BMB/BI/CH174 Spring 2017

Problem set 1: Nuclear pore complex

Due: 4/13/17 at the beginning of class
Office hour: 4/12/17, 6-8pm, Broad 300
100 points total

1. *Stuwe et al.* (2015, March) Architecture of the nuclear pore complex coat. -27pts

    a. Crystal structure of the yeast CNC.
        i. What did they do in getting better diffracting crystals? And why would that help in improving the resolution? -4pts
        ii. How did the authors confirm their molecular replacement solution was right? Briefly explain. -5pts
        iii. Make a high resolution pymol surface figure with the proteins involved in forming the CNC triskelion. Make sure you label them and you will be graded based on the quality of your figure. -3pts
    b. Structural comparison and docking with the EM reconstructions.
        i. What was the major difference between the CNC crystal structure and the human CNC EM reconstruction? Why was that? And what was the major conclusion? -3pts
        ii. Upon docking the 32 copies of CNC to the cryoelectron tomographic reconstructions of the human NPC, Nup120 was identified to be the only membrane contact point. What are two ways that Nup120 can interact with the membrane and how can you test them? -4pts
        iii. How would you confirm the interaction between $Nup84^{CTD}$, Nup133 and Nup120 upon docking, shown in Figure 4D? -3pts
    c. What is the different between NPC coat and other membrane coat? -2pts
    d. What is the major advance of this paper? -3pts

2. *Stuwe et al.* (2015, October) Architecture of the fungal nuclear pore inner ring complex. -36pts

    a. Nic96 and Nup192.
        i. Briefly describe how they found Nic96 is the sole IRC attachment site for CNT. -3pts
        ii. What are the similarities and differences between Nup188 and Nup192? -3pts
        iii. How did they determine the pre-ribosomal export defect of $Nup192^{Tail}$ domain in Figure 2K? Explain the experimental setup and result with details. -4pts
        iv. What kind interactions are there between Nic96 to CNT and IRC? -2pts

v. What is the importance of Nup192^{Tail} domain? Briefly explain the different experiments they did to make that conclusion. -4pts
vi. In the yeast experiments in Figure 2, 3 & 5, what was the purpose of using Nup57-GFP? -2pts

b. Crystal structure of the CNT
i. What did the authors see when they were trying to crystallize short channel Nup fragments? What kind of transport mechanism did those structures suggest? And why do you think they can get so many different structures? -5pts
ii. What kind of biochemical and in vivo data did they have in the paper that suggested the CNT structure can only be elucidated with CNT-Nic96^{R1} complex? -4pts
iii. What was an important feature they see and how was that important in determining the final transport mechanism? -3pts

c. What is the functional implication that all FG repeats emanate from the same region of the coiled-coil assembly? And why is that important? -3pts
d. What is the major advance of this paper? -3pts

3. *Lin et al*. (2016). Architecture of the symmetric core of the nuclear pore. -37pts
a. In summary, what was the approach they have in getting the architecture of the symmetric core of the nuclear pore? -2pts
b. What was the indispensable component for forming the NPC symmetric core protomer containing the CNC hexamer and the IRC or the CNC hexamer and the Nup188 complex? How was that determined? -3pts
c. What is the relationship between Nup145N and Nup145C? How is that important in reconstituting the symmetric core protomer? -3pts
d. How did they confirm the sequence of Nup145N that binds to Nup170 is conserved and critical in humans? -3pts
e. How was the full length structure of Nup170 and Nup192 determined? What is the importance of the serine-glycine linker? -3pts
f. Placing different complexes into the cryoET map.
i. Briefly outline how the different complexes and Nups were placed into the cryo-ET map and how many copies of each component were found. Make a flow chart. -8pts
ii. What are the symmetry operations observed? -2pts
iii. Briefly describe the overall architecture and the composition of one NPC spoke. -4pts
iv. What are the contact Nups between the pore and the membrane? -2pts
v. How much of the total NPC mass was counted after placing the different structures? And what are the missing components of the whole pore? -4pts

g. What is the major advance of this paper? -3pts