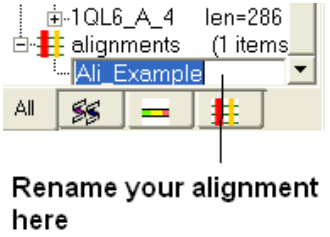
- Check the title box in the view options.



To rename an alignment:

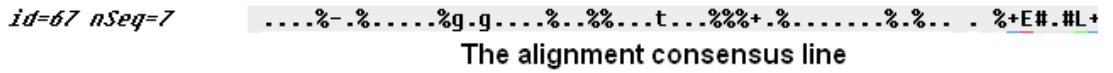
- Right click anywhere in the alignment or on the alignment tab and a menu will be displayed.

- 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.

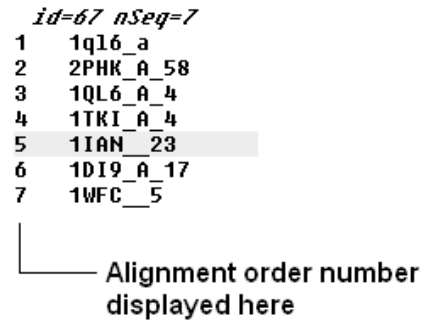
- Check the box labeled 'consensus' in the view options.



If you have a large alignment it may be convenient to show the number of each sequence

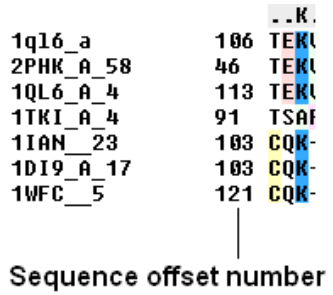
To number your alignment:

- Check the 'order' box in the view options.



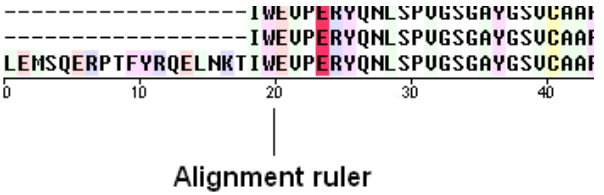
To view the sequence offset number for each of your sequences in an alignment:

- Check the 'offset' box in the view options.



To view the sequence ruler:

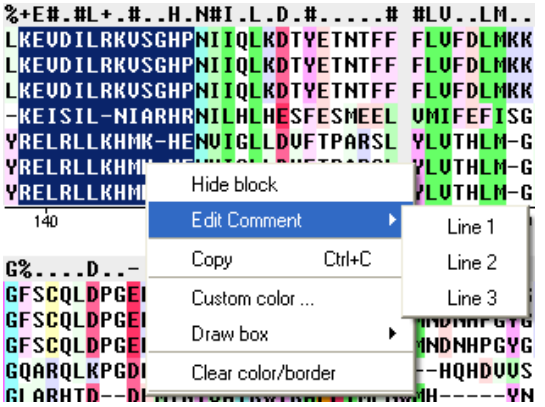
- Check the 'ruler' box in the view options.



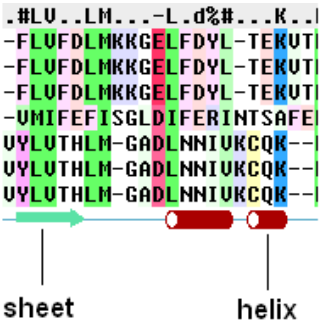
To view secondary structure.

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.

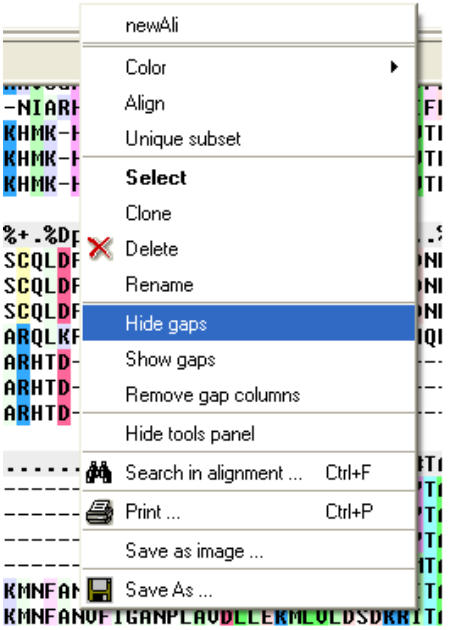


10.4.8. Alignment Gaps

To make an alignment clearer you may wish to HIDE gap regions.

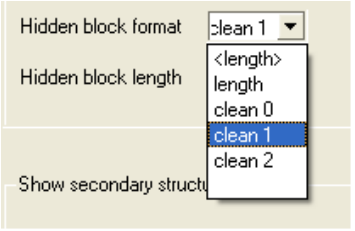
To hide all gap regions:

- 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.



The different hidden block formats are shown here:

length: displays the length of the gap

```
S.. 27 #%D
SDT    VKD
SDT    VKD
SDT    VKD
SIE    AMD
SSE 27 AVD
SSE 27 AVD
SSE 27 AVD
```

length: displays the length of the gap in .

```
S..< 27 >#%D
SDT<    >VKD
SDT<    >VKD
SDT<    >VKD
SIE<    >AMD
SSE< 27 >AVD
SSE< 27 >AVD
SSE< 27 >AVD
```

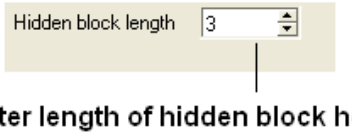
clean0: displays no indication of a gap

```
S..#%D
SDTVKD
SDTVKD
SDTVKD
SIEAMD
SSEAVD
SSEAVD
SSEAVD
```

clean1: displays grey panel in the gap position.

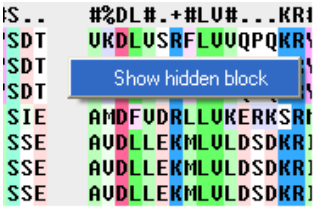
clean2: displays a wider grey panel in the gap position

NOTE: The width of the hidden panel can be changed as shown below.



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.



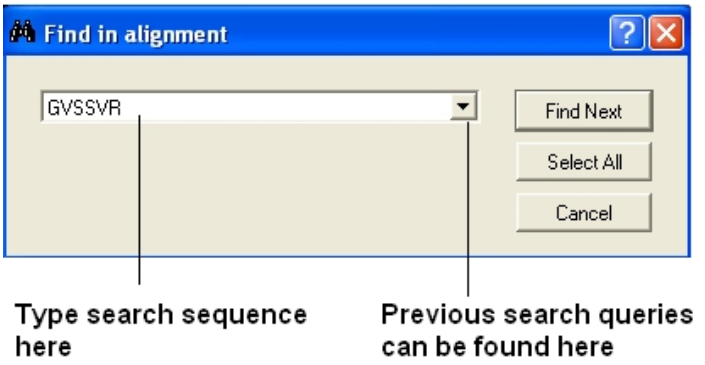
To show all gaps:

- Right click on the alignment away from a gap region and a menu will be displayed.
- Select the "Show gaps option.

10.4.9. 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.

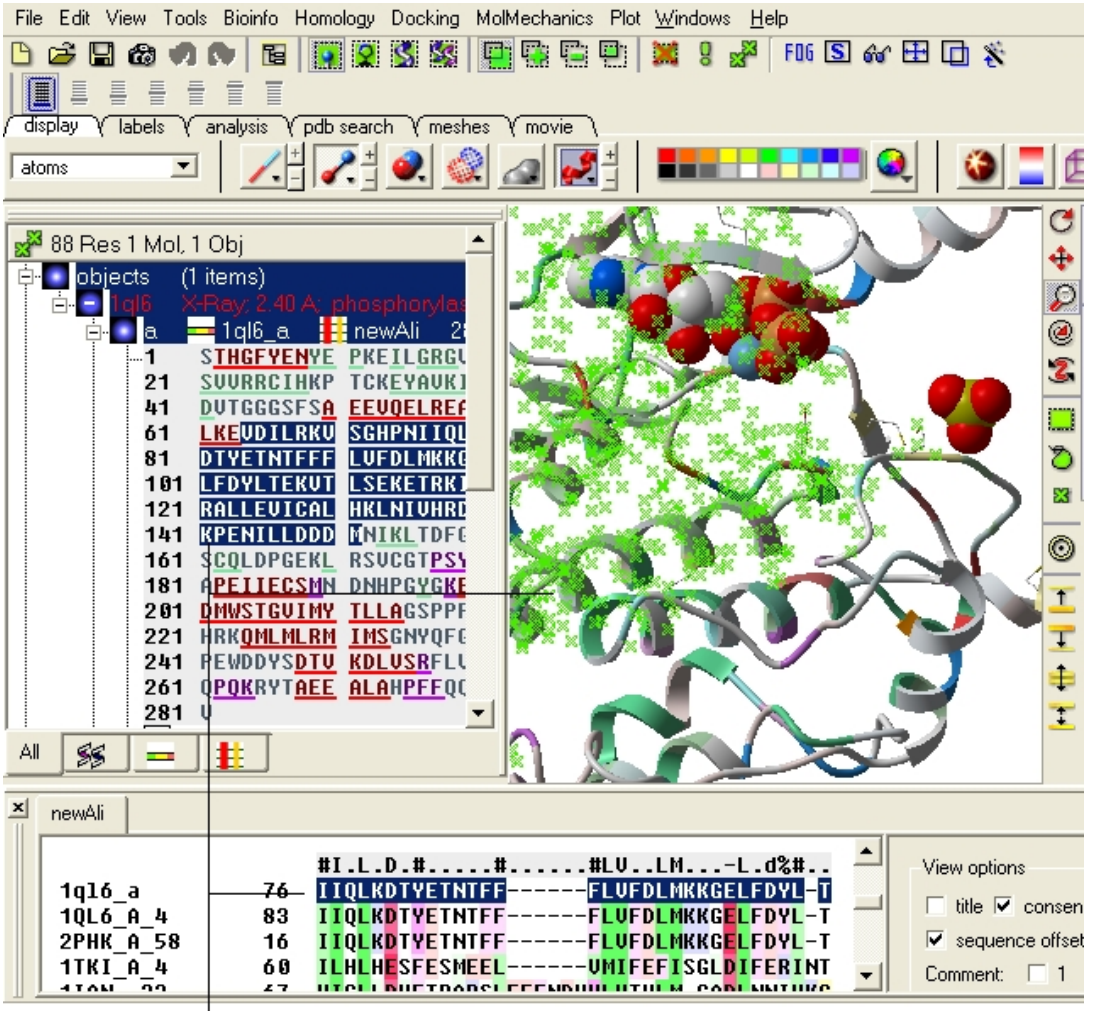
- Right click on the alignment away from any hidden gaps and a menu will be displayed.
- Select the "Search in alignment option" and a data entry box as shown below will be displayed.



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`.

10.5. 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.

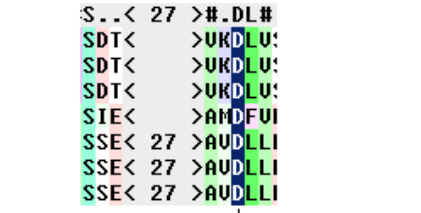


Selection is displayed in the ICM workspace (blue) the alignment window (blue) and the 3D graphics display (green cross).

10.5.1. Basic Alignment Selections

To select a single column of an alignment:

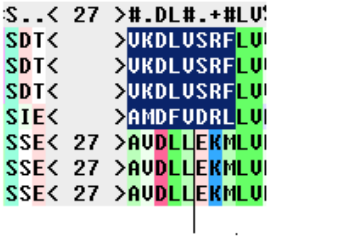
- Double click.



One column selected by double clicking

To select parts of an alignment:

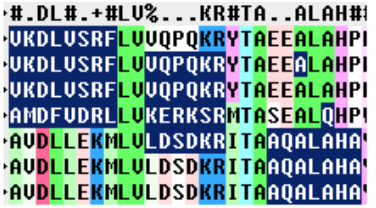
- Click and drag over the region you wish to select.



Click and drag over to select

To select multiple discontinuous parts of an alignment:

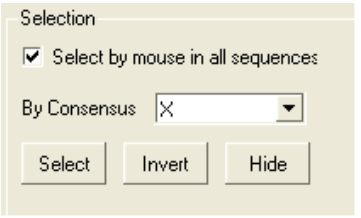
- Click and drag whilst holding down the control key.

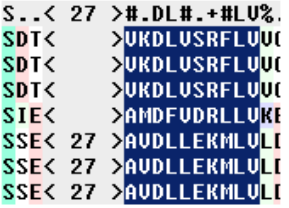


Click and drag whilst holding down the control key.

To enable the easy selection of all sequences in an alignment:

- Check the box labeled "Select by mouse in all sequences".





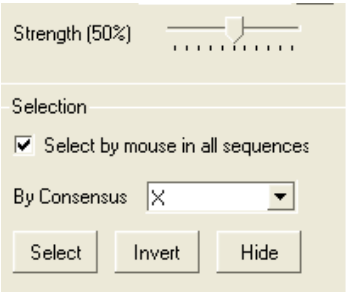
All sequences are selected using the mouse

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.

10.5.2. Select by Consensus

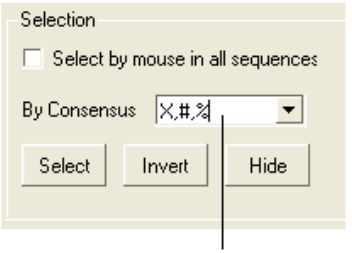
This is a very useful tool, for example, you may want to color the consrved regions of your structure in the 3D display windowa different color to the rest of the structure. This tool allows you to select the conserved regions in the sequence alignment. Once the selection has been made it can be used 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 seperated 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.

11. Molecular Modeling

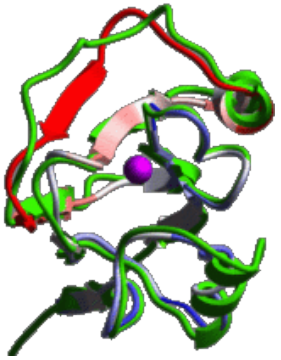
11.1. Molecular Modeling Introduction

Molecular modeling is an essential method for predicting the main structural features of a protein. From these models it is possible to derive useful functional information about the proteins concerned and also use them for molecular docking (`see docking section of manual`) or VLS (`see virtual ligand screening section of manual`).

The basis behind molecular modeling is to use as much information as possible derived from solved structures in the PDB and apply them to the wealth of newly generated gene sequences, derived from many genome programs. All the available parameters are considered. Whenever there are variables that are too uncertain to derive from experimental data, you can use powerful prediction algorithms such as the ICM program to find the most probable solution. With today's need for high−throughput, molecular modeling is often one of the best approaches to define priorities for researchers and corporations.

ICM has an excellent record in building accurate models by homology. The procedure will build the framework and shake up the side−chains and loops by global energy optimization. You can also color the model by local reliability to identify potential errors in your model.

ICM also offers a fast and completely automated method to build a model by homology and extract the best fitting loops from a database of all known loops. It just takes a few seconds to build a complete model by homology with loops.



11.2. Getting Started

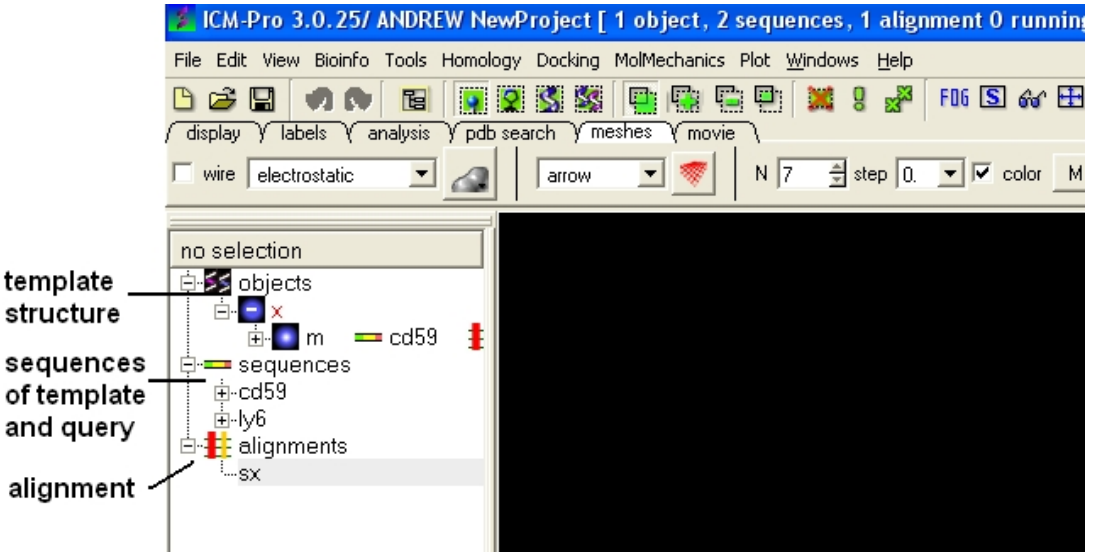
The three items you need for ICM protein molecular modeling are:

1. An alignment (see `alignment section`) of your protein sequence against a template structure from the PDB. This is a *.ali file in ICM.

2. A template structure from the PDB converted (See `convert object`) into an ICM object.

3. A sequence file of your query sequence for the structure you wish to construct and a sequence file for your template. Note ICM automatically extracts this information from the alignment or template structure.

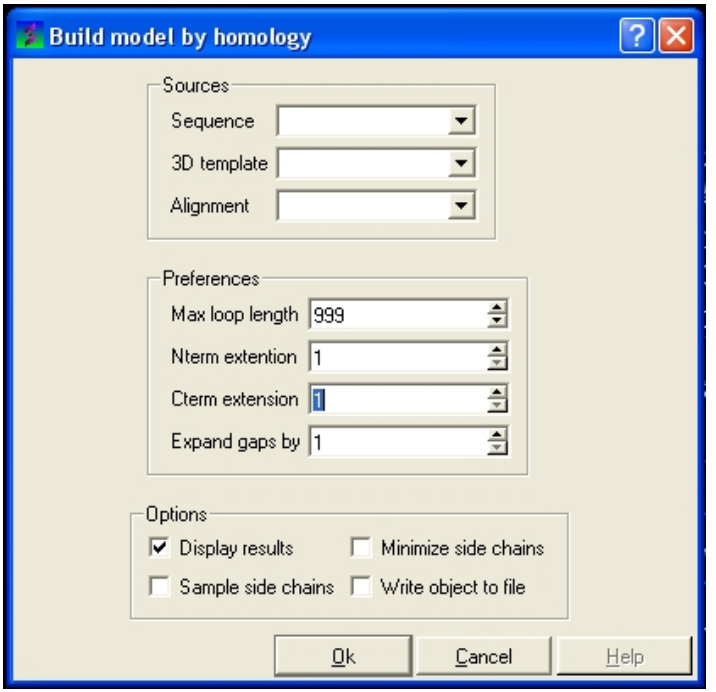
Your graphical user interface window should look something like this:



11.3. Build Model

To build a molecular model:

- Click on the 'Homology' menu at the top of the graphical user interface.
- Select Build Model and a data entry box will be displayed as shown below.



This data entry box is split into 3 sections, the first is 'sources' where you need to specify your query sequence, template and alignment. The second section is called 'preferences where penalty information for the model needs to be entered and the third section is called 'Options'. Each will be described in detail below.

To construct your model follow these steps:

- Enter the name of your QUERY (ie the sequence of the model you wish to build − NOT the template) sequence in the data entry box labeled 'sequence. If you click on the arrow next to this box a list of sequences loaded in ICM will be displayed click on your QUERY sequence. The names of the sequences are also listed in the workspace panel on the left of the graphical user interface.

- Enter the name of your template structure in the '3D template' data entry box. Once again the name of your template structure can be found by clicking the down arrow or in the workspace panel.

- Enter the name of your alignment in the 'Alignment' data entry box.

You could build your model now as ICM has enough infromation but it may be wise to take a look at some of the preferences and change them accordingly. However in most cases the default values provided are sufficient to produce a good quality model.

To change the preferences either type the number you wish or use the up and down arrows next to the data entry boxes.

Max loop length (default= 999) − longer loops are dropped Nterm extension (default=1) − the maximal length of the N−terminal model sequence which extends beyond the template Cterm extension (default=1) − the maximal length of the C−terminal model sequence which extends beyond the template Expand gaps by (default=1) − additional widening of the gaps in the alignment. End gaps are not expanded

Now all you need to do to build your model is to select some options. Check the box if you would like ICM to perform that option.

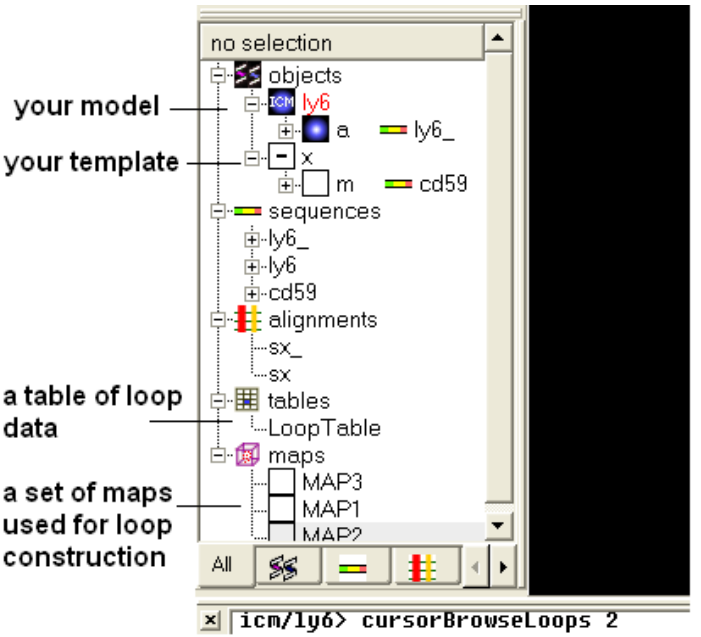
The options are:

Display results − displays your model in the 3D graphics window Minimize side chains − performs minimization on the side−chains Sample side chains − performs monte−carlo optimization on the side chains Write object to file − writes your new model as an ICM object

To build your model:

- Click OK

Once your model is built a new object will be seen in your workspace panel. This is your model (see below).



A table of the loop data will also be displayed showing the RMSD from the template.

