Mol* Tutorials

Introduction

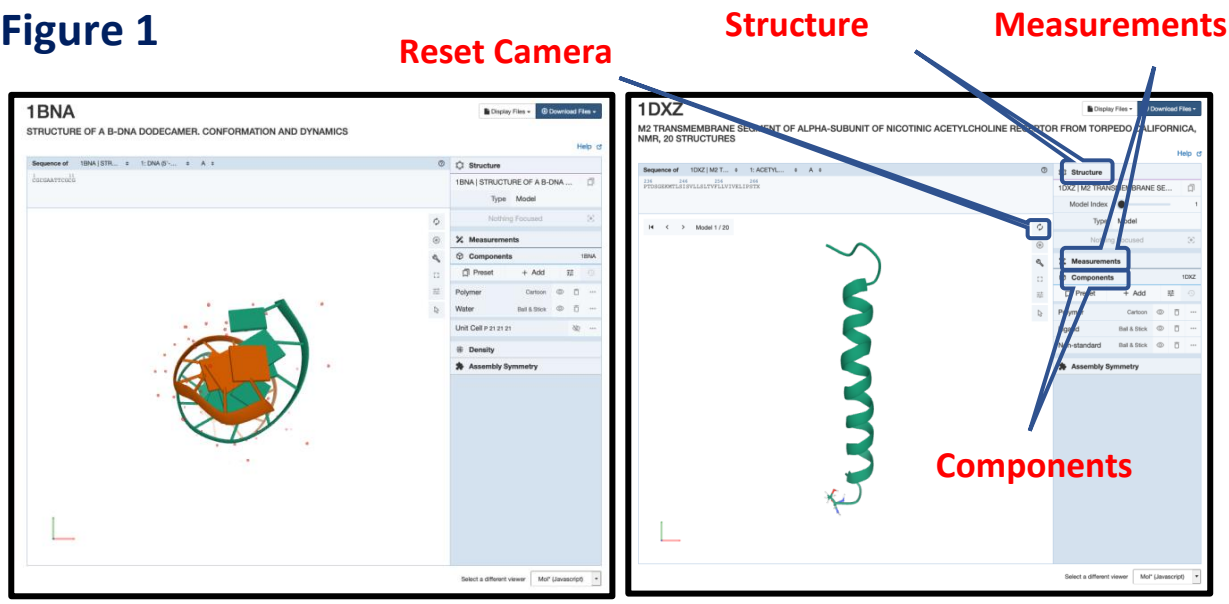
This tutorial on Mol* provides instructions on how to use and apply many of the viewer functions. The subjects of the tutorial are alpha helices (1DXZ & 2YMK), beta sheets (2BEG & 4M43), hemoglobin (2DHB), and B-DNA (1BDNA).

The ensuing instructions and example questions are components of the Bioinformatics Exercises that are integrated as learning resources across the suite of Biochemistry textbooks published by Wiley & Sons, inclusive of *Essential Biochemistry* (Pratt & Cornely), *Fundamentals of Biochemistry* (Voet, Voet & Pratt), and *Biochemistry: An Integrative Approach* (Tansey).

Please share any updates or suggestions by email to rmogul@cpp.edu.

Tutorial Questions & Instructions

Figure 1



A. Basic Operations

1. **To access Mol*, go the 3D View tab at the PDB ID home page.**

2. **To begin, review the RCSB 3D View User Guide:**
   - https://www.rcsb.org/pages/help/3dview - structure-view

