Protein Collision on DNA

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Outline

I. Introduction and background

II. DNA curtain assay

III. Protein movement and collision on DNA

IV. Summary

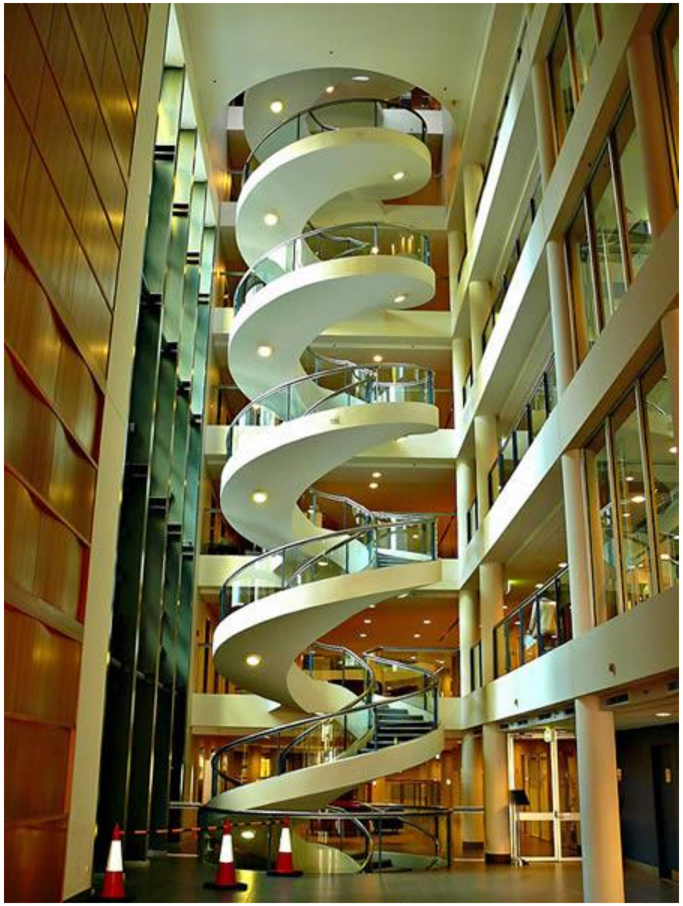
V. Beyond DNA curtain

Biophysics

- Biophysics is not easy because it contains a wide range of academic areas

- Physicists encounter huge barriers to study biology, and biologists feel frustrated when they study physics!
  → Hard to get familiar with the other discipline

- Studying biophysics is like a spiral staircase
  - *While you are walking on the step, it seems that you are just rotating around the same path. But at a certain time, you can get to higher position and see a different view*



Biophysics

What is the biophysics?

- Biophysics Society says

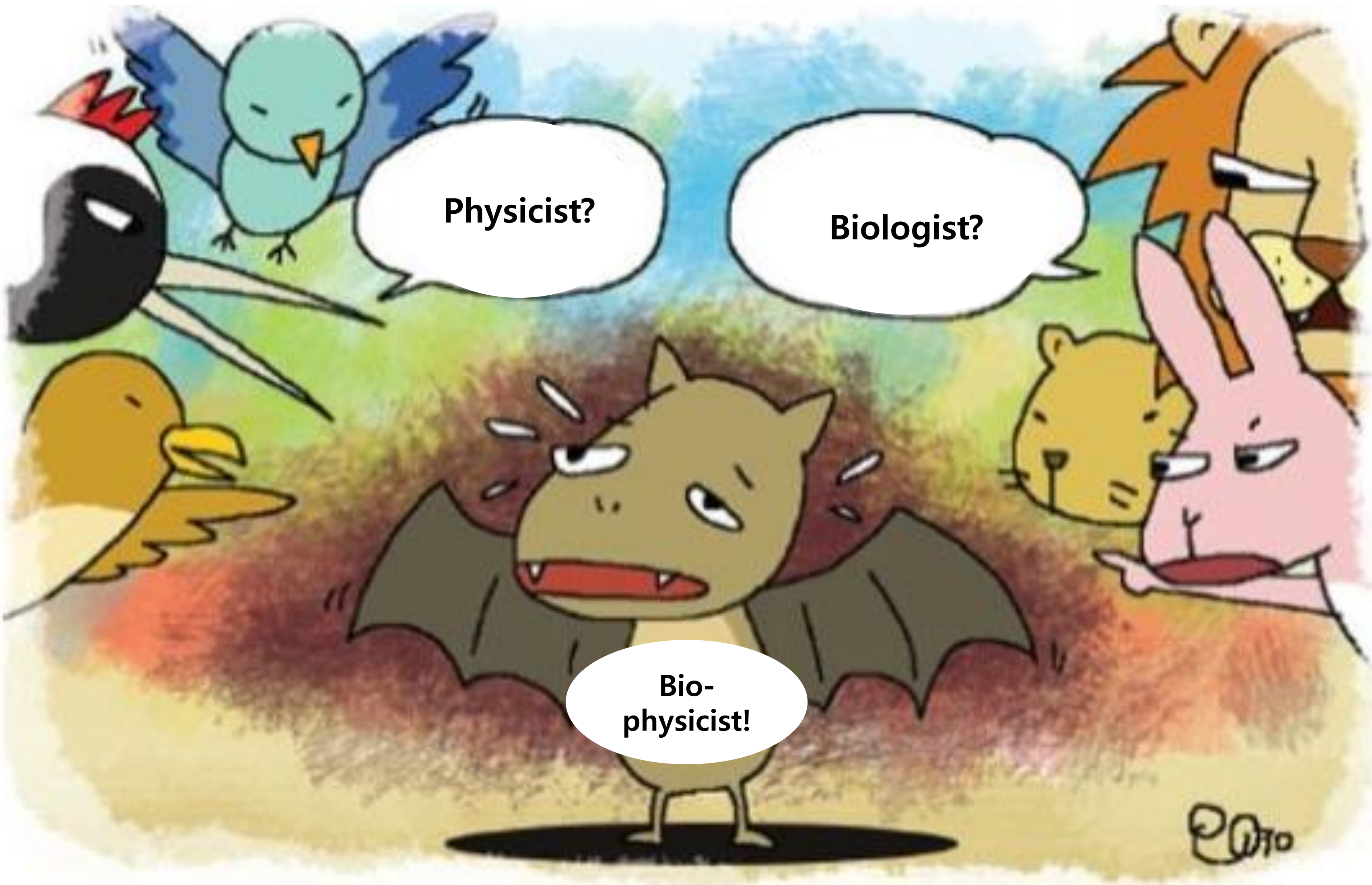
  “Biophysics is a bridge between biology and physics”

  “Biology studies life in its variety and complexity. It describes how organisms go about getting food, communicating, sensing the environment, and reproducing. On the other hand, physics looks for mathematical laws of nature and makes detailed predictions about the forces that drive idealized systems. *Spanning the distance between the complexity of life and the simplicity of physical laws is the challenge of biophysics*. Looking for the patterns in life and analyzing them with math and physics is a powerful way to gain insights.”

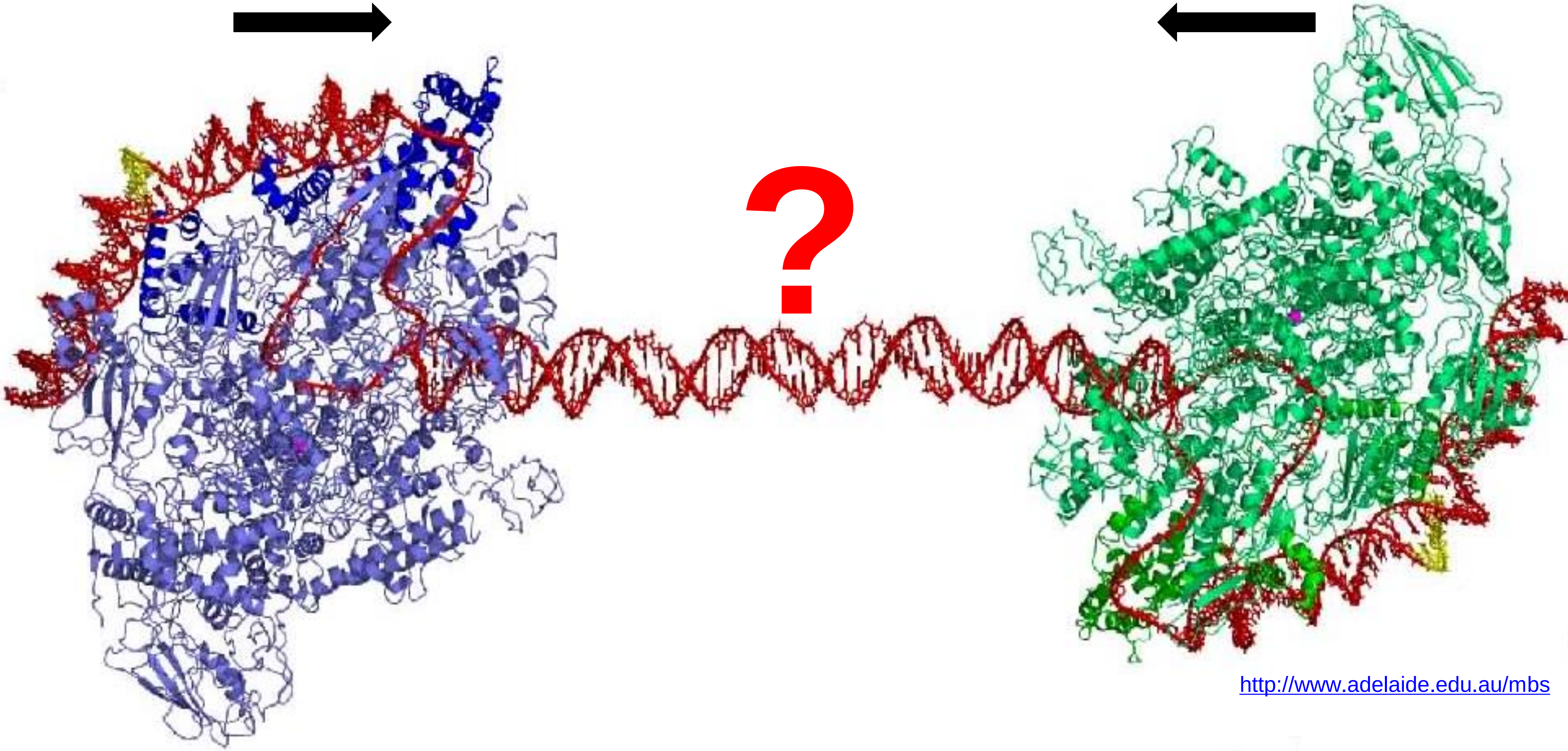
- Wiki says

  “Biophysics or biological physics is an interdisciplinary science that *applies the approaches and methods of physics to study biological systems*. Biophysics covers all scales of biological organization, from molecular to organismic and populations. Biophysical research shares significant overlap with biochemistry, physical chemistry, nanotechnology, bioengineering, computational biology, biomechanics, and systems biology.”

Biophysics

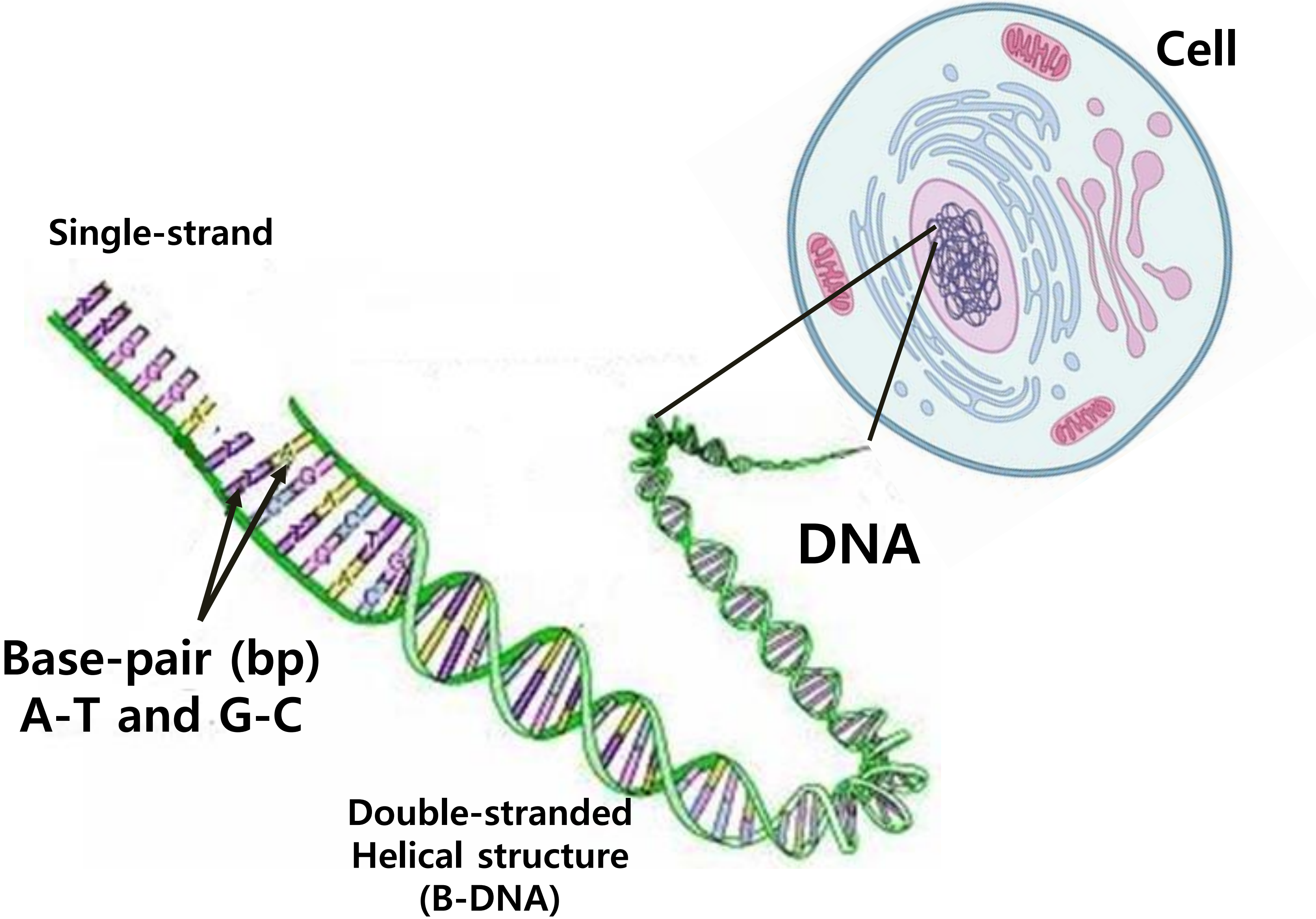


Collision

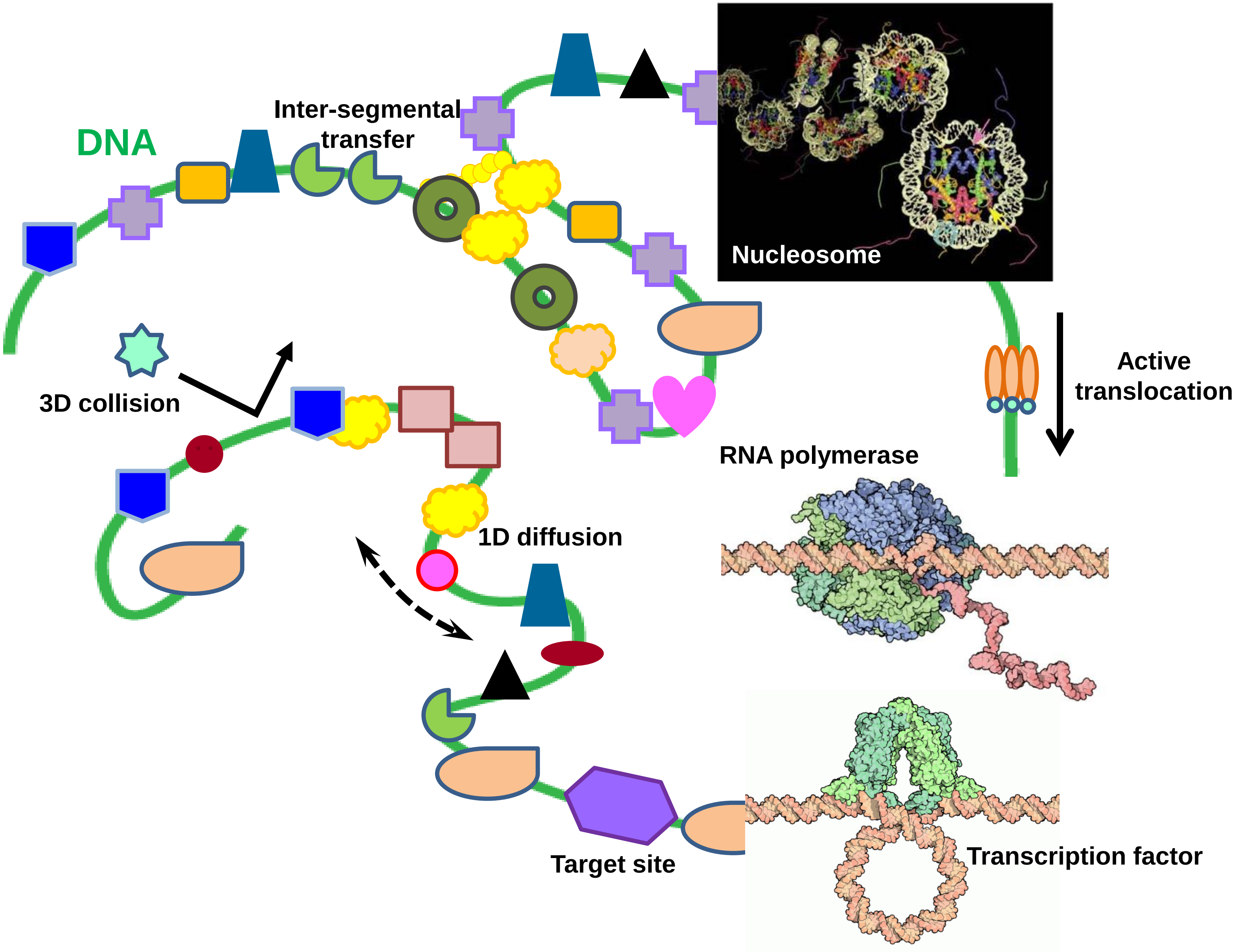


http://www.adelaide.edu.au/mbs

Biological Background



Significance of Protein Collision



Limit of Traditional Biological Methods

➢ Traditional biological techniques

1. Use a large amount of biomolecules in test tubes
2. Measure the average effect of biomolecules or biomolecular reactions

