FtsK:
A “Formula 1” Molecular Motor

Or

Translocation, Rotation, Sequence Detection, and Protein Detection by a Single Enzyme

Omar A. Saleh

Materials Department and Biomolecular Science and Engineering Program, UCSB

FtsK: A ~~"Formula 1"~~ Nascar "Molecular Motor

Or

Translocation, Rotation, Sequence Detection, and Protein Detection by a Single Enzyme

Omar A. Saleh

Materials Department and Biomolecular Science and Engineering Program, UCSB

Acknowledgements

Primary collaboration:

Jean-François Allemand, ENS, Paris

François-Xavier Barre, CGM, Gif-sur-Yvette

Conversations and assistance:

Members of the Bensimon/Croquette lab

Sequence detection:

Sarah Bigot, CGM, Gif-sur-Yvette

François Cornet et al., LMGM, Toulouse

Outline: Four aspects of FtsK, ~15 minutes each

1. The Biology

   A primer in bacterial cell division

2. Linear translocation

   A bit of technique, a bunch of speed

3. Rotational motion

   Exploiting the physics of twisted DNA

4. Sequence detection

   Or how to stop FtsK

Outline

1. The Biology

   A primer in bacterial cell division
2. Linear translocation

   A bit of technique, a bunch of speed
3. Rotational motion

   Exploiting the physics of twisted DNA
4. Sequence detection

   Or how to stop FtsK

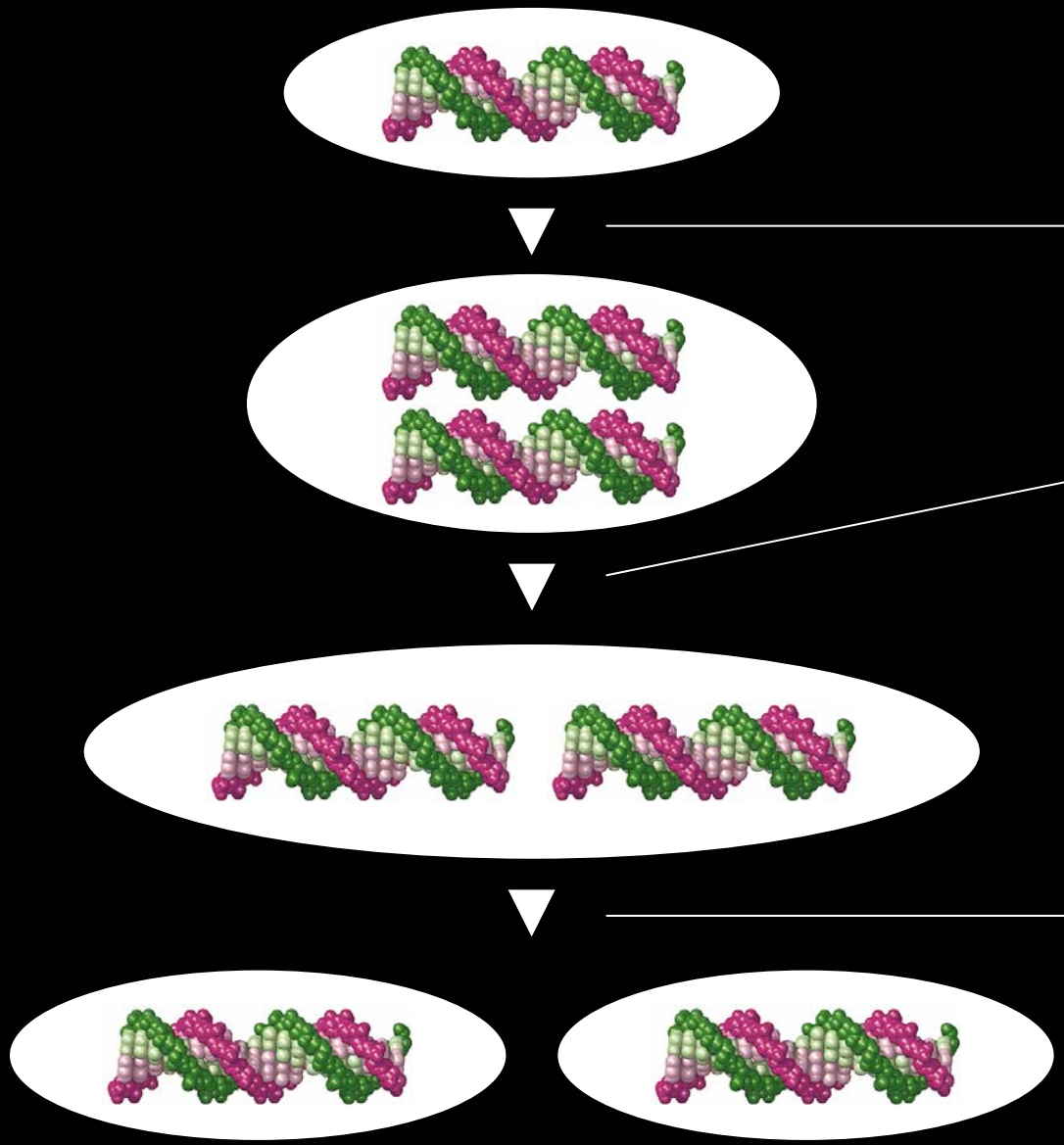
A biological question

Transmission of genetic material from generation to generation requires first that DNA is copied, then that it is physically transferred to the daughter cells

How do these things happen?

Largely through the efforts of motor proteins

The cell cycle relies on motor proteins



DNA replication:
Helicases, DNA polymerase

DNA segregation:
Kinesins, dynein, FtsK

Cell division:
Myosin, kinesins

Details of the cell cycle vary among the three kingdoms

Eukarya:

Replication/segregation/division separated

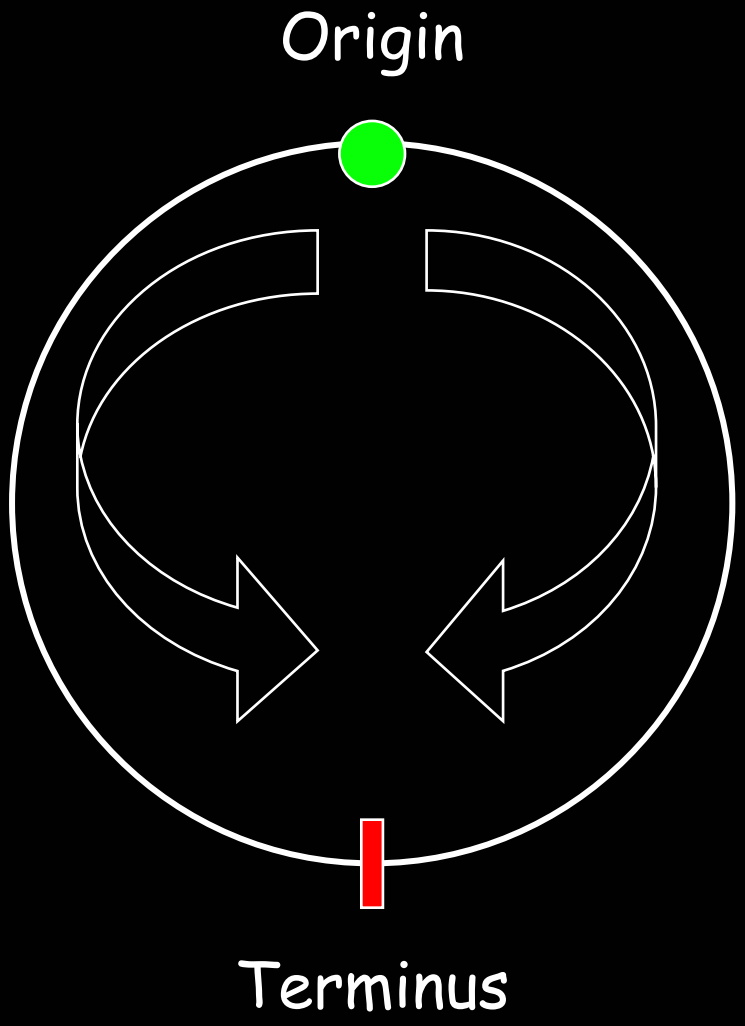
Bacteria:

Replication/segregation/division simultaneous

Archaea:

A mixture of eukarya and bacteria

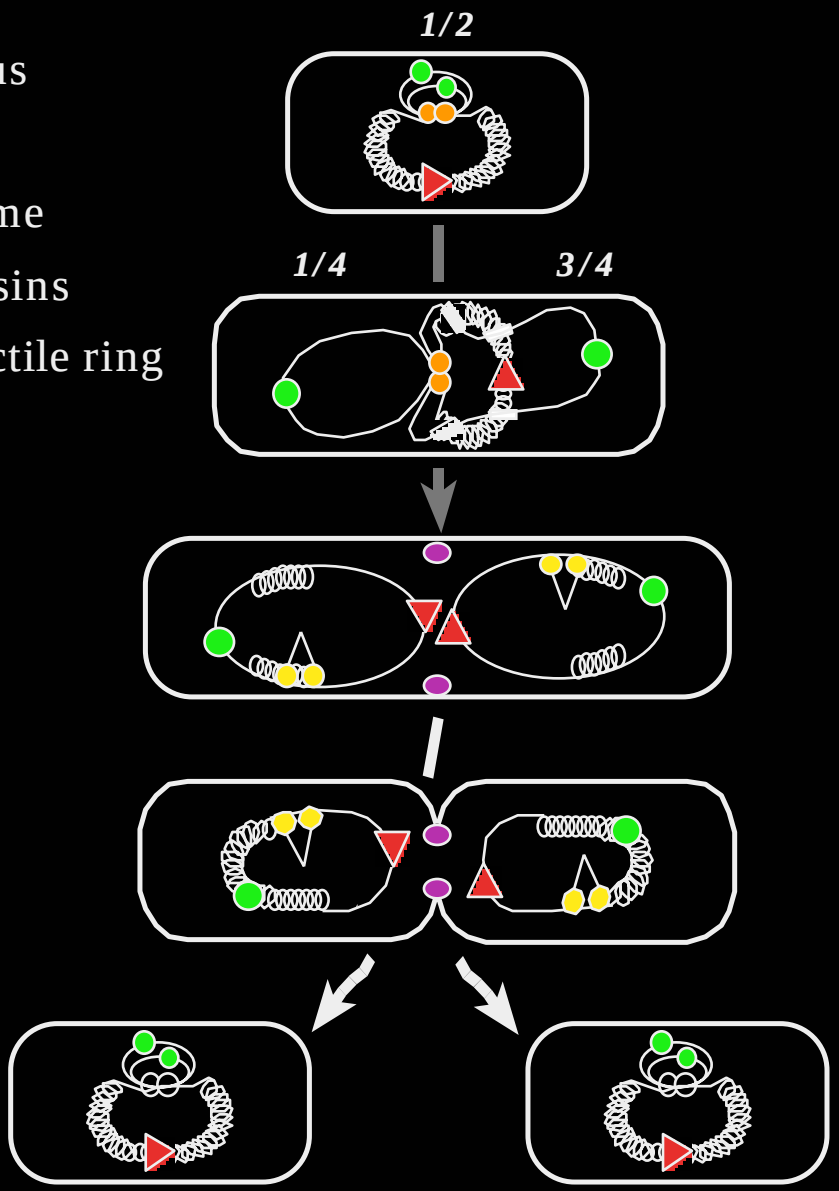
E. coli has a single, circular chromosome



- 4.5 million base pairs (compare ~3 Gbp in humans); contour length ~1.5 mm inside a ~1 μm cell
- Replication begins at a unique origin, proceeds bidirectionally to a terminus

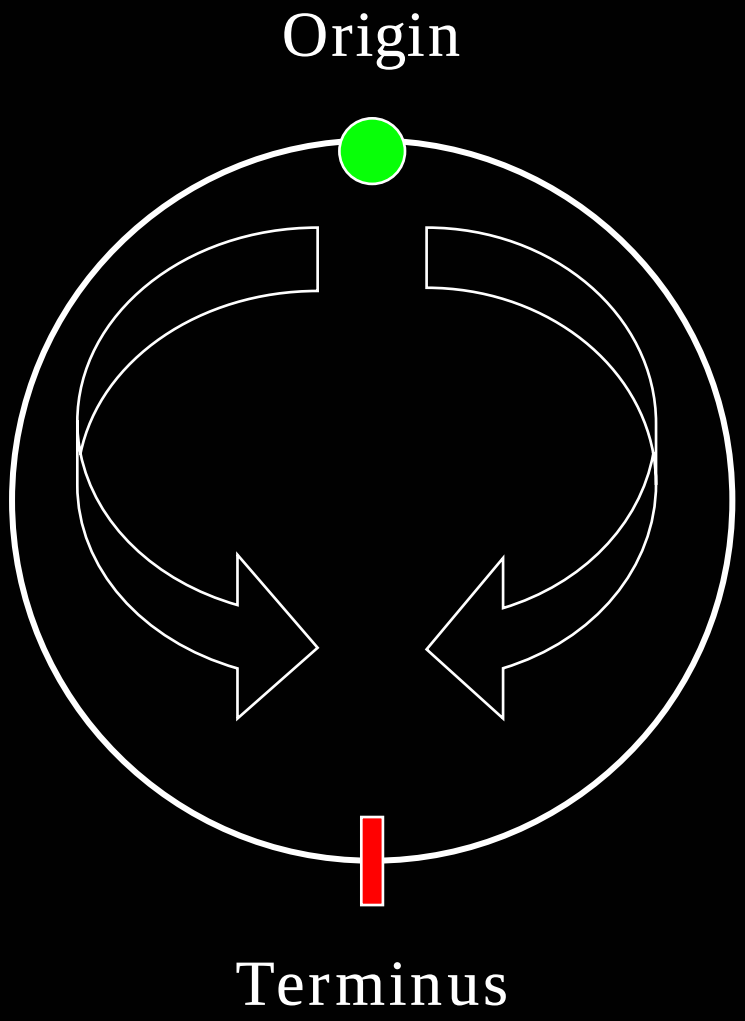
The *E. coli* cell cycle (idealized)

terminus
origin
replisome
condensins
contractile ring



1. Replication begins
2. Origins moved to poles
3. DNA condensed
4. Ring contraction forms septum
5. Cell divides

Timing of *E. coli* cell division



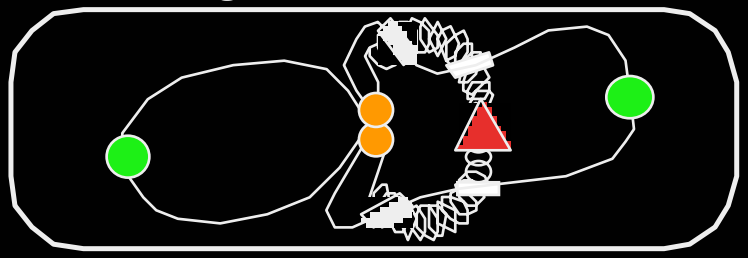
It takes ~40 minutes to replicate the chromosome, but *E. coli* can divide every 20 minutes!

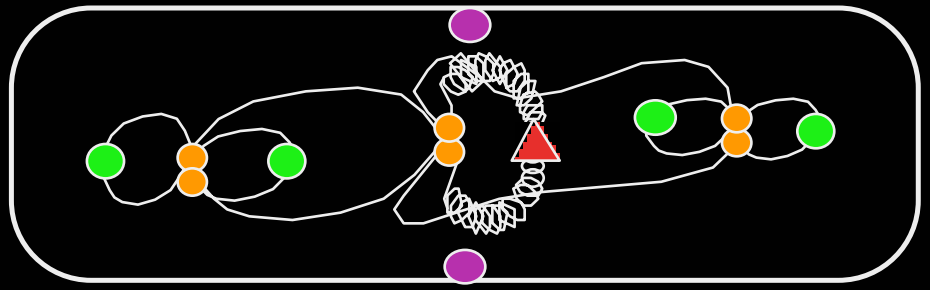
How?

►Multiple rounds of replication can be active simultaneously

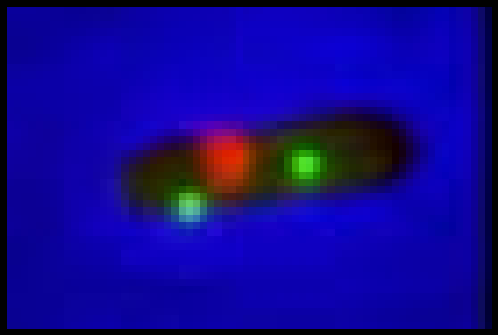
The messy reality: Everything happens at once

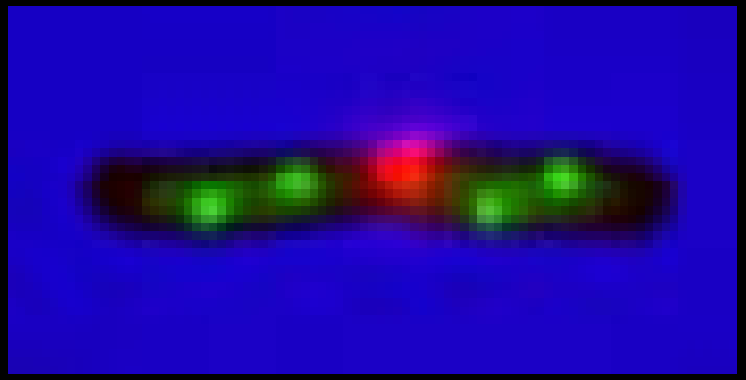
terminus
origin
replisome
contractile ring





Green fluor: origin
Red fluor: terminus





Images from Sherratt lab

How is this coordination achieved?

Apparently through sequential mid-cell positioning of the origin, replication complexes, and the cell division plane...

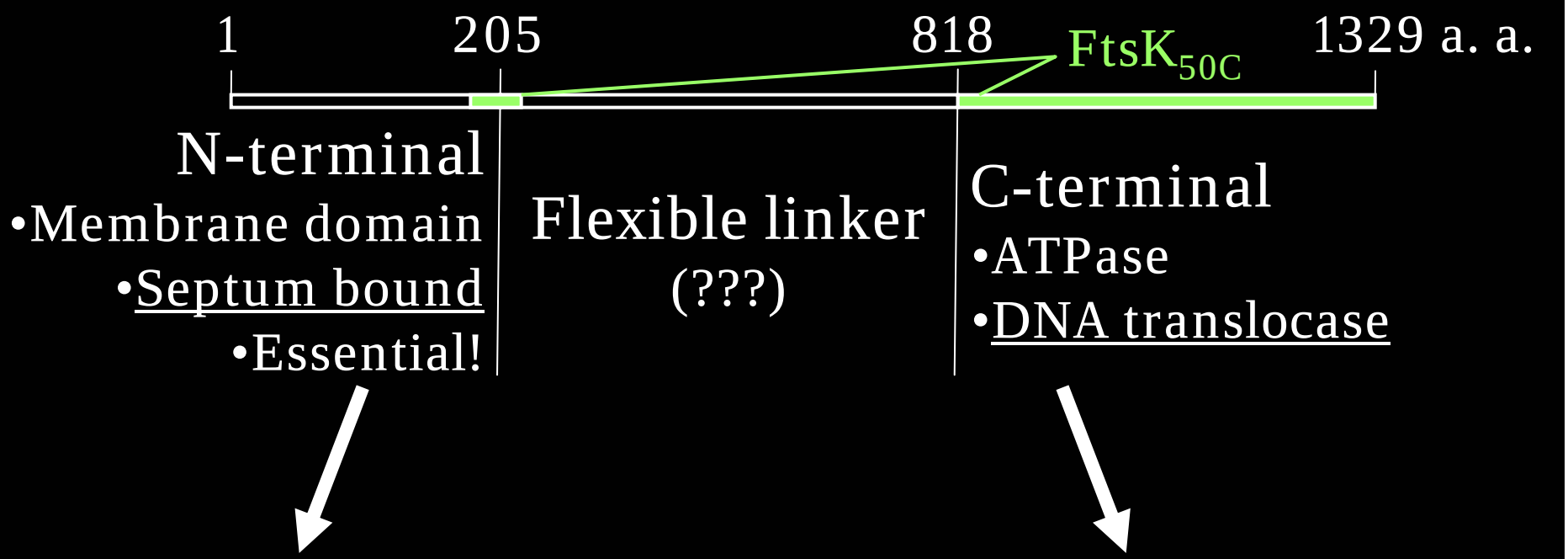
What happens if something goes wrong?

