Course content

Topics (lectures)**:**
1. **Introduction** (1)
2. **Structure** (2-5)
3. **Single molecule mechanics** (6-9)
4. **Collective/emergent properties** (9-11)
5. **Student presentations** (11-13)

Course structure:
1. **Introduction to topic**
2. **Awardees (1-2 per week)**
    - **History, first-person, second-person accounts (C)**
    - **Article, analysis of scientific work (E)**
3. **Discussion of topic, outlook**

Lecture 6: Introduction to single molecule mechanics

Today's goal: Force spectroscopy, measurements & theory, history

- Force-extension curves
- Random walk models

PBOC Chapter 8.3

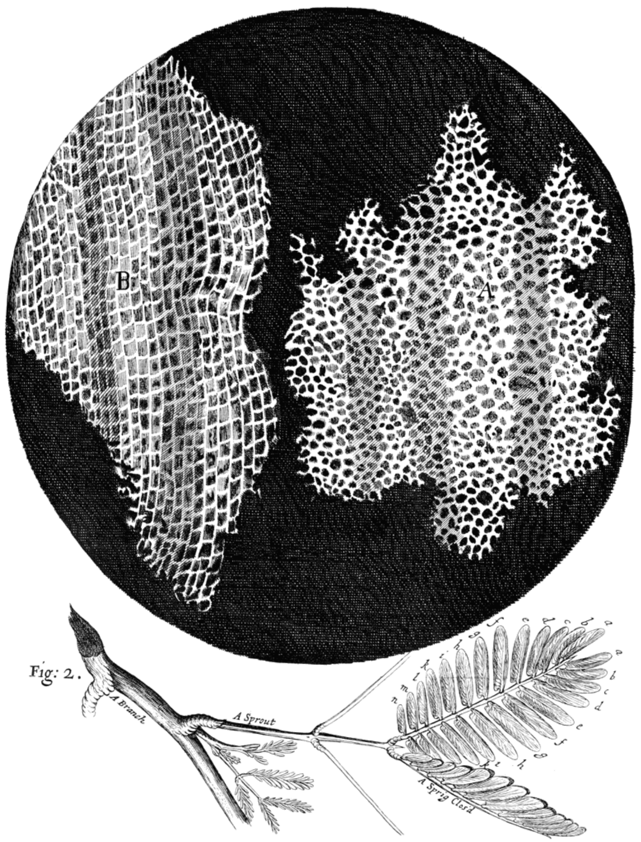
- History of nanocharacterization
- STM: Nobel 1986
  https://www.nobelprize.org/prizes/physics/1986/press-release/
- STM: the world's smallest movie
  https://www.youtube.com/watch?v=oSCX78-8-q0
- AFM
- Arthur Ashkin, optical tweezers (16:00-25:39)
  https://www.nobelprize.org/prizes/physics/2018/ashkin/lecture/
- Taekjip Ha, helicases via FRET + optical trapping
  https://www.ibiology.org/biophysics/single-molecule-technologies/#part-3

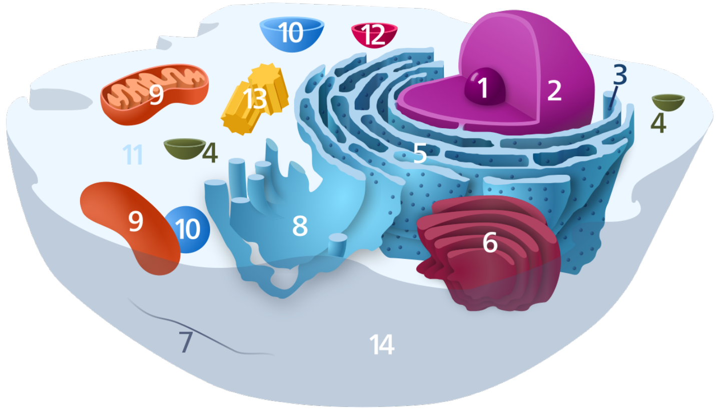
"The operative industry of Nature is so prolific that machines will be eventually found not only unknown to us but also unimaginable by our mind."

