Module II: Single Molecule Mechanics

25.3	Tue (6, GT)	Introductory unit: Single molecule mechanics	Read PBoC 8.3
27.3	Thu (GT)	Open discussion, Q&A	Ask questions
1.4	Tue (7)	Paul Hansma	Read paper
3.4	Thu (GT)	Discuss paper analysis	Bring completed worksheet
8.4	Tue (8)	Carlos Bustamante	Read paper
10.4	Thu (GT)	Discuss paper analysis	Bring completed worksheet
15.4	Tue (9)	Steven Block Outlook: Single molecule mechanics	Read paper
17.4	Thu (GT)	Discuss paper analysis	Bring completed worksheet
29.4	Tue (10)	Discussion: Single molecule Introductory unit: Collective/emergent properties	Read pdf

Previously

Paul Hansma

Development of AFMs to monitor individual protein molecules, in liquids (1990-2000)

Awarded in 2000:

“For pioneering contributions to the development of biological scanning probe microscopy and for the molecular resolution imaging of biological molecules in aqueous solutions.”

Carlos Bustamante

Study of DNA, RNA, and protein molecular mechanics (1990-2000)

Awarded in 2002:

“For his pioneering work in single molecule biophysics and the elucidation of the fundamental physics principles underlying the mechanical properties and forces involved in DNA replication and transcription.”

Imaging Crystals, Polymers, and Processes in Water with the Atomic Force Microscope

B. DRAKE, C. B. PRATER, A. L. WEISENHORN, S. A. C. GOULD, T. R. ALBRECHT, C. F. QUATE, D. S. CANNELL, H. G. HANSMA, AND P. K. HANSMA Authors Info & Affiliations

SCIENCE • 24 Mar 1989 • Vol 243, Issue 4898 • pp. 1586-1589 • DOI: 10.1126/science.2928794

Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM

MATTHIAS RIEF, MATHIAS GAUTEL, FILIPP OESTERHELT, JULIO M. FERNANDEZ, AND , HERMANN E. GAUB Authors Info & Affiliations

SCIENCE • 16 May 1997 • Vol 276, Issue 5315 • pp. 1109-1112 • DOI: 10.1126/science.276.5315.1109

Direct Mechanical Measurements of the Elasticity of Single DNA Molecules by Using Magnetic Beads

STEVEN B. SMITH, LAURA FINZI, AND , CARLOS BUSTAMANTE Authors Info & Affiliations

SCIENCE • 13 Nov 1992 • Vol 258, Issue 5085 • pp. 1122-1126 • DOI: 10.1126/science.1439819

Reversible Unfolding of Single RNA Molecules by Mechanical Force

JAN LIPHARDT, BIBIANA ONOA, STEVEN B. SMITH, IGNACIO TINOCO, JR., AND CARLOS BUSTAMANTE Authors Info & Affiliations

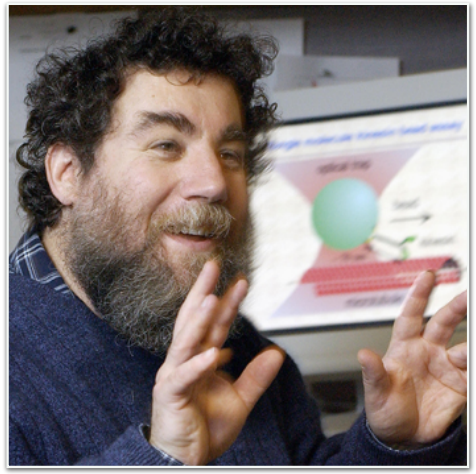
SCIENCE • 27 Apr 2001 • Vol 292, Issue 5517 • pp. 733-737 • DOI: 10.1126/science.1058498

Today's agenda

- Steven Block

- Outlook
  - Genome editing
  - Single molecule technologies in cells

Lecture 9 - On the work of Steven Block



Steven Block

Study of bimolecular complexes for DNA, RNA and intracellular transport

Awarded in 2008:

“For his originality in the direct measurement of forces and motions in single biomolecular complexes undergoing the nucleoside triphosphate hydrolysis reactions that drive intracellular transport, cell motility, and DNA and RNA replication.”