FtsK to the rescue!

What is FtsK?

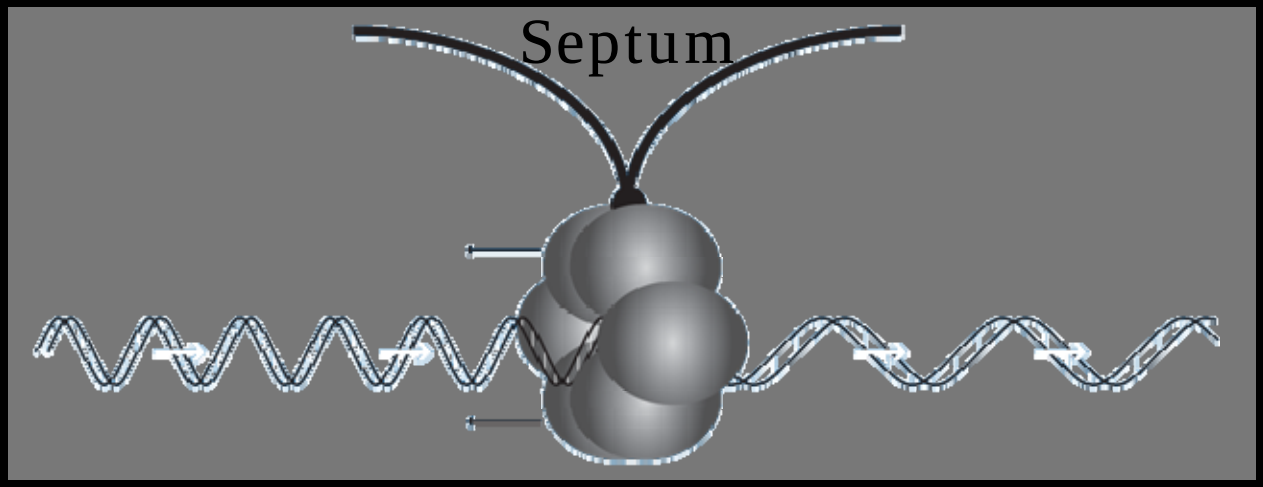
A protein, present in nearly all bacteria, that helps coordinate the cell cycle by physically linking cell division to chromosome segregation

FtsK's structure:
Two proteins in one!



$FtsK_C$ is a motor domain that forms DNA-threaded hexameric rings

$FtsK_N$ is a membrane domain localized to the septum, and essential for cell division



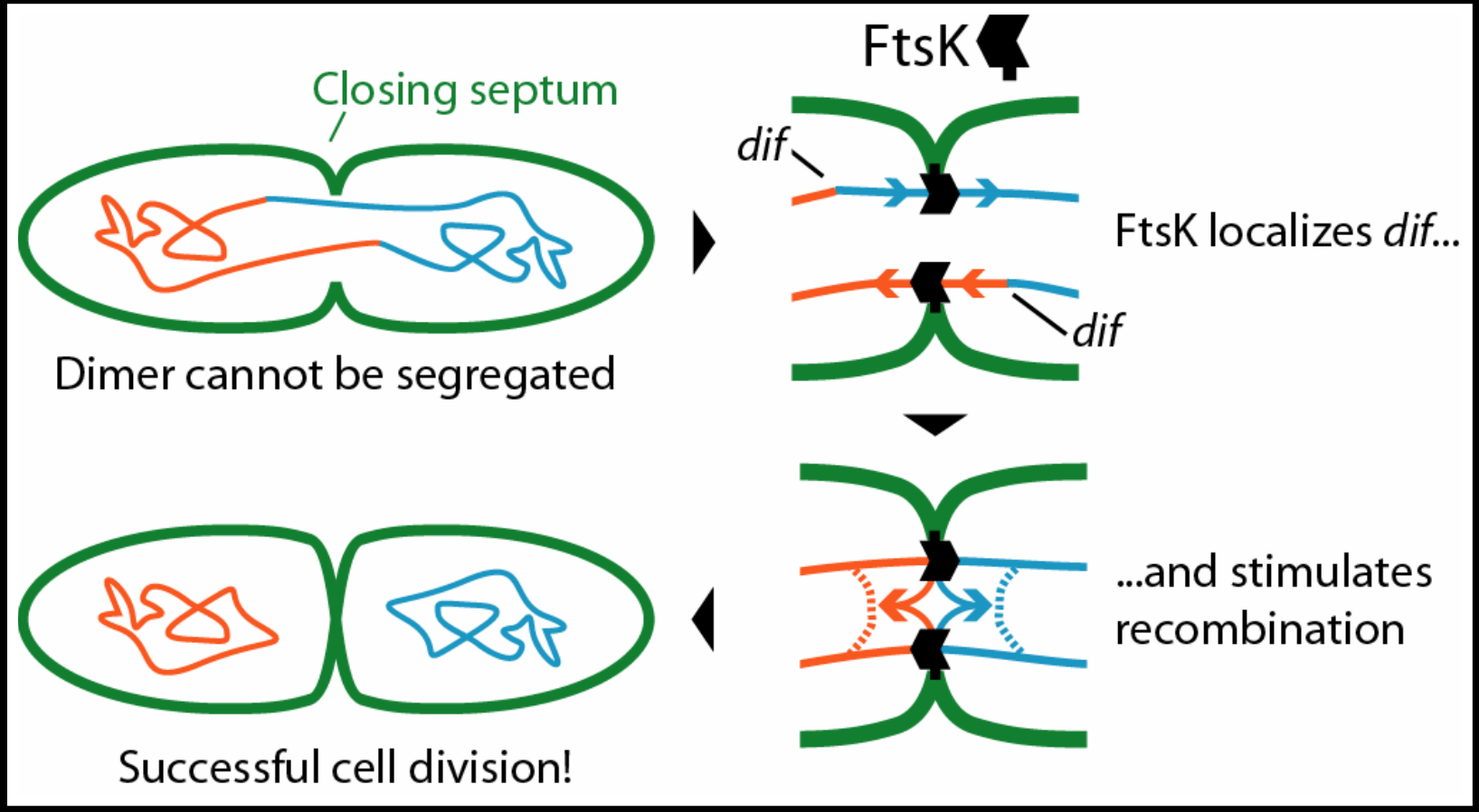
Hexameric enzymes (frequently substrate-threaded) are common to the AAA+ family, with roles in DNA replication, protein folding/unfolding/degradation, membrane fusion, etc.

See Vale (2000); Ogura and Wilkinson (2001)

$FtsK_C$'s *in vivo* activity: Pumping DNA in order to correct chromosome-segregation errors

1. $FtsK_C$ positions, then helps resolve, chromosome dimers
2. $FtsK_C$ appears to clear misaligned chromosomes from the closing septum

Chromosome dimer resolution



- Recombination at *dif* is carried out by XerC/D, but only in the presence of $FtsK_C$
- How does it get to *dif*?

  In vivo expts suggested DNA sequence

Outline

1. The Biology

   A primer in bacterial cell division
2. Linear translocation

   A bit of technique, a bunch of speed
3. Rotational motion

   Exploiting the physics of twisted DNA
4. Sequence detection

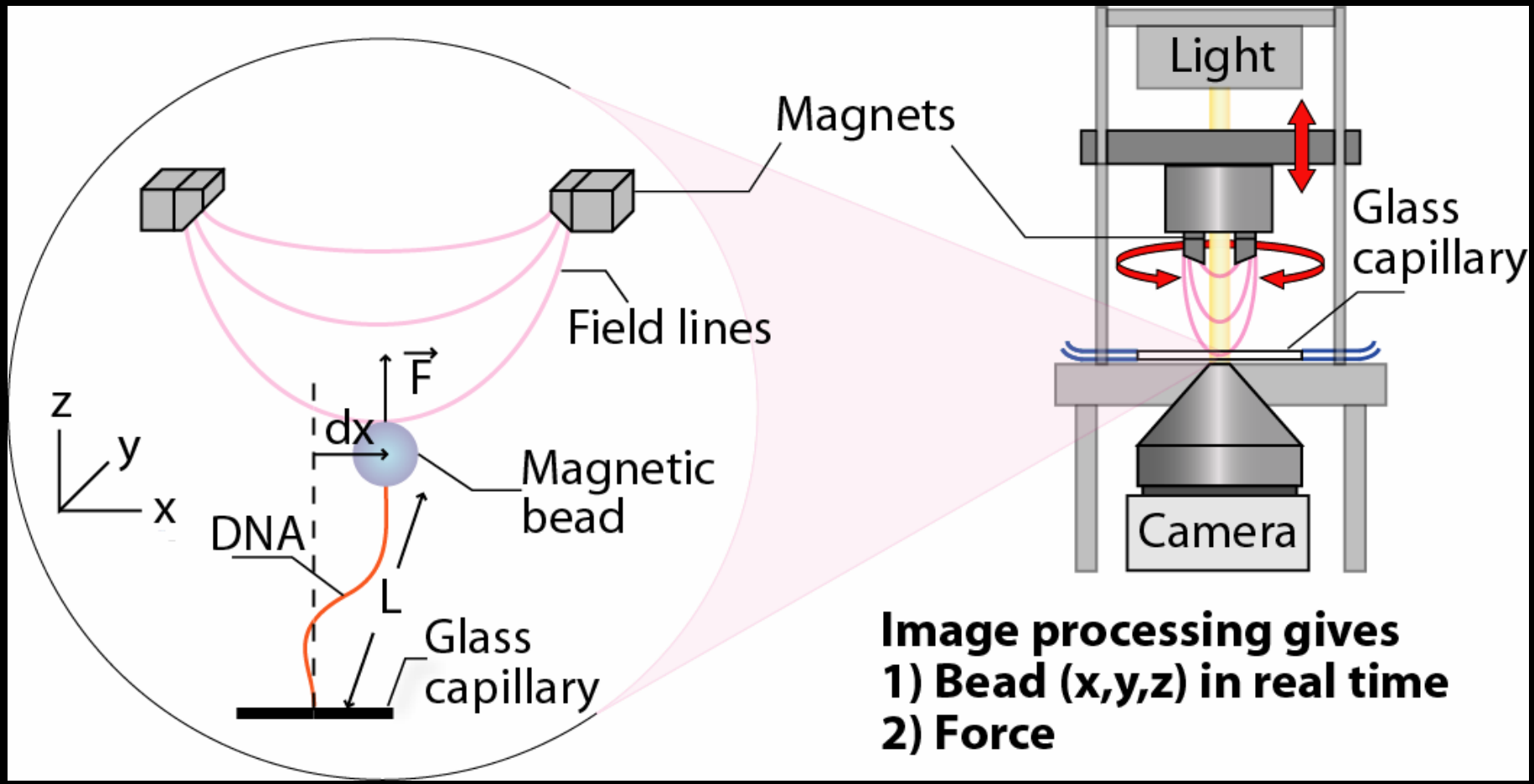
   Or how to stop FtsK

Our plan

Using direct and quantitative single molecule techniques, characterize FtsK as a motor protein (how far? how fast? how does it move? etc.)

How? Use magnetic tweezers to isolate and manipulate a single DNA molecule

The magnetic tweezer

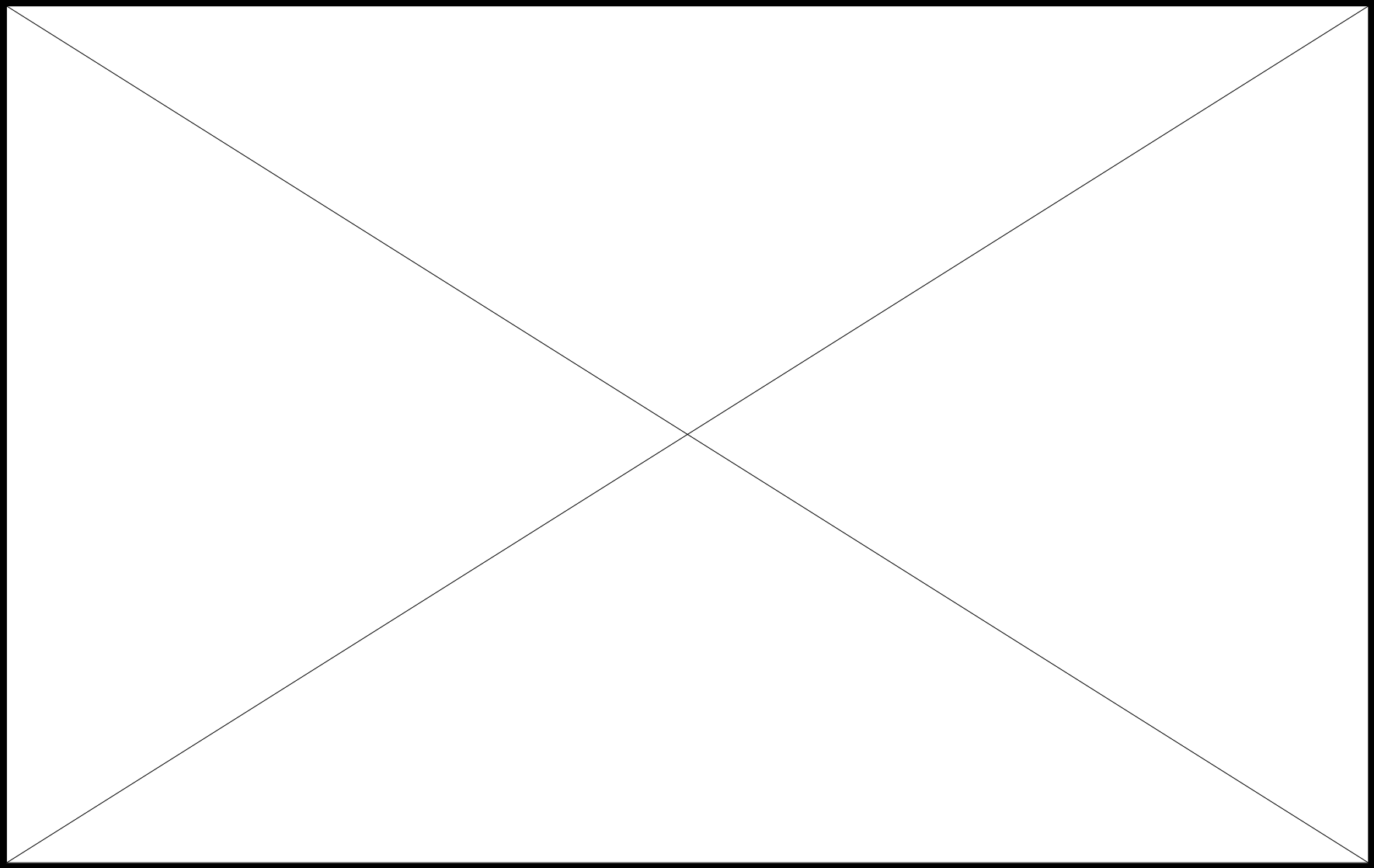


Experimental output: DNA length vs. time

Tracking the 3D bead position gives us:

1. The end-to-end extension of a single DNA molecule vs. time- so we can study any protein that affects extension
2. The force stretching that DNA molecule

Measuring the force

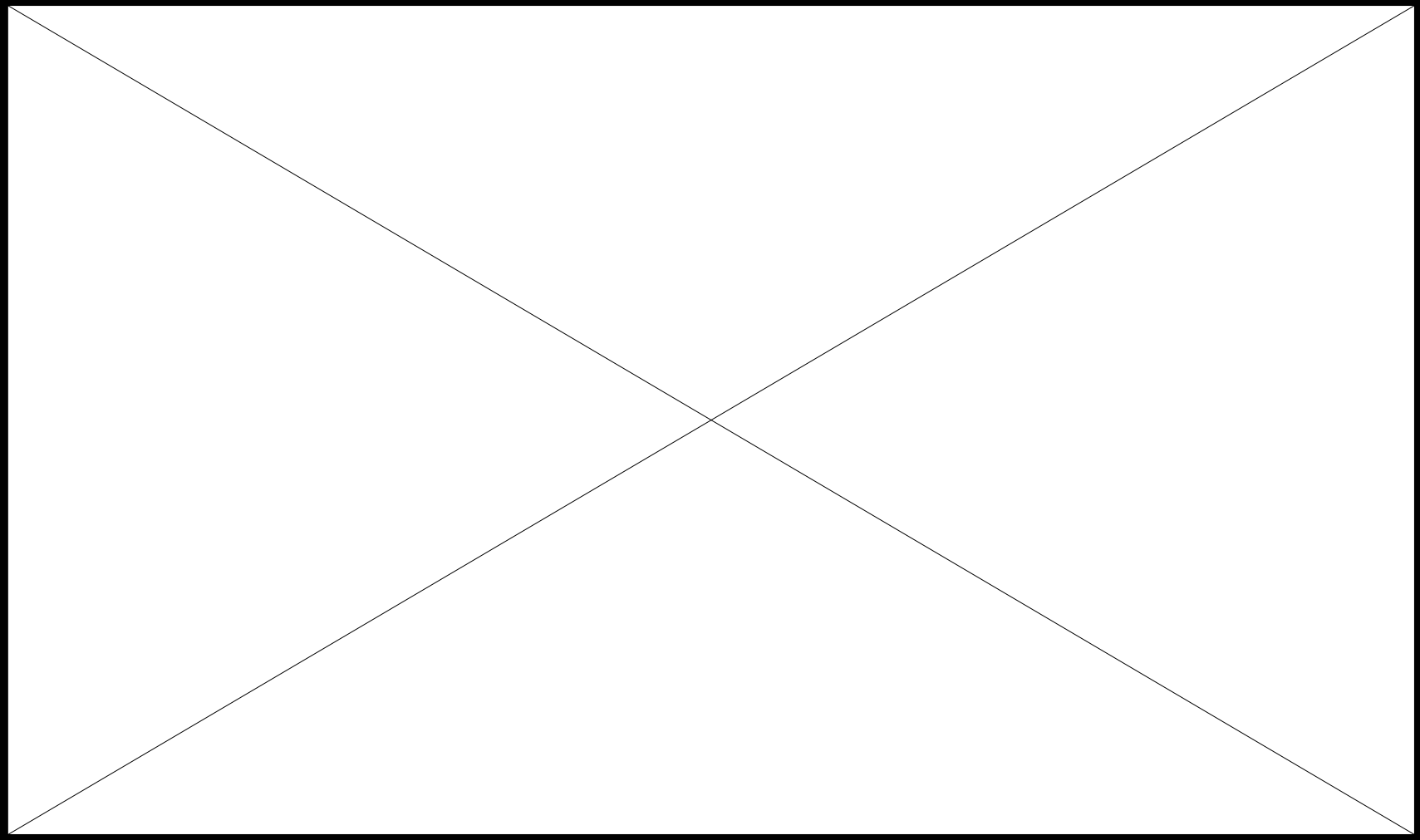


Animation by G. Charvin

Back to FtsK

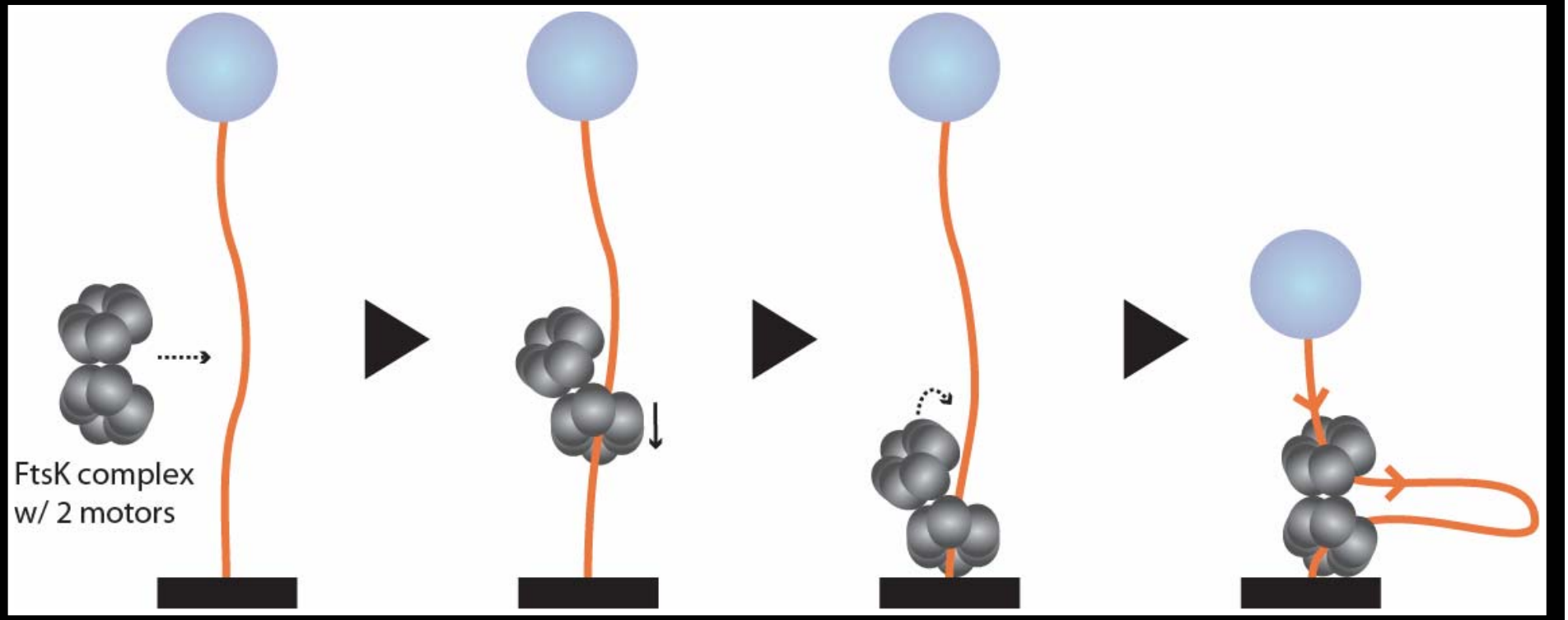
Our basic experiment: Set up a bead/DNA complex, add $FtsK_{50C}$ and ATP, and watch what happens