3. **Take note of the following right-side menu options (Figure 1):**

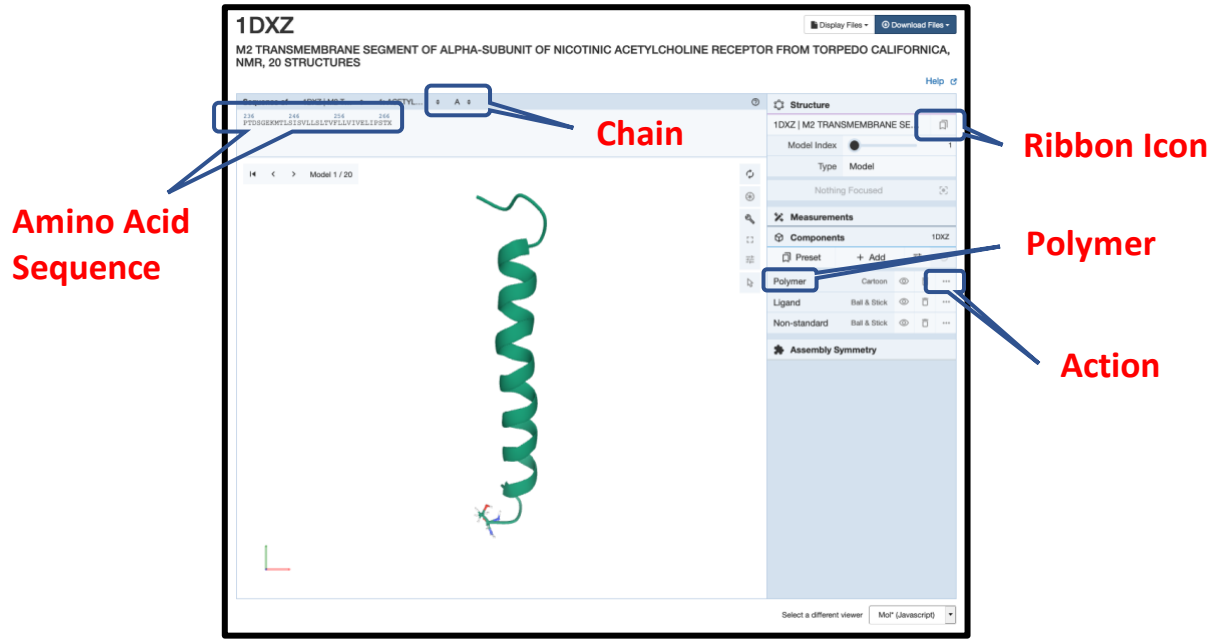
Mol* Tutorials

- **Structure**: Provide differing view options for the molecules in the crystal/structure.
- **Measurements**: Provides measuring options such as the distance between atoms and torsional/dihedral angles (angle between 4 connected atoms…).
- **Components**: Parts of the structure as defined by user selections and pre-selected groups (*e.g.*, backbone, specific amino acids or bases, atoms, etc.).

4. **Now, familiarize yourself with the basic functions of zooming, rotating, and translating the structure, and resetting the camera:**
    - *What does scrolling the mouse do to the structure (when the mouse in the structure window pane)?*
    - *What does moving the mouse around while left-clicking do to the structure?*
    - *What does moving the mouse around while right-clicking do to the structure?*
    - *What does the Reset Camera do to the view?*

B. Basic Operations For Proteins

Figure 2



Example: View hydrophobicity scale.

- **For PDB entry 1DXZ, the amino acids side chains within the helical part of the structure are _____.**

A. mostly polar
B. mostly charged
C. all aliphatic
D. mostly nonpolar

Answer: D
Feedback: The amino acids are mostly nonpolar and contain both aliphatic, aromatic, and heteroatom-containing side chains.

Mol Instructions*

- For this problem, the entire 'polymer' can be colored using a hydrophobic scale.
- On the right-hand menu, in the "Polymer" subsection, click on the "Action" button, which are the three-dots in a row (see **Figure 3**).
- Click on "Set Coloring."
- Click on "Residue Property."
- Click on "Hydrophobicity."
- In this rendering, the most hydrophobic amino acids (or residues) will be green, and the most hydrophilic amino acids will be red.
- Within the helix, most of the amino acids have which property?

Example: View amino acid side chains.

- **For PDB entry 1DXZ, which of the following amino acids is ~1 turn away from Met 243? Take note, Mol* lists Met 243 as "Met 8 [auth 243]", which indicates the internal numbering of the amino acids by Mol* (Met 8), and the author's numbering scheme ([auth 243]).**

A. Leu 245
B. Ile 247
C. Leu 251
D. Thr 244

Answer: B
Feedback: Among these choices, Ile 247 is ~1 turn away from Met 243, which is consistent with the specific measurement of 3.6 amino acids/turn.

Mol Instructions*

- The 1˚ structure (or amino acid sequence) of the protein is provided at the top of the application window (see **Figure 3**).
- Select Met 243 by clicking on the appropriate 1-letter abbreviation.

