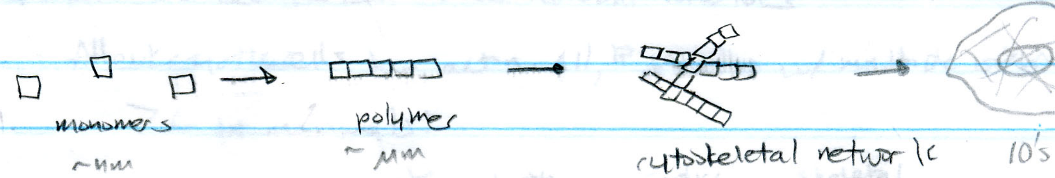
Introduction to cytoskeletal biology

- Cytoskeleton plays a large role in governing mechanics of cells
- Cytoskeleton is multiscale :



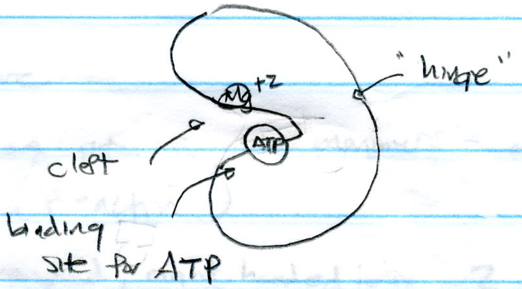
- critical to examine each of the scales to understand cell mechanics

Actin Cytoskeletal Filaments

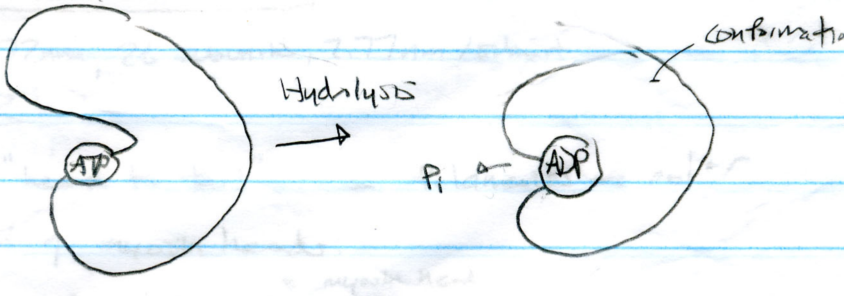
1.1 Actin

1.1.1 Monomer structure

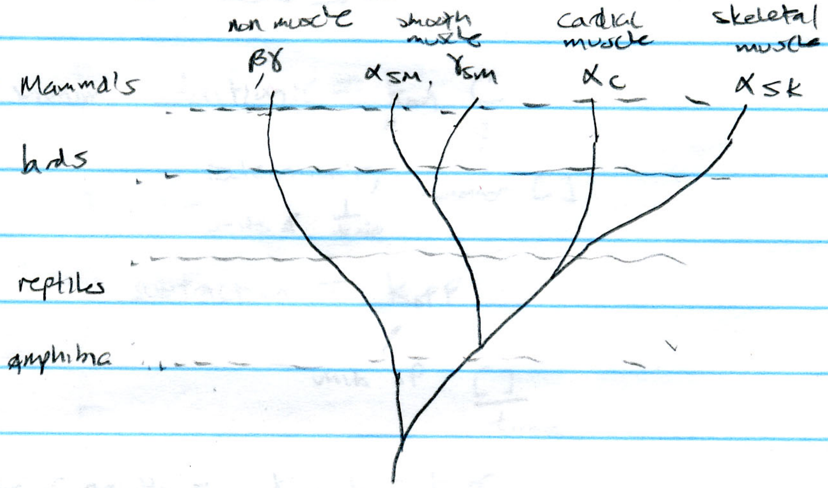
- globular protein, so called globular (G-actin)
- 375 amino acids, 42 kDA
- Two domains :



