Power at the Nanoscale: Speed, Strength and Efficiency in Biological Motors

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Winton Symposium

University of Cambridge

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Howard Hughes Medical Institute

The Cell Interior

Cells are neither isotropic nor homogeneous.

They posses polarity and many processes, from cell motility to internal transport require:

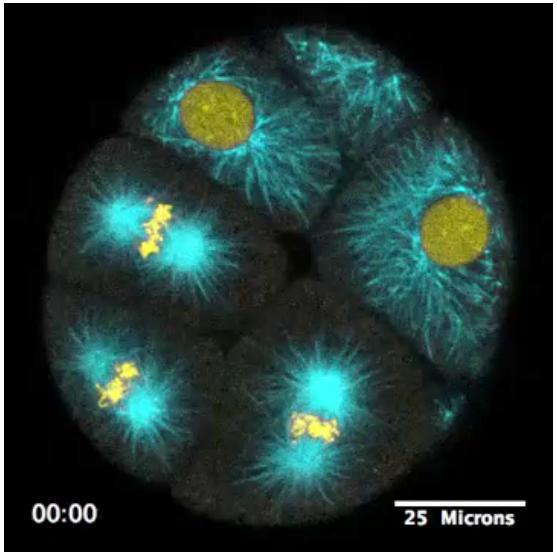
directional movement of molecular species

- through the cytoplasm,
- or across membranes into distinct compartments, often against chemical gradients.

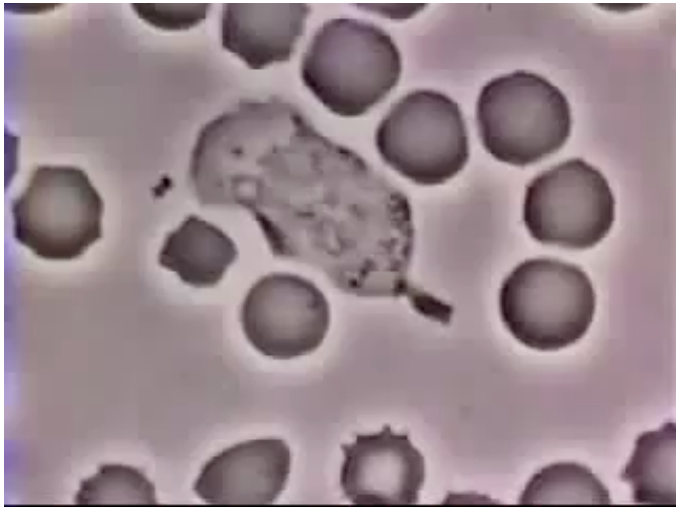
These processes cannot be accomplished by mere diffusion. They require *active* mechanisms.

Active Biological Processes

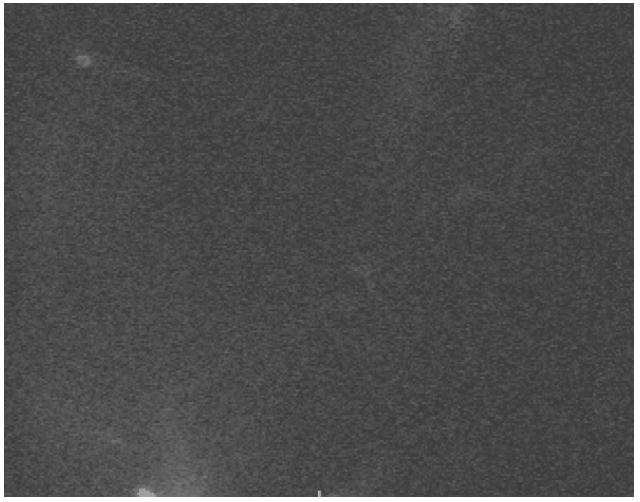
Chromosome segregation during mitosis



Crawling neutrophil chasing bacteria



Bacteria using flagella to swim around



1. **Left.** Adapted from Physical Biology of the Cell, Second Edition. David Rogers at Vanderbilt Universit
2. **Center.** Adapted from http://www.celldynamics.org/celldynamics/gallery/index.html
3. **Right.** Turner, L., Ryu, W.S. and Berg, H.C. Real-time imaging of fluorescent flagellar filaments. *J. Bacteriol.* 182, 2793-2801 (2000).

Molecular Motors

These directional movements are performed by tiny machine-like devices that operate as *molecular motors*.

Converting chemical energy in the form of bond-hydrolysis or chemical gradients into force and/or torque and displacement.

Molecular Motors

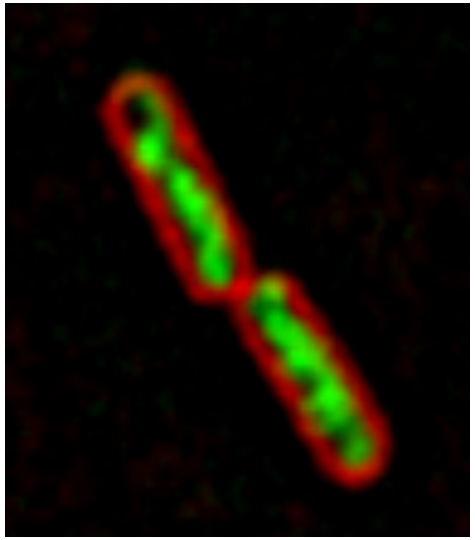
Enzymes that couple the catalysis of a downhill chemical reaction to the performance of a mechanical task.

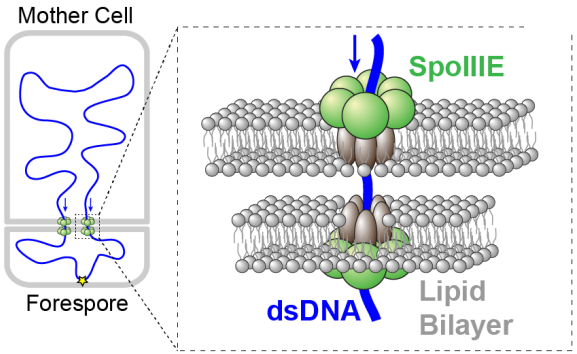
- Energy transducers: convert chemical free-energy into mechanical work:

- Force
- Torque

Molecular Motors

Sporulation in *B. subtilis*

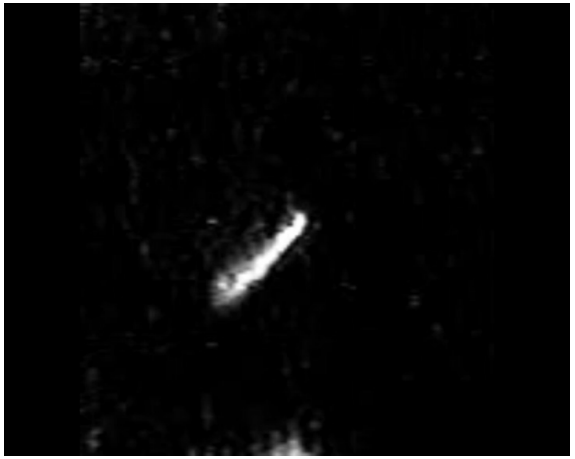


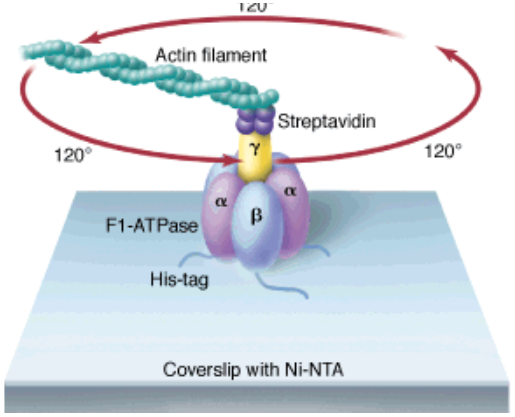


SpoIIIE

Fleming et al., *Gen. Dev.* (2010).

ATP production

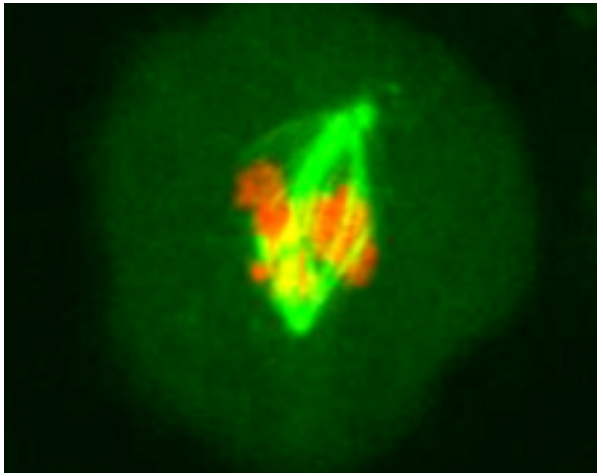


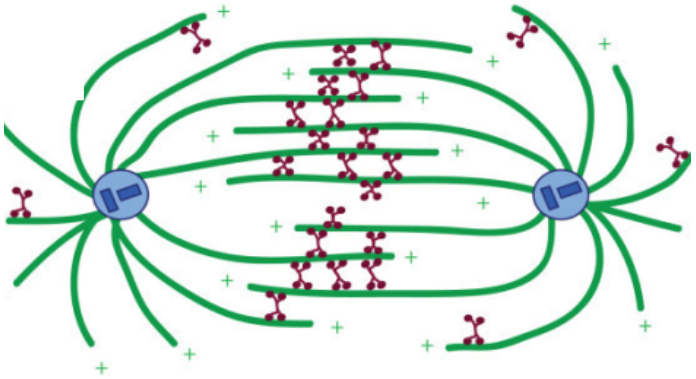


F1 ATPsynthase

Noji et al., *Nature* (1997).

Eukaryotic Chromosome Segregation



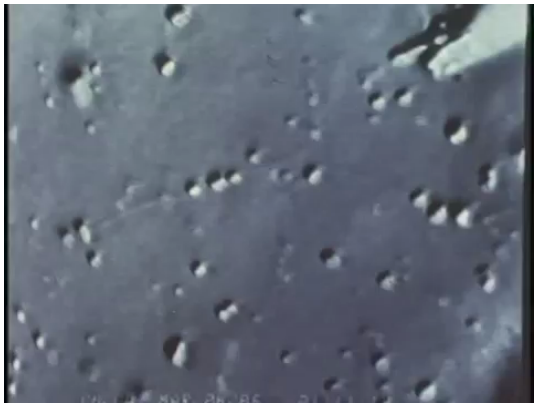


Eg5 (Kinesin-5)

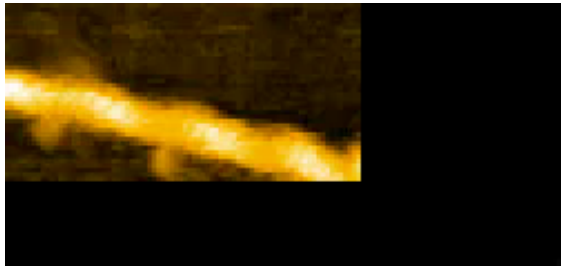
Valentine et al., *Cell Division* (2006).

Molecular Motors

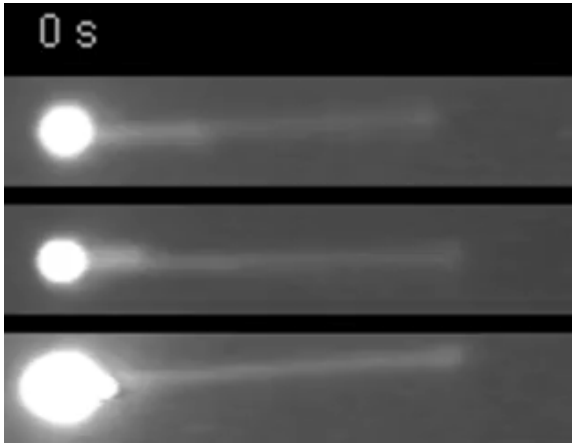
Vesicular transport

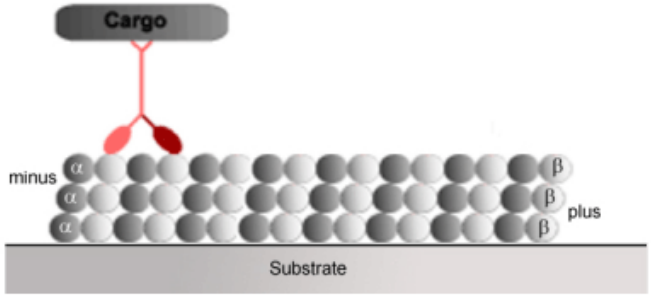


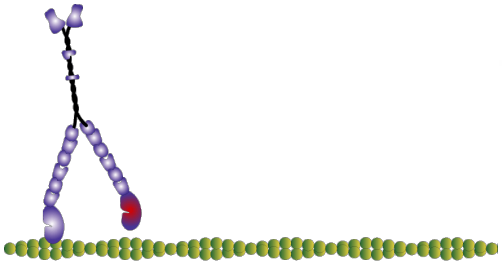
Intracellular transport

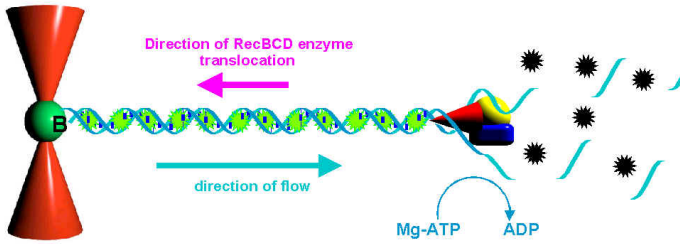


DNA unwinding









Kinesin-1 on microtubules

Movie credit: Bohm Lab (Leibniz Institute) and Vale Lab (UCSF)

Myosin V on actin

Movie credit: Warshaw Lab (University of Vermont) and Ando Lab (Kanazawa University)

RecBCD Helicase

Movie credit: Kowalczykowski Lab (UC-Davis)

The Mechanical Paradigm

"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."

[*De Viscerum Structura*, Marcello Malpighi (Malpighi, 1666)]

DE VISCERVM STRVCTVRA EXERCITATIO ANATOMICA

MARCELLI MALPIGHII

Philoſ. & Medic. Bononien. in Meſſanenſi Academia Medicinæ Primarij.

Accedit

Diſſertatio eiuſdem de Polypo Cordis.

BONONIÆ,

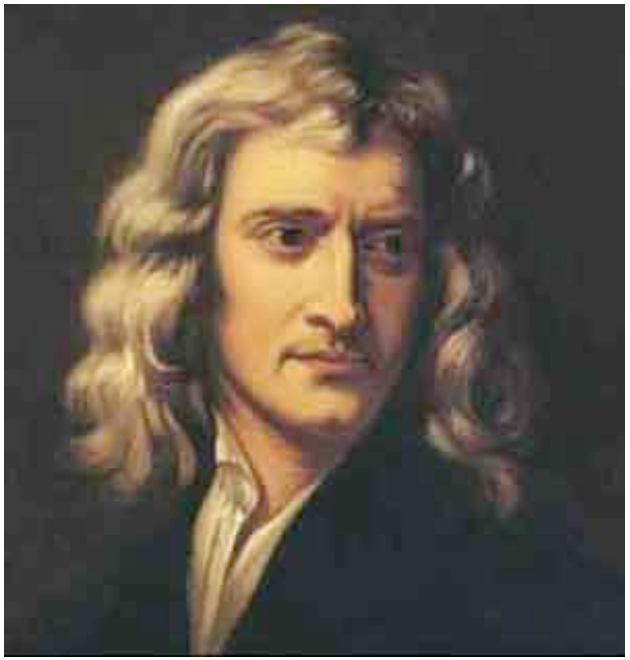
Ex Typographia Iacobi Montij. MDCLXVI.
Superiorum permiſſu.

Marcello Malpighi

(1628 – 1694)

Galileo Galilei

(1564 – 1642)

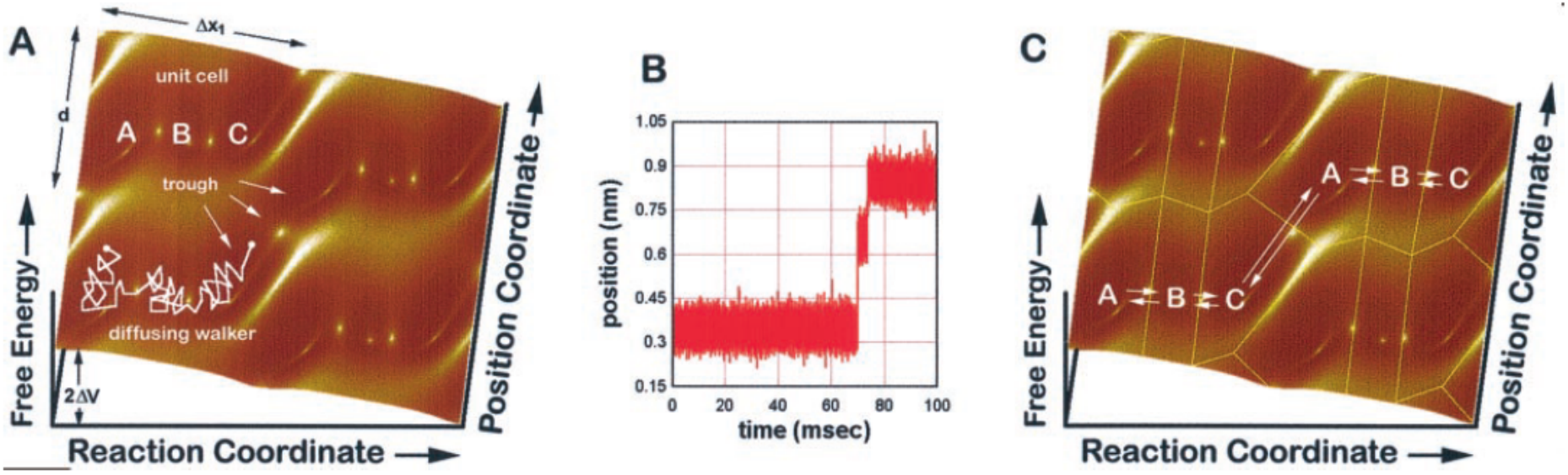


Isaac Newton

(1642 – 1727)

The Mechano-chemical Space

Operating at energies just above those of the thermal bath, these motors experience large fluctuations, and their behavior and physical description must be necessarily stochastic.



Keller and Bustamante, 2000

How to Study These Machines?

Direct single molecule manipulation methods:

optical tweezers,
AFM,
magnetic tweezers

have made it possible to study molecular motors.

The variables that are more easily detected by these methods: *force, torque, displacement, and time* are also the ones of greatest functional value to understand the operation of these motors.

What We Want to Know

Type of operation: Power Stroke or Brownian Ratchet

Step size: displacement/molecule of fuel consumed

Maximum Force (or torque) output (Stall Force or torque):
- *thermodynamic efficiency*

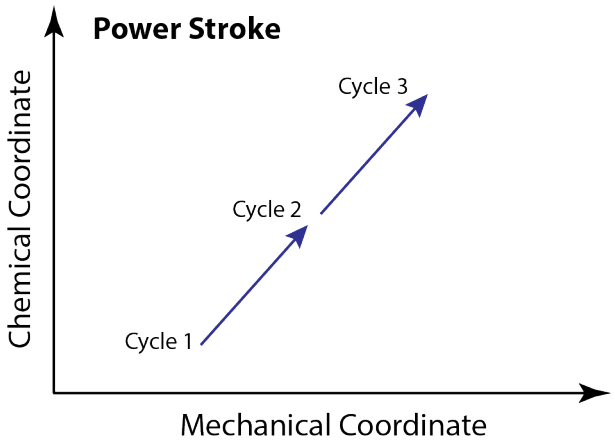
Mechano-chemical cycle

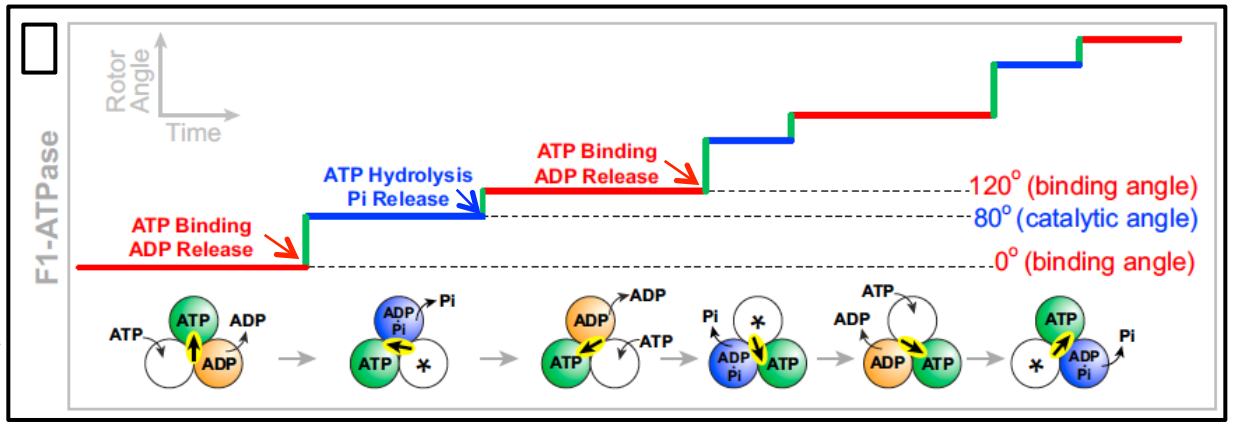
Velocity vs Force behavior: Identifying the transduction step

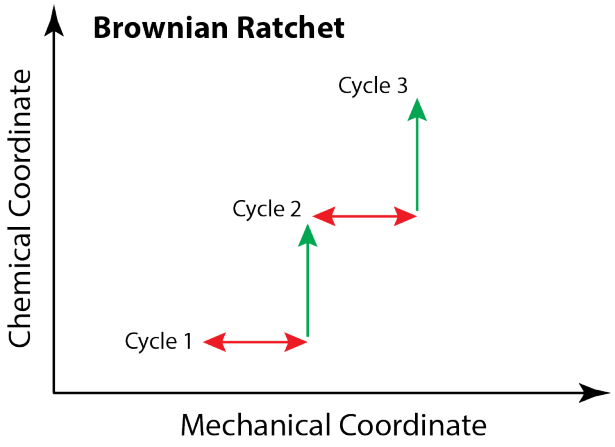
How are their parts regulated and coordinated

