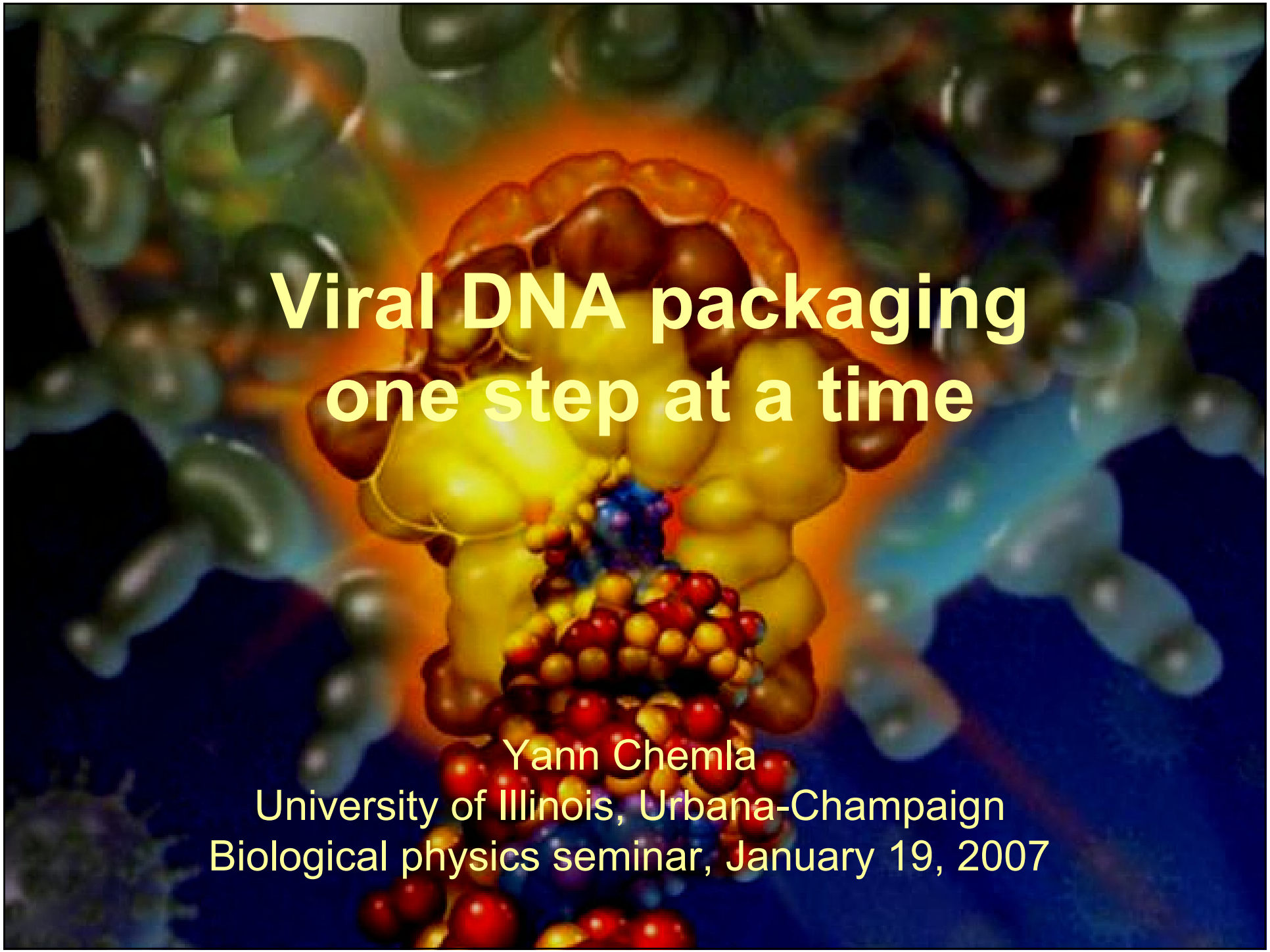