- ATP can become hydrolyzed : T form or D form

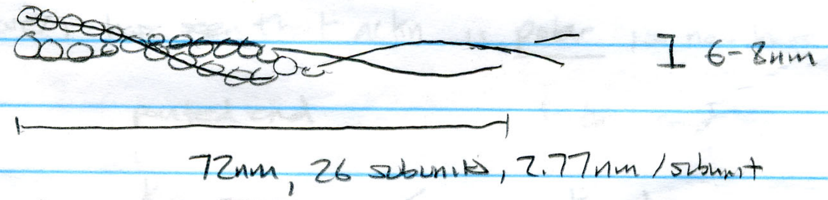


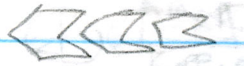
- Similar "forms" of actin can be found w/in our DNA - isoforms
  - allows different genes to encode different isoforms
  - different isoforms can serve different functions
  - All eukaryotic cells have actin, all, usually w/ multiple isoforms, >6 in mammals:



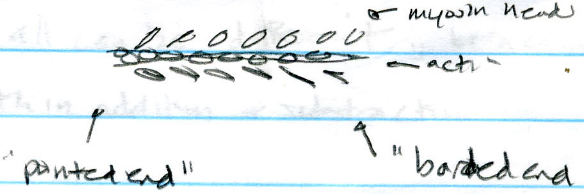
2 - Polymer structure

- Monomer has binding sites for other monomers - can polymerize into filaments (F-actin)
- Structure: two stranded, right handed helix, 2 protofilaments





- monomers bind "head to tail", so filaments are polar
- can see by binding myosin heads:



1.3 Actin polymerization kinetics + treadmilling

- In order to understand csk structure + regulation, need to understand actin polymerization kinetics.
- Imagine model filament:

$$\square\square\square\square\square \rightleftharpoons \square$$

- Rate of monomer addition = $k_{on}\ C$

ratecontant, units of $\frac{1}{time}$ ↗ k_{on}

↑ C: monomer [ ]

- " subtraction = k_{off}

↑ units of $\frac{[\ ]}{time}$

- Net rate of growth = $k_{on}\ C - k_{off}$
- At eq, $k_{on}\ C - k_{off} = 0$

$$C_{crit} = \frac{k_{off}}{k_{on}}$$

↑ critical concentration, [ ] of monomers necessary s.t. end is neither growing or shrinking

- However, have seen that actin is <u>polar</u>, so now have 4 relevant constants,

pointed end		barbed end
$k_{on,p}$	$<$	$k_{on,b}$
$k_{off,p}$	$<$	$k_{off,b}$

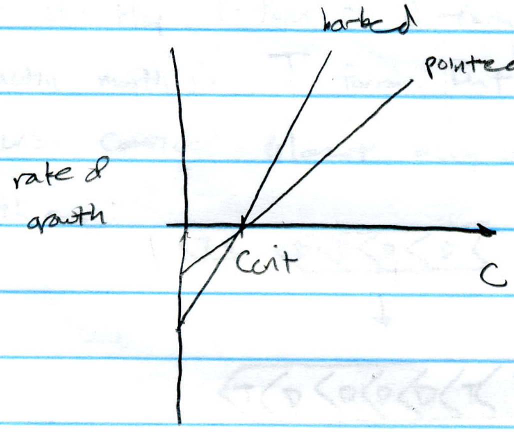
- In general ↗ all can be different, In actin, barbed end dynamics are faster, both in addition + subtraction

From energetic considerations, can show that critical [ ] is same at both ends:

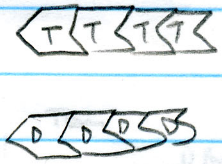
$$C_{crit} = \frac{k_{off,p}}{k_{on,p}} = \frac{k_{off,b}}{k_{on,b}}$$

-- ~~Plot~~ rate of growth at barb = $k_{on,b}\, C - k_{off,b}$

point = $k_{on,p}\, C - k_{off,p}$



- above Ccrit, get growth at both ends, but much faster at barbed!
- But also know actin can exist in ATP or ADP bound form, so can have

