Announcements

- Today is the last pre-lab discussion.
- Chapter 11C will be the last lab.
- Chapter 11C post-lab write-up can be completed during the lab session. Due end of lab day.
- A template and tutorial are available on the website.
- Lab Section on Monday (B1) has the Laboratory Report for Chapter 10 due on Wednesday next week, not Monday. But the Laboratory Report for Chapter 11 is still due Monday.

Announce Concepts Procedure Hazards Tips Clarification End

Chapter 11C: *in silico* protein mutagenesis

Objectives:

- Re-familiarize with using the protein modeling software PyMOL
- Perform an amino acid mutation to change a non-covalent bond between the protein and ligand (substrate/cofactor)
- Evaluate how that change in interaction would affect binding (potency) by measuring the distance and calculating the energy
- Align the protein structures from two different species and evaluate if they are structurally similar

Announce Concepts Procedure Hazards Tips Clarification End

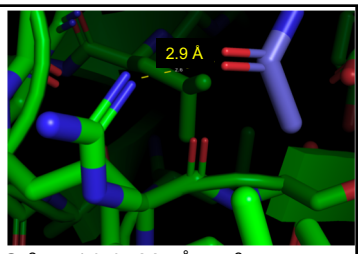
During the 11C lab:

- You will be assigned a PDB code by your TF
- Use PyMOL to find the ligand (substrate/cofactor). Do not choose an inorganic or metal cofactor. Also, you can check the protein data bank site with your PDB code to find the ligand and its structure.
- Perform the mutagenesis on your computer in lab
- Measure distances between atoms in a non-covalent interaction
- Align the protein structures from two different species

PDF tutorials will be available to help linked on the website:

- Install PyMOL (follow the announcement)
- Complete the Laboratory Report in class

For calculating free energy:



Recall Coulomb's Law:

$$E = k\frac{q_1\ q_2}{D\ r}$$

where k is Coulomb's constant, 9 x 10^9 J · m · C^{-2} or 14.4 eV · Å · e^{-2}

q_1 and q_2 are the electric charges in the bond

D is the dielectric constant

r is the length of the bond

Example: Salt-bridge q_1 = +1e; q_2 = –1e

Distance of 2.88 Å

Use k in eV = 14.4 eV·Å·e^{-2}

eV = 1.6 x 10^{-19} J

Use D = 10 for protein interior

$$E = \frac{-1e^2\ \text{X } 14.4\ \text{eV·Å·}e^{-2}}{10 \text{ x } 2.88\ \text{Å}} = -0.5\ \text{eV per molecule} \text{ x } 6.022 \text{ x } 10^{23}\ \text{molecules/mol}$$

$$= -3 \text{ x } 10^{23}\ \text{eV per mole charges} \text{ x } 1.6 \text{ x } 10^{-19}\ \text{J/eV}$$

$$= -4.8 \text{ x } 10^4\ \text{J/mol}$$

$$= -48\ \text{kJ/mol} \quad \text{or } -10\ \text{kcal/mol}$$

What is the change in the binding affinity due to loss of your chosen interaction?

Interaction with ligand in the wild-type enzyme *versus* Interaction with ligand in the mutant enzyme

$WT{\cdot}L \rightleftharpoons WT + L$

K_d of WT

$$^{WT}K_d = \frac{[WT][L]}{[WT \cdot L]}$$

$MUT{\cdot}L \rightleftharpoons MUT + L$

K_d of MUT

$$^{MUT}K_d = \frac{[MUT][L]}{[MUT \cdot L]}$$

These values are difficult to measure without going to the lab:

All we have is
– Energy of the interaction in the wild-type enzyme
– Energy of the interaction in the mutant enzyme

Announce > Concepts > Procedure > Hazards > Tips > Clarification > End

What is the change in the binding affinity due to loss of your chosen interaction?

