

Task 1

Visit the PDB site: <http://www.rcsb.org>

Search the structure DNA binding domain of the human p53 protein

1. Input “p53” to the search field.
2. At “Query Refinements” select “Homo Sapiens”.
3. Select the “Polymer type” → “mixed” option.

How much results do we have?

Select the **4HJE** structure (You may have to enlarge the number of proteins shown).

When was this structure released?

Where was it published?

What additional information can be found on the page?

What is the UNIPROT ID of the protein?

Which additional structures belong to this UNIPROT ID?

(Hover the mouse over the UNIPROT ID near the green line and click. Here select the “Show All PDB-UniprotKB mappings...” options)

The screenshot shows the RCSB Protein Data Bank website for the structure P04637 (P53_HUMAN). The page is displayed in a Mozilla Firefox browser window. The main content area shows the following details:

- Molecule:** DNA (5'-D>(*TP*TP*TP*CP*CP*TP*AP*GP*AP*CP*TP*TP*GP*CP*CP*A P*AP*TP*TP*A)-3')
- Polymer:** 1 Type: dna Length: 21
- Chains:** E
- Molecule:** DNA (5'-D>(*AP*TP*AP*AP*TP*TP*GP*GP*GP*CP*AP*AP*GP*TP*CP*TP*A P*GP*GP*AP*A)-3')
- Polymer:** 2 Type: dna Length: 21
- Chains:** F
- Molecule:** PROTEIN (P53 TUMOR SUPPRESSOR)
- Polymer:** 3 Type: protein Length: 219
- Chains:** A, B, C
- Organism:** Homo sapiens
- Gene Names:** TP53 P53
- UniProtKB:** Protein Feature View | Search PDB | P04637

Experimental Details:

- Method:** X-RAY DIFFRACTION
- Exp. Data:** N/A
- Resolution [Å]:** 2.20
- R-Value:** 0.205 (obs.)
- R-Free:** n/a
- Space Group:** C 2
- Unit Cell:** Length [Å] Angles [°]
a = 117.50 a = 90.00
b = 67.90 β = 105.50
c = 108.80 γ = 90.00

Source:

Polymer	Scientific Name	Taxonomy
Polymer: 1	Synthetic construct	Taxonomy
Polymer: 2	Synthetic construct	Taxonomy
Polymer: 3	Homo sapiens	Common Name: Human Expression System: Escherichia coli

Ligand Chemical Component:

Identifier	Formula	Name	View Interactions
ZN	Zn ²⁺	Zn	ZINC ION

The pop-up window shows mappings for P04637 to P53_HUMAN, with options to show all PDB-UniprotKB mappings.

Here on the macromolecules part you can see a “+” sign.
 Click it.
 A new page opens, where you can see a somewhat similar page.

What informations can you find here?

What other structures belong to this protein?

Are there any mutations annotated to this protein?

In which database did we see a similar type of representation with (somewhat) similar data?

Task 2

Introduction to Chimera. Launch the Chimera application!

Fetch the structure of myoglobin (**2HHB**)!

File

Fetch by ID

Type the PDB code

Fetch!

Try to move the structure with the mouse buttons!

Default Mouse Button Assignments		
Mouse button	Modifier	Action
Btn1 (left button)		Rotation
Btn2 (middle button)		XY Translation
Btn3 (right button)		Scaling
Btn1	Ctrl	Picking (selection)
Btn1	Ctrl-Shift	Addition to (removal from) selection

Lets view the options in the **Actions** menu:

Actions Menu Items	
Menu Item	Description
Atoms/Bonds	Controls the display and representation of atoms and bonds.
Ribbon	Controls the display and representation of ribbons.
Surface	Controls the display and representation of molecular surfaces.
Color	Colors selected objects. Color target can be limited to object types indicated by the radio buttons.
Label	Labels selected atoms. The residue submenu labels residues containing the selected atoms.
Focus	Focuses the view on the selected atom(s), zooming and translating if necessary.
Set Pivot	Sets the center of rotation based on the selected atom(s) without adjusting the view.
Inspect	Launches the Selection Inspector .
Write List	Writes a list of the currently selected objects to a parsable text file.
Write PDB	Writes the coordinates of the currently selected atoms to a PDB file.

Go back to the web, and try to find your favorite protein in the PDB. If you found it load it inside Chimera.

Selection:

(You can either use your favorite protein, or myoglobin)

Selection can be done with the corresponding menu, or with the mouse in the graphical interface.

