BMB174 2017
Problem set 5: Protein trafficking
Due: June 1st
100 points

Giraudo et al. [30 points]

1. Background [10 points]: understand the SNARE complex and fill in this form

	Localized to membrane? (Y/N)	If membrane localized, synaptic vesicle or presynaptic terminal membrane?	Part of 20S particle? (Y/N)	Which SNARE? v- or t- or neither (n)
VAMP(synaptobrevin)				
Syntaxin				
SNAP-25				
NSF				
α-SNAP				
synaptotagmin				

2. The authors tried to answer a question of how does 'burst' of secretion happen upon receiving a signal, their experimental design was based on a model in which fusion machinery is kept at 'on' state, while fusion is kept 'off' by another set of machineries. Describe the model of "constitutively" operating mechanism, and answer why certain predicted proteins/protein functions are required for a 'burst' of exocytosis and why did they chose these proteins as carrying the predicted functions [5 points].

3. SNARE-mediated cell-cell fusion assay. A major method used in this paper is cell-cell fusion assay. Describe how the experiment is set up to quantify fusion activity, why do they call SNARE proteins being 'flipped'? What would you expect to see in Fig. 1D if adding Cpx-I to media instead of expressing Cpx-I-GPI on either cells? [5 points]

4. Suppose there is a neurotoxin that binds specifically to Syt-I and prevents its refolding in the presence of calcium, what would you expect to see in Fig. 3A and 3E? [5 points]

5. According to the experiments, what protein/proteins function as to 'clamp' the fusion machinery, and what 'triggers' it in the proposed 'constitutive' fusion mechanism model? [5 points]

Devaraneni et al. [30 points]

1. One of the goals of this study is to characterize the topology of the nascent protein and its interactions within the RTC (ribosome-Sec61 translocon complex) at different times of type II SA biogenesis. How are the authors able to study RTCs with nascent chains at specific lengths? (4 points)

2. The authors performed photocrosslinking in combination with affinity pull-down to determine the molecular environment of the nascent chain as it increases in length.

   i. Briefly describe the method being used (3 points)

   ii. The authors placed the probe at different residues and tested for crosslinking between L28 and Sec61 at different lengths of nascent chains in Figure 1.

   What did they conclude from the observed patterns? (3 points)

3. One of the major experiments arguing for "head-first" insertion (as well as TM inversion) is fluorescence collision quenching.

   i. Why is fluorescence collisional quenching an appropriate technique to test the hypotheses of "head-first" insertion and hairpin-looped insertion? Why were Val2 and Leu44 the residues chosen for analysis in these experiments? (4 points)

ii. One of the key steps after "head-first" insertion is the inversion of TMD to assume the type II orientation. Describe the evidence for "head-first" insertion and for the subsequent TM inversion. (4 points)

4. In Figure 5C and Figure 7D, the same experiment was performed on type II and type I constructs, respectively.

i. Describe how the data in Fig. 5C fit the type II insertion model and how Fig. 7D fits the type I insertion model. (4 points)

ii. In both plots there is a dip at 103 aa in chain length. How is this explained? (4 points)

iii. Does type I insertion proceed similarly to type II insertion? If so, at what point do they diverge? (4 points)

Rao et al. [25 points]

1. The authors designed a set of substrate constructs, describe how they are designed, and explain in Fig. 4, how did they measure the capture efficiency for each construct? What did they observe and what are the conclusions? [4 points]

2. To understand how TA was transferred from Sgt2 to Get3, the authors reconstituted an *in vitro* substrate transfer system. [8 points]

i. What is the biophysical method they use? What would you expect to see from the measurement if (1) transfer happens efficiently towards one direction, (2) transfer happens inefficiently/unsuccessful, or (3) transfer is a fast reversible process? What did they actually observe? [4 points]

ii. What is the role of Get4/5 in this process, does their measurements agree with what is known? [3 points]

iii. Assume you don't know the role of ATP hydrolysis during this process, and you would assume the reaction may happen during this transfer step, to test whether ATP-bound state of Get3 or Get3 ATP-hydrolysis is required for TA transfer from Sgt2, what additional experiments can you do using FRET? [1 point]

3. The last step TA transfer is from Get3 to membrane embedded receptor Get1/2, which results in TA insertion into ER membrane. To do this, the authors reconstituted an *in vitro* transfer system. [7 points]

i. Describe how the experiment was set up, and how they measured the transfer efficiency. [4 points]

ii. They found that CTE constructs transfer were slower, what are the possible factors that contribute to this effect? What experiments did they do to test their assumptions? [3 points]

4. Summarize the proposed mechanisms during the entire TA transferring process, specifically how does sequence affect transfer at each step? [6 points]

Mariappan et al. [15 points]

1. Based on your understanding of their experiments, please summarize the sequential events in the process of TA protein's delivery to ER as the authors proposed. [3 points]

2. Fig. 2b proves that Bat3 complex is required by TRC40 substrate capturing, briefly describe how experiments were designed in Fig. 2b, you can make such conclusion by comparing which lanes? How do they think Bat3 completes its functions? [5 points]

3. In Fig. 3d and 3e, authors used an elegantly designed assay to prove the existence of substrate-Bat3-ribosome intermediate after TRC40 depletion. Describe how it is done, and you can make such conclusion by comparing which lanes? [4 points]

4. What kinetic information can be inferred from Fig. 4b by comparing WT and ΔTMD Sec61β? How does this affect the model they proposed? [3 points]