Time to choose!

blue = protein folding, structure/function
green = mechanics, forces, elasticity
yellow = imaging methods development
orange = non-imaging methods development
pink = dynamics, motility
purple = complex systems, collective behavior

Lectures 7, 8, 9

• 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)

• Steven M. Block: Optical tweezers, molecular motors (kinesin, RNA polymerase) (1990-2000)

"*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.*" (2008)

Why study single molecules?

Previously:

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

-- Nicanor Parra

Ensemble experiments hide the true dynamics of individual molecules.

"Molecular trajectories" of single molecules are more readily interpretable in mechanistic frameworks.

By exerting forces and measuring displacements, simultaneously measure dynamics and energy landscape.

How: Optical tweezers

State of the art

Published: 31 December 1987

Optical trapping and manipulation of single cells using infrared laser beams

A. Ashkin, J. M. Dziedzic & T. Yamane

Nature **330**, 769–771 (1987) | Cite this article

Use of optical traps for the manipulation of biological particles was recently proposed, and initial observations of laser trapping of bacteria and viruses with visible argon-laser light were reported[1]. We report here the use of infrared (IR) light to make much improved laser traps with significantly less optical damage to a variety of living cells.

…

Damage-free trapping and manipulation of suspensions of red blood cells of humans and of organelles located within individual living cells of spirogyra was also achieved, largely as a result of the reduced absorption of haemoglobin and chlorophyll in the IR. Trapping of many types of small protozoa and manipulation of organelles within protozoa is also possible. The manipulative capabilities of optical techniques were exploited in experiments showing separation of individual bacteria from one sample and their introduction into another sample. Optical orientation of individual bacterial cells in space was also achieved using a pair of laser-beam traps. These new manipulative techniques using IR light are capable of producing large forces under damage-free conditions and improve the prospects for wider use of optical manipulation techniques in microbiology.

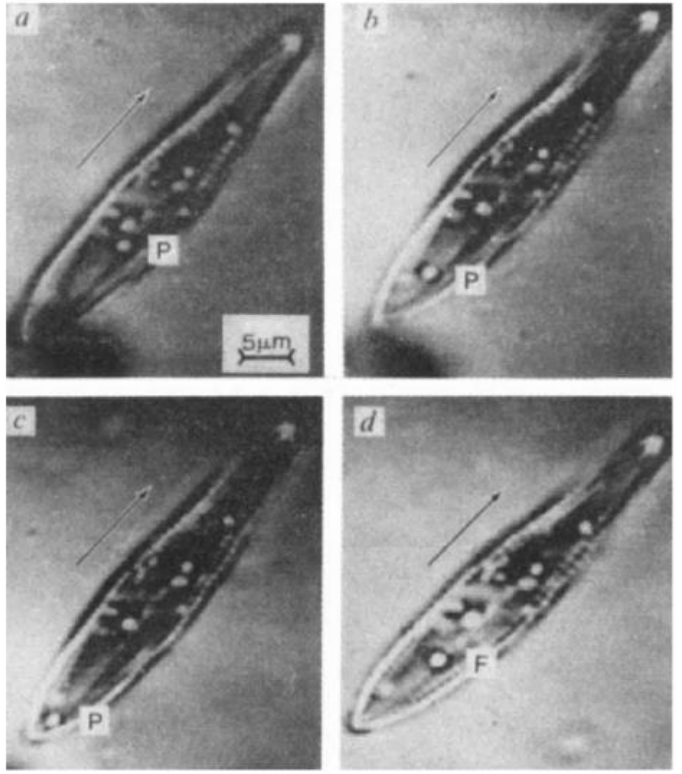


Fig. 4 *a–d*, The manipulation of an optically trapped organelle within the interior of a protozoan as it moves along freely on a glass surface in the direction of the arrow. In photographs *a–c*, an organelle, originally trapped at P (*a*), is being dragged by the advancing protozoan to the rear of the cell (*c*). In (*d*) the organelle pulls free of the trap and snaps back to its final position at F.

Why study single molecules?

Carlos Bustamante
https://www.ibiology.org/biochemistry/optical-tweezers/
Why single molecules, how are they different from bulk? (0:00-13:30)
Q: How does this relate to the previous module, “Molecular structure”?

Multimodal populations of molecules
How does gel electrophoresis separate molecules of different sizes?
DNA elasticity: measure with optical tweezers. Highly non-linear spring.
Single DNA molecules (13:30-27:00)
Q: How does this relate to the previous module, “Molecular structure”?
How does this relate to the previous awardee’s methods? Optical tweezers vs AFM?

Central Dogma
https://www.ibiology.org/biochemistry/optical-tweezers/ (27:00-38:00)
https://www.ibiology.org/talks/optical-traps/#part-3 (0-20:00)

RNA polymerase, transcribing DNA to make RNA
Ribosome, translating mRNA to make protein
Noise and measurement limits
DNA packaging motor
Stall forces of motors

Guiding questions

- Pay attention to the sources, their attributes and “genre”
- 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?

