BI/BMB/CH 174 2017
Problem Set 3: Chaperones, 100 points

Due: 5/9/17 at the beginning of class
Office Hour: 5/8/17, 6-8pm at Broad 300

1. **Bardwell et al. -25pts**
    a. What are the two classes of proteins that facilitate folding *in vivo*? And give a few examples of each. -3pts
    b. What was the initial observation that led to the search of factors that catalyze disulfide bond formation *in vivo*? -2pts
    c. For the MalF fusion constructs the authors used to select mutants, they observed different phenotypes in different minimal growth mediums. What could be the reasons behind that? Explain. -4pts
    d. Briefly explain how the experiment in Figure 4 was done. And what did the authors find about AP with this experiment? -4pts
    e. What is the difference between OmpA and β-Lactamase folding? -2pts
    f. In the discussion, the authors suggested, "the defect in assembly of F pili in *dsbA* mutants may reflect the existence of pilin proteins that contain essential disulfide bonds." How can you test this now? Propose two different experiments. -4pts
    g. Propose an experiment to test the presence of the topological model proposed in Figure 8. -2pts
    h. Using available PDBs of *E.coli* DsbA, make a pymol figure of the protein that shows the appropriate active site residues and label them accordingly (provide the PDB ID). -4pts

2. **Glover and Lindquist. -25pts**
    a. What were known about Hsp104 before this paper was published? -3pts
    b. What is the main message from Figure 1? And why was it important? -2pts
    c. In determining the Hsp40 and Hsp70 homologs that were important in Hsp104-mediated refolding, answer the following questions:
        i. How did they find the Hsp40 homolog? -1pt
        ii. How did they find the Hsp70 homolog? -1pt
        iii. Why did they do two different experiments? -2pts
        iv. Briefly explain the experimental logic behind Figure 4. -3pts
    d. What is the main message coming out from Figure 5? And why was cold buffer used to stabilize the unfolded luciferase? -3pts
    e. Briefly talk about what was going on with the different lanes and curves in Figure 6C. And what did they find about Hsp104? -4pts
    f. The authors used both unfolded luciferase and β-galactosidase as substrates for refolding, what was the rationale behind this? And what did the results say about the Hsp104 refolding system? -3pts
    g. What is the final proposed working model of Hsp104-mediated refolding? -3pts

3. **Leitner et al. -25pts**
   a. In the summary of identified crosslinks, why are there less heterotypic and homotypic crosslinks in the presence of ATP than in apo or ATP-AlFx conditions for bTRiC? -2pts
   b. Why does crosslinked TRiC migrate faster in SDS-PAGE and slower in Native-PAGE as shown in Figure 1D and 1E? -2pts
   c. Besides XL-MS that was used in this paper, what are two other methods that could possibly be used to test the proposed subunit order? Make sure you explain why you think your proposed method would work. -4pts
   d. As suggested in the combinatorial analysis of distance constraints, "the previously proposed TRiC subunit arrangement only a minor fraction (10-13%) of the observed crosslinks". Why do you think there's such a huge difference in crosslinks observed? -2pts
   e. In refining the structural model representing the XL-MS determined subunit arrangement against the X-ray diffraction data, what is the major problem that could greatly affect the model proposed? -3pts
   f. In the XL-MS-based TRiC structure, there were interring interactions observed between the N-termini of CCT1 and CCT8. They proposed "these unique structural features help to correctly establish the subunit topology in TRiC by stabilizing the ring-ring interface". Propose two separate experiments to test this idea. -4pts
   g. What are some differences and similarities between lysine crosslinking and cysteine crosslinking that were used in this paper? -4pts
   h. Throughout the paper, the authors made a lot of comparisons of their XL-MS model to the crystal structure of TRiC that was solved by Dekker et al. This is currently one of the controversies in this field. With the knowledge you have on these two methods, which model do you think is right? Reasonable and logical answers are accepted. -4pts

4. **Berkovits and Mayr. -25pts**
   a. What are the four evidences that CD47 with long 3'UTR localizes differently than CD47 with short 3'UTR? -8pts
   b. What is the role of SET in the UDPL model? How was that proved in the paper? -3pts
   c. What was done in Figure 3B? What did the authors conclude from those data? -2pts
   d. What was found in Figure 3C? How does that agree with previous data? -2pts
   e. What are the different cellular functions of CD47-LU and CD47-SU? And how did the authors test these functions? -5pts
   f. This paper and other suggested that SET binds to positively charged amino acids. Suggest one experiment that could determine the binding motif of SET. -2pts
   g. What is the take home message of this paper? -3pts