Power Stroke vs. Brownian Ratchet

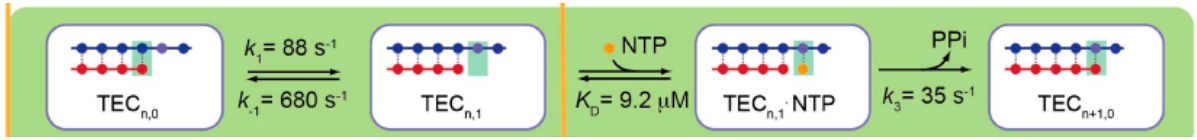
- Power stroke motor: F_1-ATPase



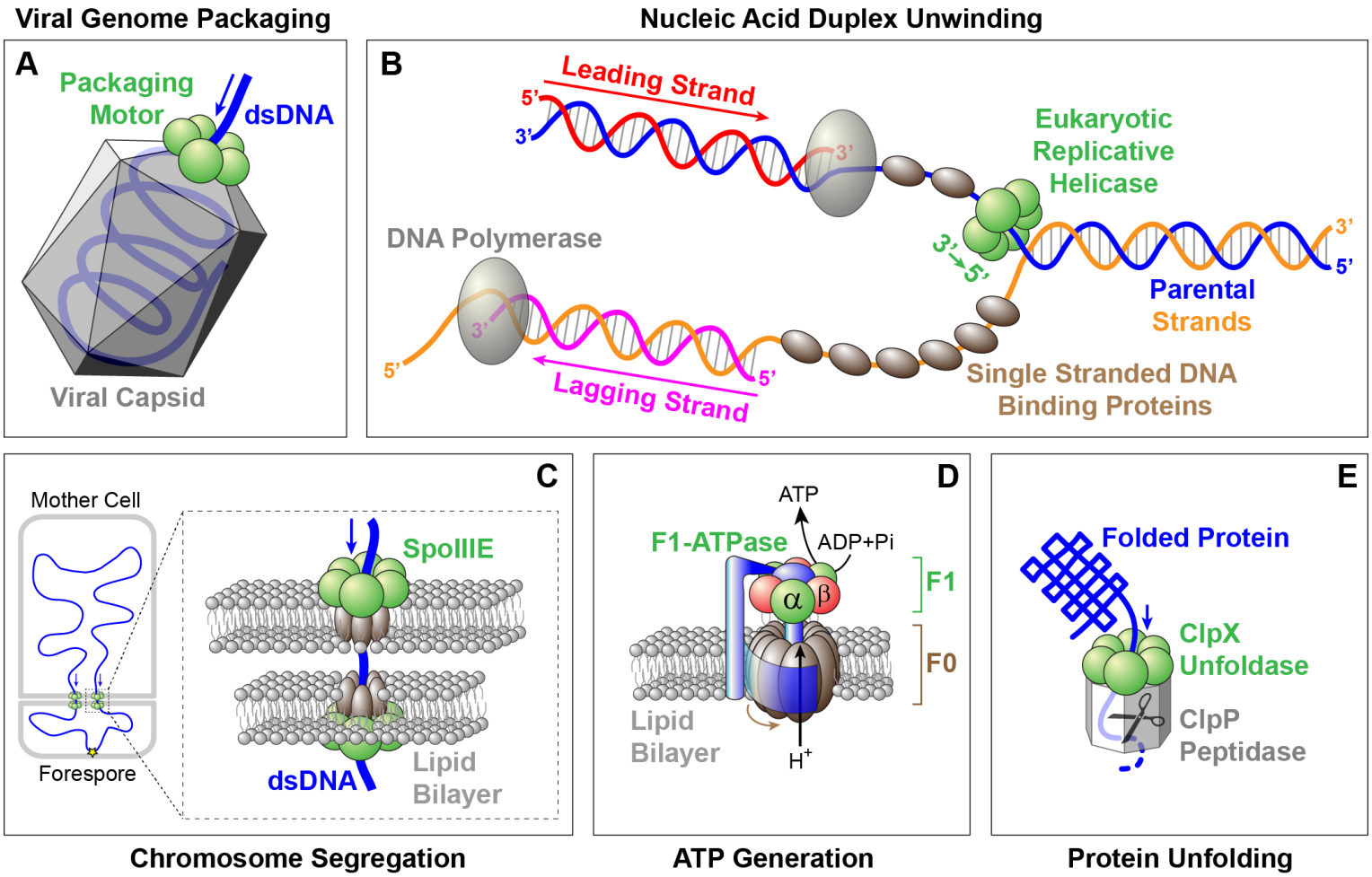




- Brownian ratchet motor: RNA polymerase

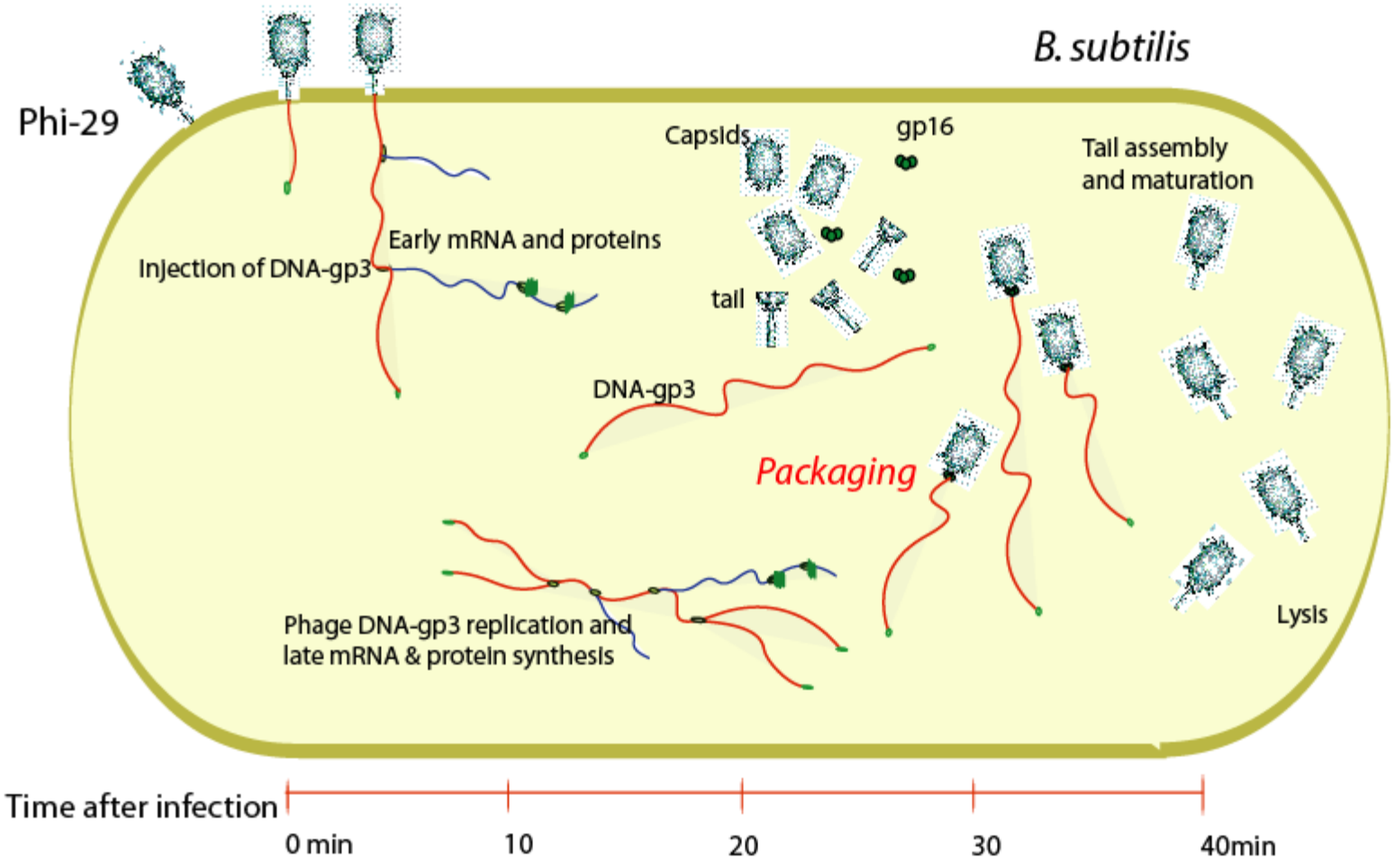


Ring-shaped Molecular Motors

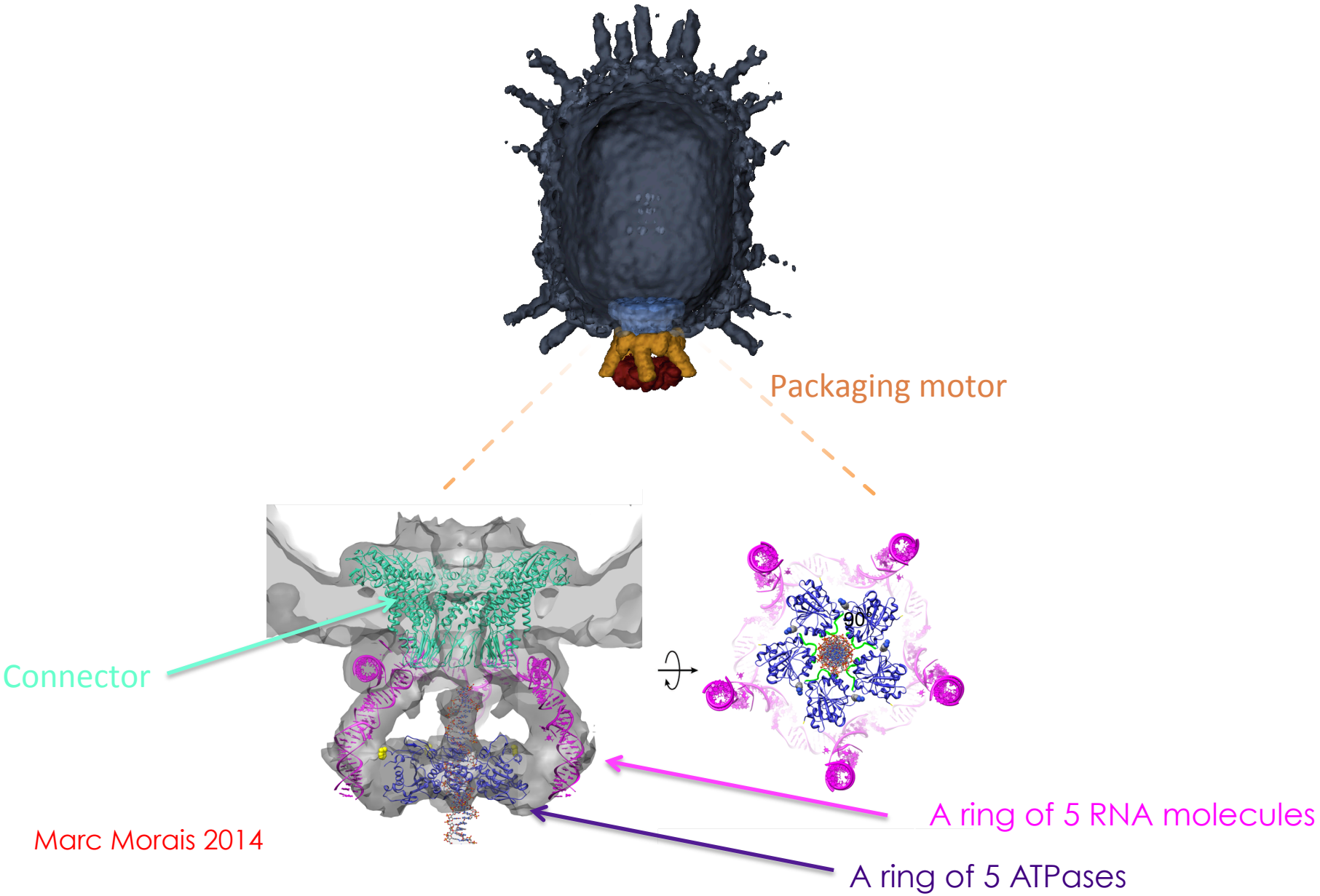


Liu, Chistol, Bustamante (2014)

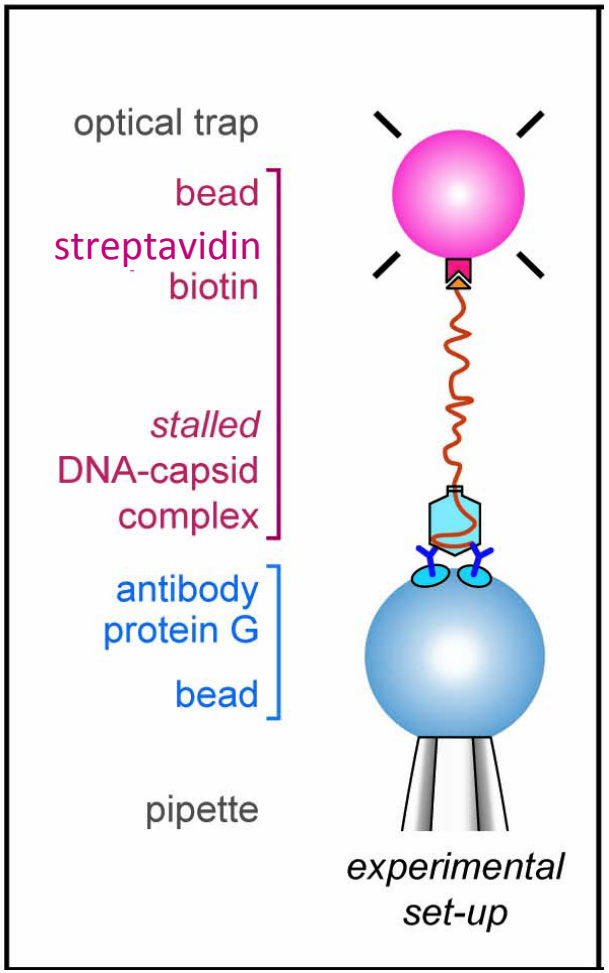
Replicative Cycle of a Virus



Bacteriophage ϕ29 Packaging Motor

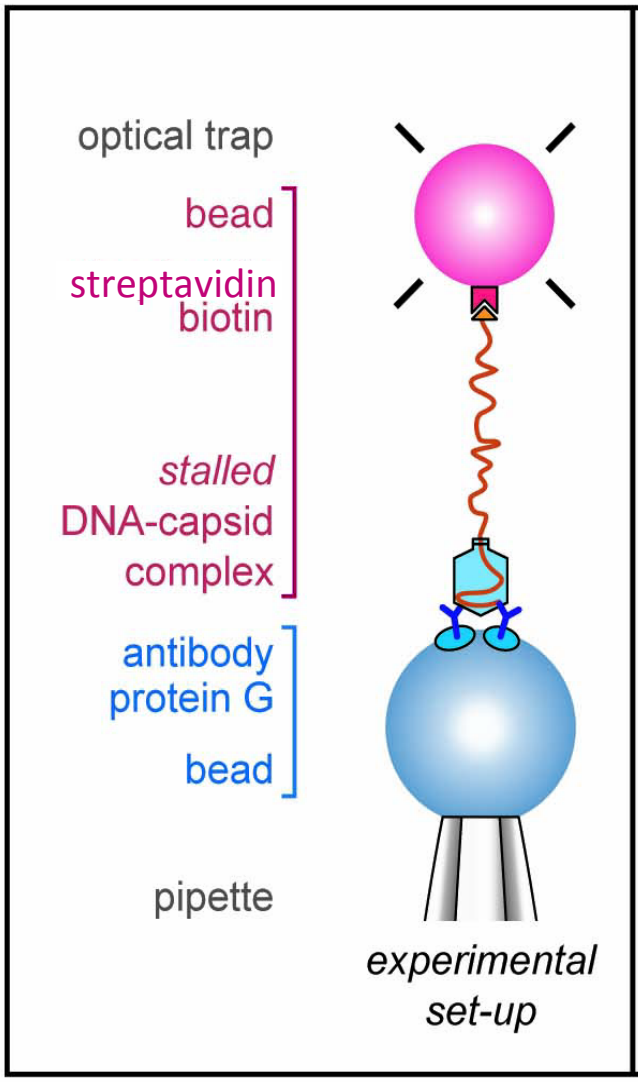


Experiment Set Up

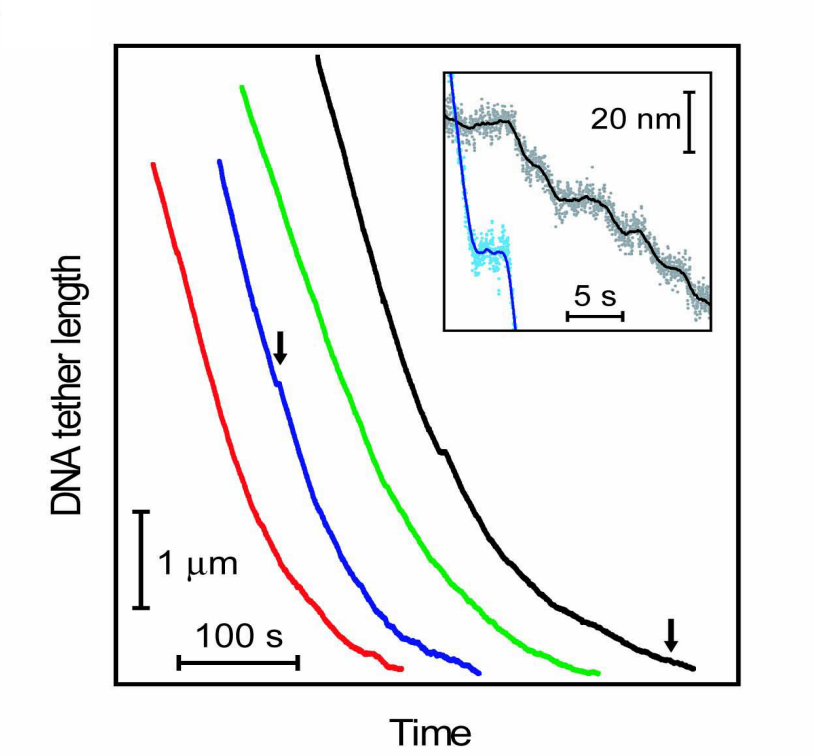


Packaging with no force feedback

Single Molecule Trajectories

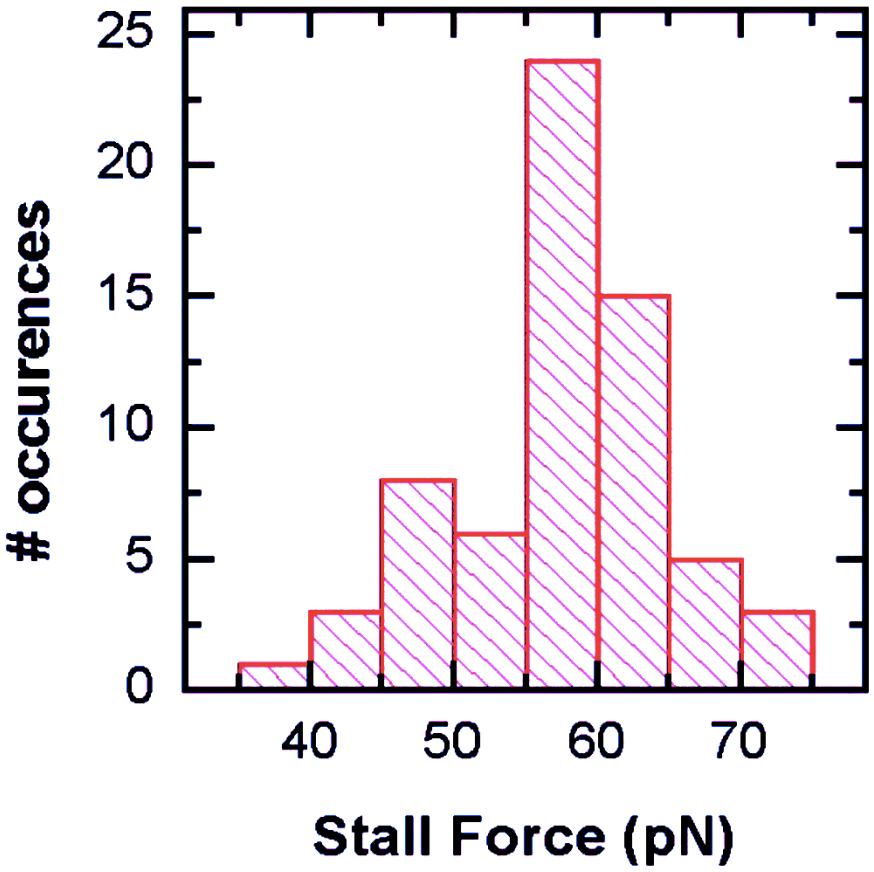


Initial slope: 100 bp/s



Smith *et al. Nature*, 2001

A Powerful Motor

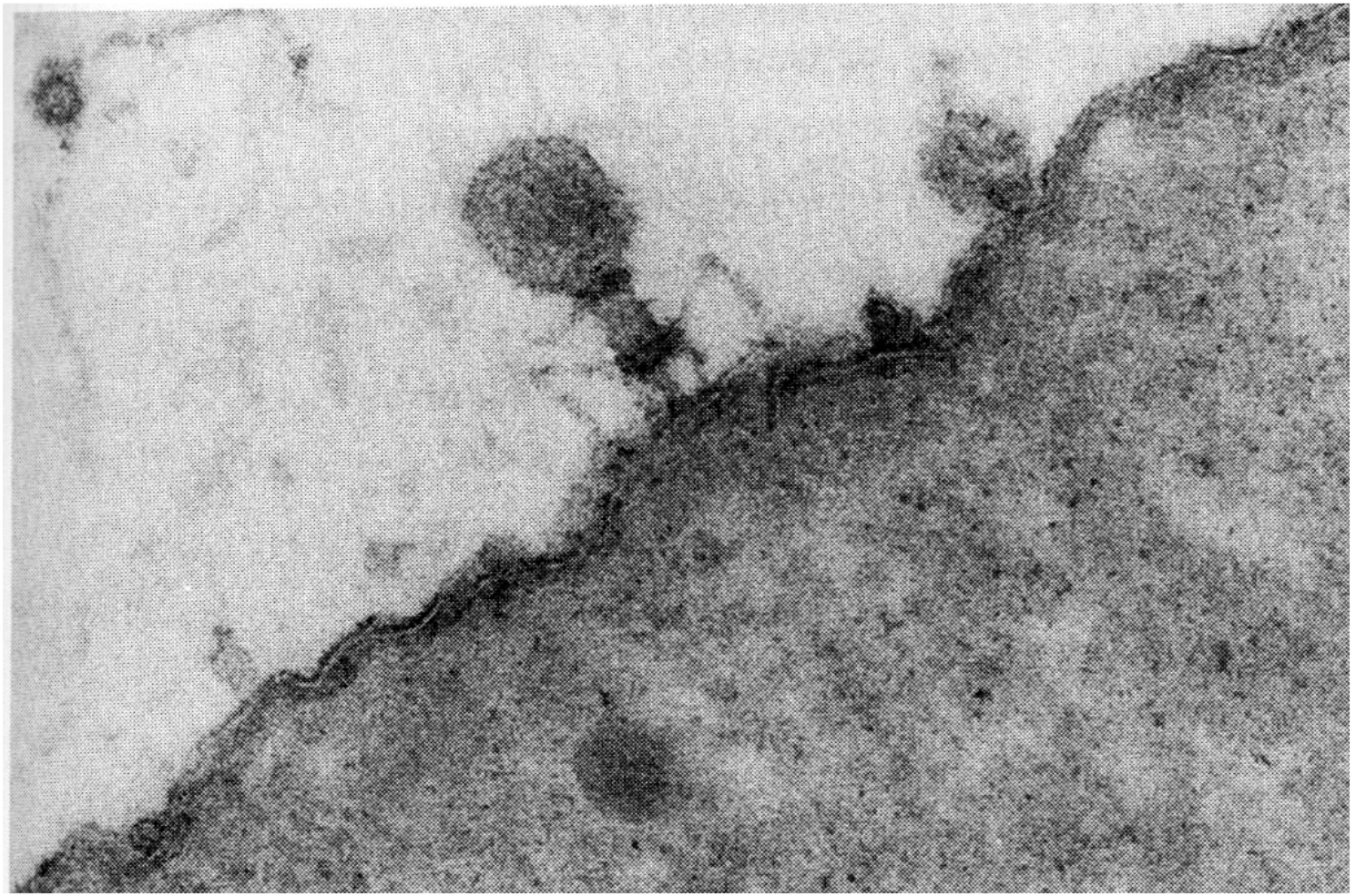


Average stall force = 55 pN

Maximum force measd. > 70 pN

The Internal pressure in the capsid reaches ~ 30 atm

How is this Pressure Used?



