A Bit About Symmetry, Ligand Binding & Mutation

SYMmetry

Many proteins have more than one subunit. For example, hemoglobin is an alpha-beta tetramer. Sometimes when proteins crystallize, only part of an oligomeric protein will reside in the crystal asymmetric unit. The asymmetric unit can simply be described as the unique portion of the crystal. The rest of the oligomer is still in the crystal, but it is in a "crystal symmetry related" location and therefore not unique. For example, there may be a crystallographic 2-fold symmetry axis (see Figure 1 below) that relates the two halves of the protein.

Figure 1. 2-fold rotation symmetry. A 2-fold rotation is a 180 degree rotation around a particular axis. In the hemoglobin example, the rotation was about the Y axis, and the crystal unit cell (the repeated unit that makes up the crystal and generally contains more than one asymmetric unit) consists of a box with 90
degree angles between X, Y and Z axes. For this case, XYZ is transformed to -XY-Z, as illustrated below. In the diagram, the Y axis is perpendicular to the page.

This sort of symmetry is beyond the focus of our course. However, one consequence will be important to many of you: when crystal symmetry is required to generate a full molecule, only the coordinates for the asymmetric portion of the molecule will be found in the corresponding PDB file. DeepView can be used to generate the full molecule through using what is known as a 'transformation matrix'. A transformation matrix can simply be described as a set of coordinates that takes a point in space and transforms it to another point in space that is related in a symmetrical fashion. In Figure 1 the point (x,y,z) was regenerated at (-x,y,-z) by applying the following transformation matrix:

<table>
<thead>
<tr>
<th>Rotation about</th>
<th>Rotation about</th>
<th>Rotation about</th>
<th>Translation along</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>-1.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>y</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>z</td>
<td>0.00</td>
<td>0.00</td>
<td>-1.00</td>
</tr>
</tbody>
</table>

where:

Xnew = (-1)(Xold) + (0)(Yold) + (0)(Zold) + 0.0*
Ynew = (0)(Xold) + (1)(Yold) + (0)(Zold) + 0.0
Xnew = (0)(Xold) + (0)(Yold) + (-1)(Zold) + 0.0

*Note: The last operation is a translation along the respective axis.

In newer structures there is explicit information about how to generate the entire molecule, e.g.:

REMARK 350
REMARK 350 GENERATING THE BIOMOLECULE
REMARK 350 COORDINATES FOR A COMPLETE MULTIMER REPRESENTING THE KNOWN
REMARK 350 BIOLOGICALLY SIGNIFICANT OLIGOMERIZATION STATE OF THE
REMARK 350 MOLECULE CAN BE GENERATED BY APPLYING BIOMT TRANSFORMATIONS
REMARK 350 GIVEN BELOW. BOTH NON-CRYSTALLOGRAPHIC AND
REMARK 350 CRYSTALLOGRAPHIC OPERATIONS ARE GIVEN.
REMARK 350
REMARK 350 BIOMOLECULE: 1
REMARK 350 APPLY THE FOLLOWING TO CHAINS: A
REMARK 350 BIOMT1 1 1.000000 0.000000 0.000000 0.000000
REMARK 350 BIOMT2 1 0.000000 1.000000 0.000000 0.000000
REMARK 350 BIOMT3 1 0.000000 0.000000 1.000000 0.000000
REMARK 350 BIOMT1 2 1.000000 0.000000 0.000000 0.000000
REMARK 350 BIOMT2 2 0.000000 -1.000000 0.000000 264.98200
REMARK 350 BIOMT3 2 0.000000 0.000000 -1.000000 198.73650

Where the symmetry operator in the last three lines would be applied in DeepView.

However, some structures were determined before explicit information was required to be included when depositing a structure in the PDB. One example is the the structure for oxyhemoglobin (1HHO). The
The following information is included instead:

REMARK 4 ............ COORDINATES ARE
REMARK 4 GIVEN FOR THE ALPHA-1-BETA-1 DIMER AND THOSE FOR
REMARK 4 ALPHA-2-BETA-2 ARE OBTAINED BY ROTATION OF 180 DEGREES
REMARK 4 ABOUT Y. THE Y AXIS (MOLECULAR DIAD) IS A CRYSTALLOGRAPHIC
REMARK 4 TWO-FOLD AXIS.

