Overview:
This seminar focuses on molecular mechanisms of cytoskeletal components. The course will examine the actin cytoskeleton and the microtubule cytoskeleton. A sample of topics include 1) the core building blocks: actin and tubulin; 2) nucleators: Arp2/3 and gamma tubulin/gamma-TuRC/Augmin; 3) motors: myosin, kinesin and dynein; 4) regulators: formins and microtubule plus end binding proteins; 5) destabilizers: cofilin and stathmin; and 6) kinetochore-microtubule attachments complexes. Primary literature will be examined, presented and critiqued. Each topic will examine a molecular/mechanistic that correlates structure with mechanism. Emerging techniques in cell biology and structure will be discussed including single molecule fluorescent techniques (PALM, FIONA, speckle microscopy), optical trapping, single particle electron microscopy, x-ray crystallography and small angle X-ray scattering. The course is intended to familiarize cell biologists with molecular mechanisms and protein structure, promoting proficiency in viewing, evaluating and presenting structure models using molecular graphics programs in order to design and implement structure-based experiments. The seminar aims to develop presentation skills, scientific writing, as well as manuscript evaluation and critique.

Methodology:
As a seminar course, we will examine primary literature. Participation from all members is critical. Each week, papers will be presented to the group by one or two assigned members. During the presentation, the paper will be critiqued as a group. People presenting the paper will present the material via Powerpoint or equivalent program. Aside from presenting figures from the paper, the presenters may be asked to show structure figures and movies they have created and rendered using molecular graphics software. Additional papers assigned for the class will be examined in a round-table format. Be prepared to discuss the paper and the presenter should have familiarity with any supplemental material.

In order to familiarize students with manuscript preparation techniques and the practice of reviewing papers for a journal, we will go over aspects of 1) writing cover letters to the editor for manuscript submission and 2) how to write a review of a manuscript for an editor. The student will prepare two cover letters, directed at the editor for two of the papers presented in the course. These cover letters will be presented to the class prior to going over the paper. At two points in the semester, a list of manuscripts will be posted. Students should choose one paper from each list and serve as a mock referee. Students
are also welcome to propose a paper for review that focuses on a specific area of interest to them that overlaps with topics covered in class. The review will be due at assigned dates shown below.

At the final class (Saturday, Dec 5th), each student will give a mini-presentation on a recently published cytoskeletal paper that probes molecular/cellular mechanism. Papers should selected by the student and approved by the instructor by Nov 24th. This manuscript should be distinct from the two manuscripts that the student based their two mock reviews on.

**Grading:**

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<tr>
<th>Component</th>
<th>Percentage</th>
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<tr>
<td>Participation Throughout Course</td>
<td>45%</td>
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<tr>
<td>Presentations (2 x 15%)</td>
<td>30%</td>
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<td>Cover Letters (2 x 2.5%)</td>
<td>5%</td>
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<tr>
<td>Reviews: 1st (Midterm)</td>
<td>7.5%</td>
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<td></td>
<td>2nd (Final Exam)</td>
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<td>Final Mini Presentation</td>
<td>5%</td>
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**Text:**

No text required, this course examines primary literature that will be available on PubMed and available for download from the class Sakai site.

**Exams:**

The midterm exam and the final exam will take the form of the mock manuscript reviews outlined above. The midterm review will be due Tuesday, October 13th and the final exam review will be due on Saturday, December 5th.
Building Blocks

1: Course Overview, Tuesday, August 18

Introduction to Protein Structure Determination
Introduction to the Protein Data Bank, pdb files
Introduction to PyMOL Molecular Graphics Program

2: Tubulin, Thursday, August 20

Nogales E, Wolf SG, Downing KH.
Structure of the alpha beta tubulin dimer by electron crystallography.

Dimitrov A, Quesnoit M, Moutel S, Cantaloube I, Poüs C, Perez F.
Detection of GTP-tubulin conformation in vivo reveals a role for GTP remnants in microtubule rescues.

3: Actin, Tuesday, August 25

Otterbein LR, Graceffa P, Dominguez R.
The crystal structure of uncomplexed actin in the ADP state.

Ponti A, Machacek M, Gupton SL, Waterman-Storer CM, Danuser G.
Two distinct actin networks drive the protrusion of migrating cells.

4: Bacterial Actin, Thursday, August 27

Garner EC, Campbell CS, Weibel DB, Mullins RD.
Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog.
Nucleators, Minus End

5: γ-TuRC / Patronin, Tuesday, September 1

Kollman JM, Polka JK, Zelter A, Davis TN, Agard DA.
Microtubule nucleating gamma-TuSC assembles structures with 13-fold microtubule-like symmetry.
Nature. 2010 Aug 12;466(7308):879-82

Goodwin SS, Vale RD.
Patronin regulates the microtubule network by protecting microtubule minus ends.

6: Centrioles I, Thursday, September 3

Structural basis of the 9-fold symmetry of centrioles.
Cell. 2011 Feb 4;144(3):364-75.