The activity of FtsK on nicked DNA



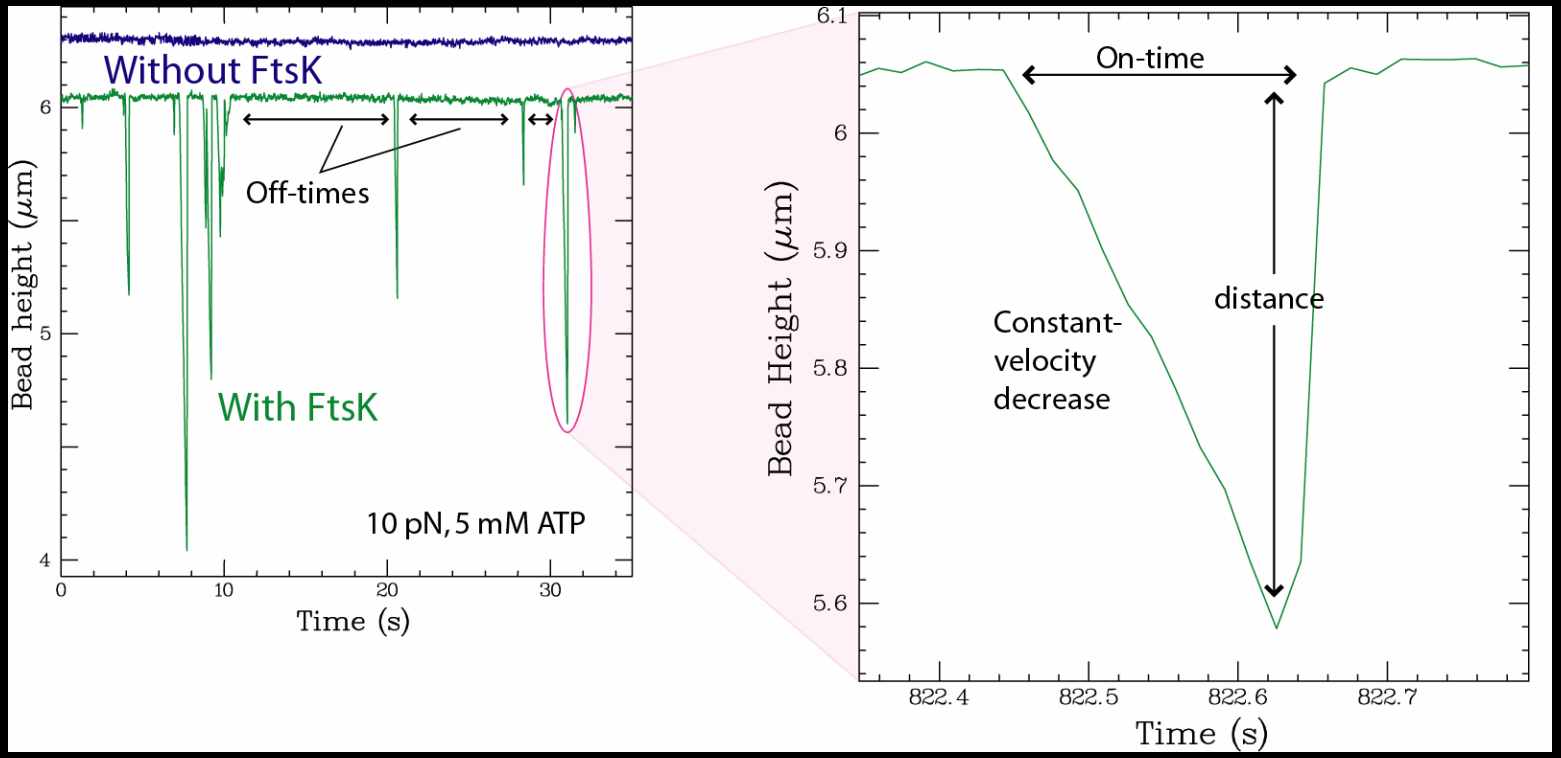
Animation by J.-F. Allemand

Events are due to loop-extrusion by a single motor



Loop extrusion can occur from either extremity, or from specific DNA sequences

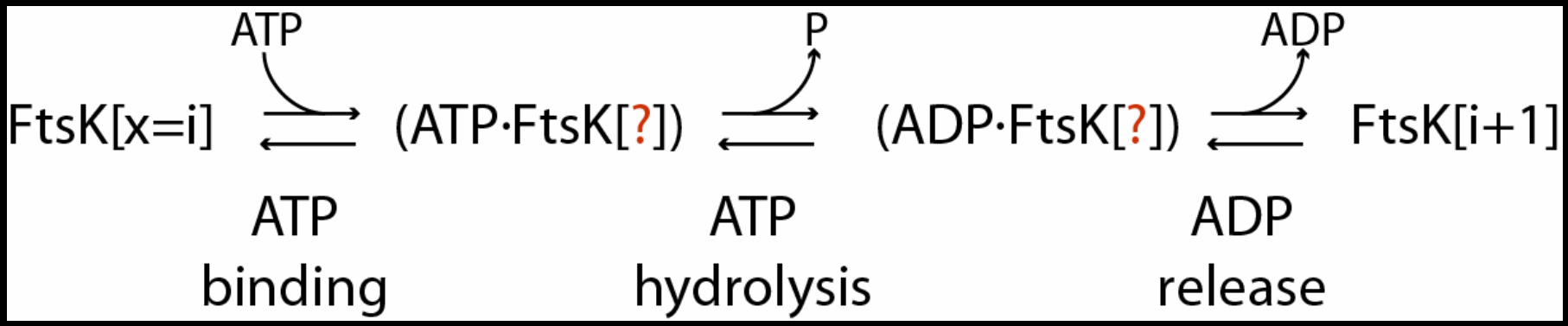
Clean events + one motor = many ways to study FtsK translocation



- Force and/or ATP dependence of velocity, on-times, off-times, distances
- Noisy stepping
- Rotational motion
- Sequence dependence

Details:
Saleh et al, EMBO Journal (2004)
Saleh et al, Nat Struct Mol Bio (2005)
Bigot et al, EMBO Journal (2005)

Mechanochemistry: The biochemical framework of translocation



Minimal enzymatic cycle

ATP binding, hydrolysis, release; leading to one forward step of motion

How does it vary with ATP?

Velocity vs. ATP

- Follows Michaelis-Menten kinetics:

$$V = V_{max} \frac{[ATP]}{[ATP] + K_M}$$

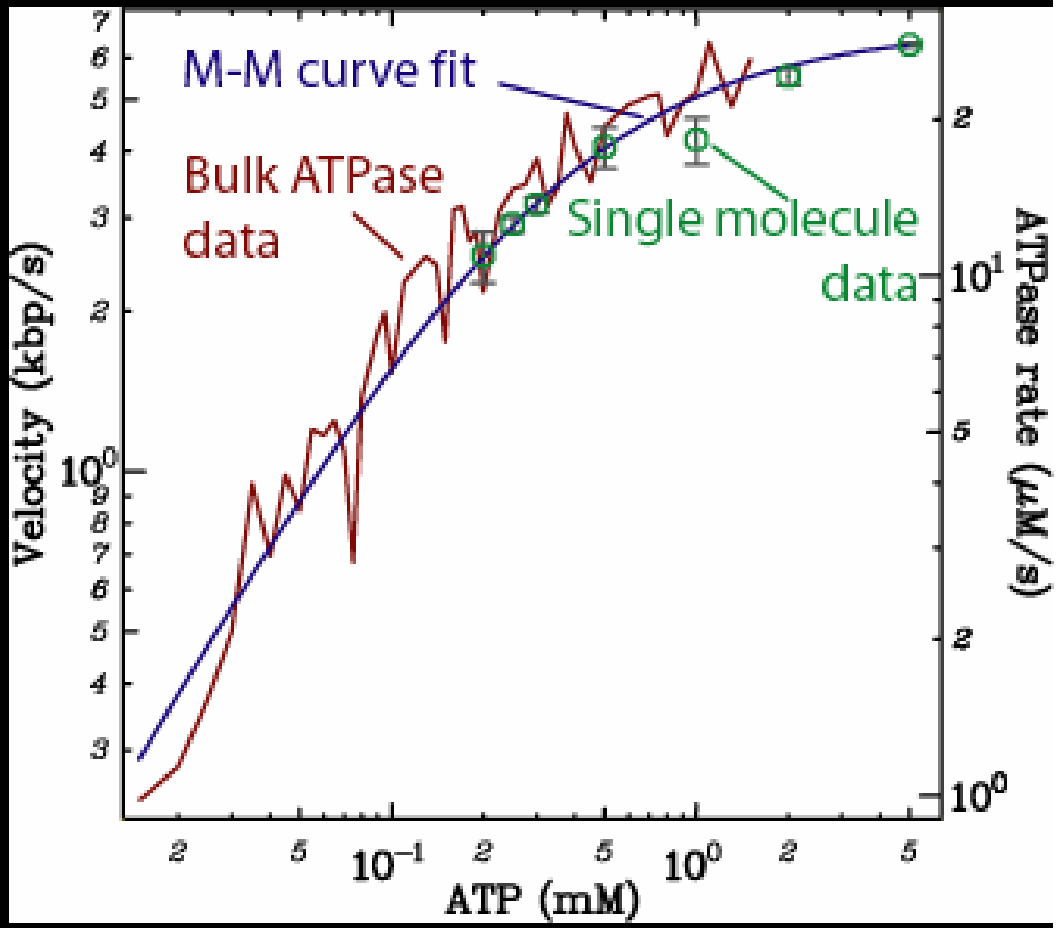
V_{max} = 6.7 kbp/s,

K_M = 0.3 mM

- Incredibly fast:

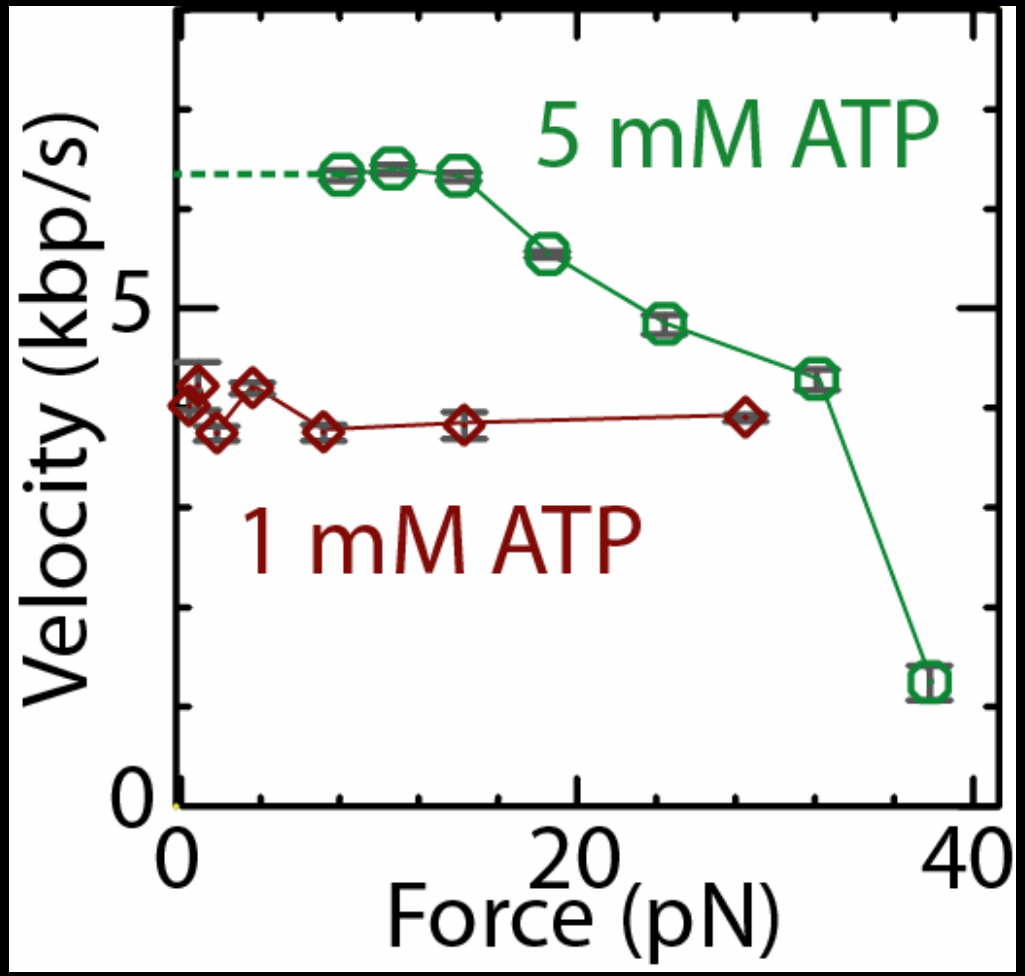
7 kbp/s is fastest known for a DNA motor

...but why?



5 pN force

Force/velocity curves



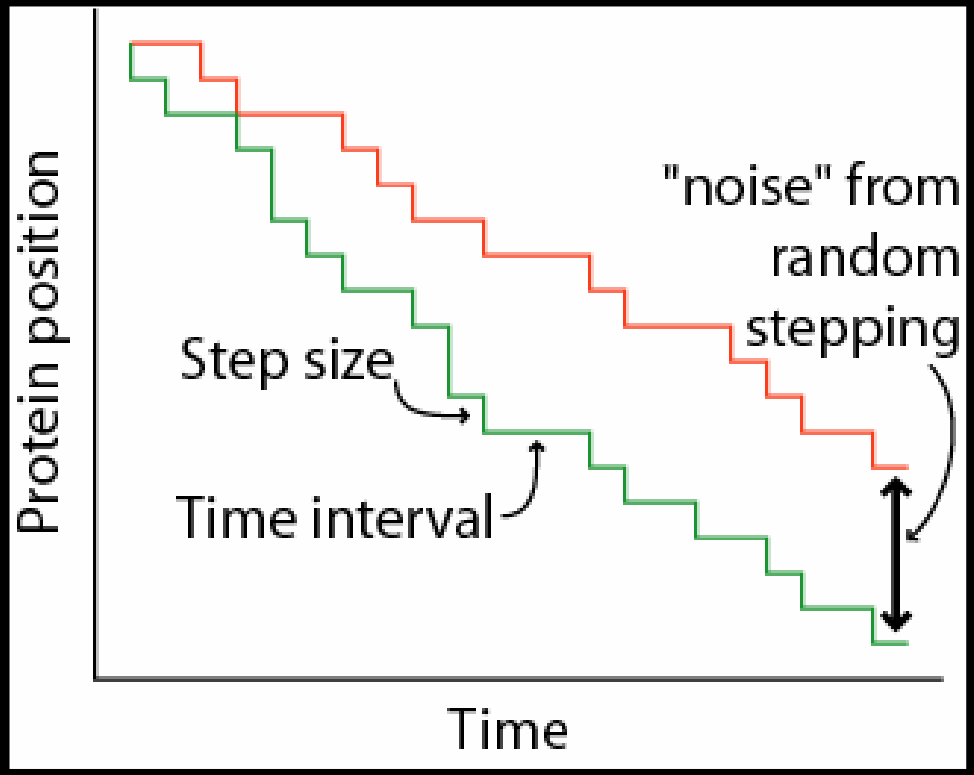
- 0 < F < 17 pN: Velocity constant
- F > 17 pN: slows; 'stall' near 40 pN
- F < 0: anomalous!

How does it go so fast-
big step or fast turnover?

We want to know the size of the translocation step...but we see no discrete steps in the traces. Is there any other way to estimate the step size?

►Use the measurement noise!