Crystallographers (such as the Montfort and Horton groups on campus) will interpret this to mean that the
following transformation matrix should be applied. This is the same as we saw above (see Figure 1 and
above text for details):

-1.00 0.00 0.00 0.00
0.00 1.00 0.00 0.00
0.00 0.00 -1.00 0.00

where:

Xnew = (-1)(Xold) + (0)(Yold) + (0)(Zold) + 0.0
Ynew = (0)(Xold) + (1)(Yold) + (0)(Zold) + 0.0
Xnew = (0)(Xold) + (0)(Yold) + (-1)(Zold) + 0.0

LIGAND BINDING

Many three dimensional protein structures contain a ligand, whether it be its natural ligand or one that
mimics its natural ligand. On the other hand, some structures do not contain a ligand at all or may not be
able to be produced with its natural ligand. Many times, structural biologists would like to see the effects of
ligand binding on the three dimensional structure of a protein and thus turn to molecular modeling as a tool.
Many ligands can be found directly in a PDB file of a protein that has already been deposited in the PDB.
These ligands can then be 'cut out' from that PDB file and used in conjunction with your protein in a
modeling experiment. If a ligand of interest is not readily available from another PDB file, it can be
generated using other modeling software that is beyond the scope of this class.

DeepView allows one to model ligand binding using several layers and then merging these layers so that
the resulting structure will contain both the protein and the ligand of interest.

MUTATION

A protein's function is directed by the three dimensional fold and thus the proper placement of specific
residues. Many times, functional studies are performed on a protein through the use of mutation. Many
residues are critical to function and if eliminated or changed, the environment around that residue is
changed and may cause the protein to become inactive or less active. In more sparse cases, the mutation
effects the protein allosterically (i.e. mutation at one local place effects another place globally in the
protein). In even more sparse cases, a mutation can change the entire global fold of the protein or cause a
secondary structure switch while still rendering it active. The use of mutation can also have positive
consequences such as making a protein more active. Protein redesign results such as these are often used
for industrial and pharmaceutical purposes.
Three-dimensional protein structures are often used by many biophysical chemists to suggest places for mutation for both functional and evolutionary studies as well as protein redesign. Their hypotheses can then be tested through molecular modeling before pushing through with the physical experiment in the lab (i.e. creating the mutant protein, producing and purifying the mutant protein, and determining the consequence of mutation through structural and functional studies). While molecular modeling programs can not predict physical activity of the mutated protein (this must be done using an activity assay designed specifically for your protein of interest), it can usually predict if the mutated protein will still fold into a three-dimensional structure.

DeepView can be used to model mutations into a specific protein. It allows you to browse a rotamer library in order to change amino acids sidechains, and thus quickly evaluate the putative effect of a mutation before actually doing the lab work. The program predicts any steric clashes as well as modified hydrogen patterns with surrounding residues resulting from the mutation if applicable. The program also includes an Energy Minimization program package that allows you to evaluate the energy of a structure as well as repair distorted geometry's. Energy minimization is routinely done after modeling a mutation or after a process called 'structure refinement'. In general, it repairs distorted geometry's by moving atoms to release internal constraints. In other words, it 'makes room' for a residue. It can not, however, pass through high energy barriers and stop in a local minima. Although energy minimization is provided with DeepView, we will not be using this feature of the program due to time.

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**Tutorial Exercise**

An introductory lesson and the Gale Rhodes Tutorial on DeepView was completed previously and so we will not reiterate the basics of this program. Instead, we will focus on a few commands that will help with generating whole intact molecules and superimposing one structure on another; modeling ligand binding; and creating mutations.

**Tutorial Exercise Objectives:**

- Generate a whole intact molecule of oxyhemoglobin using crystallographic symmetry
- Superimpose two structurally related molecules of hemoglobin (the oxy and deoxy conformations)
- Obtain a ligand from one structure (NP4 complexed with Histamine) and place it into another (myoglobin)
- Mutate a residue in a selected structure (myoglobin)

**PDB's needed to accomplish PART I, II & III:**

- 1HHO (oxyhemoglobin)
- 2HHD (deoxyhemoglobin)
- 1MBN (myoglobin)
- 1IKE (NP4 complexed with Histamine)

**PART I: SYMMETRY**

**A. BUILDING WHOLE INTACT MOLECULES**

Here we will generate a whole intact molecule from a subset of subunits in the asymmetric unit of a crystal, using crystallographic symmetry. We will use oxyhemoglobin as an example. Human oxyhemoglobin
crystallizes in a form that has only one alpha and one beta chain in the crystal asymmetric unit. Here we will build the intact tetramer, the form which it exists in nature.