LoopTable

	1_Loop	2_Conf	3_Rmsd	4_Nof	5_Type
1	a_ly6.a/57:58	1RRR	0.46	3	1
2	a_ly6.a/9:10	RRR3	0.58	3	1
3	a_ly6.a/32:33	L1R322LLR2	0.54	2	1
4					

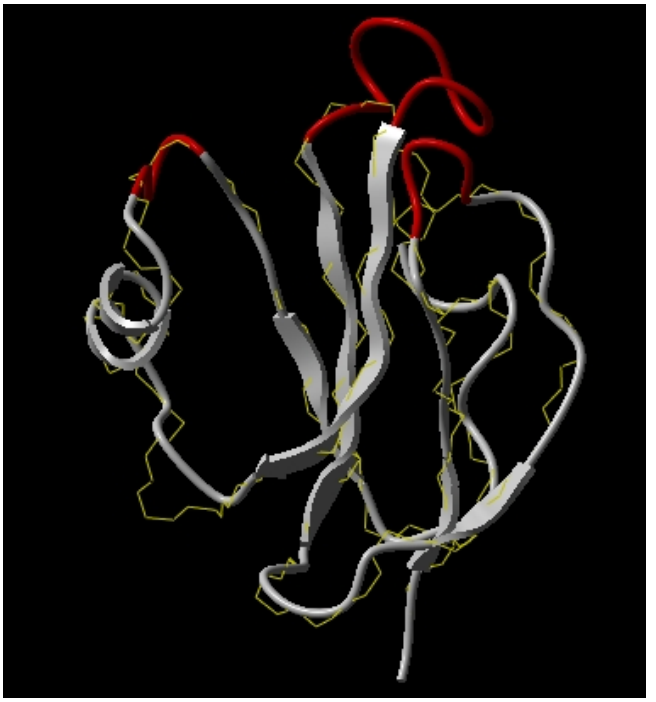
11.4. Loop Modeling

Building an accurate model of a loop is very tough. However with small loops ICM has been very succesful. ICM was used to design two new 7 residue loops and in both cases the designs were successful. Moreover, the predicted conformations turned out to be exactly right (accuracy of 0.5Å) after the crystallographic structures of the designed proteins were determined in Rik Wierenga's lab.

Once you have built a model (see section `Build Model`) the loop regions or (inserted fragments) can be viewed by:

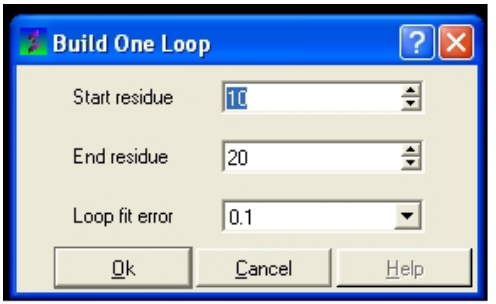
- Click on the 'Homology' menu.
- Select 'Display Loops'

The loop regions in the model will be displayed in red. Information regarding the RMSD in the loops are displayed in the Loop Table. If your template structure is still loaded this will be displayed in yellow as shown below.



To build a new loop to an existing structure or to improve your already modeled loops:

- Read your modeled structure into ICM. Or continue immediately after using build model.
- Select the 'Homology' menu.
- Select 'Build one loop' and the following data entry table (below) will be displayed.



- Enter the residue number at which you wish to start the loop modeling in the 'Start residue' data entry box − Note only one loop at a time.

- Enter the residue number at which you wish to finish loop modeling in the 'End residue' data entry box.
- Enter the value of the loop fit error
- Click OK and a new loop will be constructed.

12. Small Molecule Docking Using the ICM Graphical User Interface

As demonstrated in several recent papers, short flexible peptides can be successfully docked ab initio to their receptors. This method is a blend of the peptide folding with the grid potentials representing the receptor. A similar method can be applied to any chemical. A chemical can be built from a 2D representation and optimized. The "drugable" pockets can be predicted with an algorithm based on the contiguous grid energy densities.

This section is concerned with predictions of interactions of drugs or small biological substrates (less than about 600−700 Da) to pockets of larger, more rigid, receptors (typically, protein molecules, DNA or RNA).

12.1. Docking Introduction

For accurate ligand docking, the goal is to have an adequate three−dimensional model of the receptor pocket you are planning to dock ligands to. If this is the case then ICM docking has been shown to be very accurate in a number of independent assesments.

However, there are a number of pitfalls which need to be overcome to achieve accurate ligand docking. The pitfalls are that your model is not accurate overall, does not reflect the induced fit, or alternative conformations of the receptor binding pocket are missed.

12.1.1. Receptor from a PDB File

If you have only a single entry with your receptor, convert the protein to an ICM object, delete water molecules and irrelevant chains. However, if you have a choice between several templates, take the following into account:

- X−ray structure is preferable to an NMR structure
- High resolution X−ray structure (less than 2.1A) is much better than, say 2.5A . Watch out for high−B−factor regions and avoid them; sometimes crystallographers deposit fantasy coordinates with high−B−factors.
- Place polar hydrogens and choose correct form of histidine.
- A bound conformation of the receptor is preferable, however if you use an apo−model, an NMR structure or a model by homology, the side−chains in a pocket may be incorrect. Frequently they stick out and prevent a ligand from binding. Those stubborn side−chains can be 'tamed', (i) manually; (ii) by a side chain simulation with elevated surfaceTension; or (iii) by an explicit flexible docking calculation with a known ligand.

12.1.2. Receptor from Homology Modeling

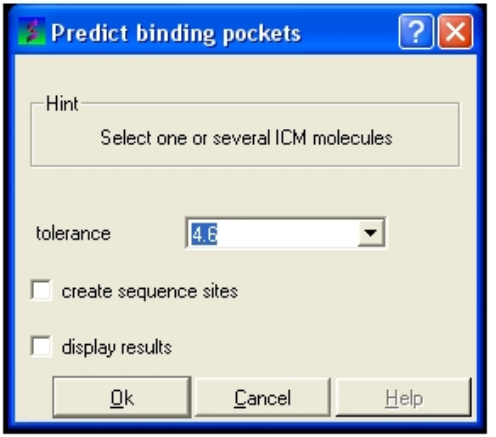
A model by homology can be built with the build model command (see `molecular modeling` section of this manual).

12.1.3. Identifying Binding Pockets

If a binding pocket is not known in advance, use icmPocketFinder or icmCavityFinder (for closed pockets).

icmPocketFinder can be accessed by

- Click on the menu Tools/icmPocketFinder



- Enter a tolerance level (4.6 is the default value and we recommended you to use this).
- 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.
- Click OK to run icmPocketFinder

NOTE: A button for icmPocketFinder can be found on the Setup Receptor option in the docking menu.

12.1.4. What to dock. Ligand, Ligands, a Database or a Library.

Usually a good start is to try to dock the known ligand(s) to the receptor model. You may also want to dock a library of compounds in order to identify lead candidates. In this case the main pitfall is that the library is too restricted, molecules are not chemically feasible or not drug−like.

12.1.5. Converting a Chemical from the PDB.

The protein data bank, due to unprecedented ignorance, for the last 15 years has not been storing any information about covalent bond types and formal charges of the chemical compounds interacting with proteins! This oversight makes it impossible to automatically convert those molecules to anything sensible and requires your manual interactive assignment of bond types and formal charges for each compound in a pdb−entry. Therefore, if you apply the convert command to a pdb−entry with ligands, the ligands will just become some crippled incomplete molecules that can not be further conformationally optimized.

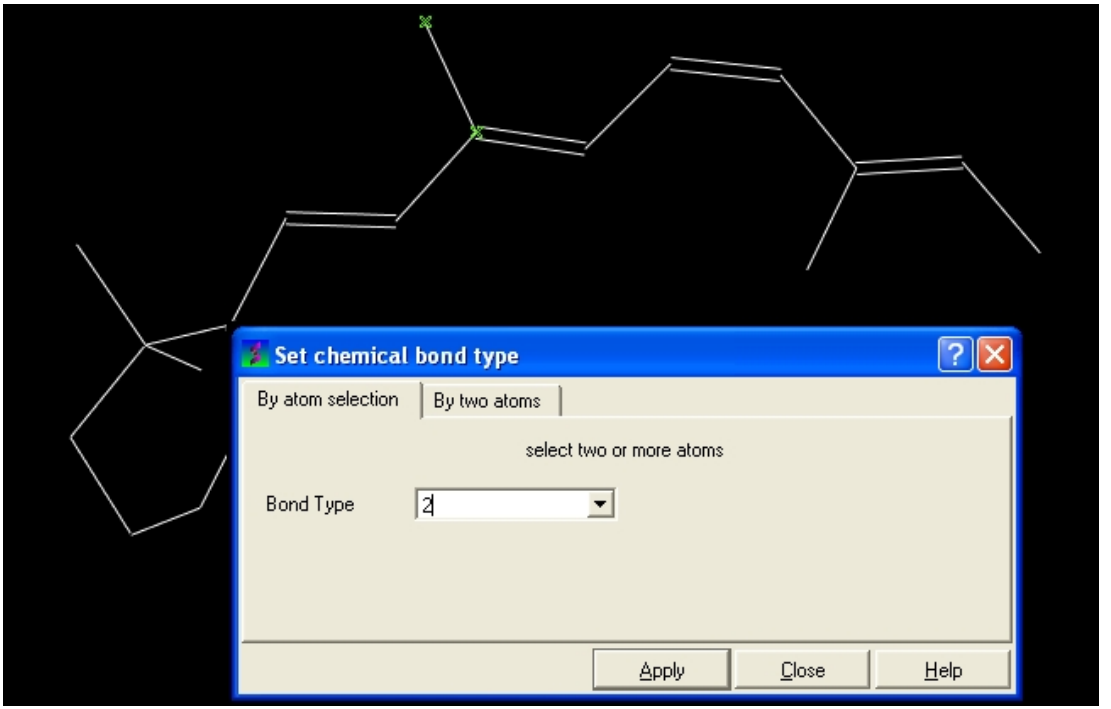
Therefore, follow these steps to convert a chemical properly from a pdb form to an a correct icm object.

- Display the molecule in wire chemistry style mode by right clicking on the Wire Representation button (see `Wire Representation section`).

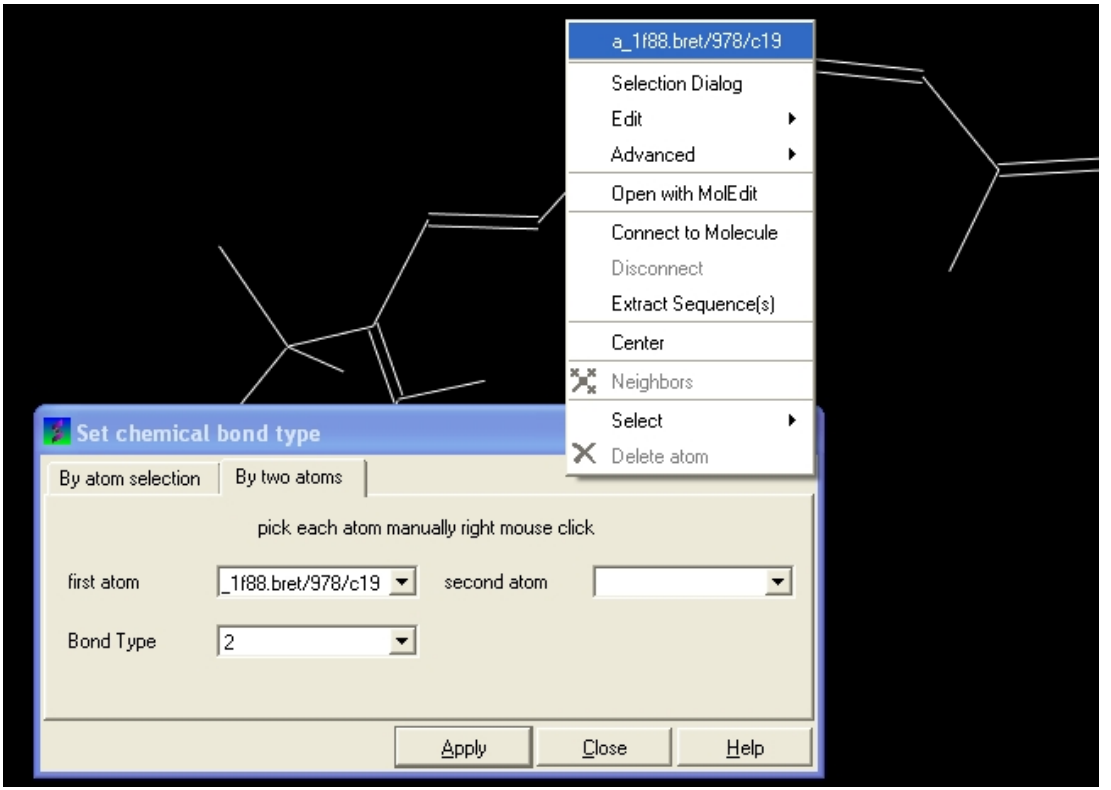
To change the bond types in your ligand:

- Click on MolMechanics/Edit Structure/Set Bond Type and the Set chemical bond type data entry box will be displayed.

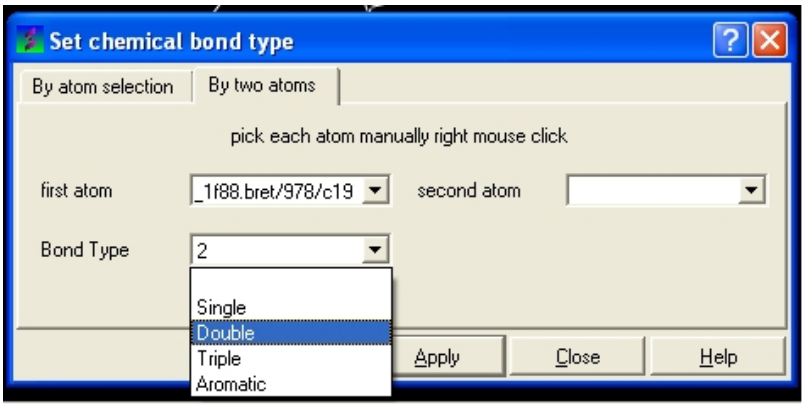
You can either select (see `selection menu section`)the atoms you wish to change graphically using the rectangular or lasoo selection button OR



You can select the **By two atoms** tabs and right click on the atoms you wish to change and then selecting the atom descriptor with the left mouse button as shown below.

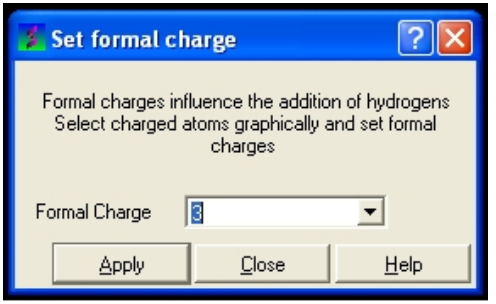


- Select the desired bond type either single, double, triple or aromatic.



To set the formal charge of a compound:

Click on MolMechanics/Edit Structure/Set Formal Charge and then select the appropriate charge.



12.1.6. Overview of the Docking Procedure

Follow these steps to perform ICM small molecule docking

1. Receptor in ICM format
2. Ligand in ICM format
3. Establish a docking project name
4. Setup receptor for docking
5. Setup a ligand for docking
6. Setup electrostatic maps

After the receptor maps are built, you will start a docking simulation. The goal of the flexible docking calculation is prediction of correct binding geometry for each binder. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that includes five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand. During this process a stack of alternative low energy conformations is saved (one of the choices in the Docking menu).

NOTE: Each docking step is described in more detail later on in this chapter.

12.1.7. Some facts about ICM docking:

- An average docking time is 20 seconds to 3 minutes per ligand per processor.
- ICM docking is probably the most accurate predictive tool of the binding geometry today.

- The time per ligand was chosen to be the smallest possible to allow screening of very large data sets. To increase the time spent per ligand, change the Docking_thoroughness parameter.

12.2. Docking Getting Started

ICM ligand docking procedure performs docking of the fully flexible small−molecule ligand to a known receptor 3D structure. Before setting up the docking project, an ICM object of the receptor has to be created. In most cases, x−ray structure of the receptor is initially in the PDB format. Thus, it has to be converted to the ICM format. This process involves addition of the hydrogen atoms, assignment of atom types and charges from the residue templates (icm.res) and imposition of internal coordinates tree (icm−tree) on the original pdb coordinates. To convert a pdb structure into icm object is through GUI as follows:

- Load pdb file into ICM by clicking File/Read Molecule/PDB
- Convert loaded structure into an ICM object by clicking MolMechanics/ICM−convert/Protein.

NOTE: It is recommended that "optimize hydrogens" option is selected. To accelerate the procedure, disable the 3D graphics window (side menu Clear/No Graphics) When the procedure finishes, converted object is the 'current' object in icm. You can check the results by displaying the converted structure.

12.3. Docking Project Setup

Follow these instructions in order:

12.3.1. Set project name

Start the docking project setup by defining the project name:

- Click on Docking/Set project name
- Enter a unique name into the Project name data entry box. Avoid spaces and leading digits in the name. All files related to the docking project will be stored under names, which start from the project name. Most customized parameters will be saved in the table file under the project name as well:

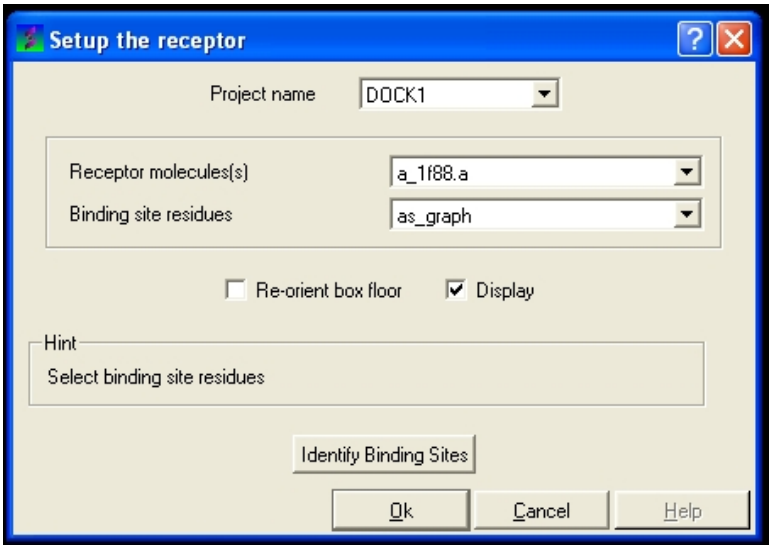
- Click on the 'OK' button.

Now set up the receptor. Go to `Receptor Setup`

12.3.2. Receptor setup

The next step is to set up the receptor for docking.

- Click on Docking/Receptor setup



- Enter the project name in the Project name data entry box. If the project name was established in the same ICM session then it should automatically appear in this box.

NOTE: Other docking project names that you have entered can be found by clicking on the arrow besides the Project name data entry box.

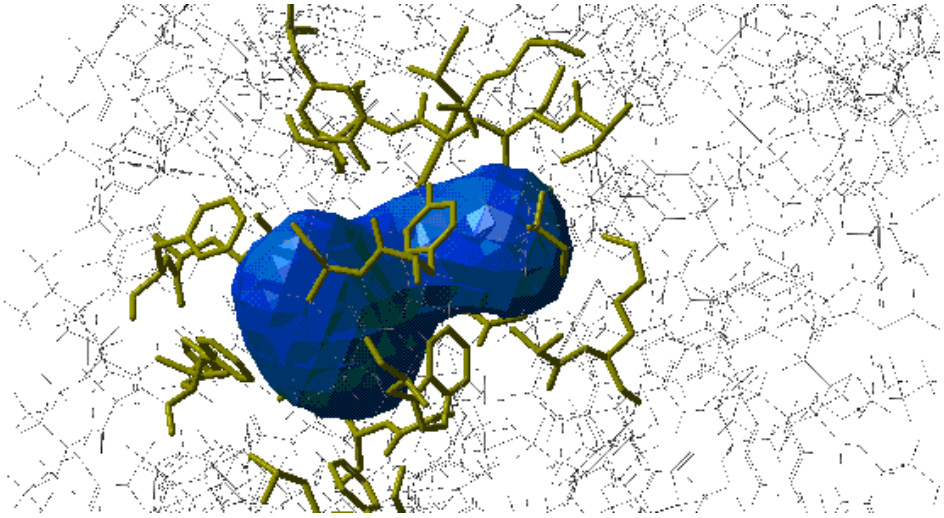
- Enter the receptor molecule in the Receptor molecule(s) data entry box. In most cases a_* will do − all molecules in the current object will be included. The receptor molecule can also be found by clicking on the arrow next to the data entry box. A list of potential receptors will be displayed. Click on the receptor you wish to use for your docking experiment.

- Enter the ICM binding site residues Binding site residues data entry box. Define the binding site residues, either manually e.g. a_/123,144,152 for selection by residue numbers, or graphically using lasso tool (don't forget to set selection level to residue). If the residues are selected using the lasso tool then type as_graph in the Binding site residues data entry box. This selection is used solely to define boundaries of the docking search and the size of the grids and doesn't have to be complete, selecting some 4 residues delimiting the binding site is sufficient. Receptor setup dialog also lets you run binding site identification routine to quickly locate putative binding sites on your receptor.

NOTE: Potential ligand binding pockets can be identified using ICMPocketFinder or by clicking on the Identify Binding Sites button in the Setup the receptor data entry window.

- Click on the **OK** button.

After the receptor setup is complete, the program normally displays the receptor with the selected binding site residues highlighted in yellow xstick representation.