Thermodynamic Efficiency of the Motor

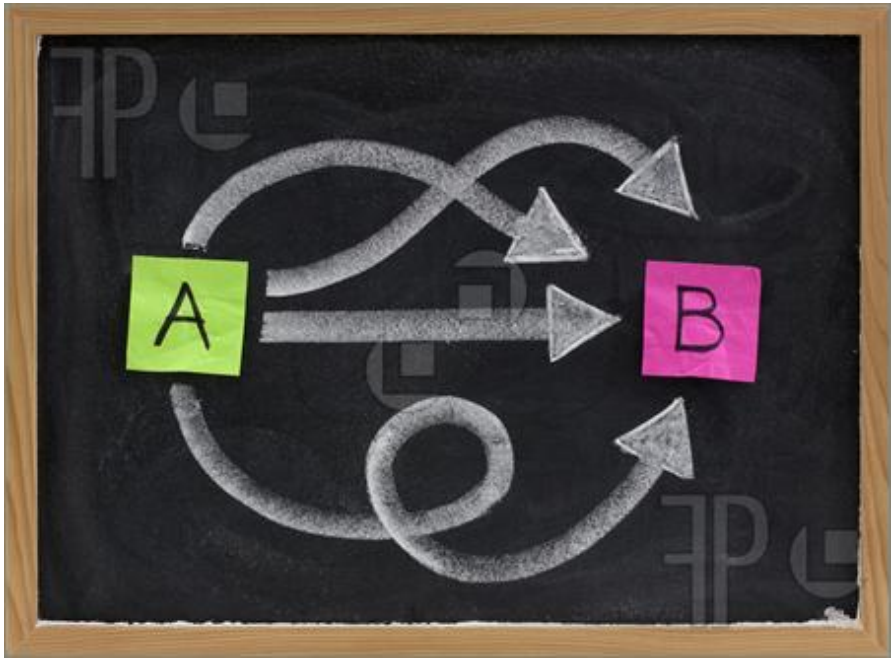
Efficiency = Work done near stall/Energy of ATP hydrolysis

Work done near stall: 70 pN x 2.5 bp x 0.34 nm/bp = 60 pN nm

ΔG is – 14Kcal/mol or 23.3 $k_B T$ or 95.7 pN nm

Efficiency at Stall ~ 63%

What is the Origin of the Large Thermodynamic Efficiency of these Machines?



What Controls the Amount of Dissipation in a Non-equilibrium Process

Formulation of Generalized Friction Coefficient Based on Linear Response Theory

Excess work relative to equilibrium

$$\langle \mathcal{P}_{\mathrm{ex}}(t) \rangle_{\Lambda} \approx \zeta(\lambda) \dot{\lambda}^2$$

The friction coefficient is defined as:

$$\begin{aligned} \zeta(\lambda) &\equiv \beta \int_0^{\infty} \langle \delta F(0)\, \delta F(t) \rangle_{\lambda} \mathrm{d}t \\ &= \beta \langle \delta F^2 \rangle_{\lambda} \tau_{\mathrm{relax}}(\lambda) \ . \end{aligned}$$

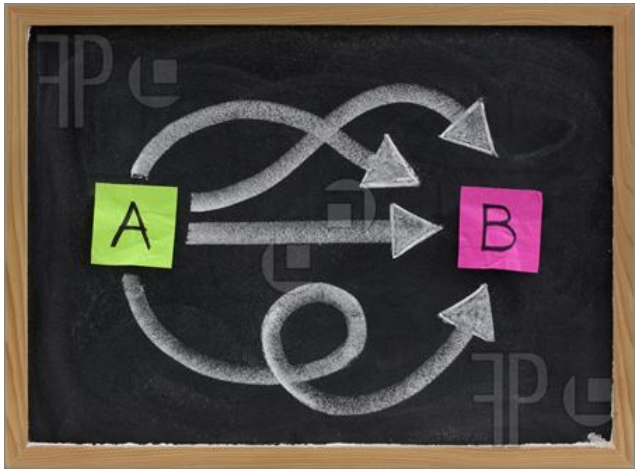
$$\tau_{\mathrm{relax}}(\lambda) \equiv \int_0^{\infty} \langle \delta F(0)\, \delta F(t) \rangle_{\lambda}\, \mathrm{d}t / \langle \delta F^2 \rangle_{\lambda}$$

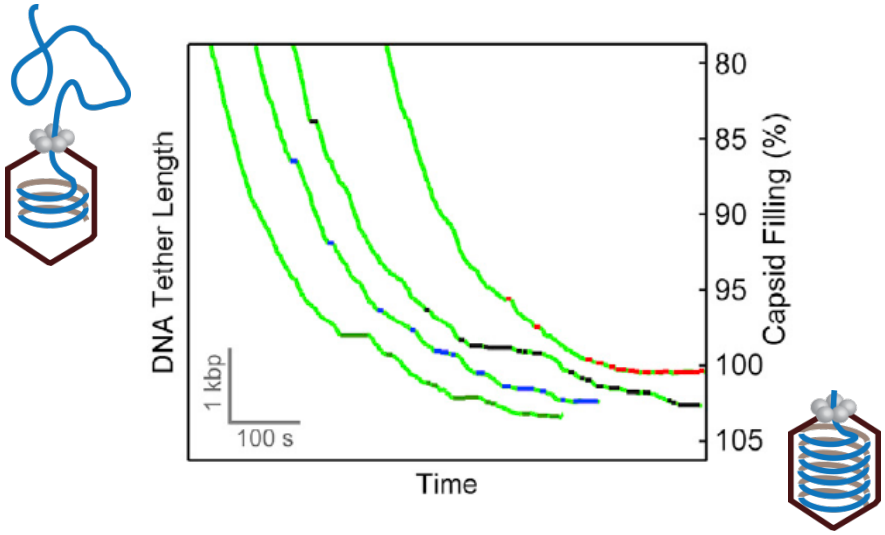
Protocol with the minimum dissipation is obtained by minimizing the excess work

$$\dot{\lambda}_{\mathrm{MD}} \propto \zeta(\lambda)^{-1/2}$$

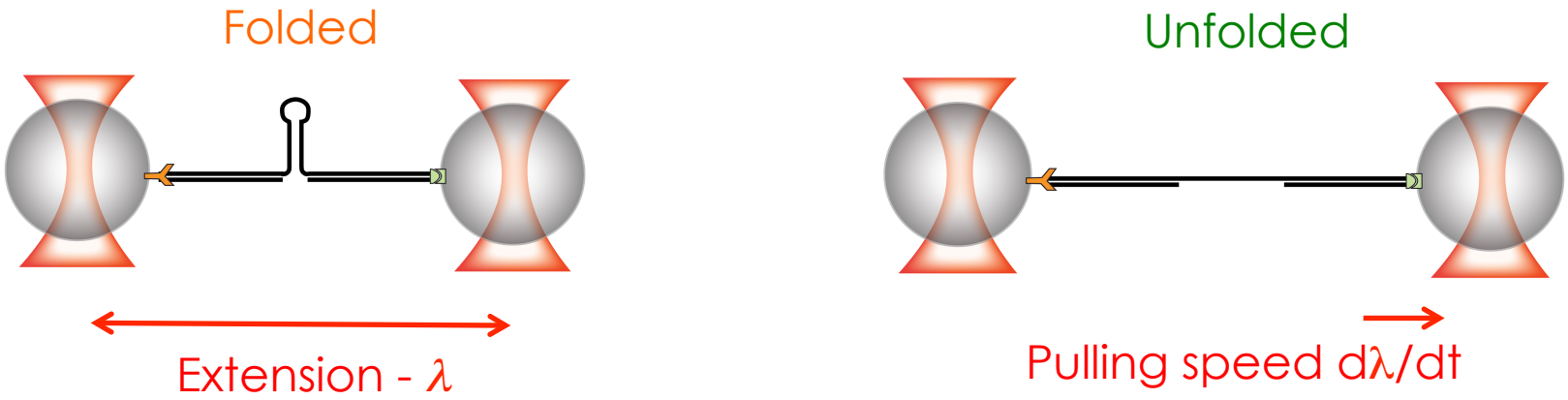
Sivak & Crooks 2012. Phys. Rev. Lett.
Sivak & Crooks 2016. Phys. Rev. E

Consider a Biological Processes Driving a System From State A to State B Through a Specific Path

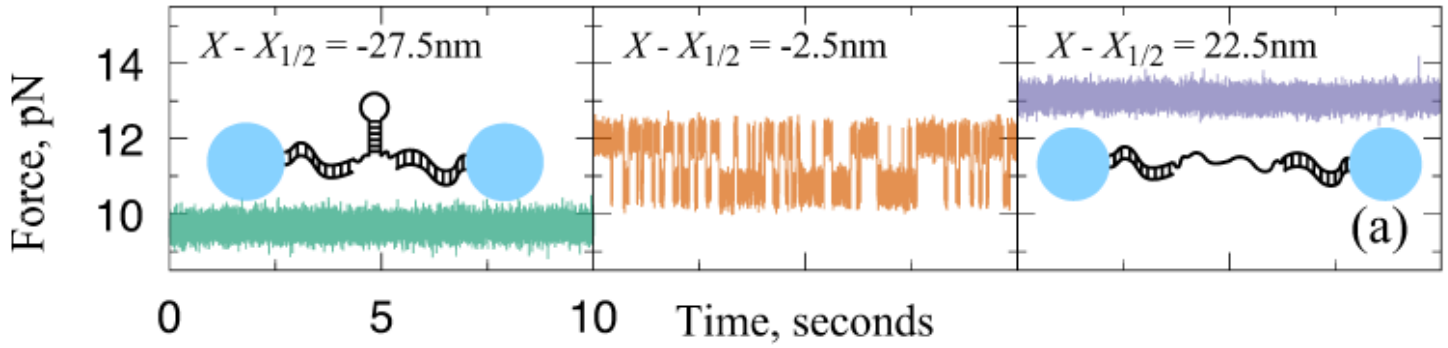


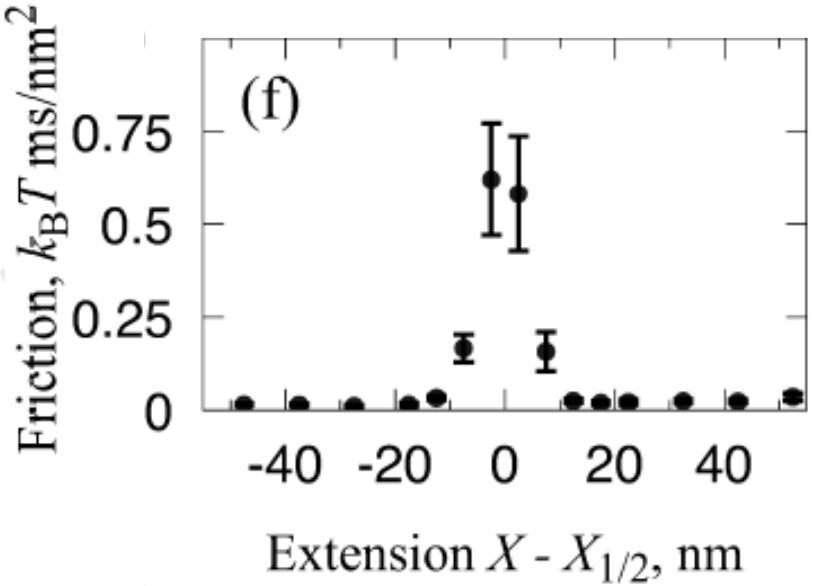


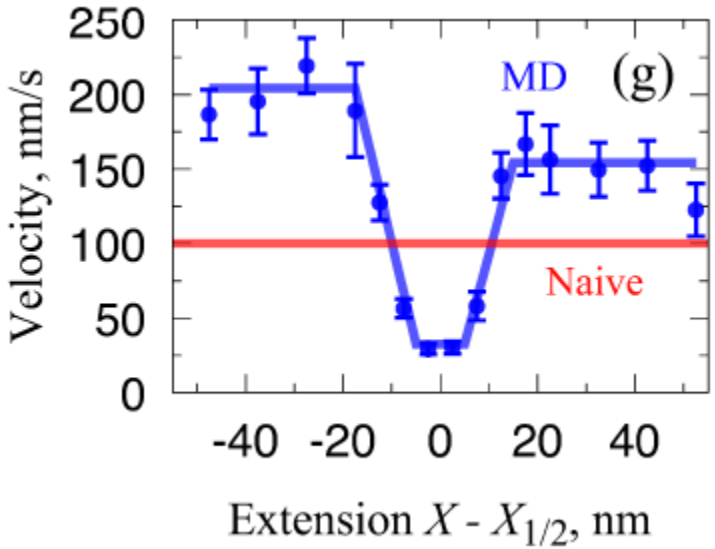
We Addressed This Problem with a Simpler Two-State System



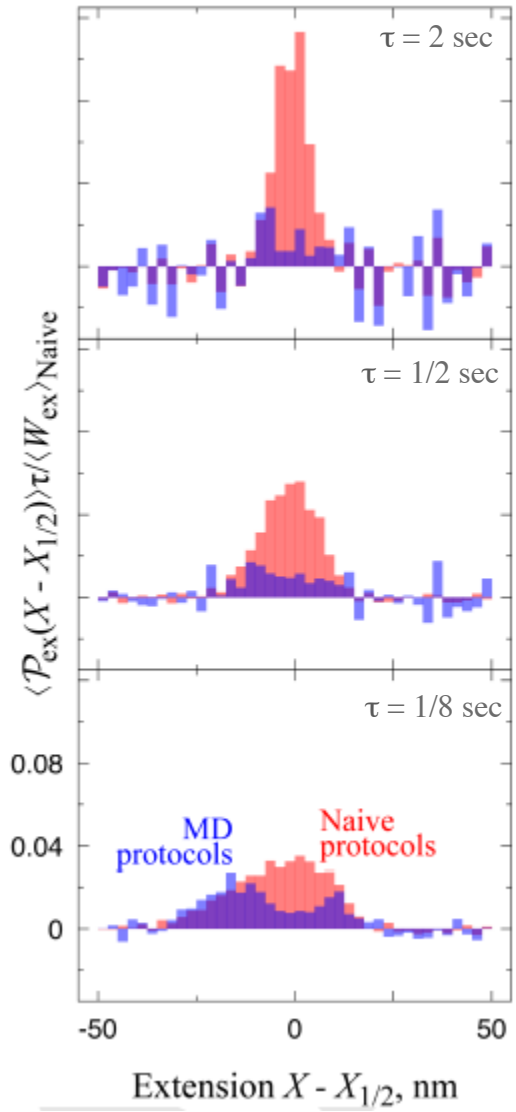
Determining the Friction Coefficient and MD Protocol Experimentally





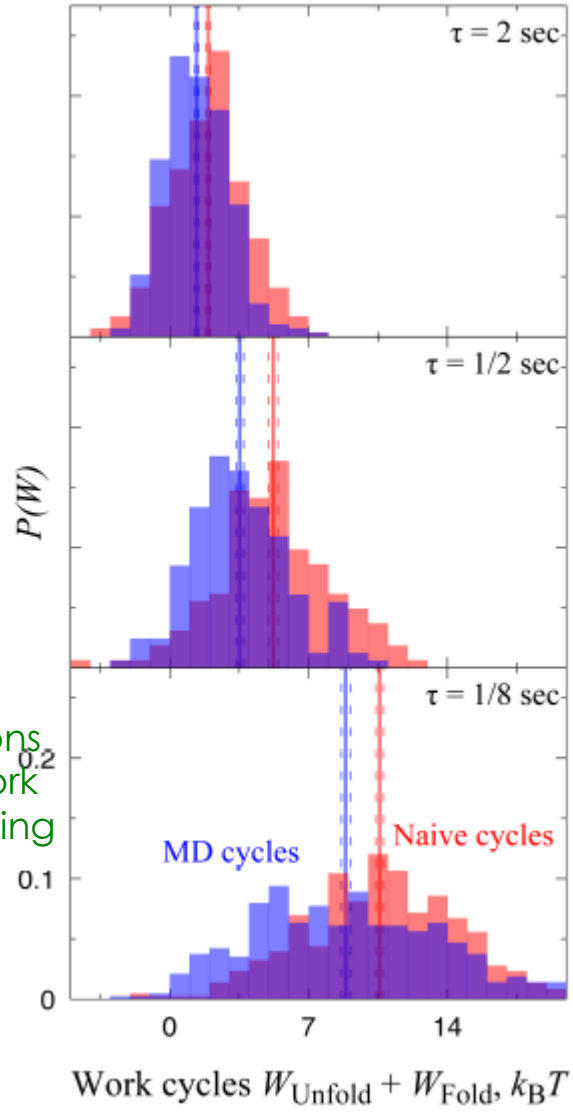


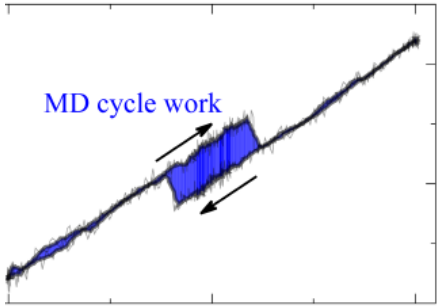
The MD Protocol Indeed Dissipates Less Energy than the Naïve One



Less hysteresis is Associated with MD protocols

Major contributions to the excess work occur in the hopping regime

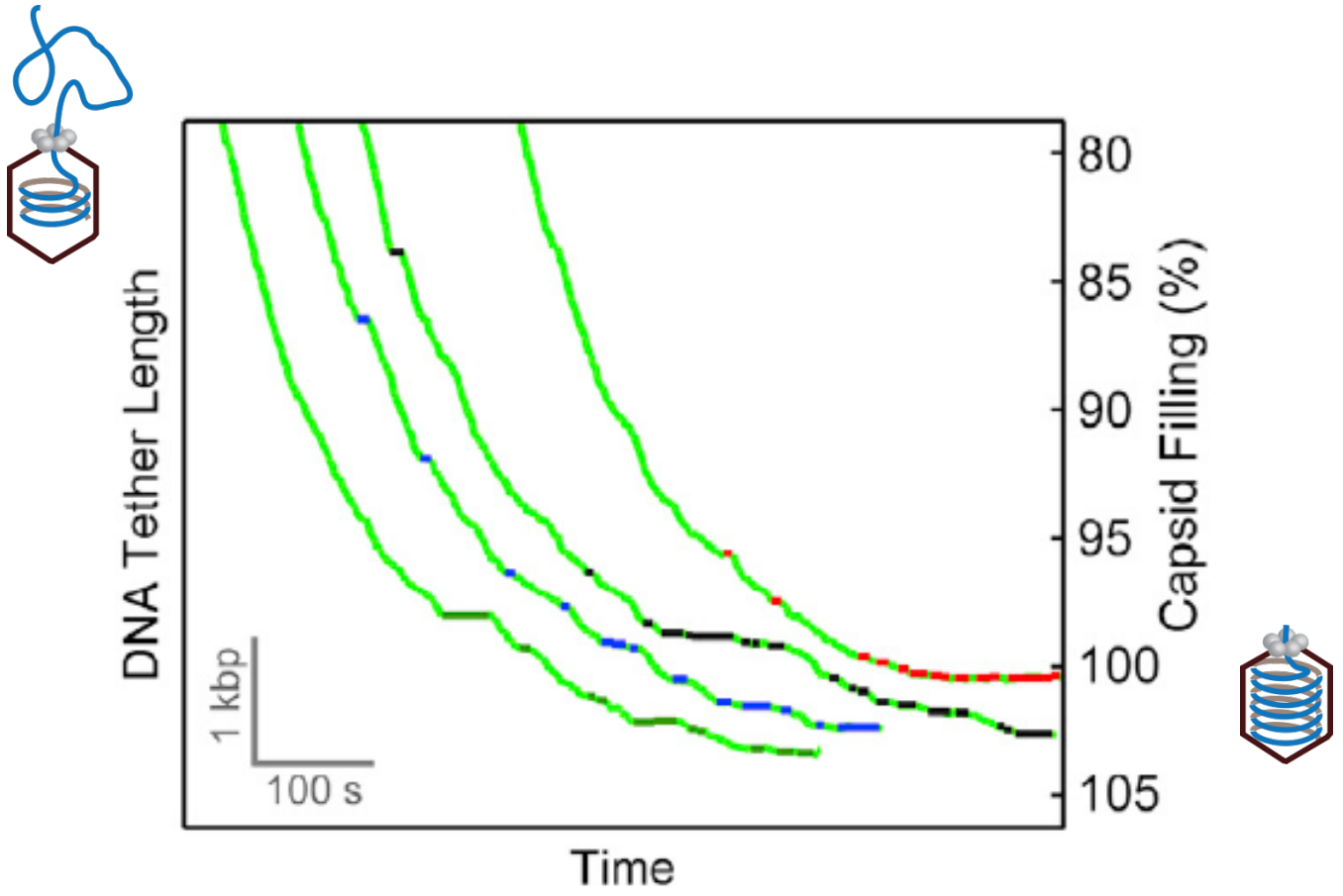




Less cycle work is required in the MD protocols. The further from equilibrium, the greater the benefit relative to naïve realizations

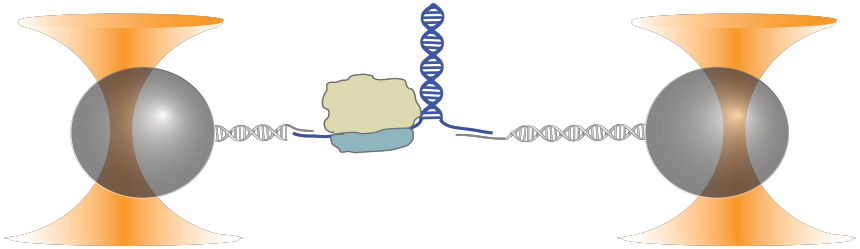
Thermodynamic efficiency

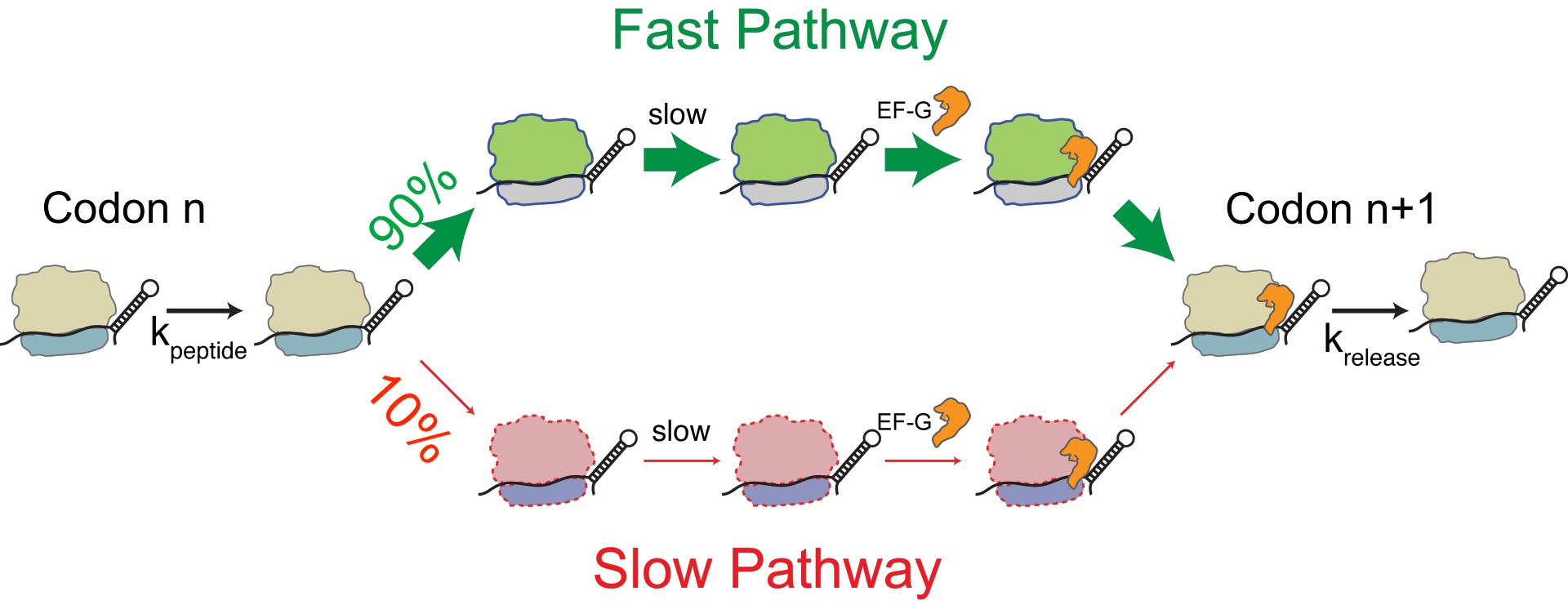
- Have molecular machines been evolved to harness energy into useful work, minimizing dissipation of heat?