Marcello Malpighi, *De Viscerum Structura*, 1666

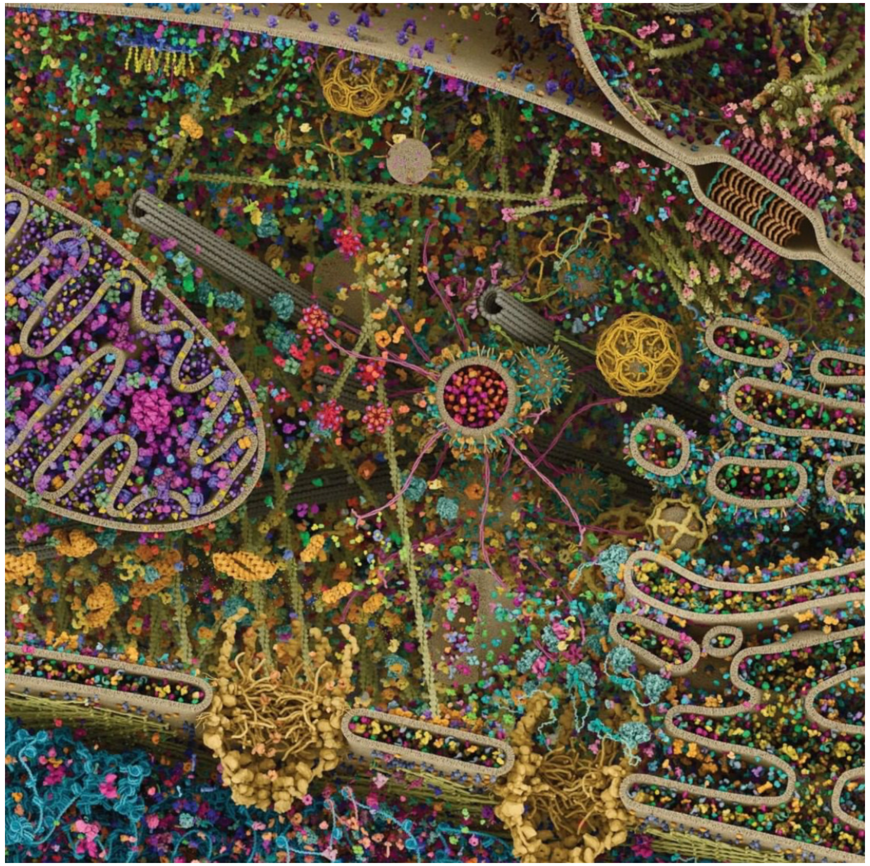
Models of the cell

Fig: 1.





magnetic resonance and cryo-electron microscopy data



"[…] great many little Boxes."

Robert Hooke
Micrographia, 1665

Small bags containing a concentrated solution of macromolecules undergoing second-order reactions.

[…] a factory that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein machines.

Bruce Alberts, The Cell as a Collection of Protein Machines: Preparing the Next Generation of Molecular Biologists, *Cell* **92** (1998)

Why study single molecules?

"There are two breads.
You eat both, I eat none.
Average consumption:
One bread per person."

Nicanor Parra

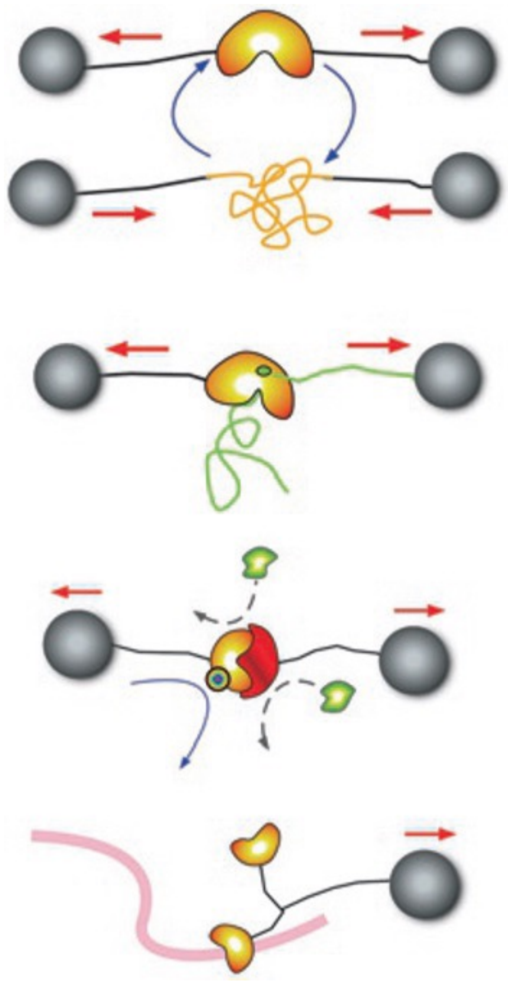
Averaging events in ensemble experiments hides the true dynamics of the actors involved.

The "molecular trajectories" recorded by single molecule experiments are more readily interpretable in the mechanistic framework.

By exerting forces and measuring displacements of single molecule, it is possible to monitor simultaneously the dynamics and the energetic landscape of a system.

Why study single molecules?

Measure forces exerted by:



