

Biol643, Molecular Mechanisms of the Cytoskeleton: Microtubules, Actin and Intermediate Filaments

Course Hours:3

Overview:

This seminar focuses on the structure and molecular mechanisms of cytoskeletal components. The course will examine the actin cytoskeleton, the microtubule cytoskeleton, intermediate filaments and bacterial cytoskeletal homologs. A sample of topics include 1) the core building blocks: actin and tubulin; 2) nucleators: Arp2/3 and gamma tubulin/gamma-TuRC; 3) regulators: formins and microtubule plus end binding proteins; 4) destabilizers: Kin1 and stathmin; 5) motors: myosin, kinesin and dynein; and 6) kinetochore-microtubule attachments complexes: the Dam1 complex and the Ndc80 complex. Primary literature will be examined, presented and critiqued. Each topic will focus on a structural paper and a complementary cell biology/biophysics paper that correlates structure with mechanism. This course is intended to familiarize cell biologists with protein structure, promoting proficiency in viewing, evaluating and presenting structure models using molecular graphics programs in order to design and implement structure-based experiments.

Methodology:

As a seminar course, we will examine primary literature. Participation from all members is critical. Each week, a structure paper 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 structure paper will present the material via Powerpoint or equivalent program. Aside from presenting figures from the paper, the presenters will 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 supplementary 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. One review paper will count as a midterm exam and the second review paper will constitute the final exam.

Towards the end of the semester, there will be a molecular graphics Picture/Movie competition. Students should choose their favorite pdb structure related to cell biology and create both an artistic picture and a movie that highlights and conveys specific scientific information. A caption should be included. A grand prize winner will be selected.

Grading:

Participation Throughout Course	50%
Structure Presentations (2)	30%
Cover Letters (2)	5%
Reviews: 1st (Midterm)	5%
2 nd (Final Exam)	5%
Picture & Movie Competition	5%

Text:

No text required, this course examines primary literature.

Exams:

The midterm exam and the final exam will take the form of the editorial reviews outlined above. The midterm review will be due Monday, October 20th and the final exam review will be due on Monday, December 8th.

Week 1: Monday, August 25th

Course Overview

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

Building Blocks

Tubulin

Present:

[Nogales E, Wolf SG, Downing KH.](#)

Structure of the alpha beta tubulin dimer by electron crystallography.
Nature. 1998 Jan 8;391(6663):199-203. Erratum in: Nature 1998 May 14;393(6681):191.

Roundtable:

[Wang HW, Nogales E.](#)

Nucleotide-dependent bending flexibility of tubulin regulates microtubule assembly.
Nature. 2005 Jun 16;435(7044):911-5.

Week 2: Actin, Monday, September 8th

Present:

[Otterbein LR, Graceffa P, Dominguez R.](#)

The crystal structure of uncomplexed actin in the ADP state.
Science. 2001 Jul 27;293(5530):708-11.

Roundtable:

[Ponti A, Machacek M, Gupton SL, Waterman-Storer CM, Danuser G.](#)

Two distinct actin networks drive the protrusion of migrating cells.
Science. 2004 Sep 17;305(5691):1782-6.

Week 3: Intermediate Filaments, Monday, September 15th

Present:

[Strelkov SV, Herrmann H, Geisler N, Lustig A, Ivaninskii S, Zimbelmann R, Burkhard P, Aebi U.](#)

Divide-and-conquer crystallographic approach towards an atomic structure of intermediate filaments.
J Mol Biol. 2001 Mar 2;306(4):773-81.

Roundtable:

[Ausmees N, Kuhn JR, Jacobs-Wagner C.](#)

The bacterial cytoskeleton: an intermediate filament-like function in cell shape.
Cell. 2003 Dec 12;115(6):705-13.

Week 4: Bacterial Tubulin Homologs: FtsZ, Monday, September 22nd

Present:

[Löwe J, Amos LA.](#)

Crystal structure of the bacterial cell-division protein FtsZ.
Nature. 1998 Jan 8;391(6663):203-6.

Roundtable:

[Osawa M, Anderson DE, Erickson HP.](#)

Reconstitution of contractile FtsZ rings in liposomes.
Science. 2008 May 9;320(5877):792-4. Epub 2008 Apr 17.

[Loose M, Fischer-Friedrich E, Ries J, Kruse K, Schwille P.](#)

Spatial regulators for bacterial cell division self-organize into surface waves in vitro.
Science. 2008 May 9;320(5877):789-92.

Week 5: Bacterial Actin Homologs: ParM, Monday, September 29th

Present:

[van den Ent F, Møller-Jensen J, Amos LA, Gerdes K, Löwe J.](#)

F-actin-like filaments formed by plasmid segregation protein ParM.
EMBO J. 2002 Dec 16;21(24):6935-43.

Roundtable:

[Salje J, Löwe J.](#)

Bacterial actin: architecture of the ParMRC plasmid DNA partitioning complex.
EMBO J. 2008 Jul 24

[Garner EC, Campbell CS, Weibel DB, Mullins RD.](#)

Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog.
Science. 2007 Mar 2;315(5816):1270-4.

Nucleators

Week 6: γ -Tubulin, γ -TuRC, Monday, October 6th

Present:

[Aldaz H, Rice LM, Stearns T, Agard DA.](#)

Insights into microtubule nucleation from the crystal structure of human gamma-tubulin.
Nature. 2005 May 26;435(7041):523-7.

Roundtable:

[Moritz M, Braunfeld MB, Guénebaut V, Heuser J, Agard DA.](#)

Structure of the gamma-tubulin ring complex: a template for microtubule nucleation.
Nat Cell Biol. 2000 Jun;2(6):365-70.