The position of the probe ie the initial position where sampling will begin can be changed at this point in the procedure. Follow the instructions in the command line section as shown below. The size of the box in which the electrostatic maps are constructed can be changed at this point or later on see section “review and adjust binding site”.

```
icm/1f88> if( type( as_graph ) == "aselection" ) tempsel = as_graph & a_1f88.a
icm/1f88> if( type( as_graph ) == "unknown" ) tempsel = as_graph & a_1f88.a
icm/1f88> dockSetupReceptor "DOCK1" a_1f88.a tempsel no no yes "none"
Two following receptor setup steps are:
1. adjustment of the initial ligand position; 2. adjustment of the box size/position.
1. If necessary, re-orient the red probe. Hold SHIFT for global rotation.
Press 'ENTER' to continue or to skip this step.
```

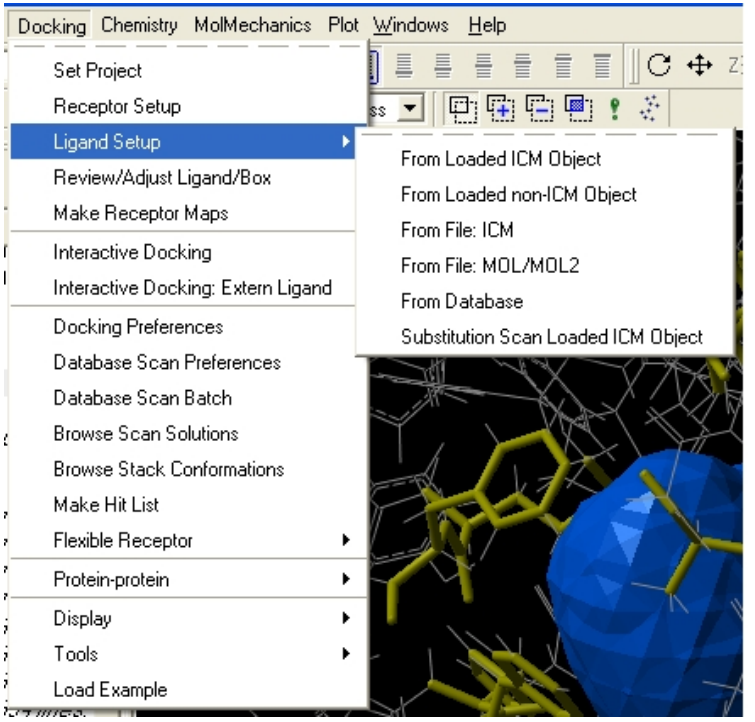
Now set up the ligand(s). Go to `Ligand Setup`

12.3.3. Ligand Setup

To setup your ligand for a docking experiment it must be converted into an ICM object but it can be loaded from a number of different locations.

- Click on the menu Docking/Ligand setup and then select one of the following options depending on the current location of your ligand molecule – From Loaded ICM Object, From Loaded Non−ICM Object, From File: ICM, From File Mol/Mol2, From Database or Substitution Scan Loaded ICM Object. Explanations for each of these scenarios are described below.

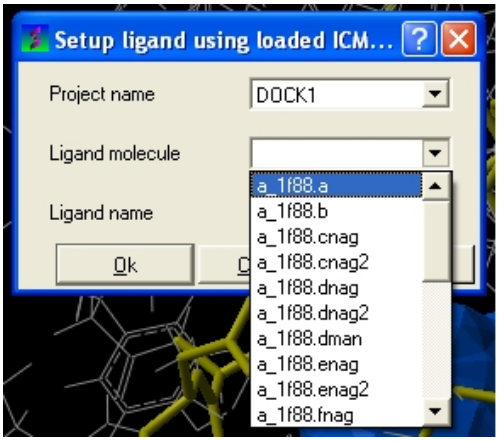
NOTE: The options from Database or Substitution Scan Loaded ICM Object are used in Virtual Ligand Screening, this section only describes the docking of 1−100 compounds.



12.3.4. Ligand setup from a loaded ICM object

If your ligand is already loaded into ICM and converted into an ICM object then

- Click on the menu Docking/Ligand setup/ From Loaded ICM Object and a data entry box will be displayed as shown below.



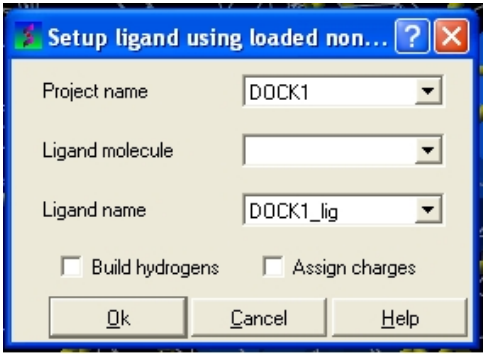
- Enter the name of the docking project followed by the ligand molecule name and you can also change the name of the ligand if you wish.

Now go to `Review Adjust Binding Site`

12.3.5. Ligand setup from a loaded Non−ICM object

If your ligand is already loaded into ICM but is not already converted into an ICM object then:

- Click on the menu Docking/Ligand setup/ From Loaded Non−ICM Object and a data entry box will be displayed as shown below.



- Enter the name of the docking project followed by the ligand molecule name and you can also change the name of the ligand if you wish.

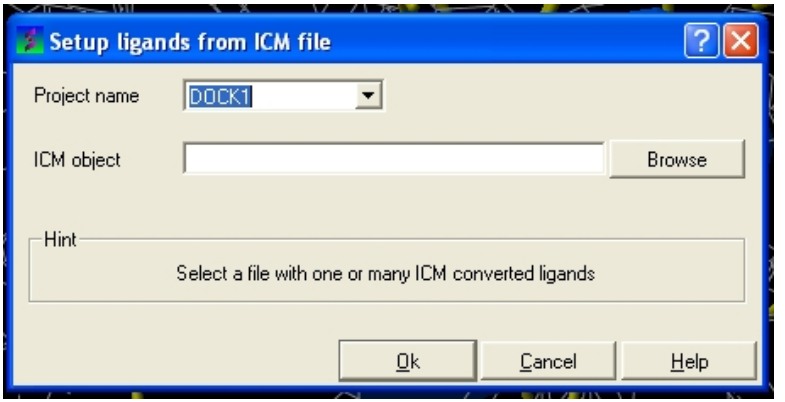
If you wish hydrogens to be added to your compound or charges to be assigned then click on the appropriate boxes in the display panel. ICM will then convert your ligand to an ICM object.

Now go to `Review Adjust Binding Site`

12.3.6. Ligand setup from File

If your ligand (s) is saved and converted to an ICM object but is not yet loaded into ICM then you need to use this option.

- Click on the menu Docking/Ligand setup/ From File :ICM and a data entry box will be displayed as shown below.



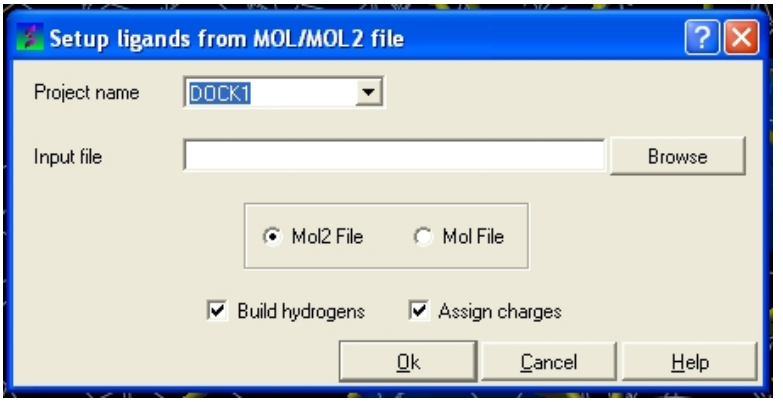
- Enter the name of the docking project.
- Click OK

Now go to `Review Adjust Binding Site`

12.3.7. Ligand setup from MOL/MOL2 File

If your ligand is a MOL or MOL2 file then

- Click on the menu Docking/Ligand setup/ MOL/MOL2 File and a data entry box will be displayed as shown below.



- Browse for your ligand file.
- Select whether your ligand is in MOL or MOL2 format.

If you wish hydrogens to be added to your compound or charges to be assigned then click on the appropriate boxes in the display panel.

- Click OK

Now go to `Review Adjust Binding Site`

12.3.8. Review and adjust binding site

ICM makes a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompasses the residues expected to be involved in ligand binding, however you may wish to alter the size of the purple box or the position of the ligand probe (red spot).

- Click on the menu Docking/Review/Adjust Ligand/Box
- A data entry window will be displayed as shown below.

- Select the option Adjust/ligand position/orientation and/or Adjust box position/size

Follow the instructions in the command line display.

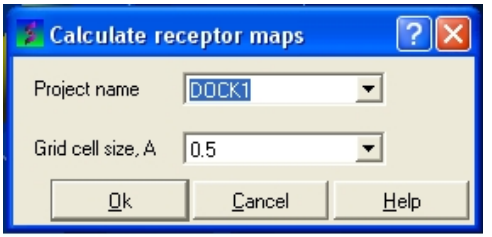
NOTE: Always check that the correct project name is displayed in the data entry window.

Now go to `Make Receptor Maps`.

12.3.9. Make Receptor Maps

The next step is to construct energy maps of the environment within the docking box.

- Click on the menu Docking/Make Receptor Maps



NOTE: Always check the correct project name is displayed in the data entry window.

- Select the resolution of the map by entering a value into the grid cell size data entry box. We recommend a value of 0.5 for both accuracy and speed of calculation.

NOTE: Calculation of the maps may take a few minutes.

Now begin the `docking procedure`.

12.4. Begin the Docking Simulation

Once the receptor, ligand and maps have been correctly set up then the docking procedure can begin.

- Click on the menu Docking/Interactive Docking

There are three check boxes that may be selected.

OPTION 1 Calc REBEL score will return a score after docking for the electrostatic free energy.REBEL stands for Rapid Exact−Boundary ELectrostatics. The energy calculated by this method consists of the Coulomb energy and the solvation energy.

OPTION 2 Use current lig pos will start the docking simulation from the position at which the system is set up.

OPTION 3 Display run will show you the position of the ligand as it samples the conformational space during the simulation. Although this is fun to watch this significantly slows down the docking operation.

12.5. Docking Results

This section explains how to view and analyse your docking results.

12.5.1. Viewing Your Docking Results

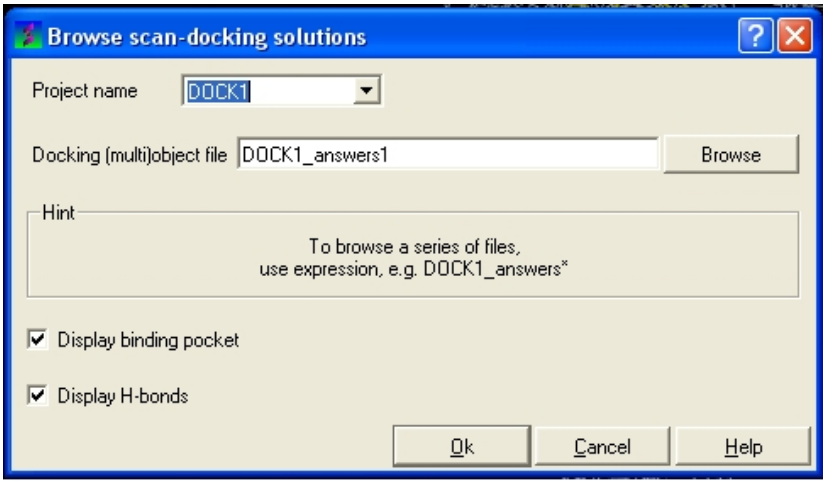
The results of the batch docking job are saved in the folling files

PROJECTNAME_answers*.ob #icm−object file with best solutions for each ligand
PROJECTNAME_*.cnf # icm conformational stack files with multiple docked conf.
PROJECTNAME_*.ou # output file were various messages are stored.

Multiple conformations accumulated during the docking of the ligand can be visualized and browsed in ICM

- Select menu Docking/Browse Stack.

The Browse scan−solutions data entry window will be displayed:



- Select the correct project name for the docking simulation results you wish to browse.
- Enter the name of the icm object file in the Docking (multi)object data entry field. This file will be called PROJECTNAME_answers*.ob .The browse button can be used to search for the correct file.

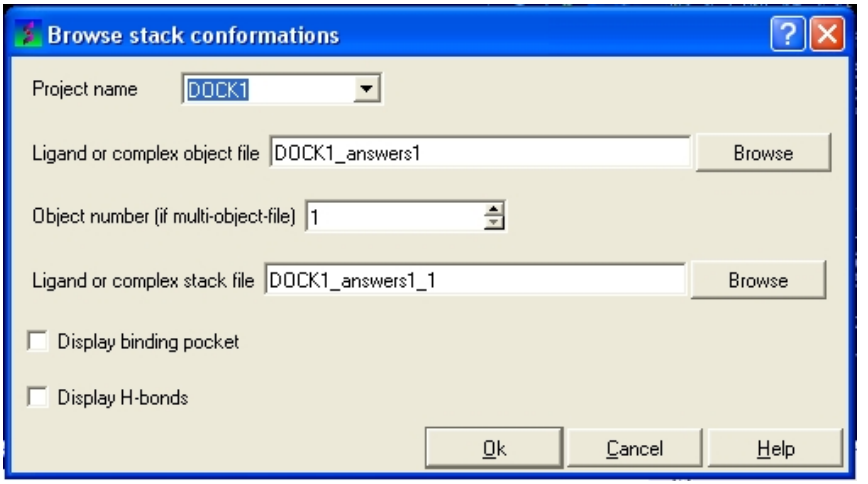
You can display the binding pocket or the H−bonds by selecting the appropriate boxes in the Browse scan−solutions data entry window (shown above).

NOTE: If you are looking at the results immediately after the docking run then all the data fields (such as Docking (multi)object and Project name)should already be correctly entered.

12.5.2. To view the multiple positions of your ligand in the docking simulation.

- Select menu Docking/Browse Stack Conformations

The Browse Stack Conformation data entry window will be displayed.



- Select the correct project name for the docking simulation results you wish to browse.
- Enter the name of the icm object file in the Docking (multi)object data entry field. This file will be called PROJECTNAME_answers*.ob .The browse button can be used to search for the correct file.
- Enter the name of the icm conformational stack files with multiple docked conformations into the Ligand or complex stack file data entry box. This file will be called PROJECTNAME1_1.cnf . The browse button can be used to search for the correct file. The second solution in the stack can be viewed by changing the number 1 at the end of the file name to 2 (PROJECTNAME1_2.cnf) and so on for each solution in the stack. You can display the binding pocket or the H−bonds by selecting the appropriate boxes in the Browse scan−solutions data entry window (shown above).

13. Virtual Ligand Screening

Virtual Ligand Screening can be used for screening as many compounds as you desire depending on the anount of computer power you have available. ICM−VLS has been successfully used by the pharmaceutical industry and academia for identifing drugs and inhibitors for a wide variety of disease.

13.1. VLS Introduction

Virtual Ligand Screening (VLS) in ICM is performed by docking a database of ligands to a receptor structure followed by an evaluation of the docked conformation with a binding−score function. Best−scoring ligands are then stored in the multiple icm−object file. The set−up of the VLS process is largely identical to the set−up for the small molecule docking simulation (see `Small Molecule Docking section`).

13.2. VLS Getting Started

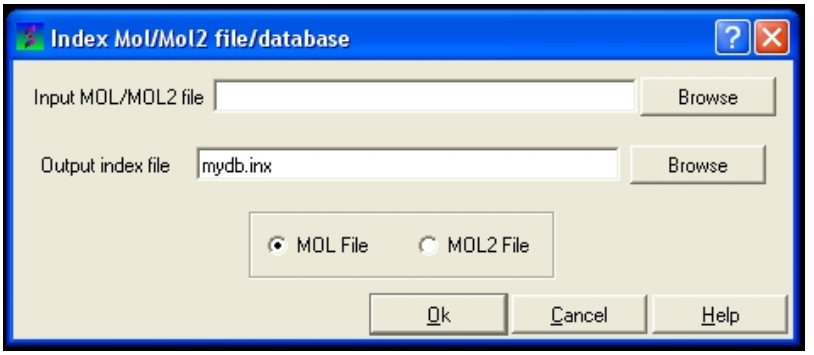
Follow the instructions in the `small molecule docking section` manual from `docking project setup option` to the `calculate maps option`. **Remember** to select the **From** Database option in the **Ligand** Setup** menu.

13.2.1. Database File Format

In most cases the ligand input file will be an SDF or MOL2 file. These files need to be indexed by ICM before they can be used in VLS runs. The index is used to allow fast access to an arbitrary molecular record in a large file such as an SDF file which in some cases contains over one million compounds.

To index an sdf file:

- Click on the menu Docking/Tools/Index Mol/Mol2 File/Database to generate the index. The following data entry box will be displayed.



- Enter the name of your Mol/Mol2 file and enter the name you wish to call your index file.

- Select whether your file is in Mol or Mol2 format.

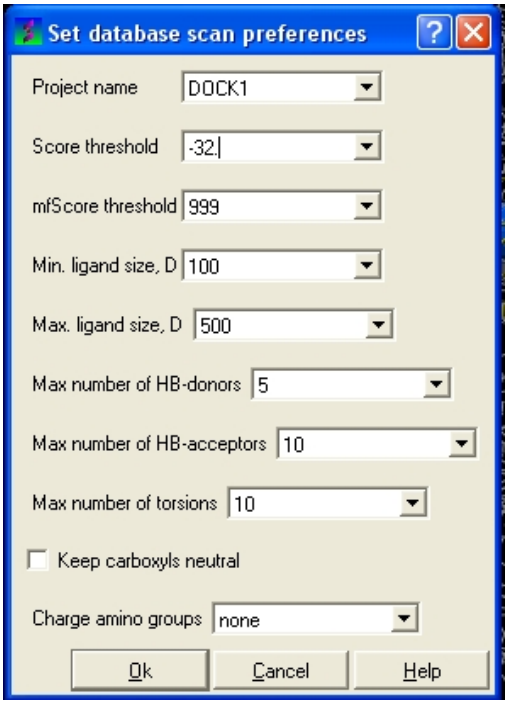
NOTE: Remember to set up the SDF/MOL2 file as a ligand source (menu Docking/Ligand Setup/From Database).

13.2.2. VLS Preferences

NOTE: It is important to setup the VLS preferences before undertaking VLS run.

VLS preferences can be setup by:

- Selecting the menu Docking/Display/Database SPreferences



Different options are available to select by clicking the down arrow next to the data entry field. These options are described here:

Score Threshold:

An important parameter of the VLS run is the score threshold. Docked conformations for a particular ligand will only be stored by ICM VLS procedure if its binding−score is below the threshold. The choice of the threshold can be done in two ways: based on the scores calculated by docking known ligands. Generally, a value somewhat above typical score observed for known ligands is a good guess. If no ligands are known, a pre−simulation can be run using ~1000 compounds from the target database. Using the resulting statistics for the scores, the threshold should be set to retain ~1% of the ligands.

Potential of mean force score:

Potential of mean force calculation (pmf) provides an independent score of the strength of ligand−receptor interaction. The pmf−parameters are stored in the icm.pmf file.

Other selection criteria:

Other selection criteria which can be changed include

Minimum/Maximum Ligand Size you wish to be screened Maximum number of H−bond donors Maximum number of H−bond acceptors Maximum number of torsions An option to keep carboxyl groups neutral An option to charge certain amino groups

13.2.3. Running VLS Jobs in PBS UNIX Cluster Environment

Before VLS jobs can be run make sure you follow the instructions in the manual entitled `Small Molecule Docking` from docking `project setup menu` to the `calculate maps menu`. Remember to select the "From database" option in the `Ligand Setup menu`. Jobs on the Linux cluster are run through PBS queuing system. Several scripts are provided to facilitate submission of vls jobs. To submit a single job, use pbs script 'pbsrun', which is a pbs wrapper for rundock qsub $ICMHOME/pbsrun −v"JOBARGS=−f 1 −t 1000 −o MYPROJECT"

NOTE: The rundock arguments go in the quotes after JOBARGS= . The qsub command is a part of PBS.

Other rundock arguments are:

−l # change the length of MC docking, default is 1.

−L # dock selected compounds from the database

−n # change the run name in the output files

−a # force docking and saving of all compounds

−s # save stack conformations

−j # dock several ligands in parallel

−o # redirect output to _from−to.ou

To submit multiple jobs, there is a simple shell script 'pbsscan' which executes multiple qsub's for database stripes: $ICMHOME/pbsscan MYPROJECT 1 6000 1000 −submits 6 jobs, 1 to 1000; 1001 to 2000 ... 5001 to 6000. Currently this script only supports default rundock arguments, copy/edit to change. The command qstat is a part of PBS and can be used to check the status of the jobs. In addition, $ICMHOME/scanstat script can be used to monitor the progress of the VLS jobs. It analyses the *.ou rundock output files. $ICMHOME/scanstat *.ou

To delete the jobs, use PBS command qdel: qdel 1234 # deletes job number 1234

13.2.4. Parallelization

If the database size exceeds several thousand compounds, it is desirable to run a number of VLS jobs in parallel to speed up calculations. Use −f and −t options of rundock to start multiple jobs on different parts of the database, e.g.

rundock −f 1 −t 10000 −o rundock −f 10001 −t 20000 −o rundock −f 20001 −t 30000 −o ..

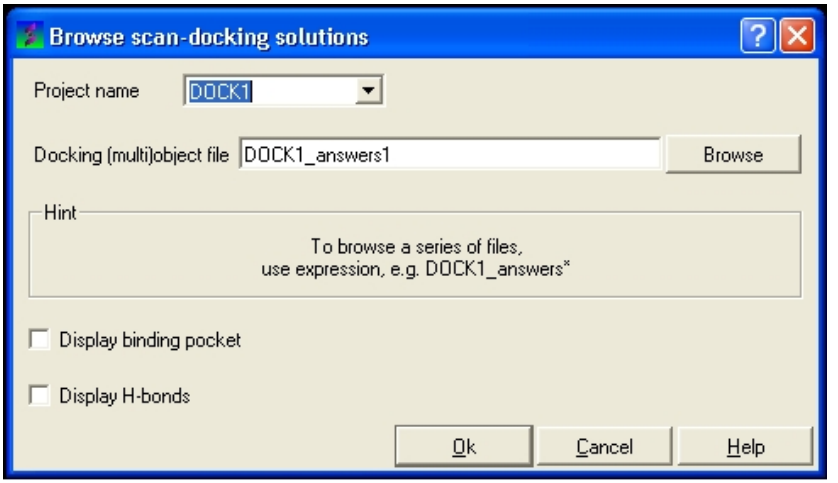
13.3. VLS Results Analysis

Once the compounds are docked, if the VLS option is installed, the procedure evaluates the score and stores it in the 'comment' of the ligand object.

