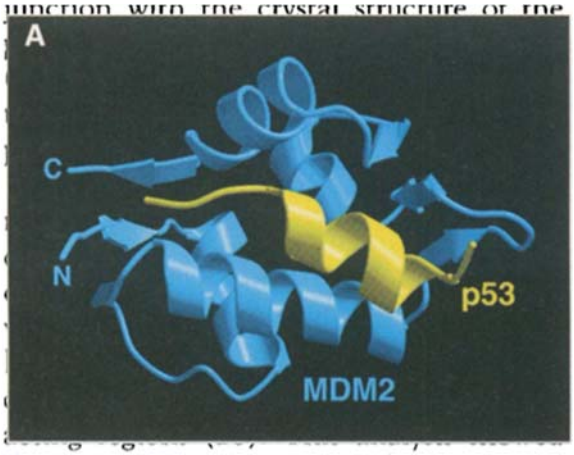
"Protein-Protein interfaces; Chimera (I)"

In this exercise, we will use a protein called MDM2 as an example. MDM2 is an important protein in cancer—it has the ability to bind the transactivation domain of tumor suppressor protein p53, down regulating its ability to activate transcription. Many researchers have sought to develop anti-cancer drugs that target the binding site of MDM2, blocking p53 from binding, and thus up regulating the tumor suppressor activity of p53. Several of these drugs are currently in clinical trials[1].

The first high-resolution atomic structure of MDM2 bound to p53 came from an X-ray crystal structure by Kussie et al. (1996)[2]. This structure shows a small helical region of p53 that binds a hydrophobic cleft in MDM2. To get these proteins to co-crystallize well, constructs with only residues 17-125 of MDM2, and residues 15-29 of p53, were used. Only the atomic structures of residues 25-109 could be resolved, however.



The X-ray crystal structure of MDM2-p53 (PDB ID: 1YCR)

Viewing and visualizing PDB files:

To find a given PDB structure, open a browser and go to http://pdb.org. In the search window, type in the PDB ID, which will bring you to the entry page. This page has a great deal of useful information about the protein sequence and structure.

To view the contents of the deposited PDB file containing the atomic coordinates, click on the **Display Files** drop-down in the upper right and choose **PDB File**. The full contents (text) of the PDB file should be viewable in your browser.

[1] Hoe, K. K., Verma, C. S., & Lane, D. P. (2014). Drugging the p53 pathway: understanding the route to clinical efficacy. Nature Reviews Drug Discovery, 13(3), 217–236. doi:10.1038/nrd4236.

[2] Kussie, P. H., Gorina, S., Marechal, V., Elenbaas, B., Moreau, J., Levine, A. J., & Pavletich, N. P. (1996). Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science, 274(5289), 948–953.

To view the molecular structure in 3D, open the **Chimera** application and choose **File > Fetch by ID …** Type the PDB ID into the text field and click **Fetch**. The ribbon structure of the protein should appear, which you can rotate in 3D by clicking and dragging.

Procedure:

Part A: Let's investigate how the p53 helix interacts with the MDM2 receptor.

Finding interacting residues:

1. Load the structure using **File** > **Fetch by ID** … The ribbon structure of the protein complex should be shown
2. Select the p53 helix by Control-clicking (holding the CNTL key and clicking with the mouse) on p53, then press the up-arrow key to select the entire chain.
3. Find the receptor residues interacting with p53 using **Tools > Surface/Binding Analysis>Find Clashes/Contacts**.
    a. In the "Atoms to Check" panel, click on the "Designate" buttons with the correct options so as to calculate interactions between the p53 helix (selected) and other atoms in the model.
    b. In the "Clash/Contact Parameters" panel, click on "Contact" (not "Clash"). Then click on "Apply" below.
    c. You should see a number of atoms in the helix and receptor selected. To select the *residues* involved, press the up-arrow key.
    d. Visualize these residues using **Actions** > **Atoms/Bonds** > **show**. It may be helpful to **Actions** > **Color** > **by element**.
    e. Answer the **questions** for **Part A** (**page#3**).

Part B: Measuring surface area changes upon binding

1. Select the entire complex by control-clicking on any residue, and pressing the up-arrow key until the entire structure is selected.
2. Show the entire surface using **Actions** > **Surface > show**.
3. Calculate the total volume and surface area using **Tools > Surface/Binding Analysis > Measure Volume and Area**. The values are reported in units of $Å^3$ and $Å^2$, respectively.