Introduction: Kinesin

State of the art - at the beginning of the ‘90s:

- Discovery of kinesin (1985)

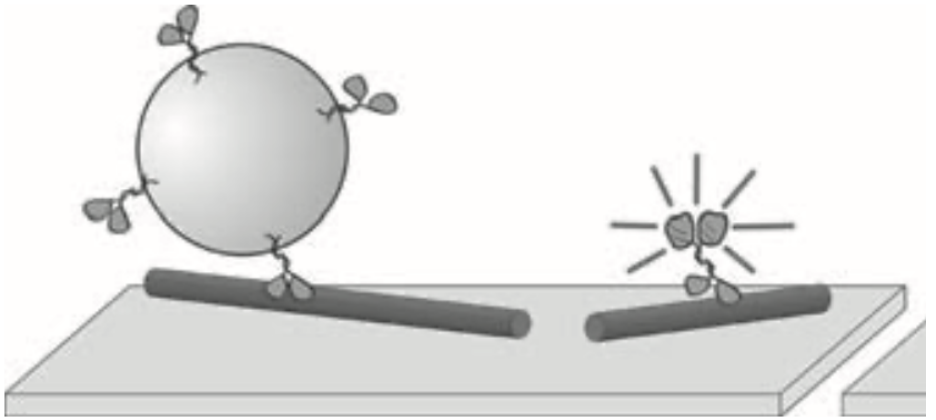
- Many members of the kinesin superfamily, functions:
  - transport of vesicles or other cargo
  - regulating microtubule growth and shrinkage
  - mediating chromosome-microtubule interactions

- Kinesin walks in one direction: towards the microtubule plus end

Cell, Vol. 42, 39–50, August 1985, Copyright © 1985 by MIT

Identification of a Novel Force-Generating Protein, Kinesin, Involved in Microtubule-Based Motility

Ronald D. Vale,*†‡ Thomas S. Reese,* and Michael P. Sheetz,*†§



Main tool:

- Walking assay: Kinesin-coated, fluorescently labelled beads are imaged when moving on fixed microtubules

Guiding Questions

- What was the scientific breakthrough?
- Can you identify a key insight(s) needed for the breakthrough?
- How do the findings align with or challenge existing models?
- Can you put this work in the context of others in the course? Compare/contrast.
- What are some potential implications of their findings?
- Pay attention to the sources, their attributes and “genre”

MOLECULES MOVEMENT AND MOTORS

October 14, 2011

RADCLIFFE INSTITUTE
FOR ADVANCED STUDY
HARVARD UNIVERSITY

Steven Block: the impact

Published: 22 November 1990

Bead movement by single kinesin molecules studied with optical tweezers

Steven M. Block, Lawrence S. B. Goldstein & Bruce J. Schnapp

Nature **348**, 348–352 (1990) | Cite this article

5132 Accesses | **823** Citations | **4** Altmetric | Metrics

Published: 21 October 1993

Direct observation of kinesin stepping by optical trapping interferometry

Karel Svoboda, Christoph F. Schmidt, Bruce J. Schnapp & Steven M. Block

Nature **365**, 721–727 (1993) | Cite this article

8667 Accesses | **1540** Citations | **34** Altmetric | Metrics

Published: 13 November 2005

Direct observation of base-pair stepping by RNA polymerase

Elio A. Abbondanzieri, William J. Greenleaf, Joshua W. Shaevitz, Robert Landick & Steven M. Block ✉

Nature **438**, 460–465 (2005) | Cite this article

9033 Accesses | **661** Citations | **23** Altmetric | Metrics

Direct Measurement of the Full, Sequence-Dependent Folding Landscape of a Nucleic Acid

MICHAEL T. WOODSIDE, PETER C. ANTHONY, [...], AND STEVEN M. BLOCK +3 authors Authors Info & Affiliations

SCIENCE · 10 Nov 2006 · Vol 314, Issue 5801 · pp. 1001-1004 · DOI: 10.1126/science.1133601

