Problem Set #6 Name _____
7.06 - Spring 2015

Section _____

Question 1
In class, we listed a range of different binding affinities/disassociation constants (K_D's) for actin monomer interactions with other monomers, dimers, or polymers (≥ trimer). These are summarized below:

Monomer-monomer interaction – 100,000 μM
Monomer + dimer – 100 μM
Monomer (ATP) + polymer (+ pointed end) – 0.12 μM
Monomer (ATP) + polymer (- barbed end) – 0.6 μM
Monomer (ADP) + polymer (+ pointed end) – 1.7 μM
Monomer (ADP) + polymer (- barbed end) – 1.9 μM

You have identified several different mutants of actin that shows very different binding affinities than wild type actin.

- **Mutant #1** reverses the affinities (K_D's) for the pointed and barbed ends.

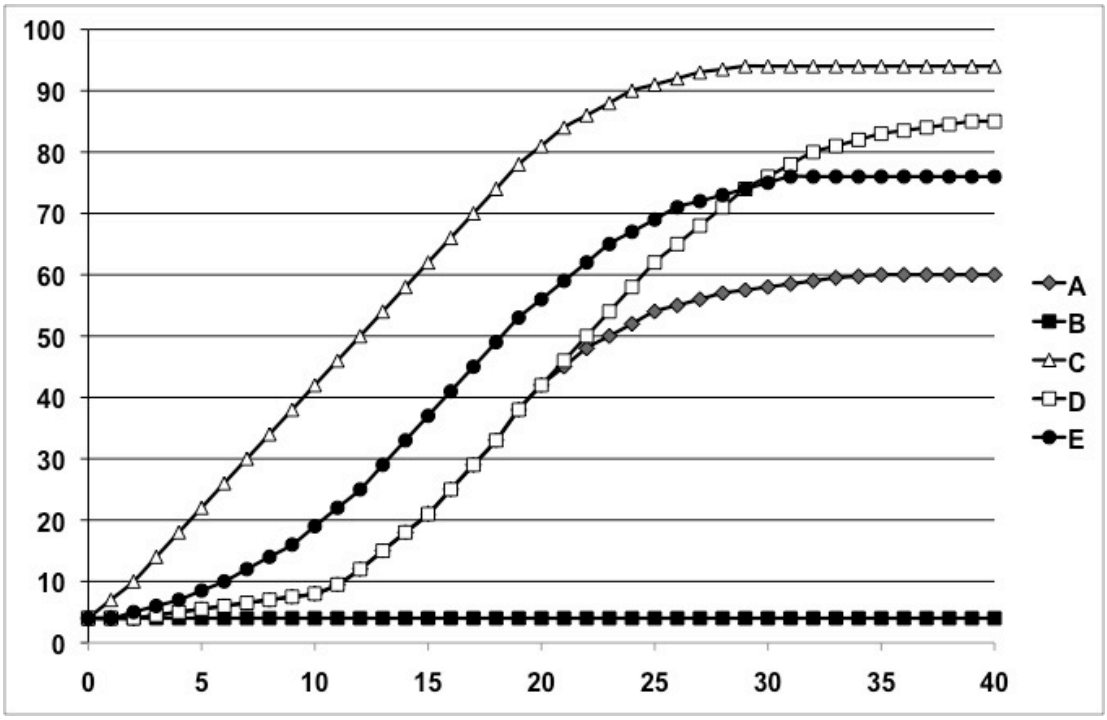
- **Mutant #2** displays 0.12 μM affinity (K_D) for each of the six binding associations listed above.

- **Mutant #3** displays 100 μM affinity (K_D) for each of the six binding associations listed above.

- **Mutant #4** displays similar affinities (K_D's) as wild type for the associations of monomer with polymer, but has 2 μM affinity for monomer-monomer and monomer-dimer interactions.

- **Mutant #5** can't undergo ATP hydrolysis

You first test each of these in the in vitro pyrene actin assembly assay (in the absence of other proteins). Wild type corresponds to Curve A. The Y axis is fluorescence intensity. The X axis is time in minutes.



A. (5 points) For each mutant, choose the pyrene assembly curve that best matches what you would be predicted to observe. Each curve can correspond to more than one sample (or not match any sample).

Mutant #1 –

Mutant #2 –

Mutant #3 –

Mutant #4 –

Mutant #5 –

B. (10 points) You next conduct a microscopy-based analysis of fluorescent actin polymers for each of the mutants. You let assembly proceed until the reaction reaches steady state. You then observe the polymers. For each of the mutants, indicate whether you would observe “longer filaments”, “shorter filaments”, or “no change” compared to wild type. In each case, explain why.