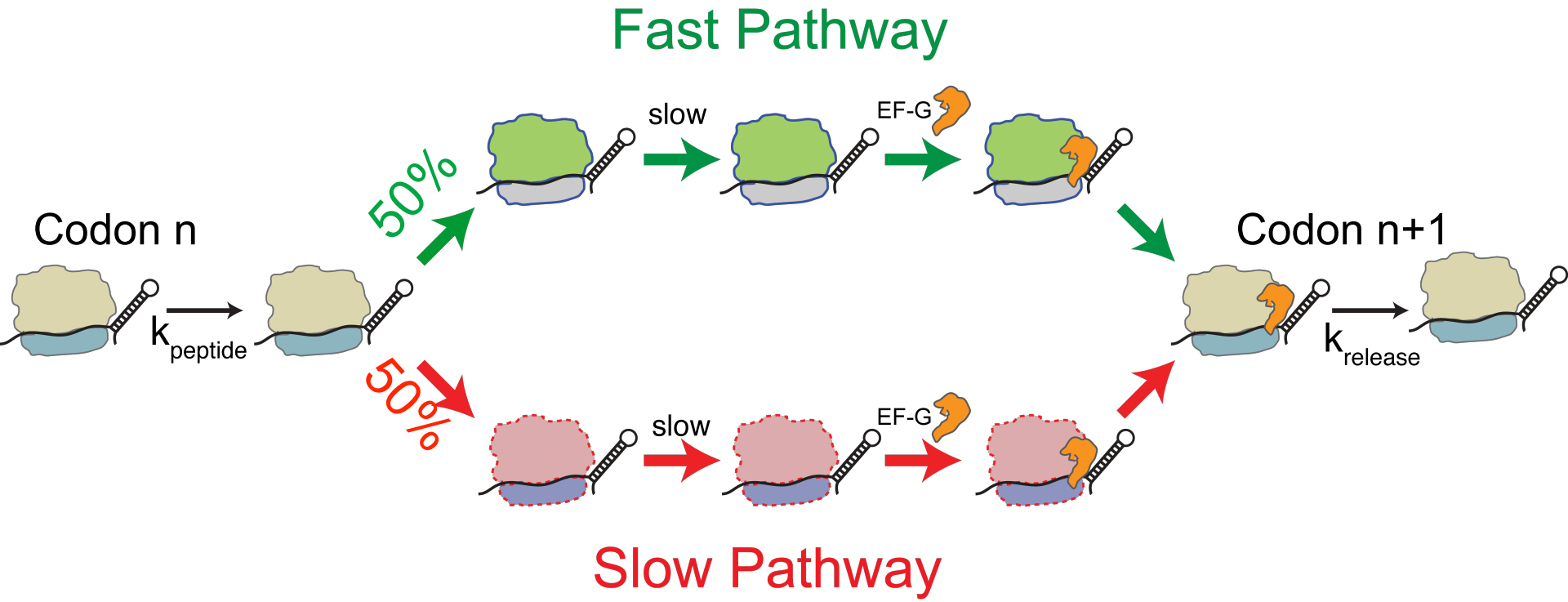
Liu *et al. Cell* (2014)

In front of a Weak Hairpin the Ribosome Translocates Predominantly via the Fast Pathway



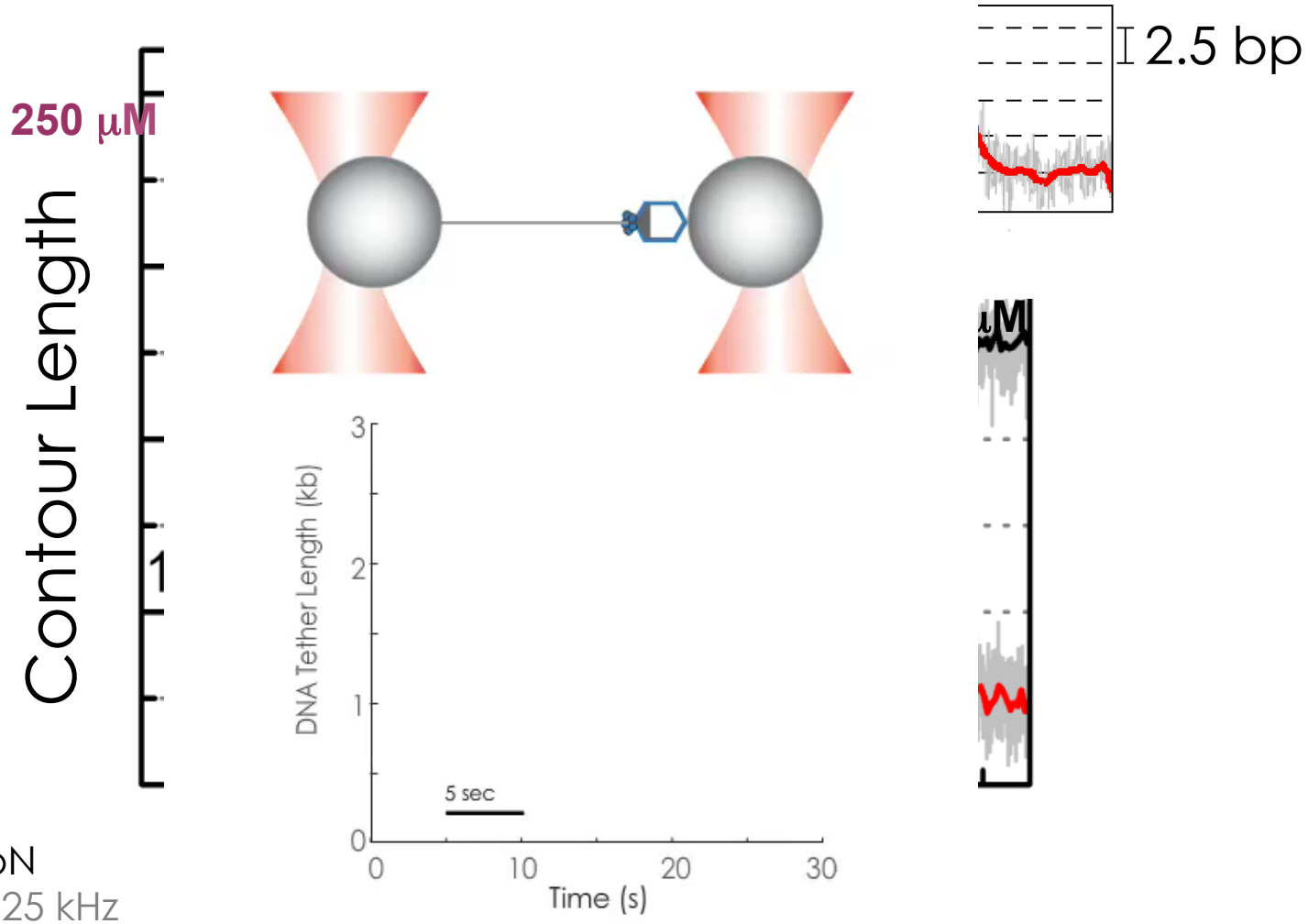


In Front of a Strong Hairpin the Ribosome Translocates Predominantly via the Slow Pathway



Stronger secondary structures bias ribosomes into a slower pathway perhaps to give the ribosome time to unwind the hairpin without multiple attempts?

Molecular Trajectories



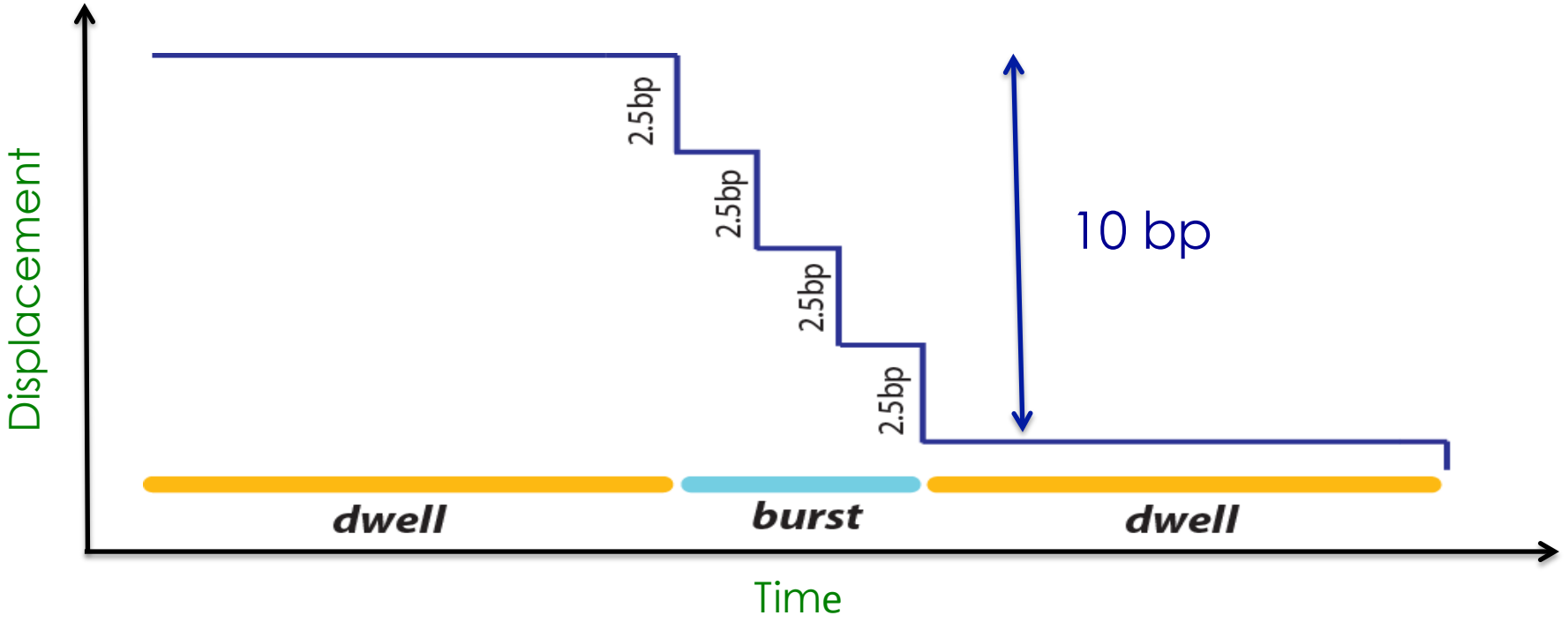
F = 6-8pN
50Hz, 1.25 kHz

Moffitt *et al. Nature* 457, 446-450 (2009)

ϕ29 Packaging Motor

The motor operates in cycles of dwells and 10 bp bursts

Each 10 bp burst is made up of four 2.5 bp steps:

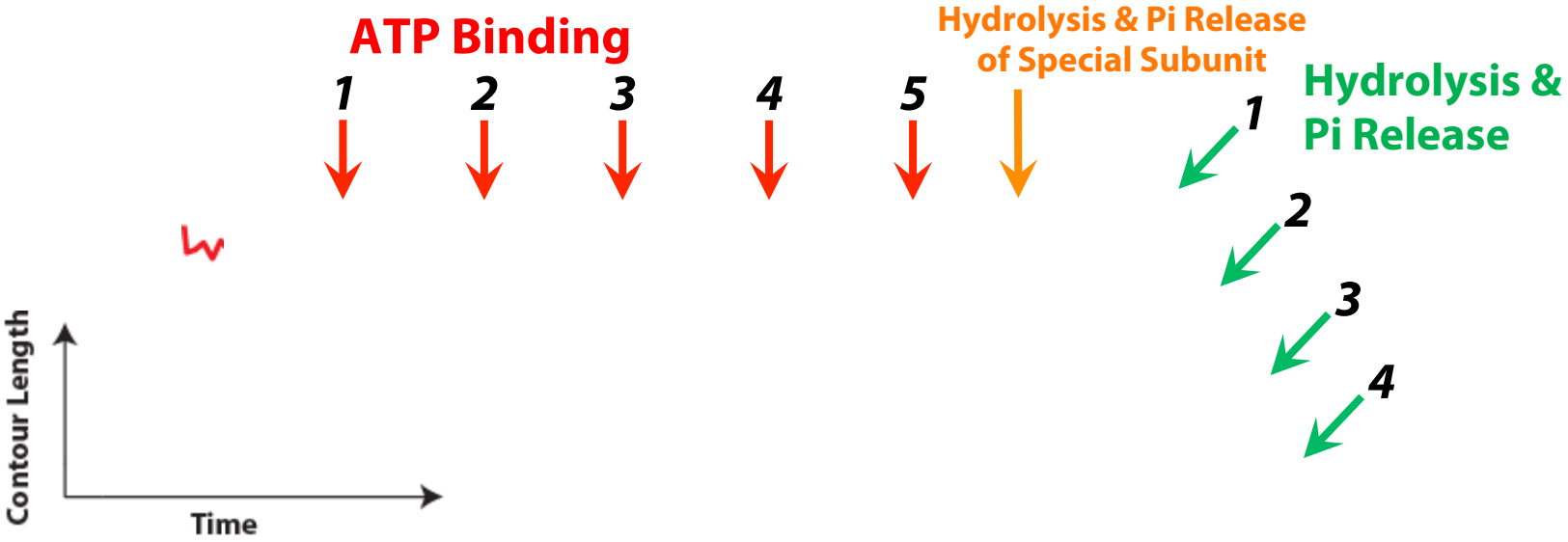


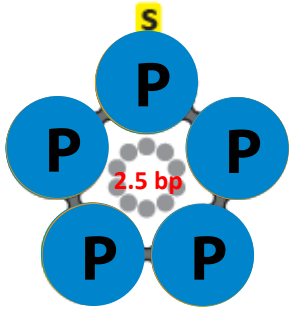
One of the subunits is distinct and does not perform a mechanical task but a regulatory one

Moffitt *et al. Nature* (2009)

What we Have Learned About this Motor

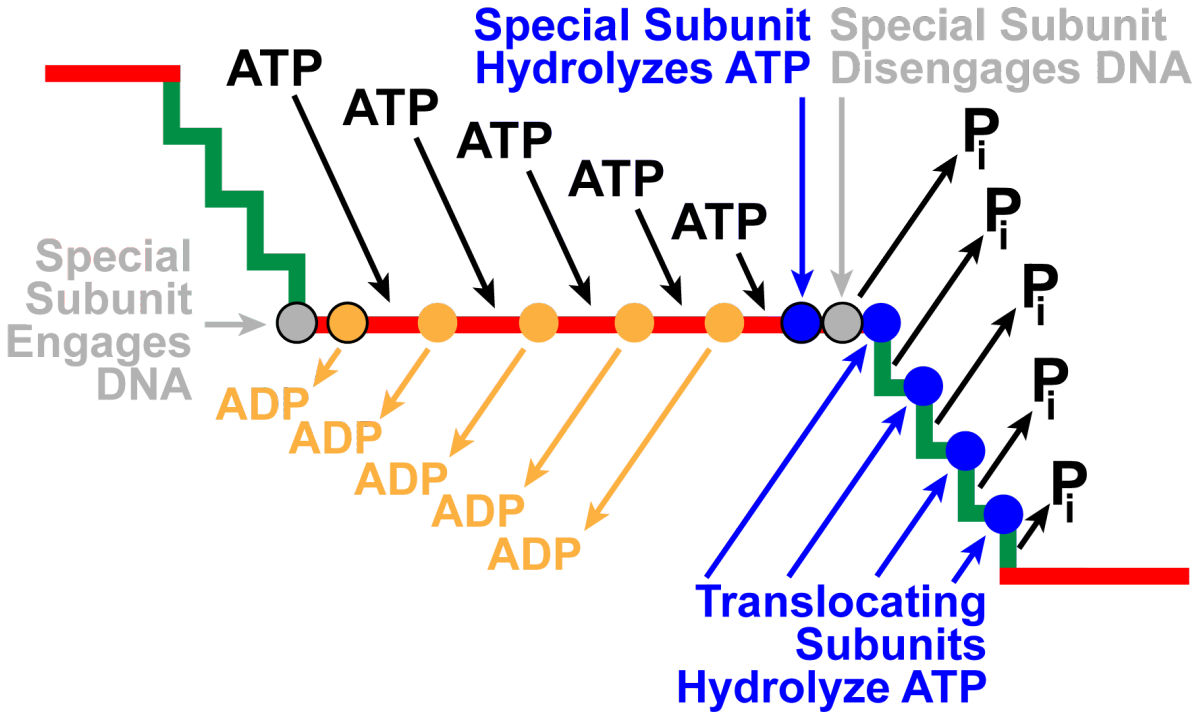
1. A very powerful motor: it can generate forces up to 70 pN
2. The power stroke coincides with Pi release
3. The ATPase activities of subunits are highly coordinated
4. Packaging occurs in cycles of alternating dwells (avg. duration ~ 80 ms during which ATP binding occurs) and bursts (10 bp, made up of four 2.5 bp steps)
5. The motor makes critical electrostatic contacts with the DNA every ten phosphates, while translocation involves steric contacts between motor and DNA
6. The electrostatic contact has both load-bearing and regulatory functions
7. One subunit is "special": it doesn't carry out a mechanical task


ATP Binding
1
2
3
4
5
Hydrolysis & Pi Release
of Special Subunit
1
Hydrolysis &
Pi Release
2
3
4
Contour Length
Time


S
P
P
P
2.5 bp
P
P

The Complete Mechano-chemical Cycle

Even though only 4 subunits translocate the DNA the 5th subunit also binds and hydrolyzes ATP.



Chistol et al. Cell, 2012

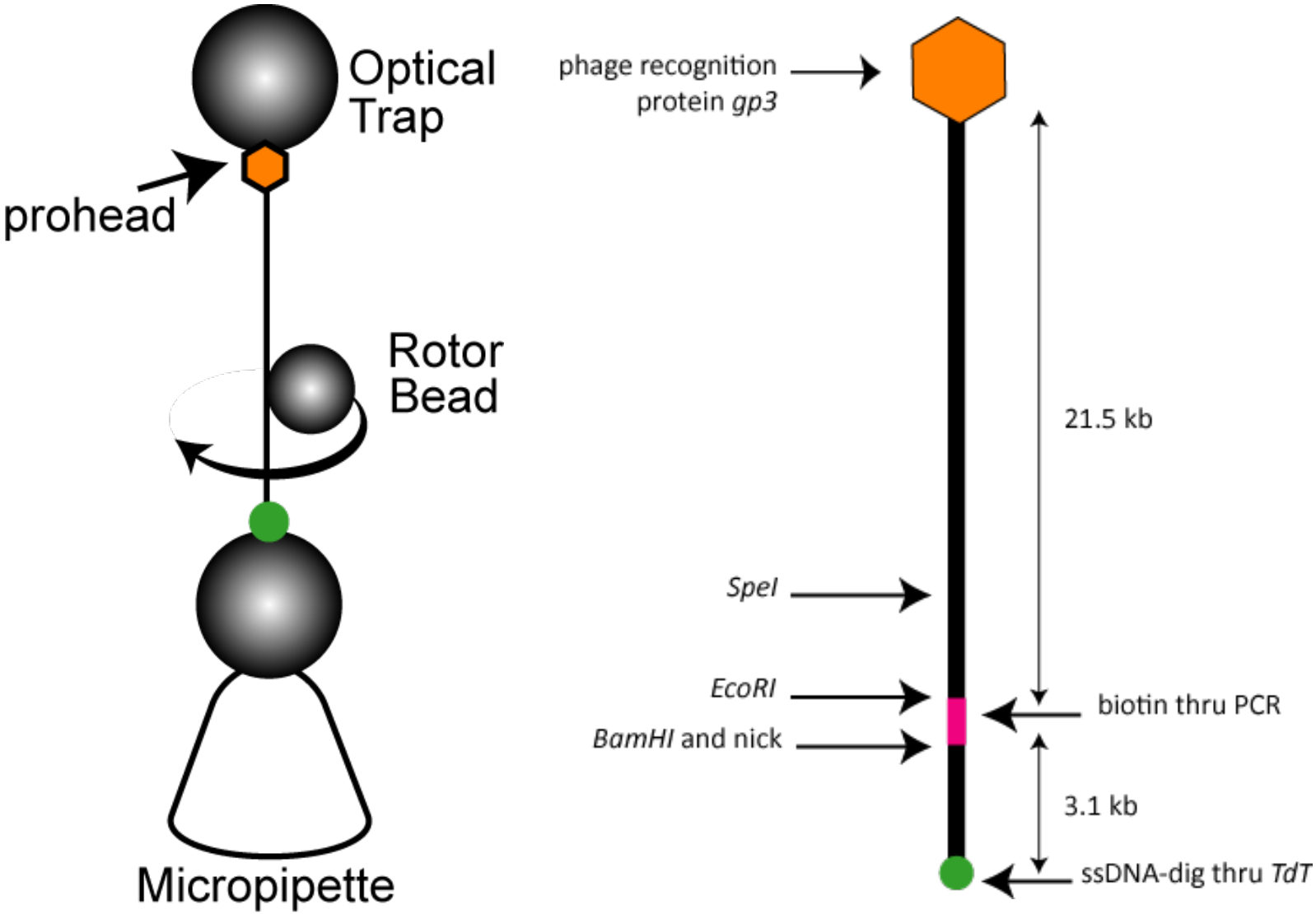
What Breaks the Symmetry of the Ring?

And is the special subunit the same cycle after cycle or does it change?

We found the answer while asking a completely different question:

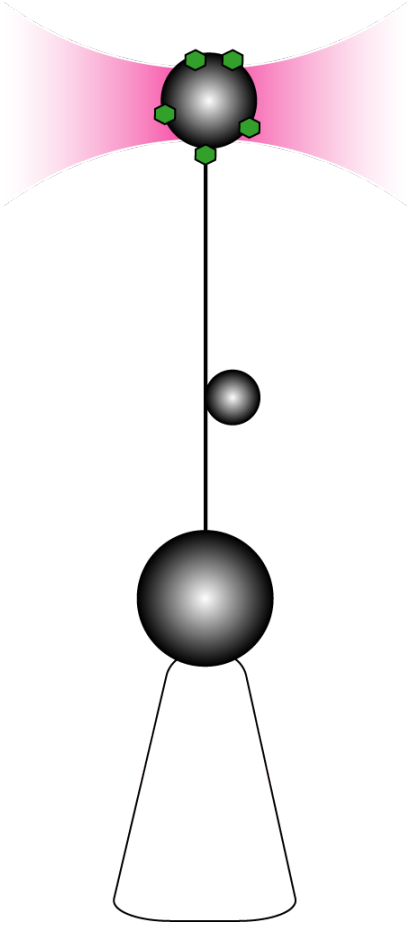
Does the motor rotate the DNA while it translocates it?

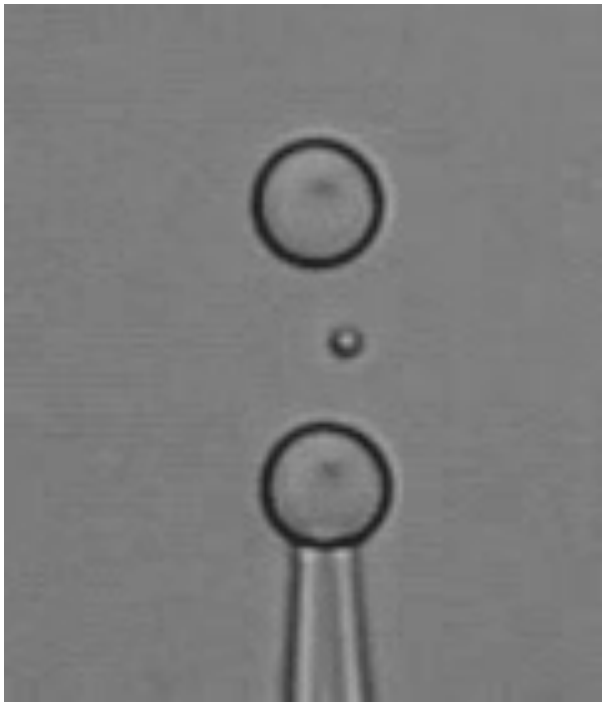
Does the Motor Rotate the DNA?



Does the motor generate torque?