protein folding
unfolding

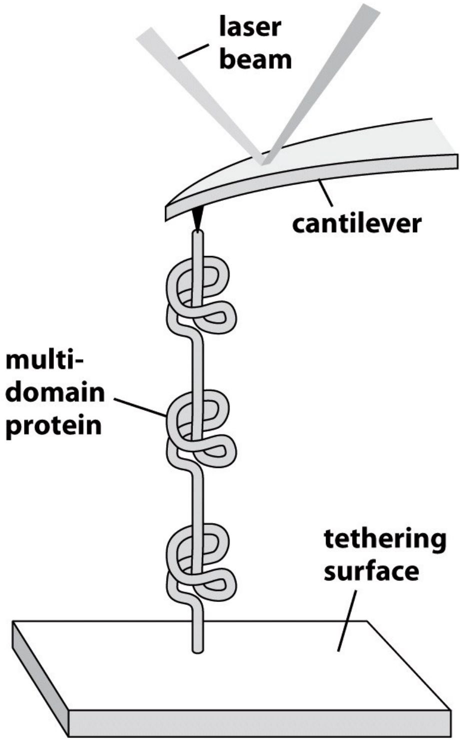
DNA binding
proteins

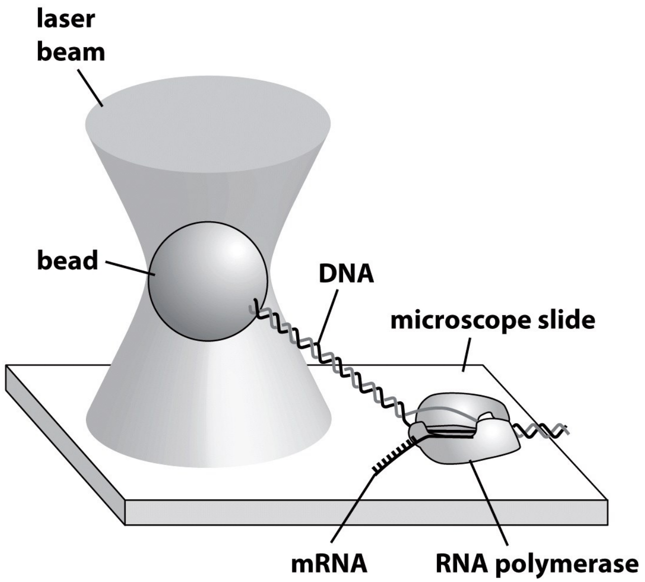
receptor-ligand
binding

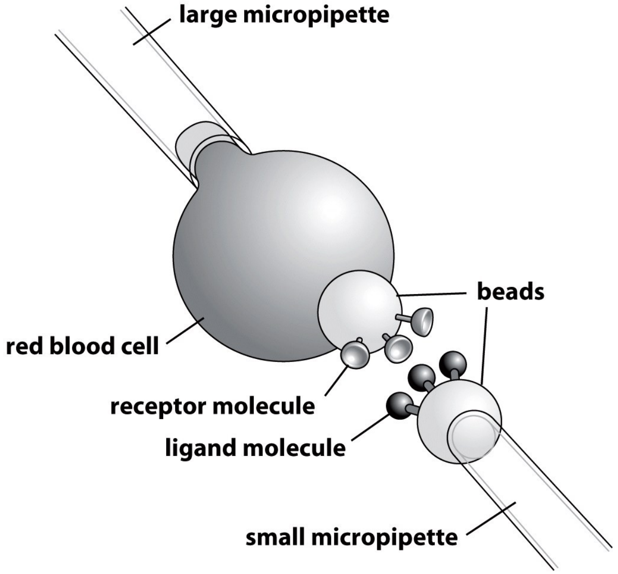
motor proteins

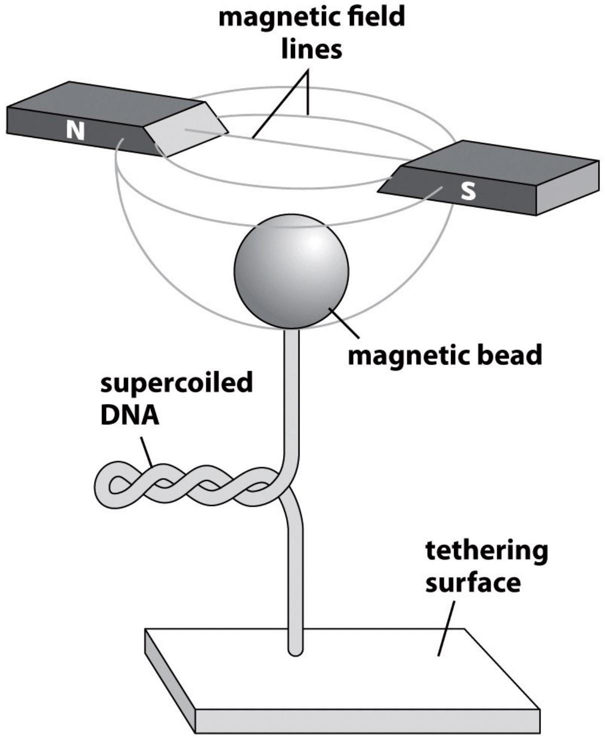
Examples:
Packing DNA into a virus
Moving cargo along cytoskeleton
DNA replication, transcription

How to study single molecules?









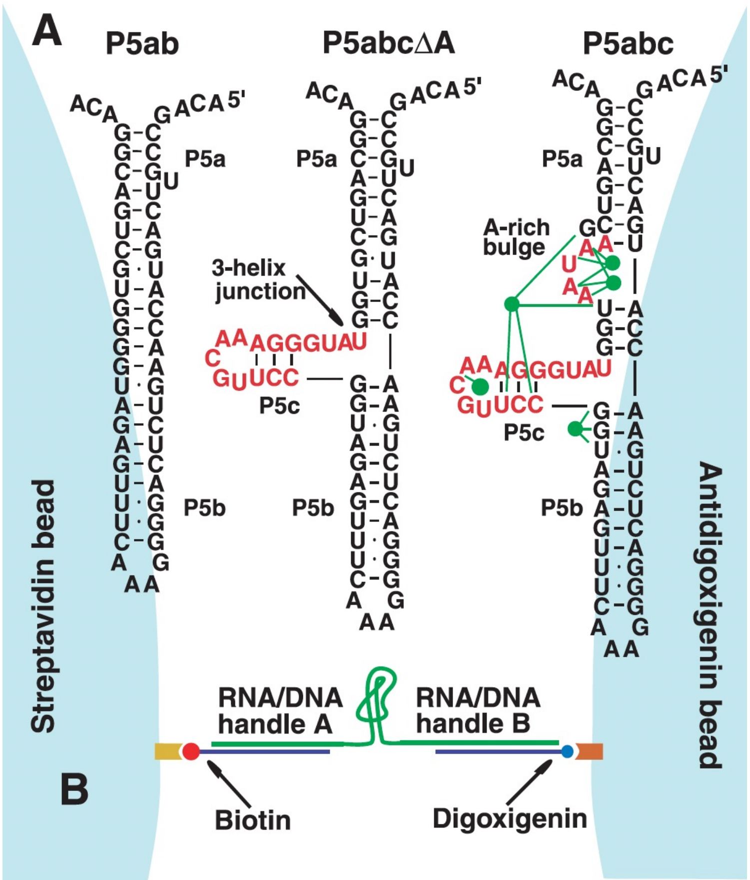
How to study single molecules?