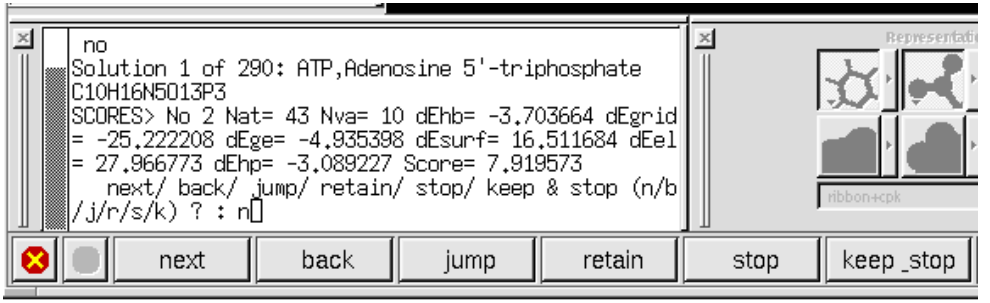
13.3.1. Viewing docked structures

To view the poses of the best energy highest scoring compounds (determined by the value in Scorethreshold in the `Preferences` table).

- Select the menu Docking/Browse Scan Solutions.
- Enter the name of the docking project.
- Browse for the appropriate object file usually called PROJECT_NAME_answers1.ob
- Check whether you wish to display the binding pocket or hydrogen bonds.
- Click OK.



You can browse the solutions by clicking the buttons at the bottom of the GUI and selecting either **NEXT(or** type 'n'), **BACK** (or type 'b'), **JUMP** (or type 'j'), **RETAIN** (or type 'r'), **STOP** (or type 's'), **KEEP_STOP** (or type 'k').



NOTE: When browsing scan answers, the SCORE>... line appears for each object viewed, containing the value of the score and it's component terms. It can also be extracted from the icm object in shell using Namex(a_1.) function, and Field() can be used to get particular component or the total: Field(Namex(a_1.) "Score=" 1). The SCORE lines also appear in the output file and can be extracted by simple unix grep command grep SCORE *.ou

NOTE: An easy way to view the socres and the compounds is to `construct a hitlist` as described later in this section.

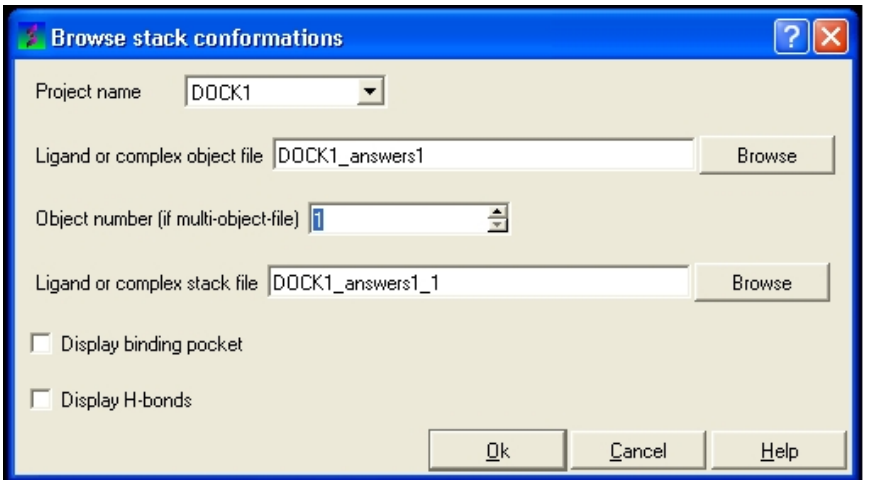
13.3.2. Viewing the conformation stack

To construct conformational stacks of the top 40 energy solutions for each ligand docked by VLS use the −s argument in the rundock procedure.

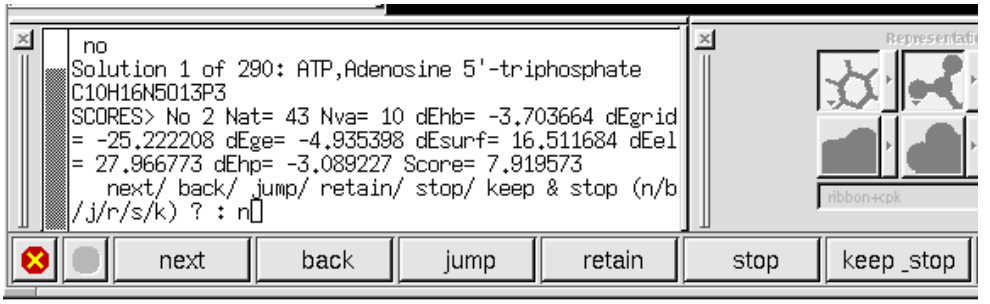
```
rundock −s
```

To view the different poses for your compound in the energy stack (*.cnf files).

- Select the menu Docking/Browse Stack Conformations
- Enter the name of the docking project.
- Browse for the appropriate object file usually called PROJECT_NAME_answers1.ob
- Enter the object file number (usually 1 unless it is a multi−object file)
- Enter the ligand stack file *.cnf
- Check whether you wish to display the binding pocket or hydrogen bonds.
- Click OK.



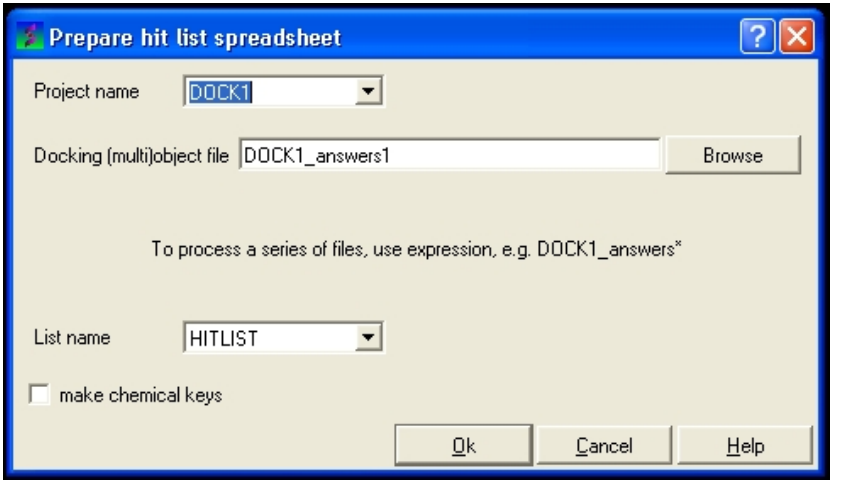
You can browse the solutions in the stack by clicking the buttons at the bottom of the GUI and selecting either **NEXT** (or type `n`), **BACK** (or type `b`), **JUMP** (or type `j`), **RETAIN** (or type `r`), **STOP** (or type `s`), **KEEP_STOP** (or type `k`).



13.3.3. constructing a HITLIST

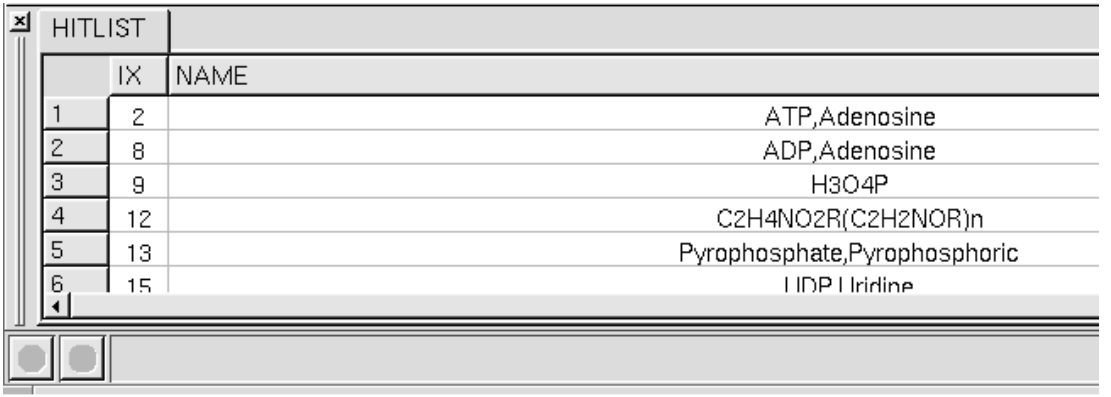
To construct a table of scores and energies for each of your docked compounds in the database:

- Select the menu Docking/Make Hit List
- Choose options (the 2D option will insert molecular drawings into the table)



- Enter the name of the docking project.
- Enter the `PROJECT_NAME_answers*.ob`
- Enter a name for the hitlist in the List name data entry box
- Select whether you wish to construct chemical keys to the compounds. This will enable distances to be calculated from the HITLIST table.
- Click OK.

A file called `HITLIST_NAME.tab` will automatically be saved and displayed



13.3.4. Viewing compounds in your HITLIST

To view compounds in your hitlist:

- Click on the compound name in the HITLIST table
- Select on the side of the table
- Display docked structures and
- Display hydrogen bonds.

The receptor and ligand will be displayed in the ICM graphical viewer.

13.3.5. Sorting the compounds in your HITLIST

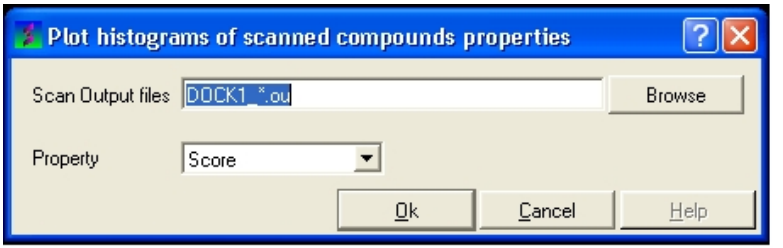
Compounds can be sorted according to their SCORE etc. See the `tables section` of this manual for more information about manipulating tables.

13.3.6. How to Plot Histograms and Scatterplots of VLS Data

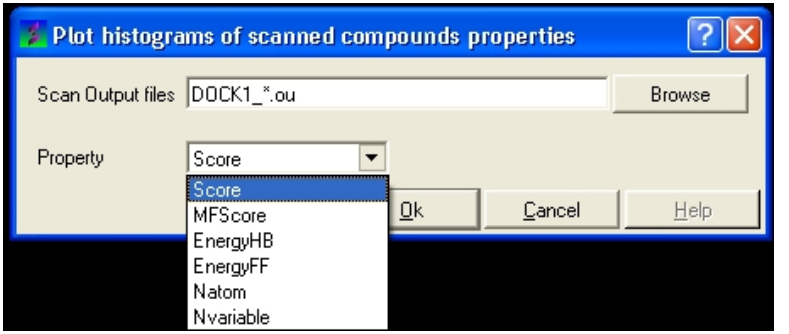
The hitlist contains many columns with numerical data. ICM can build interactive plots with the table columns (`See Tables section`). However, there are some easy to use plotting options in the docking menu which is described here.

13.3.7. To construct a histogram of your VLS data

- Select the menu Docking/Tools/Scan Results Histogram



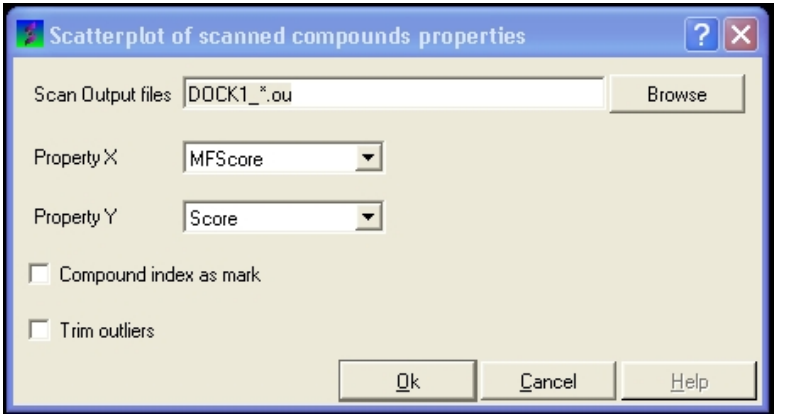
- Enter the name of the VLS output file (*.ou) you wish to construct a histogram for.
- Select which paramater you wish to plot against frequency (see below).



- Click OK and a def.eps file will be saved with a picture of your histogram.

13.3.8. To construct a scatterplot of your VLS data

- Select the menu Docking/Tools/Scan Results Scatterplot



- Enter the name of the VLS output file (*.ou) you wish to construct a scatterplot for.
- Select which paramater you wish to on the X axis.
- Select which paramater you wish to on the Y axis.
- Click OK and a def.eps file will be saved with a picture of your scatterplot.

14. Making a Movie

ICM enables users to easily make a movie. Here we will describe how to make and convert a series of frames and scenes into a movie. A movie is an excellent means of communicating results obtained in ICM such as Monte Carlo and docking simulations. The resulting movie can easily be transfered into other applications such as Microsoft Powerpoint.

NOTE: We recommend that all movie making is undertaken on the Windows platform. The Windows platform allows a series of images to be compressed into a movie file format. Unfortunately this facility is not available on other platforms such as UNIX. If you make a movie on a platform other than windows a series of picture files will be saved.

14.1. Movie Making

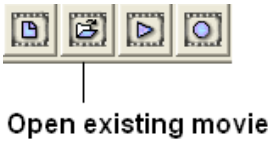
Before starting to make a movie:

1. First set up and make a directory into which you wish to store the movie.

2. Read the PDB files and objects you wish to include in the movie

A previously saved movie can be opened by:

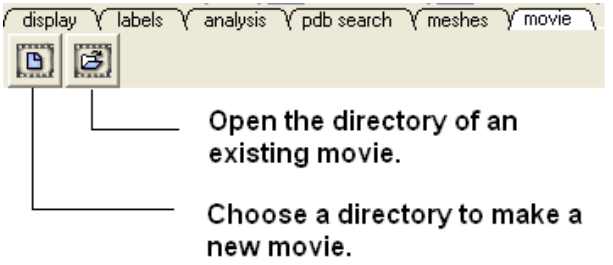
- Clicking on the movie open button shown below.



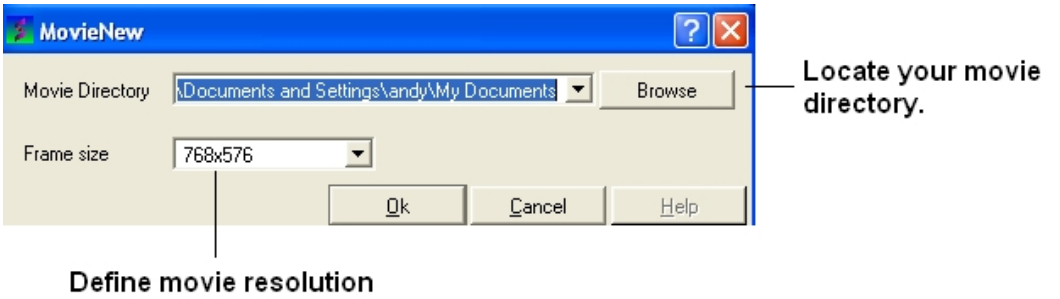
14.1.1. Movie Files and Resolution Setup

To start making a movie:

- Select the movie tab.



- Click the button to choose a new movie directory (See Figure Above).



- Browse for your movie directory.
- Select which resolution you desire for your movie by selecting the appropriate frame size.
- Click OK.

14.1.2. Defining a Movie Scene

The first step is to make the first scene.

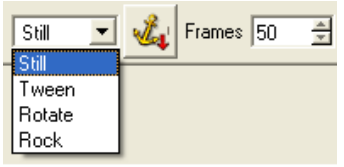
There are four choices of scene – `still`, `tween`, `rotate` or `rock`.

Select which scene you would like to start your movie with and follow the instructions for whichever one of the four scenes you choose.

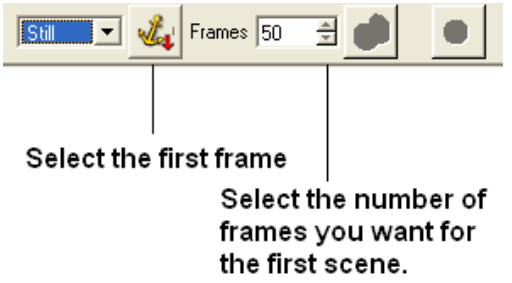
14.1.3. Still

To make a still scene:

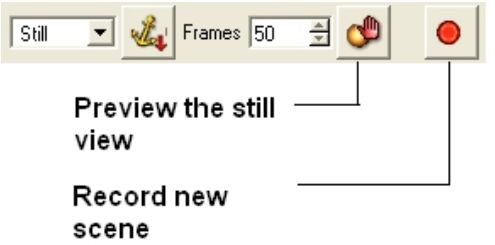
- Select the still option from the drop down list shown below.



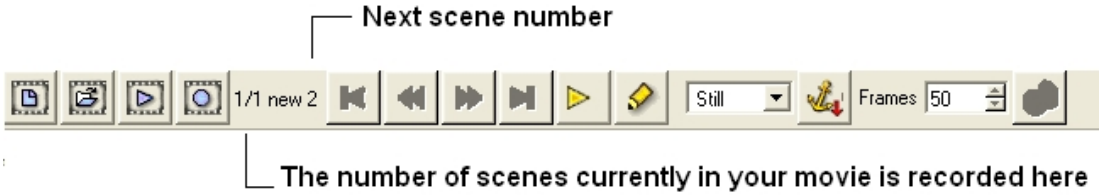
- Move the object to the starting position.
- Click on the "Define first view" button (see figure below).
- Type in the data entry box how many frames you desire for the scene.



- If you wish to preview the still view click the "preview" button (see figure below)
- If you are happy with the scene, click the red record button.



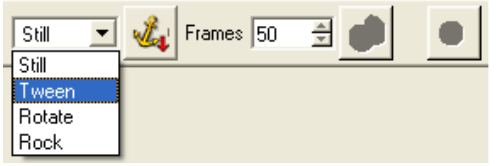
The number of scenes you have recorded so far is displayed in the video panel (see figure below).



14.1.4. Tween

To make a "tween" scene (moving your object from one point to another):

- Select the tween option from the drop down list shown below.



- Move the object to the starting position.
- Click on the "Define first view" button (see figure below).
- Type in the data entry box how many frames you desire for the scene.

- Move the object to the place you wish it to be translated to..
- Click on the "Define second view" button (see figure below).

- Click on the button shown below to preview the interpolation between the two views.

- If you are happy with the scene, click the red record button.

The number of scenes you have recorded so far is displayed in the video panel (see figure below).

14.1.5. Rotate

To make a "rotation" scene:

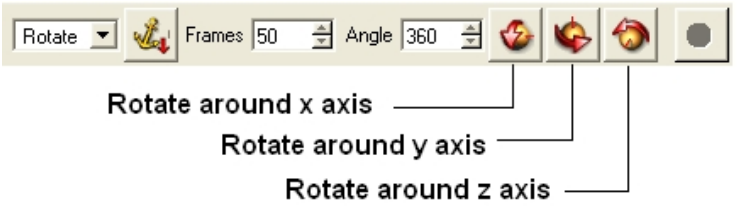
- Select the rotate option from the drop down list shown below.

- Move the object to the starting position.
- Click on the "Define first view" button (see figure below).
- Type in the data entry box how many frames you desire for the scene.

Now you have **three** preview options

1. Rotate around the x axis.
2. Rotate around the y axis.
3. Rotate around the z axis.

The buttons for each of the three options are shown below:



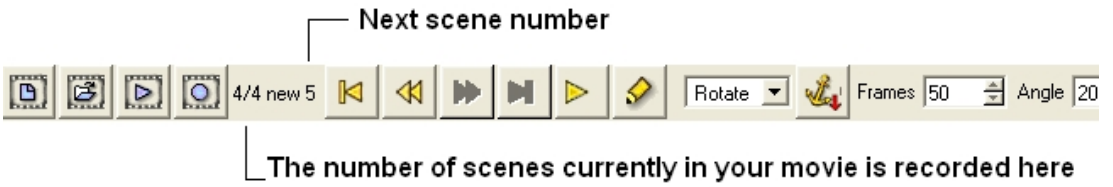
- Enter by how many degrees you wish your object to be rotated.
- Click one of the three preview options − rotate x, rotate y and rotate around the z axis.

NOTE You can play with and change the number of degree option and which kind of rotation as many times as you wish until you are satisfied with your scene.

Once you are satisfied with your scene:

- Click the red record button.

The number of scenes you have recorded so far is displayed in the video panel (see below).



14.1.6. Rock

To make your object perform a "rock" motion:

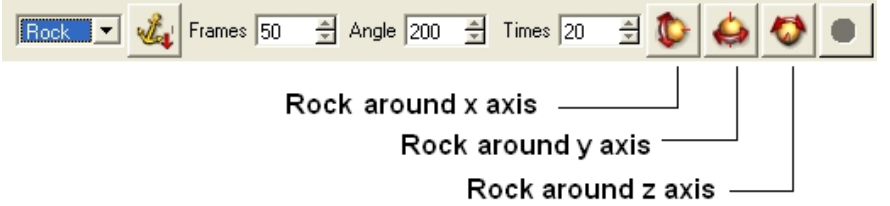
- Select the rock option from the drop down list shown below.

- Move the object to the starting position.
- Click on the "Define first view" button (see figure below).
- Type in the data entry box how many frames you desire for the scene.

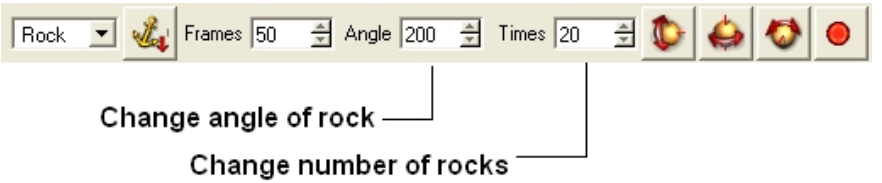
Now you have **three** preview options

1. Rock around the x axis.
2. Rock around the y axis.
3. Rock around the z axis.

The buttons for each of the three preview options are shown below:

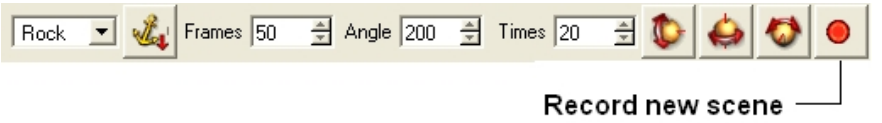


To change the angle and the number of times the rock occurs, enter the desired numbers in the data entry boxes shown below.