Single molecule mechanics

Breakthrough

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

What was the gap?
How was it overcome?

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

Entropic Elasticity of λ-Phage DNA

C. BUSTAMANTE, J. F. MARKO, E. D. SIGGIA, AND S. SMITH Authors Info & Affiliations

SCIENCE • 9 Sep 1994 • Vol 265, Issue 5178 • pp. 1599-1600 • DOI: 10.1126/science.8079175

Overstretching B-DNA: The Elastic Response of Individual Double-Stranded and Single-Stranded DNA Molecules

STEVEN B. SMITH, YUJIA CUI, AND CARLOS BUSTAMANTE Authors Info & Affiliations

SCIENCE • 9 Feb 1996 • Vol 271, Issue 5250 • pp. 795-799 • DOI: 10.1126/science.271.5250.795

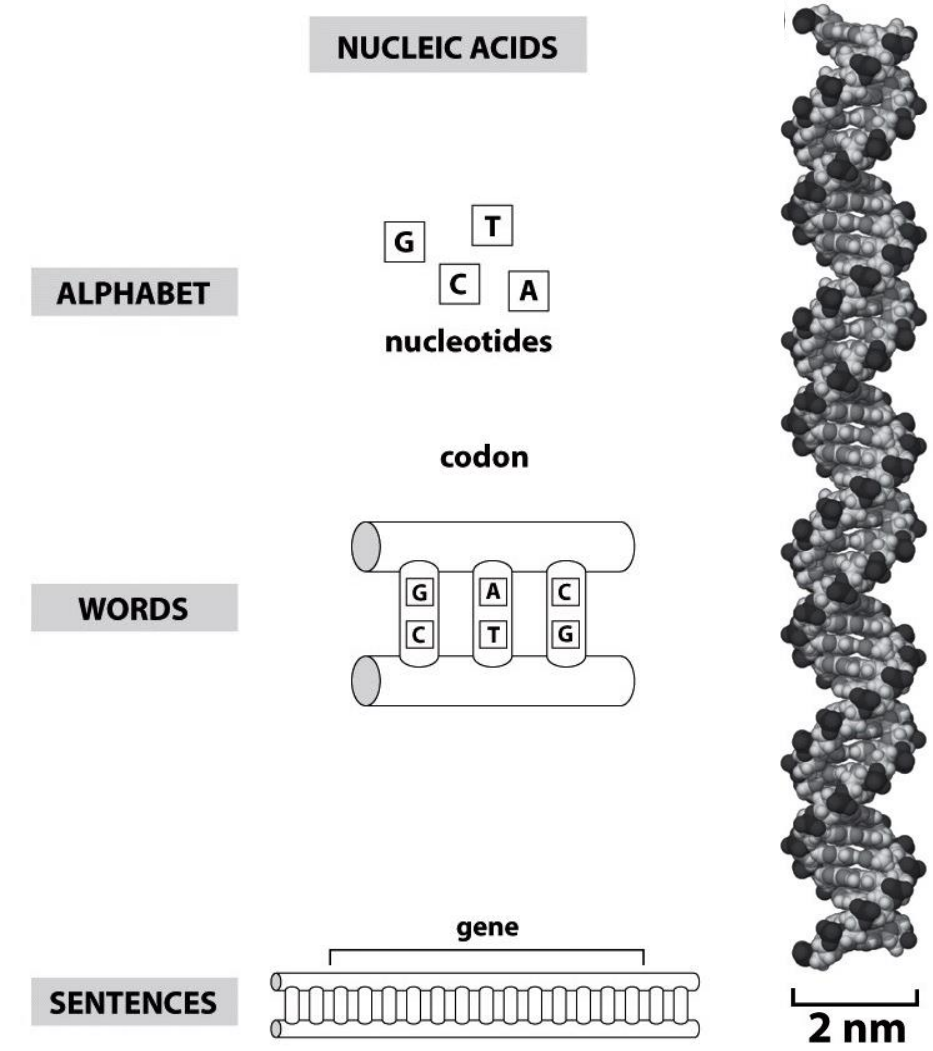
Reversible Unfolding of Single RNA Molecules by Mechanical Force

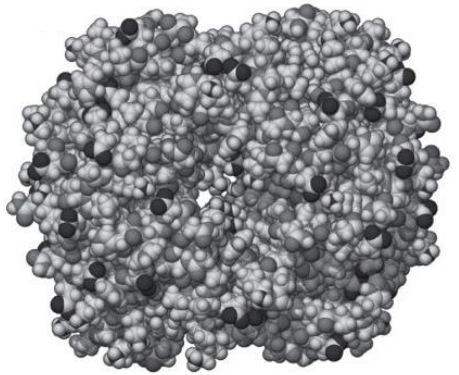
JAN LIPHARDT, BIBIANA ONOA, STEVEN B. SMITH, IGNACIO TINOCO, JR., AND CARLOS BUSTAMANTE Authors Info & Affiliations

