Structural Study of Actin Bundles

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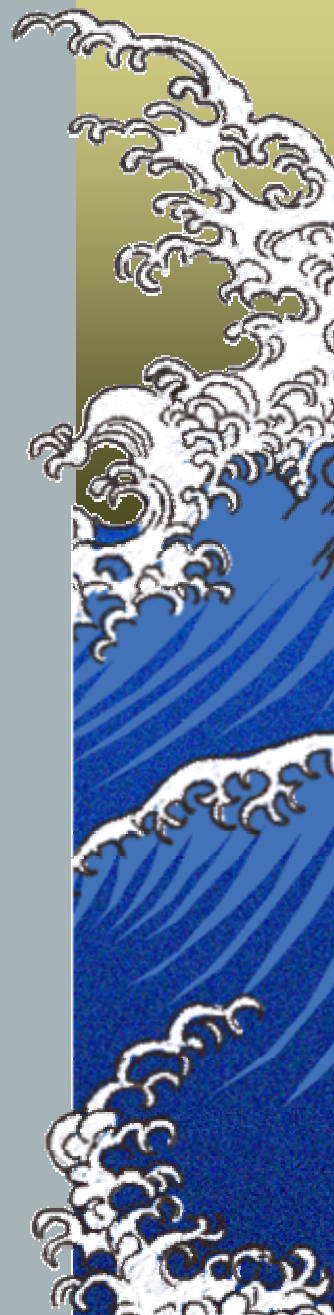
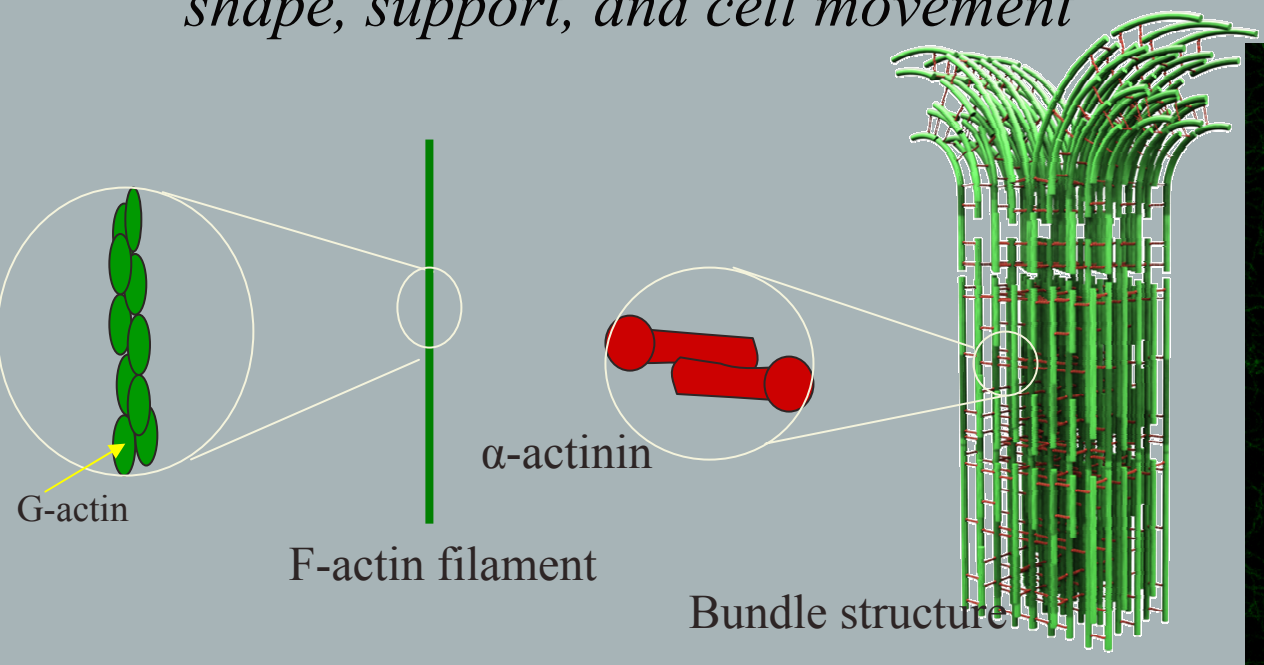
UCSB Materials Research Lab



