Announcements

• *When you are done with the computer lab, or after two hours, you may complete any remaining Chapter 3 work*

• Both Chapter 3 and Chapter 11 post-lab write-ups are due at the beginning of Chapter 4 labs (Oct 23 – Oct 29)
Chapter 11: Molecular Modeling

**Purpose of molecular modeling**
- Gain an understanding of protein-ligand interactions
- To study the active site of one protein and see if interactions are applicable to another similar enzyme
- More efficient drug design (BI422, SPRING)

*Chemists, molecular biologists, and biochemists use molecular modeling as means to understand specific protein-ligand interactions.*

This semester you will only be making observations on your protein of interest. Next semester you will be making mutations in your enzyme.
Chapter 11: Molecular Modeling

**Purpose of Week 1:**

A) Familiarize yourself with the NCBI BLAST and Protein Data Bank databases, and the protein analysis software PyMOL

B) Analyze a protein of interest and inspect enzyme-ligand interactions
Procedure: Chapter 11

- There's no formal pre-lab write-up (intro/procedures nor data collection). You may bring your PyMOL tutorial worksheet with notes to lab.
- Make sure to download, install, and run PyMOL on your computer before coming to lab.
- Complete as much of the tutorial as possible to become comfortable with the PyMOL interface and commands.
Procedure: Chapter 11A

- You will be assigned by your TF a set of enzyme keywords to search on the PDB site
- **Use Key Words for search, must have ligands, X-Ray Resolution ≤ 2.5Å**
- Sort results by resolution
- Pick structure with best resolution and good sized ligand – *get structure approved by TF*
- Make sure to record your enzyme and the PDB code somewhere into your notebook (no need to turn it in)
In discussion tutorial
on NCBI BLAST and Protein Data Bank
Procedure: Chapter 11B

- Open PyMOL on your desktop
- Load your enzyme of interest using the following command: fetch <PDB code>
  fetch 1IHY
  - Add “A” at the end of your code to load a single chain
- Look at your protein molecule and answer these types of questions in your post-lab write-up template
  - Note the overall shape of your protein/enzyme
  - Find an α-helix and β-strand
  - Find amino and carboxyl termini
  - Find your ligand and record a hydrogen bond interaction (2.5 – 4.0 Angstroms)
In discussion tutorial on PyMOL
Chapter 11:

At the end of lab, you should have:

✓ Wrote down your group number, enzyme/protein, and PDB code assigned by your TFs (in your notebook somewhere)

✓ From your PDB PyMOL exercises
  • Noted and recorded any observations required in the Chapter 11 post-lab write-up template
  • Taken screenshots of your observations

✓ Finished any remaining Chapter 3 experiments
  ✓ Ultra-filtration with final activity assay and dye-binding measurements (must make a new standard curve)
  ✓ Any previous aliquots which haven’t been assayed for protein concentration via dye-binding
Questions about Ch 11?
Purification Table: Correction Factors

- Add correction factors where appropriate & carry through subsequent steps

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (mL)</th>
<th>Volume taken for next step (mL)</th>
<th>Corrected volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>170</td>
<td>60 (used for spin)</td>
<td>-</td>
</tr>
<tr>
<td>1S</td>
<td>45</td>
<td>all</td>
<td>(170/60) x 45 = 128</td>
</tr>
<tr>
<td>1\textsuperscript{st} salt cut</td>
<td>65</td>
<td>62 (used for spin)</td>
<td>-</td>
</tr>
<tr>
<td>2S</td>
<td>55</td>
<td>all</td>
<td>(170/60)(65/62) x 55 = 163</td>
</tr>
<tr>
<td>2P</td>
<td>22</td>
<td>-</td>
<td>(170/60)(65/62) x 22 = 65</td>
</tr>
<tr>
<td>2\textsuperscript{nd} salt cut</td>
<td>57</td>
<td>50 (used for spin)</td>
<td>-</td>
</tr>
<tr>
<td>3S</td>
<td>30</td>
<td>-</td>
<td>(170/60)(65/62)(57/50) x 30 = 102</td>
</tr>
<tr>
<td>3P</td>
<td>20</td>
<td>all</td>
<td>(170/60)(65/62)(57/50) x 20 = 68</td>
</tr>
<tr>
<td>3P-D</td>
<td>22</td>
<td>15 (loaded column)</td>
<td>(170/60)(65/62)(57/50) x 22 = 75</td>
</tr>
<tr>
<td>Pooled</td>
<td>7</td>
<td>all</td>
<td>(170/60)(65/62)(57/50)(22/15) x 7 = 35</td>
</tr>
<tr>
<td>Conc’d LDH</td>
<td>1</td>
<td>-</td>
<td>(170/60)(65/62)(57/50)(22/15) x 1 = 5</td>
</tr>
</tbody>
</table>
Summary of Calculations

From enzyme activity assay:
• \([\text{Activity}_{\text{Undiluted}}] = \frac{(\Delta A_{340/min})(\text{Total Volume of Assay})(\text{Dilution Factor})}{(\text{Volume of enzyme solution in assay})(\varepsilon_{\text{app}} \text{ in mM}^{-1})} = \text{Units/mL}\)

From Bradford (dye-binding) assay:
• \([\text{Protein}_{\text{Undiluted}}] = \frac{(\text{Mass of protein})(\text{Dilution Factor})}{(\text{Volume of Protein Solution})} = \text{mg/mL}\)

Additional calculations:
• Total Activity = \([\text{Activity}_{\text{Undiluted}}](\text{Total Volume}) = \text{Units/mL} \times \text{mL} = \text{Units}\)

• Total Protein = \([\text{Protein}_{\text{Undiluted}}](\text{Total Volume}) = \text{mg/mL} \times \text{mL} = \text{mg}\)

• Specific Activity = Total Activity/Total Protein = \text{Units/mg}\)

• % Yield = \[\frac{\text{Total Activity in Given Step}}{\text{Total Activity in Crude Extract}} \times 100\]
Questions?
DISCUSSION QUIZ:

https://tinyurl.com/F19-BI421-PYMOL

Please do not close your browser until the final prompt confirms your answers have been recorded

Thank you 😊