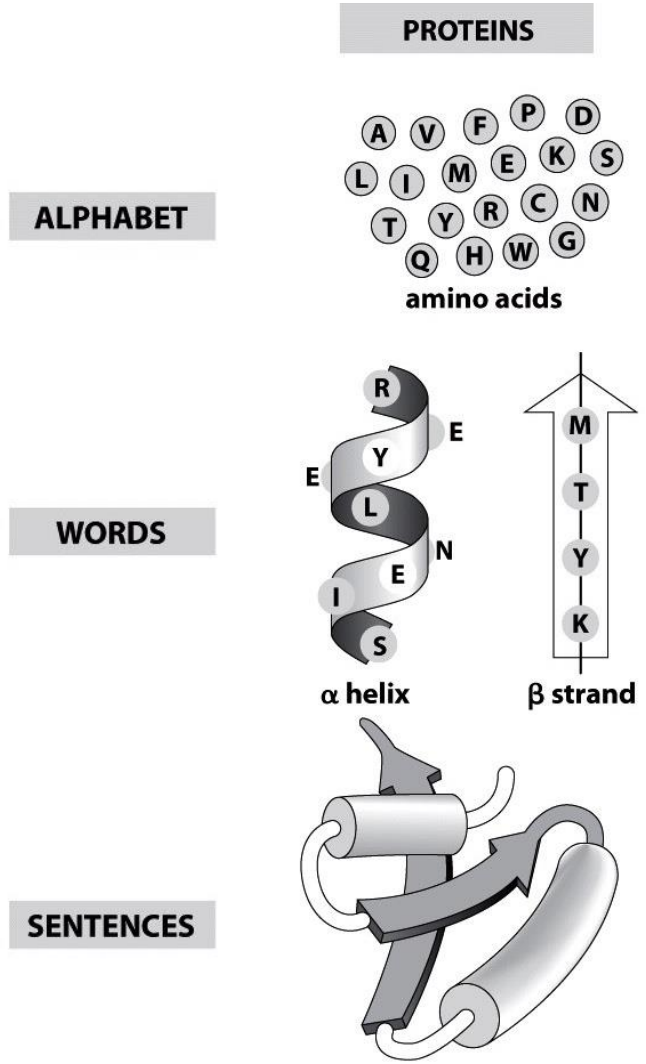
SCIENCE • 27 Apr 2001 • Vol 292, Issue 5517 • pp. 733-737 • DOI: 10.1126/science.1058498

The stuff of life

Previously: Nucleic acids and proteins are polymer languages







Single molecule force spectroscopy

Impact

What was the gap?
How was it overcome?

Published: 02 March 2000

Single-molecule studies of the effect of template tension on T7 DNA polymerase activity

Gijs J.L. Wuite, Steven B. Smith, Mark Young, David Keller & Carlos Bustamante

Nature **404**, 103–106 (2000) | Cite this article

3628 Accesses | **398** Citations | **6** Altmetric | Metrics

Single-Molecule Study of Transcriptional Pausing and Arrest by *E. coli* RNA Polymerase

R. JOHN, DAVENPORT, GIJS J. L. WUITE, ROBERT LANDICK, AND CARLOS BUSTAMANTE Authors Info & Affiliations

SCIENCE • 31 Mar 2000 • Vol 287, Issue 5462 • pp. 2497-2500 • DOI: 10.1126/science.287.5462.2497

Published: 18 October 2001

The bacteriophage φ29 portal motor can package DNA against a large internal force

Douglas E. Smith, Sander J. Tans, Steven B. Smith, Shelley Grimes, Dwight L. Anderson & Carlos Bustamante

Nature **413**, 748–752 (2001) | Cite this article

Published: 07 January 2009

Intersubunit coordination in a homomeric ring ATPase

Jeffrey R. Moffitt, Yann R. Chemla, K. Aathavan, Shelley Grimes, Paul J. Jardine, Dwight L. Anderson & Carlos Bustamante

Nature **457**, 446–450 (2009) | Cite this article

Single molecule force spectroscopy

Carlos Bustamante iBiology
https://www.ibiology.org/biochemistry/optical-tweezers/ (9:00-27:00)

Multimodal populations of molecules
How does gel electrophoresis separate molecules of different sizes?
DNA elasticity: measure with optical tweezers. Highly non-linear spring.