A motor's random stepping increases the measured noise

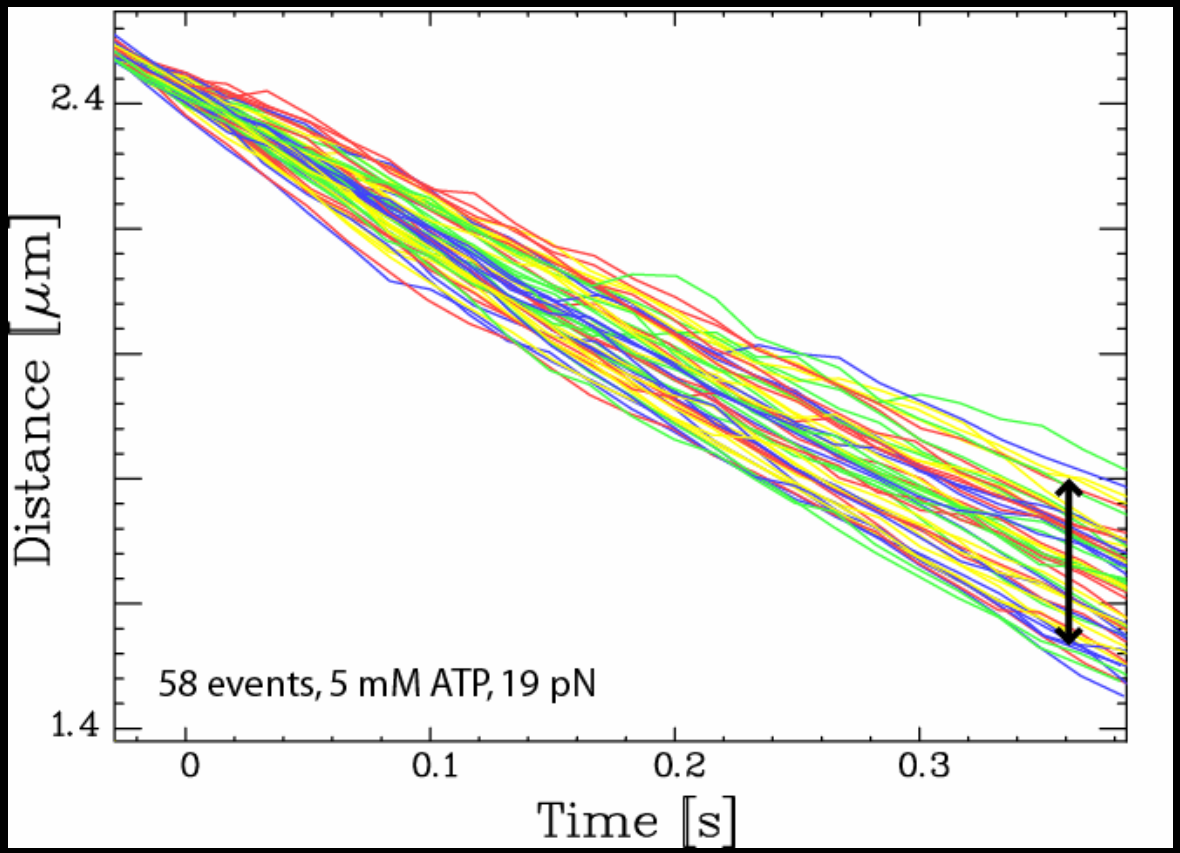


Sketch of (hypothetical) position fluctuation from identical motors

- Motor proteins advance through discrete steps, separated by random time intervals
- This randomness causes a fluctuation in position, analogous to a shot noise

Svoboda *et al*, *PNAS* **91**, 11782 (1994)

Measured traces: no steps, but there is noise

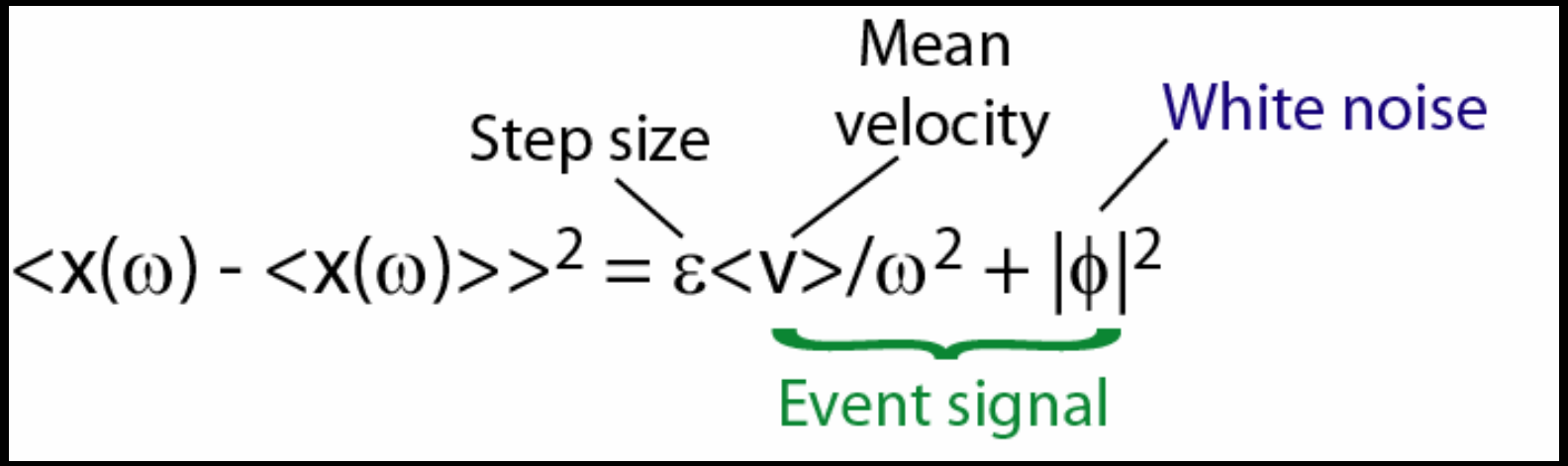


Expt. resolution does not allow direct msmt of steps, but noise is visible!

How to separate stepping noise from expt noise?

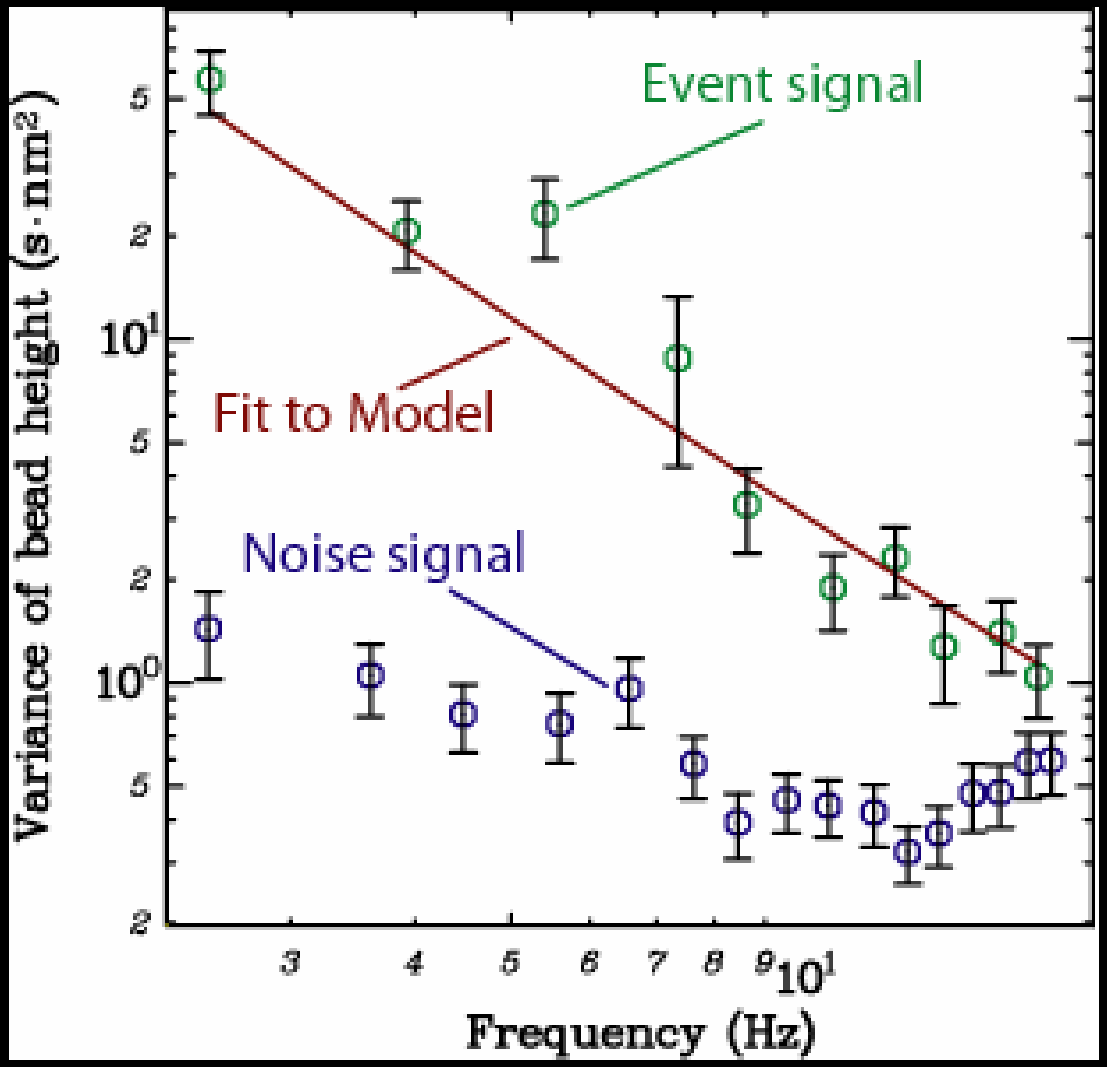
Power spectra separate noise sources

Stepping noise varies as $1/f^2$, while expt noise (Brownian motion of the bead) is white:



Charvin *et al*, *Single Molecules* **3** 43 (2002)

Fluctuation analysis



FtsK step = 12 ± 2 bp
(assuming one rate-determining step)

Each spectrum from 26 segments
5 mM ATP, 17.5 pN

12 bp (~ 4 nm) is a big step for a DNA motor

Energetic implication

- FtsK works up to ~40 pN
- Energy/step: 4 nm step × 40 pN load = 160 pN nm
- 1 ATP gives 80-100 pN nm, so FtsK uses ≥ 2 ATP/cycle

Structural implication

- DNA helical pitch ~10.5 bp; near to FtsK step...
- How does FtsK interact with DNA?
- Look at rotational characteristics

Outline

1. The Biology

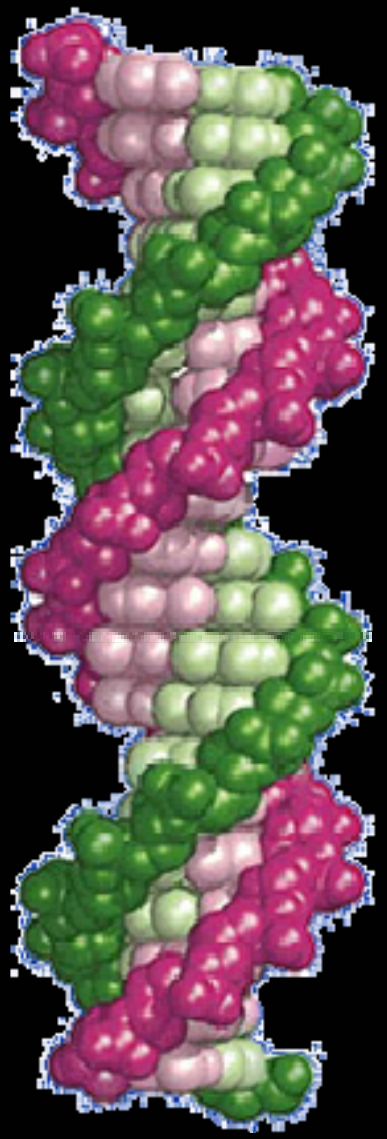
   A primer in bacterial cell division
2. Linear translocation

   A bit of technique, a bunch of speed
3. Rotational motion

   Exploiting the physics of twisted DNA
4. Sequence detection

   Or how to stop FtsK

FtsK and the double helix

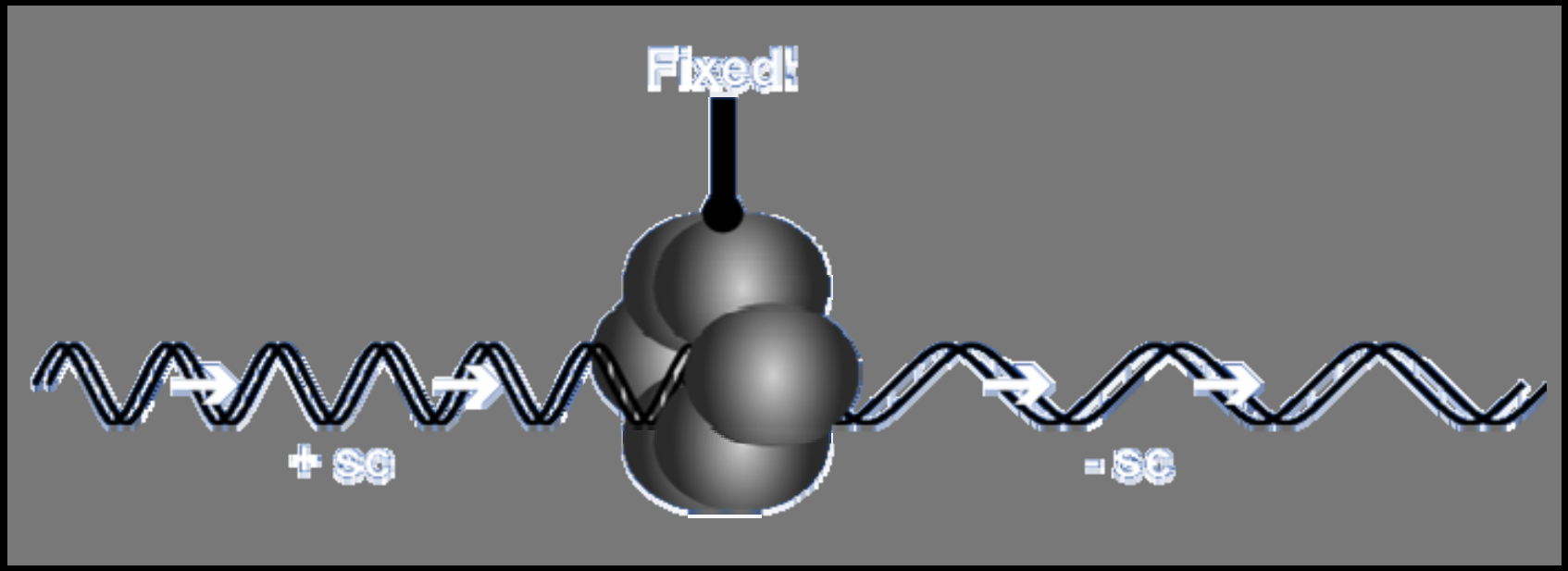


- DNA-based motor proteins tend to rotate while moving linearly due to DNA's twisted structure
- Many motors track DNA's groove (like a nut on a bolt), thus rotate one turn per pitch traveled

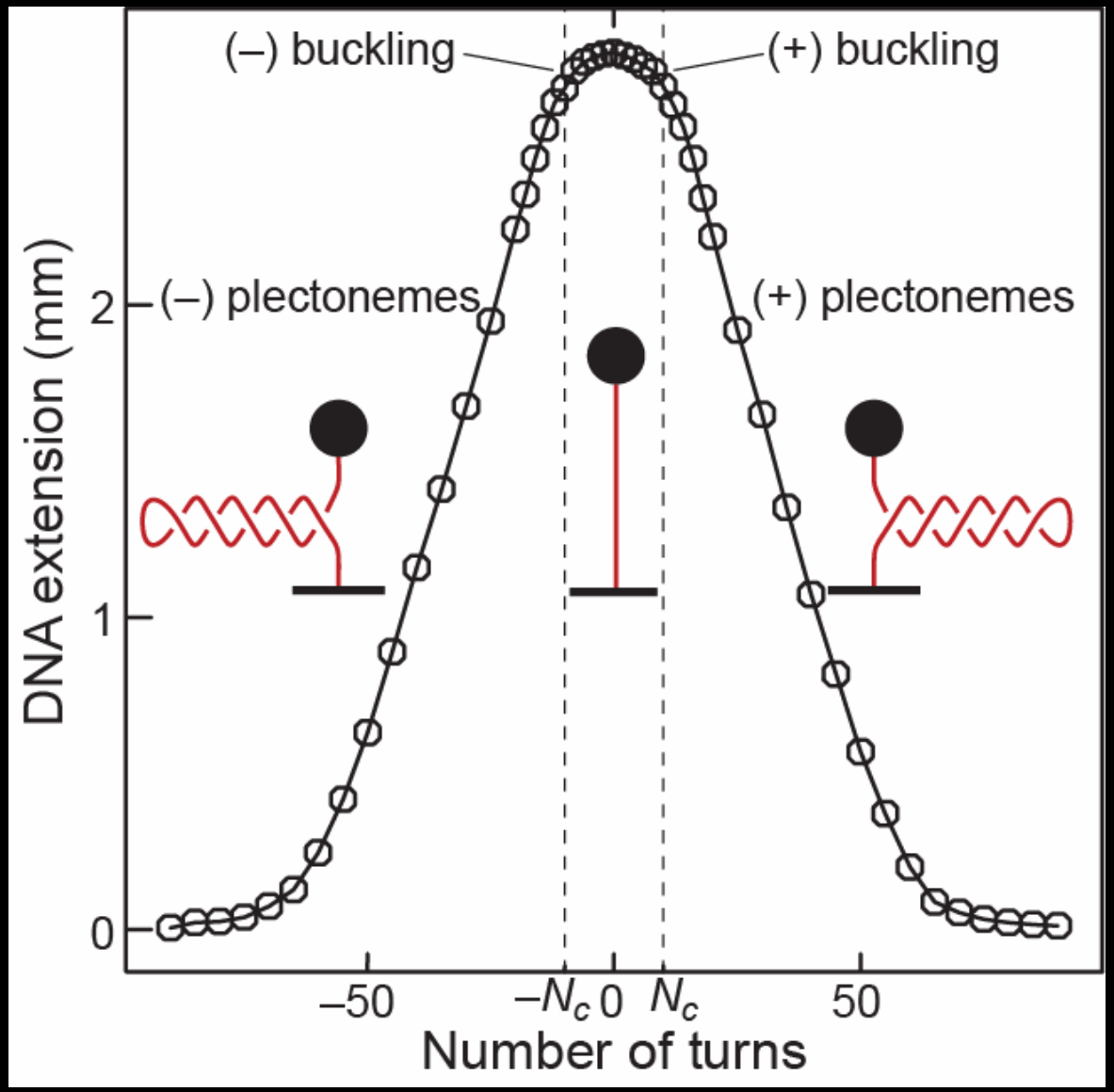
What does FtsK do?

How to measure? Invert the problem

Rather than directly look for FtsK rotation, fix the protein (already done!) and look for the effects of twist in the DNA



What happens when you twist DNA?