7: Centrioles II, Tuesday, September 8

Mennella V, Keszthelyi B, McDonald KL, Chhun B, Kan F, Rogers GC, Huang B, Agard DA.
Subdiffraction-resolution fluorescence microscopy reveals a domain of the centrosome critical for pericentriolar material organization.

8: Arp2/3 I, Thursday, September 10

Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S, Pollard TD.
Crystal structure of Arp2/3 complex.

9: Arp2/3 II, Tuesday, September 15

Svitkina TM, Borisy GG.
Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia.
Tip Proteins

10: MT +Tips, Thursday, September 17

Maurer SP, Fourniol FJ, Bohner G, Moores CA, Surrey T.
EBs recognize a nucleotide-dependent structural cap at growing microtubule ends.

11: XMAP215 Tuesday, September 22

A tethered delivery mechanism explains the catalytic action of a microtubule polymerase.

12: Formins, Thursday, September 24

Otomo T, Tomchick DR, Otomo C, Panchal SC, Machius M, Rosen MK.
Structural basis of actin filament nucleation and processive capping by a formin homology 2 domain.

13: Kinetochore, Tuesday, September 29

Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex.

Powers AF, Franck AD, Gestaut DR, Cooper J, Gracyzk B, Wei RR, Wordeman L, Davis TN, Asbury CL.
The Ndc80 kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion.

Post-translational Modifications

14: Post-translational Modifications, Thursday, October 1

Molecular basis for age-dependent microtubule acetylation by tubulin acetyltransferase.
Destabilizers

15: Spastin & Katanin I, Tuesday, October 6

Roll-MecaK A, Vale RD I.
Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin.

16: Spastin & Katanin II, Thursday, October 8

A mechanism for reorientation of cortical microtubule arrays driven by microtubule severing.

17: Stathmin, Tuesday, October 13

MIDTERM REVIEW ASSIGNMENT DUE

The 4 A X-ray structure of a tubulin:stathmin-like domain complex.

Ravelli RB, Gigant B, Curmi PA, Jourdain I, Lachkar S, Sobel A, Knossow M.
Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain.

18: Cofilin I, Tuesday, October 20

Paavilainen VO, Oksanen E, Goldman A, Lappalainen P.
Structure of the actin-depolymerizing factor homology domain in complex with actin.

Jansen S, Collins A, Chin SM, Ydenberg CA, Gelles J, Goode BL.
Single-molecule imaging of a three-component ordered actin disassembly mechanism.

19: Cofilin II, Thursday, October 22

Ghosh M, Song X, Mouneimne G, Sidani M, Lawrence DS, Condeelis JS.
Cofilin promotes actin polymerization and defines the direction of cell motility.
Motors

20: Kinesin I, Tuesday, October 27

Structure of a kinesin-tubulin complex and implications for kinesin motility.

Kaan HY, Hackney DD, Kozielski F.
The structure of the kinesin-1 motor-tail complex reveals the mechanism of autoinhibition.

21: Kinesin II, Thursday, October 29

Mori T, Vale RD, Tomishige M.
How kinesin waits between steps.

22: Dynein I, Tuesday, November 3

Structural basis for microtubule binding and release by dynein.
PMID: 22997337 [PubMed - indexed for MEDLINE]

23: Dynein II, Thursday, November 5

Lis1 regulates dynein by sterically blocking its mechanochemical cycle.

24: Dynein III, Tuesday, November 10

Urnavicius L, Zhang K, Diamant AG, Motz C, Schlager MA, Yu M, Patel NA, Robinson CV, Carter AP.
The structure of the dynactin complex and its interaction with dynein.

25: Myosin I, Thursday, November 12

Ménétrey J, Llinas P, Mukherjea M, Sweeney HL, Houdusse A.
The structural basis for the large powerstroke of myosin VI.

26: Myosin II, Tuesday, November 17

Kodera N, Yamamoto D, Ishikawa R, Ando T.
Video imaging of walking myosin V by high-speed atomic force microscopy.
27: Motor Wars I, Thursday, November 19

Derr ND, Goodman BS, Jungmann R, Leschziner AE, Shih WM, Reck-Peterson SL.
Tug-of-war in motor protein ensembles revealed with a programmable DNA origami scaffold.

28: Motor Wars II, Tuesday, November 24

Shih SM, Engel BD, Kocabas F, Bilyard T, Gennerich A, Marshall WF, Yildiz A.
Intraflagellar transport drives flagellar surface motility.

29: Motor Wars III, Tuesday, December 1

Ally S, Larson AG, Barlan K, Rice SE, Gelfand VI.
Opposite-polarity motors activate one another to trigger cargo transport in live cells.

Nakamura M, Chen L, Howes SC, Schindler TD, Nogales E, Bryant Z.
Remote control of myosin and kinesin motors using light-activated gearshifting.

30: Special Topics, Saturday, December 5

FINAL REVIEW ASSIGNMENT DUE

Recent Articles – Mini Presentations from all members