- Obtain files 1HHO (oxyhemoglobin) and 2HHD (deoxyhemoglobin) 1 of 2 ways below and place them into your personal folder:
  - On the course website under Computer Section/Shared PDB's and RASMOL Scripts
  - Go to the PDB (http://www.rcsb.org/pdb/) and retrieve the coordinate files 1HHO and 2HHD

- Start DeepView
- Open 1HHO.pdb
- Open the Layers Infos Window ('Windows' menu pulldown)
- Turn off side chains (right click on 'side' raw heading in control panel)
- Show CA trace only ('Display' pulldown menu)
- Rename Current Layer to AB ('Edit' pulldown menu)
- Color the molecule by Chains* ('Color' pulldown menu)

  *Note: Notice that there are only two chains, A (which should now be YELLOW) and B (which should now be BLUE). Yet we know that hemoglobin exists as a tetramer. To assemble the whole molecule:

- Select all ('Select' menu pulldown)
- Apply Transformation on Current Layer ('Tools' menu pulldown)
  - Change the rotation to be:
    -1.00 0.00 0.00
    0.00 1.00 0.00
    0.00 0.00 -1.00
  - And the translation to be: 0.0 0.0 0.0
  - Press OK*

  * Note: The molecule should move. The new position for the molecule represents half of the tetramer. Let's now make the other half:

- Open 1HHO.pdb again to get the original A and B chains
- Turn off side chains
  - Click on 1HHO in the Layers Info Window
    - The Control Panel should now switch to control layer 1HHO
  - Right click on the 'side' raw heading in control panel
- Show CA trace only ('Display' pulldown menu)
- Rename Current Layer to CD ('Edit' pulldown menu)
- Color the molecule's chains in Layer CD different colors than in Layer AB:
  - Select the A chain in the CD layer by clicking on the A in the control panel (left click)
  - Right click on the 'col' raw heading
  - Choose RED
  - Select the B chain in the CD layer by clicking on the B in the control panel (left click)
  - Right click on the 'col' raw heading
  - Choose GREEN

  *Note:

  * Layer AB: Chain A should still be YELLOW, Chain B should still be BLUE
Layer CD: Chain A (now considered chain 'C') should be RED, Chain B (now considered chain 'D') should be GREEN

- Select all of both layers:
  - Choose Layer AB (Layers Infos Window)
  - Select all ('Select' window pulldown)
  - Choose Layer CD (Layers Infos Window)
  - Select all ('Select' window pulldown)

- Create Merged Layer from Selection ('Edit' pulldown menu)
- Select the _merged_ layer (Layers Infos Window) Scroll down the control panel to see that all four chains are present in the layer.

- Save the current layer as a new PDB file
  - 'File' menu pulldown
    - scroll to 'Save'
    - choose 'Layer'
    - Save as: ABCDoxy.pdb

*This file will have chains A-D in a correct tetramer consisting of 2 alpha-beta dimers.

- Check your new merged.pdb to see if it worked
  - Make Layer AB, Layer CD and Layer _merged_ not visible by clicking the check under 'vis' in the Layers Infos Window
  - Open ABCDoxy.pdb ('File' menu pulldown)
  - Choose Layer ABCDoxy (Layers Infos Window)
  - Look at the structure as well as scroll down the control panel to see that all four chains are present in your newly merged structure

- Close All Layers ('File' menu pulldown)

B: SUPERIMPOSITION OF MOLECULES

Here we will superimpose deoxyhemoglobin (2HHD) upon oxyhemoglobin (now called ABCDoxy.pdb) to see the structural differences that occur upon oxygen binding to hemoglobin. The RasMol script hb2.txt highlights the differences between these structures after superimposition. The deoxy structure(2HHD) crystallized with a full tetramer in the asymmetric unit so we do not have to create the full molecule. However, the orientation is different than in the oxy crystal form.

To superimpose the two molecules:

- Open ABCDoxy ('File' menu pulldown)
- Open 2HHD (File menu pulldown)
- Turn off side chains on 2HHD
  - Click on 2HHD in the Layers Info Window.
  - The Control Panel should now switch to control layer 2HHD
  - Right click on the 'side' raw heading in control panel
- Show CA trace only ('Display' pulldown menu)
- Apply Magic Fit ('Fit' pulldown menu)
  - Use CA (carbon alpha) only
  - Layers involved:
    - ABCDoxy
    - 2HHD
**PART II: LIGAND BINDING**

In PART II, we will learn how to model ligand binding to a protein. For our purposes, we will use ligands already available in a PDB file. If a ligand of interest is not available, it can be generated using other modeling software that is beyond the scope of this course.

For this example, we will **add histamine to the myoglobin molecule**.