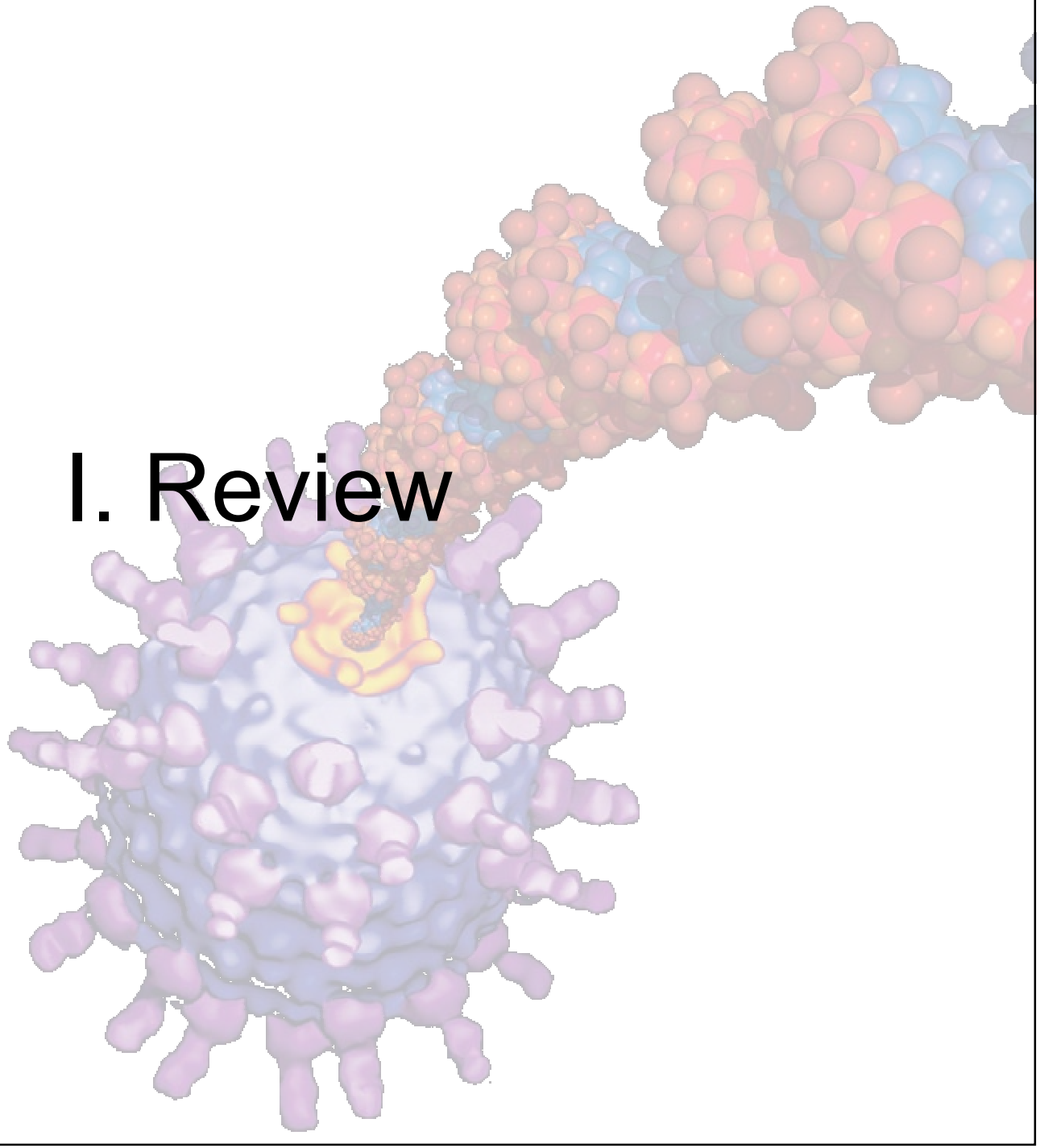