➢ Limitation of traditional methods

1. Cannot reveal the details of biomolecular properties and interactions
2. Cannot observe the biomolecular movement
3. Not easy to obtain precise kinetic information of biomolecular reactions

✓ ***New technology is demanded to overcome the limitations***

→ ***Single-molecule biophysics is the solution!!!***

Single-Molecule Biophysics

- *Observe* ***the behavior of individual bio-molecules*** *with the help of advanced physical techniques*

- **Advantages**

1. Exclude the *ensemble average effect* of massive samples
   → Reveal the hidden sub-states of biomolecules
2. Measure the kinetics of molecular reactions more precisely
   → No need for the stop flow
3. Mechanically manipulate biomolecules and examine the response to the mechanical stress
   → Apply force and torque to biomolecules
4. ***Observe biomolecular interaction in more direct way***
   → Directly visualize the molecular motion and interactions

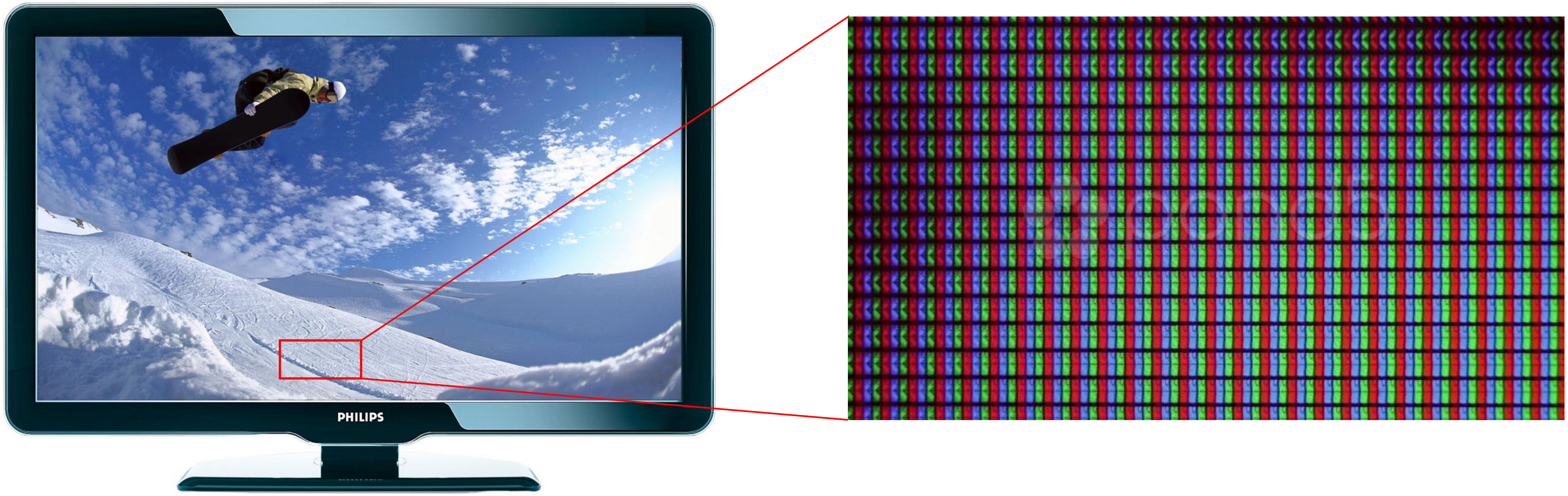
- **Disadvantages**

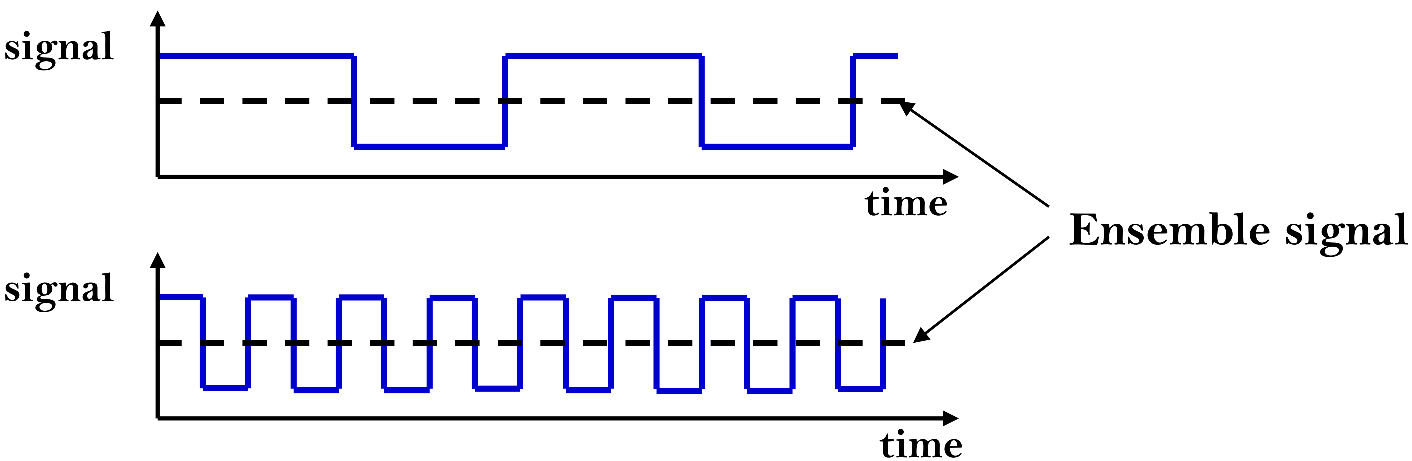
1. Gather sufficient number of data for statistical reliability
2. Modify biomolecules

Single-Molecule Biophysics

Ensemble view

Single-molecule view





Beginning of Single-Molecule Spectroscopy

VOLUME 62, NUMBER 21 PHYSICAL REVIEW LETTERS 22 MAY 1989

Optical Detection and Spectroscopy of Single Molecules in a Solid

W. E. Moerner and L. Kador[(a)]

IBM Research Division, Almaden Research Center, San Jose, California 95120

(Received 17 March 1989)

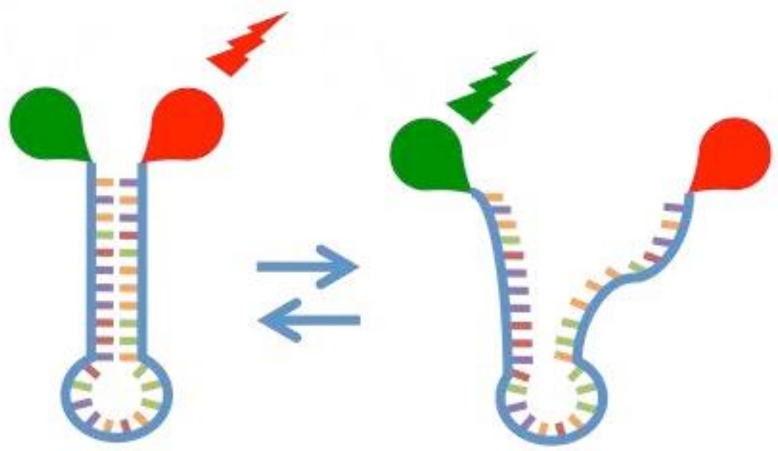
Using two different double-modulation techniques, we have observed the optical-absorption spectrum of single dopant molecules of pentacene in a *p*-terphenyl host crystal at liquid-helium temperatures. To achieve this, frequency-modulation spectroscopy was combined either with Stark or ultrasonic modulation to remove interfering background signals from residual amplitude modulation, and the number of molecules in resonance was reduced to one by operating in the wings of the inhomogeneous line. Triplet bottleneck saturation appears to be suppressed in the single-molecule regime.

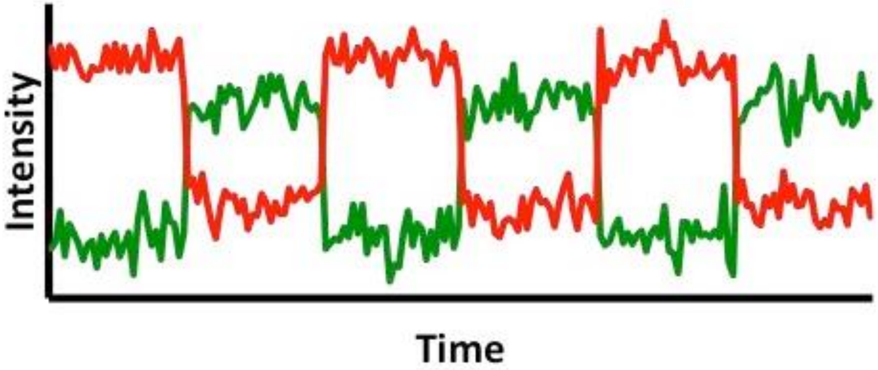
PACS numbers: 78.50.−w, 33.20.Kf, 33.70.Jg

W. E. Moerner won the Nobel prize in chemistry 2014!

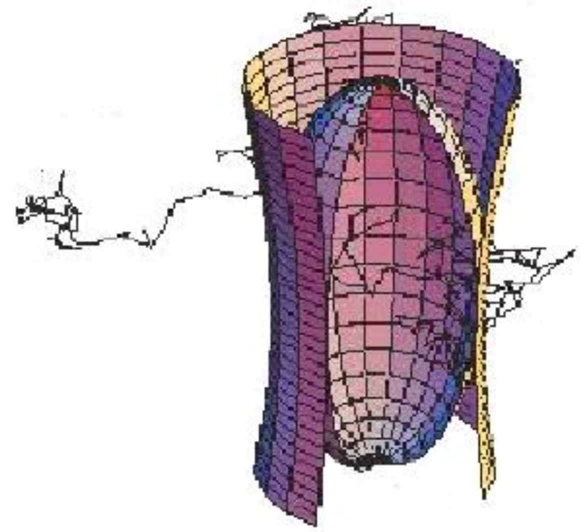
Single-Molecule Techniques: Fluorescence

Single-molecule FRET

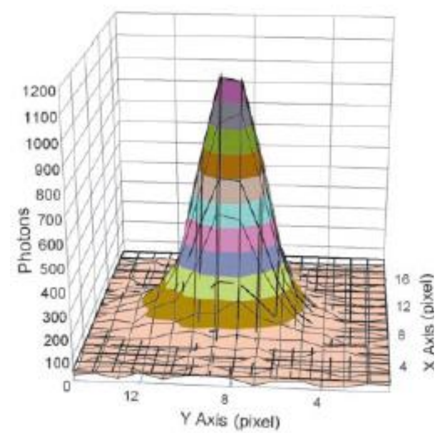


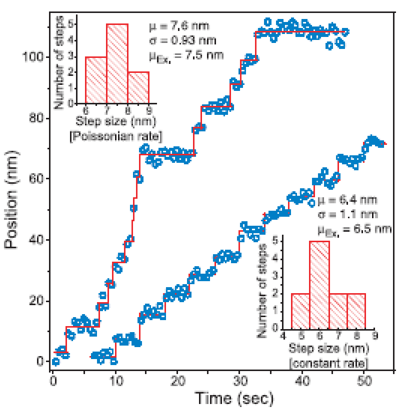


FCS (Florescence correlation spectroscopy)



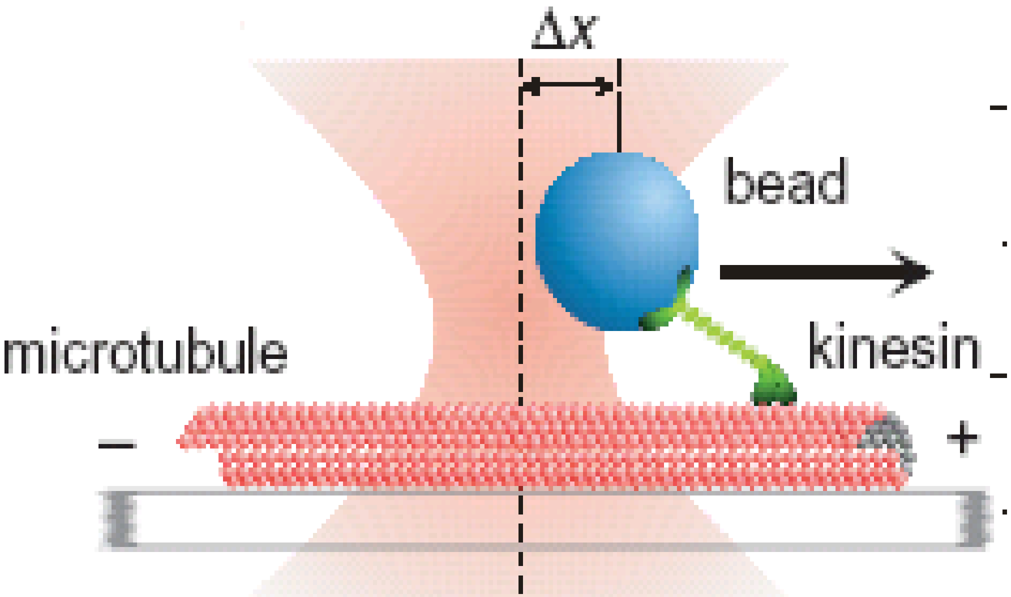
Single particle tracking



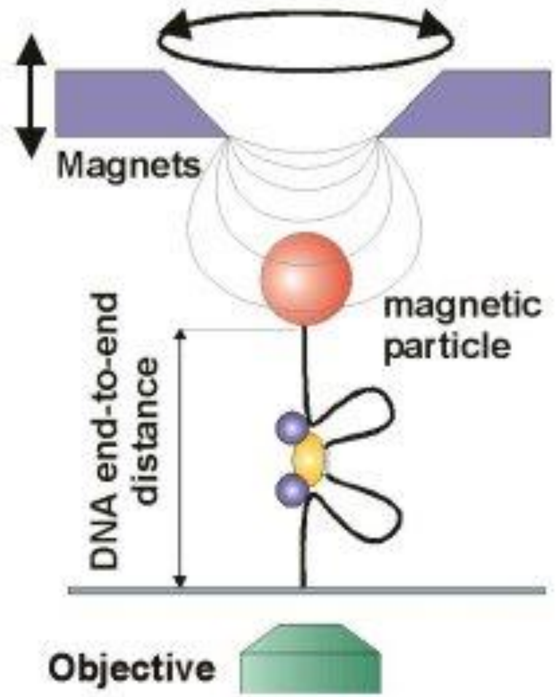


Single-Molecule Techniques: Force

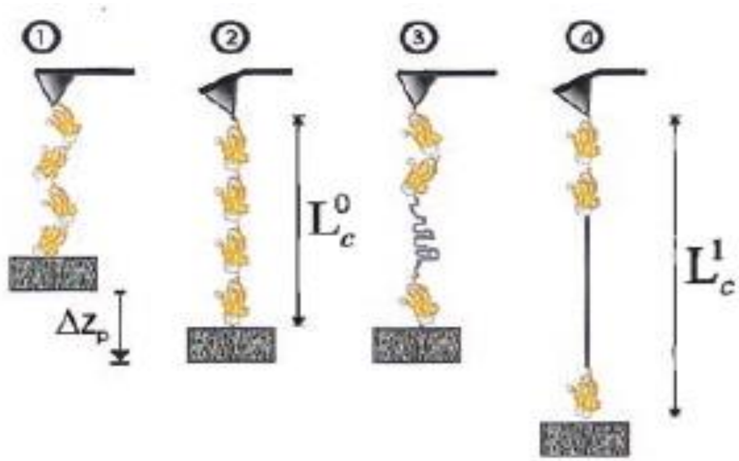
Optical tweezers



Magnetic tweezers

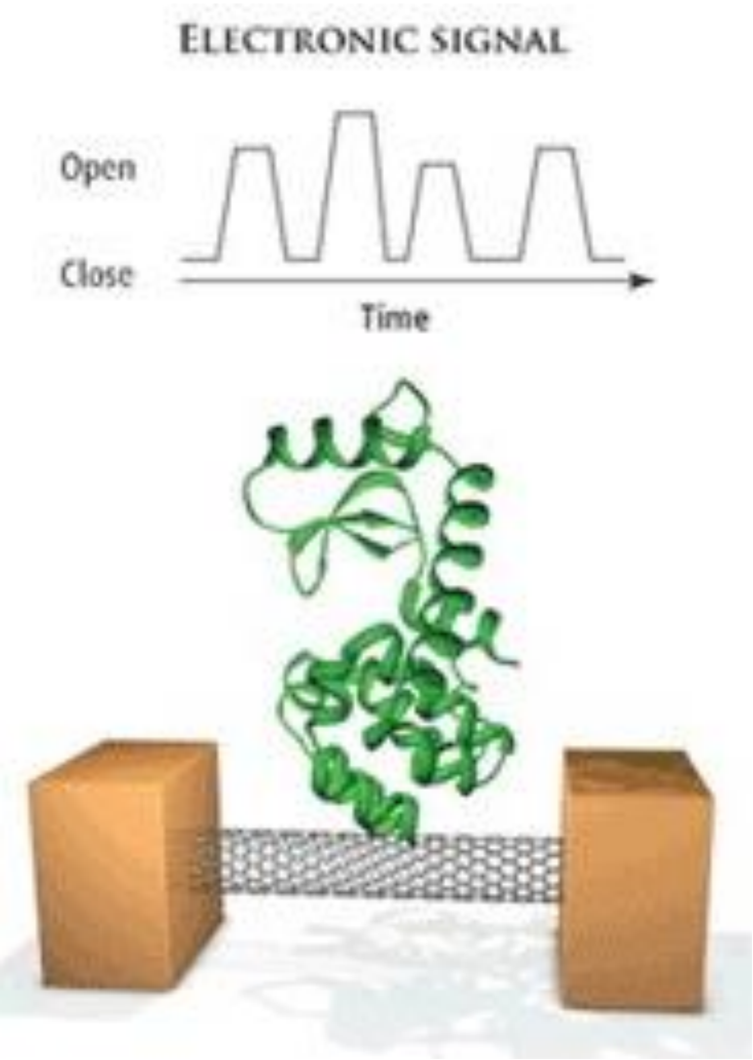


AFM (Atomic force microscopy)