- Histamine binds to the heme of the nitrophorins (NPs).
- Does histamine fit into the myoglobin binding site? Let's find out:

- Obtain the file 1IKE (NP4 complexed with Histamine) 1 of 2 ways below and place it into your personal folder if not already done so from previous lesson:
  - On the course website under Computer Section/Shared PDB's and RASMOL Scripts
  - Go to the PDB (http://www.rcsb.org/pdb/) and retrieve the coordinate files 1IKE
    - Note: If you do not already know of a structure that contains your ligand of interest then go to the PDB and search for structures with that ligand of interest.
- Edit 1IKE.pdb using a text editor (such as Text Pad)
  - leave only the histamine molecule, called HSM.
    - This will be found toward the bottom of the pdb file after HEM and before HOH. It is HETATM 1492-1499.
  - save this edited pdb as **HSM.pdb**
- Start DeepView
- Open 1MBN (myoglobin) in DeepView
- Center on the oxygen attached to the heme
- Open HSM in DeepView
- Color HSM Green
  - Click on HSM in the Layers Info Window.
  - The Control Panel should now switch to control layer HSM
  - Left click on HSM in control panel to select it
  - Left click on the box under the 'col' raw heading
  - Choose Green
- Move the histamine into the binding pocket.
  - To accomplish this (which is a little difficult at first) you will:
    - make MBN (myoglobin) immobile/mobile many times by clicking the check under 'mov' for MBN in the Layers Infos Window
    - use the slab command

**Important Note:** In this case, since the molecules have the identical sequences, the automatic ('magic') fit works. If the sequences do not have the identical sequences, then specific amino acids in common to both proteins must be specified using the 'Fit molecules (from selection)' operation under the 'Fit' menu pulldown.
PART III: MUTATION

In PART III, we will learn how to model a mutation in a protein structure. We will use myoglobin (1MBN) for an example.

- Obtain file 1MBN.pdb (myoglobin) 1 of 2 ways below and place it into your personal folder if not already done so from previous lesson:
  - On the course website under Computer Section/Shared PDB’s and RASMOL Scripts
  - Go to the PDB (http://www.rcsb.org/pdb/) and retrieve the coordinate files 1MBN
- Start DeepView
- Open 1MBN.pdb
- Center on the oxygen attached to the heme
- Learn to use the slab function
  - 'Preferences' menu pulldown
    - Display
    - Set slab to 10 angstroms
  - 'Display' menu pulldown
    - Slab (turns on slab)
  - 'Display' menu pulldown
    - Slab (turns off slab)*

*Note: The slab function can also be toggled on and off using the key pad keys 'alt+/' simultaneously

- center the rotation on the histamine
- Save the PROJECT when completed.
  - 'File' menu pulldown
    - Scroll to Save
    - Choose 'Project'
    - Save as: myo_his. pdb*

*The resulting pdb file will have both molecules in it, separated for use by DeepView.

- Check to see if your project saved
  - Use the 'File' menu pulldown to Close All Layers
  - Use the 'File' menu pulldown to open myo_his.pdb
  - Check if both 1MBN and HSM open and if HSM is in the same place as you left it!

**To use this new pdb in RasMol it is best to:

- Copy the saved pdb file to a new filename
- Edit the file using Text Pad to
  - remove the CONECT and SPDBV lines
  - keep the ATOM and HETATM and END lines!
  - renumber the new ligand to make it unique for RasMol.
- Slab (turns on slab)
  - 'Display' menu pulldown
    - Slab (turns off slab)*

*Note: The slab function can also be toggled on and off using the key pad keys 'alt+/-' simultaneously

- Find **Val 68** in the distal pocket
- Color it **GREEN**
- Select the 'mutate' button in the Tool Bar menu and the select Val 68 in the structure
- Choose **Glu** from the menu presented (this change occurs in some myoglobins, I believe)
  - The lowest energy rotamer will be presented
  - Other allowed rotamers can be selected using the arrows underneath the mutation button
  - **Clashes** with other atoms are indicated with pink dashed lines
  - **Hydrogen bonds** are indicated with **green** dashed lines
- Save the mutation and rotamer of interest by selecting the mutation button again.

**At this point an energy minimization** could be performed that allows you to evaluate the energy of a structure as well as repair distorted geometry's. Energy minimization is routinely done after modeling a mutation or after a process called 'structure refinement'. In general, it repairs distorted geometry's by moving atoms to release internal constraints. In other words, it 'makes room' for a residue. It can not, however, pass through high energy barriers and stop in a local minima. Although energy minimization is provided with DeepView, we will not be using this feature of the program due to time.