Vocabulary

- ***F-actin***: *Filamentous protein (10μm long); part of the cytoskeleton; consists of G-actin subunits;*
- ***α-actinin***: *"linker" protein; found in stress fibers, and pseudopodia; used to link F-actin fibers to create bundles*
- ***Bundles***: *cytoskeletal structures important in providing shape, support, and cell movement*



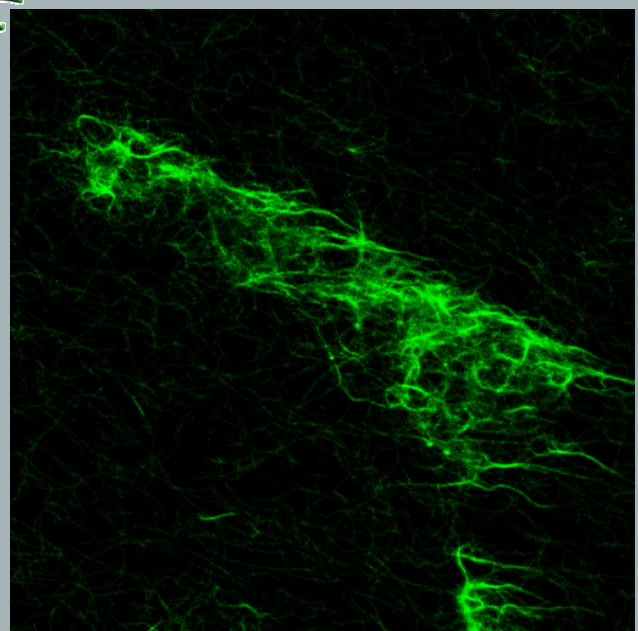


Photo by D. Aranda, 2003

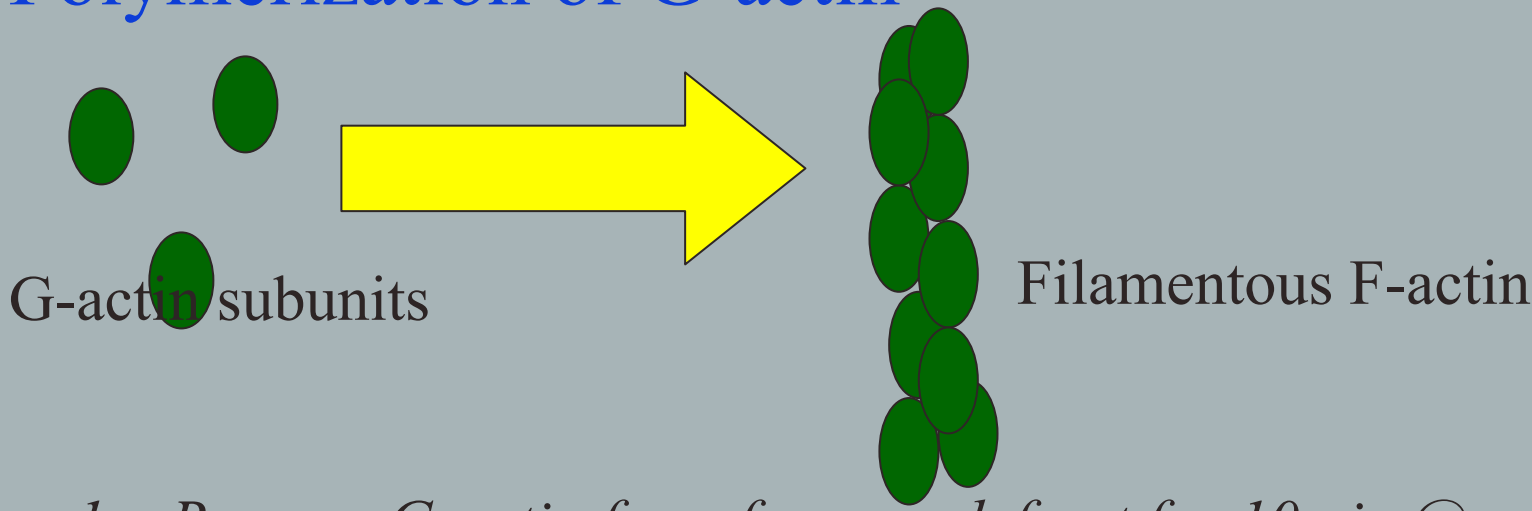
Future Applications

- *Can combine actin bundles into different shapes for use in tissue engineering*
- *Use network structure as a model for nanowires*
- *....these applications are years down the line!*