Viral DNA packaging one step at a time

Yann Chemla
University of Illinois, Urbana-Champaign
Biological physics seminar, January 19, 2007

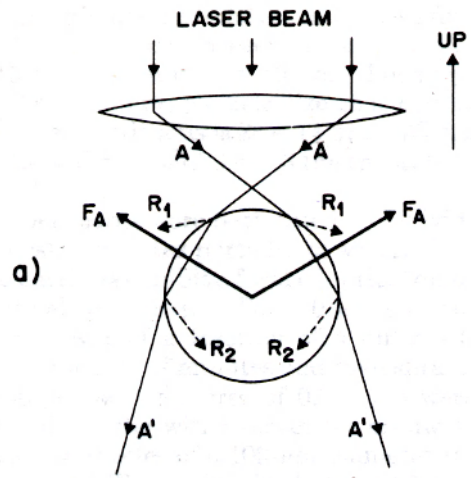
I. Review



Review of *quantitative* optical traps

Three essential elements:

1) Manipulation – spatial light gradient generates optical force to trap dielectric bead



b)

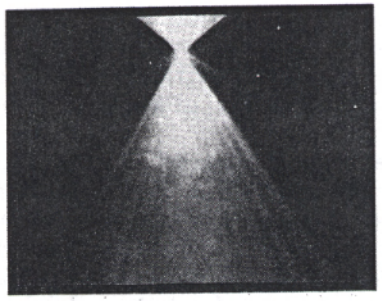
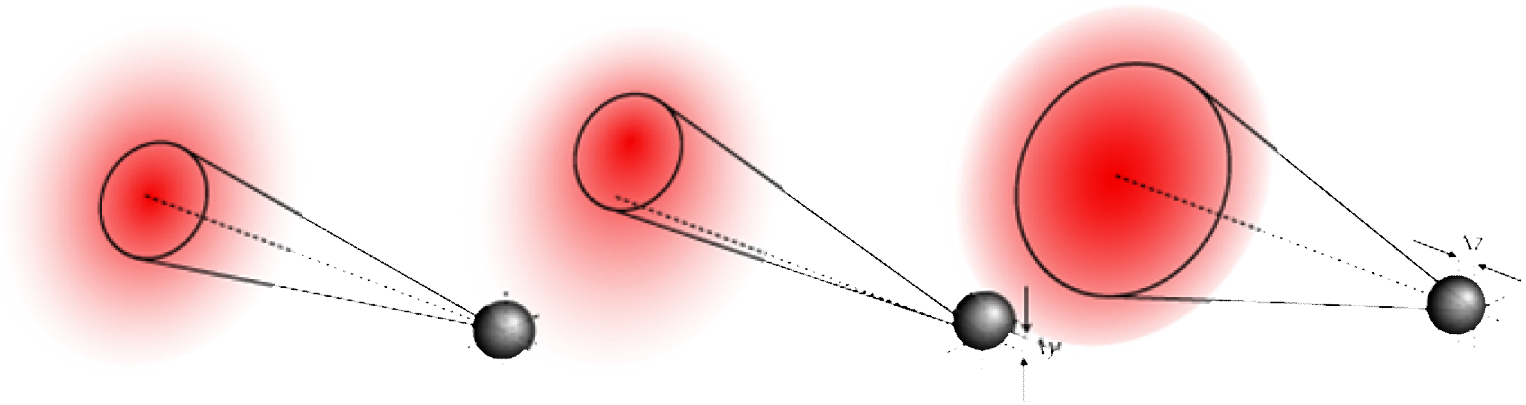


Fig. 1. a) Diagram showing the ray optics of a spherical Mie particle trapped in water by the highly convergent light of a single-beam gradient force trap. b) Photograph, taken in fluorescence, of a 10-μm sphere trapped in water, showing the paths of the incident and scattered light rays.