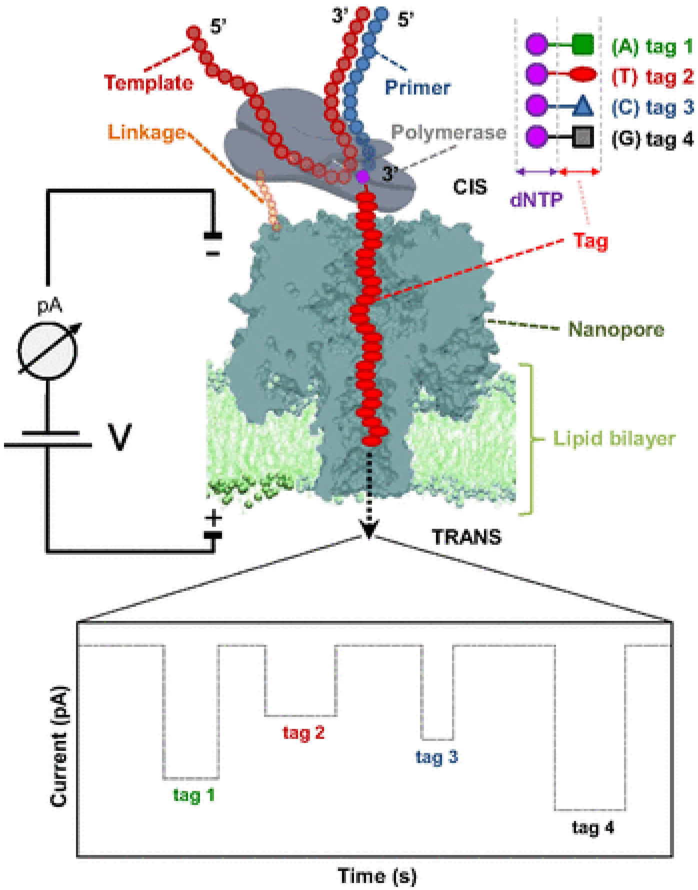


Single-Molecule Techniques: Electronic Signal

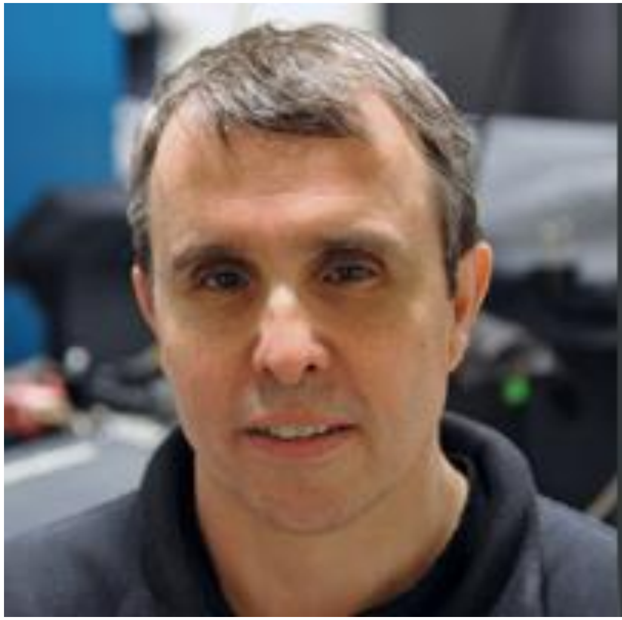
Carbon nanotube device



Membrane protein



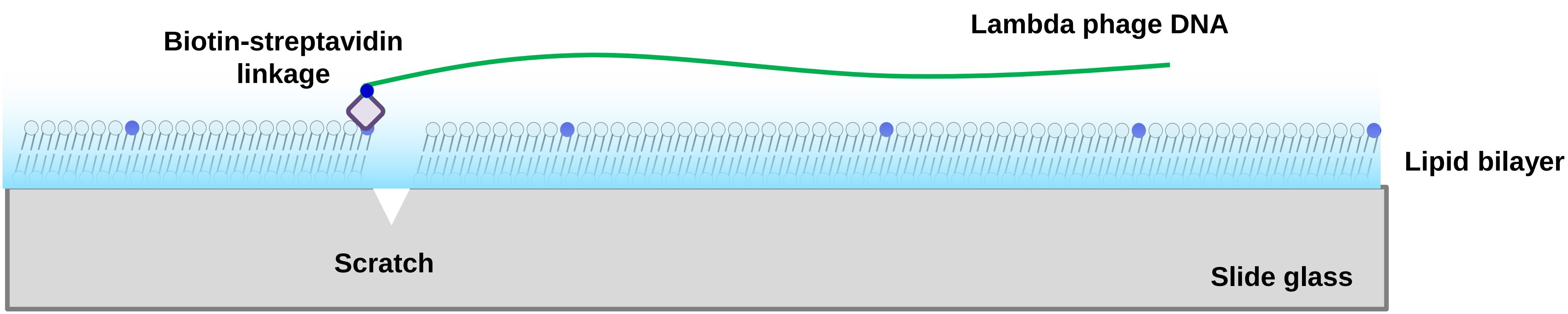
2014 Nobel Prize in Chemistry
Super-Resolution Microscopy



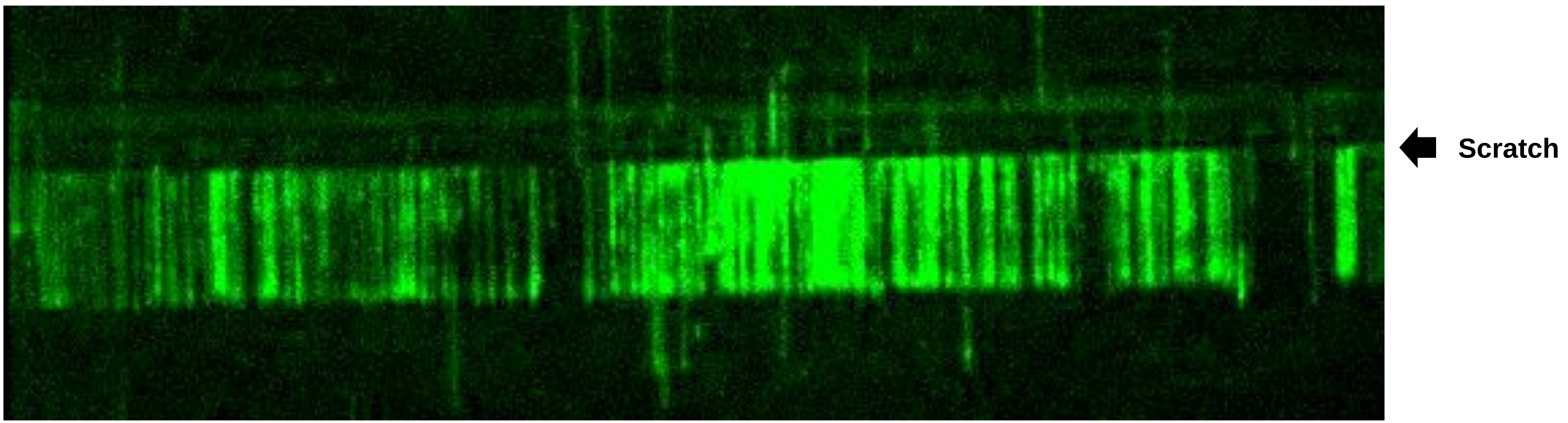
Eric Betzig **Stephan W. Hell** **William E. Moerner**

DNA Curtain Assay

DNA Curtain

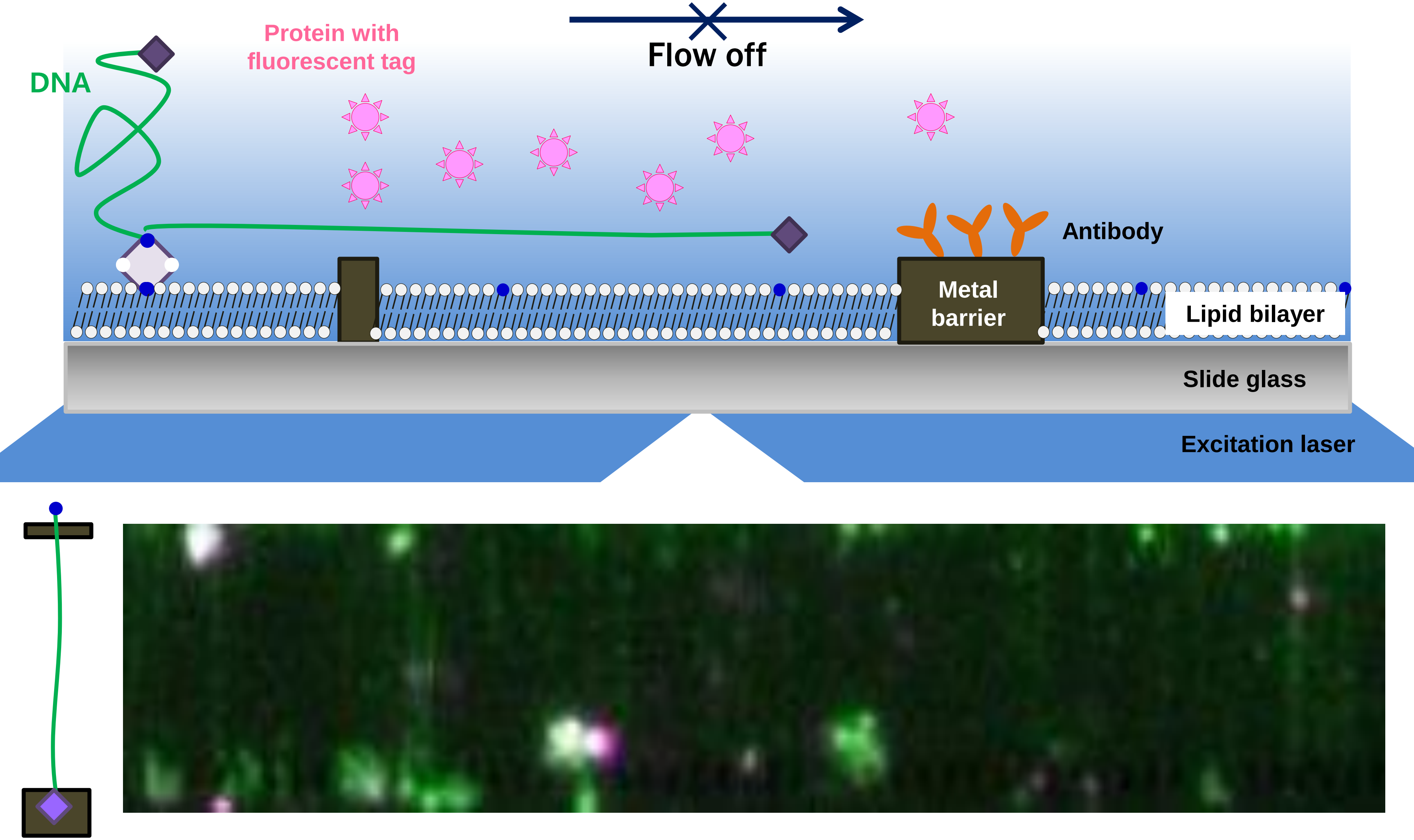


Lambda phage DNA
Biotin-streptavidin
linkage
Lipid bilayer
Scratch
Slide glass

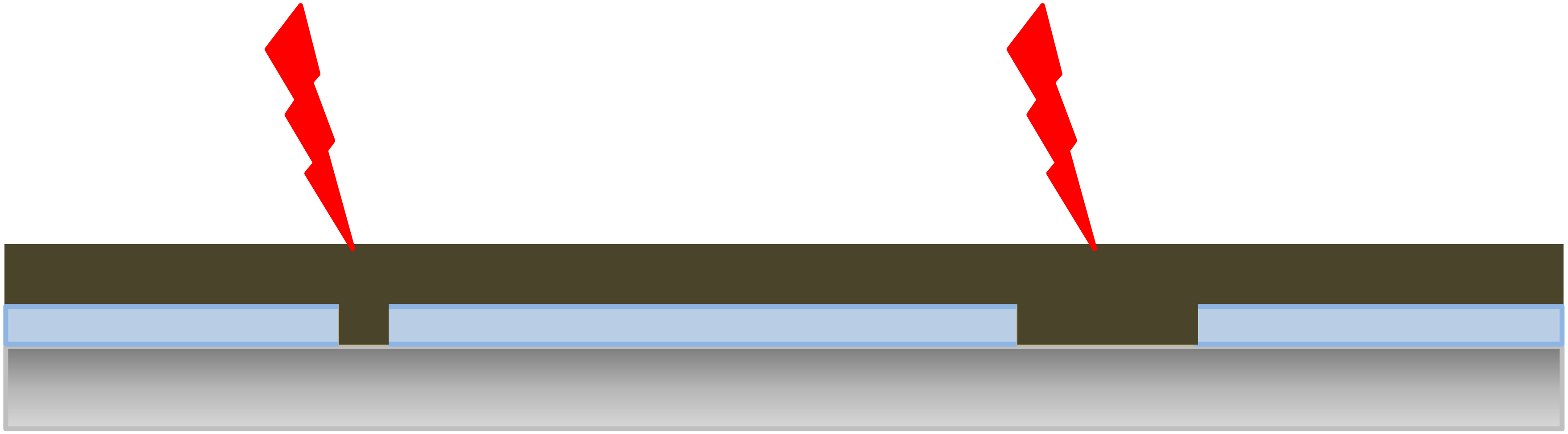


Scratch

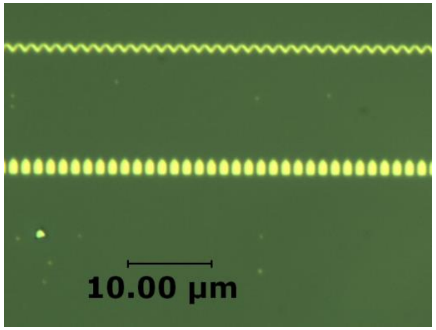
Double-Tethered DNA Curtain



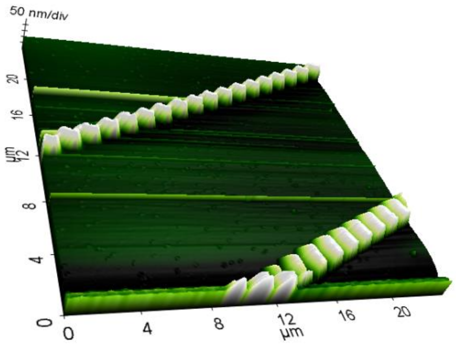
Nanofabrication for Barriers



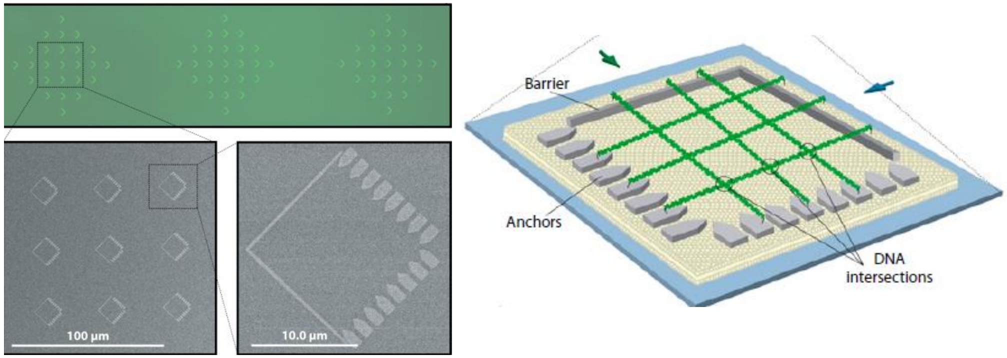
Optical image (100x)