But Right Now…

- *Study the nature of actin/α-actinin networks*
  - *Laser scanning confocal fluorescence microscopy*
  - *Fluorescence microscopy*
- *Study the structure of actin/polymer bundles*
  - *Small angle x-ray scattering*

Polymerization of G-actin



1. *Remove G-actin from freezer; defrost for 10min @ room temperature*
2. *Dilute to 1mg/ml with G-buffer and let sit for 20 min*
3. *Aliquot 10µl of G-actin and 1 µl of 1M KCl*
4. *Gently stir with tip of pipette; leave for 2.5 hours*
5. *Add 2.4 µl of 100 µM phalloidin*
6. *Actin should polymerize to 10 µm*

Formation of Actin Bundles for Microscopy

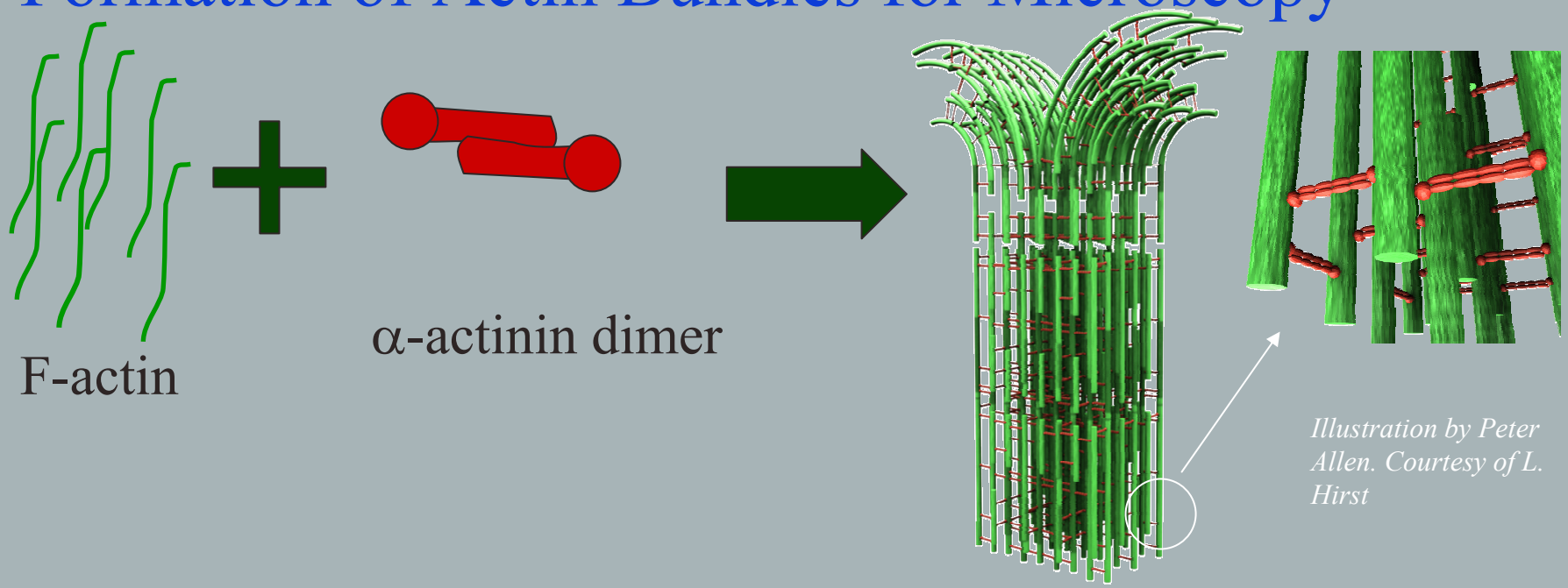
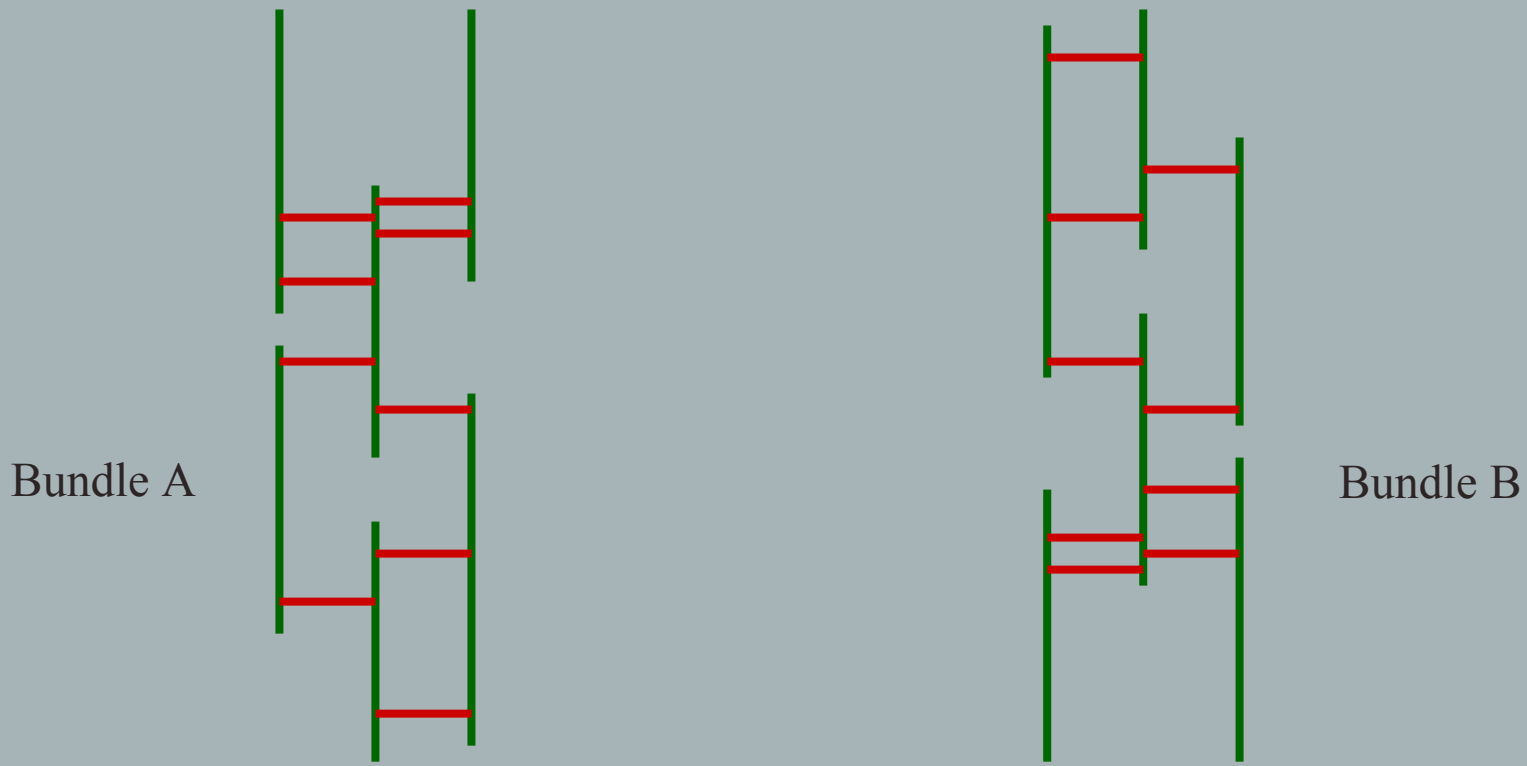


Illustration by Peter Allen. Courtesy of L. Hirst

1. Add set volume of 300mM KCl to make a final concentration of 100mM KCl
2. Add equal volume of α-actinin solution at a concentration to give a molar ratio of 1:5 (α:actin); wait 1 min
3. Add equal volume of F-actin, wait 5-30 min

