The acrosomal reaction facilitates fusion of the sperm and egg and is especially dramatic in a of organisms in the ocean including the sea cucumber, *Thyone*. Extension of the acrosomal process is driven by cytoskeletal elements and the process in *Thyone* is cartooned below.

A cartoon of the acrosomal reaction in *Thyone*. A) Resting sperm. B) Upon activation with sea water, the acrosomal process extends rapidly (C-E).

Acrosomal process is filled with cytoskeletal elements. Provide two simple experiments that would help convince me that the acrosome is built from actin filaments.

Extension of the thyone acrosomal process is now known to be driven by actin polymerization. You manage to make a fiduciary mark on the actin in the acrosomal process at the site marked in figure 1E. The mark remains in position as the tip of the acrosome continues to extend. Where is actin polymerization occurring in this system?

Given the rate at which the acrosomal process extends and the dimensions of actin monomers, the growing tip must add 400 molecules of actin/second. Given the known rate constants of actin assembly, this means that there must be at least 40 micromolar actin “monomer” available to drive the extension reaction. Actin “monomer” used to polymerize the acrosomal process is stored in the acrosomal vacuole (Fig 1A). Given the biology of this system, the actin monomer concentration in the vacuole must be at least how high?
The critical concentration for actin assembly is only ~0.2 micromolar. So a major question is how does Thyone manage to store a high concentration of G-actin in the vacuole and prevent it from spontaneously polymerizing? To answer this question, researchers developed a way to isolate the Thyone acrosomal vacuole without triggering actin polymerization. They then boiled the preparation in SDS, ran the contents out on a gel, and stained the proteins with coomassie blue. The result is shown below. Lane S4 is the pure vacuole preparation.

![Image of the gel](image)

The authors claim that the band marked “a” in lane S4 is actin. How could you prove that.

By-the-way – Mass Spec didn’t exist for proteins back then, so come up with a simpler, cheaper, faster experiment.

The lower molecular weight protein marked “P” in lane S4 is named profilin. The authors of this classic paper speculate that profilin binds to and sequesters actin monomer preventing it from polymerizing.

You have cloned profilin and can express it and purify it from bacteria. You also have plenty of pure actin. How could you prove profilin suppresses polymerization? I’ll accept written, text answers or an annotated graph.

We now know that all metazoans express profilin and it is a key regulator of actin dynamics in our cells. Our cells contain at least 100 μM actin, half of which is in the monomer “G-actin” form. Many students of the cytoskeleton think profilin is key for preventing the 50 μM G-actin from spontaneously polymerizing. Students of the cytoskeleton try very hard to measure the exact amount of profilin present inside our cells. Why do you think that would be important for understanding actin dynamics in cells?
Limulus acrosomal processes consist of a very stable, highly ordered bundle of actin filaments. They can be used as a convenient tool to study actin elongation reactions. The micrographs above show fragments of Limulus acrosomal processes to which various solutions of actin monomer or profilin-actin were added for the exact same time and then fixed and viewed by electron microscopy.

The electron micrographs on the right compare growth of 1 μM pure G-actin alone off Limulus bundles (7a) to growth of 1 μM profilin-actin complex off Limulus bundles (7b). Panel 7c shows the growth off Limulus bundles using 0.2 μM pure G-actin alone.

We've drawn graphs of the rate of polymer assembly from pure monomer several times in class. Draw a graph of actin assembly as a function of time plus-minus Limulus bundles.

From the images, what is the evidence that the actin filaments within the Limulus actin bundle are all oriented in the same direction?

Cytochalasin D is a very useful drug for studying actin. What would you expect to see if you repeated the experiment in figure 7a in the presence of cytochalasin D?

Latrunculin is another useful drug for studying actin. What would you expect to see if you repeated the experiment shown in 7a in the presence of Latrunculin?

Based upon the results in figure 7, the authors claim that profilin raises the critical concentration for pointed (−) end actin assembly. What justifies that conclusion?

Shortly after this seminal paper was published, other scientists identified a new actin binding protein called CapZ. They think CapZ binds to the (+) ends of actin and prevents growth from that end. How could you use Limulus bundles to help prove that?
Microtubule dynamics in cells.

We know that microtubules are constantly assembling and disassembling in cells. Nucleotide hydrolysis on microtubules drives turnover dynamics. We have seen in class and also in your reading that ATP hydrolysis on actin results in a property known as treadmilling. Many scientists once thought that MTs would use GTP hydrolysis to drive treadmilling of microtubules. First of all, what is treadmilling?

Draw a cartoon of what treadmilling on a single microtubule would look like. Include GTP versus GDP in the drawing.

Figure 8 is a classic experiment. The authors injected biotinylated tubulin into a cell, fixed seconds thereafter, and then compared the total microtubule distribution (left panel) to where biotinylated tubulin incorporated (right panel).

Please comment on the following conclusions and statements made about these images.

1. Incorporation of tubulin subunits along the walls of existing microtubules is irrelevant.
2. The orientation of labeled segments of MTs at the periphery of the cell implies that new tubulin polymerization at the periphery occurs off the ends of existing microtubules as opposed to spontaneous nucleation. (See next page for higher mag view)
3. The higher spatial resolution of electron microscopy could help determine whether biotinylated tubulin at the cell periphery incorporates on to existing MTs or assembles through spontaneous nucleation.
4. The majority of new microtubule polymerization occurs near the centrosome.
Higher magnification view of total microtubules (B) and biotinylated tubulin (D) at the periphery of the cell.
In the experiment depicted above, a solution of tubulin was polymerized into microtubules all the way to steady state. The sample was then diluted into buffer or into a solution containing XKCM1 and light scattering recorded over time. Obviously, XKCM1 somehow disassembles microtubules. This experiment measures the dynamics of an entire population of microtubules, but it does not tell us much about the mechanism of microtubule disassembly in the presence of XKCM1. Consider what you already know from class and your reading about microtubule assembly/disassembly dynamics. From the perspective of the microtubule, name two general ways in which XKCM1 would accelerate MT disassembly.

Begin thinking about how imaging the behavior of individual microtubules could reveal the mechanism of MT disassembly by XKCM1.
Neuronal growth cones are guided to their targets, in part, by inhibitory cues that repel them and thus steer growth cones back towards the appropriate target. One such inhibitory cue, known as Semaphorin 3A, triggers a collapse response when applied to a growth cone as depicted in the cartoon above.

Formulate a hypothesis as to how Semaphorin 3A triggers growth cone collapse.

Describe how you could use a combination of drugs and video microscopy to distinguish whether Sema3A signals to actin or microtubules or both to trigger growth cone collapse.