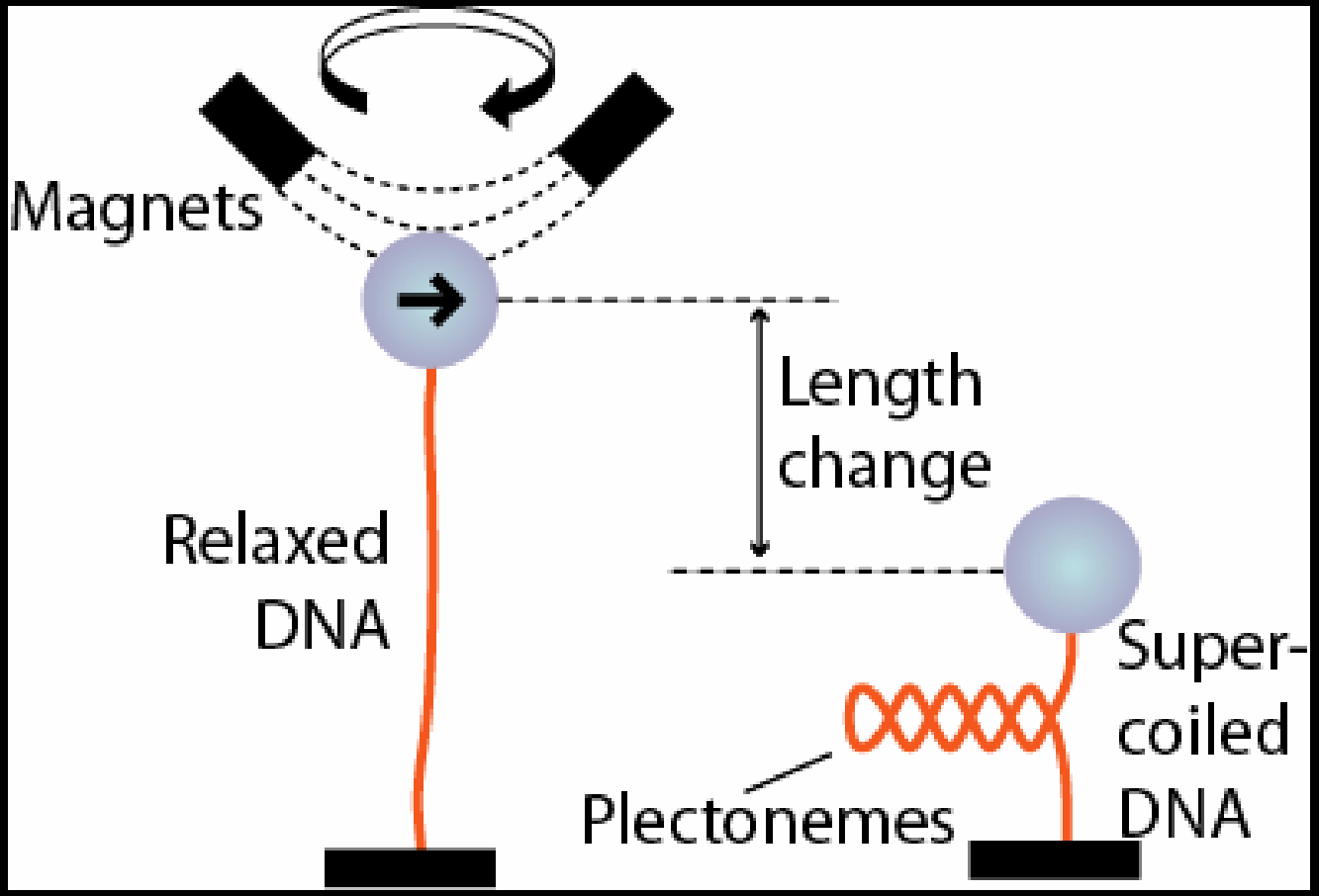


This occurs at low (<0.5 pN) stretching forces; more exotic things occur at higher forces

Twisted DNA is very important: It is the natural state *in vivo* ("supercoiled")

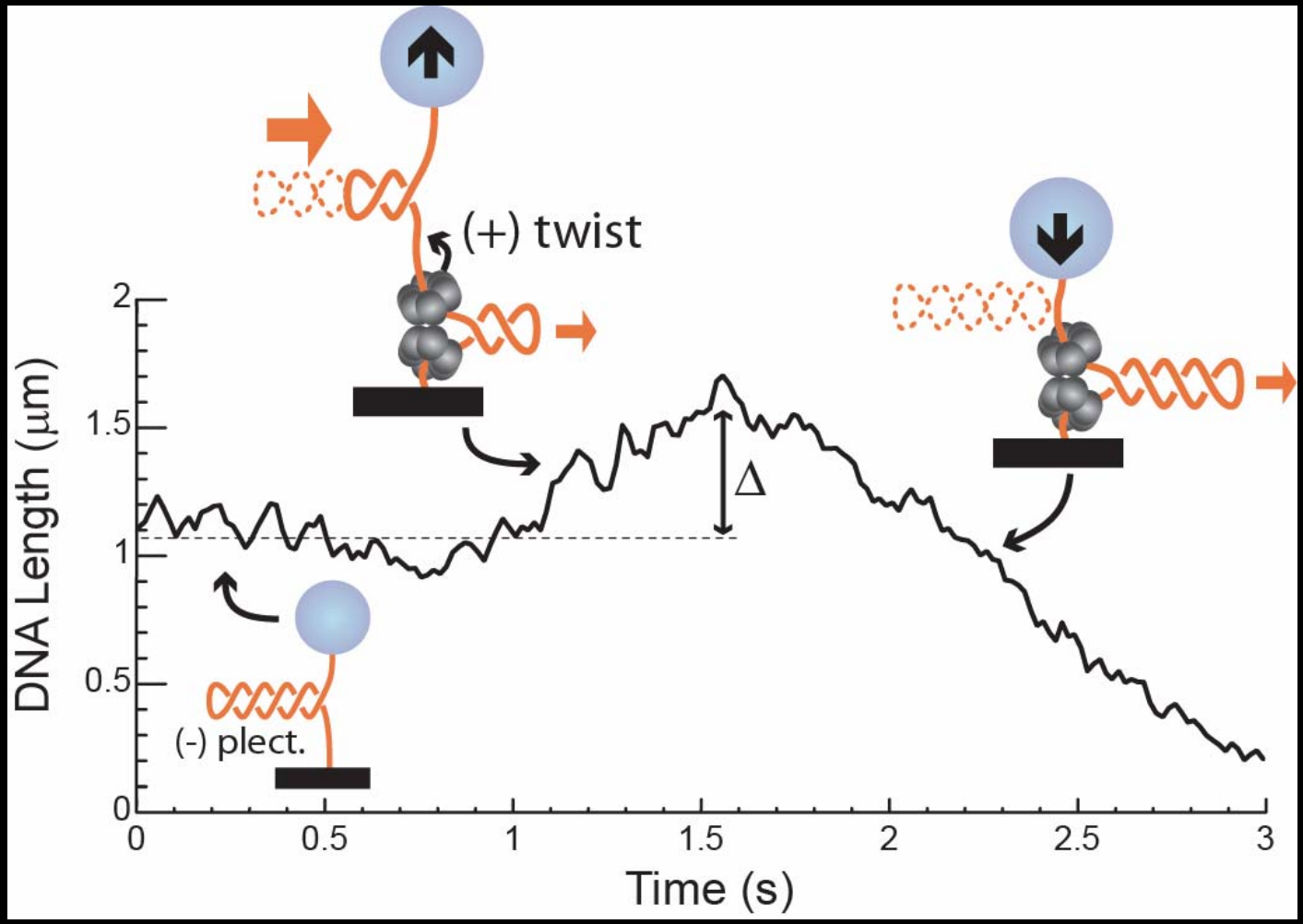
Good review of physics of twisted DNA: Charvin et al (2004)

Twist added by FtsK can add/remove plectonemes



Plectonemes act as transducers, coupling DNA's supercoiled state to its (measurable!) extension.

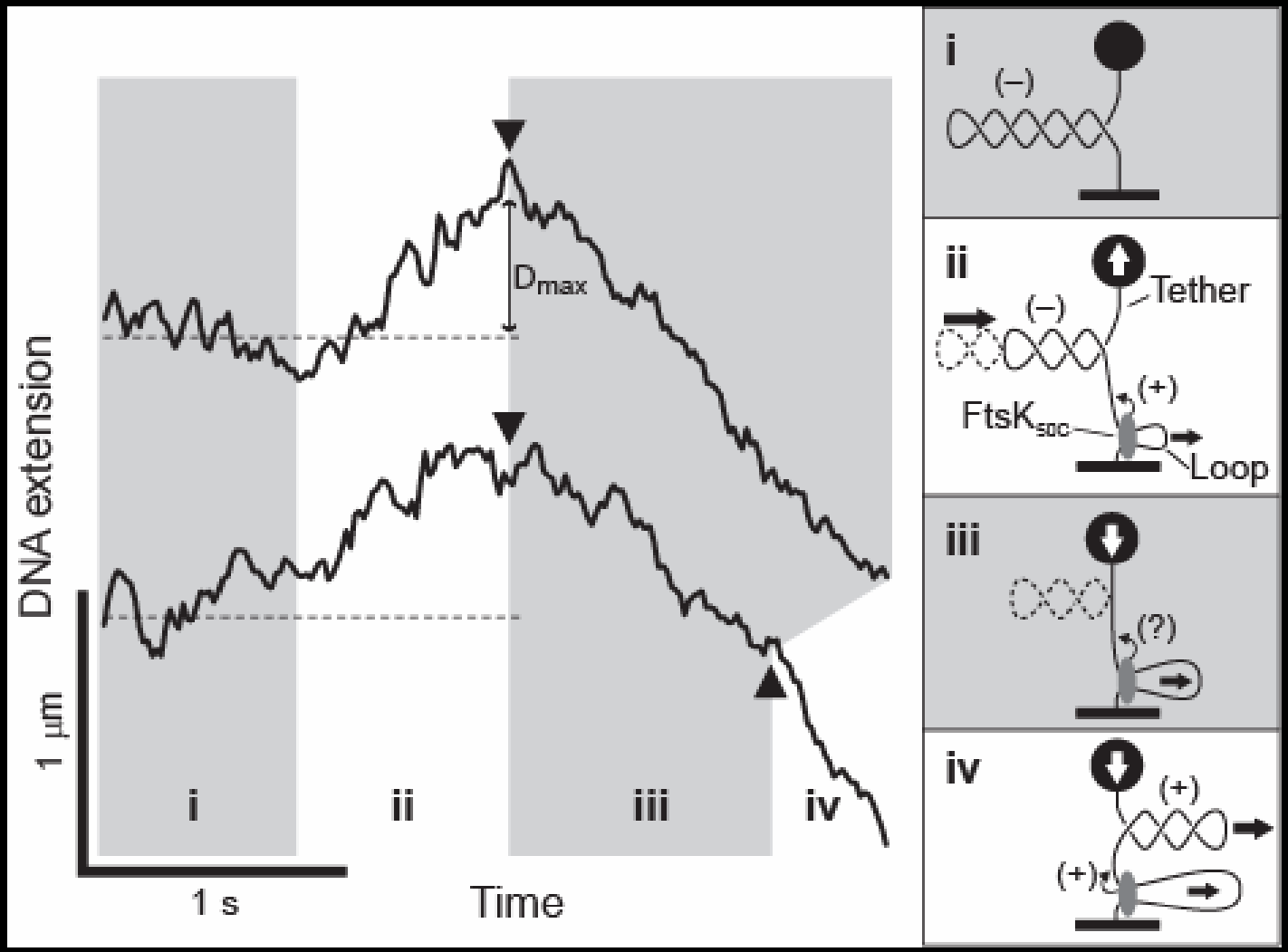
Supercoil Induction part I: Supercoil polarity



Height increases (by Δ) upon protein activity-

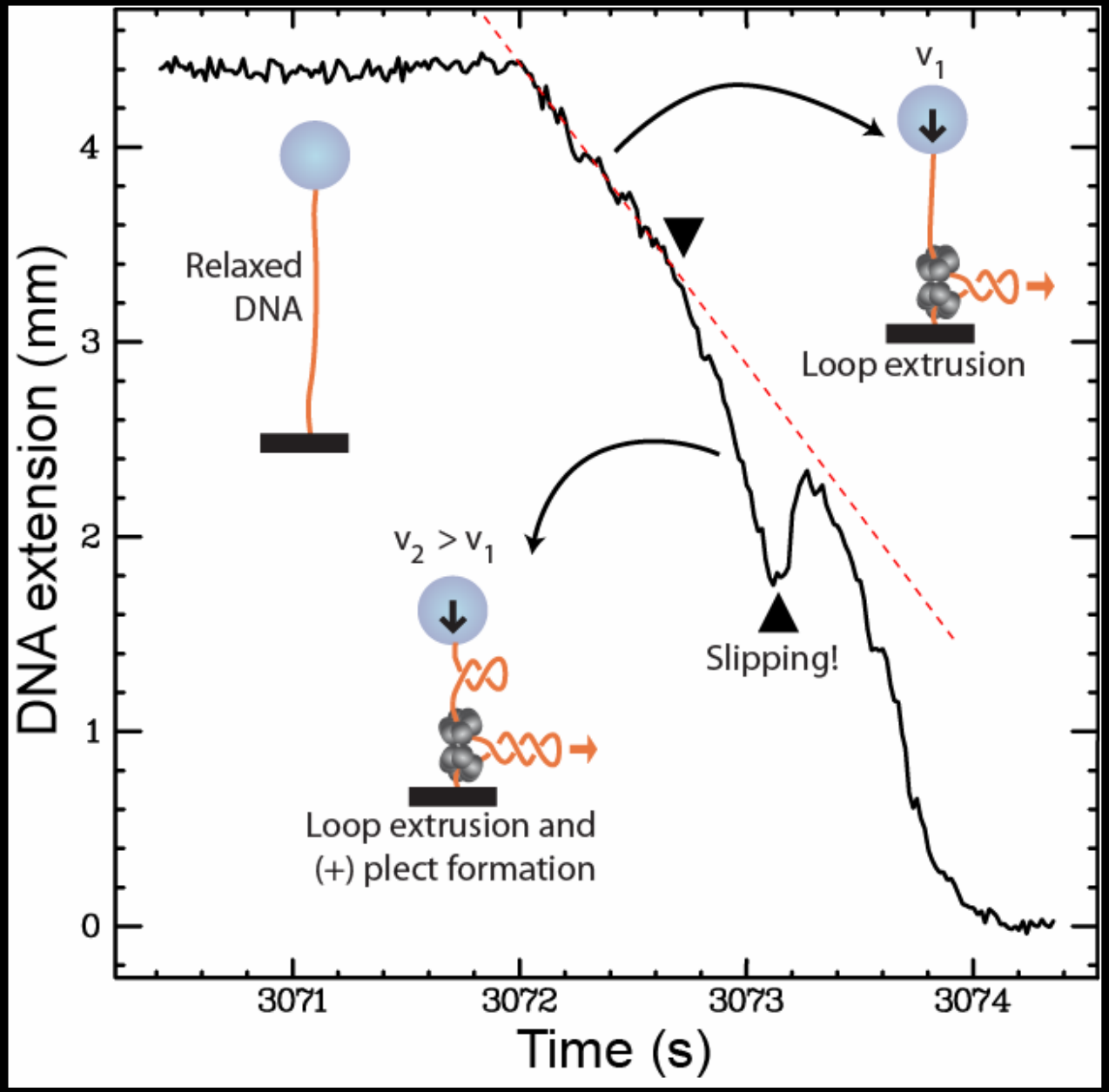
clear signal of (+) sc induction in advance of FtsK

Supercoil Induction part II: Slipping



The 2nd buckling transition is inconsistent,
unlike the 1st transition

Discrete slipping



Can look for (+) buckling transition at higher forces (better resolution)

Usually: it buckles or it doesn't (as before)

Rarely (2-3 events out of ~ 100 total): A discrete slip is seen

The slip might be entirely rotational

Analysis: Concentrate on consistent behavior on (-) sc DNA (FtsK's *in vivo* substrate)

Assuming FtsK induces a constant number of supercoils per distance traveled, we can easily convert the increase in height Δ (or the velocity before/after buckling) to a rate of sc induction.

Answer: FtsK adds 0.07 (<< 1!) sc/pitch traveled, thus it does not track DNA's groove (first known example)

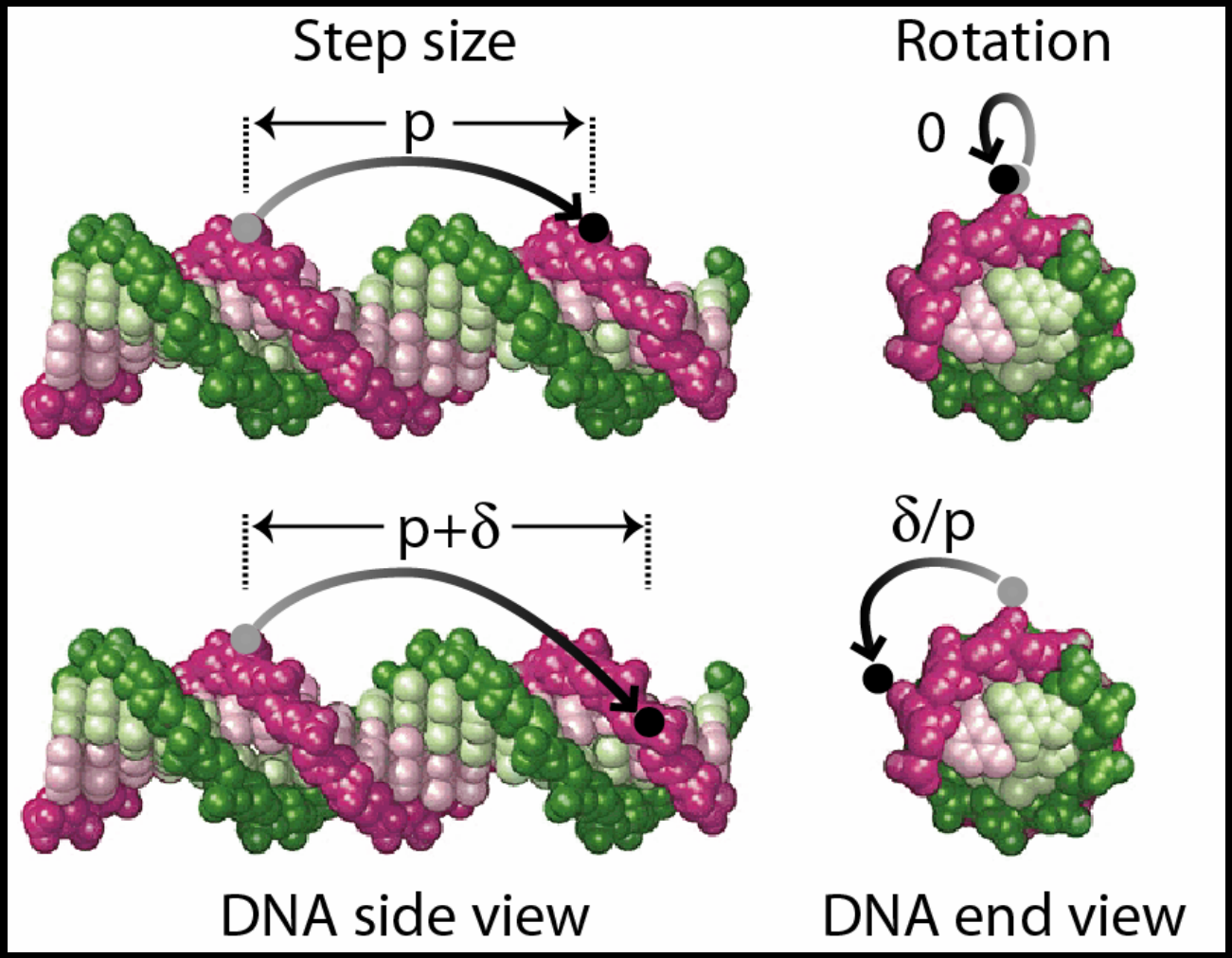
Why such a low rate?

Cells tightly control chromosomal sc density. Groove tracking plus 7 kbp/s would add ~ 700 sc/s into the chromsome, more than even the replication fork. 0.07 sc/pitch thus reduces the topological perturbation.

Given this rate, how does FtsK interact with DNA?