Question #1

- *Once the bundles are formed, do the filaments move from bundle to bundle?*



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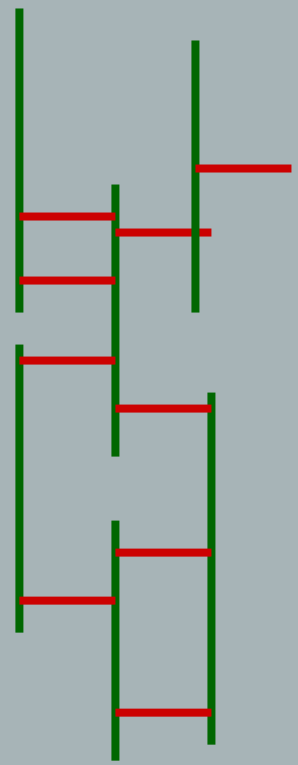
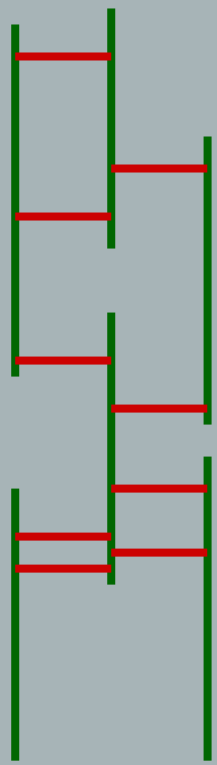


Bundle A



Bundle B

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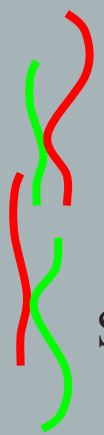

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5. Further experimentation
   - True mixing or free proteins sticking onto existing bundles?

Photo by D. Aranda, 2003

Question #2

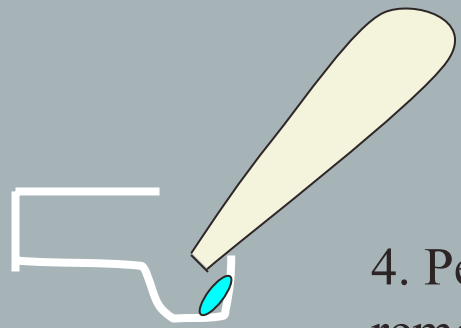
- *What structures are formed when F-actin is mixed with synthetic polymers?*
- *Provide better understanding behind F-actin and α-actinin bundle structure*