Review of *quantitative* optical traps

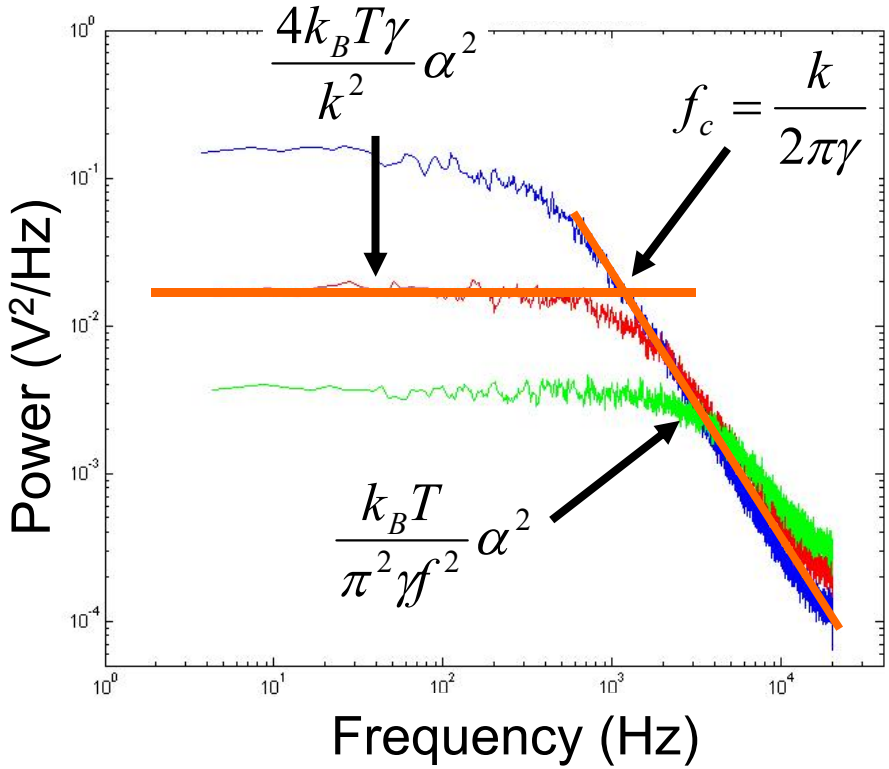
2) Measurement – sensitive detection of bead displacement by BFP interferometry



Review of *quantitative* optical traps

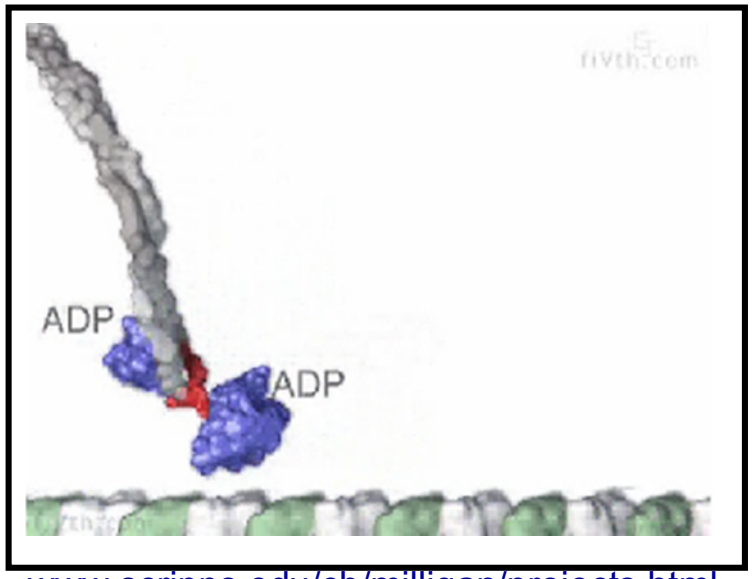
3) Calibration – conversion of raw data into forces & displacements from Brownian motion of bead