Published: 19 August 2009

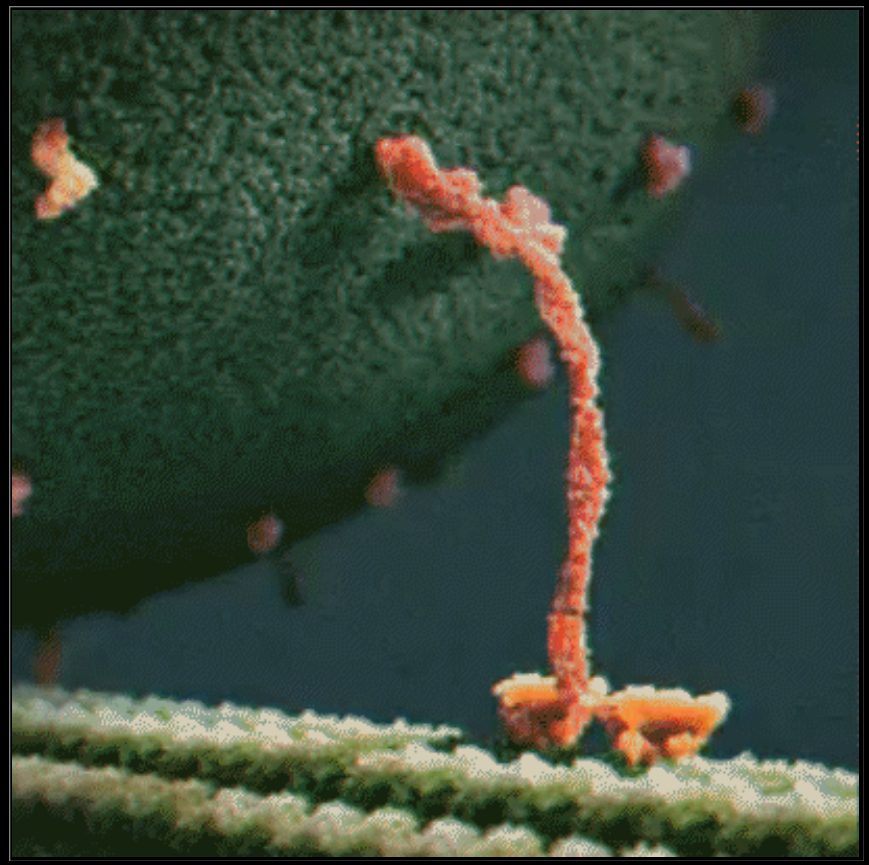
Direct observation of the binding state of the kinesin head to the microtubule

Nicholas R. Guydosh & Steven M. Block ✉

Nature **461**, 125–128 (2009) | Cite this article

1828 Accesses | **86** Citations | **1** Altmetric | Metrics

Steven Block: the impact



https://xvivo.com/examples/the-inner-life-of-the-cell/

Interview with Steven Block

Guiding Questions

- What was the scientific breakthrough?
- Can you identify a key insight(s) needed for the breakthrough?
- How do the findings align with or challenge existing models?
- Can you put this work in the context of others in the course? Compare/contrast.
- What are some potential implications of their findings?
- Pay attention to the sources, their attributes and “genre”

Single Molecule Mechanics: Future

What's next?

- Genome editing
- Bringing single molecule measurements into cells

Jennifer Doudna
Nobel Prize, 2020

TJ Ha

Xiaowei Zhuang
Breakthrough Prize, 2018

Stefan Hell
Nobel Prize, 2014

Video resources

- "Shedding Light on Motor Function, One Molecule at a Time" by Steven M. Block - Stanford University
  Molecules, Movement, and Motors: Steven M. Block and Closing Remarks - Radcliffe Institute
- Short interview with Steven Block:
  Applying physics to biology: single-molecule biophysics
- iBiology lesson on Molecular Motor Proteins, by Ron Vale:
  Ron Vale (UCSF, HHMI) 1: Molecular Motor Proteins
- … and about discovering kinesin in the first place:
  Ron Vale (UCSF, HHMI) 1: Discovering Kinesin
- Jennifer Doudna, Ted Talks: CRISPR's next advance is bigger than you think
- Taekjip Ha, TedX Talks: Eavesdropping on molecular conversations
- Xiaowei Zhuang, Breakthrough Prize: Imaging the molecular world of life

Scientific resources

- Block, S., Goldstein, L. & Schnapp, B. Bead movement by single kinesin molecules studied with optical tweezers. *Nature* **348**, 348–352 (1990). link
- Svoboda, K., Schmidt, C., Schnapp, B. *et al.* Direct observation of kinesin stepping by optical trapping interferometry. *Nature* **365**, 721–727 (1993). link
- Abbondanzieri, E., Greenleaf, W., Shaevitz, J. *et al.* Direct observation of base-pair stepping by RNA polymerase. *Nature* **438**, 460–465 (2005). link
- Michael T. Woodside et al. ,Direct Measurement of the Full, Sequence-Dependent Folding Landscape of a Nucleic Acid. *Science* **314**, 1001-1004(2006).DOI:link
- Guydosh NR, Block SM. Direct observation of the binding state of the kinesin head to the microtubule. Nature. 2009 Sep 3;461(7260):125-8. doi: 10.1038/nature08259. Epub 2009 Aug 19. PMID: 19693012; PMCID: PMC2859689. link
- A 2022 review on single molecule force spectroscopy, by Bustamante: The development of single molecule force spectroscopy: from polymer biophysics to molecular machines | Quarterly Reviews of Biophysics | Cambridge Core

Other

- An introduction to optical tweezers, from the Block lab: blocklab.stanford.edu/optical_tweezers.html
- Website for the xVivo project: amazing animations true to the science. The Inner Life of the Cell - Cell Animation