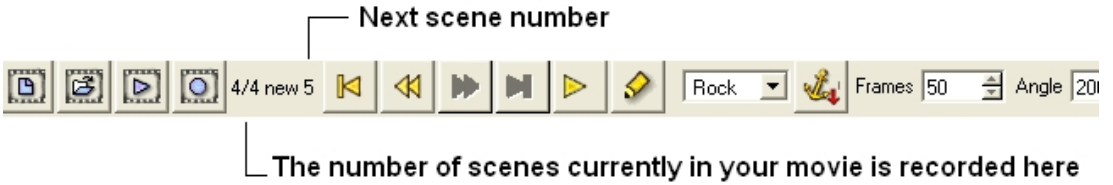


Once you are satisfied with your scene:

- Click the red record button.



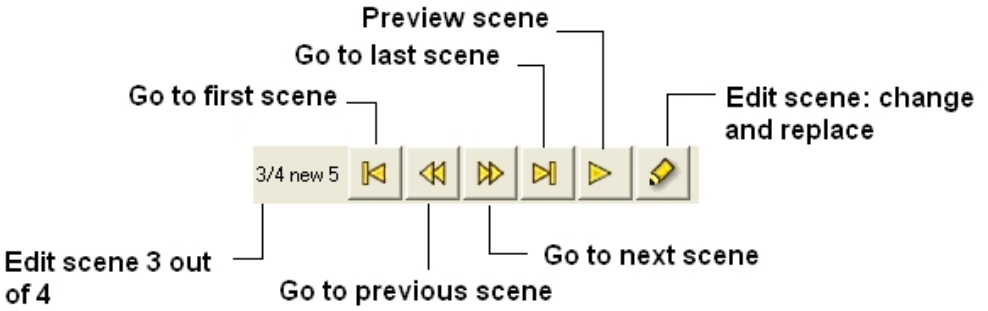
The number of scenes you have recorded so far is displayed in the video panel (see below).



14.2. Edit a Movie

To edit a movie:

- Select the scene you wish to edit by using the buttons shown below. The scene number is displayed in the movie panel.



- Click the "Edit scene: Change or replace scene" button.
- Make changes to the scene as described in the `Movie Making` section of this manual.

14.3. Preview and Export

To preview a movie:

- Click on the preview movie button shown below.

To export a movie to a series of png,gif,tiff files or an avi file:

- Click on the export movie button shown below.

- Select which format you wish to save your movie.

If you select avi a window as shown below will be displayed:

- Select which windows compression software you wish to use to make the movie.

15. Tutorials

15.1. Lesson 1: Molecular Graphics and the Graphical User Interface Tutorial

This lesson is divided into seven parts taking you through the basics of how to use the graphical user interface and the many ways in which molecules can be displayed and saved in ICM.

The ICM graphical user interface offers a rich graphical environment in which molecular images can be constructed and saved. High quality annotated structures and alignments can be easily prepared for publication purposes with the minimum amount of effort. Display your molecules in wire, CPK, ballworm, ribbon, accessible surface, transparent molecular surface, perspective, depth cueing, smooth and rugged solid surfaces. Use both hardware and side−by−side stereo. Save and print a screen image as a compact vectorized postscript file (also in stereo) in addition to a compressed bitmap. You can painlessly create movies featuring molecules dressed in solid representations such as CPK, smooth molecular surface, ball−and−stick read, display, reshape and write any 3D object in the Wavefront format. ICM molecular graphics has been used in television commercials and generated award winning molecular art.

The lessons described here cover the following subjects:

- Loading a structure into ICM and saving an ICM Project.
- Making new structures and using the connect tool.
- Displaying different structural representations.
- Basic selections.
- Surfaces and clipping tools.
- Sphere selections and electrostatic representations.
- Saving an image.

15.1.1. Lesson 1a: Loading a PDB structure and saving an ICM Project

Objective

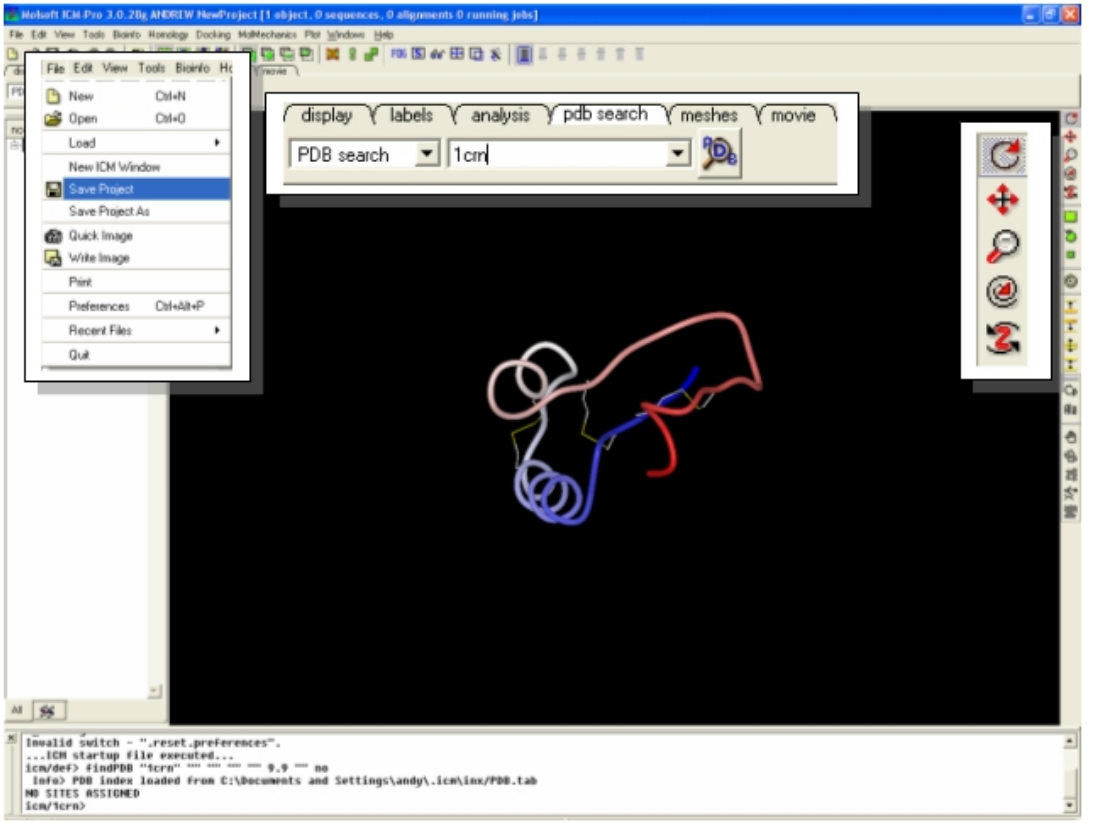
To load a structure into ICM and save as an ICM Project.

Background

The save ICM project capability is a very helpful tool and will save you time. Saving a project will allow you to quit from ICM and then return to the exact set−up at which you left off at a later date. A complete history of your ICM actions will be saved so that you can pick up exactly where you finished on your previous ICM session. Also, here, we briefly introduce the concept of loading a PDB file into ICM however this is covered in more depth in Lesson 2.

Instructions

1. Load the PDB file 1CRN using the PDB Search tab.
2. Move the structure around using the rotate and zoom options on the right hand side of the GUI.
3. Save the structure in an ICM Project . File/Save Project



Manual References (Web Links)

`Saving an ICM Project`

`Loading a PDB Structure`

15.1.2. Lesson 1b: New Molecules and Connect

Objective

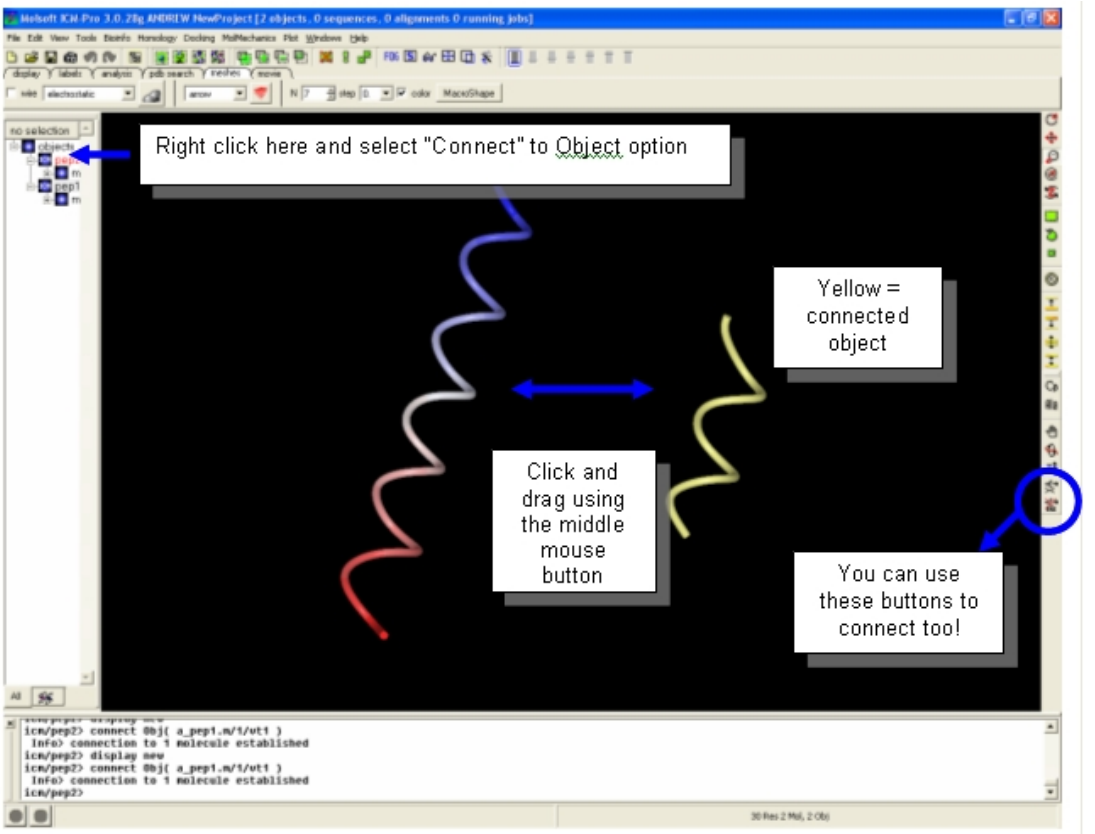
To make two new peptide structures and separate them using the connect function.

Background

The lesson described here will help you become familiar with constructing a new object, working with the ICM Workspace, and the connect option. The connect option connects selected molecules to the mouse for independent rotation (by the LeftMouseButton) and translation (by the MiddleMouseButton) with respect to the original coordinate frame.

Instructions

1. File>New> Peptide
2. Object name = pep1; One Letter Code = WAAALPAAR; N−terminus = nter; C−terminus = cooh; Click OK.
3. Repeat for second peptide but with new name pep2 and One Letter Code= WAAALPAARWWWWWWPPPPPP
4. Right click in the ICM Workspace and select the connect option. Drag the two peptides apart.



Notes and things to try

- The pep1 structure is now independent of pep2. Use your mouse to move pep1.
- To release pep1 from the connect mode hit the .Esc. key on your keyboard.
- Other ways of connecting to an object include right clicking on the object and selecting connect OR using the buttons on right hand side of the GUI.

Manual References (Web Links)

`Constructing New Objects`

`Connect and Move`

15.1.3. Lesson 1c: Graphical Representations

Objective

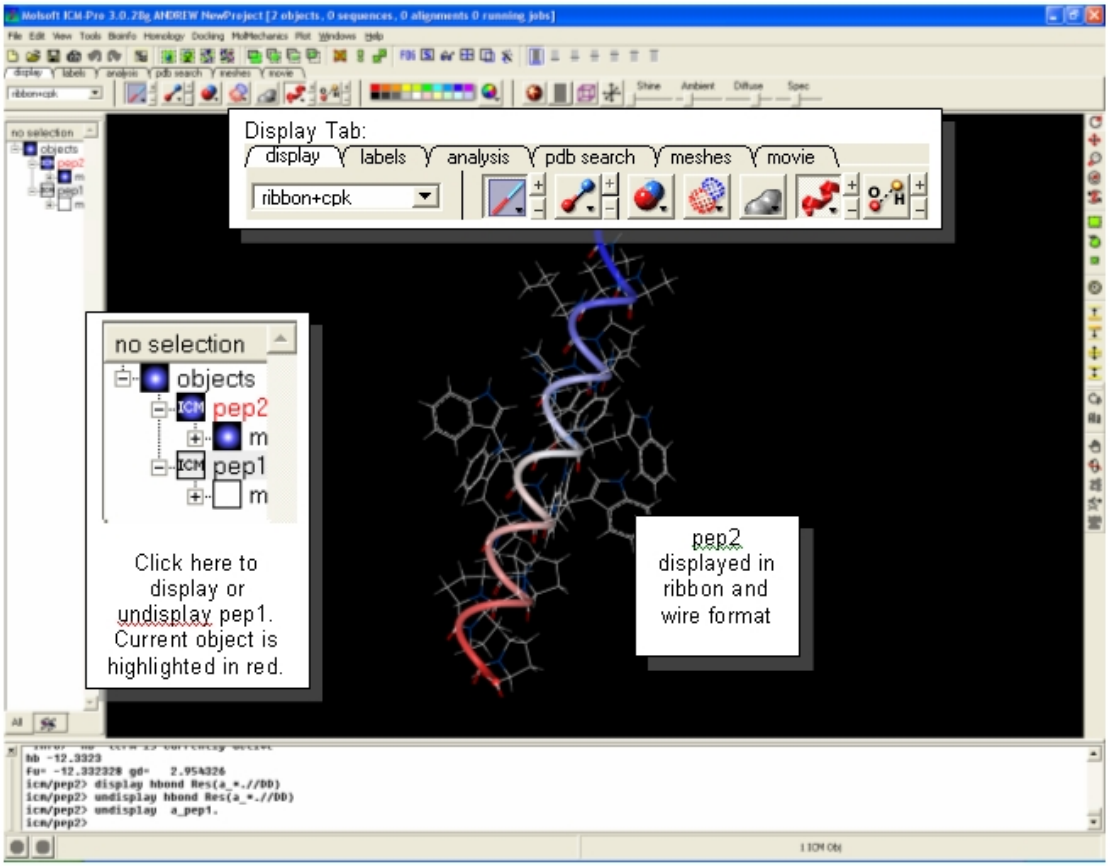
To display pep2 in ribbon and wire representation.

Background

There are many ways to represent structures in ICM. In this lesson you will become familiar with the ICM Display tab and the ICM Workspace.

Instructions

1. Undisplay pep1 using the ICM Workspace.
2. Select the display tab.
3. Select wire representation from the Display tab.



Notes and things to try

- The current object which in this case is pep2 is highlighted in red in the ICM Workspace display.
- Graphical representations such as ribbon and wire can be .toggled. on and off by clicking the button in the Display Tab.
- Try holding down on the button and a number of display options will become available.
- Try displaying pep2 in different representations using the buttons in the Display Tab.

Manual References (Web Links)

`Workspace Panel`

`Structural Representations`

15.1.4. Lesson 1d: Basic Selections

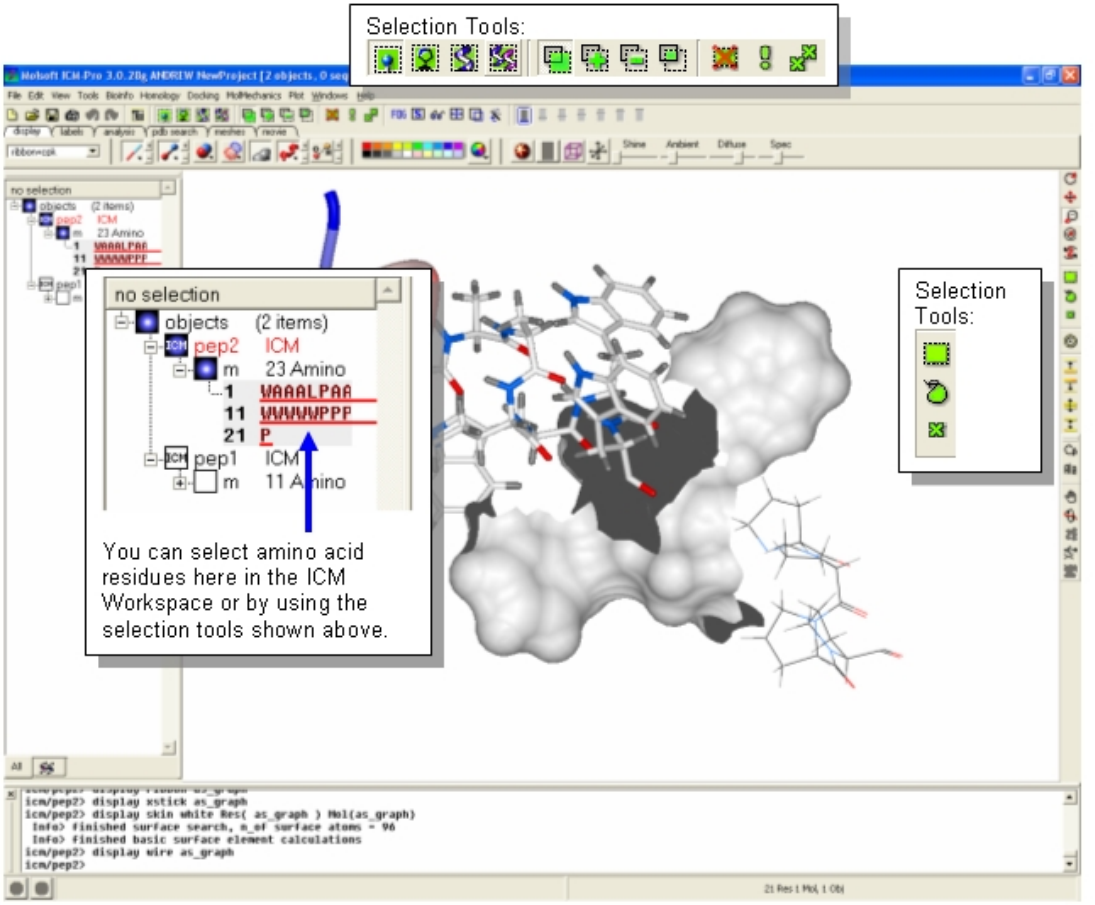
Objective To display specific regions of pep2 in different representation.

Background

For many ICM applications it is necessary to be able to select certain regions of a structure. Here you will become familiar with the basic selection tools.

Instructions

Use the selection tools (lasso, box, atom) or the ICM Workspace to display pep2: residues 1 to 5 as ribbon; 6 to 11 as xstick; 12 to17 as skin and 18 to 21 as wire.



Notes and things to try

- The basic selection tools (box, lasso and atom pick) are shown on the right of the graphical user interface.
- More advanced selections can be made with the selection tools at the top of the graphical user interface.
- Amino acid residues can be selected from the ICM Workspace.

Manual References (Web Links)

`Making Selections`

15.1.5. Lesson 1e: Surfaces and Clipping Tools

Objective

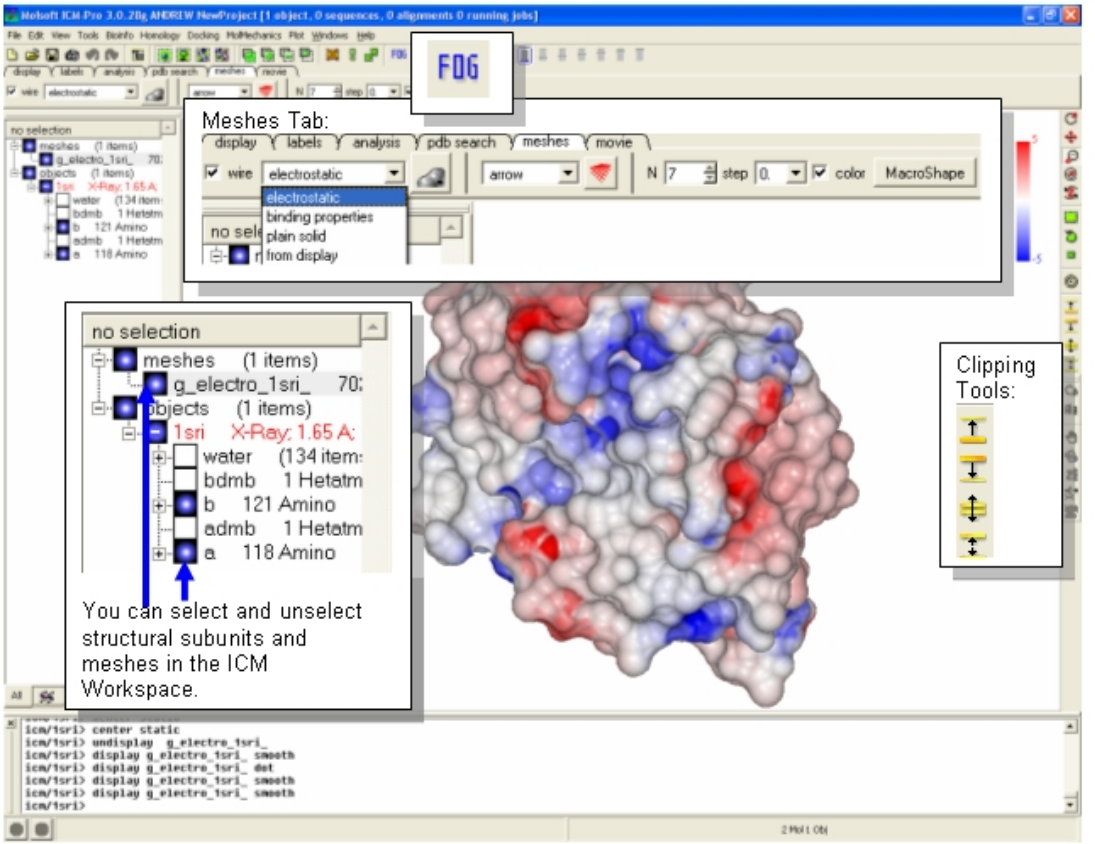
To display surface and meshes and then to cut them away using clipping tools.

Background

This lesson teaches electrostatic surfaces and the use of clipping planes. The electrostatic potential can be projected on a molecular surface for the identification of possible binding sites. You can cut away the surface using clippings planes of which there are two: the front one and the back one. The front clipping plane determines the beginning of the visible part of your object, while the back clipping plane is where the visible parts ends. The planes can be moved independently, or in concert.

