

Mini-Workshop: Protein Data Bank (PDB) and Moorhen.org

Context Many different approaches can be used to design and develop therapeutics to treat diseases like cancer. One of these approaches is known as **rational design**: If you have identified your protein target for inhibiting a specific cancer, then you can design a molecule to specifically bind at its active site to inhibit its function and activity. You can synthesize it, test it against your target protein and the cancer cells, and determine its mechanism and binding pose experimentally through structural biology techniques.

Crystallography One useful structural biology technique is crystallography (Fig. 1). Once you crystallize your protein with the molecule, perform X-ray diffraction experiments on your crystal, and use the diffraction data to build models of the protein-molecule complex, you can see their interactions at the active site with atomic resolution. This information is very useful for understanding how the compound is binding, which amino acid residues it is interacting with at the protein surface, and how your compound can be optimized to enhance its binding strength (e.i. binding affinity).

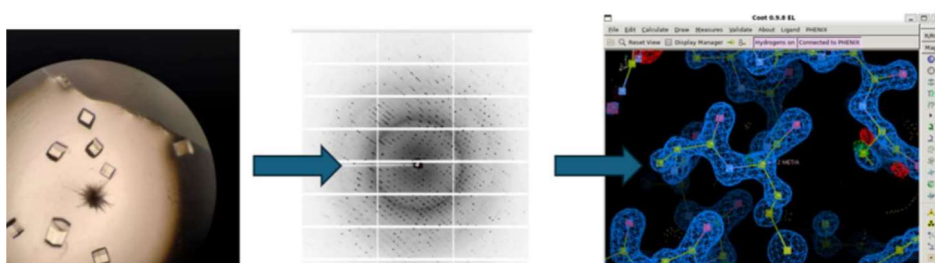


Figure 1. Crystallography technique, including (1) crystal growth and optimization (left), (2) X-ray diffraction experiments on crystals (middle), and (3) model building and refinement (right).

Protein Data Bank (PDB) and Moorhen.org When researchers solve structures for diseases and publish their work, where are all these protein structures stored? In the PDB! It is an online database of proteins, protein/molecule complexes, and other macromolecular structures. Any person can access the PDB online, download the crystallography data, and download the solved structures. What if we wanted to view the crystallography data ourselves, how should we do that? One way is through Moorhen.org, the online version of Coot, a model-building software for solving protein structures.

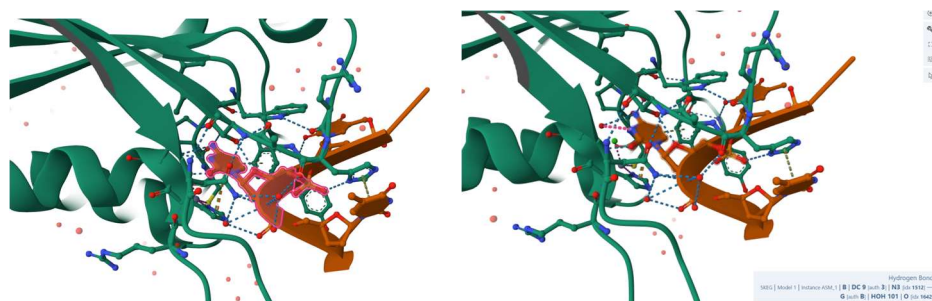


Figure 2. PDB (left), Moorhen.org (middle), and Coot (right) logos.

Skills Learn how to navigate the PDB and Moorhen.org to learn about structure characteristics and related information to allow for personal investigations.

Materials Laptop.

- Guided PDB example: 5KEG
 - o Search 5KEG (the PDB ID)
 - o Find the research authors and PI, publication DOI, structural biology method, and resolution
 - This is where you tell your students how to perform the experiment in a nice, succinct way without being wordy while being detailed enough that it is repeatable by a student who may not be familiar with crystal-growing or crystallography.
 - o Scroll down and find the types of macromolecules in this structure
 - o Go to the Structure tab and zoom in to an interesting interface, click on it, and hover above it to look at the molecular interactions taking place



- Your turn: 5F9R
 - o Determine the same things from above and check against the answer
- On your own: Find an interesting protein complex, whether if it has an interaction with a known anticancer agent or if it has an interaction with nucleic acids
 - o Determine the same things from above and share with your neighbor

- Guided Moorhen.org example
 - o Go to Moorhen.org, click on the three lines, open File, scroll down and click “Load Tutorial Data,” then click “Tutorial 1,” then click “OK”
 - o Click your space bar to advance forward in the chain of amino acids
 - o Click shift + space bar to go backwards in the chain of amino acids
 - o Blue region is where there is electron density from the diffraction experiment
 - o The model is built based on the electron density
 - o Green region is where there is electron density but with no model to explain it
 - o Red region is where there is a portion of the model in a space where there is no electron density from the experiment

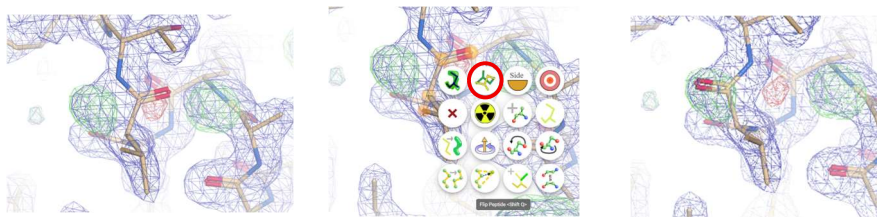


Figure 4. Fixing the positions to move into the green space and move out of the red space on Moorhen.org.

- o When solving a structure from the electron density the important part is moving the atoms in the model into areas of green and removing it from areas of red to maximize its match with the electron density (to build an accurate model of the interactions at the atomic level)

Next Steps This was an introduction to the crystallography and model-building and model refinement work that STARS at GT will be organizing for its club members and GT students this semester.

Further Reading

1. The Nobel Prize. The Nobel Prize in Chemistry 2009. *The Nobel Prize*.
<https://www.nobelprize.org/prizes/chemistry/2009/popular-information/>
2. The Nobel Prize. The Nobel Prize in Chemistry 2020. *The Nobel Prize*.
<https://www.nobelprize.org/prizes/chemistry/2020/popular-information/>

Challenge Find the DOI on 5F9R and see if the research paper specifically addresses which amino acid residues interact with certain DNA nucleobases. Go onto the PDB Structure viewer and track that interaction yourself and see if the PDB recognizes the same intermolecular reactions as the research paper reported (if it reported it).

Try Tutorial 2 on Moorhen.org and see if you can fix all the errors.