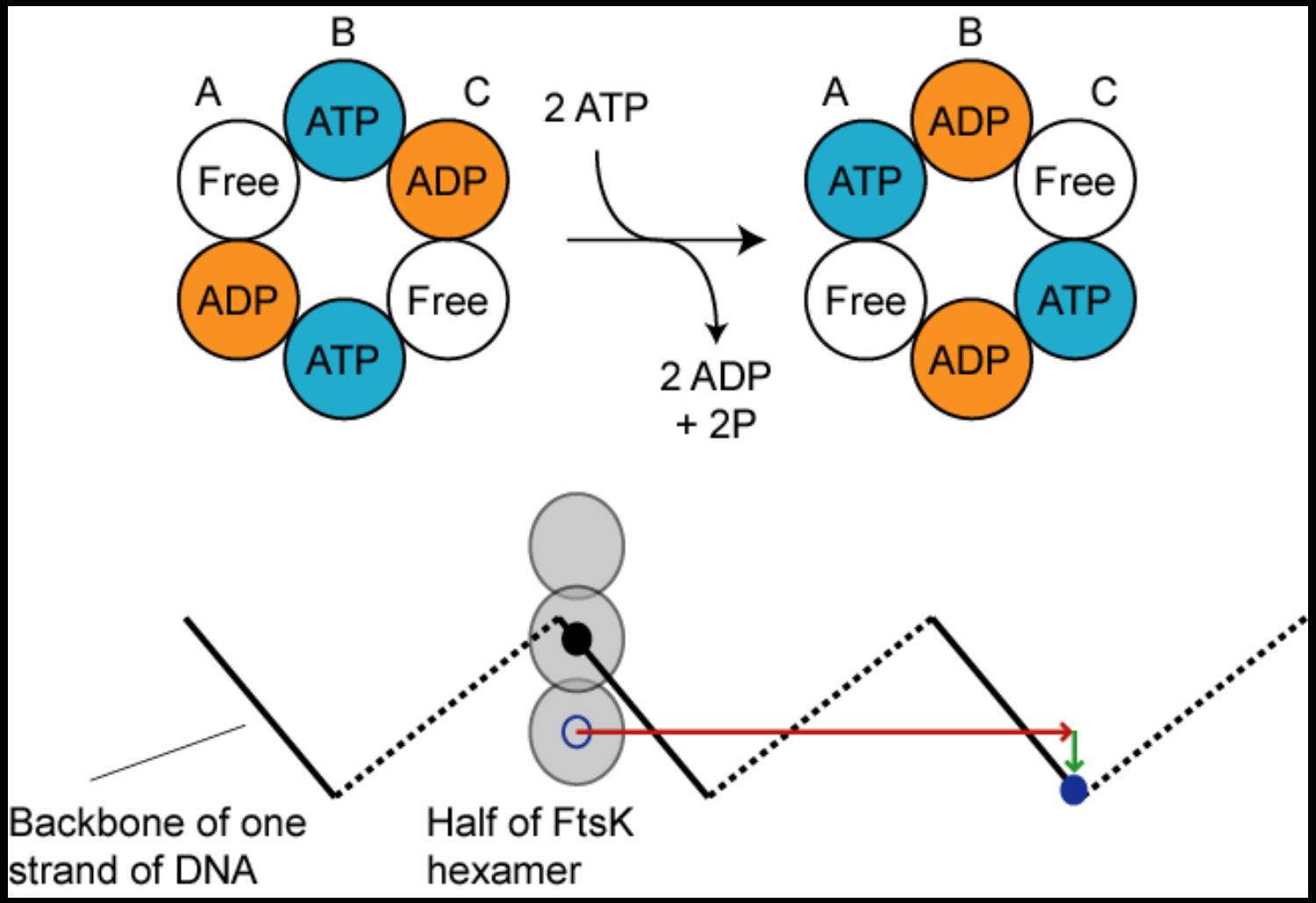
Let's go back to FtsK's step size

Does FtsK's step size define its supercoil induction rate?



In this model, 0.07 turns/pitch gives a ~11 bp step; close to our fluctuation-based estimate!

Speculative full translocation mechanism



By analogy to other hexameric enzymes:
ATP binding, hydrolysis, release occurs sequentially around ring, probably coordinating with DNA binding and release

Full disclosure:
There is an alternate mechanism

'structural step' = minimal discrete motion of the motor

'kinetic step' = distance between rate-determining states

1. **Presented model**:

   kinetic = structural = 12 bp (explains randomness and sc induction)

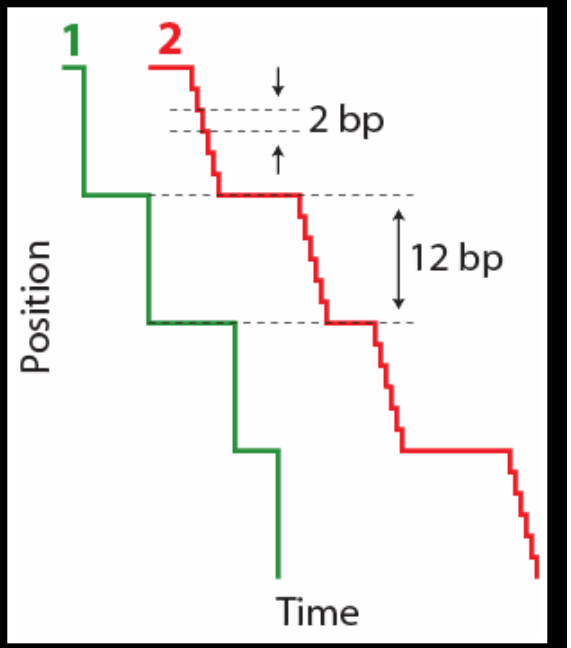
   ≥ 2 ATP per step (needed to explain stall force)

2. **Alternate model** (similar to Dumont et al, 2006):

   structural = 2 bp (gives 0.1 sc/pitch ~ 0.07)

   1 ATP per structural step (explains stall force)

   long pause every 12 bp (to explain randomness)



Experiments to differentiate the two are underway

Outline

1. The Biology

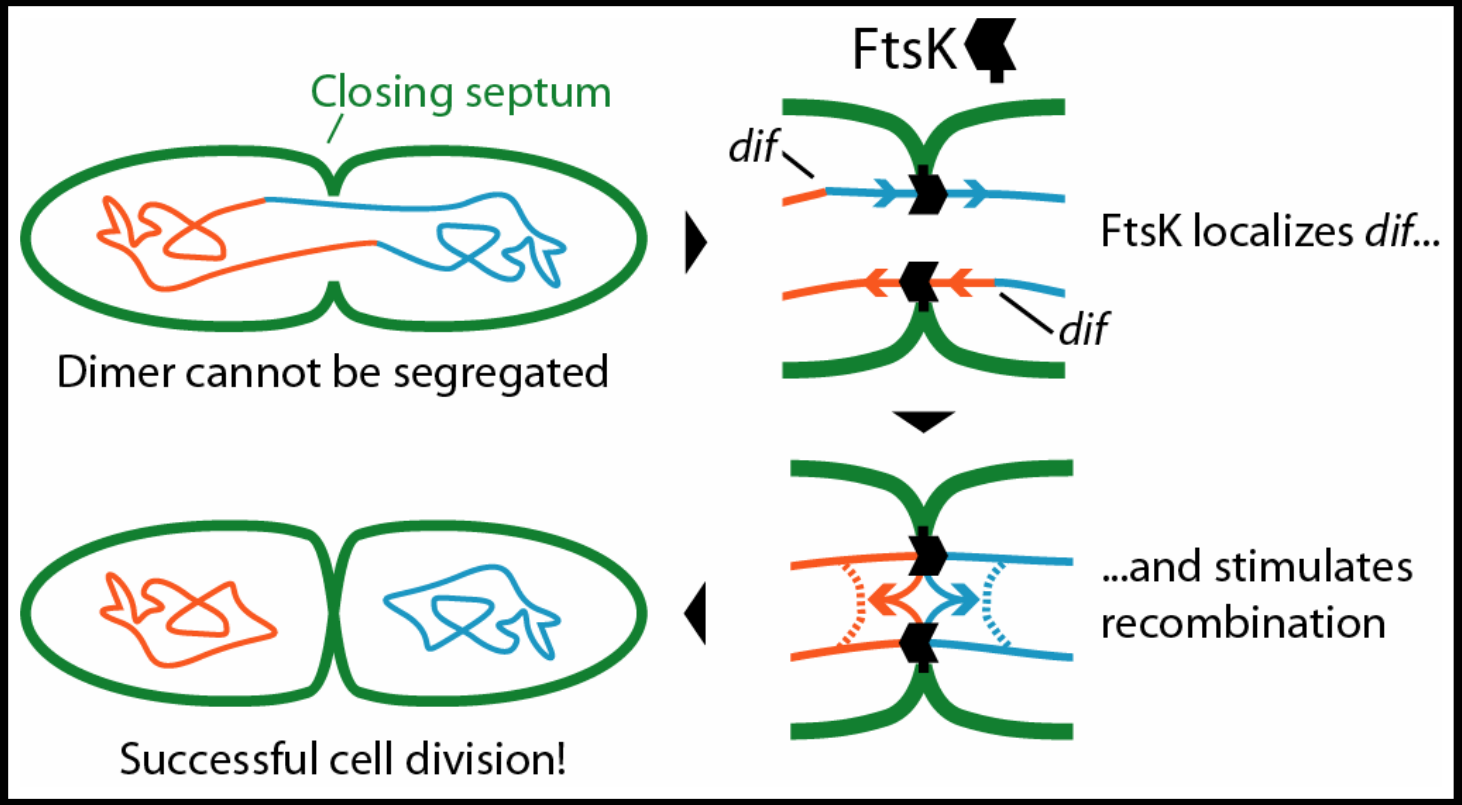
   A primer in bacterial cell division
2. Linear translocation

   A bit of technique, a bunch of speed
3. Rotational motion

   Exploiting the physics of twisted DNA
4. Sequence detection

   Or how to stop FtsK

Chromosome dimer resolution



How does it get to *dif*? *In vivo* expts suggested DNA sequence...

...confirmed by Pease et al, Science (2005):
In vitro, FtsK is directed to, oscillates around *dif*

The next question

What is the sequence that directs FtsK?

The next question

What is the sequence that directs FtsK?

- FtsK Orienting Polar Sequence (KOPS):

5’-GGGNAGGG-3’

Bigot et al, EMBO J. (2005)

The next question

What is the sequence that directs FtsK?

- FtsK Orienting Polar Sequence (KOPS):

5’-GGGNAGGG-3’

Bigot et al, EMBO J. (2005)

Levy et al., PNAS (2005): FRS = GNGNAGGG

Only difference:

degeneracy of 2nd base; not yet tested

Representation of GGGNAGGG throughout the *E. coli* genome

- Up-ticks (down-ticks) represent the presence of 5'-GGGNAGGG-3' on the top (bottom) strand
- 368 total instances of KOPS (335 properly skewed); 1 per 13 kbp
- KOPS are skewed far from *dif*, while FtsK activity confined near *dif* …role in chromosome organization?

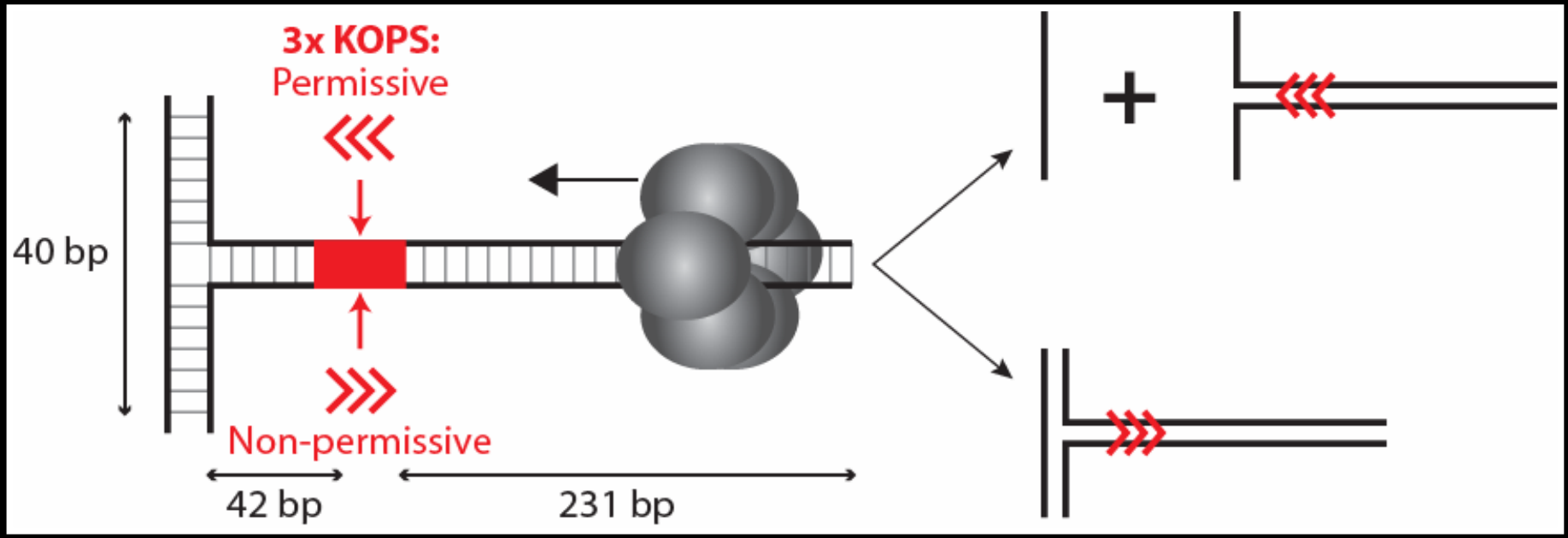
Discovering KOPS:
A story in three parts

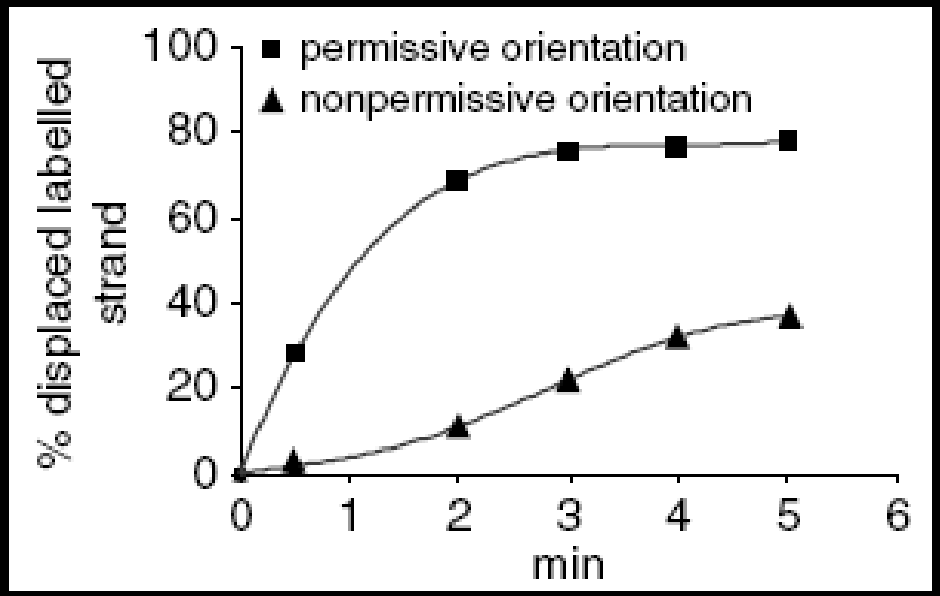
1. *In vivo* dimer resolution assay (replacing *dif* with *psi*, inverting flanking sequences) and genomic analysis identify GGGNAGGG
2. *In vitro* bulk T-dissociation assay and recombination assay demonstrate polarity and combinatorial effect of GGGNAGGG
3. *In vitro* single-molecule measurements investigate mechanism of KOPS/FtsK interaction

Discovering KOPS:
A story in three parts

1. *In vivo* dimer resolution assay (replacing *dif* with *psi*, inverting flanking sequences) and genomic analysis identify GGGNAGGG
2. *In vitro* bulk T-dissociation assay and recombination assay demonstrate polarity and combinatorial effect of GGGNAGGG
3. *In vitro* single-molecule measurements investigate mechanism of KOPS/FtsK interaction

T-dissociation assay



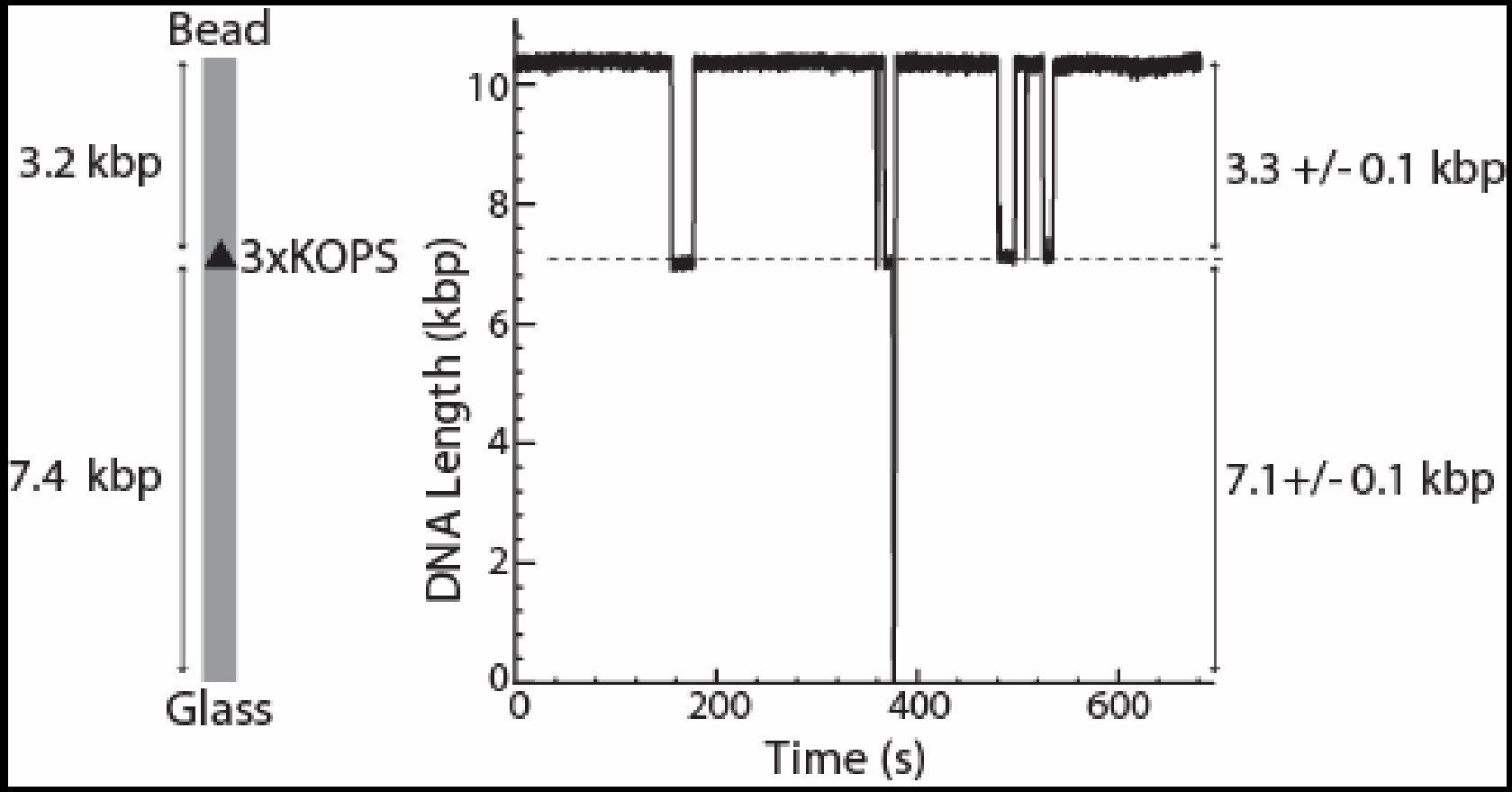


- Permissive: FtsK approaches from 5' end of G-rich strand
- Assay conceived by Kaplan and O'Donnell (2004) for DnaB

Why use 3xKOPS?
Just one KOPS had no effect

- In both *in vitro* and the *in vivo*, the effect of KOPS strongly increases by repeating the motif
- Thus the effect of a single KOPS is stochastic- repeating the sequence increases the chances of recognition by FtsK
- This also happens with RecBCD recognition of the Chi sequence (See: May 9, S.Kowalczykowski)