[Moritz M, Braunfeld MB, Sedat JW, Alberts B, Agard DA.](#)

Microtubule nucleation by gamma-tubulin-containing rings in the centrosome.
Nature. 1995 Dec 7;378(6557):638-40.

Week 7: Arp2/3, Monday, October 13th

Present:

[Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S, Pollard TD.](#)

Crystal structure of Arp2/3 complex.

Science. 2001 Nov 23;294(5547):1679-84.

Roundtable:

[Svitkina TM, Borisy GG.](#)

Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia.

J Cell Biol. 1999 May 31;145(5):1009-26.

Tip Proteins

Week 8: MT +Tips, Monday, October 20th

Present:

[Hayashi I, Ikura M.](#)

Crystal structure of the amino-terminal microtubule-binding domain of end-binding protein 1 (EB1).

J Biol Chem. 2003 Sep 19;278(38):36430-4. Epub 2003 Jul 11.

[Honnappa S, Okhrimenko O, Jaussi R, Jawhari H, Jelesarov I, Winkler FK, Steinmetz MO.](#)

Key interaction modes of dynamic +TIP networks.

Mol Cell. 2006 Sep 1;23(5):663-71.

Roundtable:

[Bieling P, Laan L, Schek H, Munteanu EL, Sandblad L, Dogterom M, Brunner D, Surrey T.](#)

Reconstitution of a microtubule plus-end tracking system in vitro.

Nature. 2007 Dec 13;450(7172):1100-5. Epub 2007 Dec 2.

Week 9: Formins, Monday, October 27th

Present:

[Xu Y, Moseley JB, Sagot I, Poy F, Pellman D, Goode BL, Eck MJ.](#)

Crystal structures of a Formin Homology-2 domain reveal a tethered dimer architecture.

Cell. 2004 Mar 5;116(5):711-23.

[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.

Nature. 2005 Feb 3;433(7025):488-94. Epub 2005 Jan 5.

Roundtable:

[Romero S, Le Clainche C, Didry D, Egile C, Pantaloni D, Carlier MF.](#)

Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis.

Cell. 2004 Oct 29;119(3):419-29.

Destabilizers

Week 10: Spastin, Monday, November 3rd

Present:

[Roll-Mecak A, Vale RD.](#)

Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin. Nature. 2008 Jan 17;451(7176):363-7.

Roundtable:

[Evans KJ, Gomes ER, Reisenweber SM, Gundersen GG, Lauring BP.](#)

Linking axonal degeneration to microtubule remodeling by Spastin-mediated microtubule severing. J Cell Biol. 2005 Feb 14;168(4):599-606.

Week 11: Stathmin, Monday, November 10th

Present:

[Gigant B, Curmi PA, Martin-Barbey C, Charbaut E, Lachkar S, Lebeau L, Siavoshian S, Sobel A, Knossow M.](#)

The 4 Å X-ray structure of a tubulin:stathmin-like domain complex. Cell. 2000 Sep 15;102(6):809-16.

Roundtable:

[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. Nature. 2004 Mar 11;428(6979):198-202.

[Gigant B, Wang C, Ravelli RB, Roussi F, Steinmetz MO, Curmi PA, Sobel A, Knossow M.](#)

Structural basis for the regulation of tubulin by vinblastine. Nature. 2005 May 26;435(7041):519-22.

Week 12: Cofilin, Monday, November 17th

Present:

[Fedorov AA, Lappalainen P, Fedorov EV, Drubin DG, Almo SC.](#)

Structure determination of yeast cofilin. Nat Struct Biol. 1997 May;4(5):366-9.

[Paavilainen VO, Oksanen E, Goldman A, Lappalainen P.](#)

Structure of the actin-depolymerizing factor homology domain in complex with actin. J Cell Biol. 2008 Jul 14;182(1):51-9.

Roundtable:

[Ghosh M, Song X, Mouneimne G, Sidani M, Lawrence DS, Condeelis JS.](#)

Cofilin promotes actin polymerization and defines the direction of cell motility. Science. 2004 Apr 30;304(5671):743-6.

Motors

Week 13: Kinesin, **Monday, November 24rd**

Present:

[Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD.](#)

Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. Nature. 1996 Apr 11;380(6574):550-5.

Roundtable:

[Mori T, Vale RD, Tomishige M.](#)

How kinesin waits between steps.

Nature. 2007 Nov 29;450(7170):750-4. Epub 2007 Nov 14.

Week 14: Dynein, **Monday, December 1st**

Present:

[Burgess SA, Walker ML, Sakakibara H, Knight PJ, Oiwa K.](#)

Dynein structure and power stroke.

Nature. 2003 Feb 13;421(6924):715-8.

Roundtable:

[Reck-Peterson SL, Yildiz A, Carter AP, Gennerich A, Zhang N, Vale RD.](#)

Single-molecule analysis of dynein processivity and stepping behavior.

Cell. 2006 Jul 28;126(2):335-48.

Week 15: Myosin, **Monday, December 8th (Time Change: 12:00 – 2:00)**

Present:

[Ménétreay J, Llinas P, Mukherjea M, Sweeney HL, Houdusse A.](#)

The structural basis for the large powerstroke of myosin VI.

Cell. 2007 Oct 19;131(2):300-8.

Roundtable:

[Yildiz A, Forkey JN, McKinney SA, Ha T, Goldman YE, Selvin PR.](#)

Myosin V walks hand-over-hand: single fluorophore imaging with 1.5-nm localization.

Science. 2003 Jun 27;300(5628):2061-5. Epub 2003 Jun 5.