To select with the mouse hold down the Ctrl key and click at a residues.

To make the selection wider or thicker use the Up and Down arrow keys. Select the whole molecule and color it in the Actions menu.

Now erase the selection by Ctrl clicking on a background area.

Select the A chain of the molecule with

Select → **Chain** → **A**

Show every atom in the A chain with sticks using the actions menu.

Now lets view the sequence of our protein. **Favorites** → **Sequence**

You can also select residues from the sequence windows by click and drag method.

Try some additional action options on selection.

We will use selection a lot, so get used to it!

Try the different representation options!

Start with some of the “**Preset**” options.

Use the “**Rainbow**” coloring, first on subunits, then on amino acids.

Tools

 Depiction

 Rainbow

Now close the session.

File → **Close session**

Task 3

Surface of structures.

Load the **1d86** molecule.

There are built-in categories within structures such as **main** and **ligand**; when nothing is selected, **Actions... Surface... show** displays the surface of **main**.

Actions... Surface... show

Actions... Surface... hide

Select... Structure... ligand

Actions... Surface... show

Actions... Surface... mesh

How well does the ligand fit?

Surface color can be specified separately from the colors of the underlying atoms. The ligand surface is tan and white because the original model color (tan) is used for surfaces of atoms not explicitly recolored by the user, and above, only the carbon atoms were changed to white. With the ligand still selected, choose **Actions... Color... all options...** to open the **Color Actions** dialog. In that dialog,

1.change the **Coloring applies to** (target) setting to

surfaces 2.click **red**

3.click **Close** (which will automatically reset the coloring target back to **all of the above**)

Lets get back to myoglobin (2HHB), so close the current session, and open it again.

Make the surface visible. (Lets ignore the error message)

Use Tools →Surface/Binding Analysis → Coulombic surface coloring and click OK. (Lets ignore the error message)

Note that this might take a while on slower computers.

What can we tell about the ligand binding surface?

Task 4

Close your current session

Fetch the structure **121p**

Open up the command line: Favorites → Command line Lets

open two additional structures with the command line. Type

„open **1r2q**” then hit enter. Open **1j2j** the same way.

Open up the Model panel. Favorites → Model panel. Lets try the options here to hide and show the loaded structures.

Now we are going to align the structures.

Click on Tools → Structure Comparison → MatchMaker

Select 121p as the reference structure and hit OK.

You can see the information in the **Reply Log** window (**Favorites** → **Reply Log**)

How similar are these structures?

Which model has two additional helices compared to the others?

As we know Chimera uses a sequence based alignment so lets

view that. Click on Tools → Structure Comparison → Match Align

Select the A chain of each loaded molecule, then hit OK.

How similar are the sequences?

Task 5

The mutations of p53 in cancer cells is very common. The most common one is the substitution of 273 arginine to a cysteine or histidine.

Create this mutation, and try to understand its effect!

- ~ Read the 4HJE structure.
- ~ *How many chains are in this structure?*
- ~ Color the chains with "Rainbow".
- ~ Show the sequence of the A chain
- ~ Find the 273th position (hover the mouse), and select it with click and drag.
- ~ Show the atoms of this residue
- ~ *Which atoms are in contact with this residue?*
 - Tools
 - Structure analysis
 - Find Clashes/Contacts
 - Designate
(We designate the selected atoms this way) For the other group select all other atoms

Click on "Contact"
Select "Select, Draw pseudobond" and "Write Information to reply log" options

Also, show the amino acids that are in contact. Select

- Broaden selection
(Remember you can also use the up arrow key) Actions
 - Show Atoms/Bonds
 - Show

Mutations

In Chimera there are no default ways to make mutations, but we can use rotamers. Select the arginine in the 273 position

- Tools
 - Structure Editing
 - Rotamers

Select Histidine.

Review how well they fit

Select the one that fits the best then click Apply

Select the mutated histidine residue and find its contacts! Explain the effect of this mutation!

Task +1

Now in a new session mutate the same amino acid again but now select one of the most unlikely rotamer.

Is there a clash?

Minimize the mutated amino acid with 50-100 steps according to the second video. Note that the minimization may take a lot of time on slower computers

Is there still a clash?

In case there is, try to eliminate it with some more minimization steps.

How similar did it become to the most likely rotamer?

If you are finished continue at:

<https://www.cgl.ucsf.edu/chimera/tutorials.html>