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.

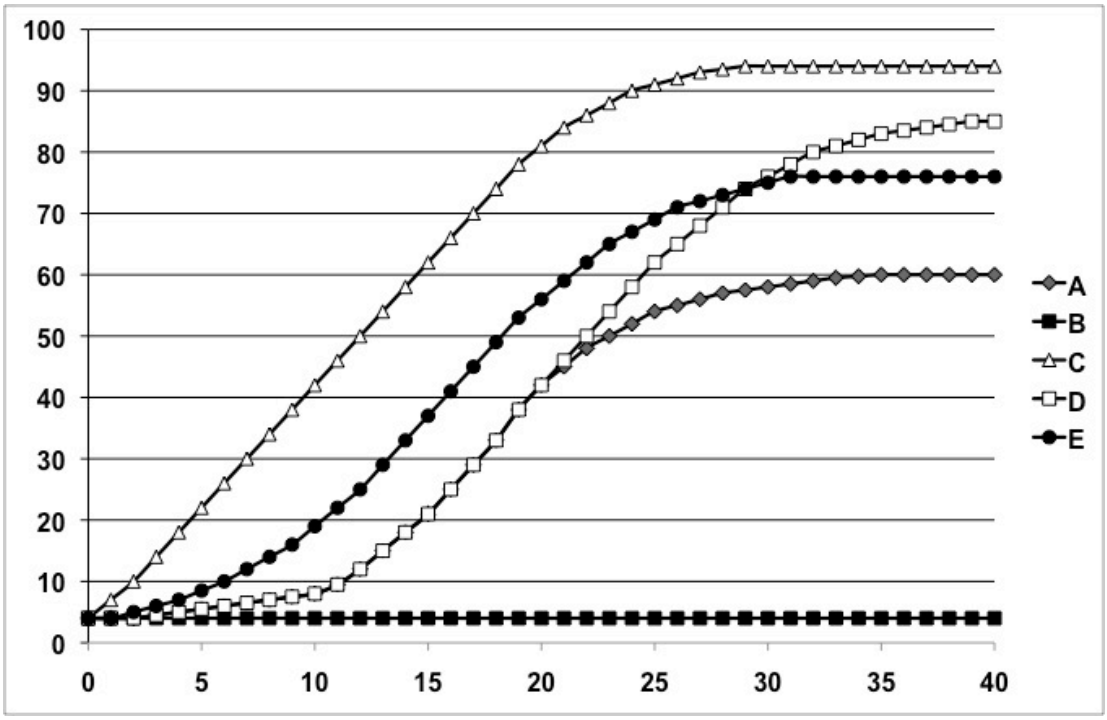
- **Mutant #2** displays 0.12 μM affinity (K_D) for each of the six binding associations listed above.

- **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 – **Curve A**

Mutant #2 – **Curve C**

Mutant #3 – **Curve B**

Mutant #4 – **Curve E**

Mutant #5 – **Curve D**

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 – **No change. Reverses which end shows favored growth, but wouldn't affect overall polymer length since net growth/loss is the same**

Mutant #2 – **Shorter filaments. You would see MANY more filaments spontaneously nucleate due to the elimination of the lag phase, and so overall filament length would be shorter**

Mutant #3 – **Shorter filaments. Assembly would be strongly disfavored. You would either see very few filaments or none at all. Anything that did form would be very short.**

Mutant #4 – **Shorter filaments. As with mutant 2, you would nucleate more filaments than normal (although not quite as many as mutant 2). This would give an overall shorter filament length for the same amount of bulk polymer.**

Mutant #5 – **Longer filaments. Absence of ATP hydrolysis would keep filament such that growth was favored and monomers wouldn't disassociate as readily.**

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.

There are two aspects of actin polarity – polarized growth, and a polar orientation of the polymer. Although these mutants all affect different aspects of growth, the inherent polarity of the filament remains. Thus, for each mutant, assuming that a polymer is formed, myosin would be able to "walk" along this polymer in a polar fashion with the correct directionality.

