True or false questions. If false explain why. (15 points)

1. The binding constant K_D describes the equilibrium shown below and is in the units M^{-1}.

True

False If false, explain why.

The K_D is for the dissociation reaction where PL dissociates into P and L. The units are M, not M^{-1}.

2. The equation the Lineweaver–Burk plot is based on assumes infinite cooperativity in ligand binding and is the most common plot used to look for cooperative binding.

True

False If false, explain why.

LB is a double reciprocal plot. Infinite cooperativeity is from the Hill plot.

3. In competitive inhibition the K_M increases as inhibitor is added.

True

False If false, explain why.

4. In uncompetitive inhibition the K_M increases as inhibitor is added.

True

False If false, explain why.

An uncompetatiev inhibitor causes the apparent KM to decrease by a factor of 1/alpha'.

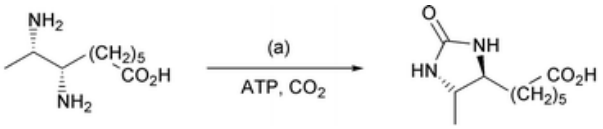
5. In the symmetry model for allostery the free T and R states are in equilibrium with the T state favoured.

True

False If false, explain why.

3. The enzyme Dethiobiotin synthetase is involved in the biosynthesis of the co-factor biotin. It uses ATP and 7,8-diaminononanoic acid (DAPA) to make dethiobiotin. Biochemists identified the residues T11 (Thr at position 11), E12 and K15 as playing a role in the active site. Three site specific mutants were generated, T11V (T11 is mutated to valine), E12A, and K15Q. These mutants and the un-mutated wild type enzyme were characterized kinetically as shown below. (10 points)

enzyme	K_M (DAPA) μM	K_M (ATP) μM	k_{cat} min^{-1}
wt	0.3	0.39	3.73
T11V	0.34	9.5×10^3	1.51
E12A	1.13	2.38	4.61
K15Q	11	701	5.4×10^{-4}



Which residue (use 3 letter code) is involved in:

DAPA binding? What data tells you this? What type of non-covalent interaction is disrupted by the mutant?

E12 – The KM in the E12A mutant goes up indicating that DAPA binds with lower affinity in the mutant. Note K15 is not involved in DAPA binding. It is involved in catalysis. Ionic interaction between E carboxylate and DAPA ammoniums.

ATP binding? What data tells you this? What type of non-covalent interaction is disrupted by the mutant?

T11 is involved in ATP binding. The KM for ATP is dramatically increased when T11 is mutated to Alanine. Hydroge nbonding interaction between side chain alcohol and ATP.

Catalysis? Explain why (explanations > 140 characters may not be graded).

K15. Mutation to Q decreases kcat by 4 orders of magnitude. Lys is likely playing a role as a catalytic base in the enzyme.

Draw a reaction coordinate diagram. Show and label the free enzyme plus free DAPA and ATP, the E-DAP-ATP ternary complex, the TS and the free enzyme plus dithiobiotin. Indicate which energy differences correspond to k_{cat} and k_{cat}/K_M.