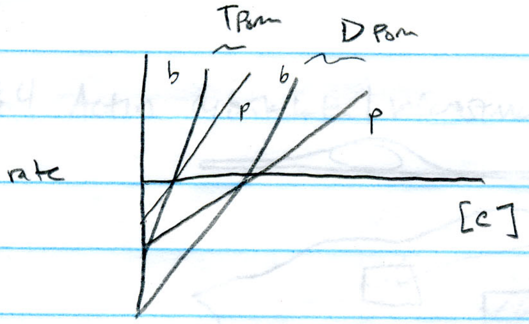


Now have 8 constants $\overset{T}{k}_{on,p}$ $\overset{T}{k}_{off,p}$, $\overset{T}{k}_{on,b}$ $\overset{T}{k}_{off,b}$, $\overset{D}{k}_{on,p}$ $\overset{D}{k}_{off,p}$, $\overset{D}{k}_{on,b}$ $\overset{D}{k}_{off,b}$

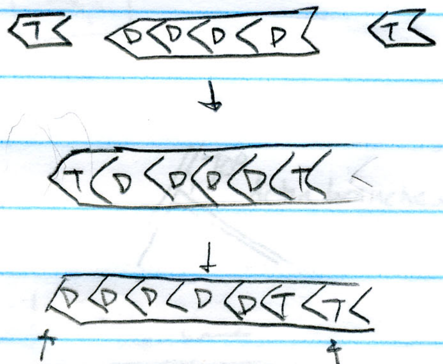
- Critical [ ] same at each end for each filament, but not same for T and D form:

$$\overset{T}{C}_{crit} = \frac{\overset{T}{k}_{off,p}}{\overset{T}{k}_{on,p}} = \frac{\overset{T}{k}_{off,b}}{\overset{T}{k}_{on,b}}, \quad \overset{T}{C}_{crit} < \overset{D}{C}_{crit}$$

$$\overset{D}{C}_{crit} = \frac{\overset{D}{k}_{off,p}}{\overset{D}{k}_{on,p}} = \frac{\overset{D}{k}_{off,b}}{\overset{D}{k}_{off,b}}$$



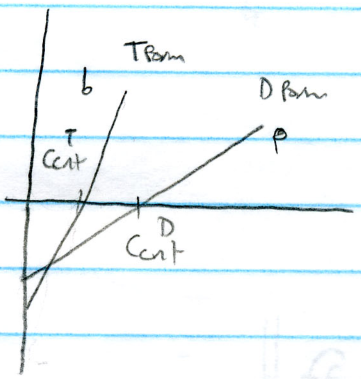
- But strictly T-form or D-form filament don't exist. In reality, G actin mostly in T-form. But when polymerized, hydrolysis quickly occurs. Consider filament only in D form; and add T-form to both ends:



hydrolysis at slow end

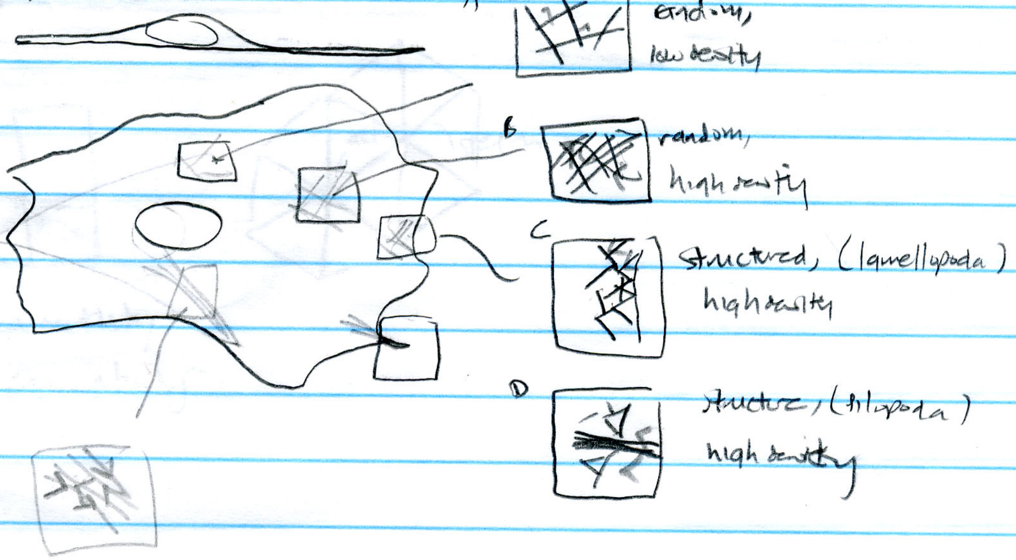
addition at barbed end before hydrolysis occurs, get one end D, other T!