Repeat these calculations for …

a. … the MDM2 receptor by itself (select the p53 helix and delete it using **Actions** > **Atoms/Bonds** > **delete**.), and
b. … the p53 helix by itself (reload 1YCR, select MDM2 only and delete it)

4. Complete the **Table** and answer the **questions** for **Part B** (**page#3**).

Name: **TUID:**

Section:

Part A:

Q1. Which residues of p53 have the most interactions with the MDM2 receptor?

Q2. Which residues would you say are making hydrophobic interactions, and which have polar interactions?

Part B:

Q1. Complete the following table:

	1YCR (entire complex)	**MDM2**	**p53**
Volume ($Å^3$)			
Surface Area ($Å^2$)			

Q2. What is the total change in surface area upon binding of the p53 helix to MDM2?

Q3. What is the total change in Volume upon binding of the p53 helix to MDM2?

Q4. Using the data from Figure 1a of Chen et al. (2013) (**page#4 of this worksheet**), make a rough estimate of the binding affinity of p53 helix (i.e. its dissociation constant K_d).

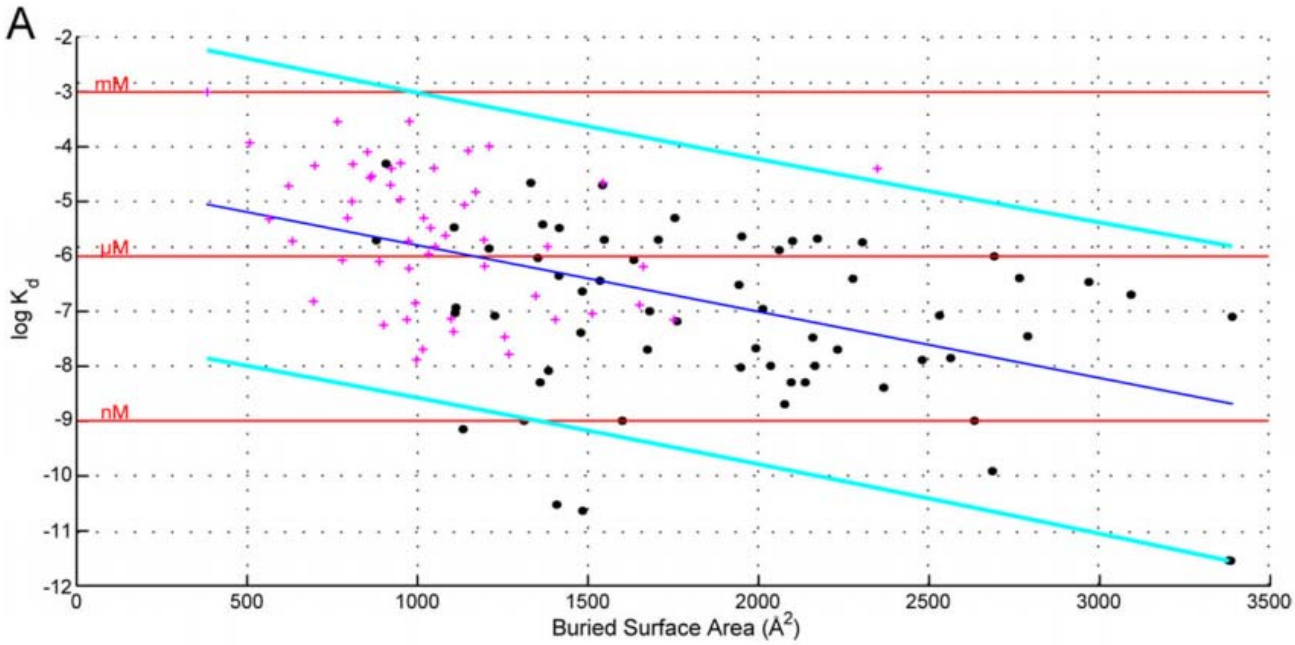


Figure 1a from Chen et al. (2013)[3]

[3] Chen, J., Sawyer, N., & Regan, L. (2013). Protein-protein interactions: General trends in the relationship between binding affinity and interfacial buried surface area. Protein Science, 22(4), 510–515. doi:10.1002/pro.2230.