Measuring the Twisting of the DNA



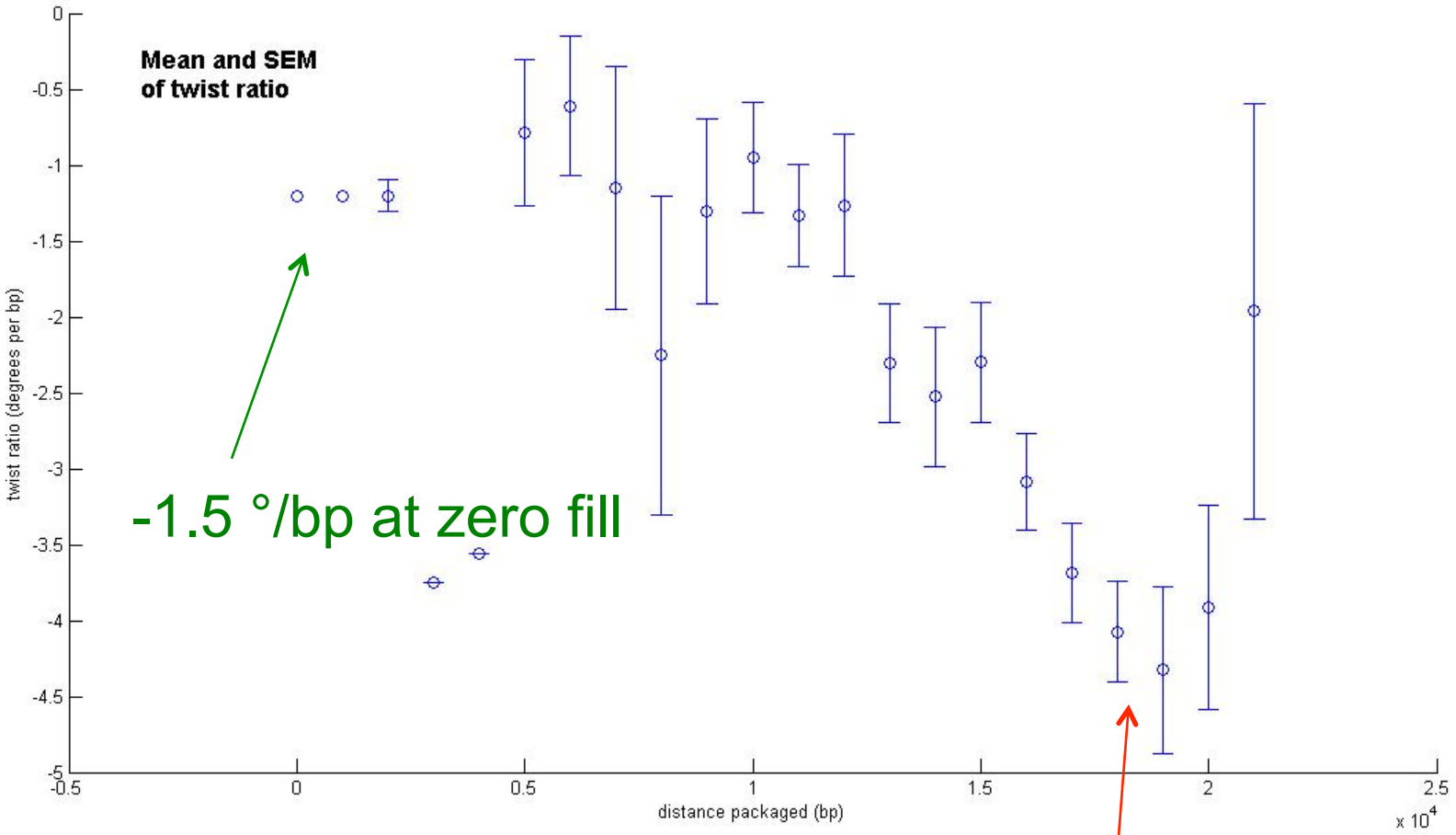


Craig Hetherington

2x
video
10 pN

Liu *et al. Cell*, **157**: 702-713 (2014)

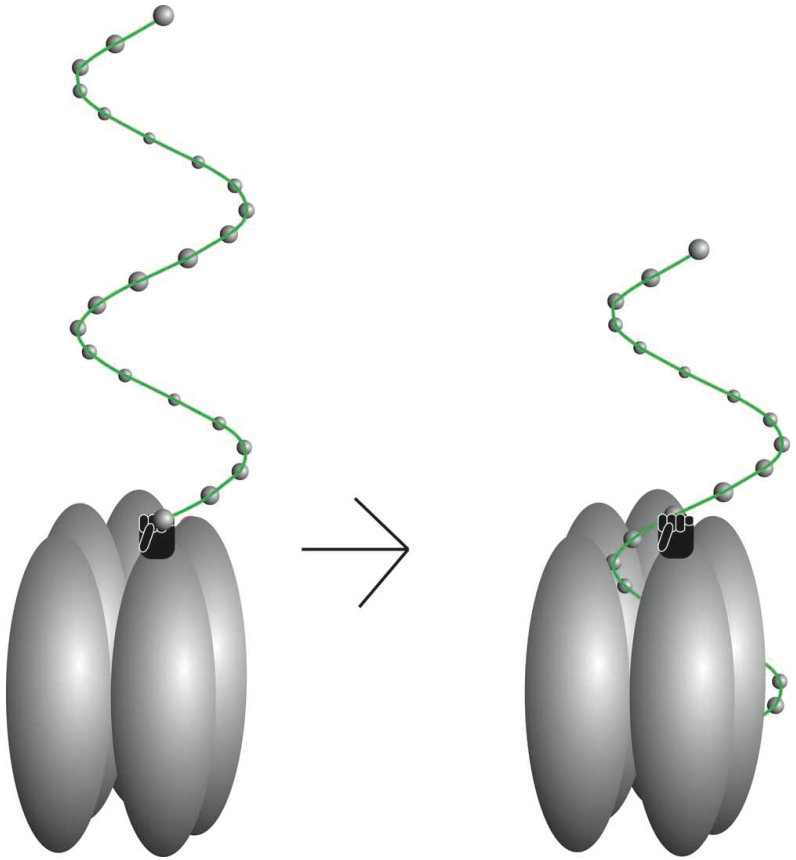
Rotation at Low Filling

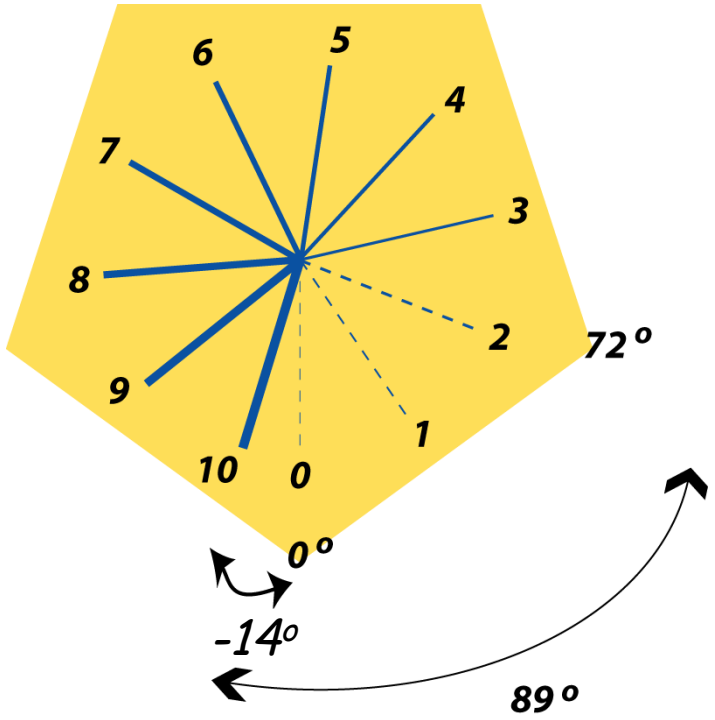


-1.5 °/bp at zero fill

-4.5 °/bp at high fill

Rotation Expected for 10-bp Step



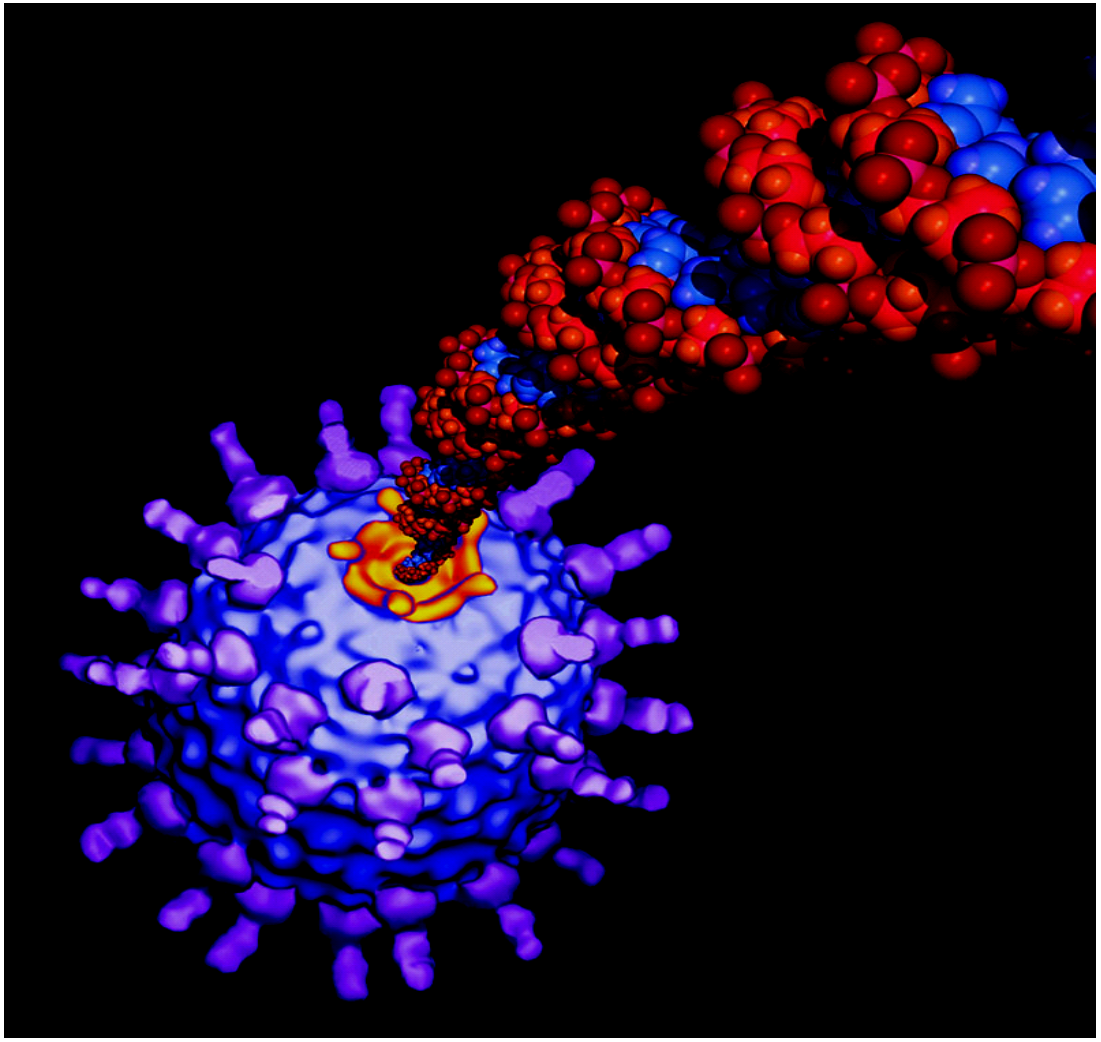


DNA pitch = 10.4 bp/turn
After a 10 bp burst, the DNA backbone has moved 346°

So the DNA is out of phase relative to the motor by -14° per burst

Current Model

Acknowledgements



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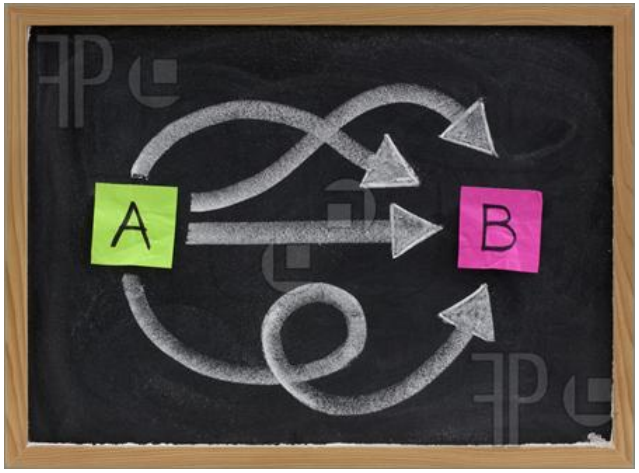
Recent Theoretical Work Formulates a Generalized Friction Coefficient to Quantify Energy Efficiency in Non-equilibrium Processes.

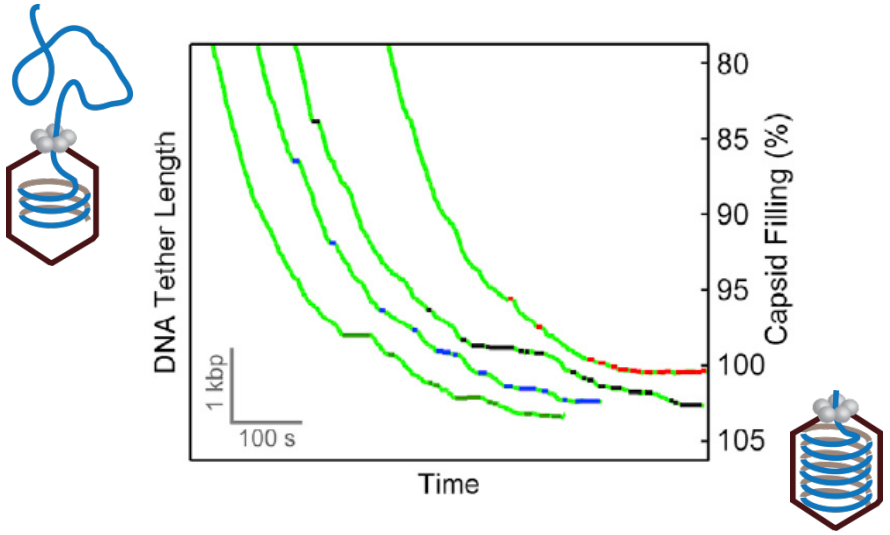
What is a Generalized Friction Coefficient?

1. Intuitive Description

2. Formal Description

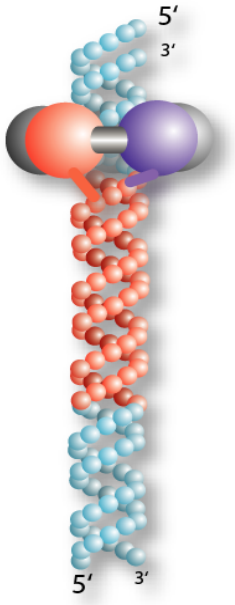
Consider a Biological Processes Driving a System From State A to state B Through a Specific Path

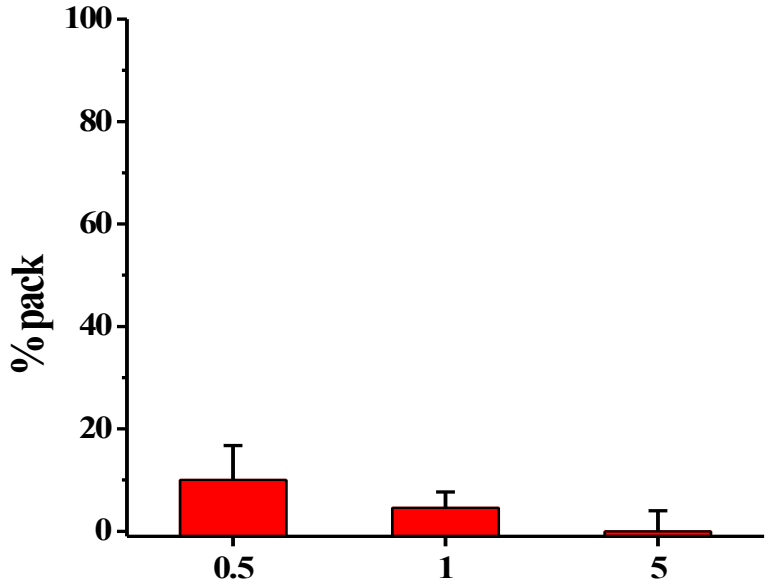




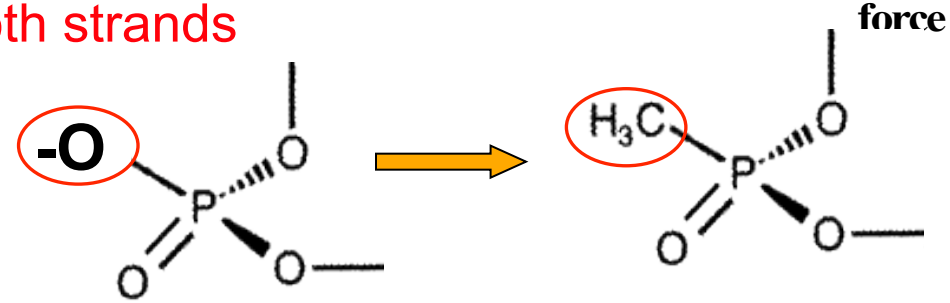
How Does the Motor Grab the DNA?

30 bp





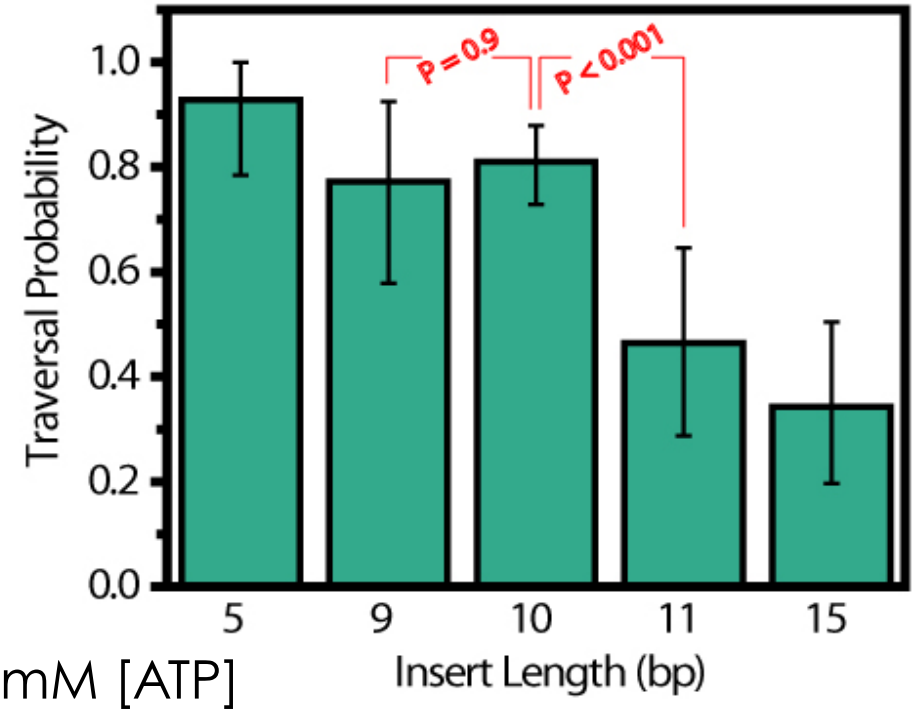
Mep30-both strands



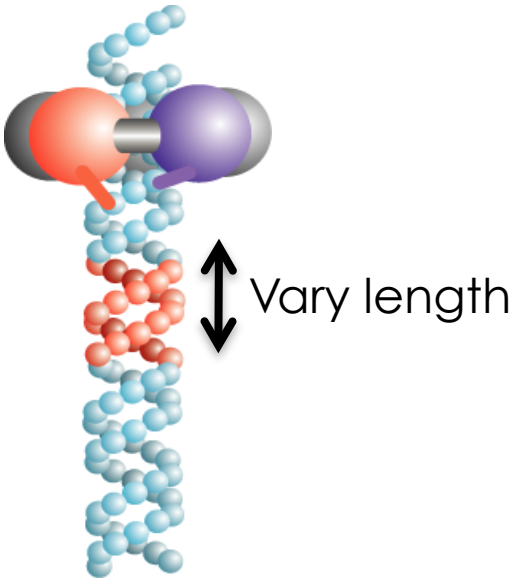
Methyl phosphonate DNA mimic

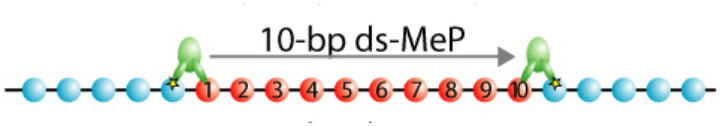
* Aathavan K. *et al. Nature,* 461, 669 – 873 (2009)