Instructions

1. File/Open/1sri
2. Display the molecule in ribbon mode (see lesson 1c).
3. Select the Meshes Tab.
4. Display the surface of the molecule by electrostatic properties.
5. Use the clipping tools to slice away the surface and reveal the ligands and ribbon display.



Notes and things to try

- Try cutting away the mesh to reveal the ribbon structure and ligands using the clipping tools.
- Right clicking on the meshes section of the ICM Workspace allows other options to be accessed.
- Try using the fog option.

Manual References (Web Links)

`Structural Representations`

clippingtools{Clipping Tools}

15.1.6. Lesson 1f: Spherical Selections

Objective

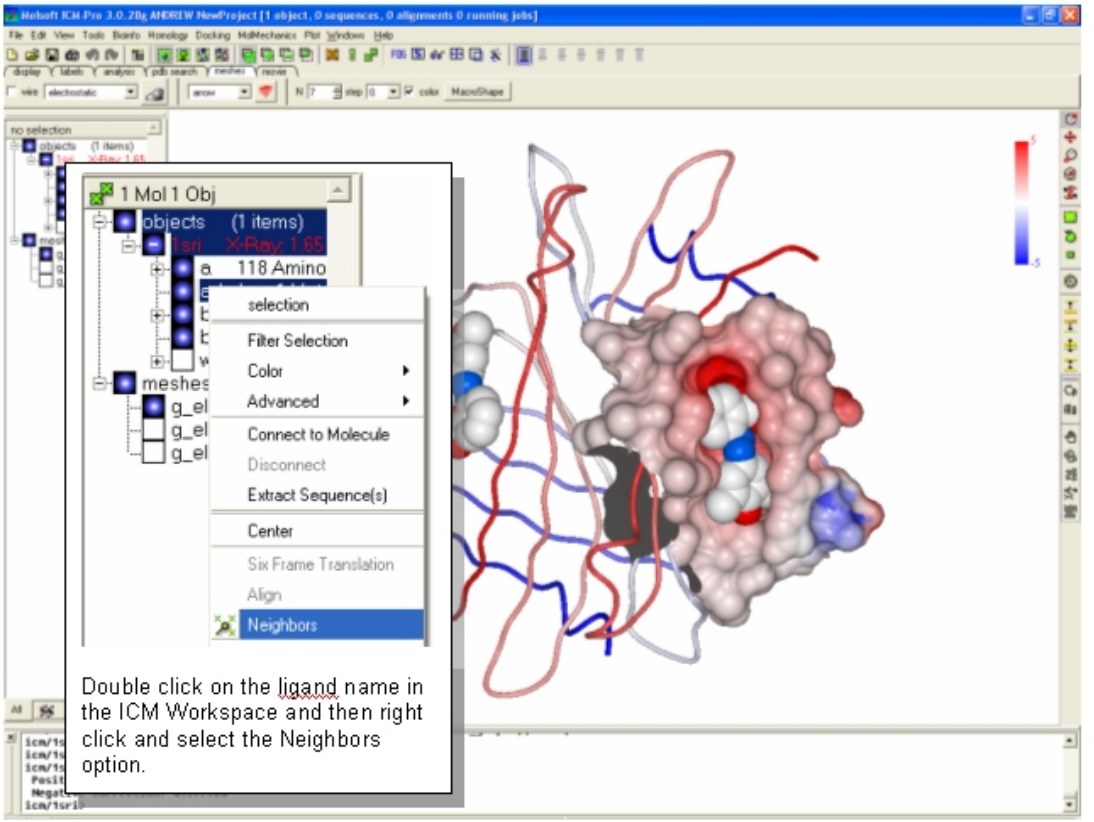
Display the electrostatic properties surrounding the ligand binding site.

Background

A more advanced selection tool is described here whereby a sphere of a specified radius is made around an atom(s). This is useful for making selections around a ligand binding pocket for example.

Instructions

1. This lesson still uses the 1sri structure from the previous lesson. Undisplay all meshes from the previous lesson by clicking on the blue square in meshes section of the ICM Workspace.
2. Double click on the ligand name .admb. in the ICM workspace and it will be highlighted in blue.
3. Right click on the name and select the Neighbors option.
4. Enter 7.5 Å for the sphere radius selection. Green crosses represent selected residues
5. Display the electrostatic properties as described in the previous lesson.



Notes and things to try

- The "Neighbor" option selections can be made in other objects loaded into ICM by selecting the drop down menu in the .type. data entry box.

Manual References (Web Links)

`Selecting Neighbors`

15.1.7. Lesson 1g: Saving an Image

Objective

To save an image.

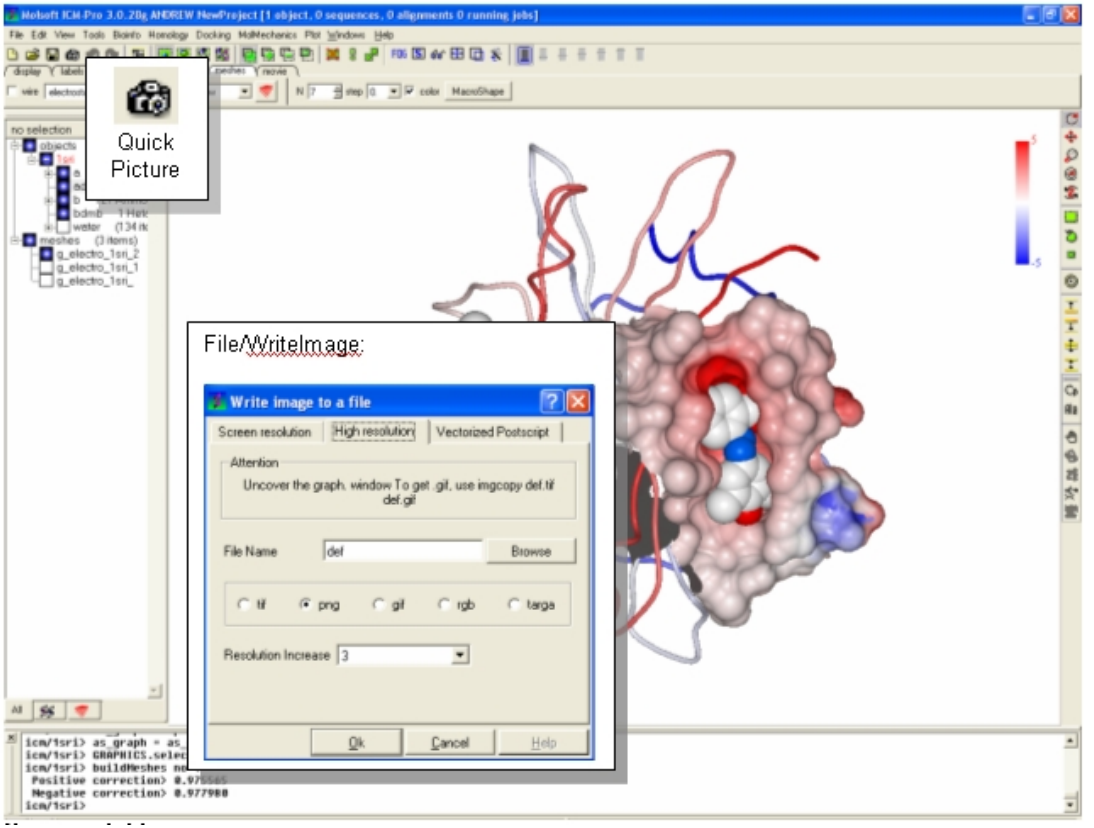
Background

There are two ways to save a publication quality image in ICM. A quick one button click approach or a more advanced approach which allows many parameters to be changed.

Instructions

1. Quick way: Click on the camera button and a default PNG image will be saved.

2. Not so quick way but just as effective: Select File/WriteImage and a number of different resolution and file type options are available.



Notes and things to try

- The quick image approach using the camera icon will save a high quality image with a default name icm.png and then subsequent pictures taken will be numbered sequentially icm1,2..n. The resolution of this image is controlled in the preferences section of ICM. Flie/Preferences

- When saving a high resolution image we recommend using a resolution increase of 3 as shown above.

- Images can be saved in a variety of formats by using the WriteImage option.

Manual References (Web Links)

`Making a Quick Picture`

`Writing an Image`

15.2. Lesson 2: Sequence−Structure and Annotation

Overview

This lesson is divided into six parts taking you through the basics of PDB structures, sequence analysis and alignments.

Topics covered include:

- Loading a PDB structure.
- Converting a PDB structure into an ICM object.
- Pairwise sequence alignment.
- Drag and drop and the ICM alignment editor.
- Multiple sequence alignment.
- Box, shade and annotate an alignment.

Background

ICM provides state of the art sequence analysis, alignment and alignment editing tools. Many sequence analysis tools can be found in the Bioinfo menu.

If you intend to use ICM alignment tools you can customize your sequence alignments in a number of ways:

- Coloring according to a number of different consensus schemes.
- Customizing your own consensus tables.
- Shading areas of interest.
- Boxing areas of interest.
- Adding comments to an alignment.
- Saving an alignment as a high quality image for publication.

- Displaying and analyzing phylogenetic trees.
- Direct selection from the alignment to the 3D object.

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.

15.2.1. Lesson 2a: PDB Searching

Objective

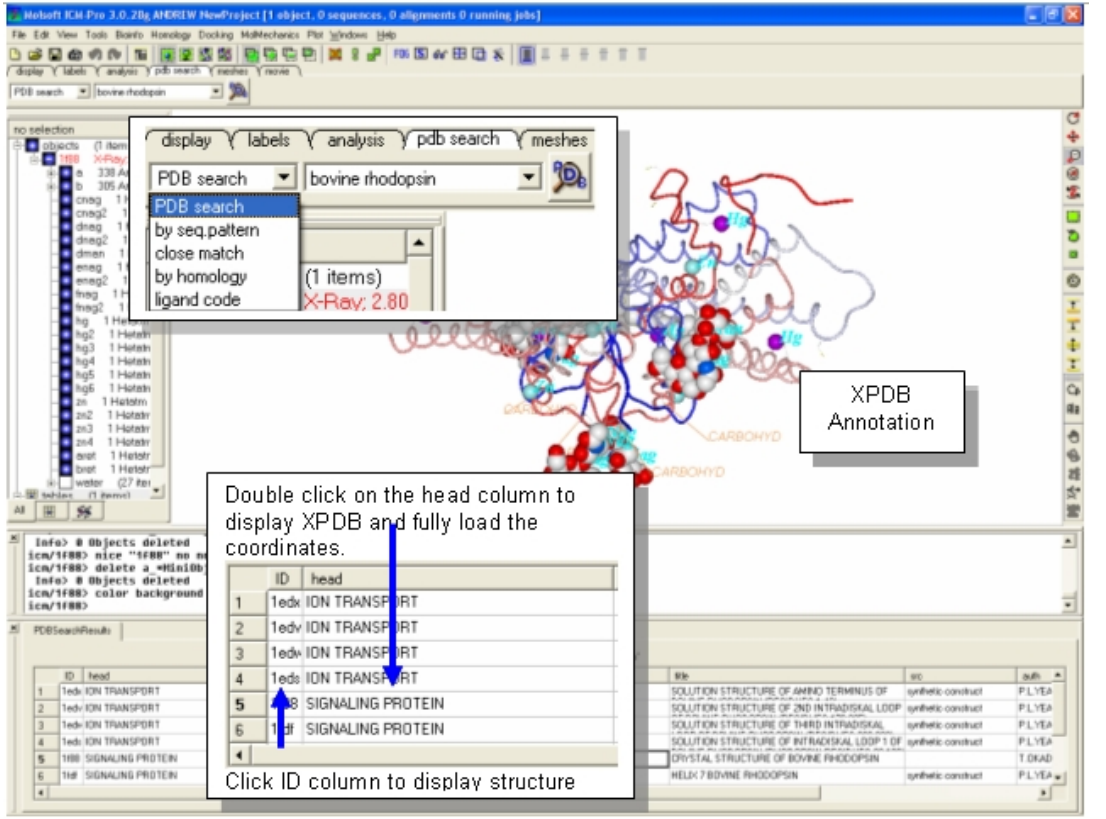
To display the crystal structure of a G−protein coupled receptor.

Background

Using ICM it is easy to quickly search and download PDB files using the .pdb search. tab.

Instructions

1. Click on the PDB Search Tab
2. Type .bovine rhodopsin. into the search box and click the button next to it. A table of hits will be displayed at the bottom of the GUI.
3. Click on the ID field of structure 1F88 to display the structure.
4. Double click on the head field to display the XPDB structure and fully download the PDB coordinates.



Notes and things to try:

- Try searching for a PDB file by sequence or homology. Use the drop down menu next to the PDB search box to define which kind of search you are undertaking.
- XPDB is a unique database developed by Molsoft which displays SWISSPROT annotation onto protein structures.
- In order to fully transfer the coordinates from the PDBSearchResults table you need to double click or else the PDB will be highlighted as a TempMinObject. Alternatively, right click menu in ICM workspace gives an option .Read Full PDB Entry.

Manual References (Web Links)

`Finding a PDB Structure`

15.2.2. Lesson 2b: Converting a PDB File into an ICM Object

Objective

To convert a PDB file into an ICM object.

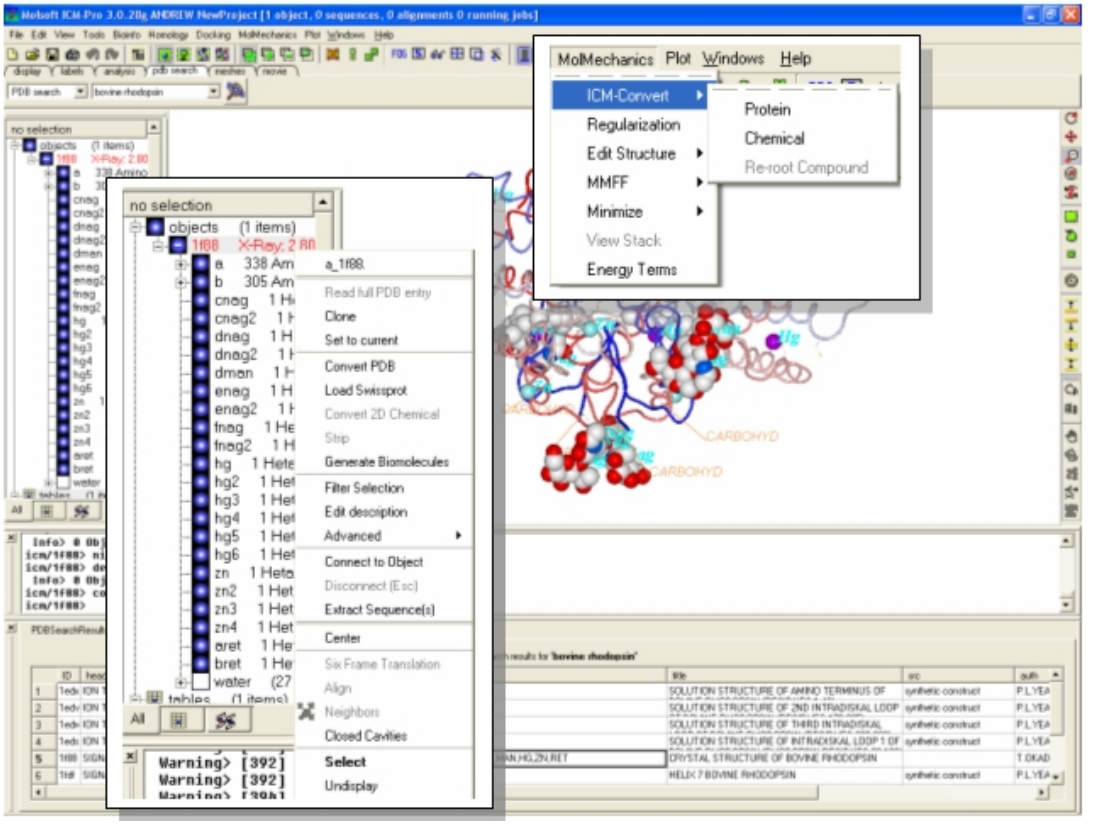
Background

Sometimes it is necessary to have a PDB file in the form of an ICM molecular object. For example, it's a convenient way to list and/or to change a torsion angle (or a series of them). It is also necessary to convert PDB files into ICM objects for ICM functions such as docking.

Instructions

To convert a PDB file into an ICM object:

- Right click on the PDB file name in the ICM Workspace and select Convert PDB. OR Select MolMechanics/ICM−Convert/Protein



Notes and things to try:

- Within the right click menu there are many other useful options such as: clone− which copies the current object; set to current − if multiple structures are loaded you can set this object to be the current one; Extract Sequence(s) . extracts the sequence of the whole object or the subunit depending where you click. Experiment with some of these options.
- The ICM workspace will tell you whether a structure is an X−Ray or an ICM object.

Manual References (Web Links)

`Converting PDB files into ICM Objects`

15.2.3. Lesson 2c: Pairwise Alignments

Objective

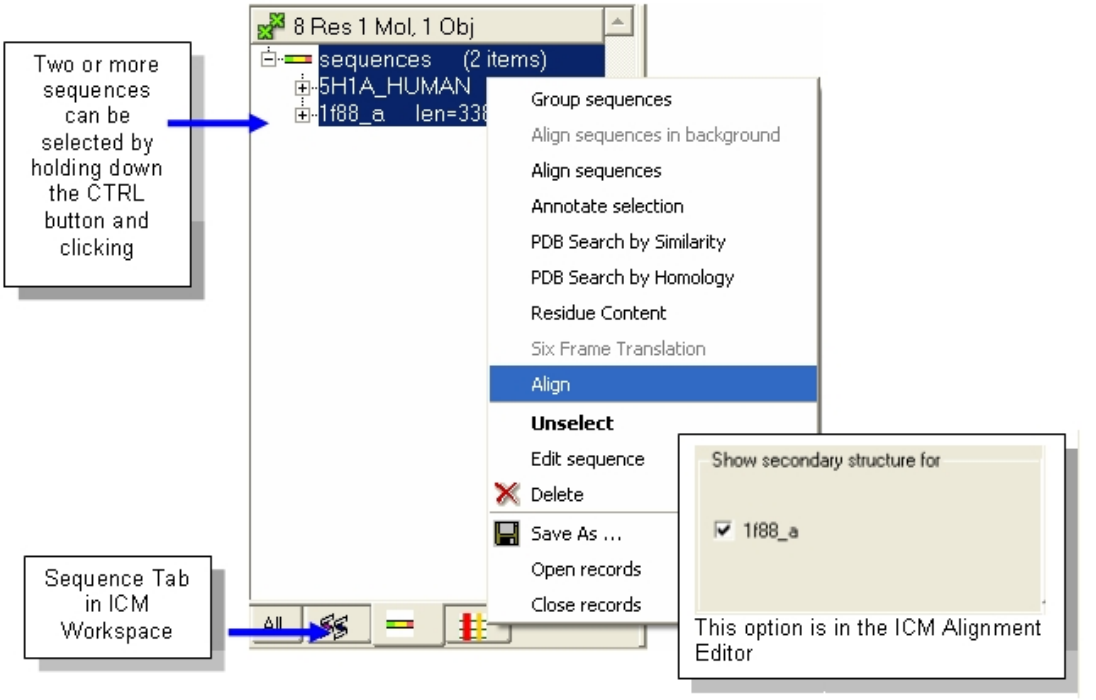
To perform a pairwise sequence alignment between rhodopsin and a GPCR sequence.

Background

Here you will become familiar with sequence manipulation and pairwise alignment.

Instructions

1. Read the PDB structure 1F88
2. File/Load/Swissprot and enter 5H1A_Human (human serotonin GPCR).
3. Now we need to extract the sequence data from subunit a of 1F88. Right click on the 1F88 subunit .a. and select the .Extract Sequences. option..
4. Click on the green/yellow/red sequences tab at the bottom of the ICM Workspace and you will see your two extracted sequences aligned.
5. Double click on one of the sequences, hold down the Ctrl key and click on the other . both sequences should now be highlighted in blue.
6. Right click on them and select Align Sequences. The alignment should be displayed in the alignment editor.
7. Check the box under the heading .Show secondary structure for. in the ICM Alignment Editor. You may have to scroll down to see this option). The secondary structure of 1f88 is then shown in the alignment.



Manual References (Web Links)

`How to Align Two Sequences`

`Alignment Editor`

15.2.4. Lesson 2d: Sequence Drag and Drop and the Alignment Editor

Objective To add another GPCR sequence to the alignment made in lesson 2c and to improve the alignment within the first helix using the ICM alignment editor.

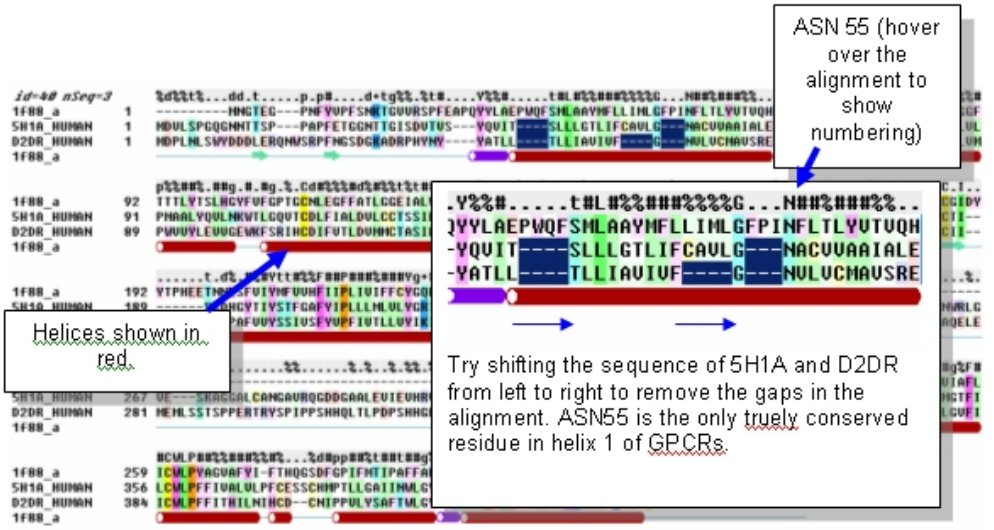
Background Here you will become familiar with sequence .drag and drop. and the ICM Alignment Editor.