Preparing Actin/polymer Bundles

1. F-actin solution and polymer are combined and allowed to sit for 30 min.

2. Bundles are spun @ 11K rpm for 30 min. Pellet is created

3. Supernatant is removed



4. Pellet is removed

5. Pellet and supernatant are placed in capillary and sealed

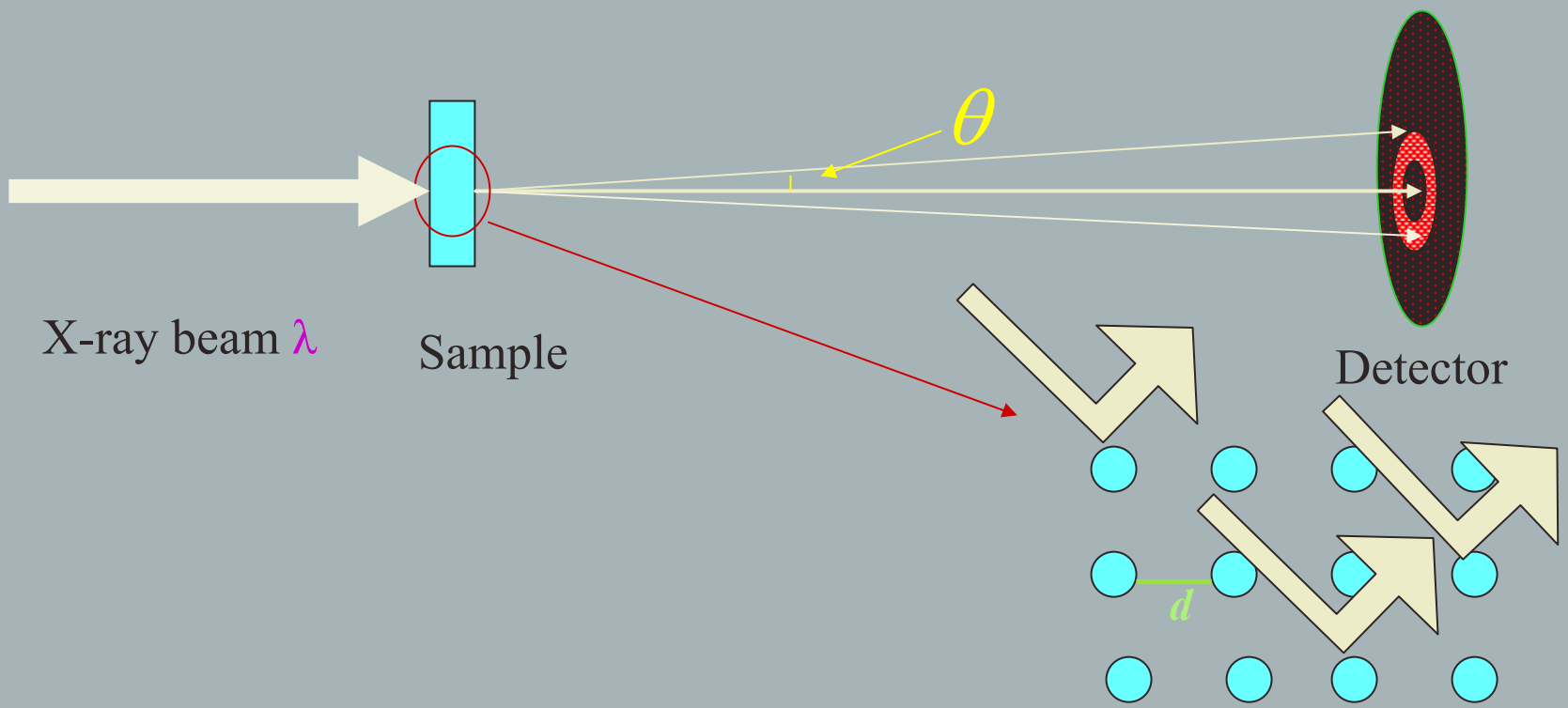
6. Samples are labeled and placed in a small angle X-ray diffraction beam for two hours

Polymers Utilized

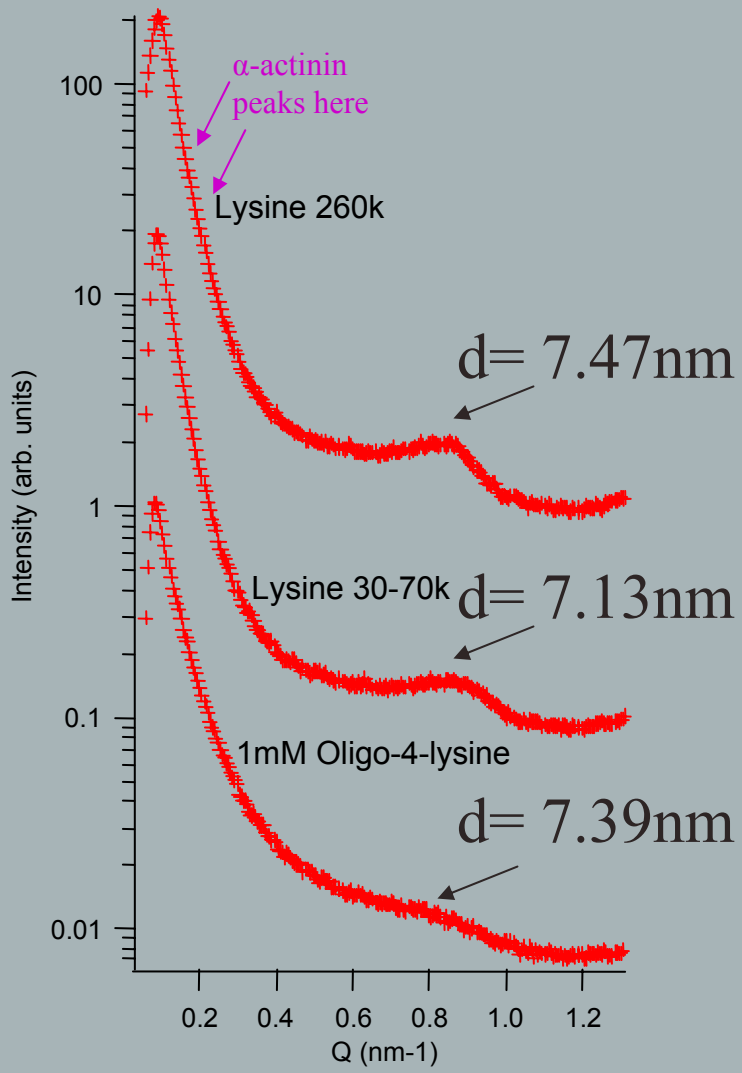
- *Lysine:*
  - *30K-70K mw*(similar weight to G-actin)*
  - *70K-150K mw*
  - *260K mw ** (similar weight to α-actinin)*
  - *Oligo-4-lysine (0.4mM, 1mM)*
- *Proteins 1,2,3*