Mol* Tutorials

- Note, when hovering over an amino acid letter, a textbox with the amino acid name and number will appear at the bottom right of the application window.
- When hovering over "M", the amino acid backbone on the ribbon diagram will also highlight.
- When clicking on "M", all of the side chains within 5 Å will appear.
- To answer the question, choose an amino acid that is "~1 turn away" from Met 243.
- Use your understanding of alpha-helices as a guide.

Example: View molecular surfaces.

- **Dermcidin, 2YMK, is a barrel-like assembly of 6 α helices that has a _____ and _____ interior.**

    A. hydrophilic; hollow
    B. hydrophobic; hollow
    C. hydrophilic; fully occluded
    D. hydrophobic; fully occluded

Answer: A
Feedback: Rendering the backbone using the hydrophobicity command and displaying the wireframe in CPK show that the interior is hydrophilic and hollow.

Mol Instructions*

- Go the PDB page for 2YMK and load the Mol* viewer.
- How many alpha helices are visible in the structure?
- If only 3 helices are visible, then click on the ribbon icon (See **Figure 3**), and select "Default (Assembly)."
- Now, 6 helices should be visible.
- As was done in Question 2, change the Polymer coloring to "Hydrophobicity."
- This should change the coloring on all chains.
- Rotate the structure until you can see down the central channel.
- Do the non-polar residues (amino acids) point towards the inside or outside of the channel?
- Do the polar residues (amino acids) point towards the inside or outside of the channel?
- Now, render the structure as a Molecular Surface.
- First, click on the "Action" button for the "Polymer" settings (see **Figure 3**).
- Then, click on "Add Representation."
- Click on "Molecular Surface."
- Rotate the structures.
- Is the central channel occluded or open?

Example: View non-covalent bonding arrangements.

Mol* Tutorials

5. **Which of the statements below is consistent with the structure of 2BEG:**
    A. The β sheets are stabilized by multiple intrachain hydrogen bonds.
    B. The β sheets are not stabilized by any intrachain or interchain ion pairs.
    C. The β sheets are stabilized by multiple intrachain ion pairs.
    D. The β sheets are stabilized by multiple interchain ion pairs.

Answer: D
Feedback: The rendering clearly shows that the β sheets are stabilized by multiple interchain ion pairs between Asp and Lys residues.

Mol Instructions*

- Go the PDB page for 2BEG and load the Mol* viewer.
- Under the "Chain" option (See **Figure 3**), choose chain A.
- From the 1˚ structure window, click on D 23.
- When clicking on D 23, all of the side chains within 5 Å will appear.
- Rotate this structure to obtain a good view of the side arm of D23.
- The side arm of D23 is bonded to which amino acid, and from which chain?
- Considering the respective charges of these amino acids, what possible non-covalent may form? Take note, the bonding information in Mol* is still being worked out, and the bonds provided in the structure may be incompletely labeled.
- Alternatively, select the respective oxygen and nitrogen atoms in the amino acid side arms (see Question 3 in Section D for instructions), and take note of the possible bonding interactions in the atom labels.
- Repeat the major steps for Chains C and D (and the other chains, if so desired).
- How are the chains (in part) held together?

Example: View the arrangement of 2˚ structures.

6. **When considering the structure for 4M43, which of the following conclusions can be made about antiparallel β sheets?**

    A. Antiparallel β sheets are mostly found in the protein interior due to the arrangement of nonpolar amino acids on both sides of the sheet.
    B. Antiparallel β sheets are mostly found in the protein interior due to the arrangement of nonpolar amino acids on one side of the sheet.
    C. Antiparallel β sheets are often amphiphilic and are mostly found on the protein exterior due to the opposing polarities of each strand in the sheet.
    D. Antiparallel β sheets are often amphiphilic and are mostly found on the protein exterior due to the opposing polarities of each face of the sheet.

Answer: D

Mol* Tutorials

Feedback: As shown in 4M43, antiparallel β sheets are amphiphilic and are mostly found on the protein exterior due to the opposing polarities of each face of the sheet.