Mutant #1 –

Mutant #2 –

Mutant #3 –

Mutant #4 –

Mutant #5 –

C. (5 points) You next conduct myosin motility assays on actin polymers assembled with these mutants. Would any mutants fail to show myosin "walking" in a polar fashion? In addition, would any mutants show opposite myosin directionality? In each case, indicate which mutants (if appropriate) and explain why this is the case.

D. (6 points) Finally, you conduct assays on listeria-based motility in cell extracts in which the only source of available actin in one of the mutants listed above (or wild type as a control). In this assay, you find that Mutant #1 and Mutant #2 both fail to show listeria-based motility. In each case, explain why this would be true. Be sure to incorporate everything you know about the features of this mutant and the mechanisms of listeria-based motility.

Mutant #1 -

Mutant #2 -

Question 2
We discussed a number of microtubule-associated proteins and compounds. For each protein listed below, state the name of the actin-associated protein that is the most functionally analogous to this protein. For each small molecule compound, state the name of the actin-associated drug that is functionally equivalent.

1. Katanin

2. Gamma-tubulin

3. Prc1

4. XMAP215

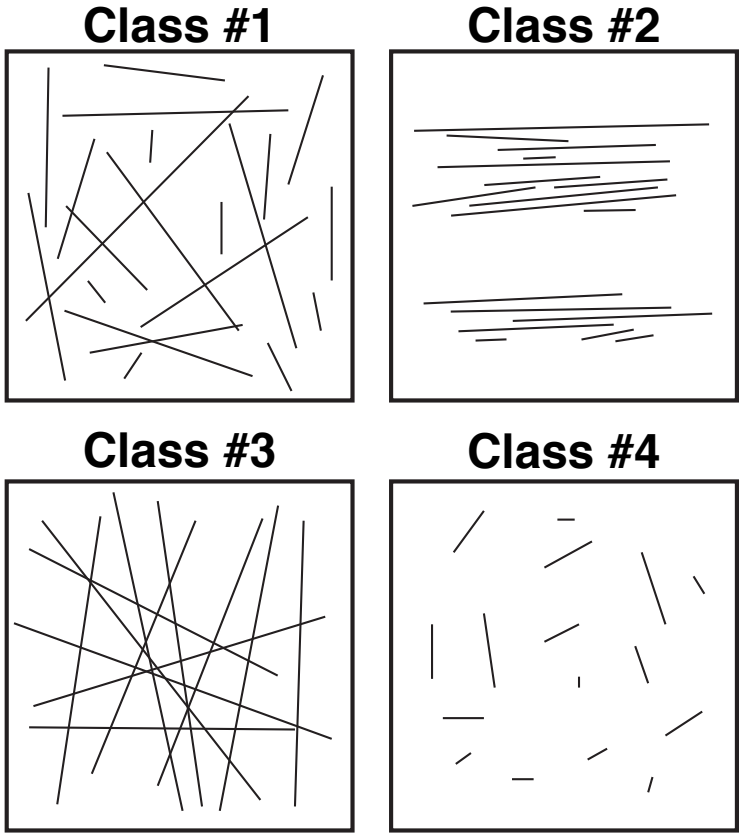
5. MCAK (kinesin-13)

6. Nocodazole

7. Taxol

Question 3

You are working on a series of microtubule associated proteins. To test their effects on microtubules, you assemble fluorescent microtubules. At t=0, you make a reaction mix that contains α/β tubulin heterodimers and GTP. You allow assembly to proceed for 20 minutes. Then, you either add buffer (no protein) as a control, or one of the proteins listed below and allow the reaction to continue to proceed for another 20 minutes. You then examine the microtubules under the microscope. You find 4 different behaviors for the different proteins, and divide these into classes. Class 1 corresponds to the buffer control.



A. In the control samples, why do the microtubules have different lengths?

B. For each of the following proteins, match these with one of the four classes (write the class number below). If addition of this protein would result in a different pattern than one of the four shown, indicate "other".

• The microtubule depolymerizing drug nocodazole

• The microtubule stabilizing compound taxol

• Katanin

• XMAP215

• XKCM1

• Prc1

• Cold (4°C)

• The non-hydrolysable GTP analogue GMPCPP

• A monomeric (one molecule acting alone) kinesin involved in the transport of cargo molecules to the plus end of microtubules in axons

• A tetrameric (four molecules bound together) kinesin that binds to anti-parallel microtubules in the spindle midzone to slide them apart from each other

C. After doing these experiments the first time, you come up with a way to label the minus end of each polymer with a different color fluorescent molecule (which is only present in the first few minutes of your assembly reaction). In the control samples, the very tip of one end of each microtubule is labeled. For each class above where more than one treatment matches that class, indicate whether this minus end labeling protocol would help discriminate between the different treatments. *Briefly* explain why for each class.