The Motor Needs to Make a Critical Contact with a DNA Phosphate Every 10 bp



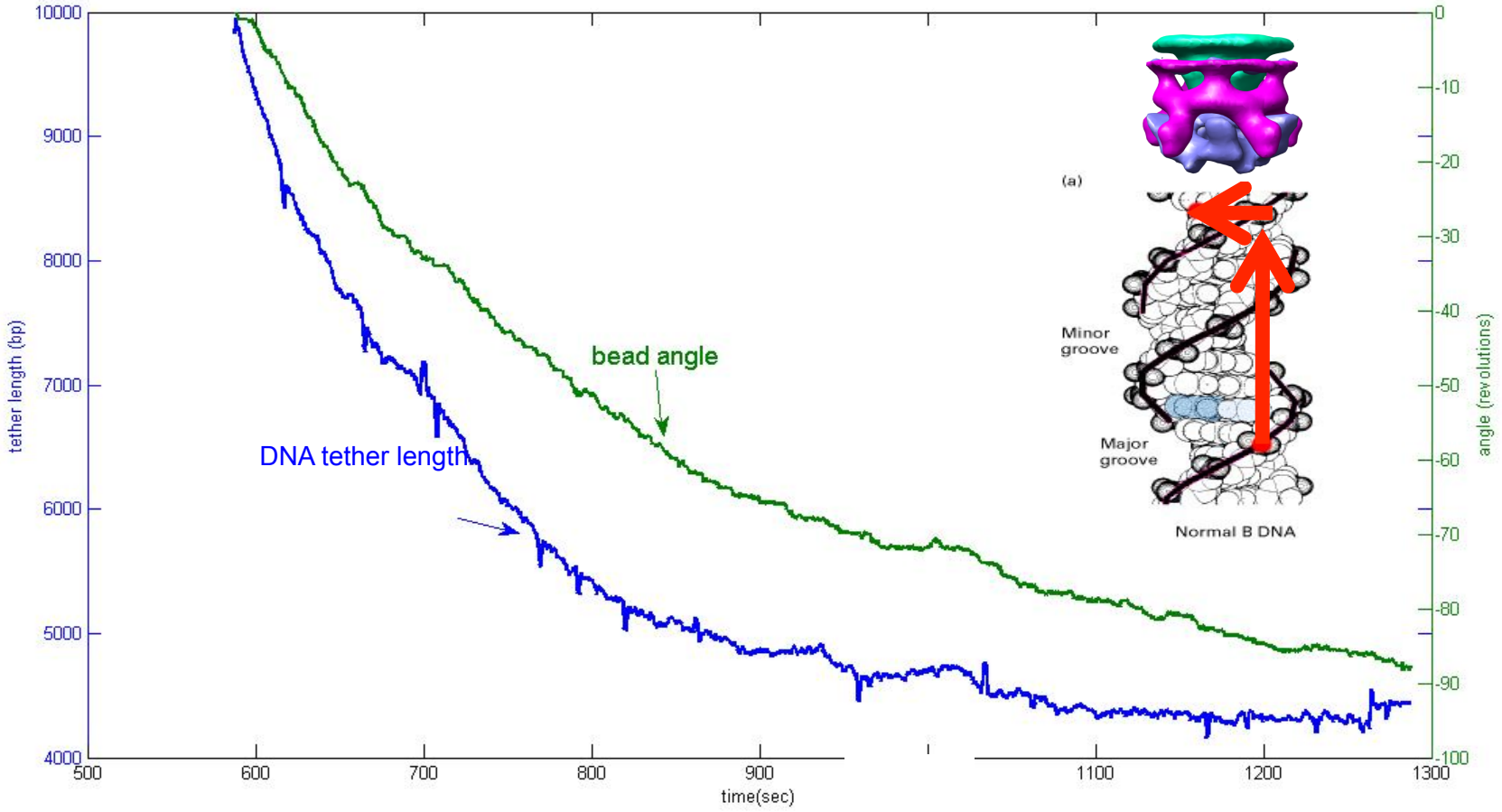
1 mM [ATP]
5 pN





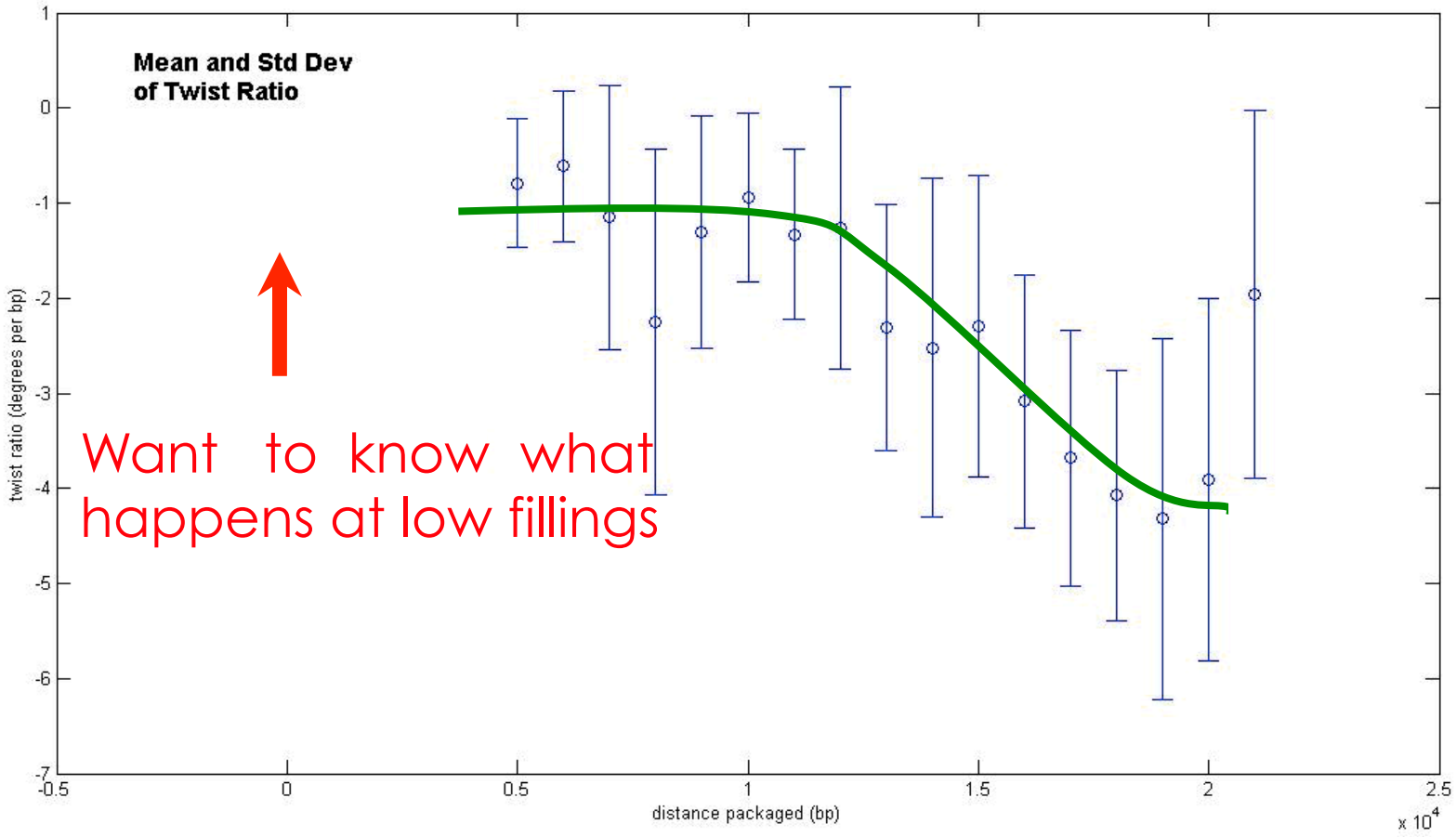
Important phosphate contacts are made by the motor every 10-bp
These contacts appear to have a regulatory function. At high res. the motor is seen to pause and stay there long in their absence.

Twist is in Direction of DNA Unwinding

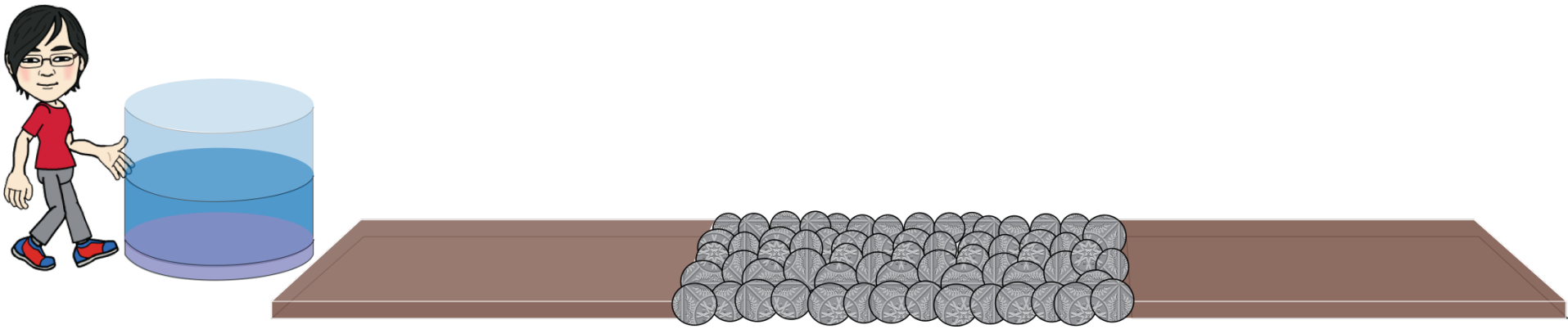


The motor can exert a max torque of ~ 10 p-nm

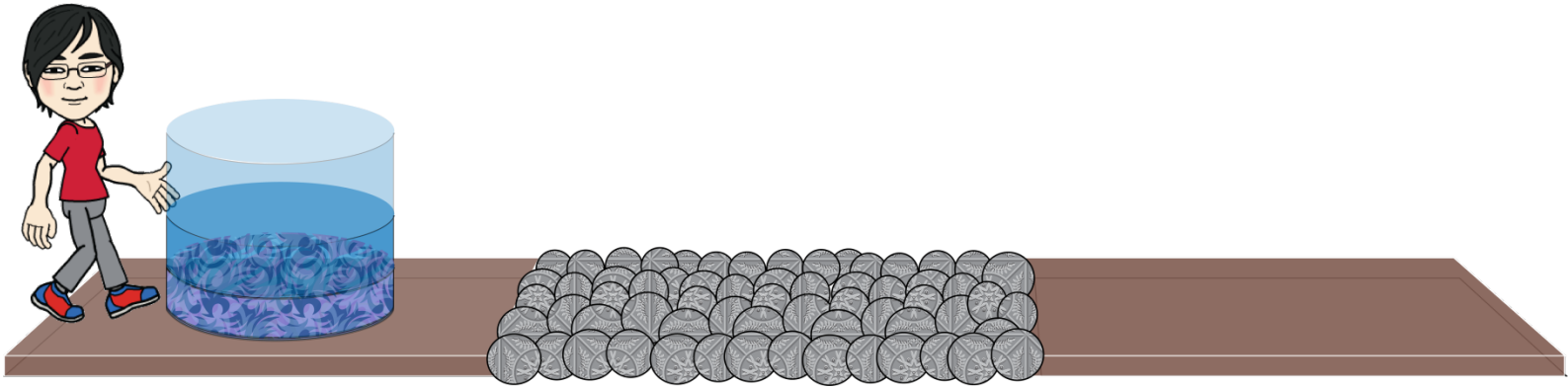
Local Twist Ratio Trend Shows Effect of



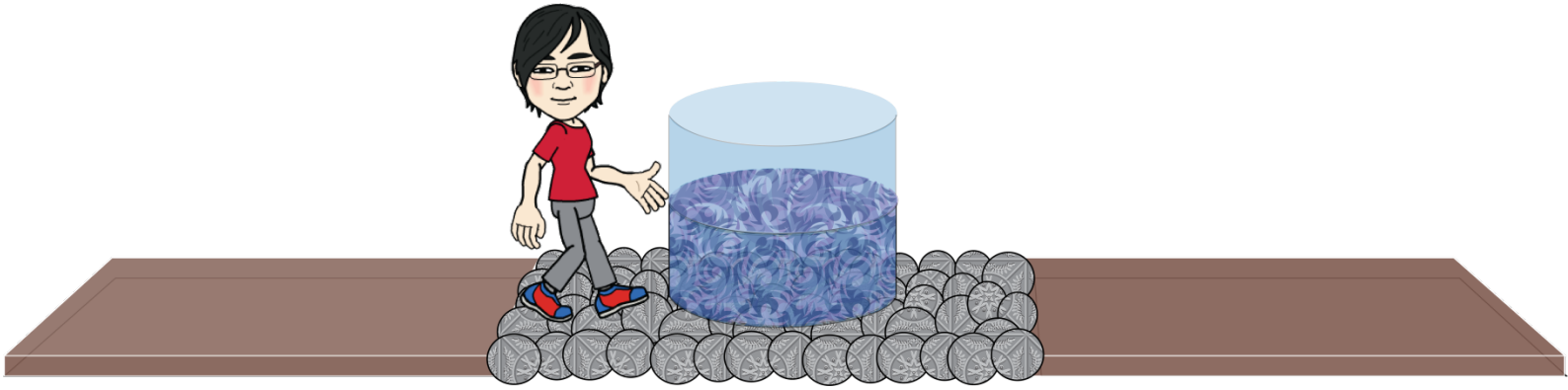
Person X is Sliding a Bucket of Water and a Paint Sediment …



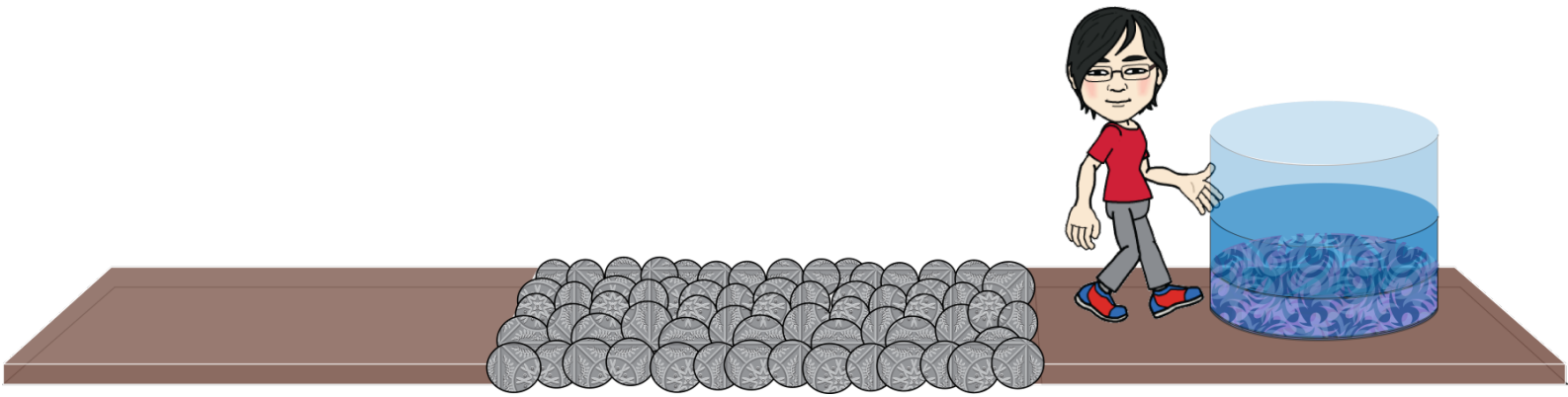
In the Smooth Surface the Paint and Water Will Mix a Little



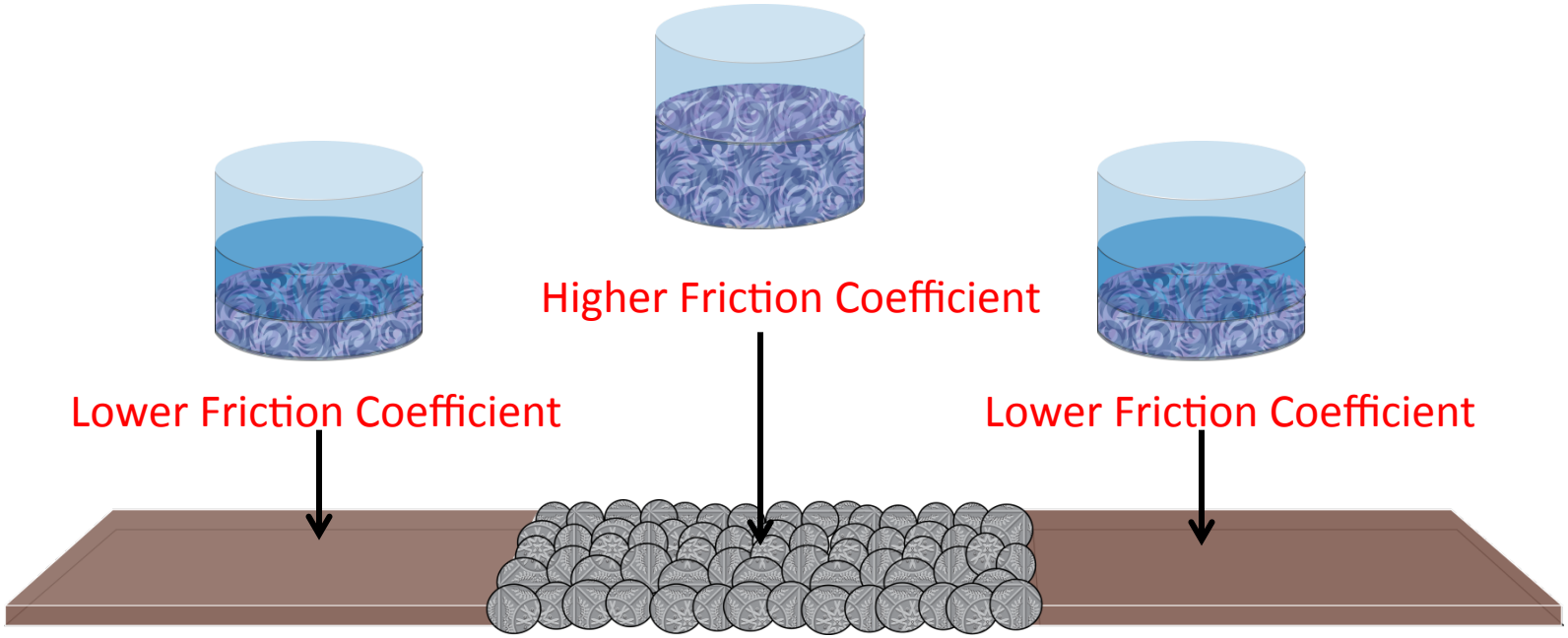
In the Rough Surface the Paint and Water Will Mix Substantially



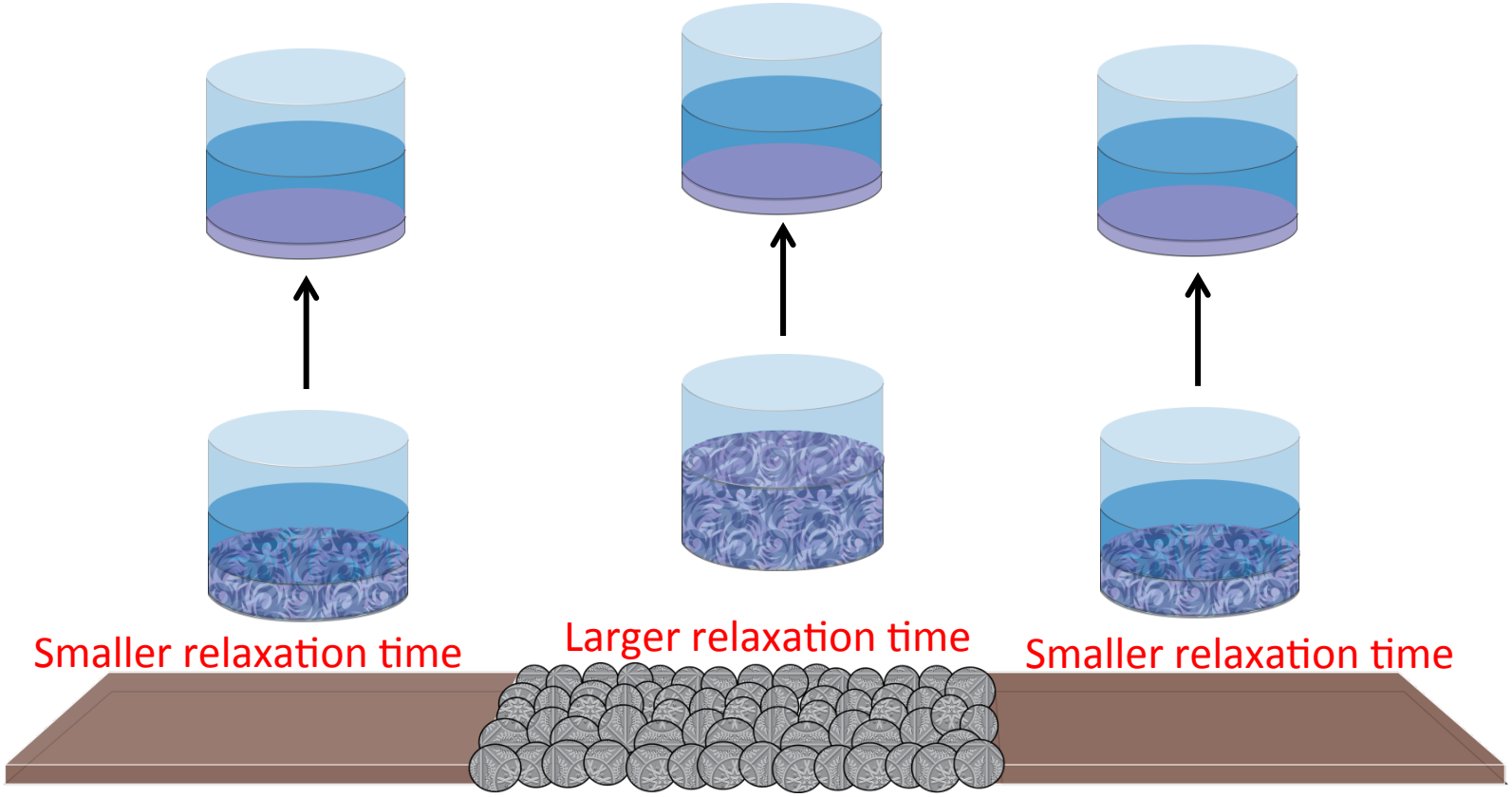
Going Back to the Smooth Surface Will Make the Paint Sediment Again



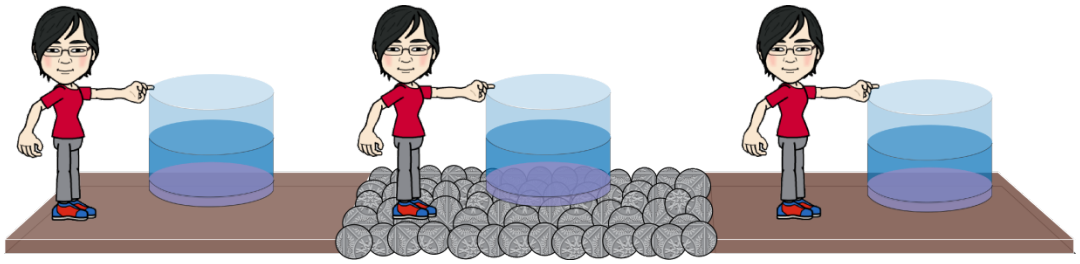
How Far From Equilibrium? It Depends on the Characteristics of the Surface



The Higher the Friction Coefficient the Larger the Relaxation Time

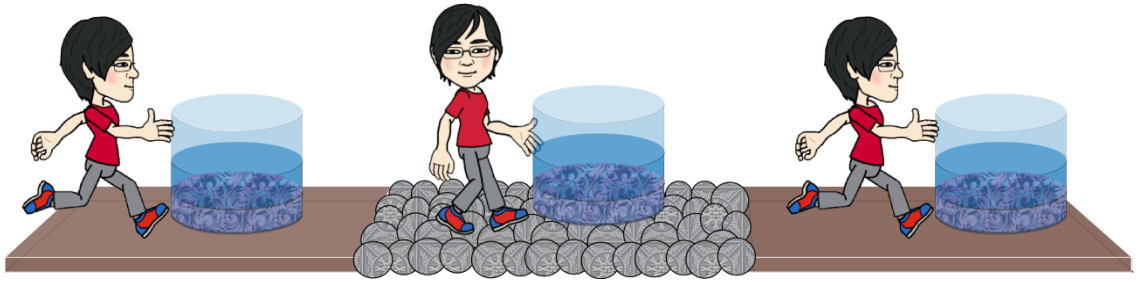


Equilibrium Thermodynamics



Naïve Protocol

Minimum Dissipation Protocol



Acknowledgements

How to Find the Optimal Path?

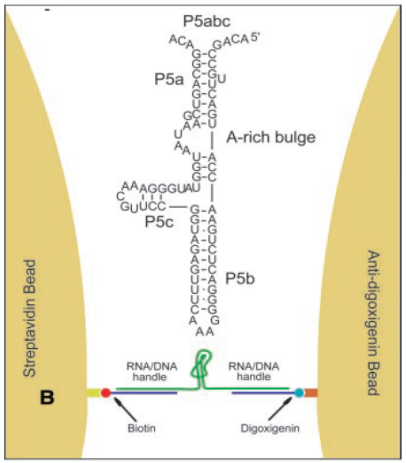
- Excess power:

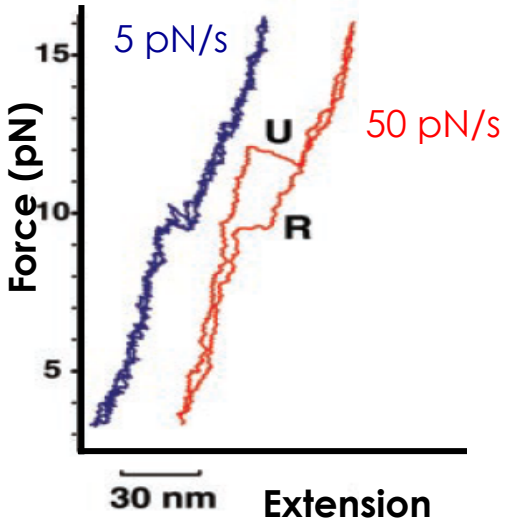
$$\mathcal{P}_{\mathrm{ex}}(t_0) = \left[\frac{d\boldsymbol{\lambda}^T}{dt}\right]_{t_0} \cdot \zeta(\boldsymbol{\lambda}(t_0)) \cdot \left[\frac{d\boldsymbol{\lambda}}{dt}\right]_{t_0}.$$

- Friction tensor:

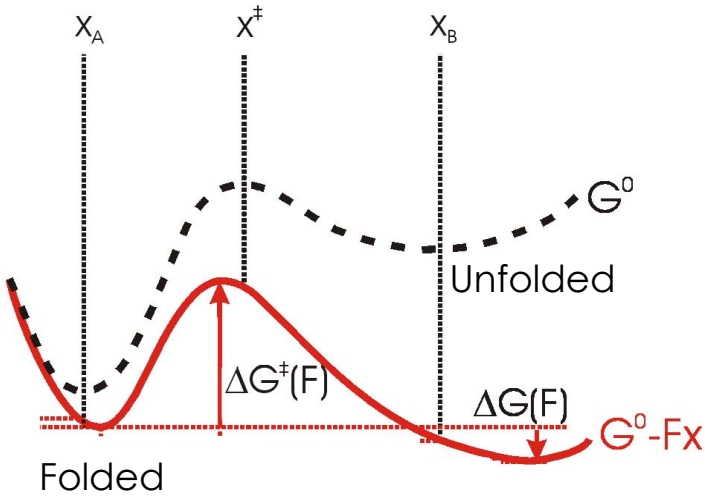
$$\zeta_{ij}(\boldsymbol{\lambda}(t_0)) = \beta \int_0^\infty dt'' \Sigma_{ij}^{(\boldsymbol{\lambda}(t_0))}(t''),$$

$$= \beta \int_0^\infty dt'' \langle \delta X_j(0)\, \delta X_i(t'') \rangle_{\boldsymbol{\lambda}(t_0)}.$$





- For a fixed control parameter velocity, excess power is greater where the friction coefficient is greater.
- In the optimal protocol, the control parameter changes slowly where the system experiences large friction.