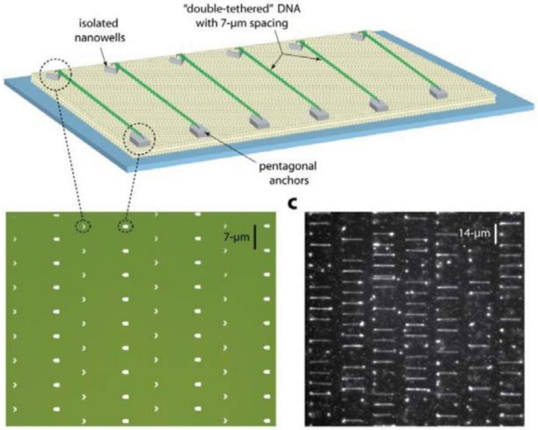
AFM image



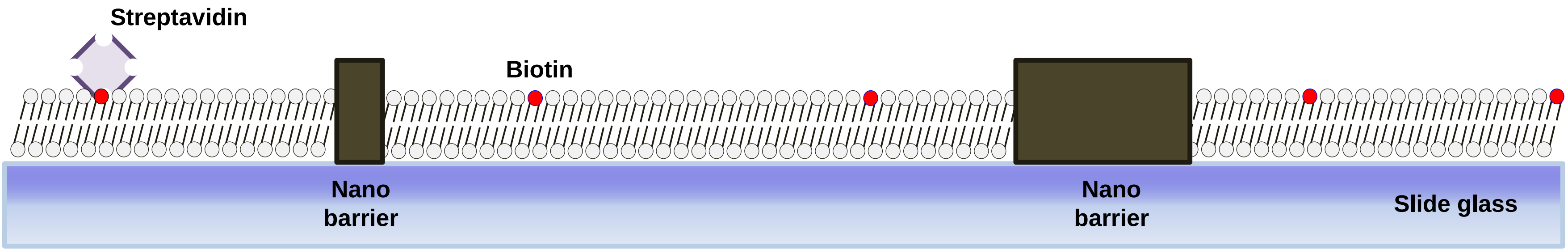
Criss-cross



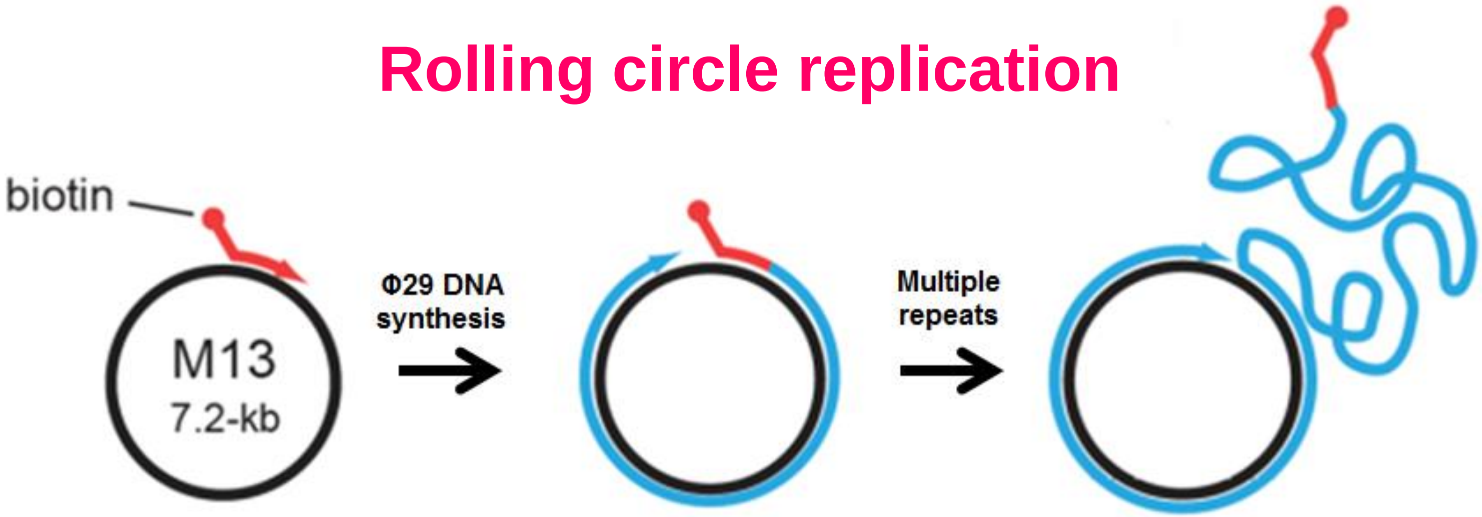
Separate curtain



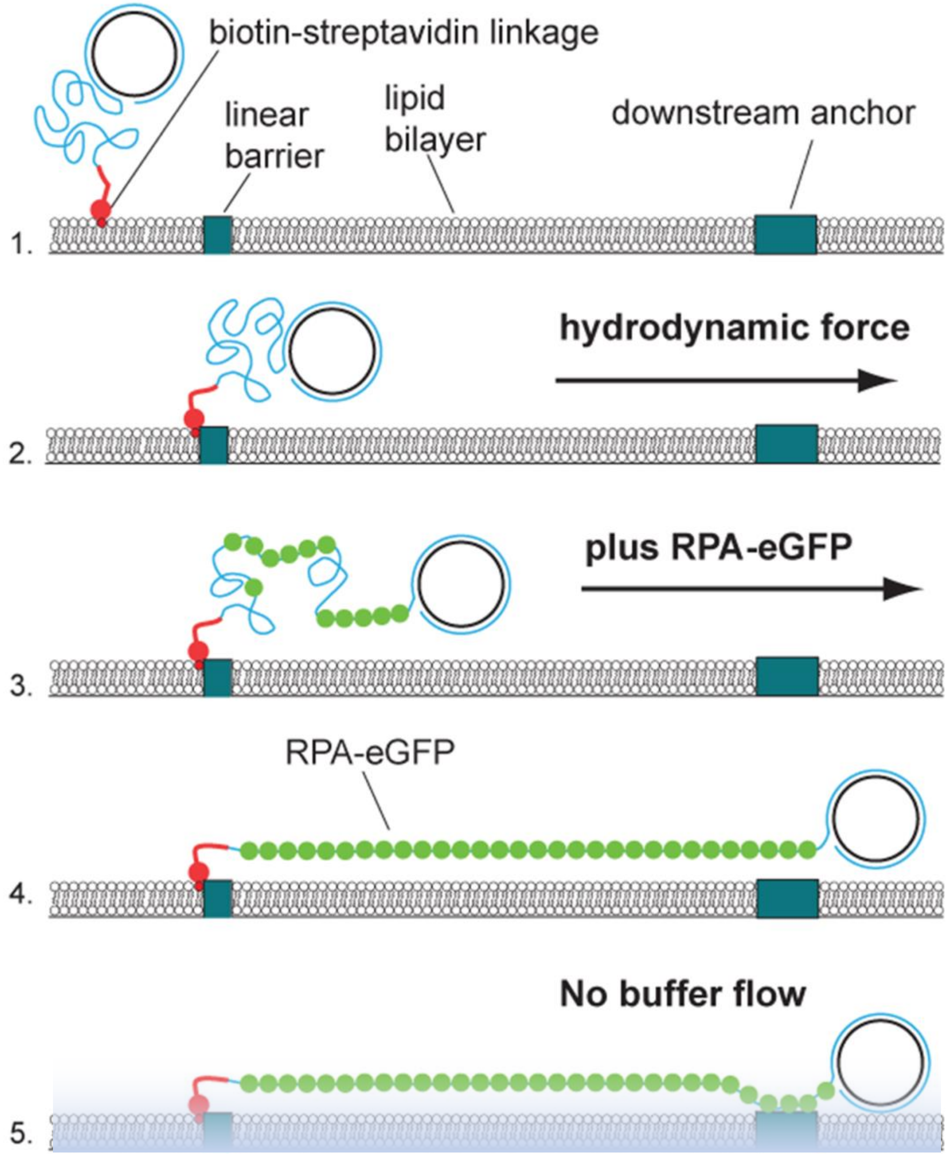
Single-Stranded DNA Curtain



Rolling circle replication

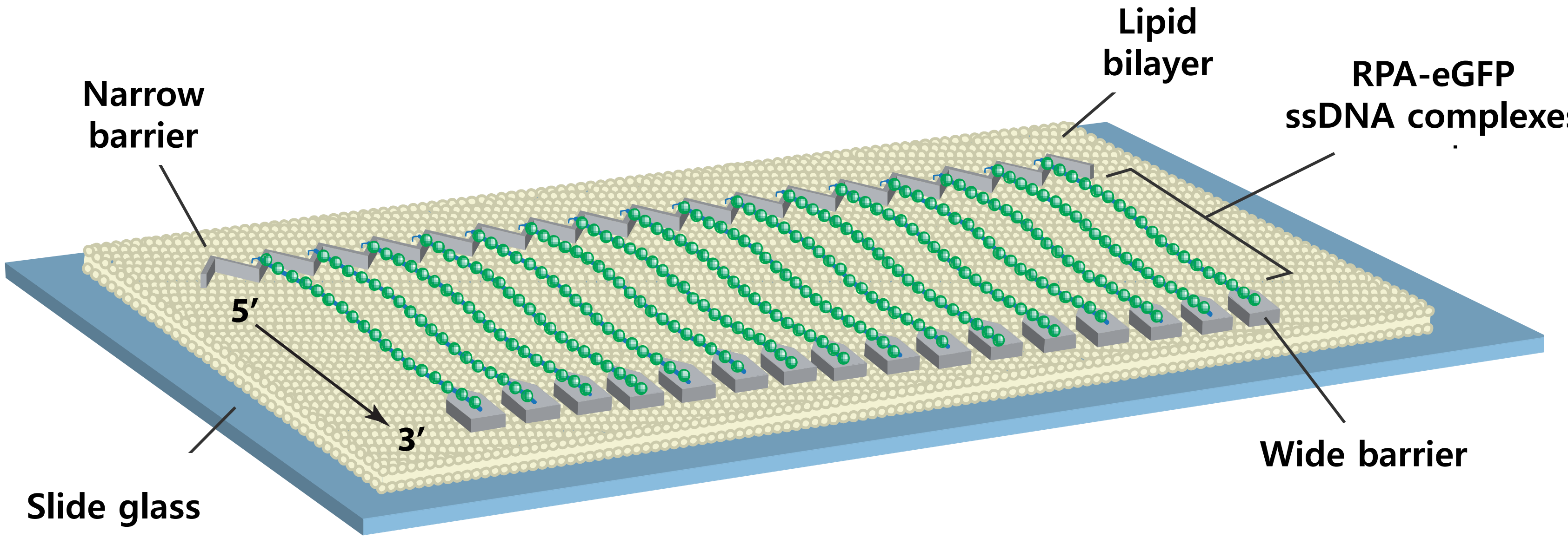


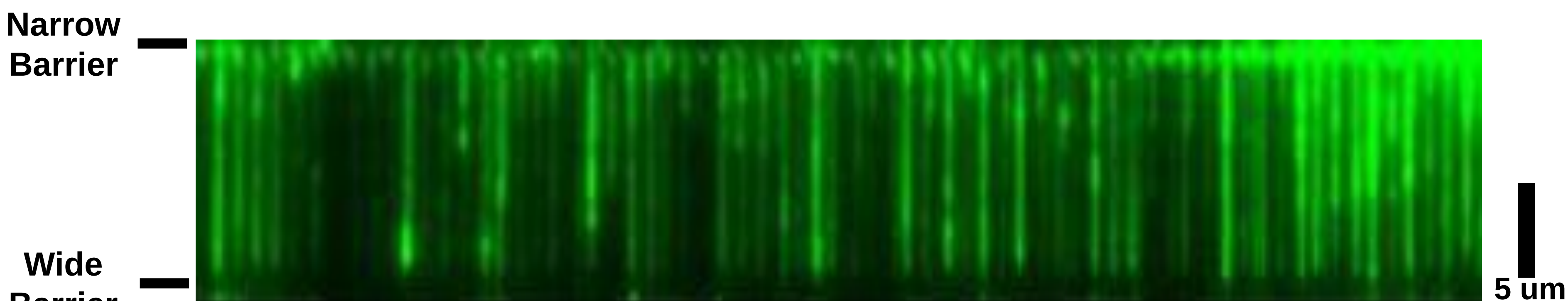
Single-Stranded DNA Curtains



Anal. Chem. 84, 7607 (2012)