Mol Instructions*

- Go the PDB page for 4M43 and load the Mol* viewer.
- View the structure using the hydrophobicity function.
- How do the amino acids alternate on most of the beta strands?
- To obtain more insights, highlight 1 beta strand as follows.
- Click on residue D 70 (Mol* numbering system).
- By default, all of the amino acids within 5 Å of D70 will now appear.
- To turns these amino acids 'off':
    - go to "[Focus] Surroundings (5 Å), and
    - click on the 'eye' icon.
- Only the atoms of D70 should be visible now.
- Repeat these steps for amino acids 71-76.
- How do the amino acids alternate on this strand?
- What type of amino acids point towards the solvent?
- What type of amino acids point towards the protein interior?

Example: View 3˚ and 4˚ structures.

6. **Representing the backbone as ribbons clearly shows that 1HRC is mostly _____, while an opaque surface rendering shows that Fe-heme is bound in a _____ binding site.**

    A. α helix; deep
    B. β sheet; deep
    C. α helix; shallow
    D. β sheet; shallow

Answer: A
Feedback: 1HRC is mostly α helical, and the opaque surface rendering shows that Fe-heme is bound in a deep binding site.

Mol Instructions*

- Go the PDB page for 1HRC and load the Mol* viewer.
- View the structure using the Molecular Surface function.
- For the "Polymer" component (**Figure 3**), click on the "Action" button.
- Select "Add Representation", and choose "Molecular Surface".
- Rotate the structure to view the surface topography and binding site.
- To change the opacity, re-select the "Action" option for the "Polymer" component.
- Choose the sub-menu "Molecular Surface Representation".

Mol* Tutorials

- Click on the “Action” button that is associated with the “Type” option.
- Slide the opacity function to ~0.5.
- Rotate the structure and observe the depth or shallowness of the binding site.

C. More Advanced Protein Operations

Figure 3

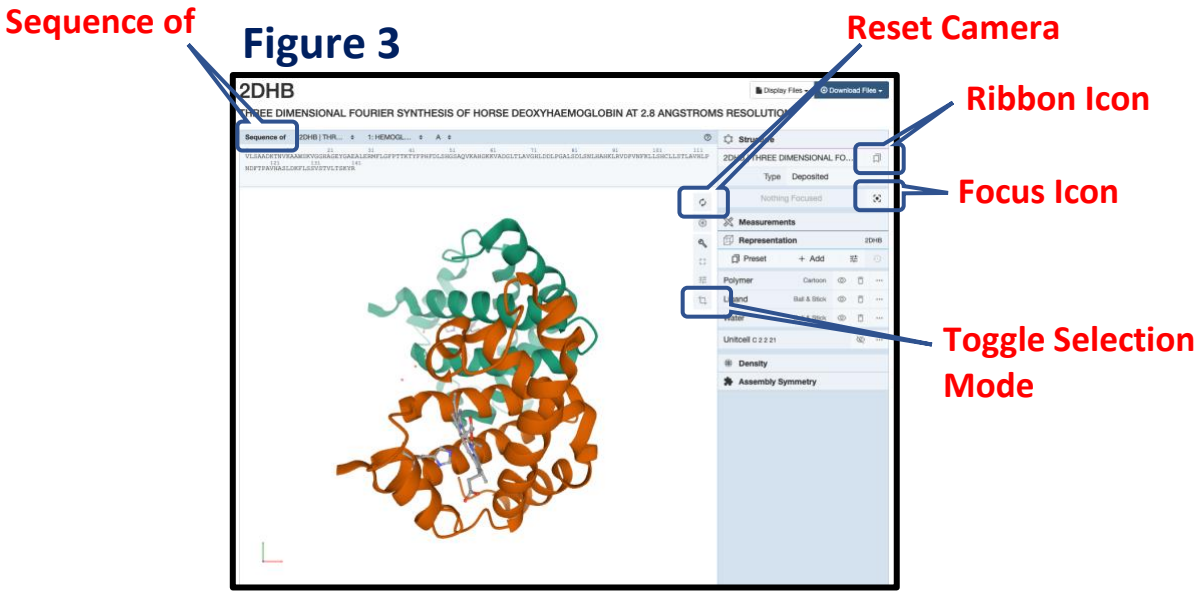
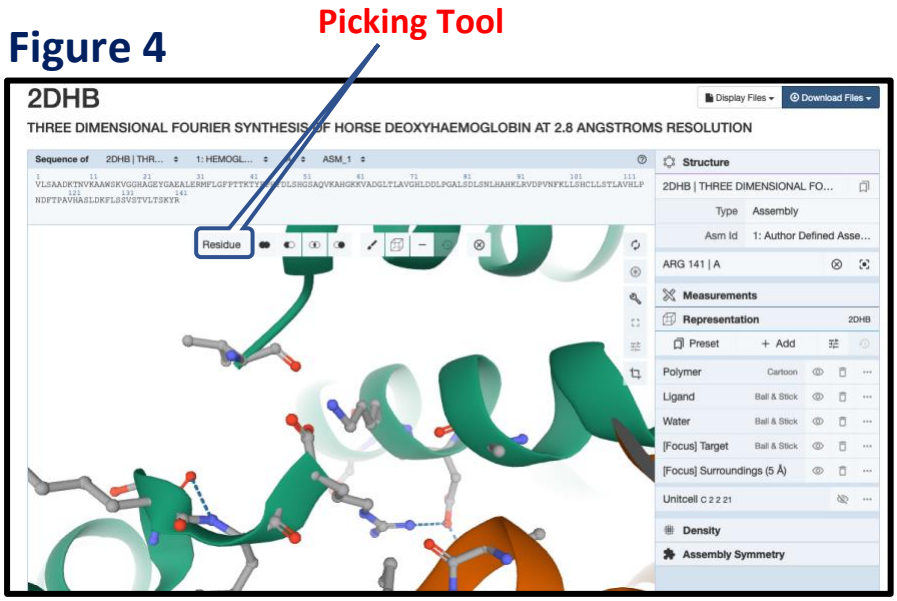