Sivak & Crooks, *Phys. Rev. Lett.* 2012

Use Trepanated Heads

"Peru has produced some of the largest samples of prehistoric trepanned individuals, since trepanation was practiced over a long period of time and across a broad geographical area. The earliest Peruvian trepanations appear on the south coast of Peru circa 400 BEC."

"The Cuzco trepanation survival rate reached 90% at one point and was accompanied by a low frequency of infection (4.5%).

Ansrushko and Verano, *J. Amer. Phys. Anthropology* (2008).

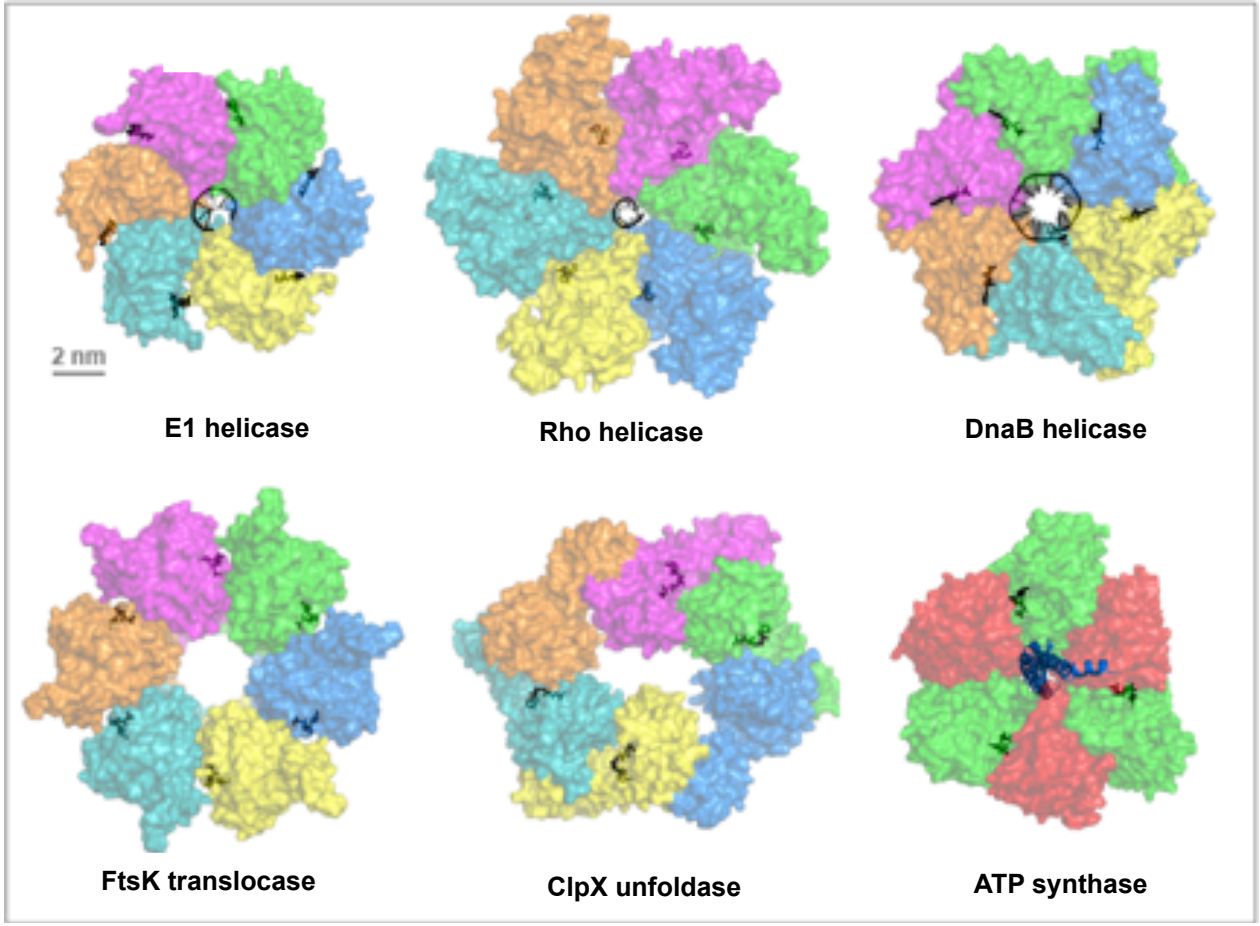
Mode of Operation

By the mode of operation, molecular motors can be classified as:

- Brownian Ratchets

- Power Strokers

Ring ATPases



Thermodynamic Efficiency of the Motor

$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G^\circ = -30.5$ kJ/mol (−7.3 kcal/mol)

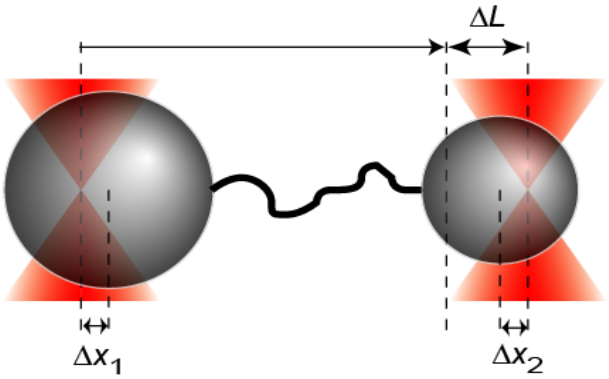
2.5 bp is 0.85 nm. At 70 pN the motor performs 60pN x 0.85 nm = 51 pN.nm of mechanical work (near stall)

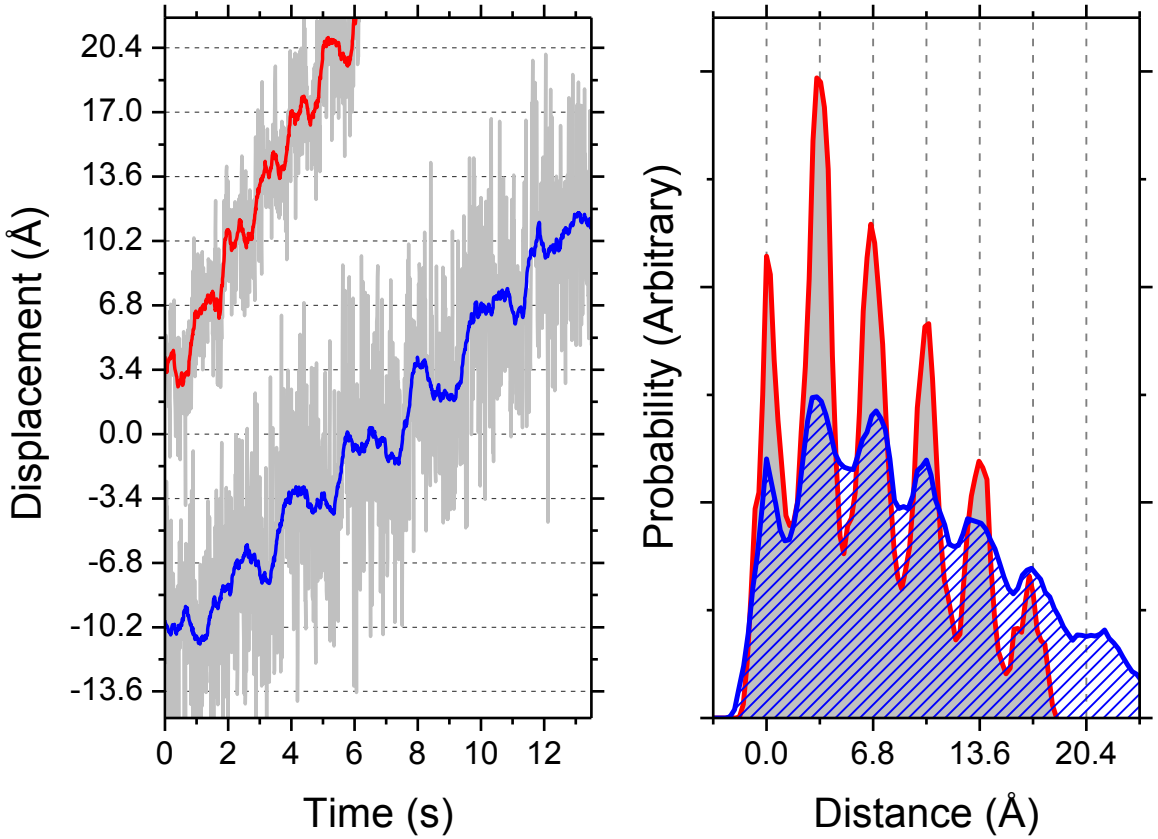
Stardard free energy of ATP is -7.3 Kcal/mol, in k_BT units = 12.2 kT = 50 pN.nm

Since ATP is 1000 times higher concentration than ADP, the ΔG is – 14Kcal/mol or 23.3 k_BT or 95.7 pN.nm

Efficiency at Stall > 53%

Packaging at Single Base Pair Resolution

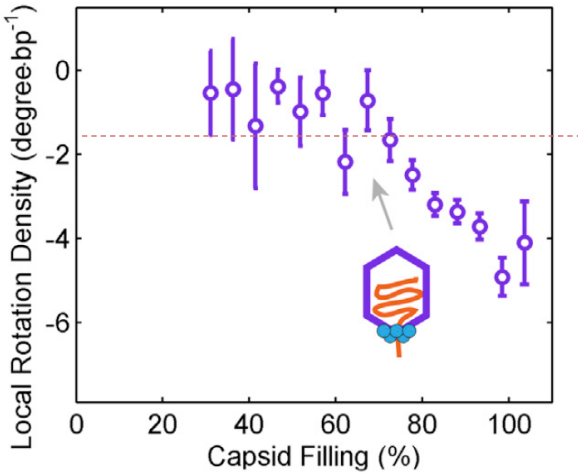




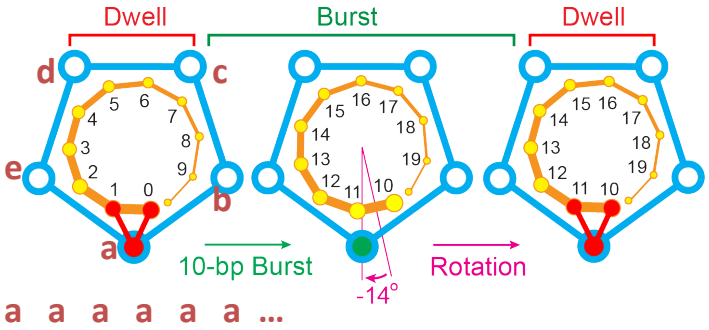
3.4 kb DNA
860 nm Beads
F = 7, 27 pN
f_{samp} = 50 Hz
f_{av} = 2, 4 Hz

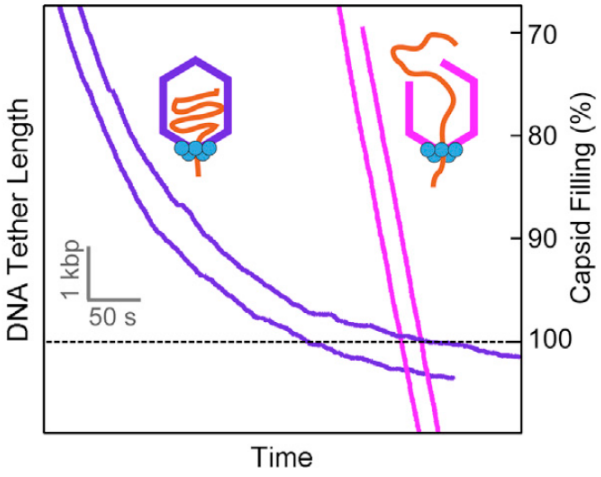
Moffitt et al. *PNAS* **103**, 9006 (2006)
Bustamante et al. *Cold Spring Harbor Laboratory*, (2007)

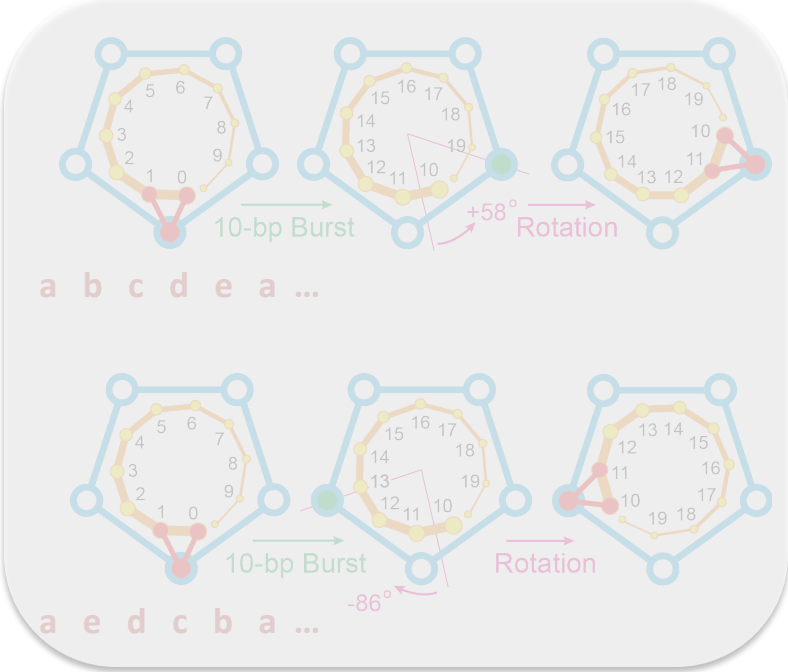
The Motor Rotates the DNA at Zero Filling



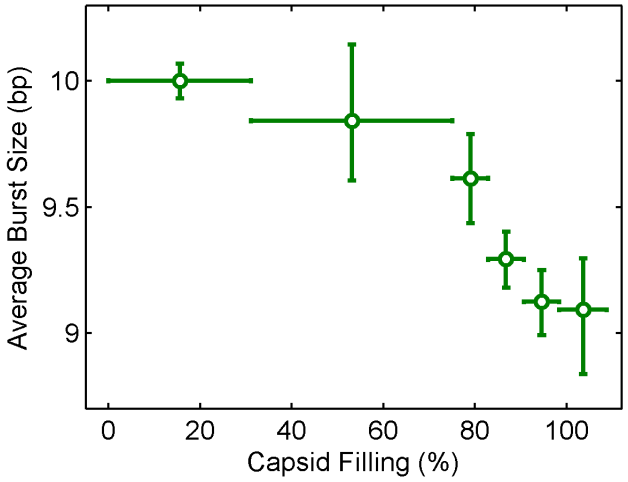
-1.5 ± 0.2 °/bp



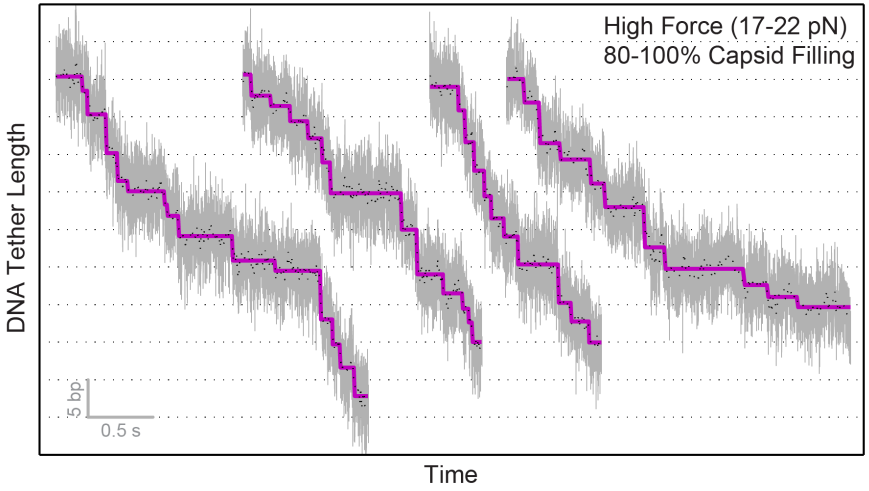


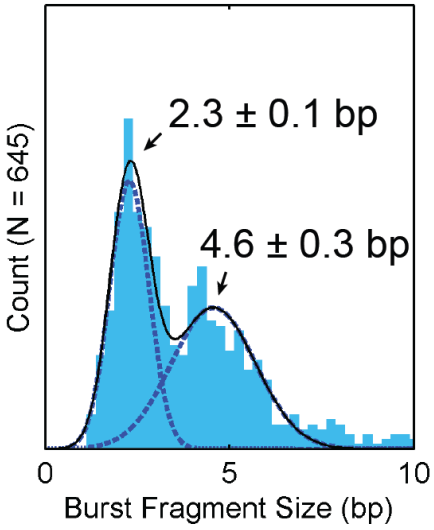


Head Filling Changes the Step Size

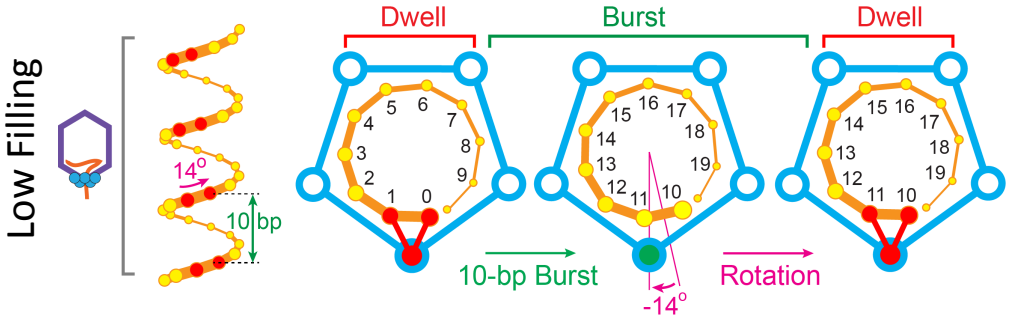


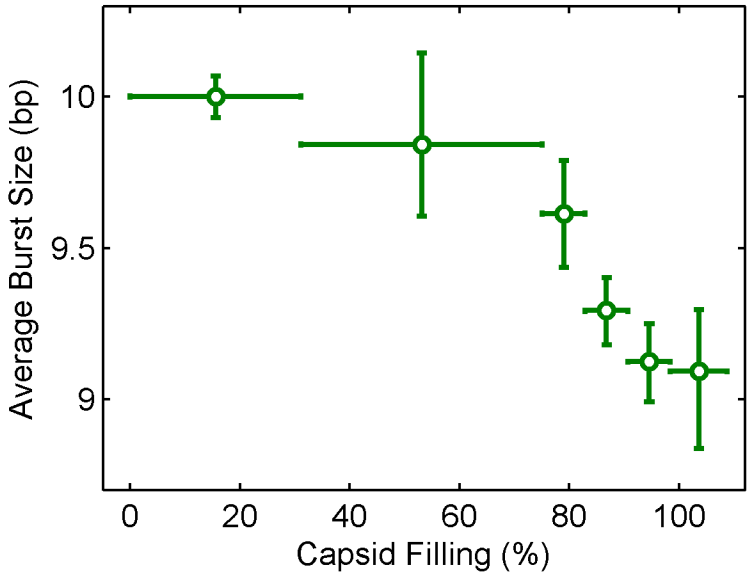
- Low filling: 2.5 bp × 4 = 10 bp
- High filling: 2.3 bp × 4 = 9.2 bp





Subunit Coordination is Maintained Throughout Packaging





Capsid filling	Burst size	Predicted rotation
0	10 bp	-1.4 °/bp
100%	9 bp	-5.3 °/bp

Φ29 Packaging Motor

The power stroke coincides with the release of Pi:

M +ATP

Operation of the 5 subunits is precisely coordinated

Power at the Nanoscale: Strength, speed and efficiency in biological nanomachines

Speed: At saturating ATP conditions phi29 reaches optimal packaging velocity (120 nm/s) to encapsulate the viral genome (19.3 kb) in less than 3 min.

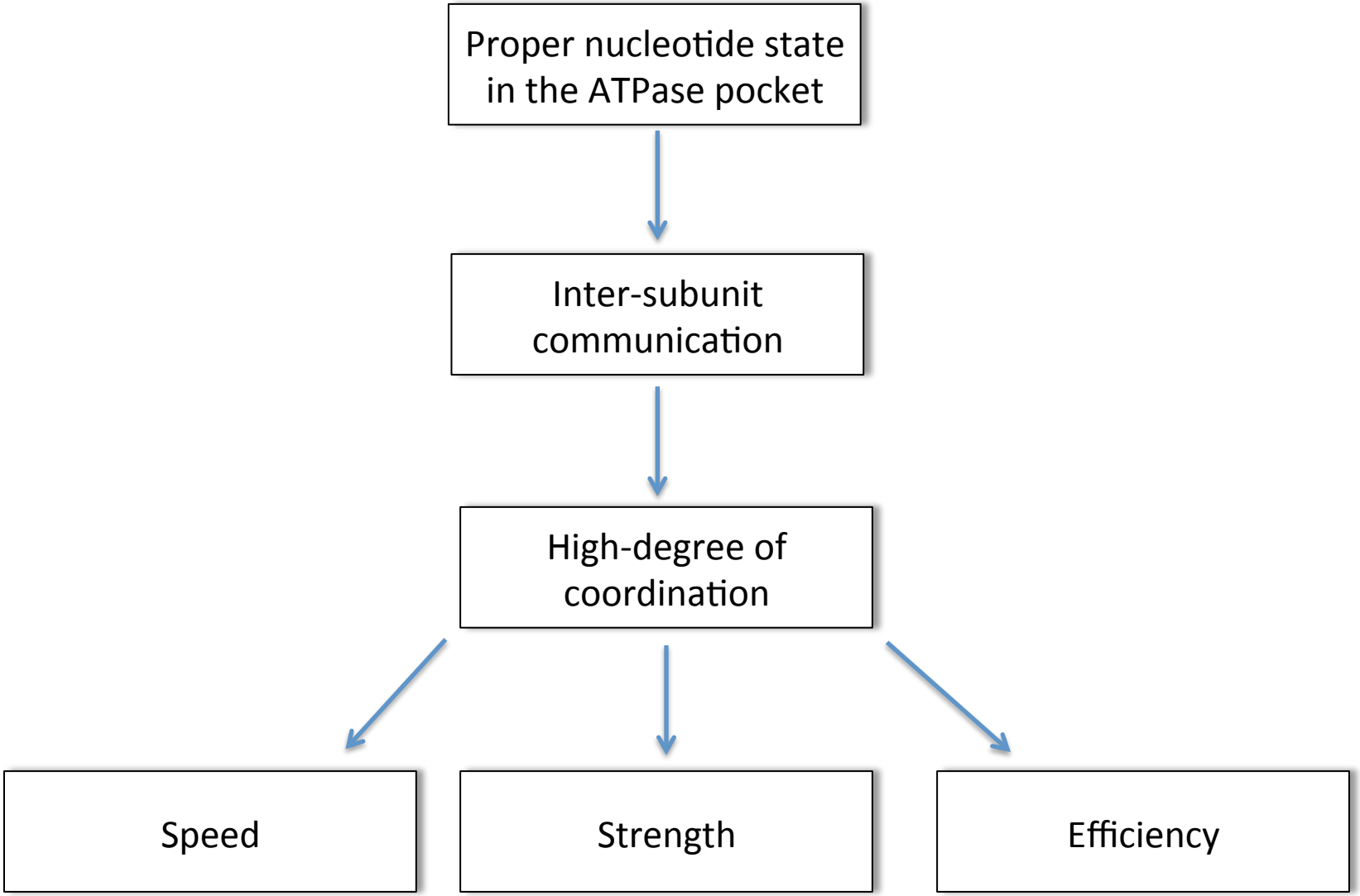
Strength: Phi29 packaging motor exerts forces in excess of 60 pN to counteract the internal pressure built at high capsid filling.