~~Best~~ So get

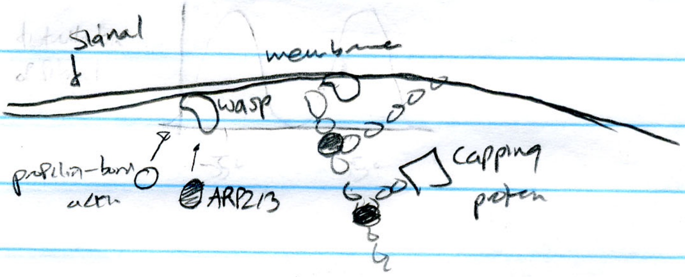


- Get treadmilling when $C_{crit}^{T} < C < C_{crit}^{D}$!
- Highly dynamic filaments mean: - able to produce a wide variety of different structures by altering kinetics
  - must be strong, but having dynamic filaments allows it to be used for motion

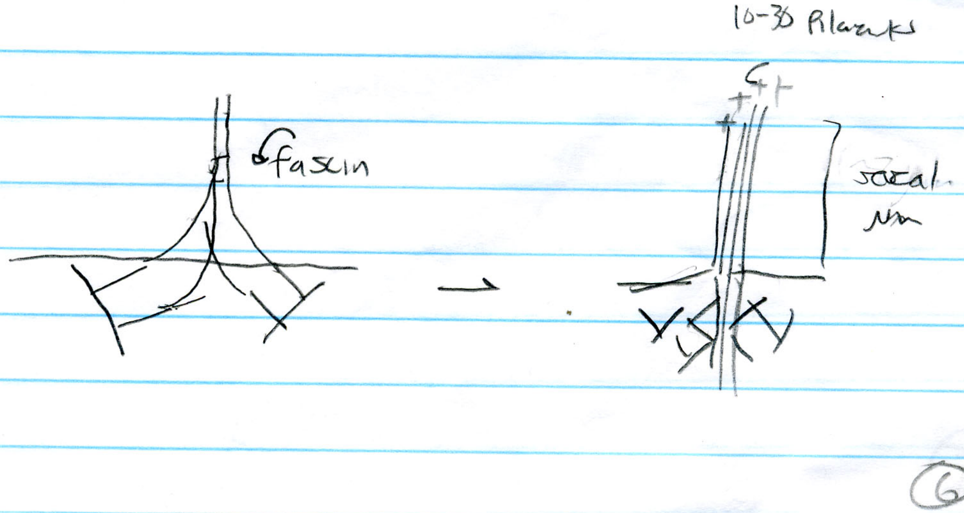
~~1.1~~.4 Actin cytoskeletal microstructure



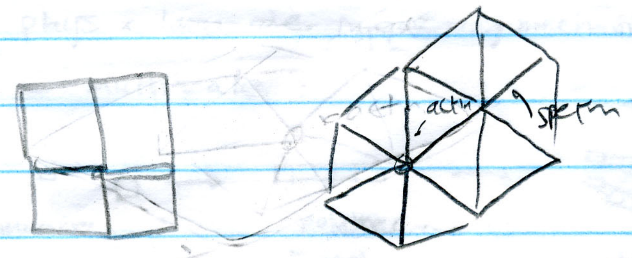
- Lamellopodia
  - 

Actin branches at 70° - ARP2/3 complex
  -
  - 

- Filopodia



Another example of structured network - RBC CSK



- four fold connectivity
- six fold connectivity