Announcements

- Today is the last pre-lab discussion.
- Exam Tuesday
- Ch 10 is due next week
- Chapter 11C will be the last lab.
- Chapter 11C post-lab write-up can be completed during the lab session. Due end of day, 1 day after your lab section (i.e. B1 due Thurs 11:59 pm..).

Chapter 11C: *in silico* protein mutagenesis

Objectives:

- Re-familiarize with using the protein modeling software PyMOL
- Perform an amino acid mutation to change a bond between the protein and ligand (substrate/cofactor)
- Evaluate how that 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

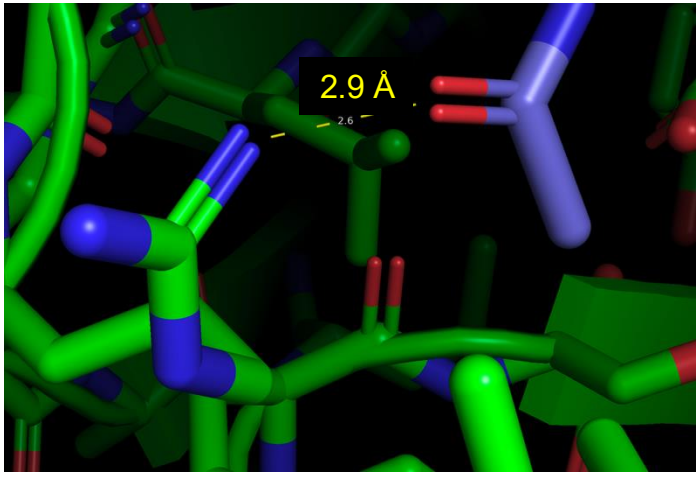
During the 11C lab:

- 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 (google drive – linked in slack) will be available to help:

- Install PyMOL
- Complete the post-lab 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

Use k in eV = 14.4 eV·Å·e^{-2}

eV = 1.6 x 10^{-19} J

Use D = 10 for protein interior

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

Distance of 2.88 Å

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

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

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

$$\Delta G = -RTlnK_{eq}$$

Where ΔG is the change in the Gibb's free energy

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

and K_{eq} is the equilibrium constant, describing the ratio of [Products] over [Reactants] at equilibrium

$$K_{eq} = \frac{[\text{products}]}{[\text{reactants}]}$$

$$\Delta G_{WT} = G_{WT\ products} - \boldsymbol{G_{WT\ reactants}}$$
$$\Delta G_{MUT} = G_{MUT\ products} - \boldsymbol{G_{MUT\ reactants}}$$

$$\Delta\Delta G_{MUT/WT} = \Delta G_{MUT} - \Delta G_{WT}$$
$$= \left(G_{MUT\ products} - \boldsymbol{G_{MUT\ reactants}}\right) - \left(G_{WT\ products} - \boldsymbol{G_{WT\ reactants}}\right)$$
$$= G_{MUT\ products} - G_{WT\ products} - \boldsymbol{G_{MUT\ reactants}} + \boldsymbol{G_{WT\ reactants}}$$

For the reactants, if the enzyme and substrate are unbound in solution, then the starting free energy can be assumed to be equal and negligible

$$\Delta\Delta G_{MUT/WT} = G_{MUT\ products} - G_{WT\ products}$$

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$$\Delta\Delta G_{MUT/WT} = \Delta G_{MUT} - \Delta G_{WT}$$

$\Delta G = -RTlnK_{eq}$ …then,

$$\Delta\Delta G_{MUT/WT} = \Delta G_{MUT} - \Delta G_{WT} = (-RTlnK_{eq\ MUT}) - (-RTlnK_{eq\ WT})$$
$$= -RT\left(lnK_{eq\ MUT} - lnK_{eq\ WT}\right)$$
$$= -RTln\frac{K_{eq\ MUT}}{K_{eq\ WT}}$$

$$\boldsymbol{\Delta\Delta G_{MUT/WT} = G_{MUT\ products} - G_{WT\ products} = -RTln\frac{K_{eq\ MUT}}{K_{eq\ WT}}}$$

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

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

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

Bond made in WT ⇌ Bond broken in WT

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

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

Bond made in MUT ⇌ Bond broken in MUT

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

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

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

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

$$WT \rightleftharpoons MUT$$

$$\Delta\Delta G_{MUT/WT} = \Delta G_{MUT\ (bond\ made)} - \Delta G_{WT\ (bond\ made)} = -RT\ln\frac{K_{d\ MUT}}{K_{d\ WT}}$$

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

= –E in MUT bond + E in WT 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

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

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

Chapter 11C

Before the lab period, you should have:

- ✓ No pre-lab/in-lab needed this week
- ✓ Ensure you have PyMOL installed on your computer before lab
- ✓ Might want to 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 over the wild type enzyme-ligand interaction
- ✓ Aligned protein structures from two different species and evaluate if they are structurally similar
- ✓ Submit the post-lab by end of the day after your lab on Gradescope

Questions?

Discussion Quiz

https://sites.bu.edu/mcneely/it-advice/pymol/