Efficiency: The motor couples energy release in the hydrolysis of 1 ATP molecule to a power stroke of 2.5 bp, which corresponds to an efficiency of ~41 % per power stroke (120 pN.nm energy released per ATP hydrolysis at a maximun force of 60 pN). However, the motor uses 5 ATP to complete a cycle packaging 10 bp, which means the motor has an average efficiency of 33% in each cycle.

Speed, strength and efficiency of the motor are ensured by a mechanism of high degree of coordination that is dependent on communication of the nucleotide's state between adjacent subunits.

At limiting ATP some subunits are found in the apo state (empty) which impairs proper inter-subunit communication and the motor's coordination.

Speed, strength and efficiency of phi29 packaging motor depend on nucleotide state of ATPase pocket



Loss of motor's coordination at low [ATP]

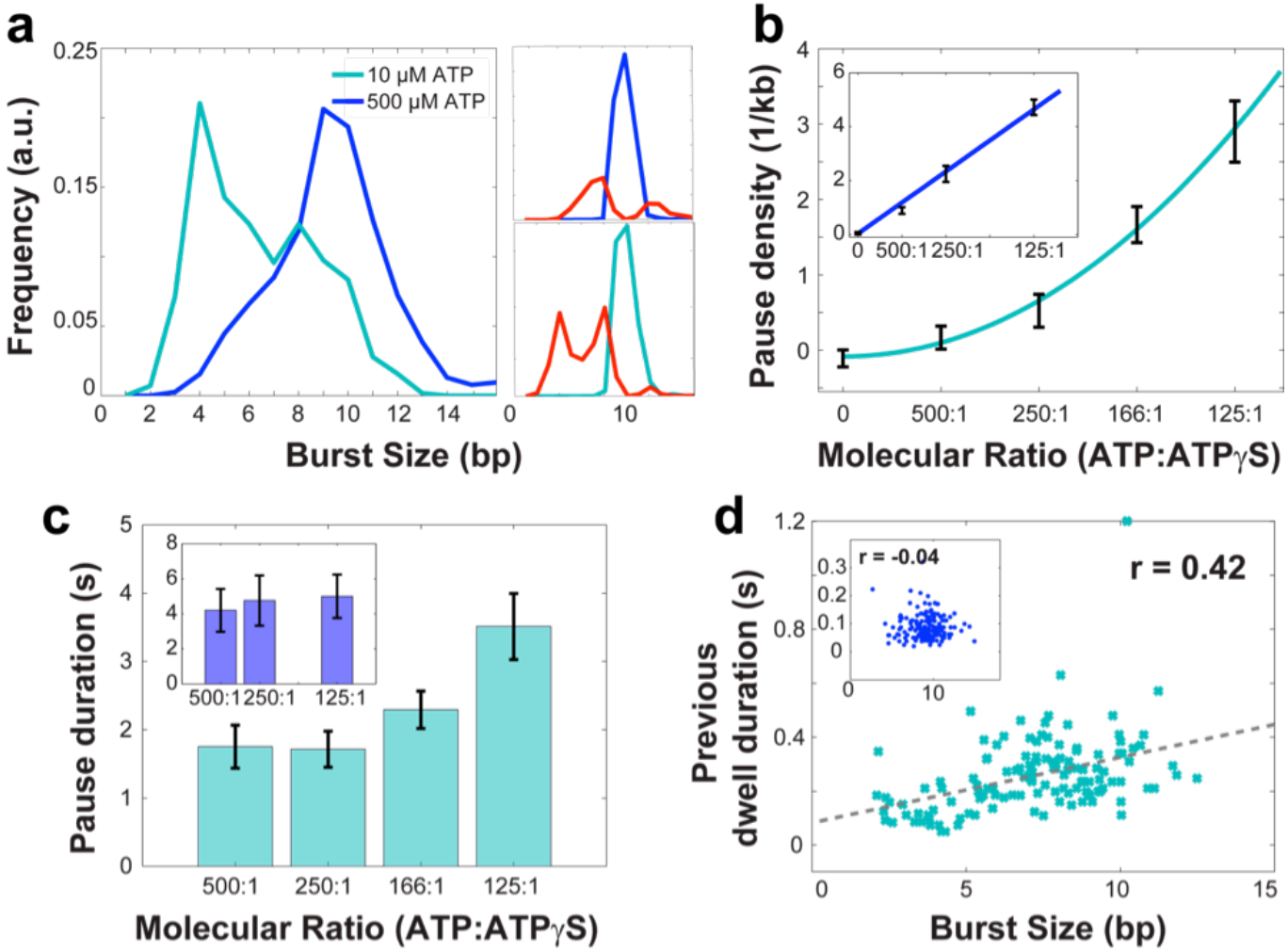


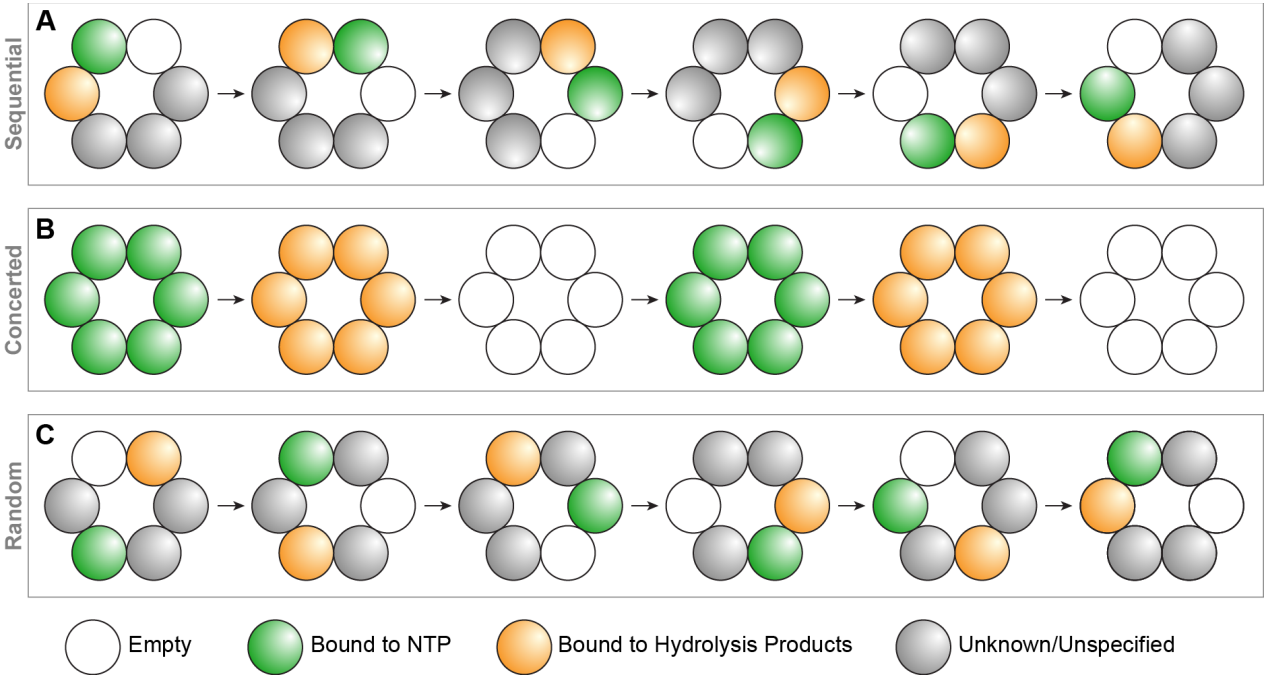
Figure . Loss of coordination at low [ATP]. a) Left. Burst distribution shows smaller burst sizes for limiting, sub-K_m conditions of ATP (K_m=35 uM). Right. Fragmented but otherwise complete cycles are identified and included in the distribution depicted here in dark blue for saturating ATP (upper) and light blue for limiting ATP (lower). Incomplete bursts are depicted in red. AT low ATP there is a larger fraction of incomplete bursts. **b)** The pause density increases non-linearly with [ATPgS] ($y=ax^{n,}$ with n=2), suggesting that more than one ATP molecule might be necessary to halt the motor. **c)** Average pause duration increases with [ATPgS] suggesting that exiting the pause might require the release of more ATPgS molecules as we increase [ATPgS]. **d)** The linear correlation coefficient R was calculated for limiting and saturating (inset) conditions of ATP. R=1 is indicative of positive correlation, R=-1 is indicative of negative correlation -anticorrelation- , and R=0 suggests no linear relationship. At limiting conditions of ATP, smaller bursts are preceded by shorter dwell durations. This supports a mechanism in which the motor fires stochastically using as many ATP molecules as it is able to bind before undergoing the hydrolysis cascade.

Catalytic cycle of ring ATPases

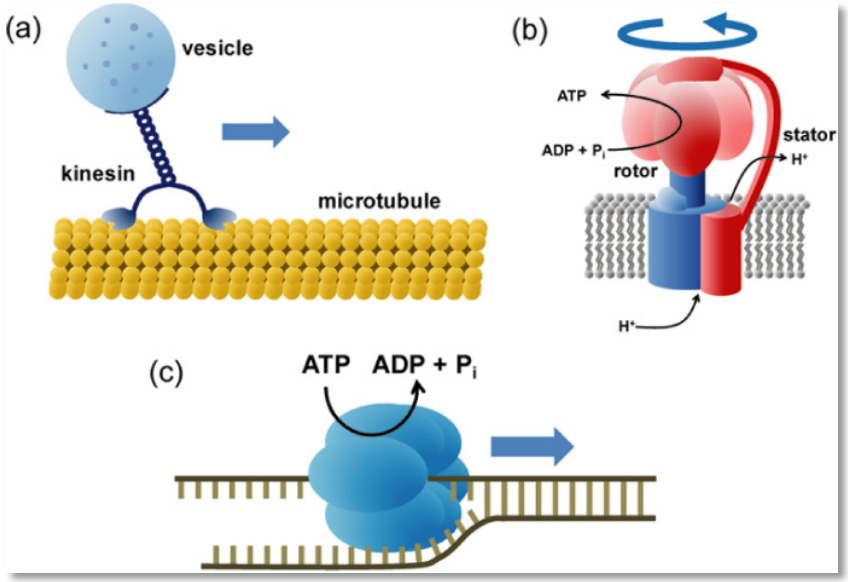
- ATP binding
- Hydrolysis
- Release of products (ADP, P_i)

Mechanochemical coupling

Intersubunit coordination



Molecular Motors Carry Out Diverse Cellular Functions

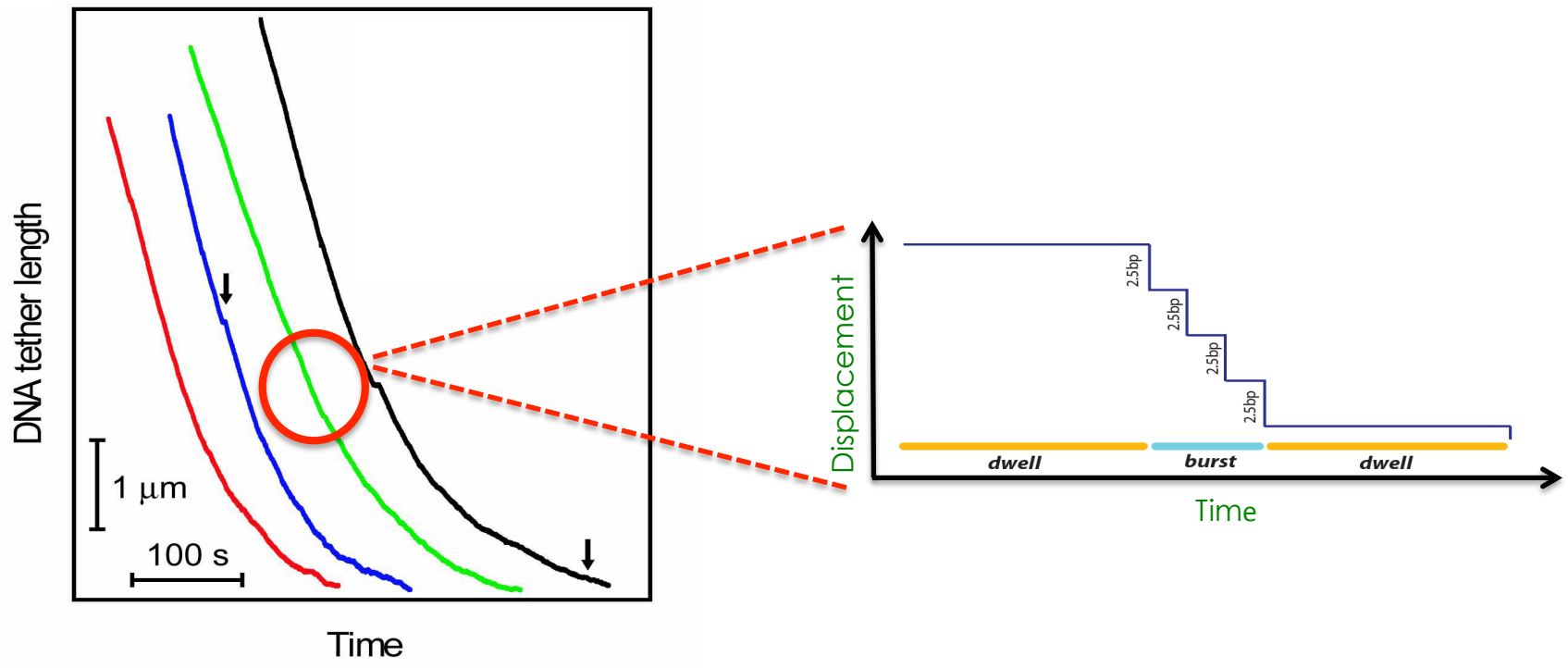


Kim & Ha (2013)

- **Cytoskeletal motors** (kinesin, dynein, myosin)
- **Rotary motors** (ATP synthase, flagella motor)
- **Nucleic acid motors** (helicase, DNA/ RNA polymerase, ribosome)
- **Polypeptide motors** (protease, translocase)

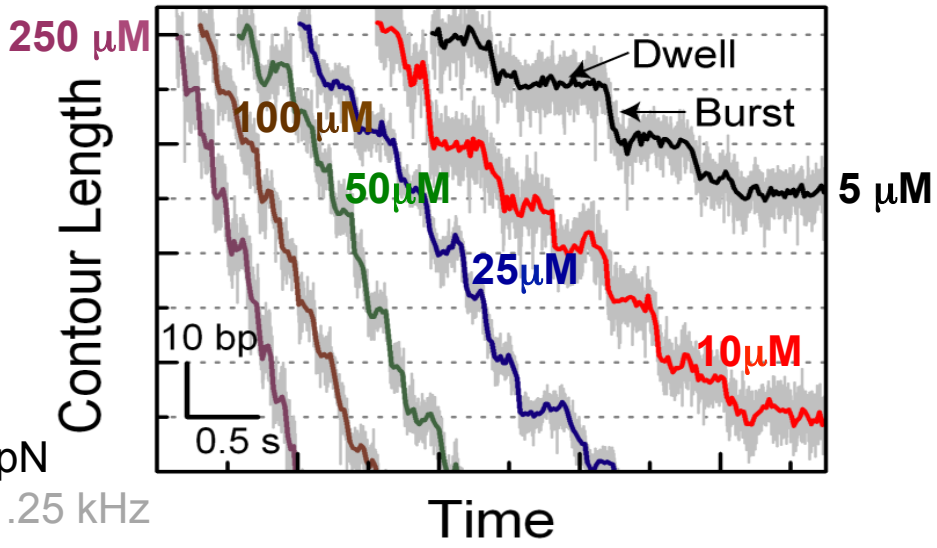
Experiment Assembly

Packaging at Single Base Pair Resolution

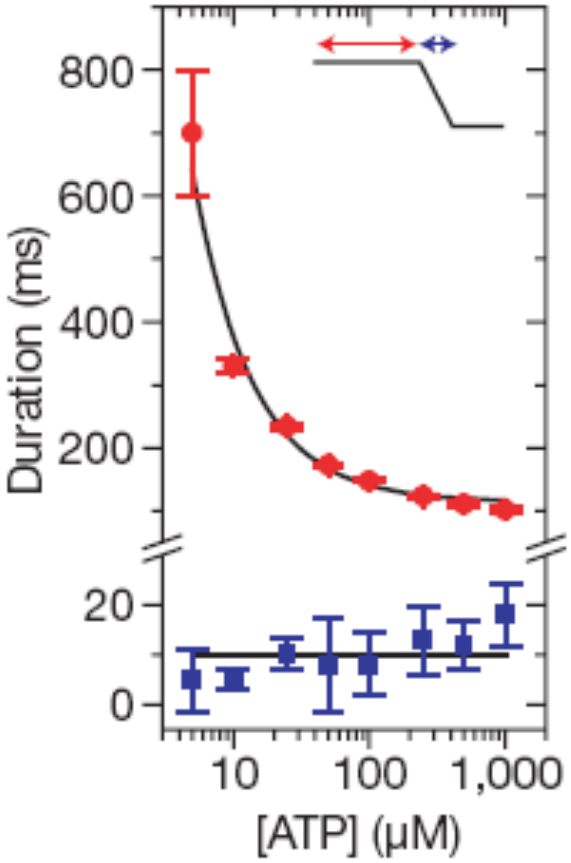


Moffitt *et al. Nature* (2009)

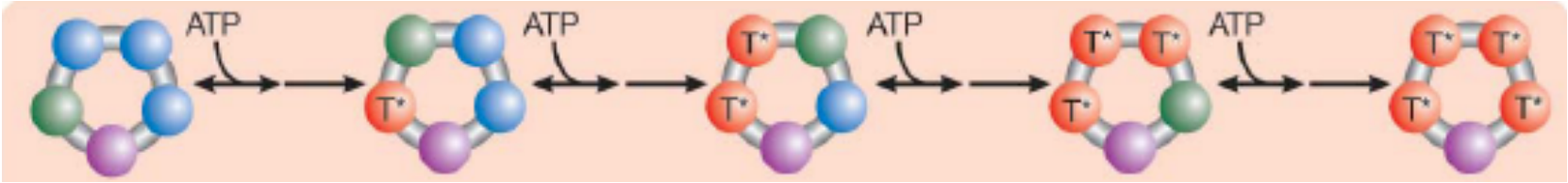
ATP Binding Occurs in the Dwell



F = 6-8pN
50Hz, 1.25 kHz



- All ATPs bind in the dwell phase



Moffitt *et al.*, *Nature* (2009)

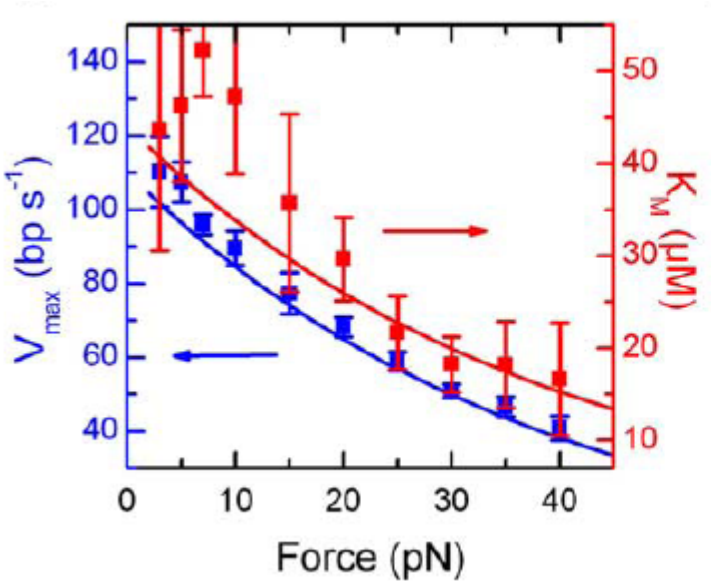
What Step Powers Translocation?

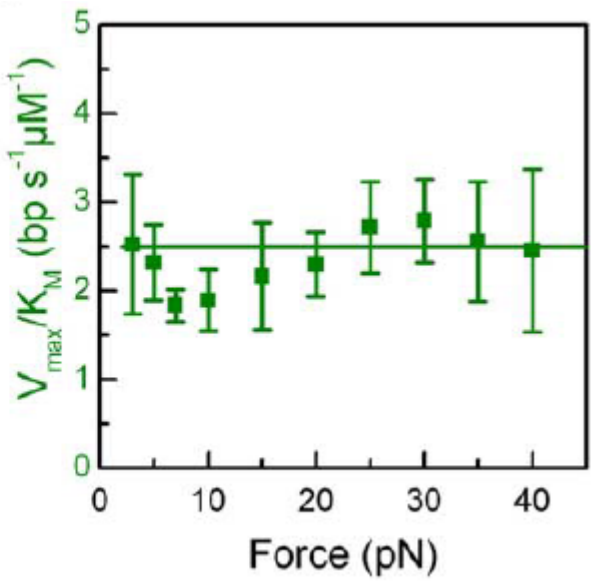
$$E \overset{k[\mathrm{ATP}]}{\leftrightarrow} T \leftrightarrow \cdots M_i \rightarrow M_{i+1} \leftrightarrow \ldots M_n \rightarrow E$$

Kinetic Block 1 — Kinetic Block 2

Location	K_M	k_{cat}	k_{cat}/K_M
ATP Docking	modified	same	modified
Connected to Binding	modified	modified	modified
Other Kinetic Block	modified	modified	same

Bustamante et al., Annu Rev Biochem (2004)





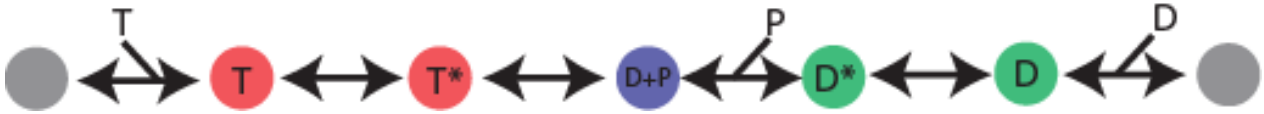
Chemla *et al., Cell* (2005)

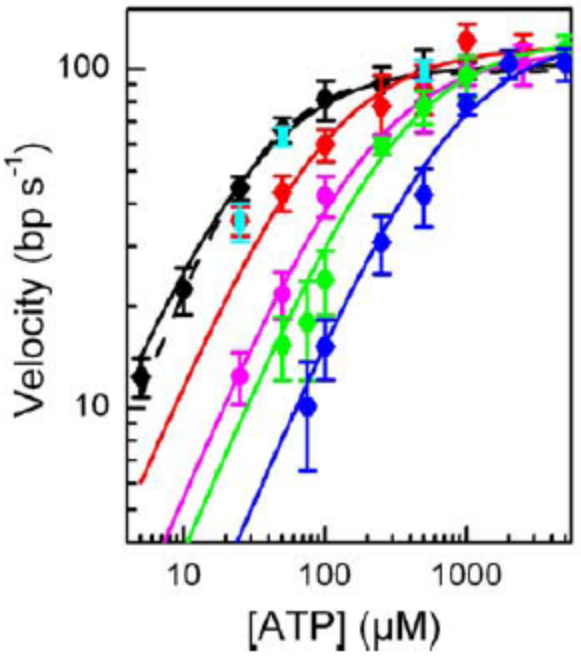
- DNA translocation is not powered by ATP docking, tight binding, or hydrolysis.

What Step Powers Translocation?

- Stall force: >57pN
- Step Size: 2.5bp = 2.5 x .34 nm/bp = 0.85nm

- Free energy generated by each step: 48 pN-nm = $12k_BT$





Chemla *et al., Cell* (2005)

Product release:

(1) Pi release is irreversible: $\Delta G_P > 7k_BT$

(2) ADP release is reversible: $\Delta G_D \approx 2k_BT$ and is a competitive inhibitor.

❑ DNA translocation is most likely powered by Pi release.