Discussion Questions (Q9) for


- Background: Overall reaction catalyzed; what part is being studied in these papers; Big picture objective for both papers and differences
- See slides 46-54 from Monday’s notes for background on experimental methods

Paper 1
- Experimental:
  - Scheme I - Why is only lower path relevant?
  - Table I: $K_a$, $k_3$, and $K_{pp}$; how measured and determined; How is back reaction prevented?
  - Table II - $k_3$, $K_{pp}$, & how obtained (Note description of E•Tyr-Ad; what does this tell us about stability of complex?)

- Results/Calc:
  - Tables I and II – which measured parameters are affected
  - Fig. 2 & Table III – calculation of G, depiction
  - Fig. 2, 3 & Table IV – Key observations of where binding energies for each are “expressed”

- In discussion section on “Enzyme Complementary to Intermediates” – Discuss effect of enzyme on (i) unfavorable equilibrium, i.e. $\Delta G^0$, and (ii) why there is a biological need to stabilize this intermediate.
- Consider mechanistic interpretations together with model in 2nd paper
- Looking back at Nature paper from wk1 discussion with Stroud – note that both Km values for ATP and Tyr as well as kcat in the Nature paper are lower than k3 and the Kd values reported here? What does that tell us about the overall reaction profile, i.e., where is the rate-limiting step overall, what enzyme form(s) accumulate? Why was it still ok to use $k_{cat}/K_m$ to examine the H-bond strengths in the Nature paper?

Paper 2
1) (a) Calculate the $\Delta \Delta G^\pm$ values for the catalytic step for all three mutations.  
(b) Are they additive? Thoughts?
2) From text, note the effects of the mutations on the Kd for PPi and for k-3 for reverse reaction. Thoughts?
3) Fig. 3 – Explain what is shown and what info is extracted from this plot.
4) Discuss implications of double mutant rate in comparison with model reaction shown in Eq 3. In particular by how much does enzyme catalyze the reaction vs the solution reaction, i.e., both in x-fold terms and lowering of $\Delta \Delta G^\pm$? Where does this fall on the range of $\Delta \Delta G^\pm$ values discussed in class notes?
5) Discuss model in Fig. 5 for how binding energies are used in TS vs GS to catalyze reaction and note comment on unpublished $\Delta H^\pm$ vs $\Delta S^\pm$ values (how would you measure these?)
6) Comment on two final factors cited: (1) entropic factor bi -> uni, (2) TS binding