Figure 4



***Example*: Obtain stoichiometry information.**

Mol* Tutorials

- **Hemoglobin (2DHB) contains ____ protomers, known as alpha (α) and beta (β), and has a stoichiometry of _____.**

    A. 2; $\alpha_4\beta_4$
    B. 4; $\alpha_4\beta_4$
    C. 2; $\alpha_2\beta_2$
    D. 4; $\alpha_2\beta_2$

Answer: C
Feedback: Hemoglobin contains 2 protomers, known as alpha (α) and beta (β), and has a stoichiometry of $\alpha_2\beta_2$.

Method A: If using Protein Workshop

- Follow the instructions in the question.

Method B: If using only the PDB page for 2DHB

- Go to the Structure Summary tab.
- On left hand side of the window, find "Global Stoichiometry."
- As listed, what is the listed stoichiometry of hemoglobin?
- Scroll down to "Macromolecule Content."
- As listed, hemoglobin has how many "Unique protein chains" (or protomers)?

Method C: If using NGL

- Go to 3D view, keep or select the NGL viewer option.
- Use the right-hand menu to render the protein structure.
- For the "Assembly" option, select "Asymmetric Unit."
- For the "Color" option, select "By Chain."
- As per this rendering, hemoglobin has how many unique chains?
- For the "Assembly" option, now select "Bioassembly 1."
- As per the chain colors, what is the total stoichiometry?

*Method D: If using Mol**

- Go to 3D view, keep or select the Mol* viewer option.
- As per the colors in the structure, hemoglobin has how many unique chains?
- Now, use the right-hand menu to change the viewing options.
- Click on the Ribbon icon (see Image A), and select "Default (Asssembly)."
- Rotate the structure to get a better view of the protein chains.
- As per this rendering, hemoglobin has how many total chains?
- As per the chain colors, what is the total stoichiometry?

***Example*: View ligand binding sites.**

Mol* Tutorials

- **As per the Mol* default rendering, the Fe-heme groups in 2DHB are bound to the protein by _____ in a stoichiometry of ___ per subunit.**

    A. only hydrophobic interactions; 1
    B. metal coordination and Pi stacking; 1
    C. only hydrophobic interactions; 4
    D. metal coordination and Pi stacking; 4
    E. only hydrogen bonding; 4

Answer: D
Feedback: As per the Mol* default rendering, the Fe-heme groups in 2DHB are bound to the protein by metal coordination and Pi stacking in a stoichiometry of 4 per subunit.

