Lab sheet#6

ExPASy Tools

Objective:

- Translation of a nucleotide sequence to a protein sequence using ExPASy.
- Analysis of protein primary structure and computing various physical and chemical parameters

A. Translation of a nucleotide sequence to a protein sequence using ExPASy web page:

1. Retrieve the FASTA format of homo sapiens X-ray repair cross complementing 1 (XRCC1) mRNA sequence (**NM_006297.3**) from the NCBI GenBank database.
2. Use the translate tool on ExPASy website:
   - Paste the retrieved XRCC1 mRNA sequence into the box.
   - Click the "**Translate sequence**" button.
   - Choose the most reliable predicted protein sequence.

B. Primary Structure Analysis of a Protein Using ProtParam on the ExPasy server:

1. Search for ProtParm, click the first link.
2. Copy the protein sequence of XRCC1 protein (**CAG33009.1**), then paste in the ProtParm webpage.
3. Click compute parameters.
4. Find out the Molecular weight of the protein.
5. How many Cysteine amino acids located in the protein sequence.
6. What is the total number of negatively charged residues.
7. Find out the Estimated half-life of the protein in mammals and yeast.

✓ **Exercise:**

2

- ✓ Find the most reliable predicted protein sequence for human TRF2 mRNA (NM_005652.4).
- ✓ What is the molecular weight, Ip and number of positively charged residues in TRF2 (NP_005643.2).

Lab sheet#7

Structure visualization using jmol

Objectives:

- Download protein sequence as PDB format using PBD ID.
- To be familiar with structure visualization program (Jmol).
- Display 3D structure of a protein, change its view, color and select motifs by writing commands.

Use Jmol program to perform the following:

Jmol is a free molecular viewer, used to create and view three dimensional structures of proteins. This viewer is linked to the Protein Data Bank (**PDB**) website, which provide biological macromolecular structures.

Note: in case of any difficulty downloading Jmol as an independent program on a desktop, you can use the web-based version (oftern refered to as "JSmol") that runs on a JavaScript platform (https://chemapps.stolaf.edu/jmol/jsmol/jsmol.htm). Make sure that Java is installed.

Visualization of ATP-binding subunit of the histidine permease from salmonella typhimurium.

Each structure hosted on the Protein Databank has a unique four character long alpha-numeric identifier, referred to as the structure's **PDB ID**.

1. Open Protein Data Bank (**PDB**) website, Search for PDB ID of the protein above, **download the structure as PDB format**, then answer the following questions:

    - Who are the authors of the PDB file?
    - From what source was this molecule isolated?
    - In which journal was the primary citation published?
    - On what date was the file deposited into the PDB?
    - How many chains are in this file?
    - Are there any heterologous groups within this PDB file? If so, which ones?

2. Open Jmol program and get the protein structure by entering PDB ID. **(file➔ load➔open from PDB).**
3. Change Style display from Atom style to **Cartoon** scheme style. **(Click right ➔style➔scheme➔cartoon).**
4. Open the console window, change the **color** of the whole structure to **grey**. **(Click right ➔ console➔type: select all➔ press enter➔ type: color grey➔ press enter).**
5. Open the protein page in protein database (NCBI) to know the different motifs of the protein and their location.
6. Select the **Walker A/P-loop motif** (39-46), and color it by blue. **(type: select 39-46 ➔press enter➔ type: color blue➔ press enter).**
7. Select the **ABC transporter signature motif** 154-163, and color it to green. **(type: select 154-163 ➔ press enter➔ type: color green ➔ press enter).**
8. Select **Walker B motif** 174-179, and color red. **(type: select 174-179 ➔ press enter➔ type: color red ➔press enter).**
9. Show the ATP **ligand** as **Ball and stick** scheme style. **(type: select ligand ➔ click right ➔style➔scheme➔ ball and stick).**
10. Change the measurement unit from **nm to Angstroms**. **(click right ➔measurements➔distance unites angstroms)**
11. Show which one is closest to **Walker A/P-loop motif** and measure the **distance** between them. **(double click on the ligand then drag the mouse towards the motif)**
12. Save the protein structure as a picture. **(right click → file → save → save as PNG)**

Here is → Video displaying the steps to visualize the protein above:

https://drive.google.com/file/d/1cD4R0XxGxRbi40LI4FJlrbIoz79_3GrW/view?usp=sharing

Exercise

1. Open Protein Data Bank (**PDB**) website, Search for PDB ID of T3R3 Human Insulin Hexamer.
   - Go down to the molecule description to see how many polymers and chains does insulin have.
2. Open Jmol program and get the protein structure by entering PDB ID. **(file➔get PDB).**
3. Change Style display from Atom style to **Cartoon** scheme style.
4. Change Style display to **Backbone 1.5** scheme style.
   (Click right ➔console➔type: Backbone 1.5➔press enter).
5. Turn cartoon style off. **(type: cartoon off➔press enter)**
6. Select sheets and color red.
   (type: select sheets ➔ press enter➔ type: color red ➔press enter)
7. Select helix and color blue.
   (type: select helix ➔ press enter➔ type: color blue➔press enter)
8. Show cysteins (Sulfur) that forms disulphide bridges "showing how the polypeptides hold together through S-S bonds". Change to wireframe 1.25 and color them yellow.

   (type: select sulfur ➔ press enter➔ type: wireframe 1.25➔press enter➔type: color yellow).

   - How many disulphide bonds are found in insulin protein?
   - Show for each disulphide bridge the position of each Cys and the chain involved.
9. Move the structure 0 360 0 0 0 0 0 0 10. **(type: move 0 360 0 0 0 0 0 0 10 ➔press enter)**
10. Save the protein structure.