$$\Delta x = \alpha \Delta V, \quad F = k\Delta x = \alpha k \Delta V$$

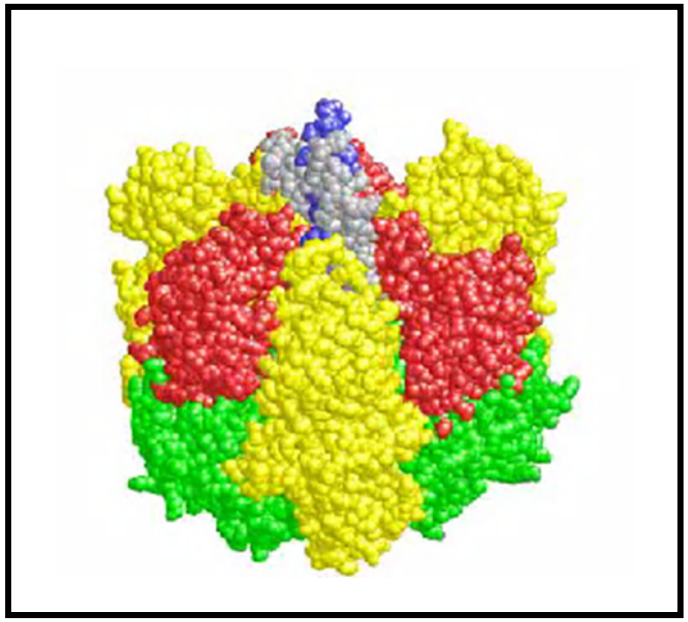


Biological application of optical traps

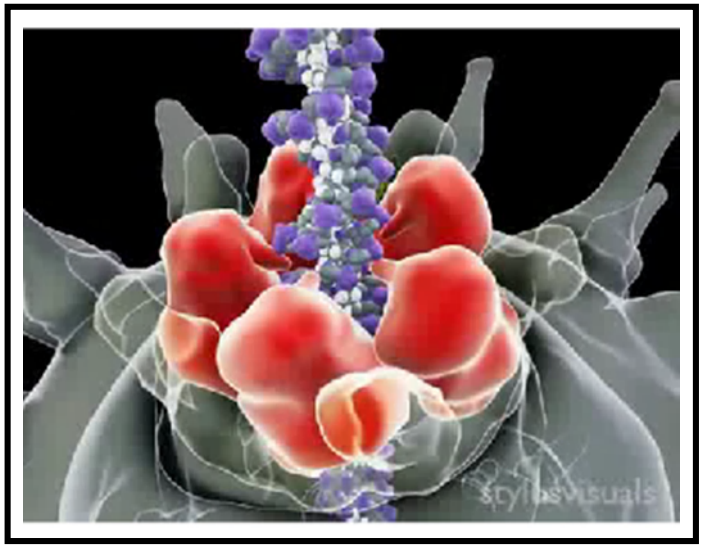
Force: 1-100 picoNewton (pN)
Distance: <1–10 nanometer (nm)