Paradigm shift (1980-1990's):
bag of concentrated solution undergoing chemical reactions
vs. factory with network of interlocking assembly lines, protein machines

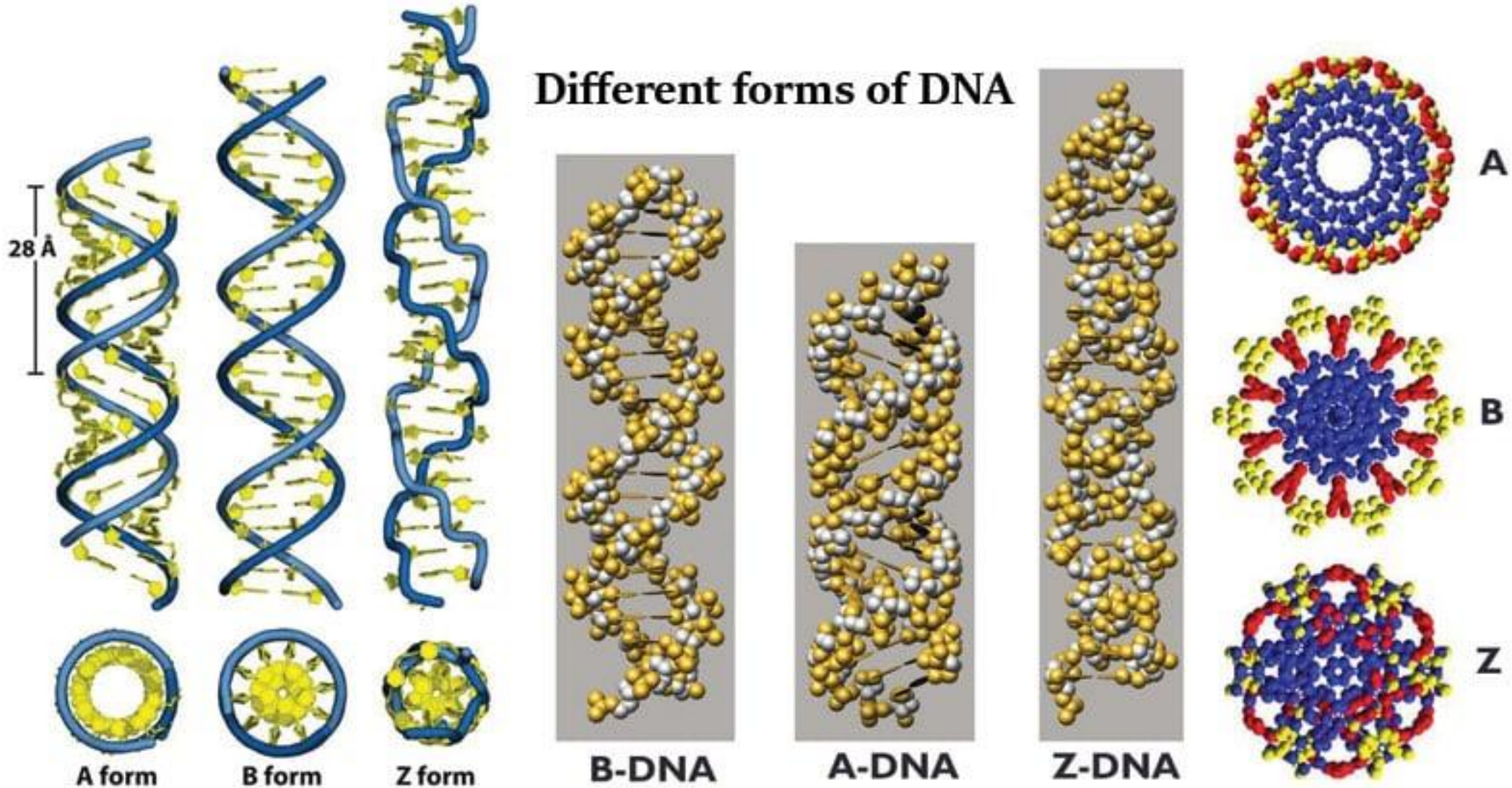
Central Dogma
https://www.ibiology.org/biochemistry/optical-tweezers/ (27:00-38:00)
https://www.ibiology.org/talks/optical-traps/#part-3 (0-20:00)

RNA polymerase, transcribing DNA to make RNA
Ribosome, translating mRNA to make protein
Noise and measurement limits
DNA packaging motor
Stall forces of motors

Carlos Bustamante

History

- Vilcek Prize in Biomedical Science (2012)
- Richtmyer Award, American Association of Physics Teachers (2005)
- Alexander Hollaender Award in Biophysics, National Academy of Sciences (2004)
- Biophysical Society Founder's Award (2004)
- American Physical Society Biological Physics Prize (2002)
- Member of the National Academy of Science (2002)
- Time magazine's America's Best (2001)
- American Physical Society Fellow (1995)


Different forms of DNA
28 Å
A form
B form
Z form
B-DNA
A-DNA
Z-DNA
A
B
Z