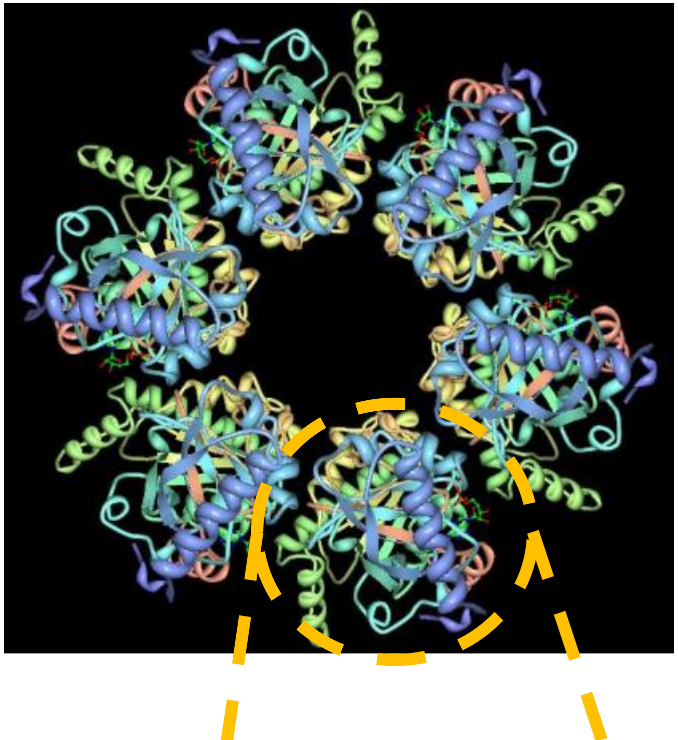
Single-Stranded DNA Curtains

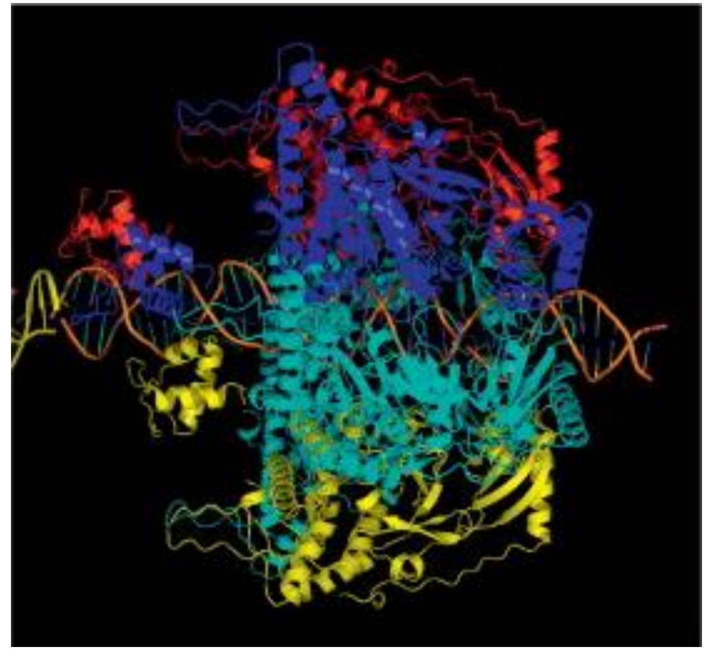


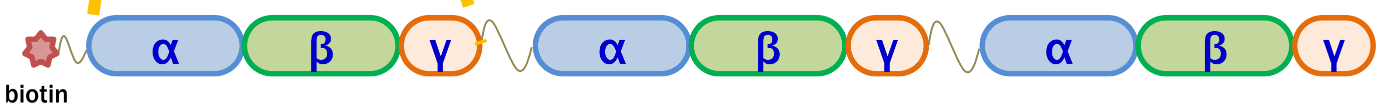


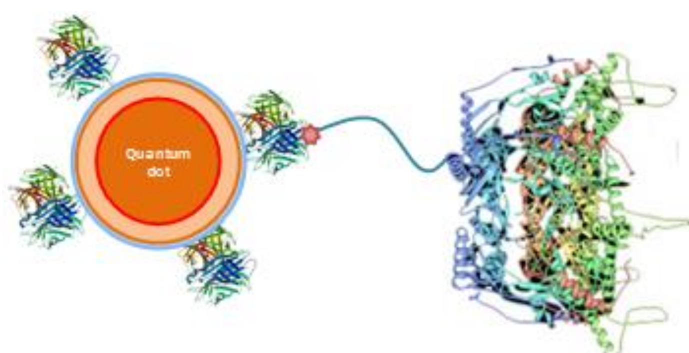
Protein Movement and Collision

DNA Motor Protein: FtsK



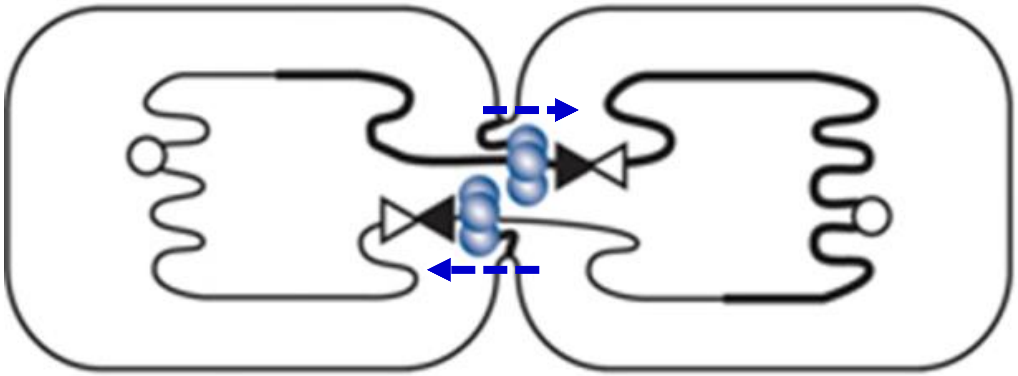


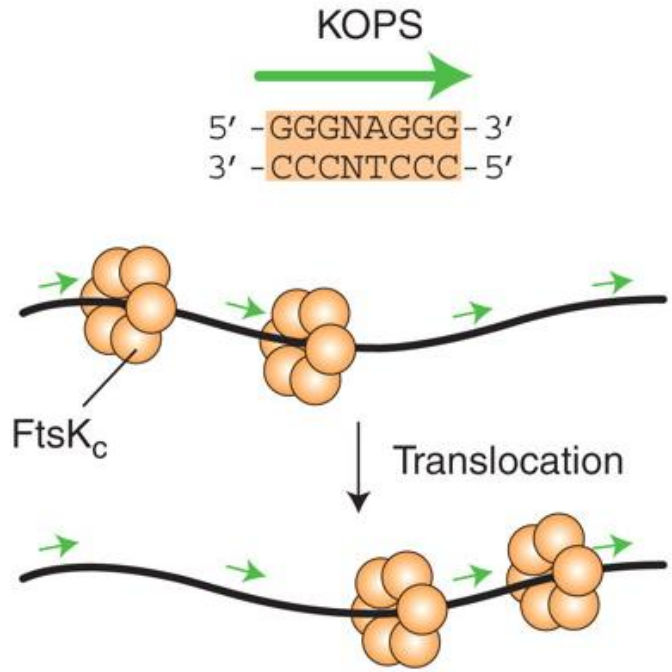


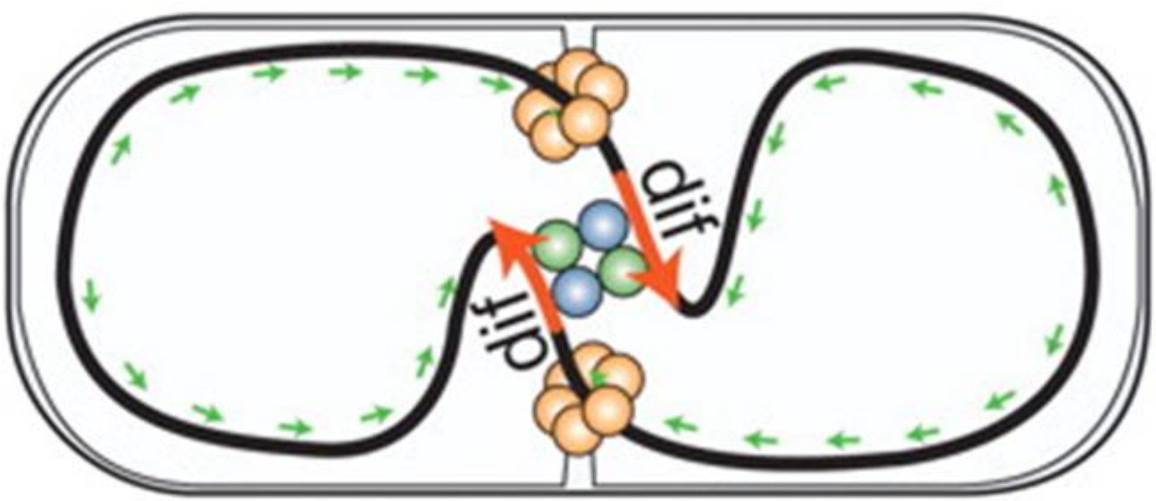


KOPS-Guided Translocation of FtsK

FtsK must move toward *dif*





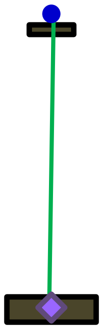


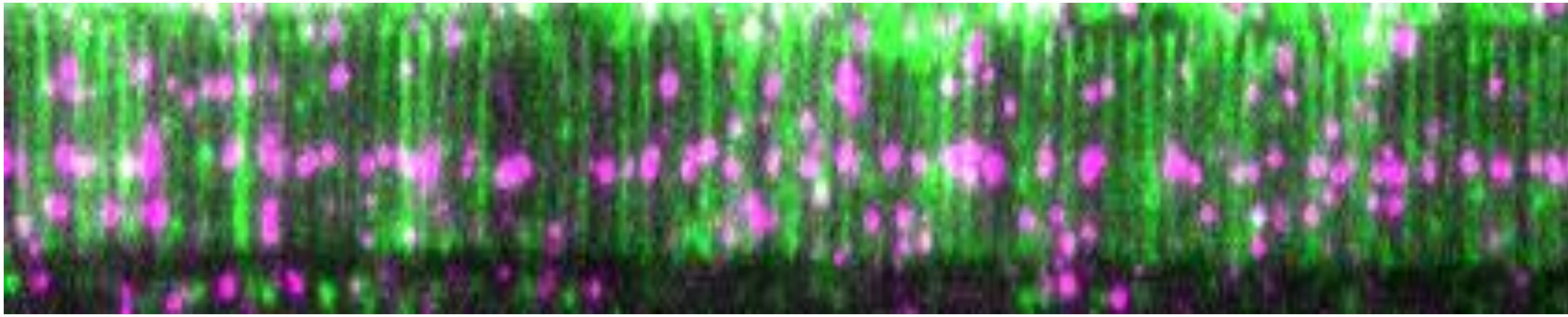
CHS Perspectives

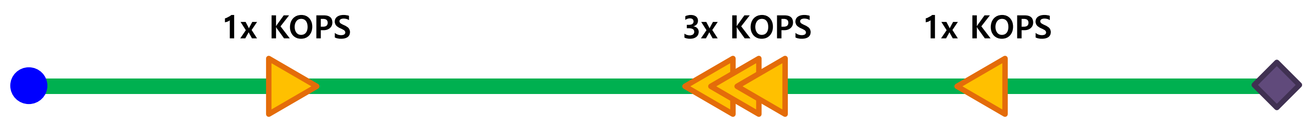
1. Single FtsK Motion on DNA

FtsK Binding without ATP

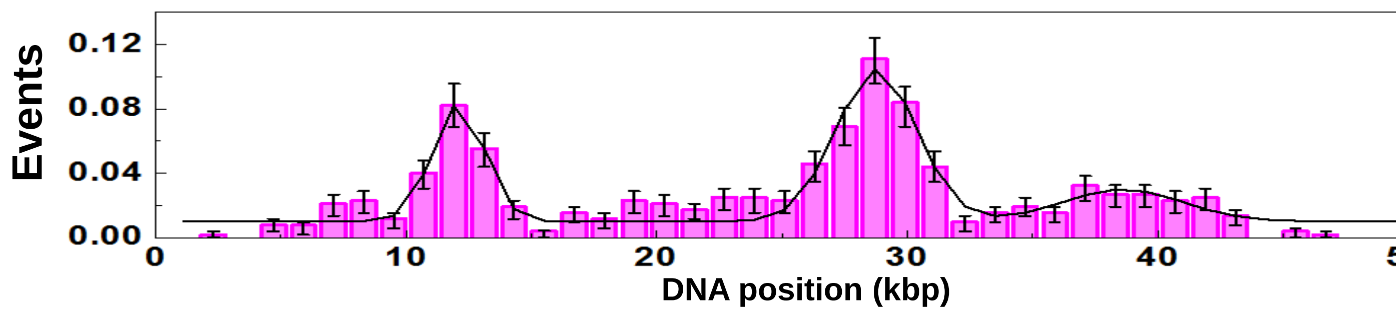
Green: DNA, magenta: FtsK



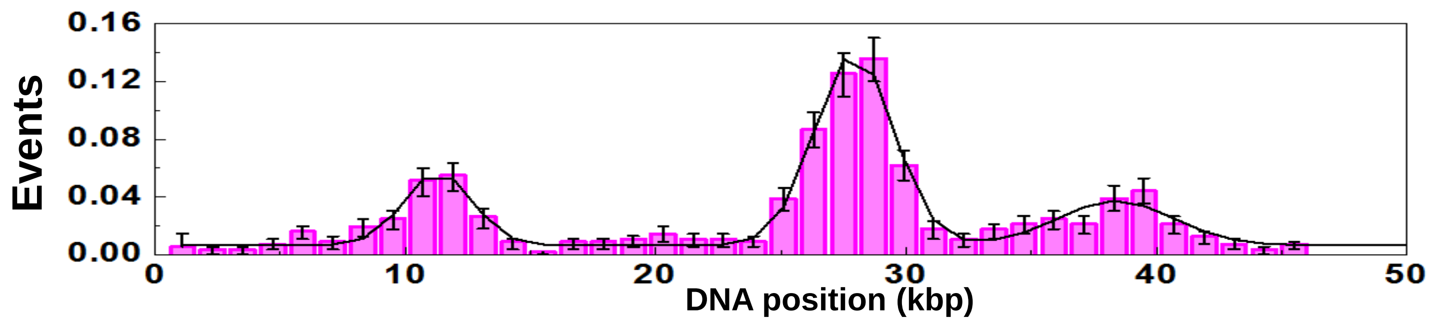




No ATP

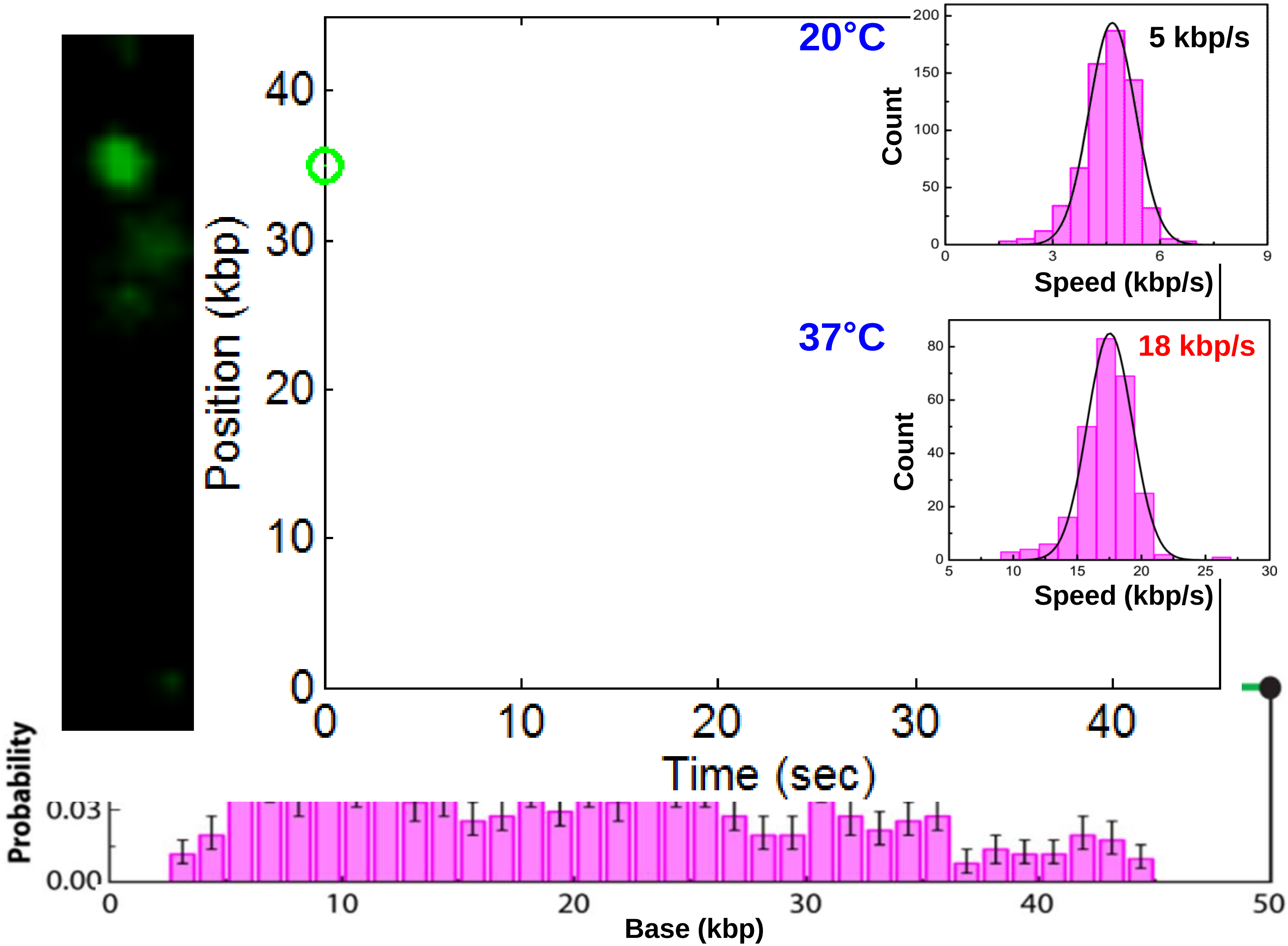


Mutant FtsK & ATP



PNAS 109, 6531 (2012)

FtsK Translocation

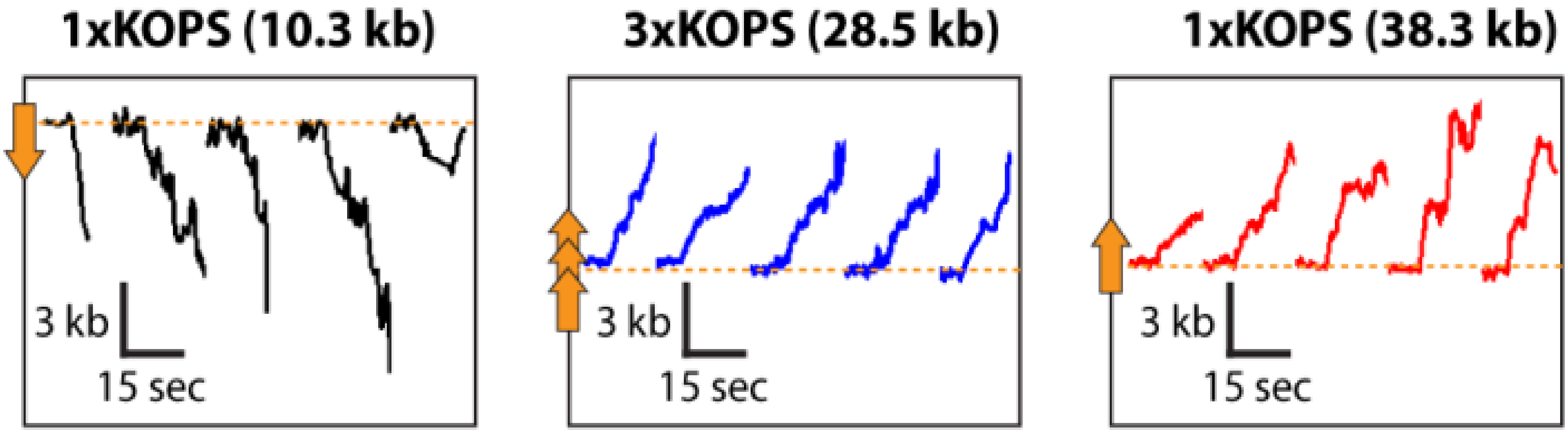


KOPS-Guided Movement

- FtsK does not recognize KOPS during translocation.

  Hypothesis: *initial binding on KOPS determines the orientation of FtsK movement.*

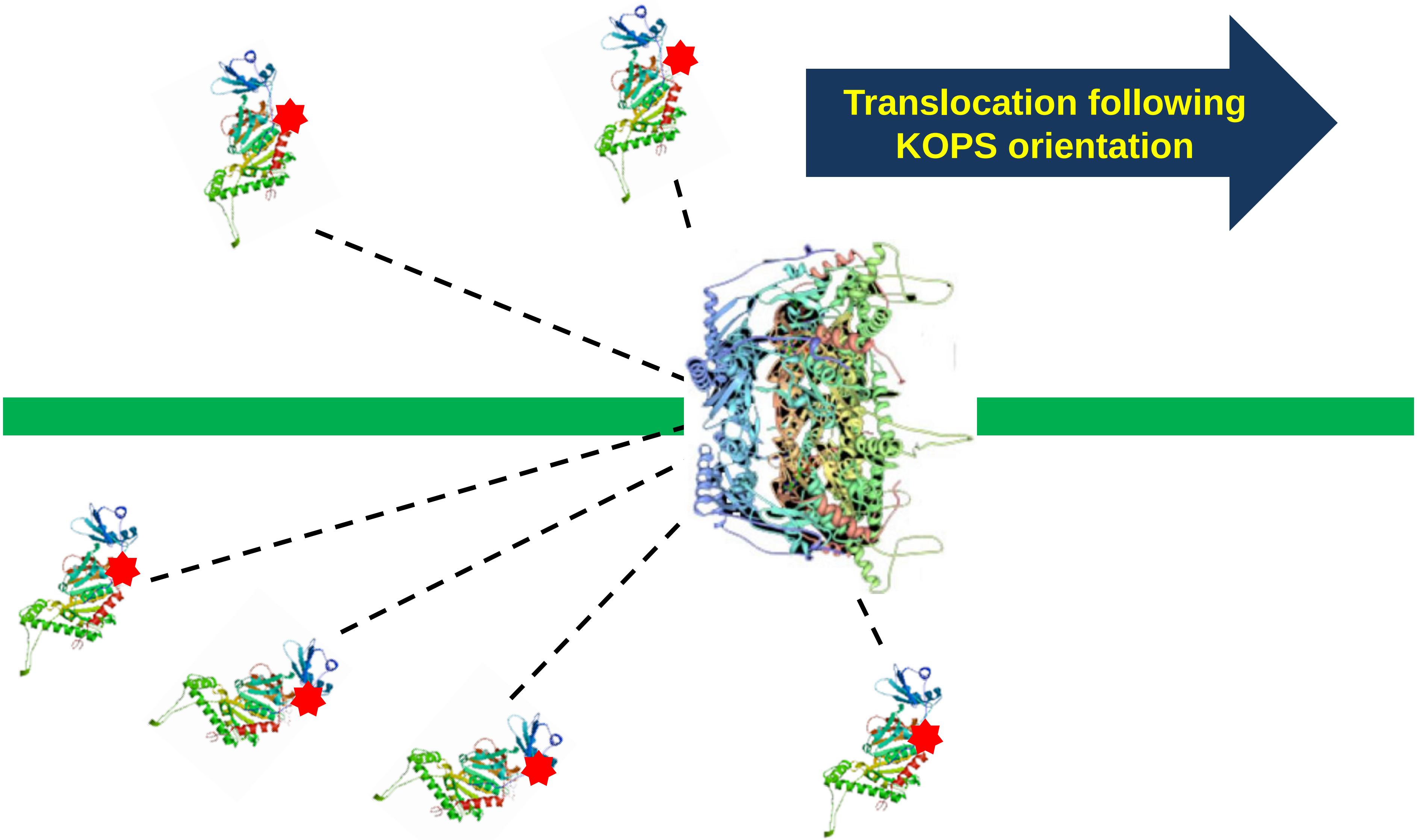
- ***ATP-chase*****:** place FtsK on KOPS with ADP, then inject ATP into the flowcell.



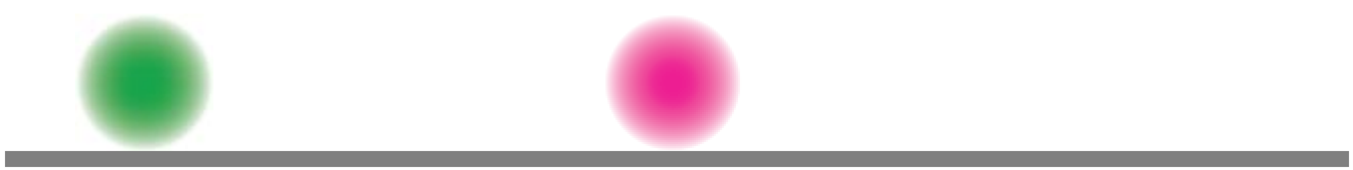
Lee et al. PNAS (2012)

KOPS guides the orientation of FtsK only when the FtsK departs from KOPS !!!