Energy of the interaction in the wild-type enzyme – Energy of the interaction in the mutant enzyme

$$\Delta G = G_{products} - G_{reactants}$$

Bond made in WT ⇌ Bond broken in WT

$$\Delta G_{WT} = G_{WT\ bond\ broken} - G_{WT\ bond\ made}$$
$$\Delta G_{WT} = -G_{WT\ bond\ made}$$

Bond made in MUT ⇌ Bond broken in MUT

$$\Delta G_{MUT} = G_{MUT\ bond\ broken} - G_{MUT\ bond\ made}$$
$$\Delta G_{MUT} = -G_{MUT\ bond\ made}$$

But Energy in both enzymes due to bond broken is the same
(assuming the enzyme's energy unbound is the same)

Announce > Concepts > Procedure > Hazards > Tips > Clarification > End

What is the change in the binding affinity due to loss of your chosen interaction?

Energy of the interaction in the wild-type enzyme – Energy of the interaction in the mutant enzyme

$$WT \rightleftharpoons MUT$$

$$\Delta\Delta G_{MUT/WT} = \Delta G_{MUT\ (bond\ made)} - \Delta G_{WT\ (bond\ made)}$$

$$= -G_{MUT\ bond\ made} - (-G_{WT\ bond\ made})$$

= –E in MUT bond + E in WT bond

= E in WT bond – E in MUT bond

$\frac{K_{d\ MUT}}{K_{d\ WT}}$ = the fold change of the dissociation constant between ligand and enzyme due to loss of one interaction from mutation

$$\frac{K_{d\ MUT}}{K_{d\ WT}} = e^{-\Delta\Delta G_{MUT/WT}/\mathrm{RT}}$$

Example: remove a H-bond
Calculate that it has –20 kJ/mol free energy
Mutant changed to something without H-bond at all; 0 kJ/mol
$\Delta\Delta G_{MUT/WT}$ is –20+0 = –20 kJ/mol
e to the +20/2.5 = ~3000 fold!!

For *RT:* use 27 °C (300K)
8.314 J/mol·K(300K)
= 2.5 kJ/mol

Announce > Concepts > Procedure > Hazards > Tips > Clarification > End

Chapter 11C

Before the lab period, you should have:

- ✓ No pre-lab/post-lab writeups this week
- ✓ Ensure you have PyMOL installed on your computer before lab
- ✓ Review your amino acid structures and properties
- ✓ Bring a three-button mouse (may be helpful for you)

At the end of lab, you should have:

- ✓ Re-familiarized yourself with PyMOL
- ✓ Performed a single amino-acid substitution
- ✓ Performed calculations to determine how much the mutated enzyme-ligand interaction is favored or unfavored over the wild type enzyme-ligand interaction
- ✓ Aligned protein structures from two different species and evaluate if they are structurally similar
- ✓ Submit the Laboratory Report by end of your lab day on Gradescope

Announce > Concepts > Procedure > Hazards > Tips > Clarification > End

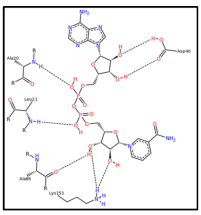
Questions?

Discussion Quiz

NAME: For GradeScope, please write your FIRST and LAST name in CAPITAL letters WITHIN the box:

Instructions: Write your answers in the boxes provided to the right.

The image below is a cartoon representation of the interactions between NADH and the amino acids in the enzyme lactate dehydrogenase (LDH).



1. Which two amino acids are interacting with the two phosphate groups in NADH? (Include the number for each amino acid)

Amino acid 1: Ala-20

Amino acid 2: Leu-21

2. Would a mutation to Tyrosine at Ala86 increase, decrease, or not affect the bond distance between LDH and NADH?

A. Increase.
B. Decrease.
C. No change.

B or C

Briefly explain your reasoning.

B: Tyr is more bulky and might push the backbone in towards the ribosyl
C: The interaction is with the backbone carbonyl, so changing the residue would not affect the backbone

3. In an effort to increase the interaction strength between NADH and LDH, Aspartate40 should be mutated to which amino acid in order to shorten the hydrogen bond distance(s) while still preserving the hydrogen bond interaction type?

A. Proline.
B. Glutamate.
C. Serine.
D. Threonine.

B