Pharmacophore Searching

In this lesson you will learn how to perform a pharmacophore search. The target of interest is the MDM2/P53 dimer. In this lesson, we will build a pharmacophore of the P53 protein and we will use this pharmacophore to identify compounds that might have the potential to replace P53 in binding MDM2.

> Task: Look up some background about the MDM2/P53 system and figure out its pharmacological relevance.

1. Open a subfolder on the desktop and name this folder "Pharmacophore_searching"
2. Open a terminal using the right button on your mouse and change directory into the recently opened folder:

   ```
   $ cd Desktop/Pharmacophore_searching
   ```

3. Download a ligand database that is already prepared fro pharmacophore searching:

   ```
   $ git clone https://github.com/UAMCAntwerpen/2040FBDBIC.git
   ```

   ```
   $ cd 2040FBDBIC/Class_03
   ```

4. Start Maestro and make sure to change the working directory to "`Desktop/Pharmacophore_searching/2040FBDBIC/Class_03`"
5. Download and prepare the MDM2/P53 complex. The PDB-code of this complex is 4HFZ.
6. After downloading, you should prepare the structure as usual. The protein is crystallised as dimer of a heterodimer. Chains C and D are comparable to A and B, so you should delete chains C and D. You also can delete all waters and the sulfate group.
7. Now is time to split the prepared structures into its two monomers. You can do this by right-clicking the prepared entry ("4HFZ - prepared"), and selecting "Split > By Chain". Two new entries are produced: "4HFZ – prepared_chainA" and "4HFZ – prepared_chainB". Chain A is MDM2 and Chain B is P53.
8. Make sure that you have selected both chains (the two circles should both be blue). Under "Tasks", look for "Develop Pharmacophore Hypothesis" and select it. This open a window in which we can define a pharmacophore model.
9. Select "4HFZ – prepared_chainB" and make sure that you specify to build a pharmacophore model from a single ligand in the workspace (you need to specify this in the "Develop Pharmacophore Model" dialog box. A large number of pharmacophore features are given, but it is up to you to define the ones of which you think might be crucial. It has been shown in numerous studies that three residues are quite important for binding of P53 to MDM2: Phe-19, Trp-23, and Leu-26.
10. After having selected the desired pharmacophore points, you can now click the "Create" button to start building a pharmacophore. This is a fast process and the generated pharmacophore is imported in the workspace.

11. Now we can start with our pharmacophore screen. Look for “Ligand Screening” task and open this window. Both an database and a pharmacophore hypothesis have to be specified:

    a. Ligands to screen: select “Phase Database” and then “Add Database > Browse”. Specify the Enamine_PPI database that was downloaded in step 3.

    b. Hypothesis: select “Add Hypothesis > Workspace”. This adds the just created hypothesis.

    Start the search.

12. After completion, all hits are incorporated in the workspace. The initial compound database was composed of compounds with high potency to inhibit protein-protein interactions (all compounds starting with “Z”). In addition, the database as spiked with siz compounds that were shown to inhibit the MDM2/P53 system (AMG232, CGM097, DS3032b, RG7388, R054337, SAR405838).

13. Have a look at the Project Table to see how well the compounds score, and that all compounds match all features. All compounds with a higher score than the worst spiked compound (SAR405838; row 317) should be considered to be hits.

Next step: save to best-ranked compounds to a file and use this as imput for a docking setup.