Mol Instructions*

- To view the binding site of 1 subunit, click on the Focus icon (see Image A), choose "PROTOPORPHYRIN IX CONTAINING FE", and select "[HEM] 142 | C."
- Rotate the ensuing structure until you find a satisfying view of the ligand binding site.
- If the image looks 'faded', click on the "Reset Camera" button (see Image A).
- In this view, how many ligands are bound to 1 subunit?
- To gain insights into some of the interactions between the protein and ligand, scroll the mouse over the dotted bonds between the Fe-heme group and protein.

Example: View non-covalent interactions between subunits.

- **Which of the following statements best describes how an ion pair forms between Arg 141 in the α_1 chain and Lys 127 in the α_2 chain (note the symmetrical arrangement of the subunits in 2DHB)?**

    A. An ion pair forms between the N-terminal ammonium of Arg 141 in the α_1 chain and the side chain ammonium of Lys 127 in the α_2 chain.
    B. An ion pair forms between the C-terminal carboxylate of Arg 141 in the α_1 chain and the N-terminal ammonium of Lys 127 in the α_2 chain.
    C. An ion pair forms between the side chain of Arg 141 in the α_1 chain and the side chain ammonium of Lys 127 in the α_2 chain.
    D. An ion pair forms between the C-terminal carboxylate of Arg 141 in the α_1 chain and the side chain ammonium of Lys 127 in the α_2 chain.

Answer: D
Feedback: An ion pair forms between the C-terminal carboxylate of Arg 141 in the α_1 chain and the side chain ammonium of Lys 127 in the α_2 chain.

Mol* Tutorials

Mol Instructions*

- Begin with the "Default (Asssembly)" view of the protein, (as in Question 1).
- Using the top selection bar ("Sequence of" – see image), select the Hemoglobin Alpha Chain and ASM_1.
- This function should choose 1 alpha chain.
- In Sequence window, select Arg 141.
- This function will 'turn on' all amino acids within 5 Å of Arg 141.
- Among the highlighted amino acids, where is Lys 127 (from the α_2 chain)?
- Which functional groups of Arg 141 and Lys 127 are in close proximity?
- To determine if they may be bonded, click on the "Toggle Selection Mode" tool (see Image A).
- In this option, use the "Picking Tool" (see Image B), and select the "Atom/Coarse Element" option.
- Now, click on the nitrogen atom in the side arm of Lys 127 (it should turn green).
- Change the representation of this atom to Spacefill by doing the following:
    - Click on "+ Add" (under Components),
    - Click on "<Create Later>,
    - Scroll down and choose "Spacefill",
    - **Click on "Create Component,**
    - ***And wait for a moment***;
    - These selections should change the nitrogen atom to a large sphere.
- Repeat Steps 10-11 for the single-bonded oxygen in the carboxylate group of Arg 141 (which is the C-terminal amino acid).
- What does the Spacefill representation mean?
- What does the overlap between the spacefill side chains indicate?

Example: View interactions between subunits.

- **In hemoglobin (2DHB), the β_1 and β_2 chains share _____ intermolecular interactions.**

    A. no
    B. multiple

Answer: A
Feedback: Surface renderings of 2DHB show no evidence of intermolecular interactions between the β_1 and β_2 chains.