KOPS-Guided Initiation

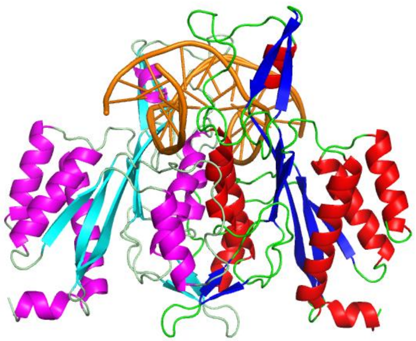


2. Collisions with Stationary Protein Obstacles



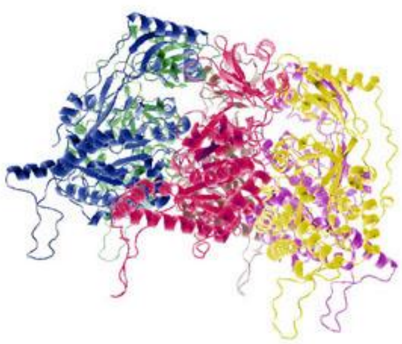
Collisions with DNA Binding Proteins

EcoRI^{E111Q}



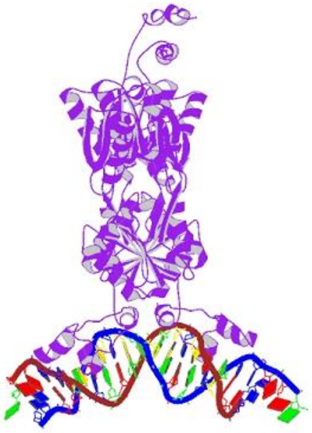
PDB: 2ckq

Mutant FtsK



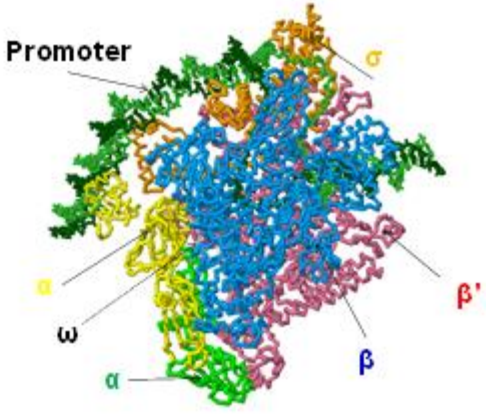
http://www.bioch.ox.ac.uk

LacI



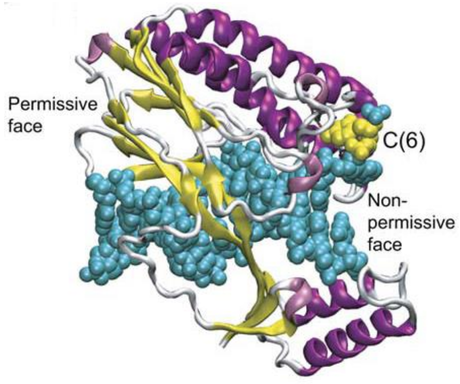
PDB: 1LBG

RNAP



Curr Opin Struct Biol. 11, 155 (2001)

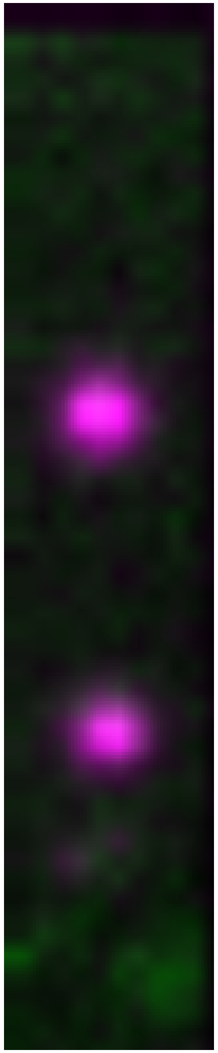
Tus

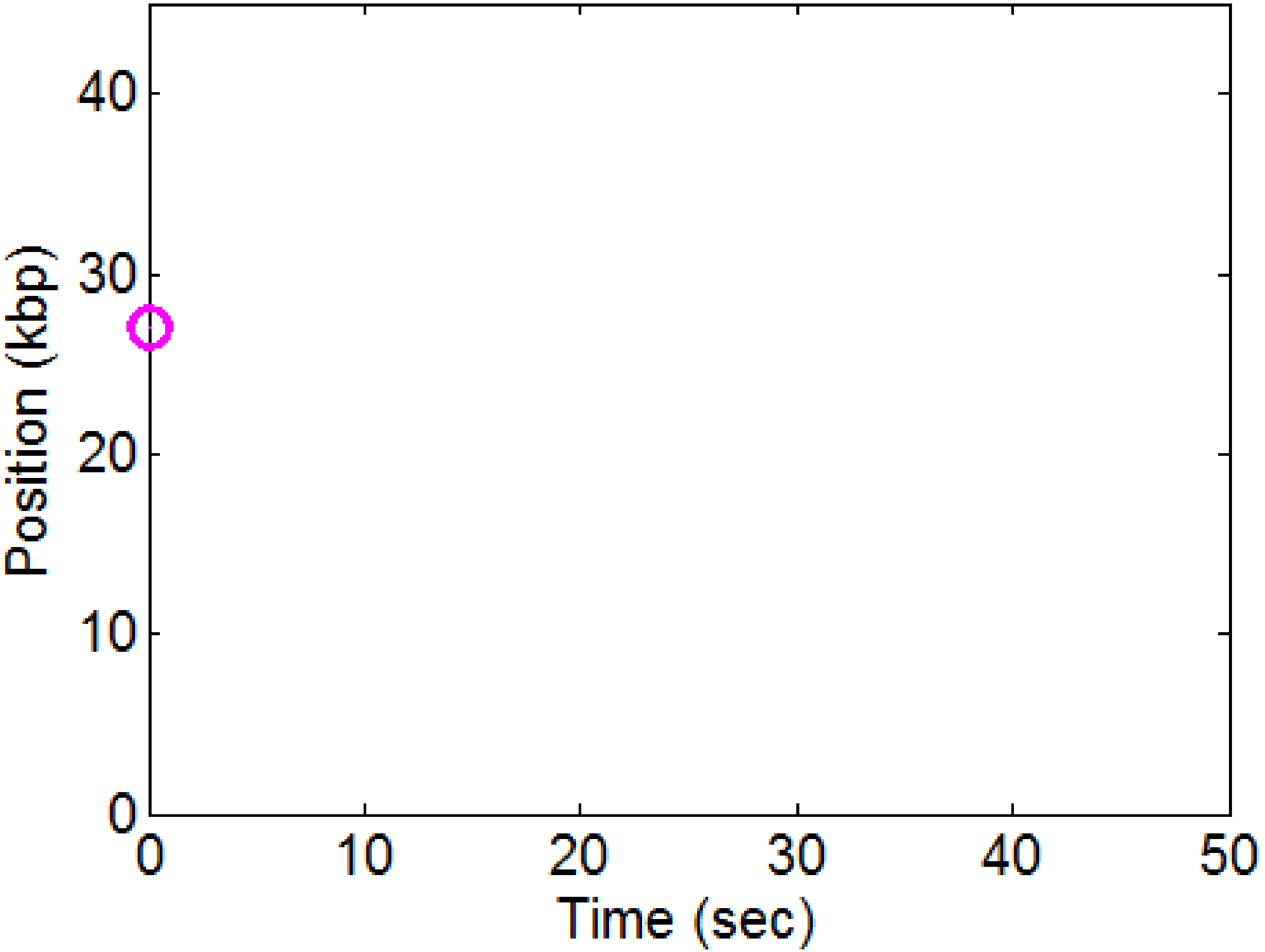


Cell 125, 1309 (2006)

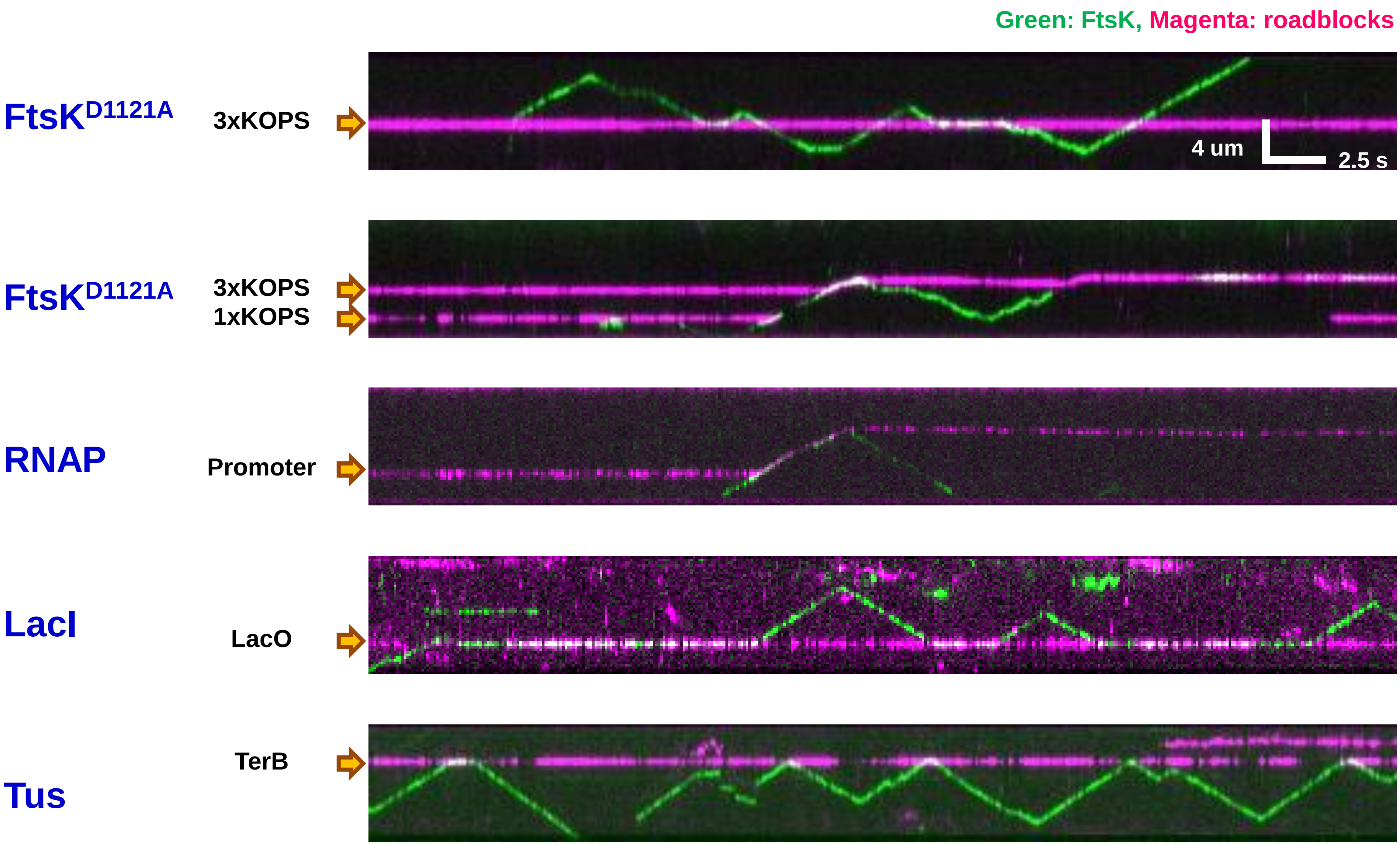
FtsK Collision with EcoRI^{E111Q}

Green: FtsK, Magenta: EcoRI^{E111Q}

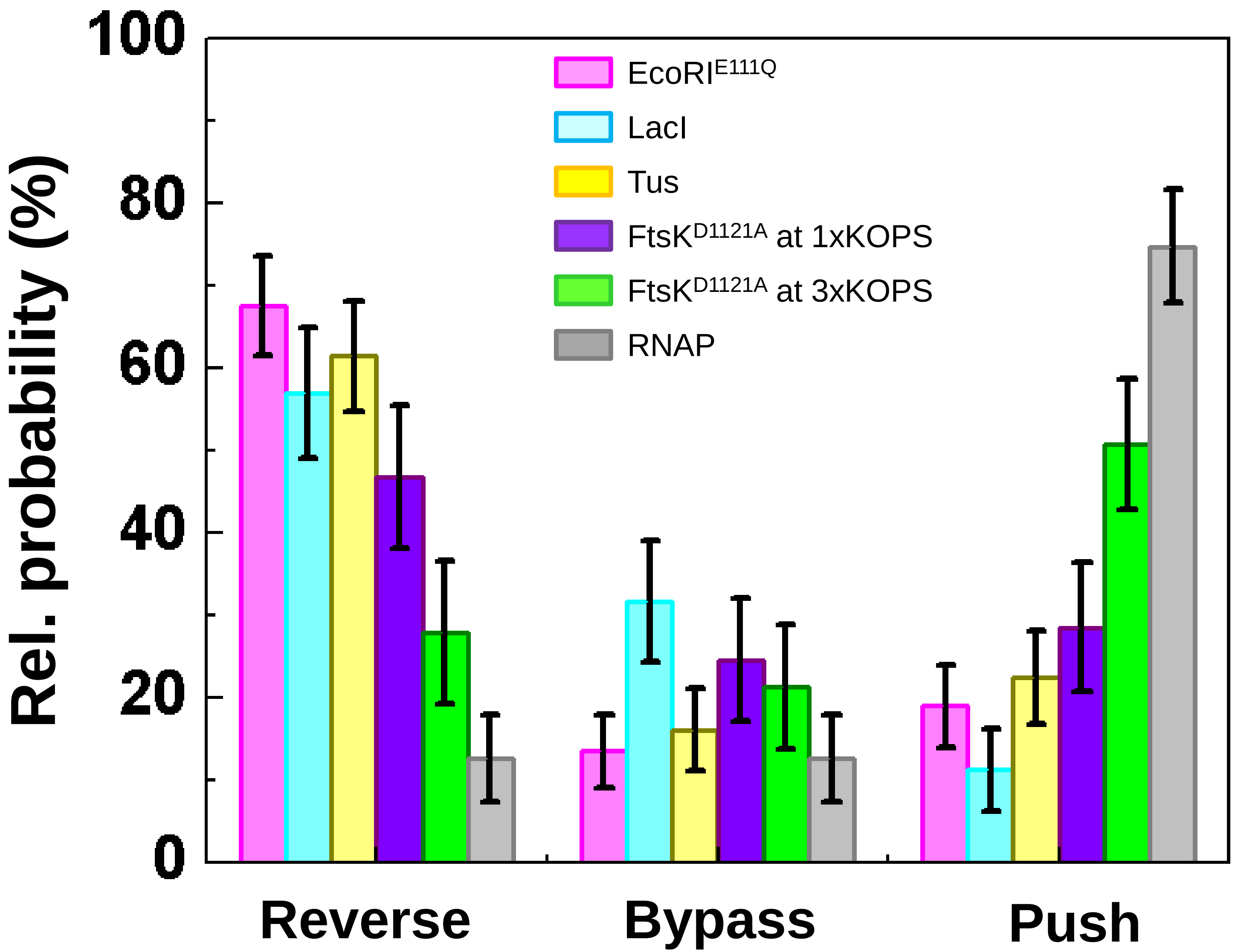




Collisions with Other Protein Roadblocks

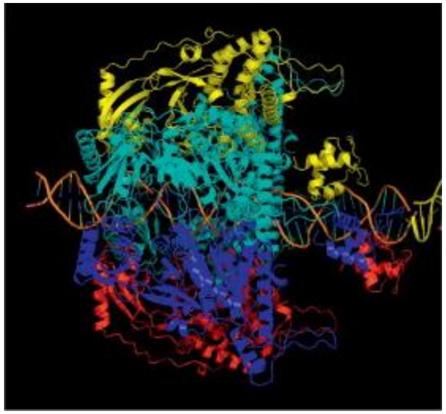


Collision Outcome for All Protein Roadblocks



Lee et al. Mol Cell (2014)

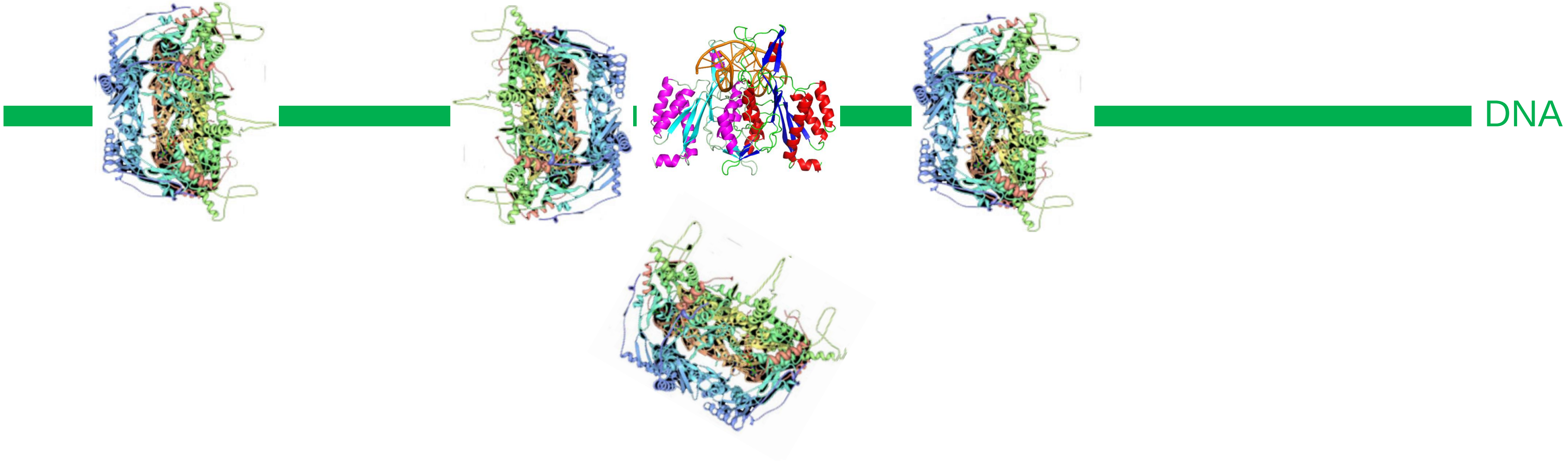
Mechanism of Bypass (or Reverse)



FtsK hexamer is wrapping dsDNA.

→ How can the FtsK bypass the protein roadblock?