Small Angle X-Ray Scattering

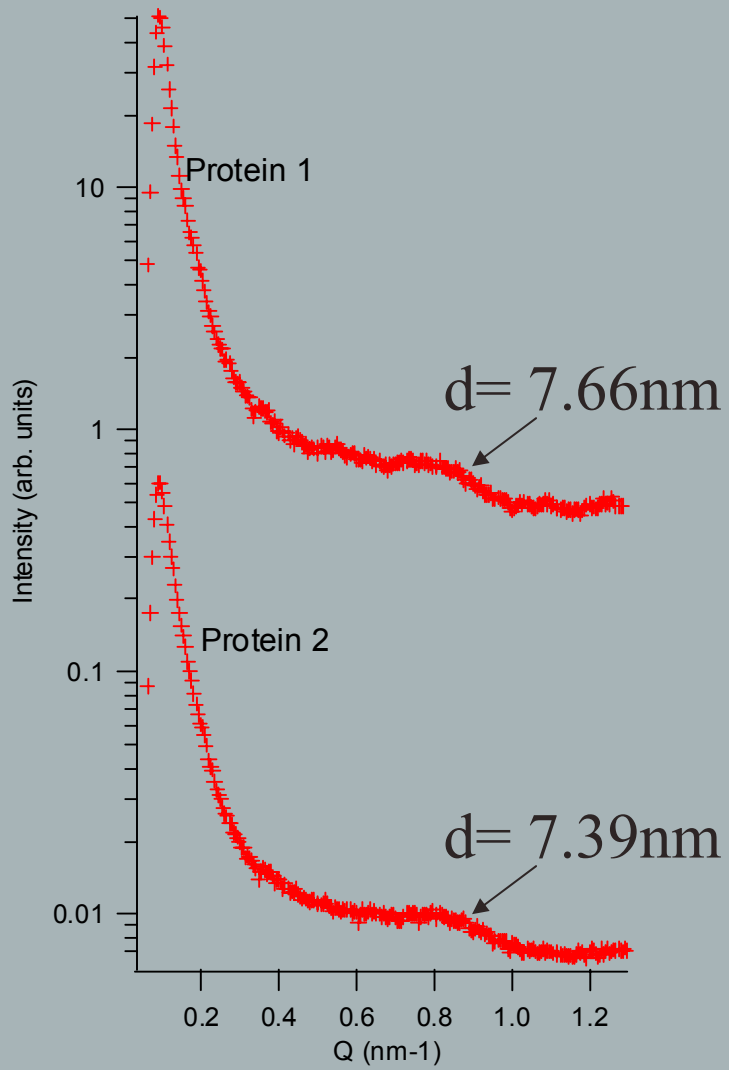
- *Bragg scattering:* $n\lambda = 2d\sin\theta$
- $Q = 2\pi/d$