Mol Instructions*

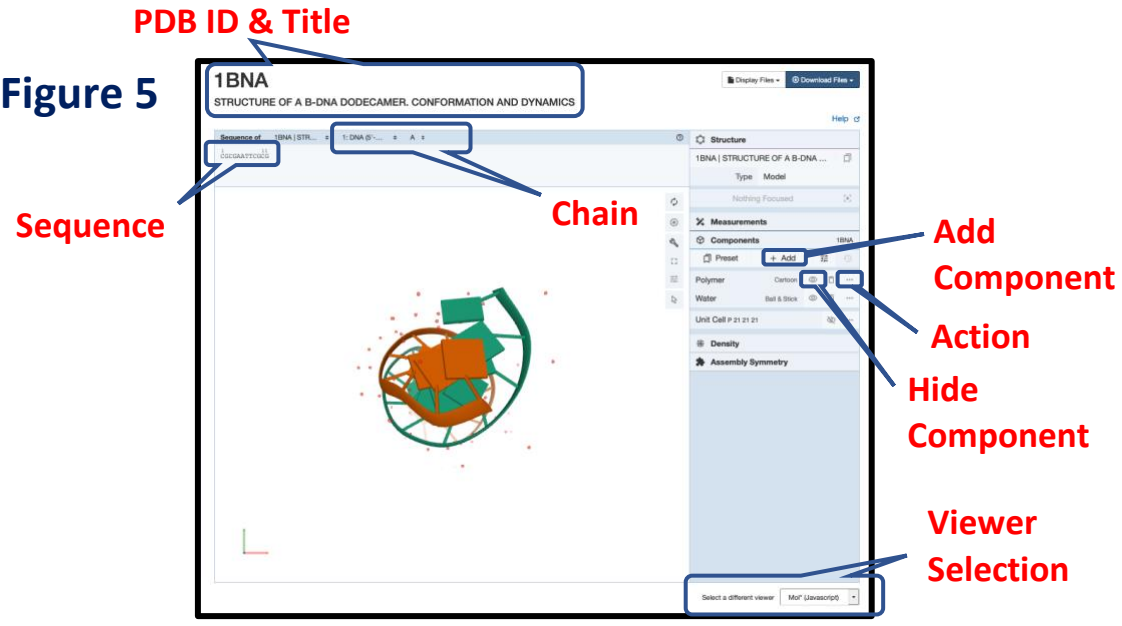
- Refresh your browser window, and start with a fresh structure.
- Start with the "Default (Assembly)" view of the protein, (as in Question 1).
- Use the top selection bar ("Sequence of") to ensure which subunits are the beta chains.
- Select the Hemoglobin Beta Chain and ASM_1.

Mol* Tutorials

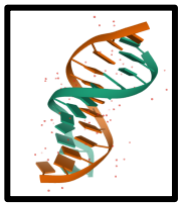
- Scroll the mouse over the Sequence window to highlight the correct subunit.
- Repeat Steps 3-5 for Beta Chain and ASM_2.
- Click on the "Toggle Selection Mode" tool (see Image A).
- Select the "Chain" option.
- Click one of the beta subunits.
- Now, change the representation to a "Molecular Surface" by doing the following:
    - Click on "+ Add" (under Representation),
    - Click on "<Create Later>,
    - Scroll down and choose "Molecular Surface",
    - **Click on "Create Component"**,
    - ***And wait for a moment***;
    - These selections should provide a 'surface' view of the subunit.
- Now, repeat steps 5-6 for the 2nd beta chain.
- Rotate the structure and observe the relations between the beta chains.
- Do the surfaces interact?

C. Basic Operations For Nucleic Acids

Figure 5



Example: Change rendering options.



- As shown in the following rendering of B-DNA, components of the DNA structure can be represented by ribbons and squares. Comparison of the following rendering options confirms that the ribbon structures represent _____.

Mol* Tutorials

A. the torsional bond angles along the DNA backbone
B. the torsional bond angles of the modified ribose components
C. the approximate positions of the phosphodiester linkages
D. the overall shape of the DNA backbone

Answer: D
Feedback: The ribbon diagram is a trace through the backbone, which highlights the general structure.

Option A: *Render as Ball & Stick*

1. Rotate the structure to obtain a view of the entire molecule (as shown in the figure above).
2. Turn off or "hide" the ribbon/cartoon component.
3. Click on the 'eye symbol' (**Figure 2**) for the 'Component' referred to a "Polymer".
4. Only the water molecules (red dots) should be visible now.
5. To view the atoms and bonds of the DNA, click on "+ Add" ("Add Component", **Figure 2**).
6. Click on "Current Selection" (next to Selection) to obtain several options.
7. Select the "Type" option, and choose "Nucleic".
8. Click on <Create Later> next to "Representation".
9. Select "Ball & Stick".
10. **Click on "Create Component.**

Option B: *Re-Render as Cartoon*

1. Turn on the Cartoon option by selecting the "Hide Component" function (eye symbol; **Figure 2**).
2. This option will overlay the Cartoon rendering option on the Ball & Stick option.