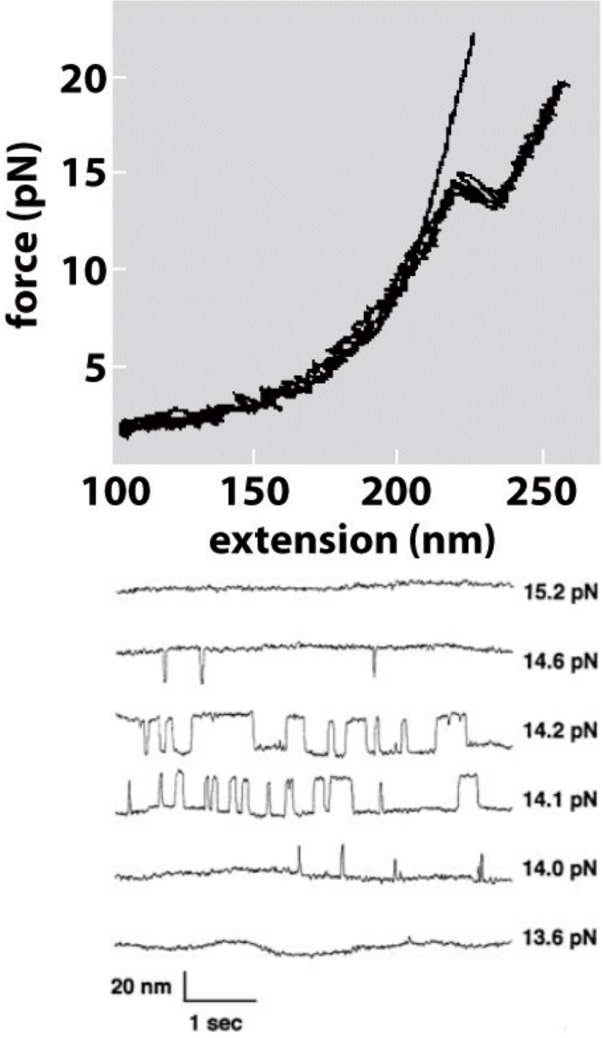
	Optical tweezers	Magnetic (electromagnetic) tweezers	AFM
Spatial resolution (nm)	0.1–2	5–10 (2–10)	0.5–1
Temporal resolution (s)	10^{-4}	10^{-1}–10^{-2} (10^{-4})	10^{-3}
Stiffness (pN nm^{-1})	0.005–1	10^{-3}–10^{-6} (10^{-4})	10–10^{5}
Force range (pN)	0.1–100	10^{-3}–10^{2} (0.01–10^{4})	10–10^{4}
Displacement range (nm)	0.1–10^{5}	5–10^{4} (5–10^{5})	0.5–10^{4}
Probe size (µm)	0.25–5	0.5–5	100–250
Typical applications	3D manipulation Tethered assay Interaction assay	Tethered assay DNA topology (3D manipulation)	High-force pulling and interaction assays
Features	Low-noise and low-drift dumbbell geometry	Force clamp Bead rotation Specific interactions	High-resolution imaging
Limitations	Photodamage Sample heating Nonspecific	No manipulation (Force hysteresis)	Large high-stiffness probe Large minimal force Nonspecific

Force-extension curves

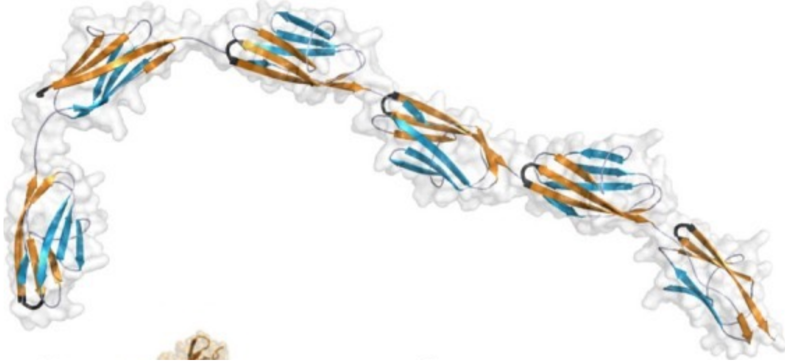
Experiments:

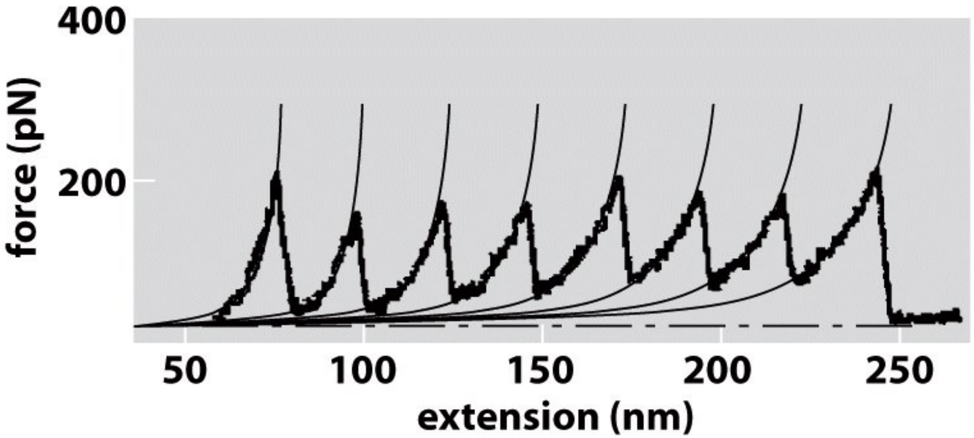
RNA





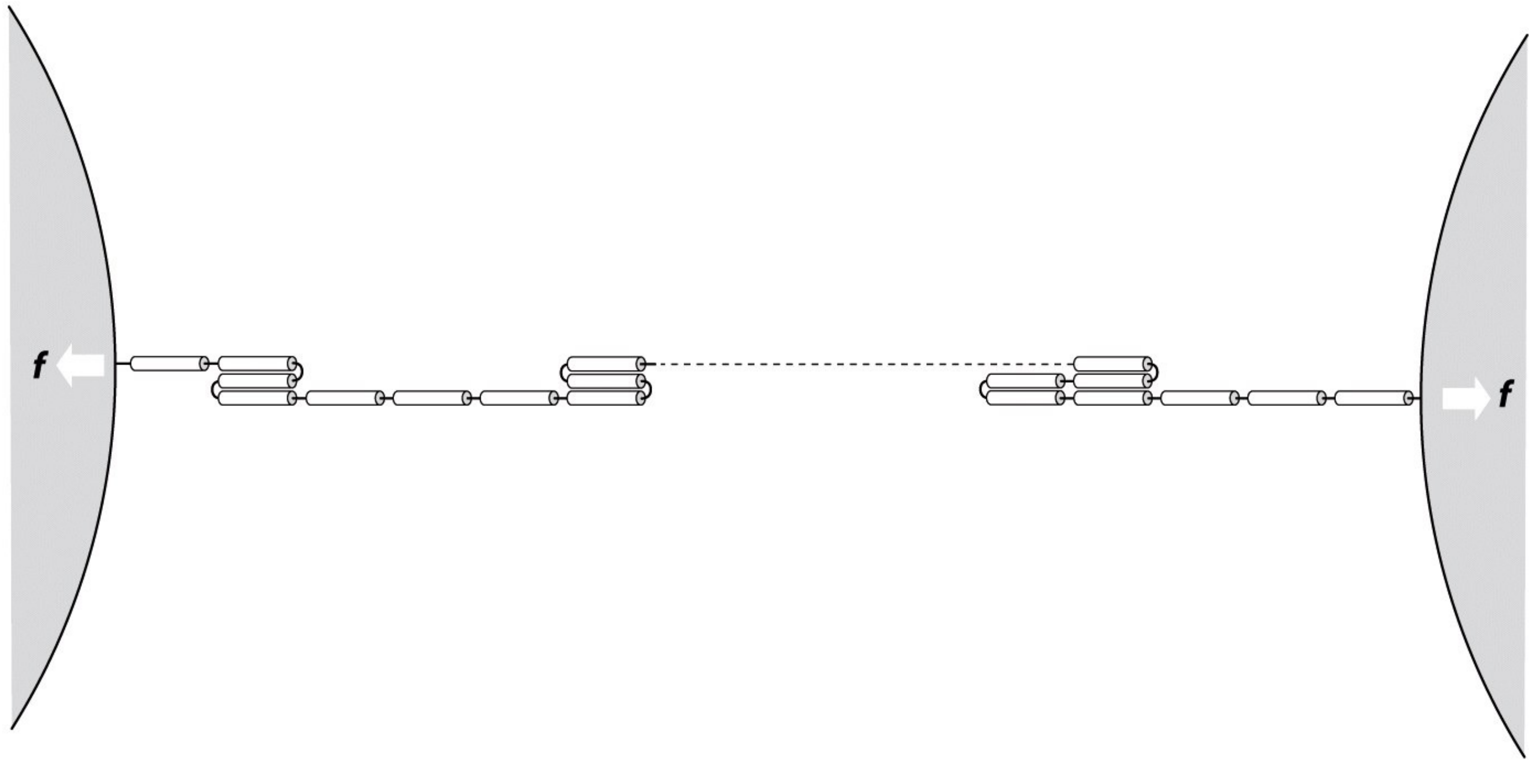
titin (human cardiac protein)