Instructions

1. File/Load/Swissprot and enter D2DR_HUMAN (Human Dopamine GPCR)
2. Click on the D2DR_HUMAN sequence in the ICM Workspace and then drag it and drop it into the ICM Alignment. A new alignment will be formed with the 3 sequences (1f88_a,

5H1A_Human and D2DR_HUMAN).

3. You may need to restore the secondary structure display. Each red bar indicates a helix. There should be 7 in total.



Notes and things to try:

- Helix 1 in GPCRs are notoriously hard to align to the only currently available template structure (bovine rhodopsin . 1f88). The ASN55 (is the only fully conserved residue in helix 1. Shift the sequence of 5H1A and D2DR along to remove the gaps in this helix. To move a block of sequences there needs to be a gap in front. To do this click and drag over the block and they will be highlighted in blue. Use the right and left arrow cursors to move.

- Try hiding gaps using the menu displayed when right clicking on the Alignment Editor.

Manual References (Web Links) `Alignment Editor` `Align Multiple Sequences`

15.2.5. Lesson 2e: Multiple Sequence Alignments

Objective To perform a multiple sequence alignment.

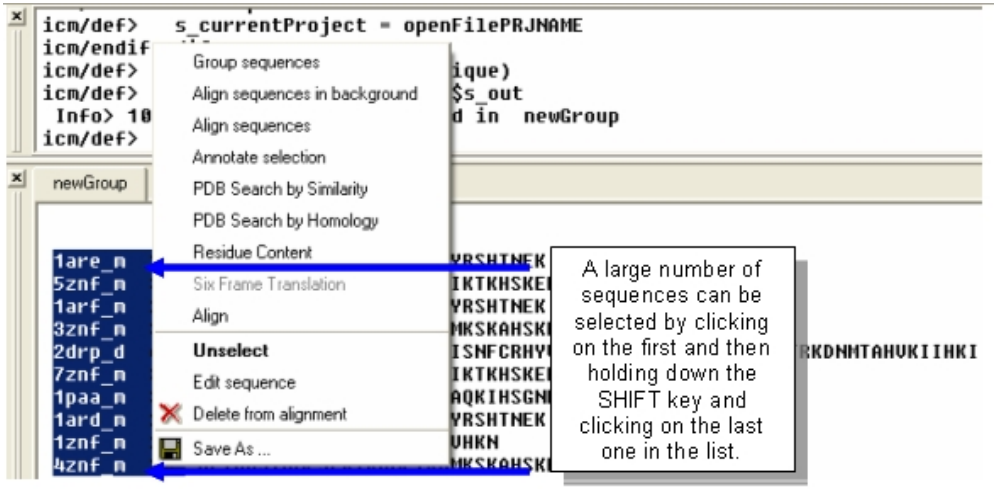
Background

Here you will become familiar with grouping sequences in the ICM Workspace and performing an alignment.

Instructions

1. Select the sequences from lesson 2d (selected=highlighted blue)
2. Right click and select delete.
3. File/Open/zincFing.seq (sequence format files needs to be specified to locate the zincFing.seq file)
4. Select the sequences tab at the bottom of the ICM Workspace.
5. Click on the first sequence and then the last whilst holding the SHIFT key. All the sequence names should be highlighted in blue.
6. Right click on the selected sequences and select the Group Sequences option.
7. The grouped sequences will be displayed at the bottom of the GUI.
8. Right click on the sequence name and select Align Sequences.

The alignment will be displayed at the bottom of the graphical user interface.



Notes and things to try:

- If you have many sequences in your multiple alignment then the option to run the alignment in the background can be chosen.
- Multiple sequence alignments can also be undertaken by choosing Bioinfo/Multiple Sequence Alignment.
- Try some other sequence manipulations at this point such as searching a sequence for homology against the PDB direct from the ICM Workspace.

- The next lesson describes some of the actions you can perform using the ICM Alignment Editor.

Manual References (Web Links) `Alignment Editor` `Align Multiple Sequences`

15.2.6. Lesson 2f: Boxing Shading and Annotating an Alignment

Objective

To box, shade and annotate an alignment.

Background

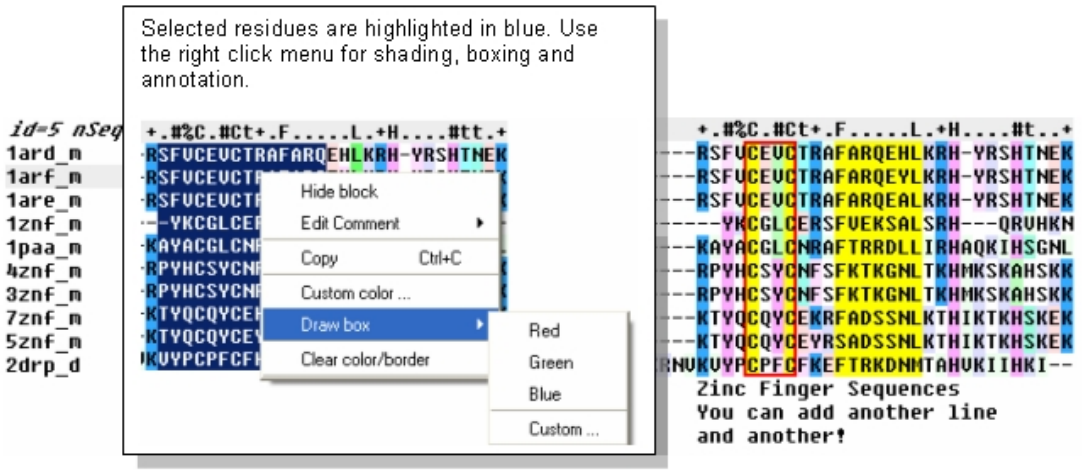
To become familiar with the ICM Alignment Editor.

Instructions

1. First let us place a red box around the conserved cysteine residues. To do this click and drag over the residues in the alignment. Selected residues will be highlighted in blue. Right click on the selected residues and select .Draw Box. and select a color.

2. Now try to shade a region of your alignment. Select the residues as before. Right click on the selection and select Custom Color.

3. Now try annotating the alignment. Select the residues in the alignment in the same way as before. Right click on the selection and select Edit Comment / Line 1. Type the text in the box provided under the alignment. Note the length of text is limited by the length of the selection you made.



Notes and things to try:

- Try saving the alignment as an image. To do this, remove any blue selection and then right click and a menu is displayed. Select save as image there are three alignment style formats to choose.
- Try searching in the alignment for a particular sequence string using the right click menu.

Manual References (Web Links)

`Alignment Editor`

15.3. Lesson 3: Homology and Modeling Tools

Overview

Lesson 3 is divided into seven parts (a−g) taking you through the basics of protein modeling.

Lesson 3a − Building a homology model.

Lesson 3b − Linked alignments and structures.

Lesson 3c − Protein health and regularization.

Lesson 3d − Crystallographic symmetry.

Lesson 3e − Crystallographic neighbors.

Lesson 3f − Superimpose structures.

Lesson 3g − Protein folding and structure prediction.

Background

ICM has an excellent record in building accurate models by homology. The procedure will build the framework, shake up the side−chains and loops by global energy optimization. You can also color the model by local reliability to identify the potentially wrong parts of the model.

ICM also offers a fast and completely automated method to build a model by homology and extract the best fitting loops from a database of all known loops. It just takes a few seconds to build a complete model by homology with loops. Some selected publications related to modeling and structure determination are listed here.

Abagyan, R.A., and Totrov, M.M. (1994). Biased Probability Monte Carlo Conformational Searches and Electrostatic Calculations for Peptides and Proteins. J. Mol. Biol., 235, 983−1002

Cardozo, T., Totrov, M., and Abagyan, R. (1995). Homology modeling by the ICM method. Proteins: Structure, Function, Genetics, 23, 403−414

Abagyan, R., and Totrov, M. (1999). Ab initio folding of peptides by the optimal−bias Monte Carlo minimization procedure. Journal of Computational Physics, 151, 402−421

Maiorov, V.N., and Abagyan, R.A. (1997). A new method for modeling large−scale rearrangements of protein domains. Proteins, 27, 410−424

Schapira, M., Totrov, M. and Abagyan, R. (2002). Structural Model of Nicotinic Acetylcholine Receptor Isotypes Bound to cetylcholine and Nicotine. BMC Structural Biology 2:1

ICM also provides powerful tools for determining crystallographic symmetry and neighbors which allows the biological environment of a protein to be viewed and understood.

15.3.1. Lesson 3a: Homology Modeling

Objective

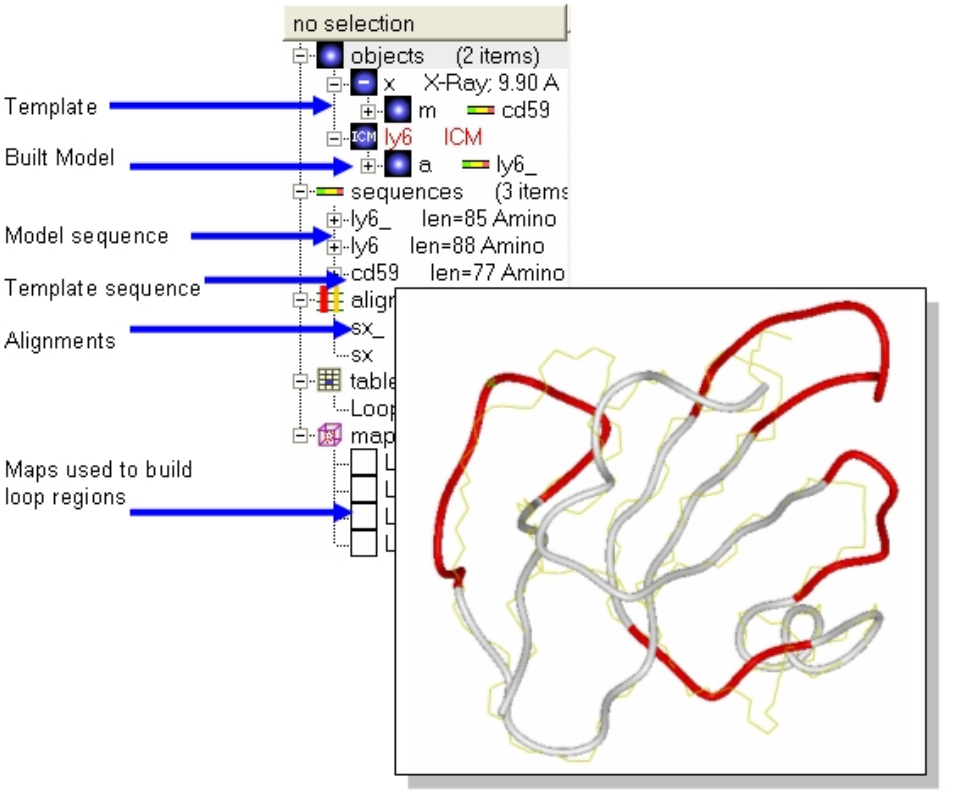
To make a protein model based on sequence homology.

Background

ICM has an excellent record in building accurate models by homology. The procedure will build the framework, shake up the side−chains and loops by global energy optimization. You can also color the model by local reliability to identify the potentially wrong parts of the model.

Instructions

1. Edit/Delete All . let us begin with a clear ICM session!
2. Homology/Load Example
3. Two sequences (ly6,CD59), one template structure (x) and an alignment (sx) should be loaded. Sequence CD59 is the sequence of the template structure called x.
4. Homology/Build Model and fill in the table using the drop down options. Warning minimize side−chains may take a few minutes.



Notes and things to try:

- The four built in loops are shown in red as default.
- Try displaying the model and the template in different colors or representations to observe any siginificant deviations between template and model.

Manual References (Web Links)

`Homology Modeling`

15.3.2. Lesson 3b: Linked Alignments and Structures

Objective

To select, display and label the conserved regions of the model.

Background

Within the ICM Alignment Editor there is a rich array of tools. Some of these tools allow selections between a linked alignment and a structure. The strength of consensus can be changed and selections can be made according to a variety of criteria. There will be an alignment symbol next to a structure in the ICM Workspace if the structure is aligned.

Instructions

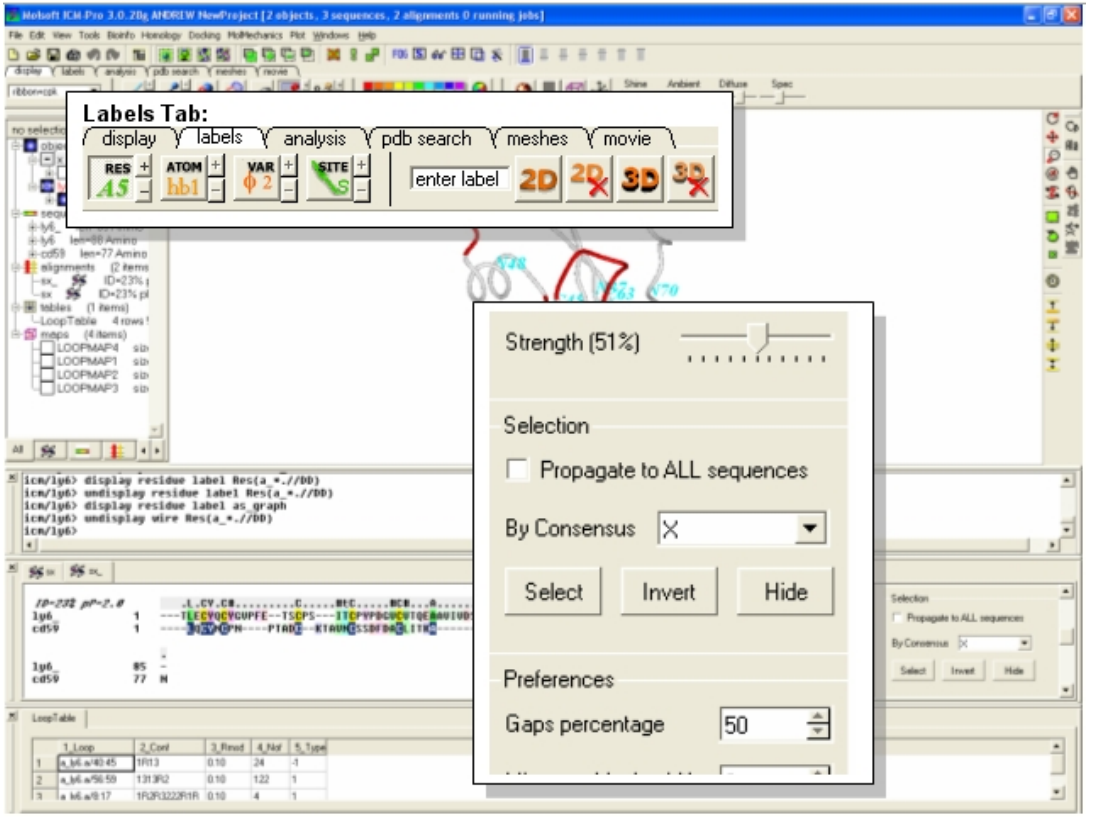
Using the alignment from lesson 3a we will display and label the conserved residues between our model and the template in CPK format.

1. Change the strength of the alignment consensus to 50% in the ICM Alignment Editor.

2. Type in the consensus you wish to select. For example if you only want to select identical residues between the template and model type in X. Other symbols (such as #) from the alignment consensus line can be entered here if desired. You may wish to play with this and the alignment consensus value.

3. Click on the Select button and the residues selected will be highlighted with green crosses.

4. To label the residues select the labels tab and select the label residue button.



Manual References (Web Links)

`Alignment Editor`

15.3.3. Protein Health

Objective

To remove clashes from a PDB structure.

Background

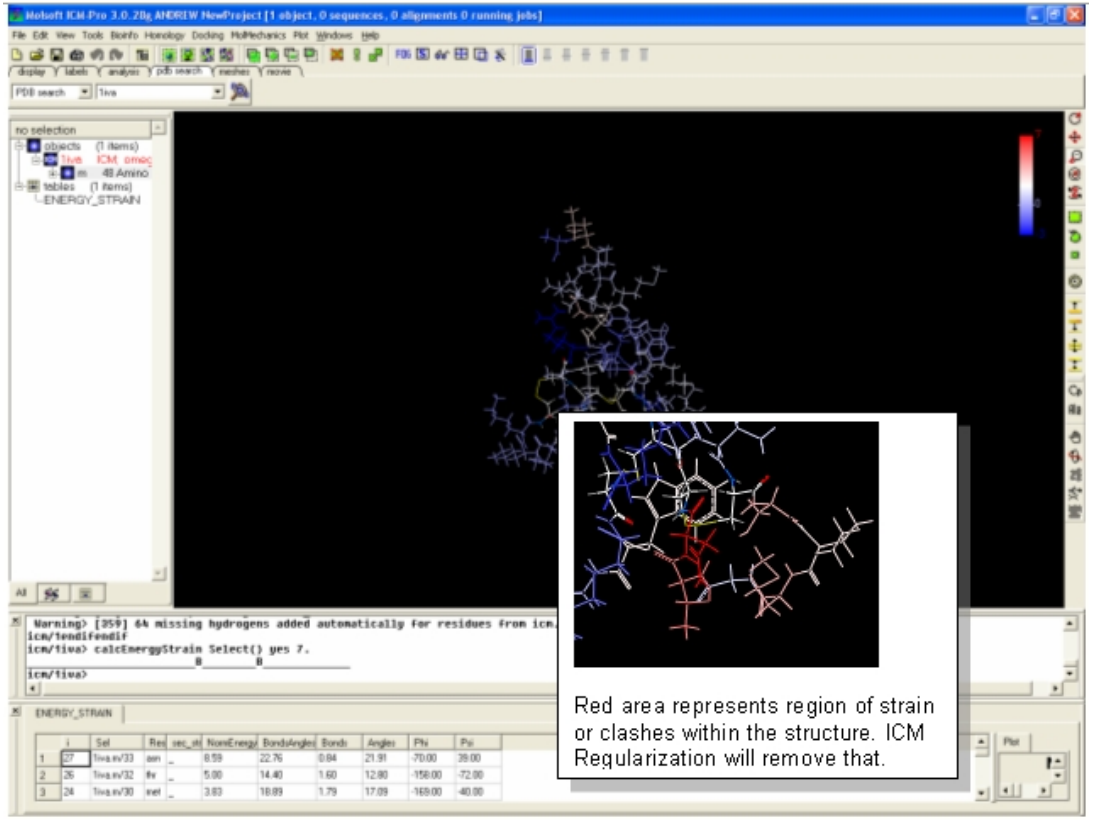
Here we will use a macro that calculates the energy strain (Protein Health) within a protein structure. The macro is based on a paper by Maiorov and Abagyan (1998). The regularization macro will remove any clashes and improve the energy of the structure.

Instructions

1. Edit/Delete All . let us begin with a clear ICM session!
2. Search and display the PDB structure 1iva (see Lesson 2a)
3. Convert 1iva into an ICM object (see lesson 2b)
4. Tools/Protein Health

   Note red coloring indicates clashes or high strain. Lets remove these clashes using the ICM regularization tool.

5. MolMechanics/Regularization
6. Color and display in wire − all clashes should have been removed.



Notes and Things To Try:

- Always use the Protein Health tool and ICM Regularization after you have constructed a protein model.
- It is always wise to check a protein structure from the PDB with the Protein Health tool and then use ICM Regularization to remove any potential problems you may identify.

15.3.4. Lesson 3d: Crystallographic Symmetry

Objective

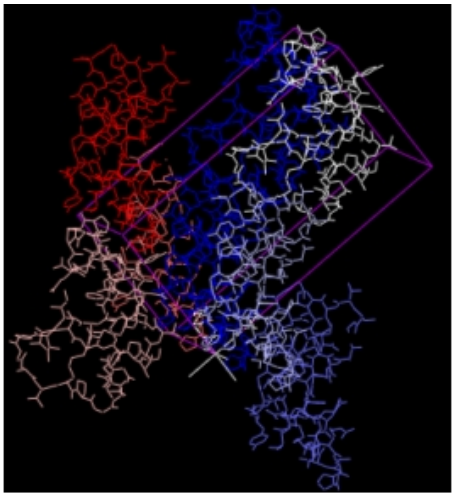
Here you will learn how to build crystallographic symmetry for a PDB structure.

Background

Most structure files contain some information regarding symmetry such as (i) crystallographic group eg. P2121 that determine N (depends on a group) transformations for the atoms in the asymetric unit and (ii) 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 ICM to generate symmetry related parts of the density or molecular objects.

Instructions

1. To generate symmetry related molecules we first need to read a PDB file into ICM (for an example choose 1crn)
2. Display the structure and select some residues around which you wish to generate the symmetry related molecules.
3. Tools/Crystallograhic Cell.
4. Select the radius around your selction from which you wish to construct the symmetry related molecules.
5. Tick the create symmetry related molecules box.
6. Select whether you want the symmentry generated molecules to be merged into the original PDB object.
7. Select whether you wish for the symmetry related generated molecules to be displayed. The crystallographic symmetry neighbors will be displayed in the Workspace Panel along with the crystallographic cell. For packing analysis and display you can color each symmetry unit a different color:
8. Select the Display tab hold down on the wire button and select the color by chain option.



As an example the crystallographic unit cell (purple box) and symmetry neighbors are shown for PDB structure 1CRN.

Manual References (Web Links)

`Symmetry`

15.3.5. Lesson 3e: Biomolecule Generator

Objective

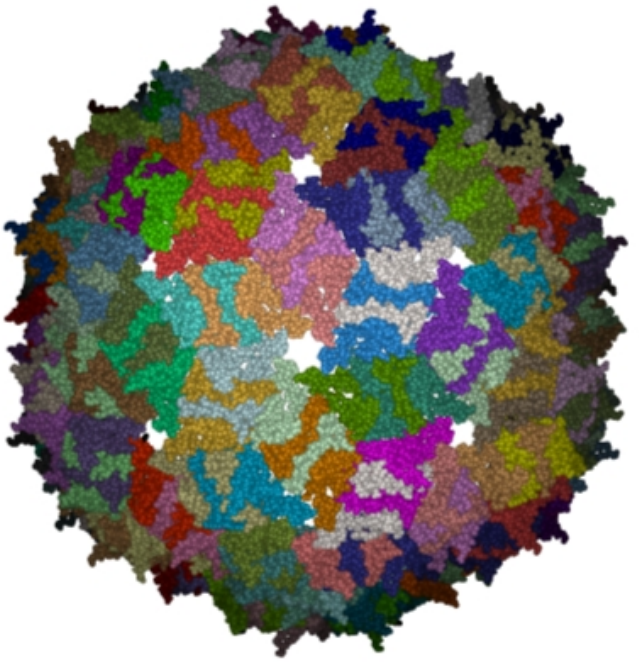
Here we will investigate the biological environment of a virus protein . PDB code 1DWN.