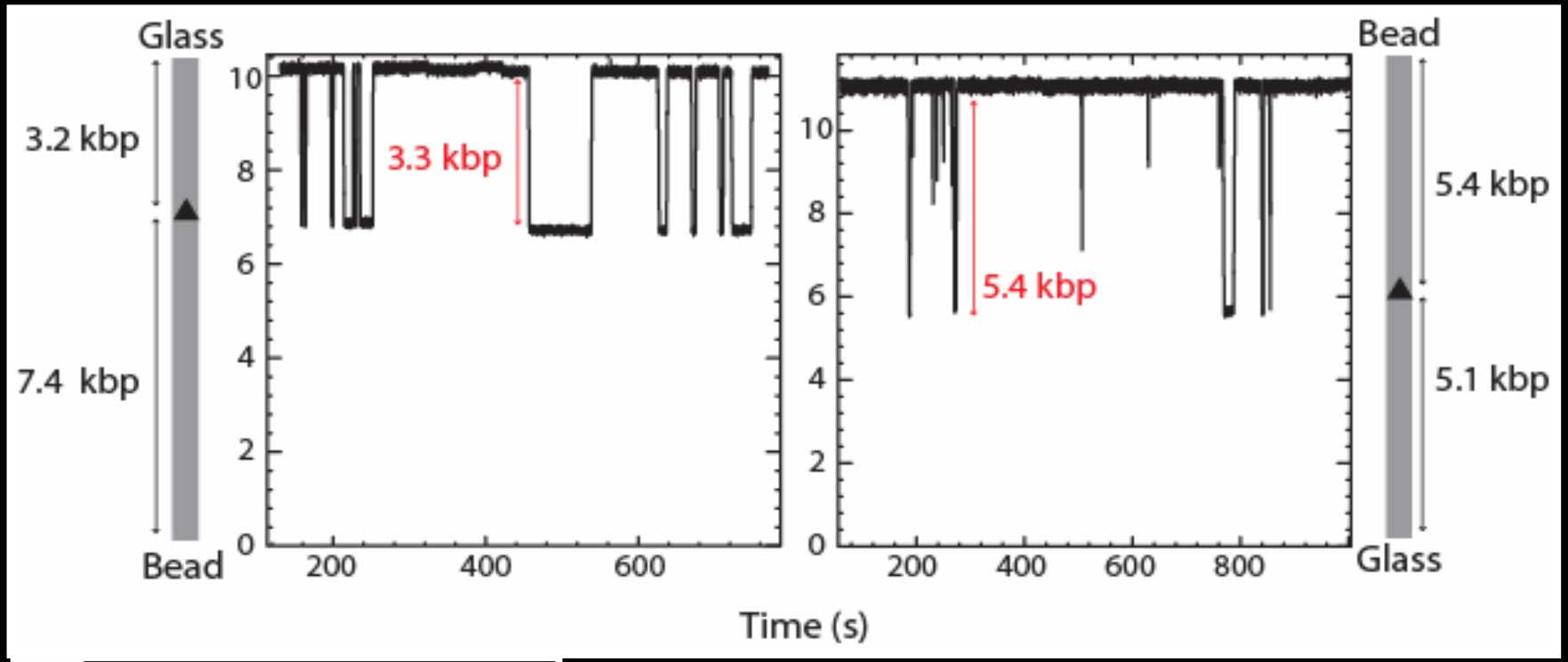
Single-molecule msmts help determine mechanism of KOPS

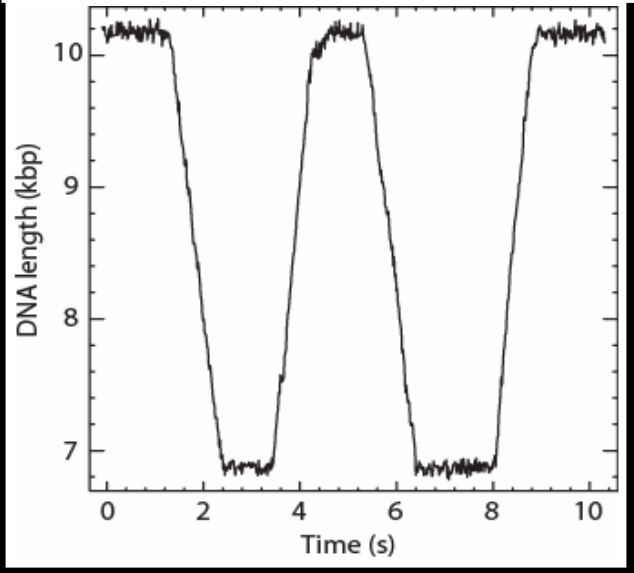


Nearly all events pause at the extremity/KOPS distance

Indicates static binding of FtsK to KOPS

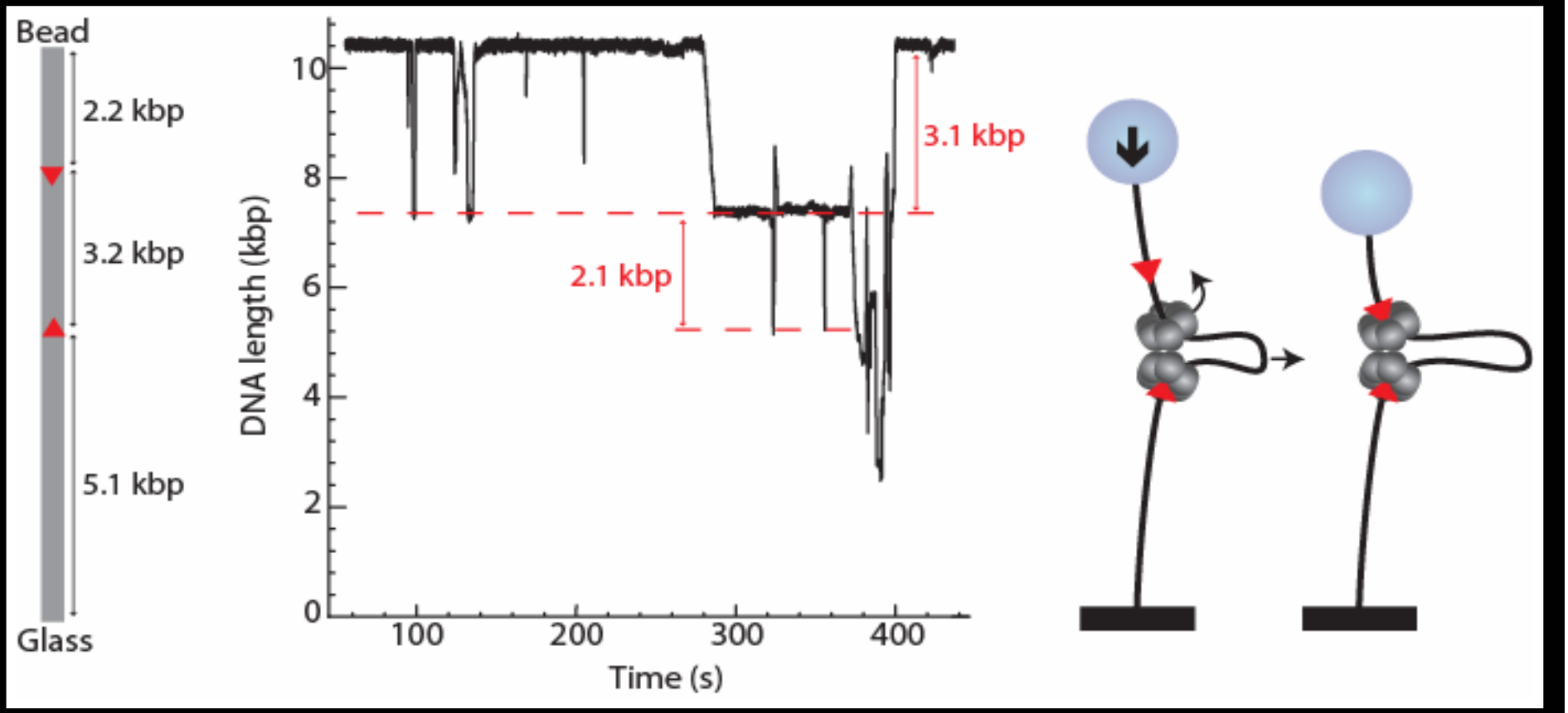
Moving/flipping the KOPS





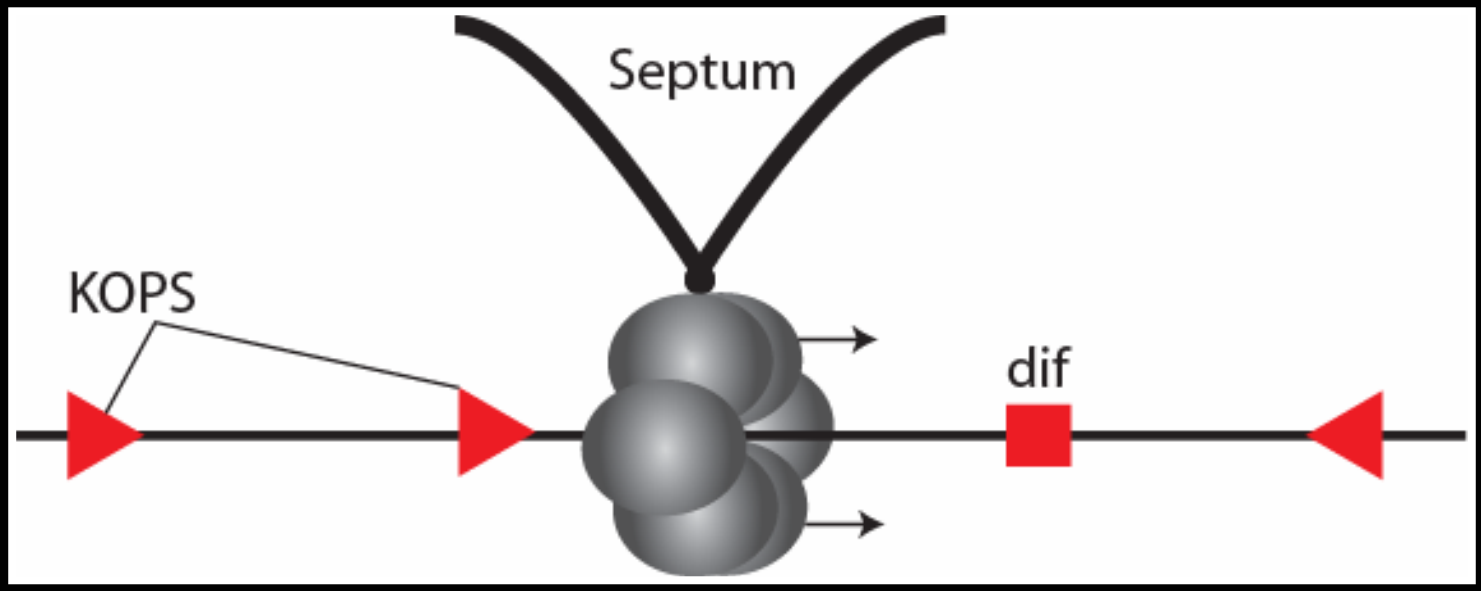
- Same result if sequence flipped, or moved
- Close-up: events have "translocate-pause-reverse trans" shape
- Pause times roughly exp. distributed (τ~5 s), w/ many outliers (>100 s)

TWO cassettes of 3xKOPS



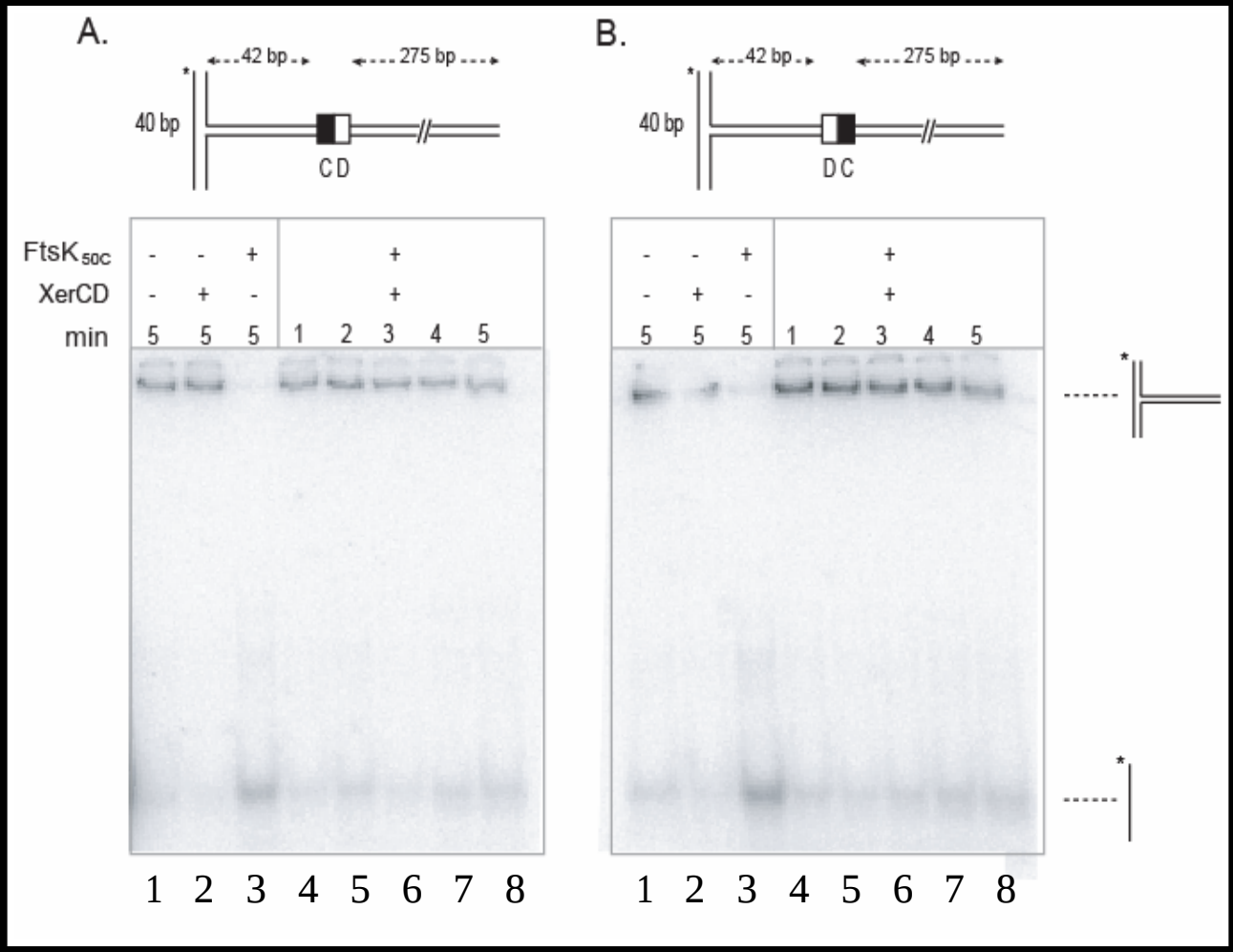
FtsK translocates from one KOPS, pauses upon hitting 2nd KOPS in anti-aligned direction

KOPS direct FtsK to *dif* by stopping translocating, anti-aligned motors



BUT: *In vivo* activity requires FtsK to be localized at *dif* (for recombination), not just pass it by...KOPS alone won't do that

Preliminary Data: How FtsK stops at *dif*



- XerC/D bound to dif stops FtsK in both orientations (lanes 4-8)
- FtsK is NOT stopped by *dif* alone (lane 3), or by other DNA-bound protein (Tus bound to *ter*; not shown)

Summary of sequence effects

- FtsK stochastically binds to, and then reverses, at GGGNAGGG sequences (KOPS) when anti-oriented and translocating, thus directing motion towards *dif*
- We cannot rule out a further effect of KOPS on the initial loading of FtsK on DNA
- XerC/D bound to *dif* (but not *dif* alone) stops translocating FtsK, regardless of orientation
- Mechanistically: sequence-specific DNA binding could be the pause state needed to link the kinetic and structural steps

Conclusion:

$FtsK_C$ does MANY thing and 1-mol expts with magnetic tweezers are uniquely able to sense them all

References

- Linear translocation:

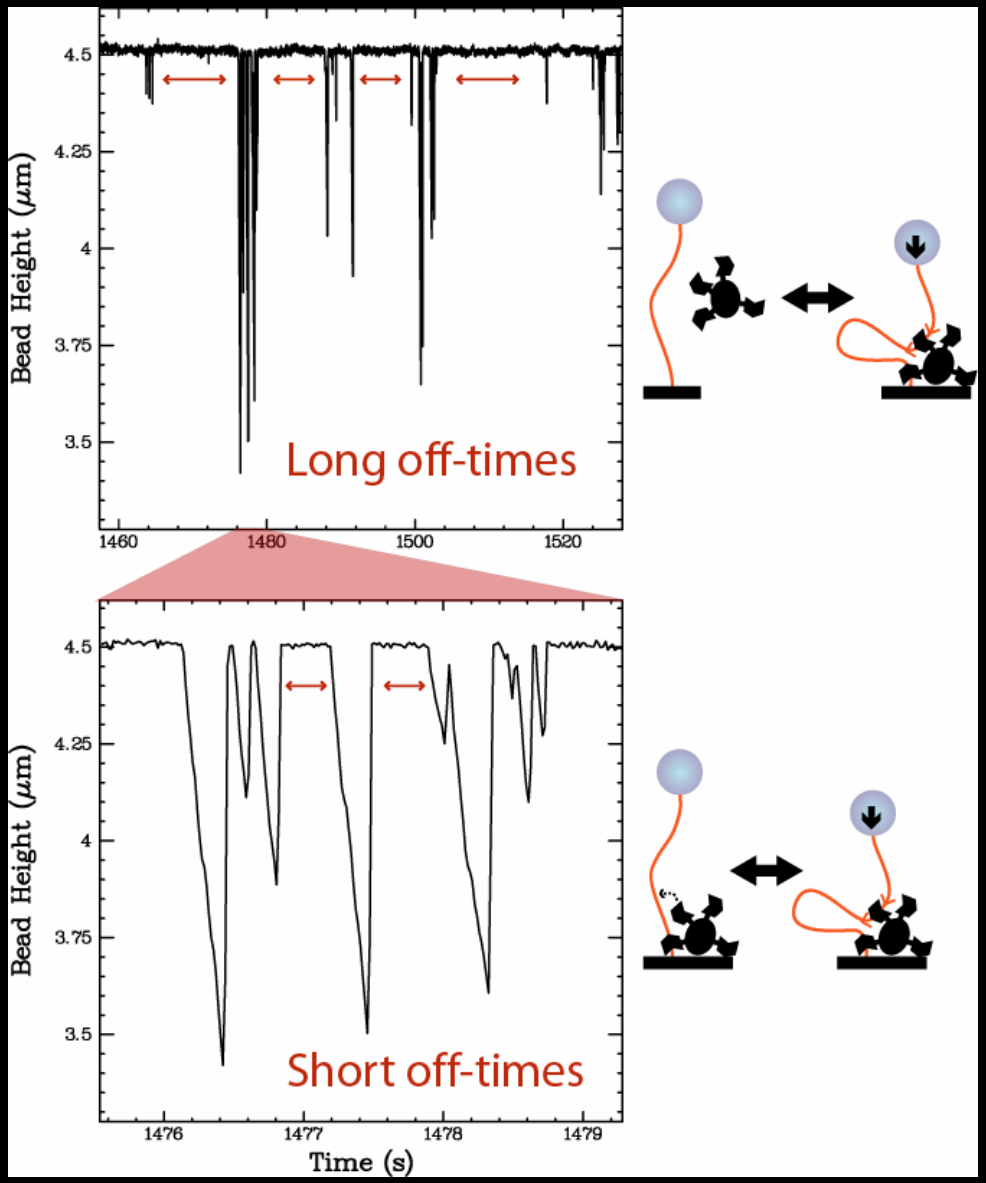
  Saleh et al., EMBO J. (2004)
- Rotational motion:

  Saleh et al., Nat Struct Mol Bio (2005)
- Sequence effects:

  Bigot et al., EMBO J. (2005)

Others: Pease et al, Science (2005); Levy et al, PNAS (2005)