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 – **Polar growth of the barbed (plus) end of the actin polymer is what provides the force to move a listeria forward in the cell (new subunit addition with the Brownian motion moving the listeria small distances to allow new incorporation – Brownian rachet). By reversing the polarity of growth, this will prevent these polymers from generating force in the proper way.**

Mutant #2 – **Normally, listeria direct actin assembly at their surface due to the presence of ActA, which recruits and activates the Arp2/3 complex. Activated Arp2/3 lowers the barrier for actin assembly locally causing actin filaments to assemble there. However, in the case of mutant 2, there is no longer any barrier to nucleation, and filaments will nucleate spontaneously throughout the cell. Since this won't be directed to the listeria surface, the listeria won't be able to show directed movement.**

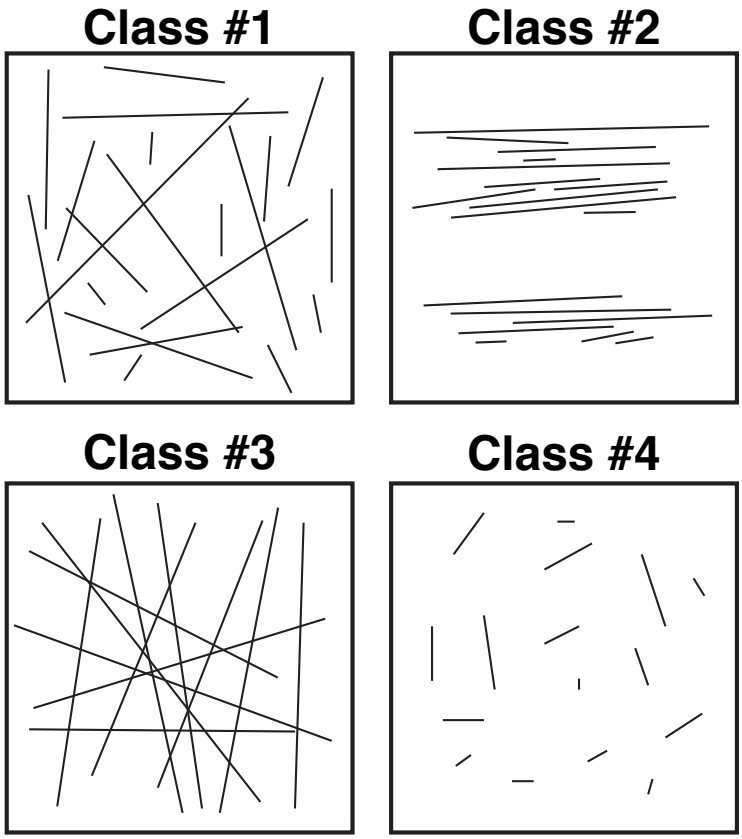
Question 2

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 COFILIN (Severing enzyme)
2. Gamma-tubulin ARP2/3, FORMIN (Nucleating complex)
3. Prc1 alpha-actinin, fimbrin, fascin (Crosslinkers)
4. XMAP215 Profilin (Polymerase)
5. MCAK (kinesin-13) Cofilin (Depolymerase)
6. Nocodazole Latrunculin A (Destabilizing drug)
7. Taxol Phalloidin (Stabilizing drug)

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?

Due to dynamic instability, these different microtubules will undergo distinct (and random) phases of growth and shrinkage.

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
Class 4

• The microtubule stabilizing compound taxol
Class 3

• Katanin
Class 4

• XMAP215
Class 3

• XKCM1
Class 4

• Prc1
Class 2

• Cold (4°C)
Class 4

• The non-hydrolysable GTP analogue GMPCPP
Class 3

• A monomeric (one molecule acting alone) kinesin involved in the transport of cargo molecules to the plus end of microtubules in axons
Class 1

• A tetrameric (four molecules bound together) kinesin that binds to anti-parallel microtubules in the spindle midzone to slide them apart from each other
Class 2

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.

Class 2 – Could distinguish parallel from anti-parallel bundles
Class 3 – These would not be distinguishable in this assay
Class 4 – Could distinguish whether it is cut in the middle (by Katanin) or depolymerized from the end