Part 1: Actin & myosin

Open Actin\_myosin.pse. This file has contains several structures including a recent high resolution CryoEM structure of actin and myosin VI by the Alushin lab at Rockefeller.

1) Actin forms one of the major cytoskeleton filaments in the cell. Actin filaments are defined by a “barbed” and “pointed” end. Can you identify which end is which in the structure?

2) Look at the “Myosin” object (this is Myosin VI, an unusual myosin that “walks” towards the pointed end – all other myosins traverse the other way). This is the motor domain, which binds actin and uses ATP to power myosin’s walking. Each of the available binding sites for myosin are occupied in this structure. Compare the Myosin X binding – Myosin X walks the other direction – are they facing the opposite way? Myosins have many more domains – can you imagine how Myosin “prefers” one direction to another?

3) Actin filaments are constantly being remodeled in the cell, for instance as they move or come into contact with other cells. One molecule that helps with this remodeling are cofilins, which “cap” actin and cause its depolymerization. Look at where the cofilins are binding. Compare that to where the myosins are binding – what does this tell you about how many molecules recognize actin?

4) Hide the myosins and hide the surface of our bare actin filament and show it as a cartoon instead. Look at where the cofilins are binding. Look at the cartoon of the cofilin-bound actin compared to the bare actin. Note the differences? How might you hypothesize cofilin “encourages” actin to depolymerize?

5) Another protein that modifies actin is the ARP2/3 complex (“ARP” stands for “Actin related protein”). ARP2/3 seeds new actin filaments, initiating polymerization. It can also cause new actin filaments to “branch” off of old ones. As individual actin filaments are very flexible, this can strengthen them. This structure of ARP2/3 has been aligned to the actin filament, though it was not solved bound to actin. It has been aligned to the actin filament. Look at the purple subunit of the ARP2/3 complex and compare it to actin. What might be a hypothesis of how ARP2/3 seeds actin filament formation (or branching)?

6) Quickly measure the diameter of an actin filament.

Part 2: Microtubules

Microtubule\_kinesin.pse this file has an CryoEM model of a microtubule bound to kinesin. Because microtubules are symmetrical (they form spirals), the individual monomers can be averaged, to improve the quality of the density. This has made them ideal systems for early methods development of EM structural studies. The model in this file is of a series of tubulin dimers in what would essentially be the middle of a stable microtubule. We don’t really know what the end of a microtubule looks like because it’s very dynamic (Luke Rice at UTSW studies microtubule dynamics).

1) Kinesins are the major motor proteins that “walk” along microtubules. Shown is the structure of the kinesin “head” (the microtubule-binding, motor-domain) bound to a tubulin dimer. A large amount of traffic (going in both directions) can be carried along a single microtubule. Looking at the kinesin and the microtubule, why do you think that is?

1) Measure the diameter and length of this section of microtubule. Compare that to the individual actin filament. Did you imagine it would be this size? How flexible do you think microtubules are as compared to actin?

2) Why do you think microtubules are called “tubules?”

3) One of the major functions of microtubules is in mitosis – they help pull the replicated DNA apart during cytokinesis. Open the raw pdb of the nucleosome (1-PyMOL\_basics/raw\_pdbs/Nucleosome\_1aoi.pdb). Drag it to near the microtubule. What does this tell you about how microtubules interact with the DNA?