Error= ± 0.5nm *100mMKCl* *1:5 ratio*



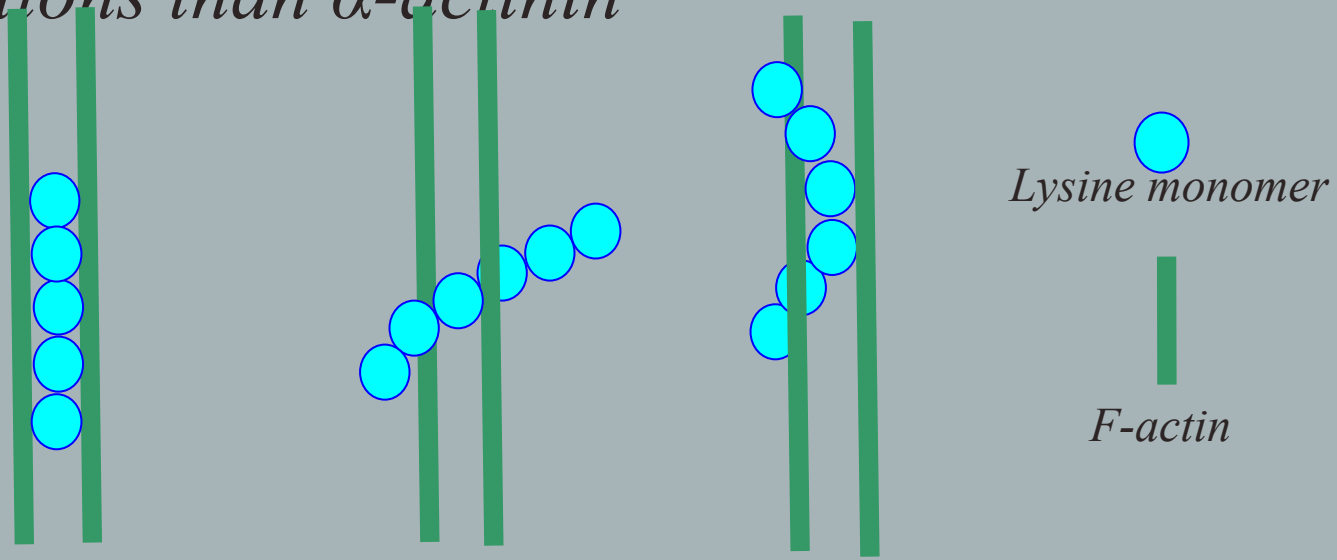
Lysine polymers and actin



Actin bundles made with Proteins 1 and 2

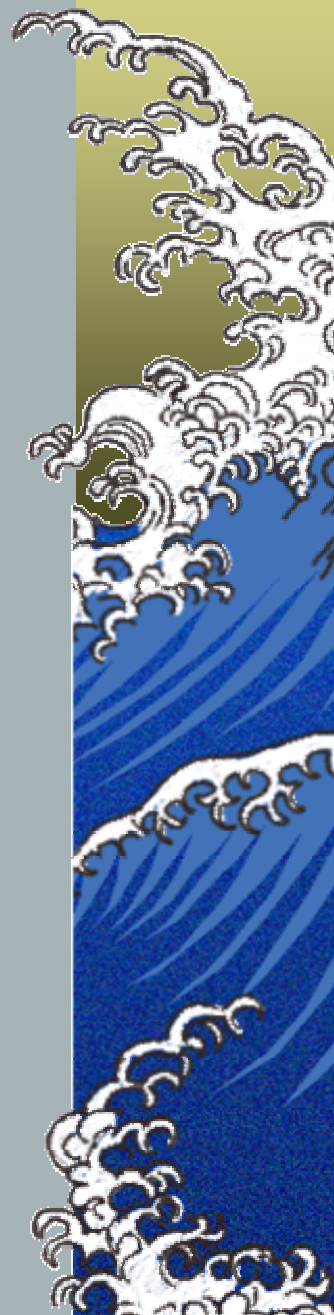
Conclusions

- *Peaks indicate filaments in bundles to be closer together (~7.5nm) than with α-actinin (35nm)*
  - *Peak position consistent with hexagonal packing*
- *Poly-lysines could be flexible and not rigid*
- *Therefore, they could have different bond formations than α-actinin*



Reflections on My Experience

- *Science is a process; the learning never ends*
- *The main branches of science (biology, physics, and chemistry) are intertwined*
- *The cell is a dynamic and complex unit of life*
- *Real science is **not** an exact science*
  - *Equipment breaks*
  - *Hypotheses are incorrect*
  - *Human error*



Thank you

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- *Safinya Group*
- *Apprentice Researchers*