1. Many adhesion molecules such as the cadherin family of cell-cell adhesion molecules display very low binding affinities. In addition, the strength of cadherin mediated adhesion is critically dependent upon its cytoplasmic tail. Name two ways in which cytoplasmic proteins could increase the adhesive activity of the cadherin extracellular segment. Include examples from integrin mediated cell-matrix adhesion to support your hypotheses.

2. L1 is an important cell-cell adhesion molecule expressed in the developing brain that is necessary for establishing proper neural connections. You have found that L1 adhesive activity is upregulated in response to an extracellular stimulus known as netrin which activates neuronal migration.

2a. First of all, how would you test whether or not cells increase expression of L1 in response to netrin?

2b. The experiment demonstrates that L1 levels remain the same +/- netrin. Provide three alternative ways in which netrin might regulate L1 activity.

2c. Describe one experiment to test one of your hypotheses for netrin dependent L1 regulation.

2d. Netrin dependent neuronal migration correlates with upregulation of L1 activity. Briefly describe how you would test that L1 function is necessary for neuronal migration.

2e. Applying netrin to neuronal precursors activates a downstream kinase (Kinase A) that phosphorylates the cytoplasmic tail of L1 at Serine residue # 700. How would you test that Kinase A is necessary for upregulation of L1 adhesive activity?

2f. How would you test that Kinase A phosphorylation at Ser700 is necessary for netrin dependent L1 activation?

3. True or false – integrins cannot signal because the integrin subunits, unlike growth factor receptors, lack domains with enzymatic activity. Explain your answer.

4. As we discussed cadherins, extracellular matrix, and integrins, we asked the question of whether the biology of these factors could be explained by their adhesive functions alone. This simple question helped guide each of these fields to do some very informative experiments. Now try it yourself. What might you ask about growth factor receptors that would lead to some novel experiments? (keep it simple and broad).

5. “We propose that myosin activation results in the exposure of cryptic sites in p130Cas and downstream signaling.” Now, explain that to a non-scientist.

6. Name two ways in which ECM can facilitate signaling.

7. Cdt1 and cdc6 recruit MCM to origins of replication beginning in anaphase of mitosis. So I guess DNA replication begins at anaphase, right?
8. Cdc6 is important for building a pre-replication complex at origins of replication. Cdc6 is phosphorylated by S-phase cyclin-cdk complexes which targets cdc6 for degradation by SCF. What terrible thing would happen if you blocked cdc6 phosphorylation in S phase?

9. Geminin is a protein that binds to cdt1 preventing cdt1 from binding to origins and replication. What terrible thing would happen if you depleted cells of geminin in S phase?

10. Geminin is high in S phase. Geminin is ubiquitinated by the APC at anaphase. Why is this important for S phase? Afterall, S phase is already over.

11. Sister chromatids must be held together to ensure proper segregation in mitosis. Cohesin is the key factor holding sisters together in mitosis. True or false – a single kinase is able to explain the release of cohesin from sister chromatids to allow segregation at anaphase. Please provide a very brief explanation. (Note, I will come back to this point with more detail on Wednesday).