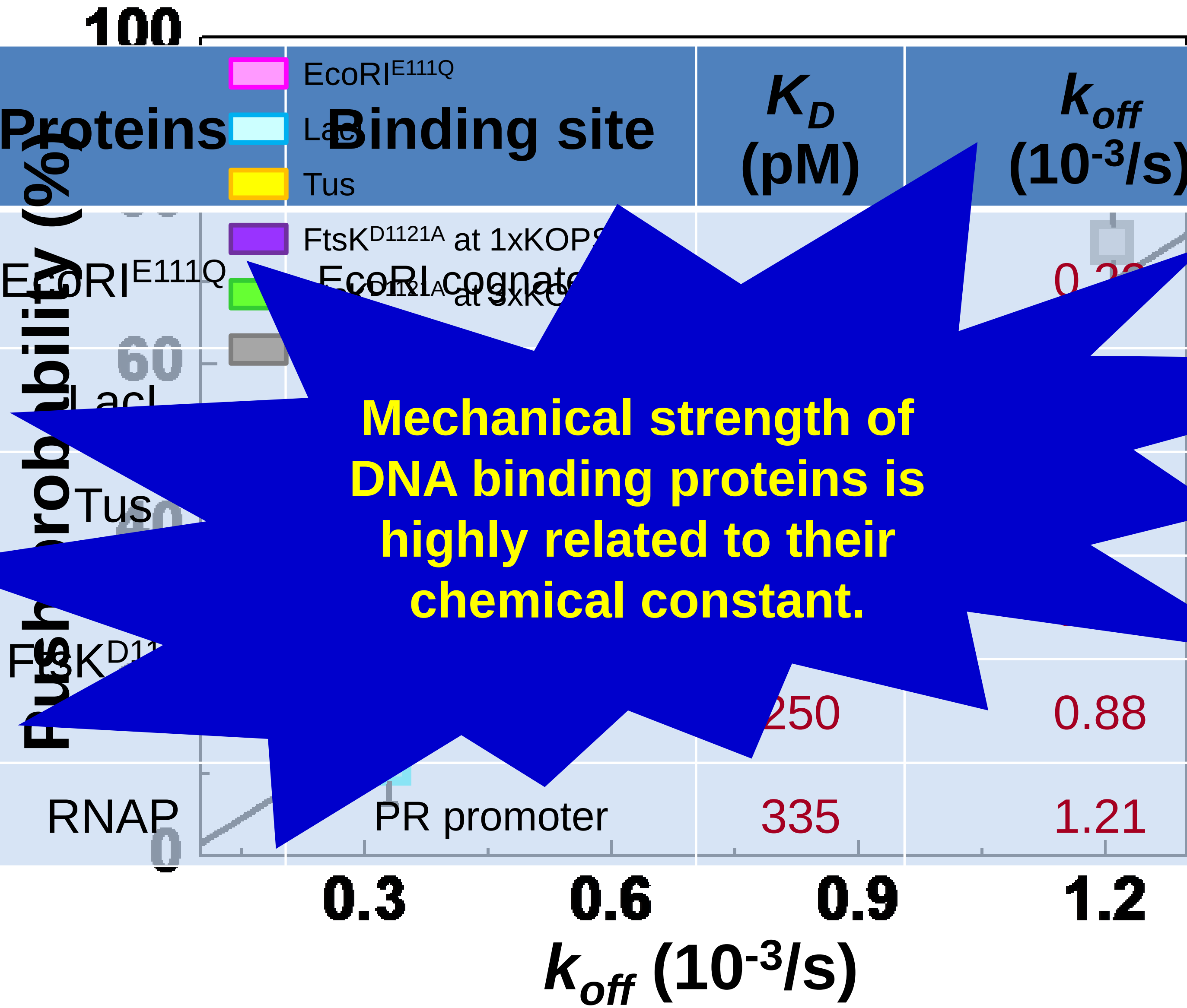
Dissociation & re-association mechanism



k_{off} (K_D) vs. Push Probability

Proteins	Binding site	K_D (pM)	k_{off} (10^{-3}/s)
EcoRI^{E111Q}	EcoRI cognate [illegible]	[illegible]	[illegible]
LacI	[illegible]	[illegible]	[illegible]
Tus	[illegible]	[illegible]	[illegible]
FtsK^{D11}[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	250	0.88
RNAP	PR promoter	335	1.21

Mechanical strength of DNA binding proteins is highly related to their chemical constant.



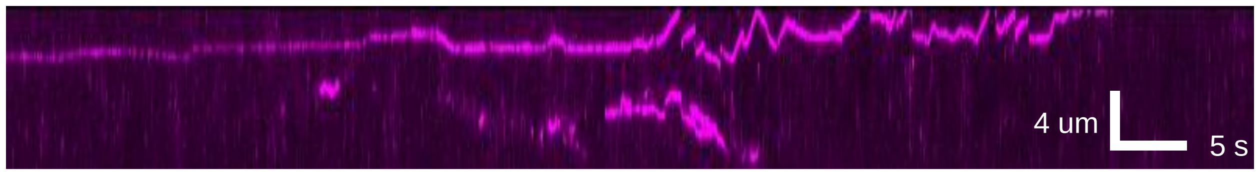
Mol Cell 54, 832 (2014)

Collision with Multiple FtsK Hexamers

- *In vivo,* FtsK hexamers are localized at the septum.
  → The local concentration of FtsK hexamers must be fairly high.
  → Multiple FtsK hexamers seem to work on the chromosomal DNA simultaneously.

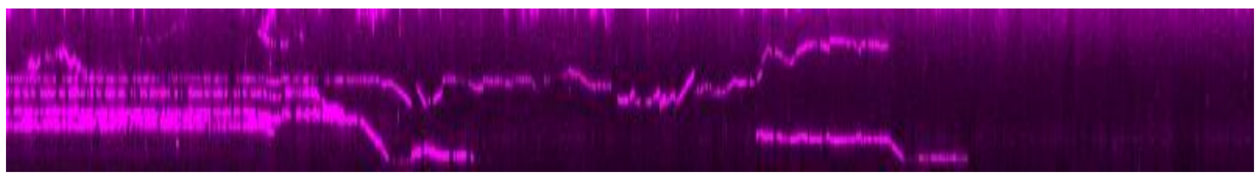
Magenta: Tus

Tus on TerB



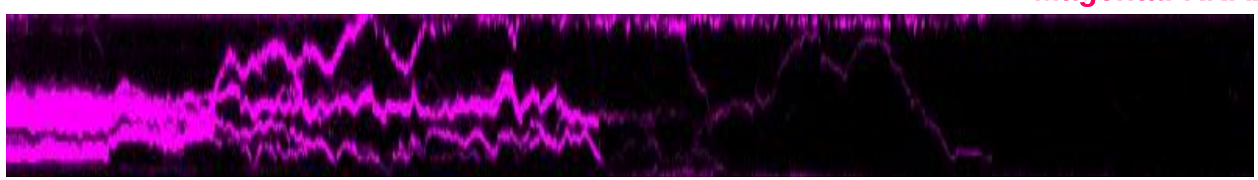
Magenta: RNAP

RNAP open complexes (- rNTPs)



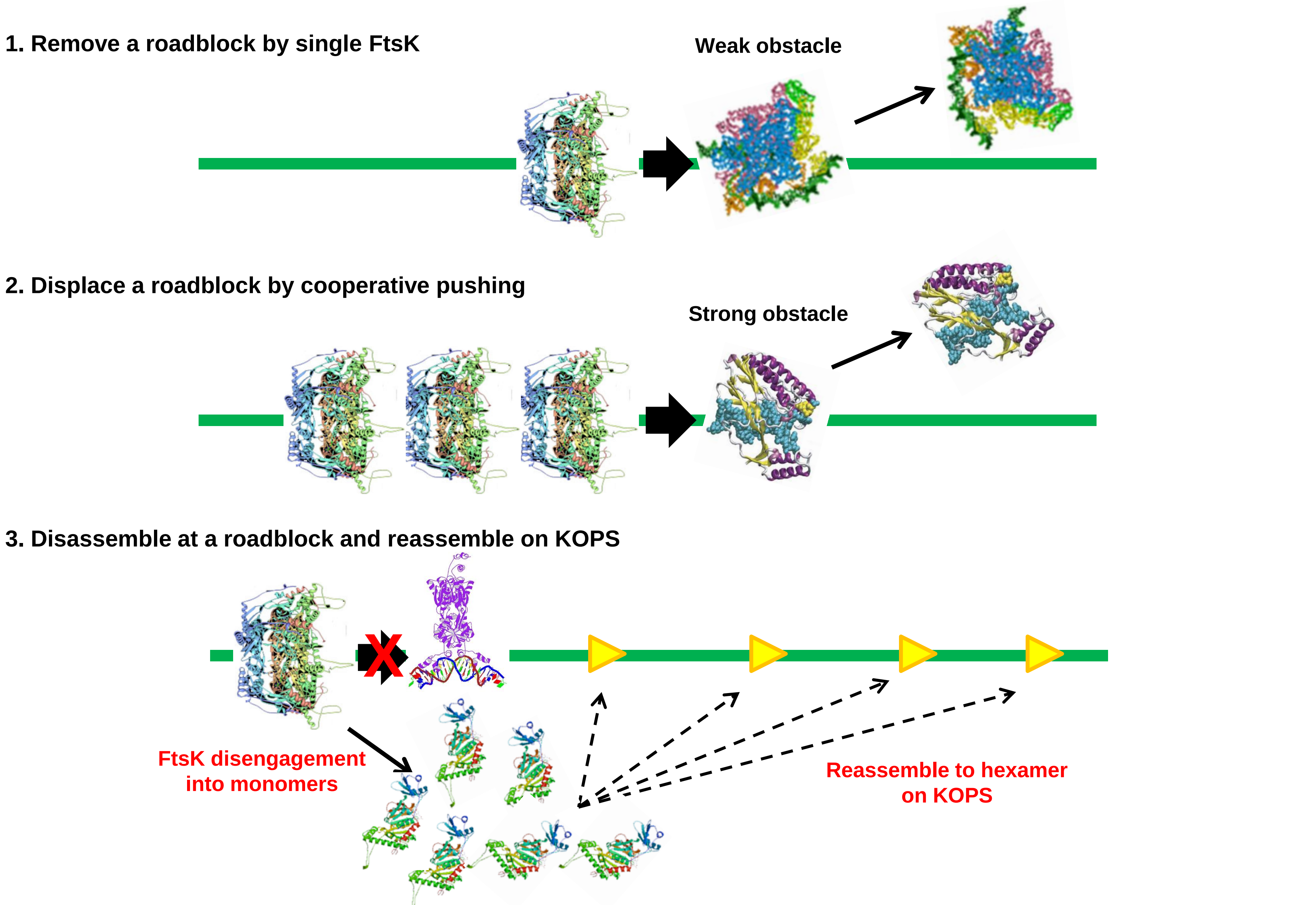
Magenta: RNAP

RNAP elongation complexes (+ rNTPs)



Multiple FtsK hexamers can remove protein roadblocks!!!

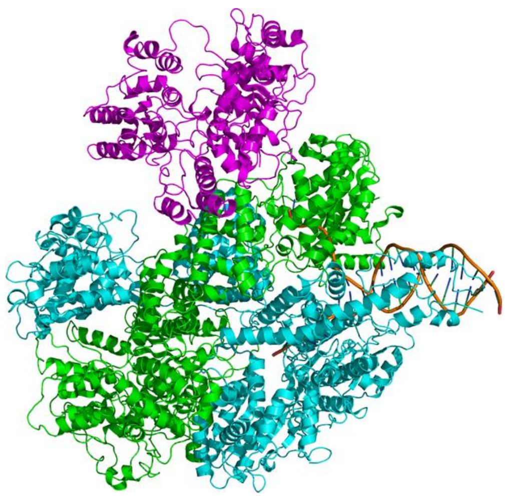
Translocation Mechanism through Crowded DNA

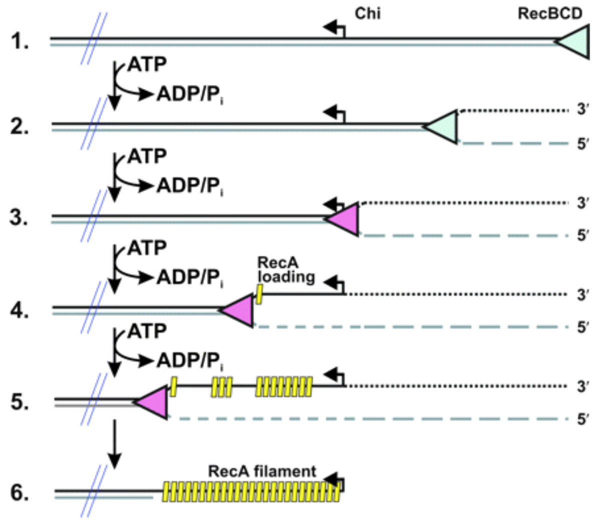


3. Collision with Another Moving Protein

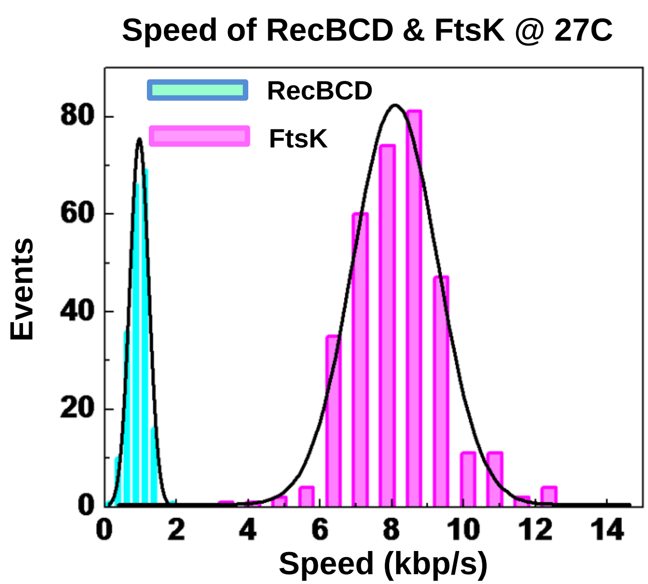
Collision with Another DNA Translocase (RecBCD)

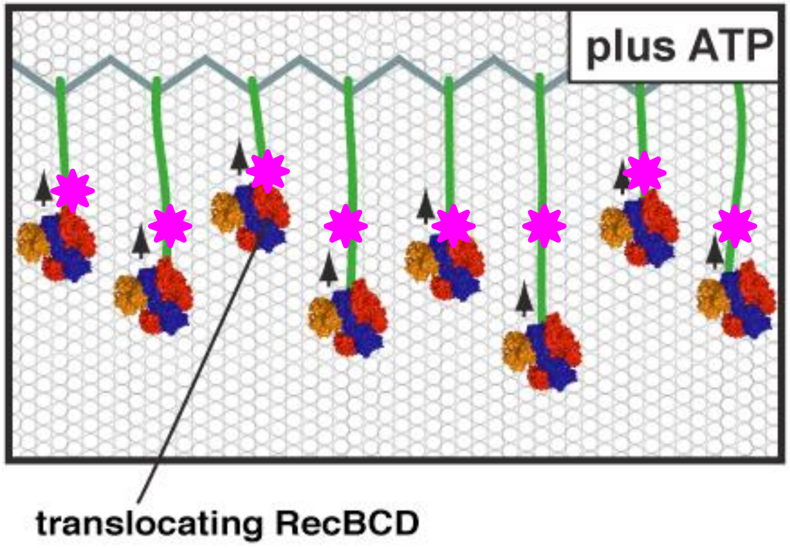
PDB: 1W36





Microbiol Mol Biol Rev 72, 642 (2008)

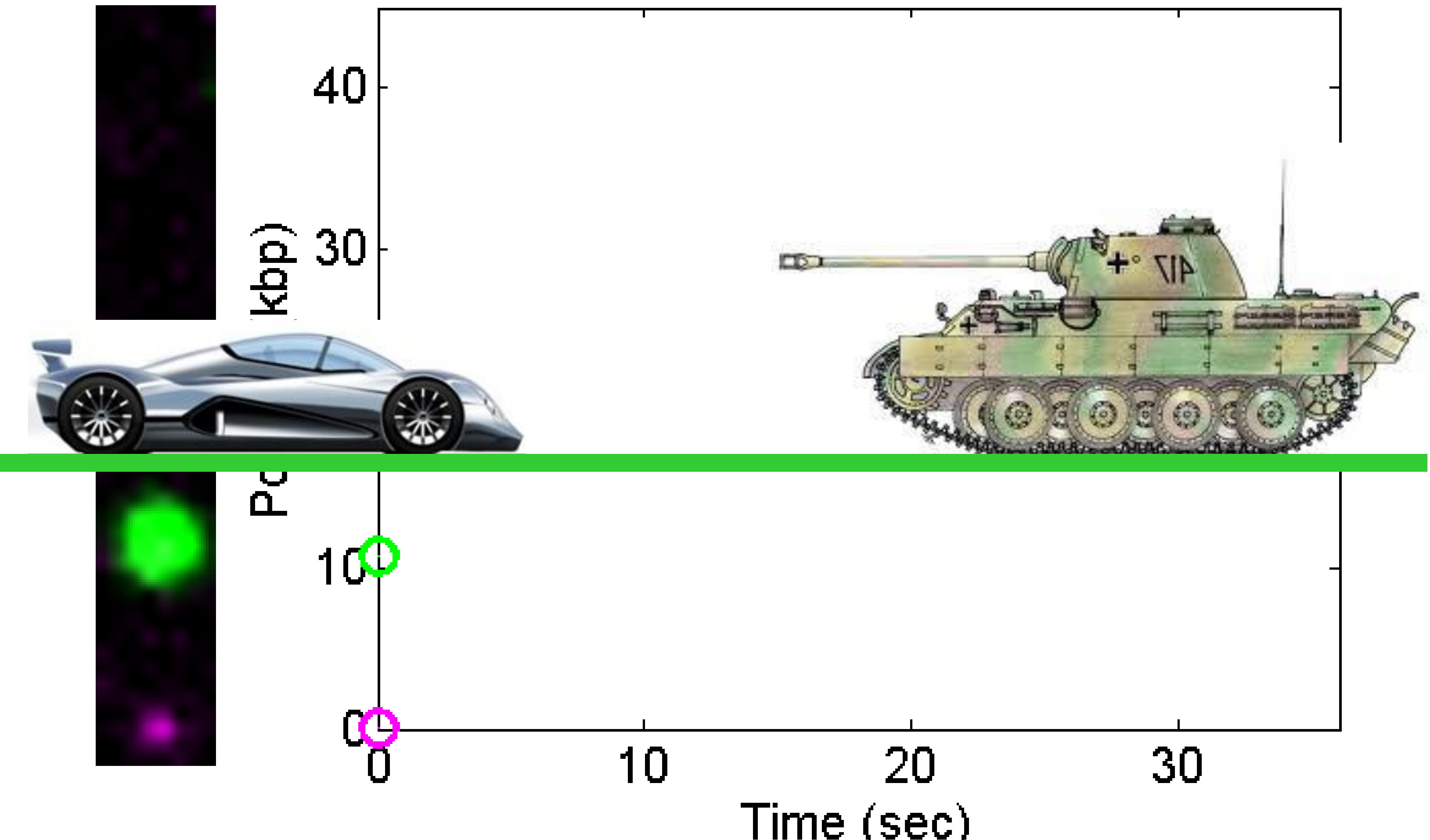




Nature 468, 983 (2010)

Collision of FtsK and RecBCD

Green: FtsK, magenta: RecBCD



Lee et al. Mol Cell (2014)

Summary

Using the single-molecule DNA curtains technique, we directly visualize the behavior of FtsK and its collision with other proteins.