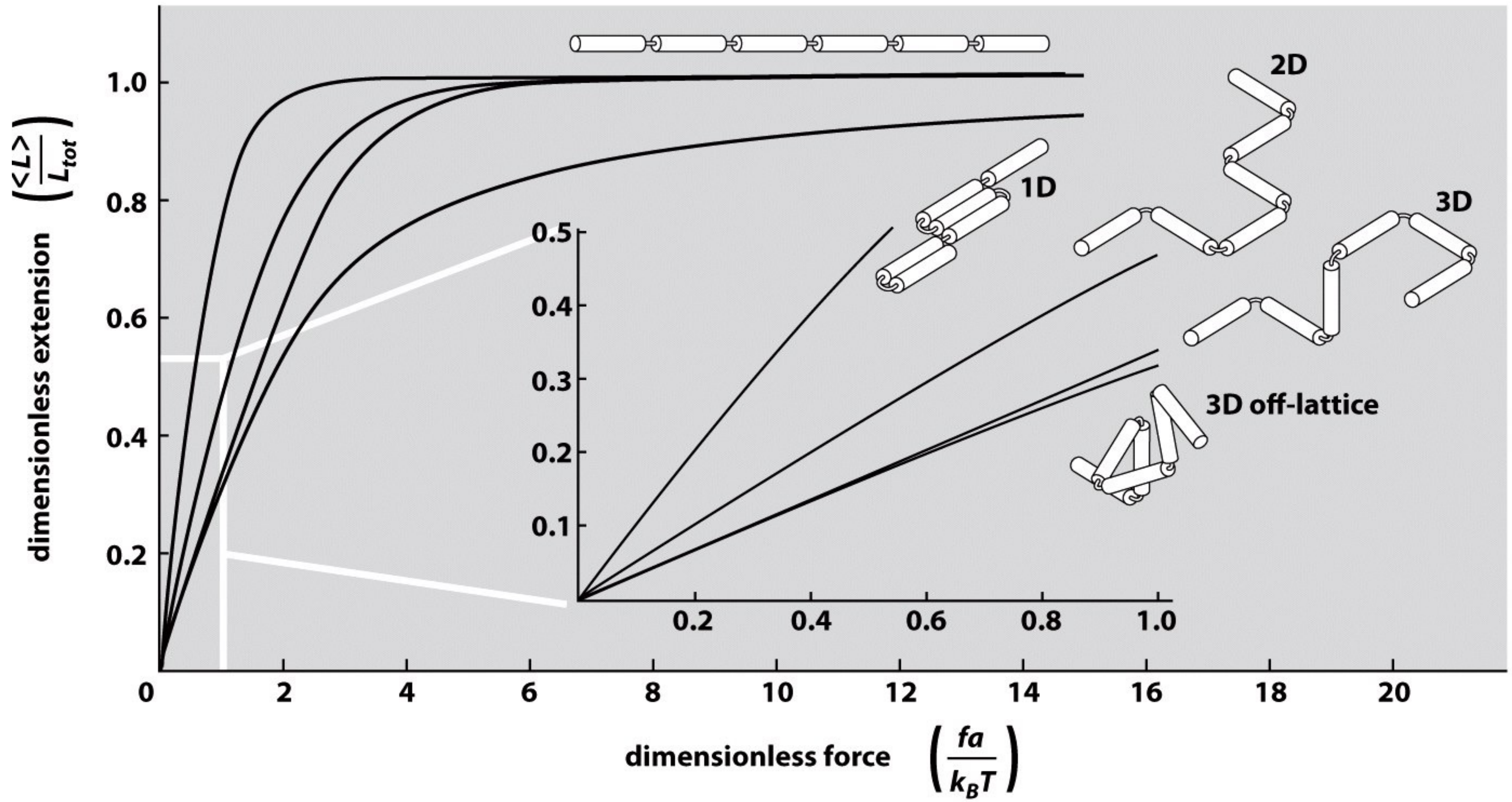
Force-extension curves

Models:



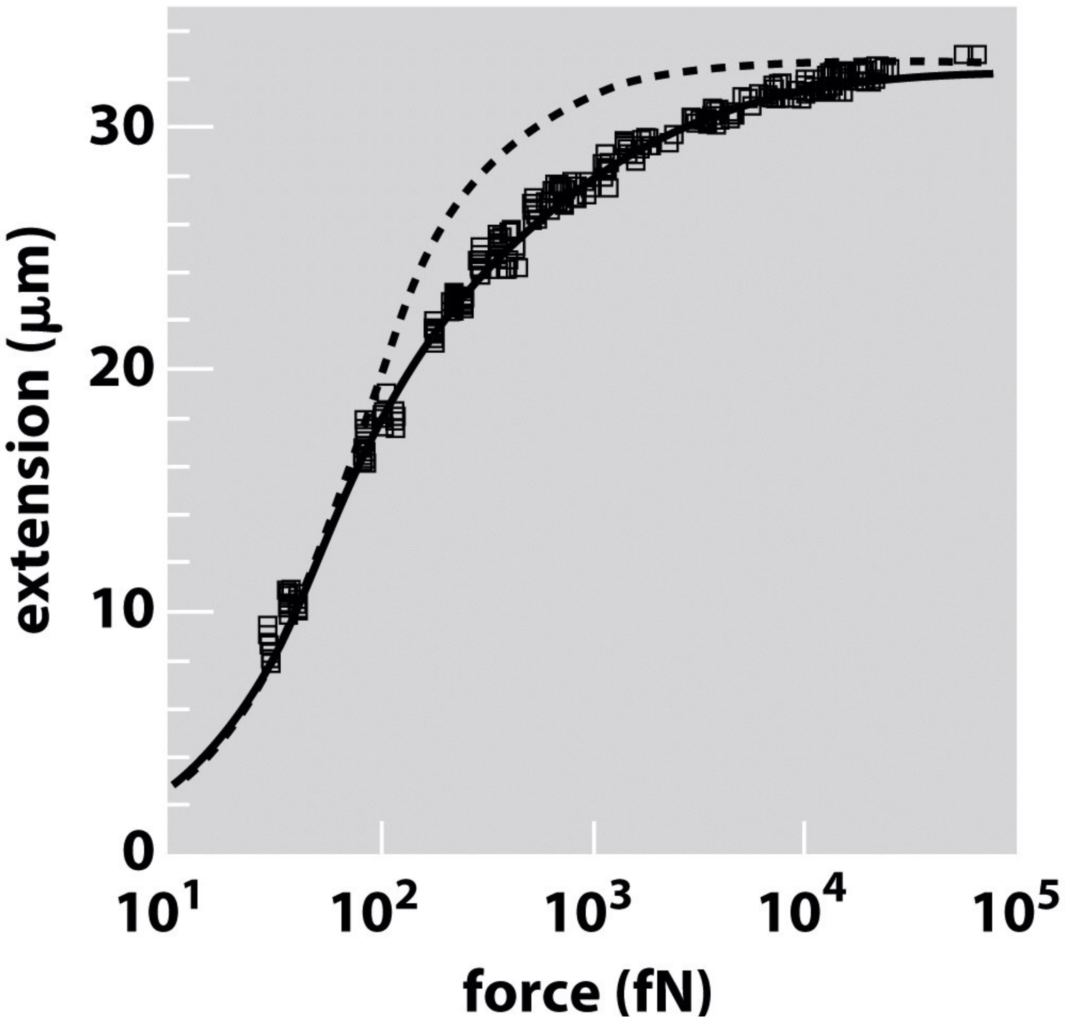
Force-extension curves

Models:

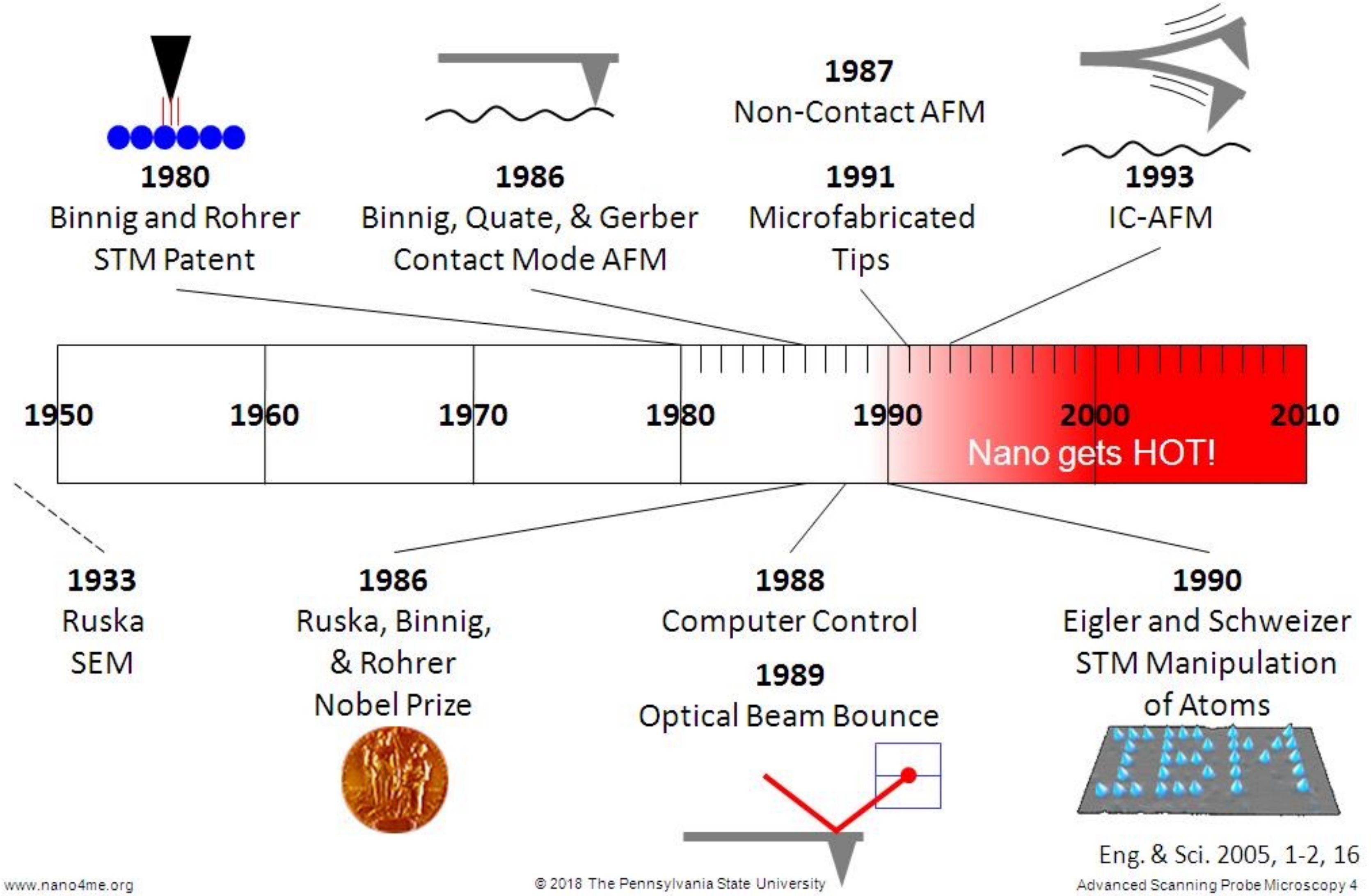


Force-extension curves

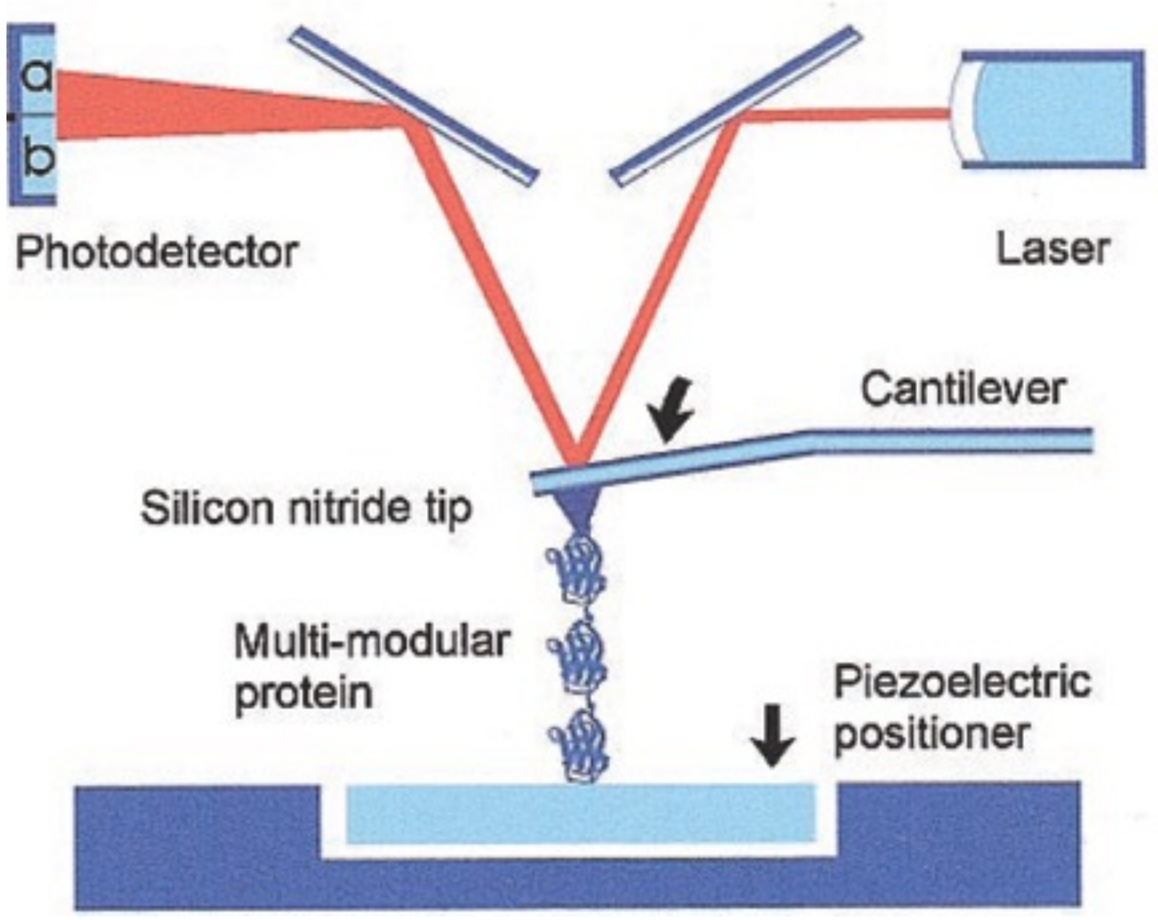
Models and measurements:



History of nanocharacterization



Atomic force microscope



- AFM cantilever probes by moving its tip along surface, or pulling on a protein
- Cantilever movement is detected with a focused laser beam that refracts into a photodetector
- The deflection of the cantilever deflects the laser correspondingly and can map the surface
- In single molecule force spectroscopy, the cantilever is pressed against a layer of proteins attached to a substrate, the tip adsorbs a single protein molecule, which is then extended.
- Extension of the molecule by retraction of the piezoelectric positioner results in deflection of the cantilever.

Lectures 7, 8

• Paul Hansma: Development of AFMs to monitor individual protein molecules, in liquids (1990-2000)
"*For pioneering contributions to the development of biological scanning probe microscopy and for the molecular resolution imaging of biological molecules in aqueous solutions.*" (2000)

• Carlos Bustamante: Study of DNA, RNA, and protein molecular mechanics (1990-2000)
"*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.*" (2002)