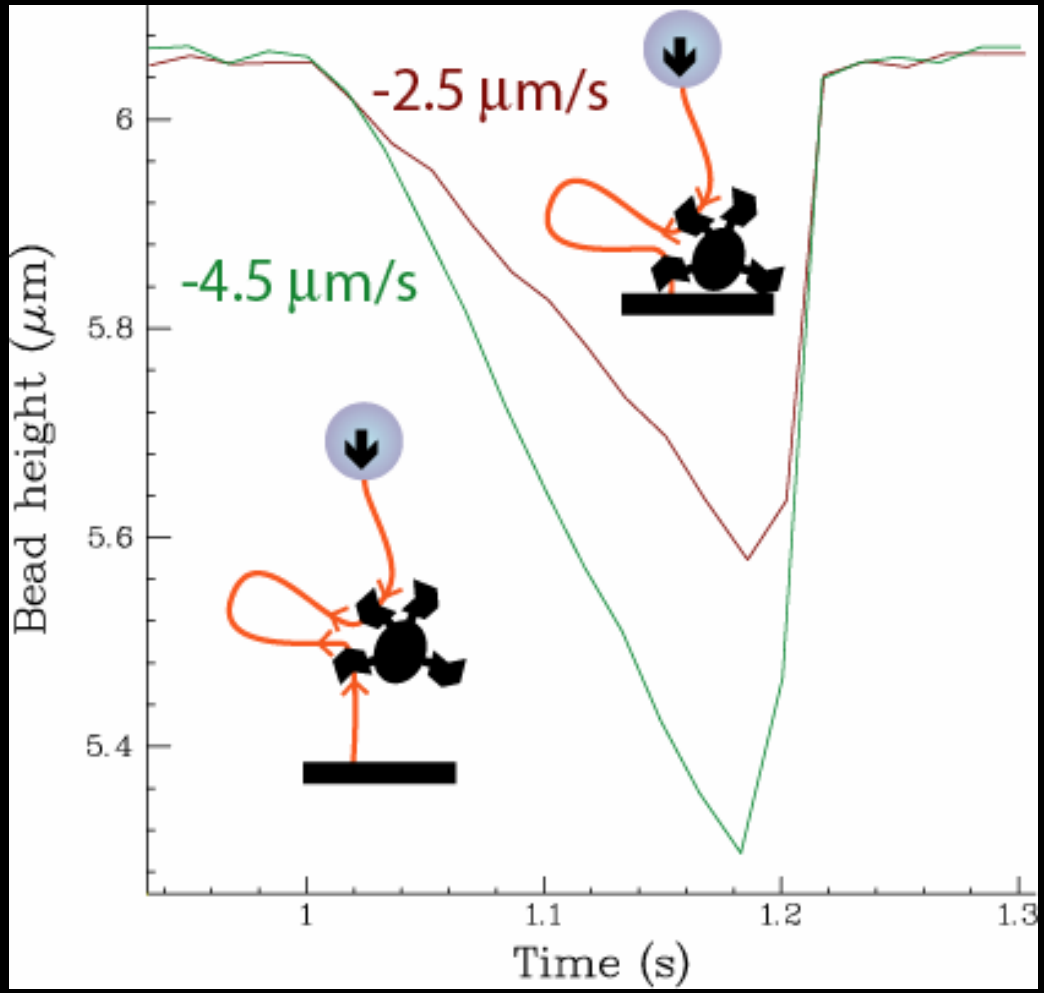
Off-times support this picture



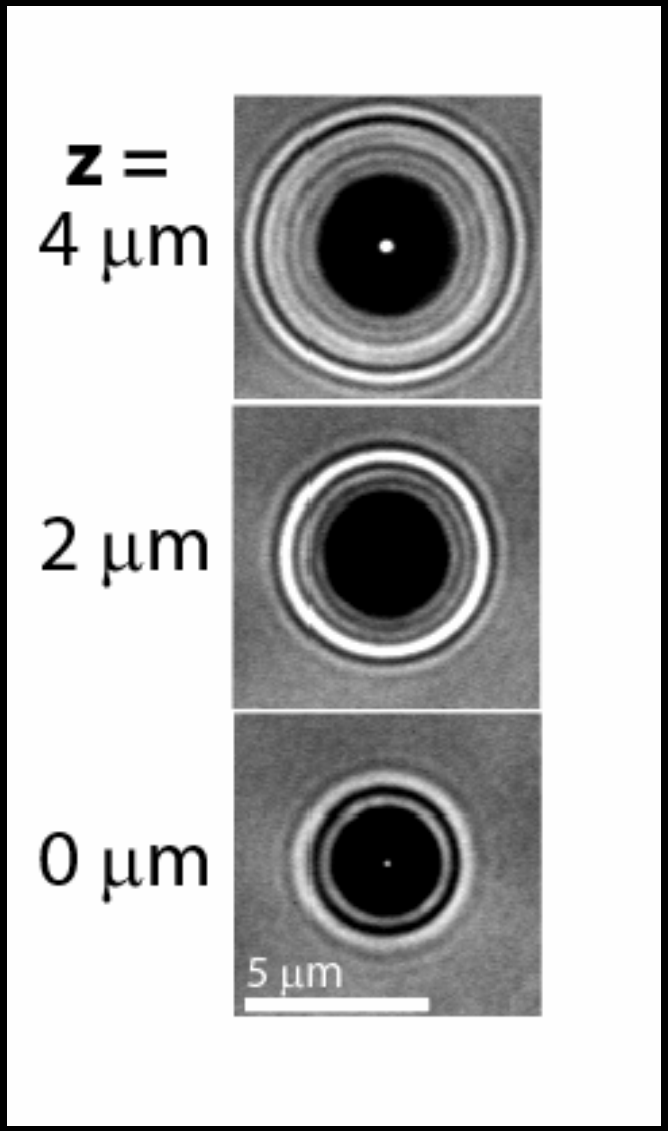
Off-time distributions indicate two independent (probably equivalent) DNA binding sites within one complex

2 motor events occur, but are rare

We observe rare events at twice the velocity… the exception that proves the rule!

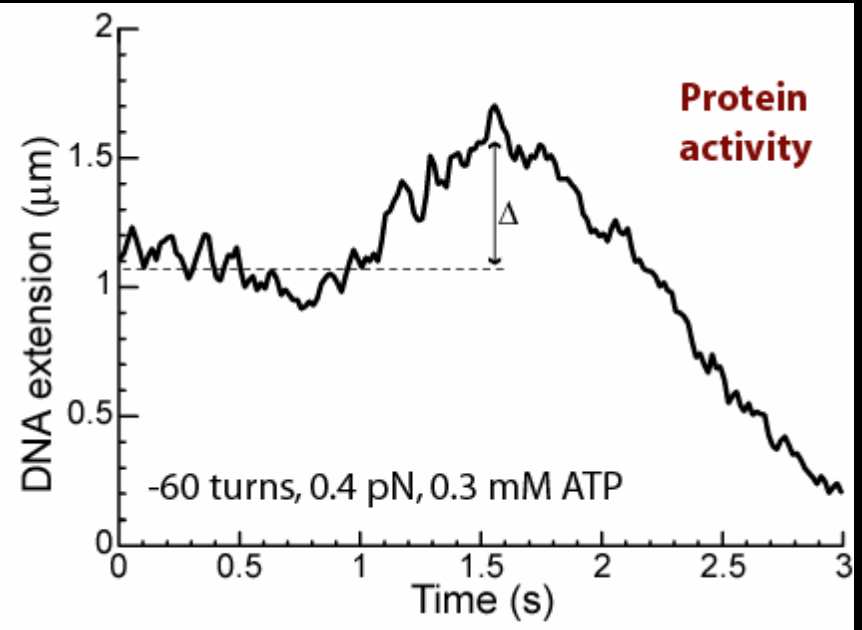


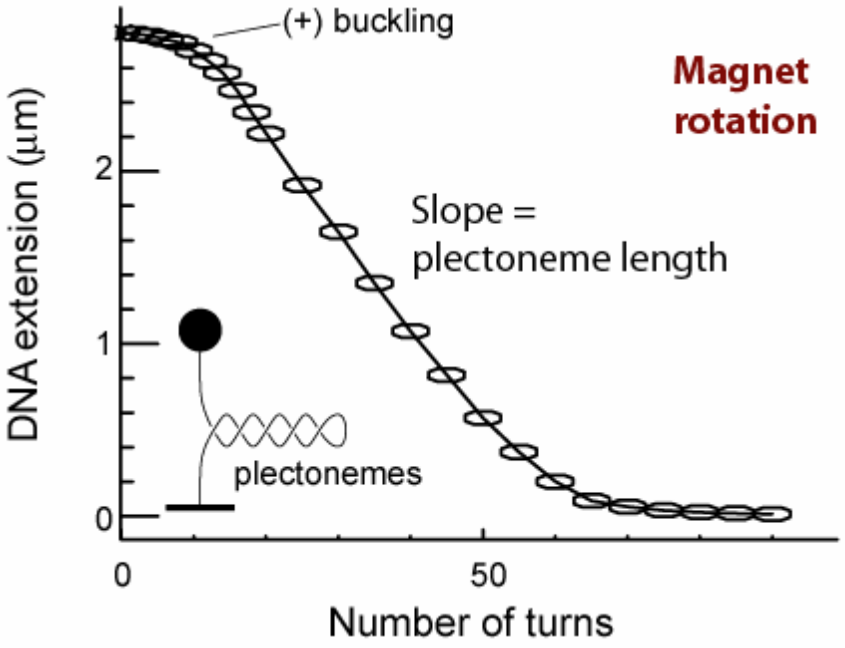
Optically tracking the 3D bead position



- Bead's image processed in real time (30-60 Hz) to give 3D position
  - x,y: correlation analysis, precision ~1 nm
  - z: comparison of diffraction ring width to reference image, precision ~3 nm
- Differential tracking w/ reference bead removes drift

Calculating the supercoil induction rate





We assume a constant # sc per distance traveled, and define:

κ = # sc induced/pitch traveled

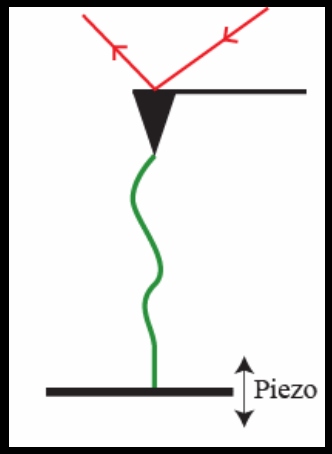
Then: # sc added in a distance d is $N = \kappa d/p$ (p=length of pitch), causing an increase in bead height $NL_p = \kappa d L_p/p$ (L_p = plectoneme length).

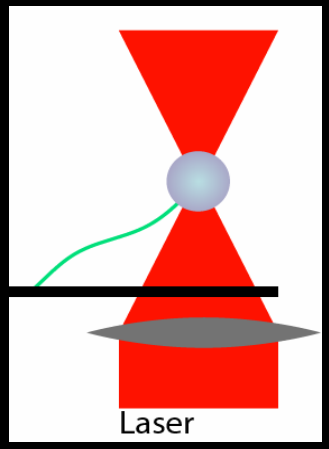
Also: $N = N_i - N_c$ (N_i = initial # of added turns, N_c = buckling point)

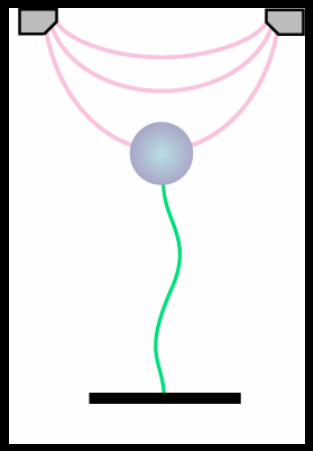
The total change in bead height is: $\Delta = NL_p - d = N(L_p - p/\kappa)$, so

$$\kappa = \frac{Np}{NL_p - \Delta}$$

The common techniques







	The Atomic Force Microscope	The Optical Tweezer	The Magnetic Tweezer
The actuator	A cantilever	A dielectric bead	A paramagnetic bead
Position detection	Photodiode	Photodiode	Video tracking
Force range	10-1000 pN	1-200 pN	0.1-100 pN
Advantages	Bandwidth, sensitivity	Bandwidth, manipulation	Simplicity, constant force, rotation
Disadvantages	Limited low-force ability	Complicated	Slow, low precision in position detection

What is a relevant force?

Force ~ Energy/Length; k_BT ~ 4 pN nm

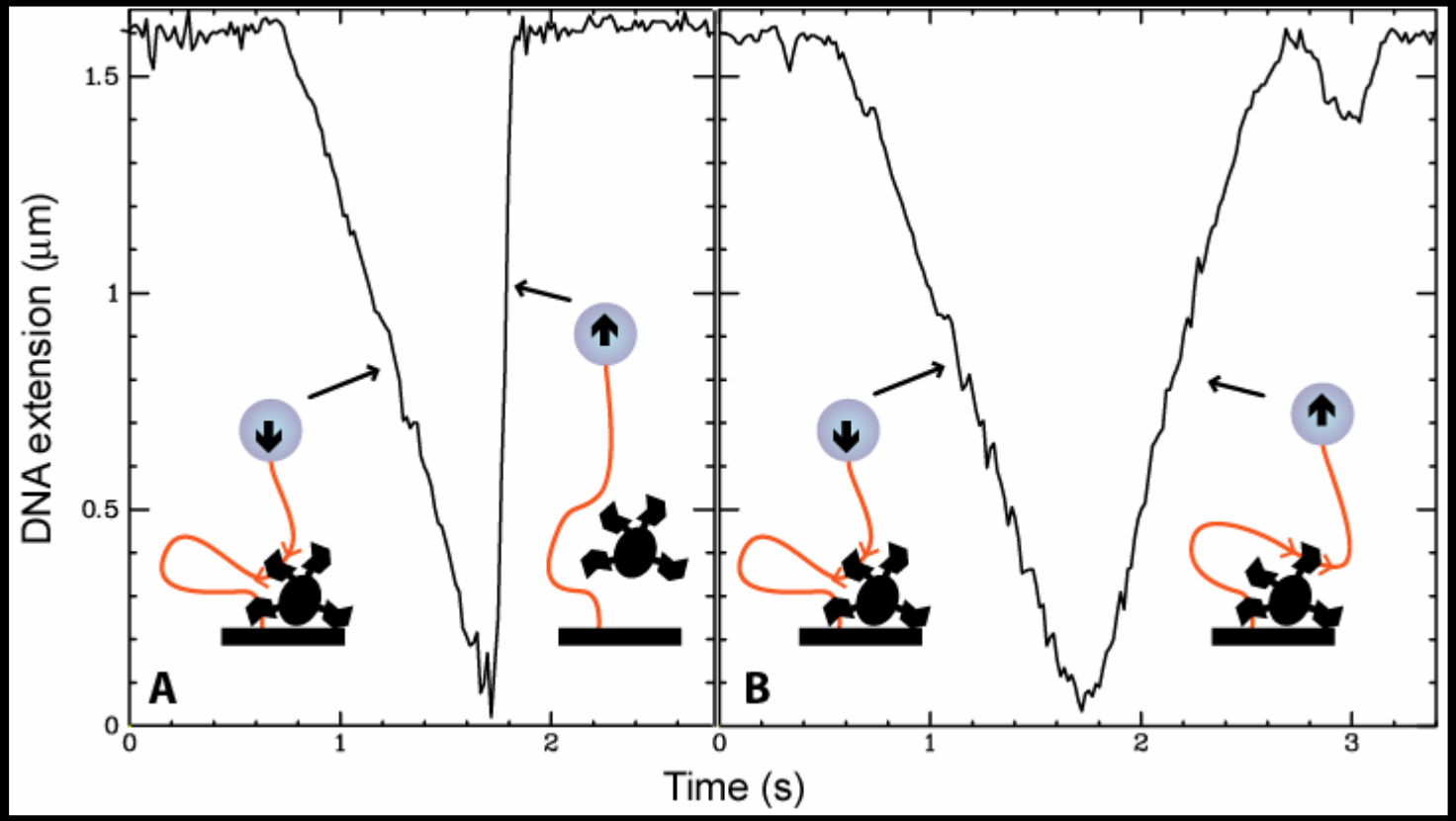
Process	Energy	Length	Force
Stretching DNA	~k_BT	50 nm	0.1 pN
Weak bonds	~k_BT	nm	4 pN
Unzipping DNA	2/3 H bonds = a few k_BT	nm	10-15 pN
Motor motion	ATP ~ 20 k_BT	1-10 nm	8-80 pN
Denaturing a protein	[many weak bonds]	nm	10-200 pN
Covalent bonds	1 eV ~ 40 k_BT	0.1 nm	1 nN

Magnetic tweezers

Optical tweezers

AFM

Not just fast...also reversible!

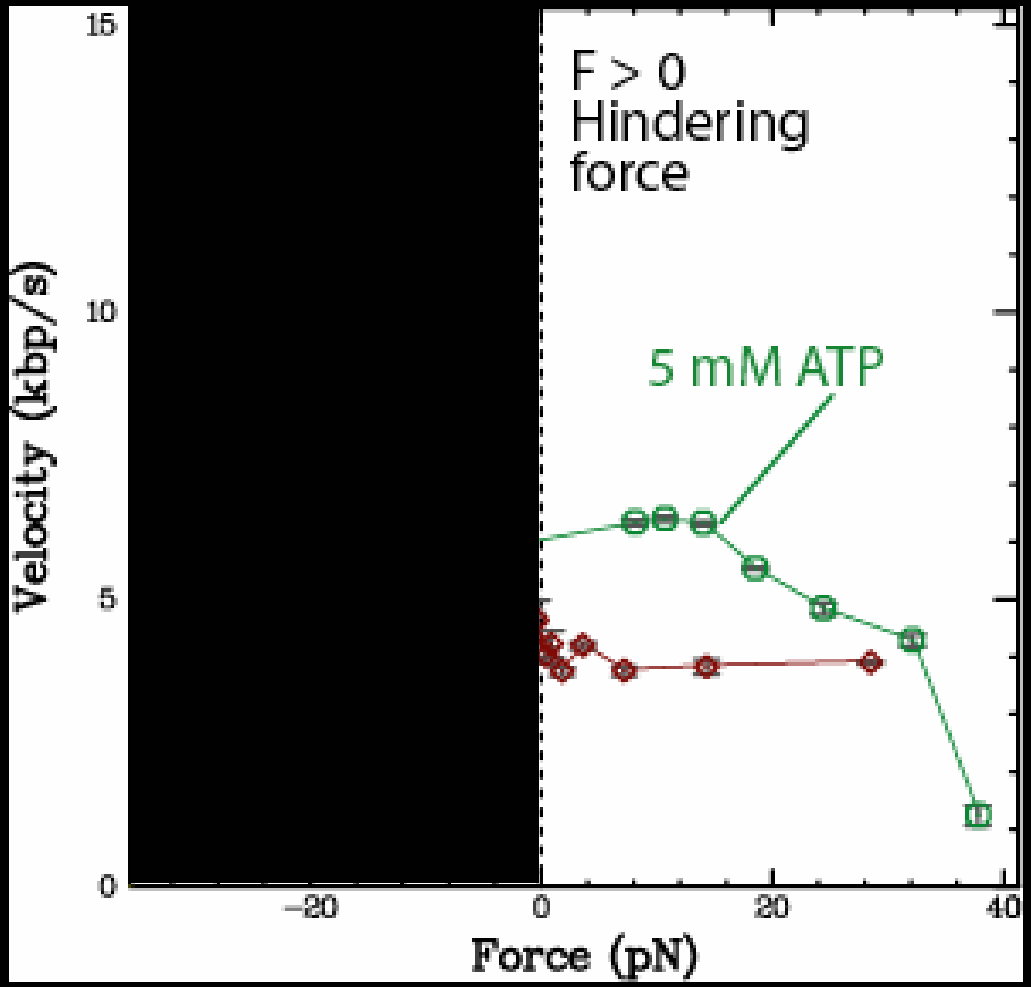


1 mM ATP
3.6 pN

Descending velocity ~ ascending velocity, thus (reversed) motor activity

This permits the study of velocity vs hindering AND assisting forces

Force/velocity curves



- 0 < F < 20 pN: Velocity constant
- F > 20 pN: slows; ‘stall’ near 40 pN