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/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.



Biomolecule generated by ICM for PDB: 1DWN Bacteriophage Pp7 From Pseudomonas Aeruginosa At 3.7 A Resolution.

Please note that right clicking on a PDB file in the ICM Workspace will tell you whether there is any biomolecule information available for that structure. If there is any information then the "Generate Biomolecules" option will be activated.

Manual References (Web Links)

`Biomolecule Generator`

15.3.6. Lesson 3f: Superimpose Structures

Objective

To superimpose two structures.

Background

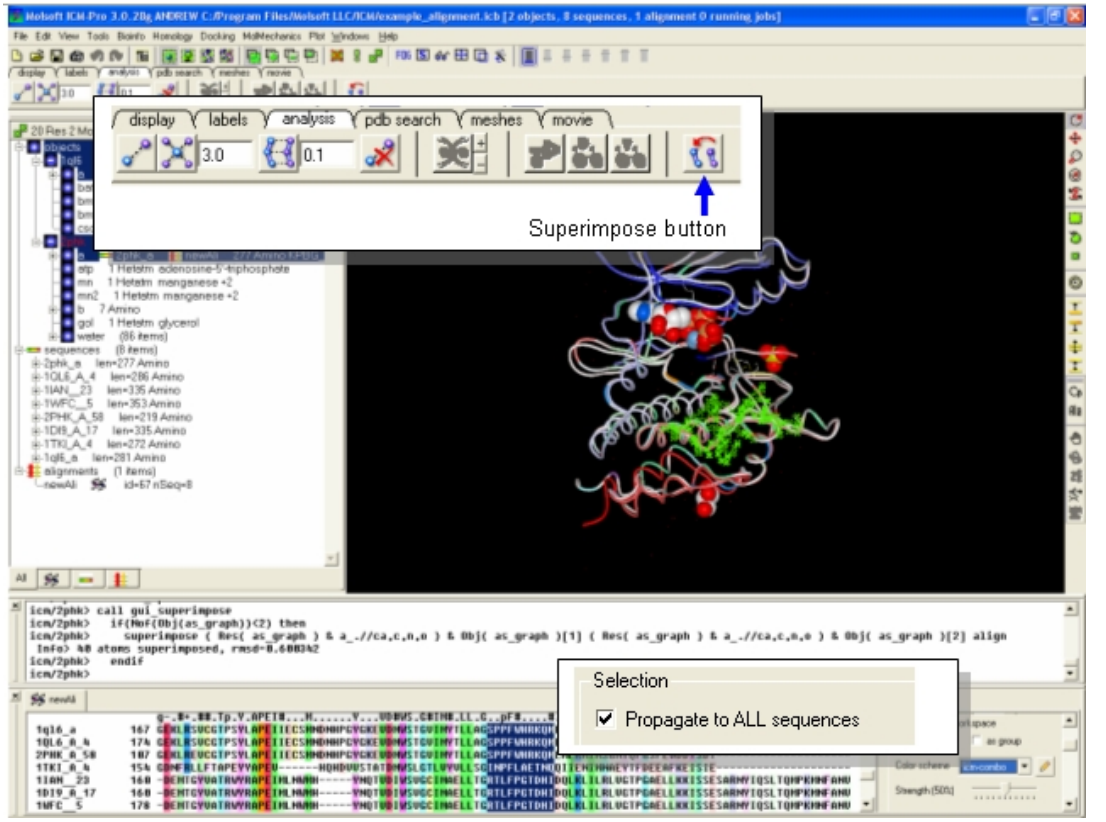
In this lesson we demonstrate the use of a superposition based upon a sequence alignment. All superposition analyzes can be performed using the button available within the Analyses tab. The example here uses protein kinase structures to superimpose.

Instructions

1. File/Open/Example_Alignment.icb
2. Read PDB 2PHK
3. Extract the sequence from 2PHK and then drag it and drop it into the alignment.

4. Select a region of the alignment around which you wish to superimpose. You can use the propogate to all sequences in the Alignment Editor to make this selection.

5. Select the analysis tab and click on the superimpose button.



Notes and Things To Try:

- Try making a superposition around the ligand binding pocket only by selecting the ligand.
- Try improving poorly superimposed regions such as loops.

Manual References (Web Links)

`How to Superimpose Two Structures`

15.3.7. Lesson 3g: Protein Folding and Structure Prediction

Objective

To use a script to perform protein folding / structure prediction.

Instructions

```
# Example folding script. Use as directed.
read libraries
s_pepname="tetrapep"
build string "se nh3+ ala his trp glu coo-" name=s_pepname # you may also use
the uncharged nter and cooh termini
write IcmSequence( a_ ) s_pepname+".se"
#build "pep16"       # alternative: your peptide sequence is in   pep16.se
file.
set symmetry exact
rename a_*. "f1"    # specifies current name. Several runs (f2,f3 etc.) are
recommended
nvar = Nof( v_//phi,psi,H,P ) # number of essential variables
                    #
#nProc =4
mncallsMC     = 10000 + Integer(0.008*nvar*nvar*nvar*nvar*nvar) # maximal
number of energy evaluations
mncallsMC     = Min(mncallsMC 40000000) # impose maximal limit
mncalls       = 170+nvar*3  # max. n_of minimization calls after each random
change
temperature   = 600   # optimal temperature for the simulation
tolGrad       = 0.01  # exit minimization when gradient is < 0.01
mcBell        = 1.0   # the default width of the MC probability distributions
mnconf        = 20 + 2*nvar   # max. n_of low-energy conformations saved in the
stack (f1.cnf file)
mnvisits      = 10 + nvar     # if stuck for >= 25 times, push it out
visitsAction  = "random" # drastic, works better then "heat"
mnreject      = 10    #
mnhighEnergy  = 15 + nvar/2   #
l_bpmc        = yes   # use biased probability
electroMethod = "distance dependent"
surfaceMethod = "atomic solvation"
set terms "vw,14,hb,el,to,sf,en"  # ECEPP/2 energy + solvation + entropy (see
icm.hdt file)
                    #
fix v_//?vt*        # exclude irrelevant virtual variables specifying
                    # absolute molecular position
set vrestraints a_/* # load preferred backbone and side-chain angle zones
                    # for the  biased probability MC
randomize v_//!omg 180.0  # create random starting conformation
vicinity = 30.0     #
betaStepPb = 2.
compare v_//phi,psi # use this variables to compare structure
montecarlo movie    # run it and record a movie.
                    # watch the movie later by:
                    # read movie "f1"; display ribbon; display movie "f1" 4.
8.
                    # analyse the best conf. in the stack by:
                    # build "tetrapep"; read stack; show stack all; load conf1
```

15.4. Lesson 4: Cheminformatics − Working with Chemical Structures

Overview

Background

15.4.1. Lesson 4a: Reading and Displaying Chemical Structures

Objective

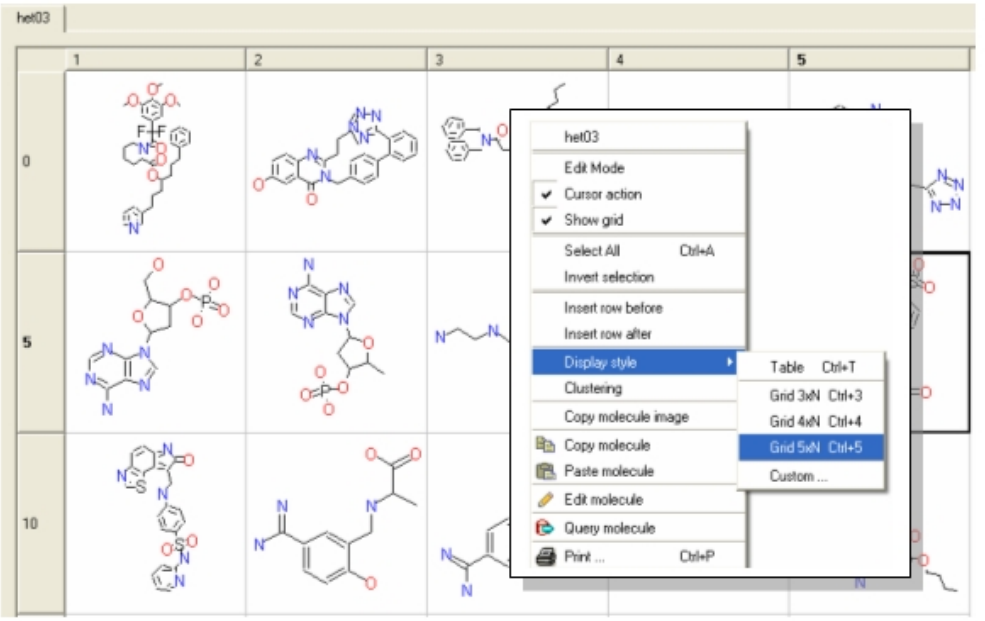
To read and display chemical structures.

Background

Small molecules are generally stored in three different file types . SDF, MOL and MOL2. An SDF file is a database of chemical structures. When chemical structure(s) are loaded into ICM they are automatically displayed in a chemical table. In this lesson we will read a SDF file that contains compounds extracted from the PDB.

Instructions

1. File/Open . locate the file called het03.sdf in the directory /home/share/training
2. The compounds will be displayed in a table called .het03. .
3. To arrange the compounds in a grid as shown below. Right click on the table and a menu will be displayed select Display Style/ Grid 5xN



Notes and Things to Try:

- Try inserting a row into the table.
- Try different ways of displaying the compounds. Try selecting different columns in the table and displaying based on this. (see section 9.1.3 of the User.s guide on how to make table selections)
- Try selecting rows or columns.
- We will learn how to edit structures in the next lesson but if you want to edit a structure you can select the Edit Molecule option and the ICM Molecular Editor will be displayed.
- You can copy and paste molecules into other applications such as word or other molecular drawing software.

Manual References (Web Links)

`Loading Chemical Structures`

15.4.2. Lesson 4b: Drawing Compounds and Molecular Tables

Objective

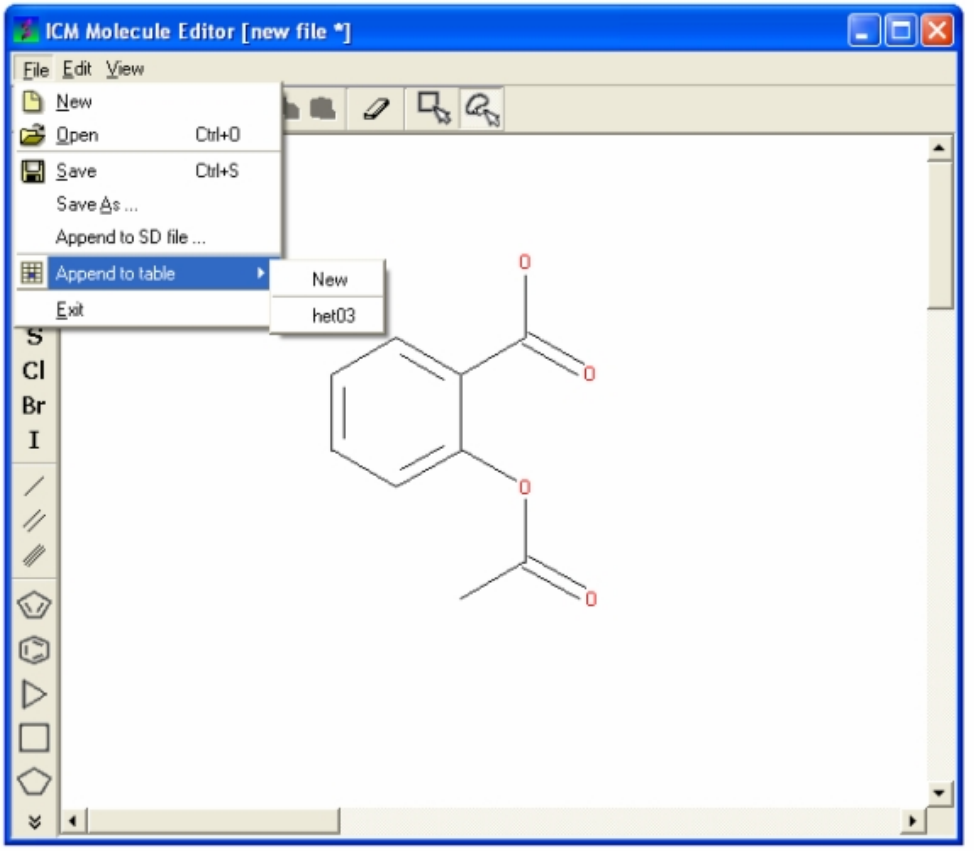
To build a compound and save it in an ICM Molecular Table.

Background

In ICM it is possible to not only edit structures that you have read into ICM but also create your own and append them to a table, file or a database of structures. All these actions take place in the ICM Molecular Editor.

Instructions

- Edit/Molecular Editor and the editor will automatically be displayed.
- Draw a compound within the editor using the rings, atoms and bonds on the left hand side of the editor. Note more advanced options can be found by right clicking on either a bond or an atom.
- Once you have finished drawing you can either save the compound as a separate file, append it to an already existing compound database or you can save it into an ICM table.
- Save it to an ICM table by selecting File Append to table and then select either New or an already loaded ICM table.
- File/Exit



Manual References (Web Links)

`Molecular Editor`

15.4.3. Lesson 4c: Chemical Similarity Searching

Objective

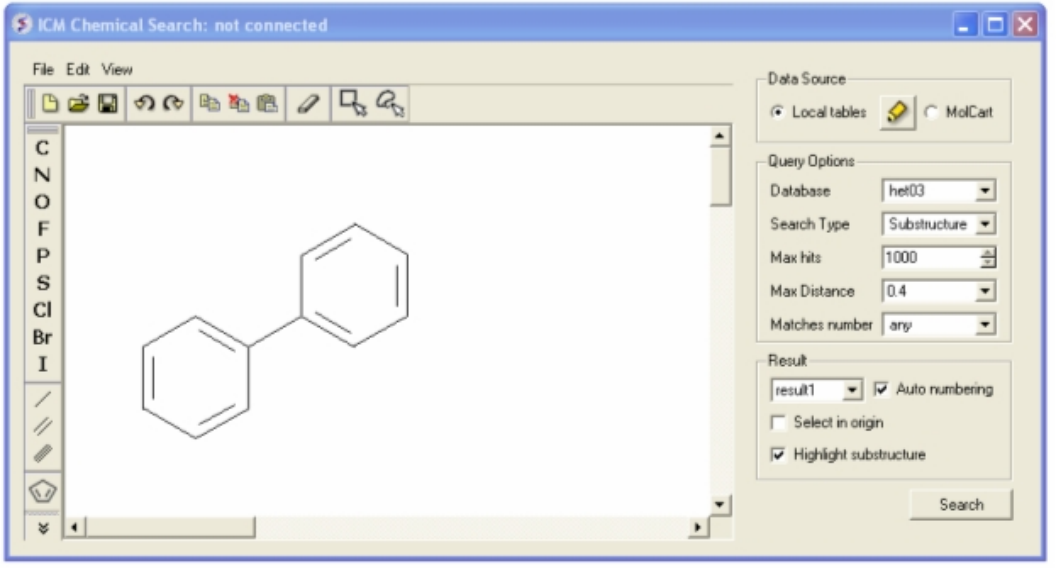
To perform a basic substructure chemical similarity search.

Background

Using ICM you can perform a compound similarity search whereby a query structure will be searched against a database of compounds. The database can be a compound database already loaded into ICM such as an SDF file or Molsoft.s very own compound database called MOLCART.

Instructions

1. Load the het03.sdf file into ICM as described in lesson 4a.
2. Right click and select the Query molecule option.
3. The ICM Molecular Editor and another menu for query search (on the right) will be displayed.
4. Edit/Select All to delete the current compound displayed.
5. Draw a structure in the editor. This will be your substructure query structure.
6. Select the option Local Tables
7. Select het03 as your database.
8. Select substructure search..
9. Select the other options as shown in the diagram below. You can experiment with different values from the drop down menu.
10. Select the Search button.
11. A new table will be constructed called result1 with your substructure search results contained in it.



Notes and Things to Try:

- Note your substructure is highlighted in red.
- Try using the FP finger print option from the drop down .Search Type.. A substructure search is a search whereby only the defined molecule in the query will be searched against the database. Whereas, a FP search which stands for fingerprint search enables any fingerprint within a structure to be searched for in the database. The "Max distance" option is available for use with the FP search and the "Matches number" option is for use with the substructure search. The option you do not require based on your search method will be blanked out. A "Max distance" value of 0 means that the search will only identify matches exactly the same as the fingerprint − the default is 0.4. The "Matches number" option allows you to stipulate how many times within a structure in the database your query can be found.

Manual References (Web Links)

`Chemical Similarity Searching`

15.4.4. Lesson 4d: Advanced Chemical Similarity Searching

Objective

To perform advanced chemical searching.

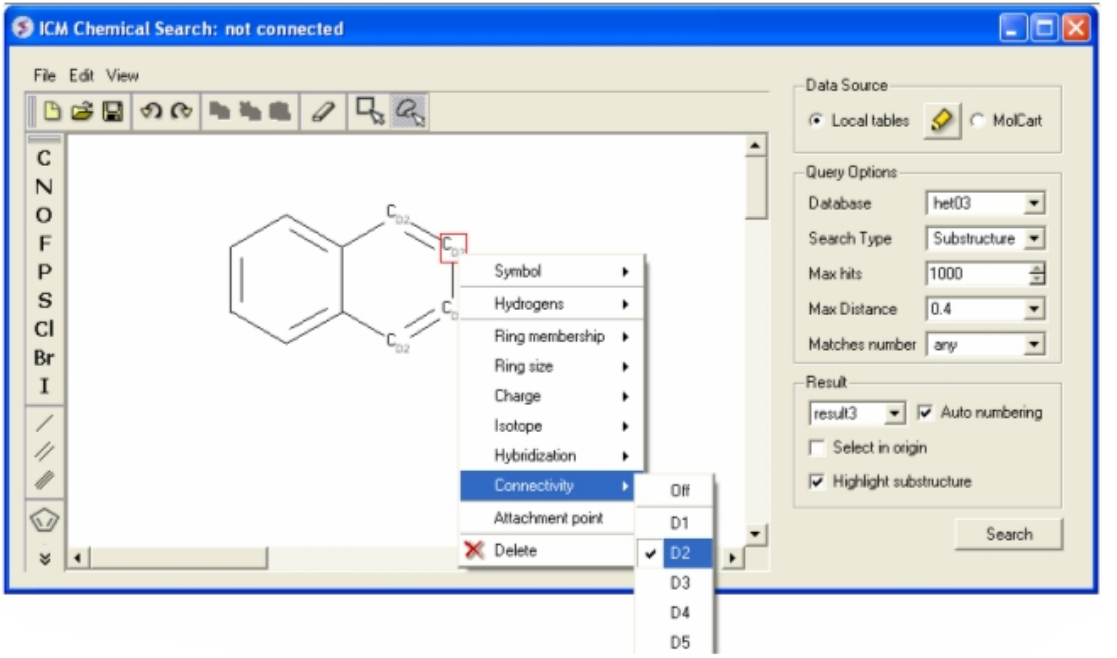
Background

You can make your similarity search more refined by selecting certain criteria when you set up your query.

Instructions

1. Repeat steps 1−5 of lesson 4c.
2. Draw your query structure but use the right click options to filter your search.

For example you can set the connectivity of an atom In the example below we have set the connectivity of the atoms in the ring to two. Therefore this search will only identify compounds with two bonds at that position.



Notes and Things to Try:

- Try filtering yout search by using the .Attachment point. and .Ring Membership. options.
- Try varying the query options.

Manual References (Web Links)

`Chemical Similarity Searching`

15.5. Lesson 5: Docking and Virtual Ligand Screening

15.5.1. Lesson 5a: Docking I

Objective

To dock biotin into the streptavidin receptor.

Instructions

1. Docking>Set Project Name (BIOTIN)
2. Docking>Load Example (streptavidin complexed with haba)

3. Select biotin in workspace window, select .neighbors. atoms on .other objects. in a 5 A radius
4. Docking>Receptor setup (Receptor molecules: a_rec.a) Adjust box size / probe position
5. Select and delete a_rec.
6. Docking>Ligand Setup>From Loaded ICM object (Ligand molecule: a_biotin.biotin)
7. Docking>Make Receptor Maps
8. Docking>interactive docking (Ligand object: a_biotin.)
9. MolMechanics>View Stack

15.5.2. Lesson 5b: Docking II

Objective

To dock a ligand into the ricin crystal structure.

Instructions

- Select tab .Pdb Search.. Type .ricin. and hit PDB button
- Double−click 1br6
- Right click on 1br6, .Clone., click OK, call it ligand.
- From the first object delete the small molecule (pt1) by right clicking on it in the ICM workspace. From the clone, delete the receptor (a), and water
- MolMechanics >ICM−Convert >Protein (Object: a_1br6., replace)
- MolMechanics >ICM−Convert >Chemical (Object: a_ligand.)
- Docking>Set Project Name (RICIN) Docking>Receptor Setup (make sure receptor a_1br6.a is selected in the receptor molecule box) .Identify binding Sites. Select second pocket, click OK
- Docking>Ligand Setup>From loaded ICM object (Ligand: a_ligand.pt1)
- Docking>Review/Adjust Ligand/Box (Box can be resized) Docking>Make receptor maps
- Docking>Interactive docking>Loaded Ligand (Ligand: a_ligand.)

15.5.3. Lesson 5c: Virtual Ligand Screening

Objective

To perform virtual screening into the ricin receptor.

Instructions

- Docking> Set Project (select RICIN) FROM LESSON 5b
- File> Open (select ricinLigands2D.sdf)
- Docking> Interactive Docking> Mol Table Ligand (Mol Table: ricinLigands2D, select .Calc ICM Score.)
- Docking> Tools> Index Mol/Mol2 file/database (Input file . select ricinLigands2D.sdf,)
- Docking> Ligand Setup> From Database (select mydb.inx, check .mol., .build hydrogens. .assign charges. and .2D to 3D. convert)
- Docking>Small Set Docking Batch
- Docking>Make Hit List (select import 2D from DB)
- Browse HITLIST table

Index