- FtsK is assembled into a hexamer on KOPS, which guides FtsK orientation at the departure.

- FtsK translocates on DNA rapidly and processively.

- FtsK can continue to translocate by bypassing or evicting protein obstacles.

- Chemical constants (K_D or k_{off}) are highly related to the mechanical binding strength of DNA-binding proteins.

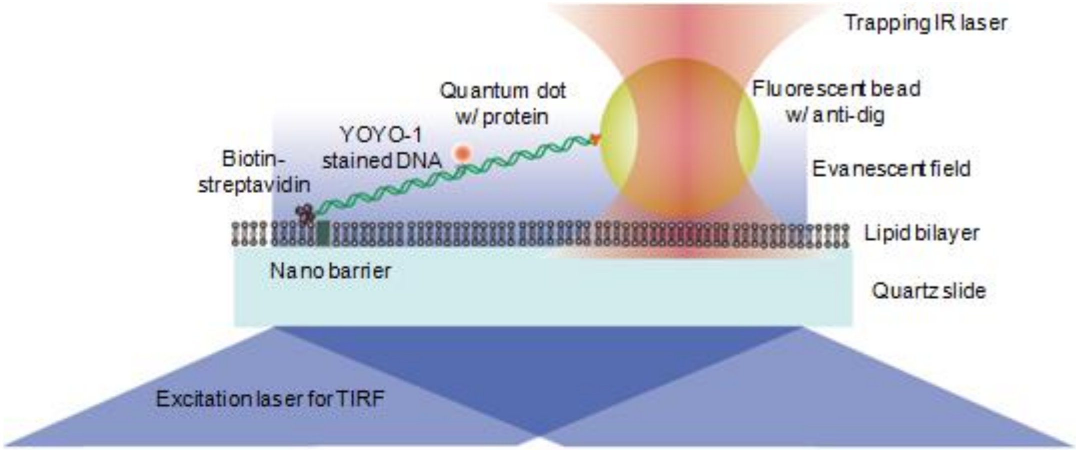
- Specific interaction between γ-subdomain and XerD prevents displacement of XerCD from the *dif* site.

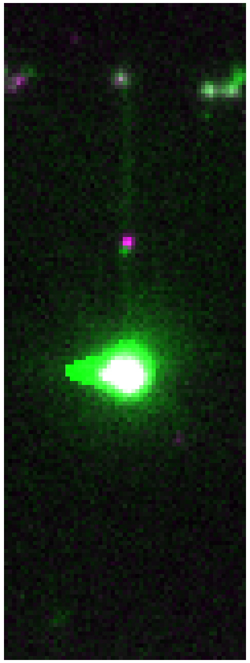
- RecBCD is more powerful than FtsK although FtsK is faster than RecBCD.

Beyond DNA Curtain

DNA Curtains + Optical Tweezers

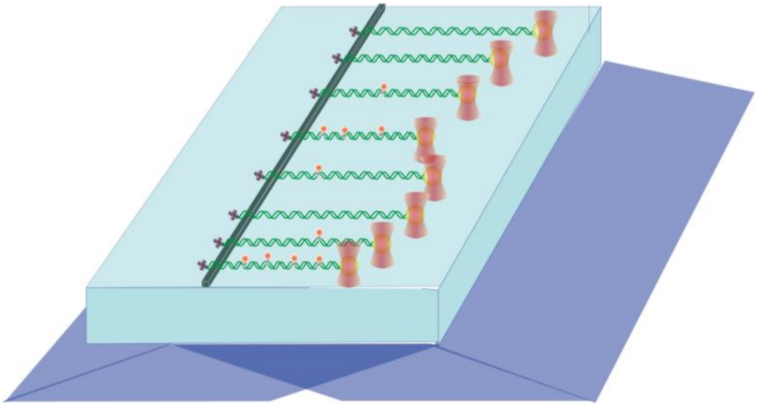
Optical tweezers + DNA Curtains

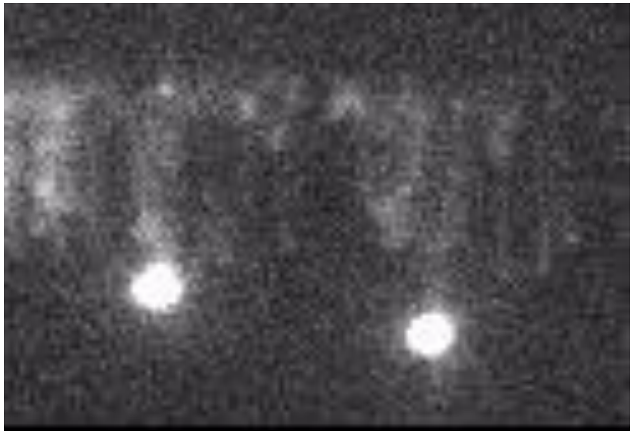




BBRC 426, 565 (2012)

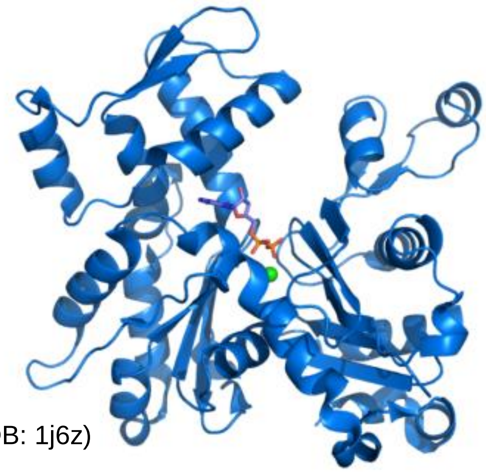
Multiple Optical-Trap & DNA Curtains





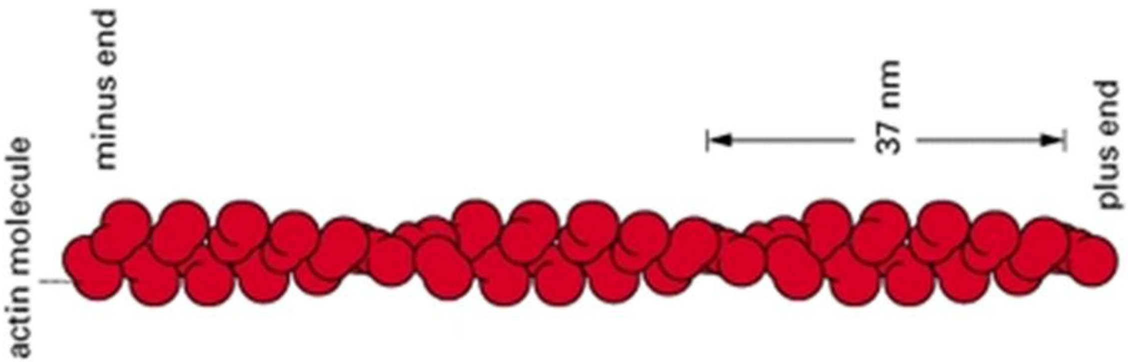
Actin & Actin Filaments

- Globular protein (42 kDa).
- Highly conserved and abundant in eukaryotic cells.
- Monomeric form (G-actin) and filamentous form (F-actin).
- Polarized polymerization: barbed (+) end is faster than pointed (-) end.



(PDB: 1j6z)

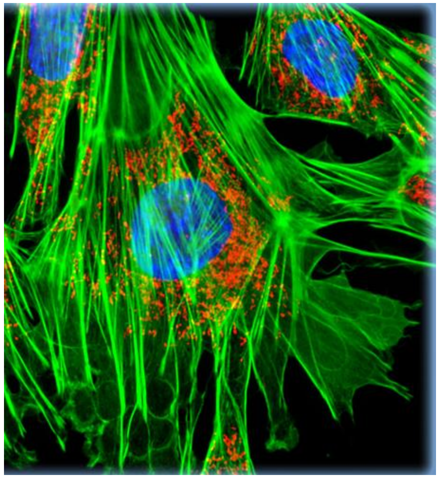
G-actin



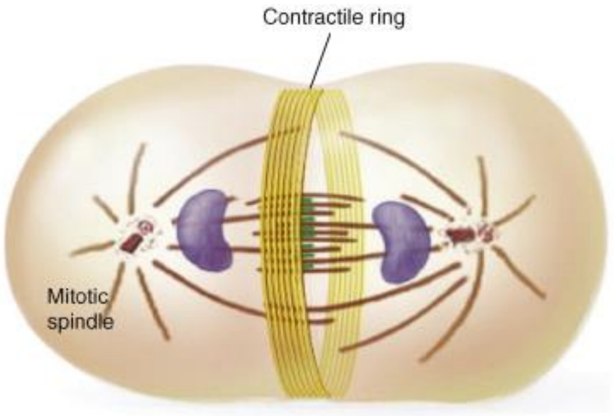
F-actin

Biological Functions of Actin Filament

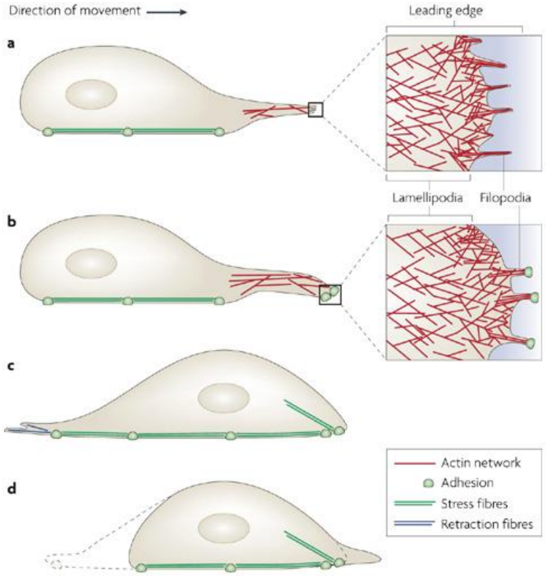
Cytoskeletal network



Cytokinesis: contractile ring

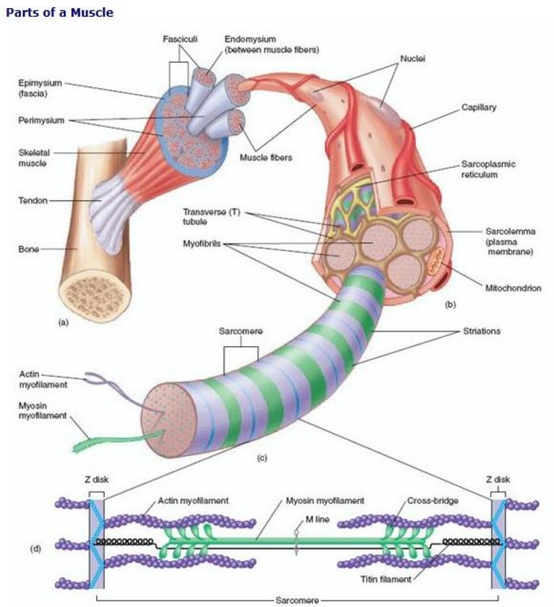


Cell motility

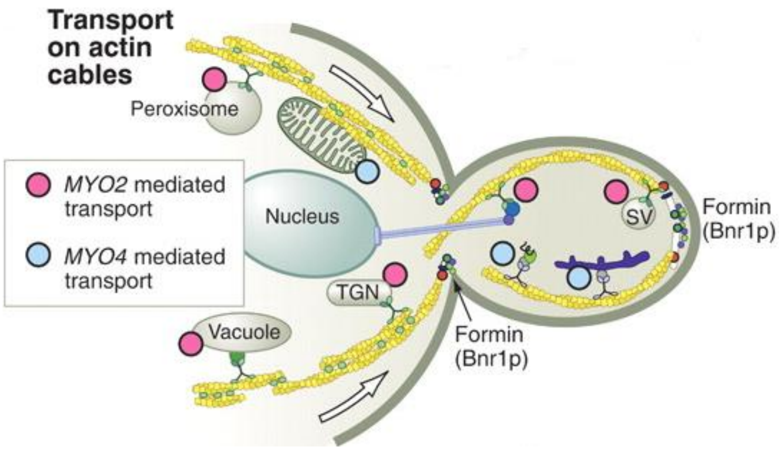


Nature Reviews | Molecular Cell Biology

Muscle contraction

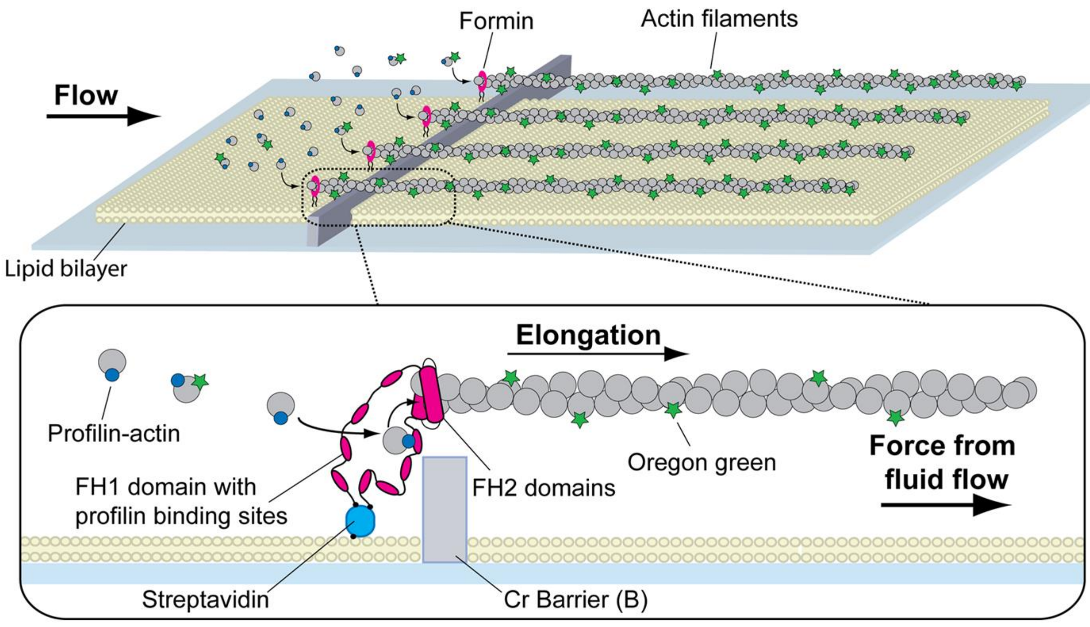


Cellular transport

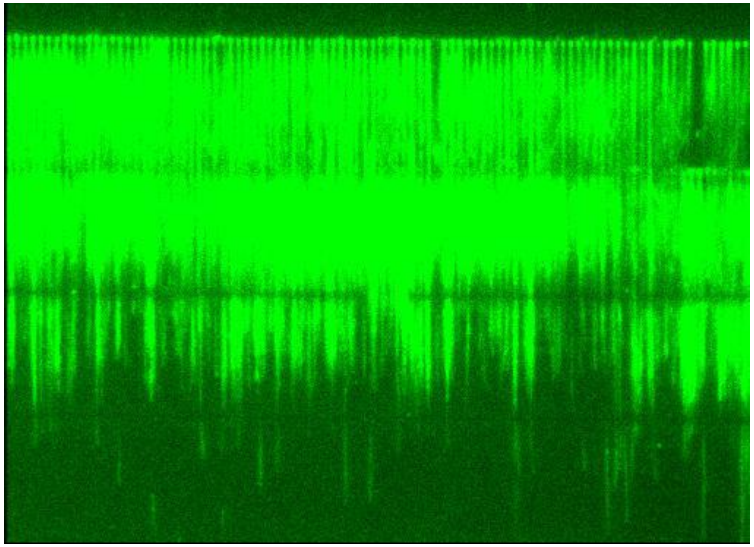


Actin Curtain

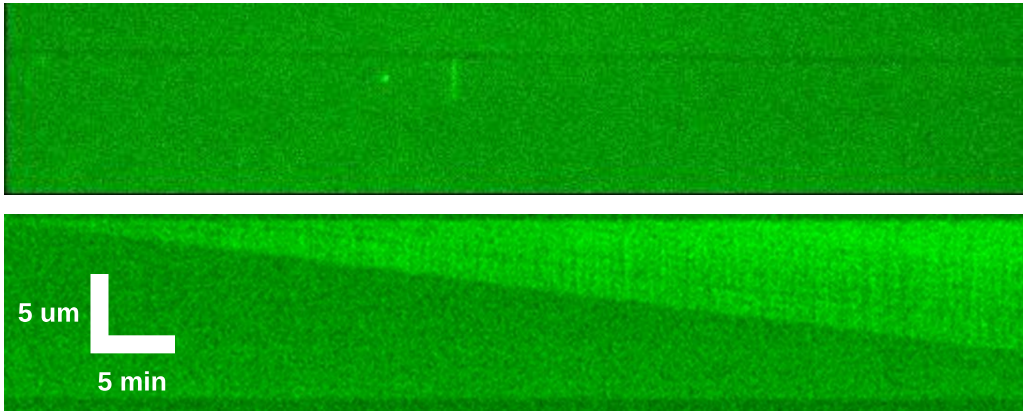
Schematics of actin curtain



Actin curtain



Polymerization of single actin filament



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Questions?