www.scripps.edu/cb/milligan/projects.html



www.cnr.berkeley.edu/~hongwang/Project

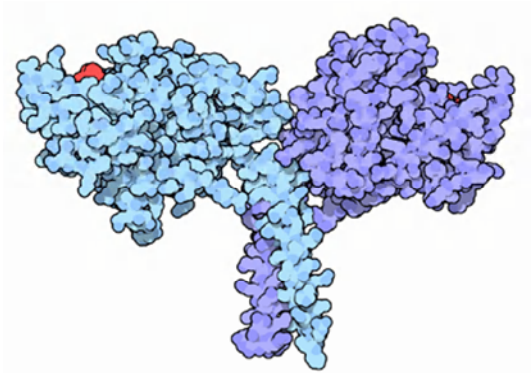


www.alice.berkeley.edu

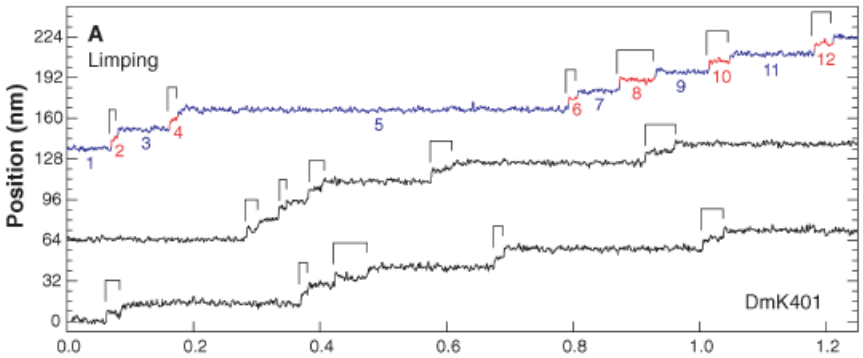
Observing individual steps

Motors move in discrete steps

Kinesin

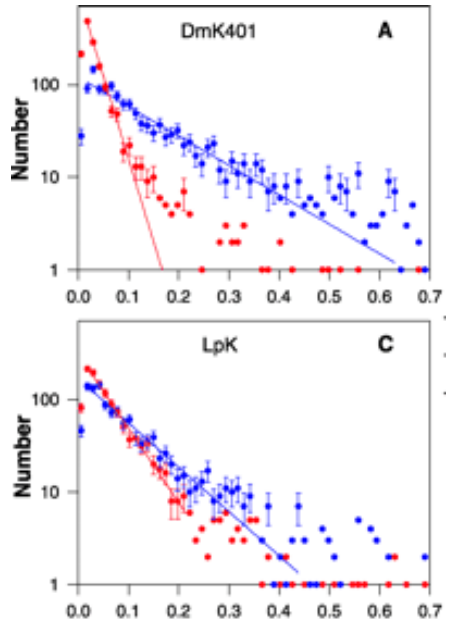


Step size: 8nm



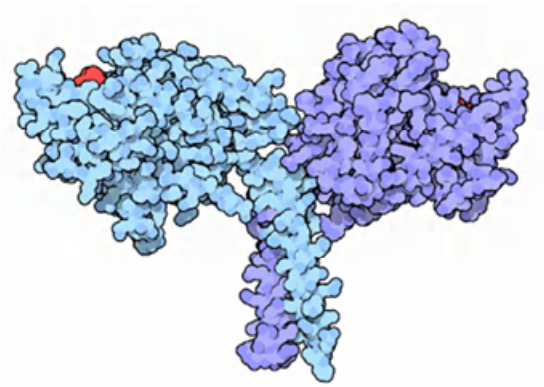
Asbury, et al. *Science* (2003)

Detailed statistics on kinetics of stepping & coordination

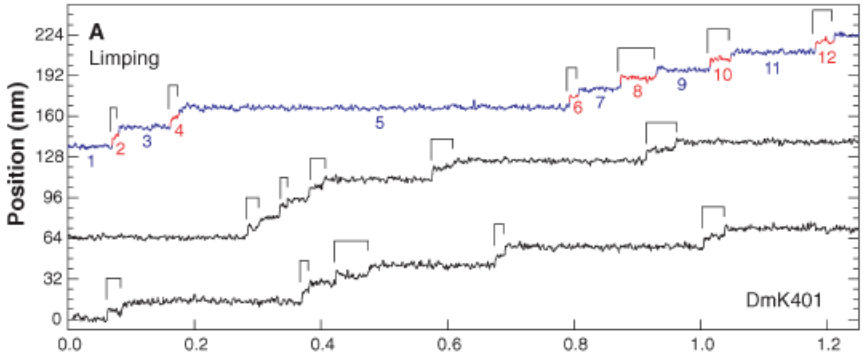


Motivation for high-resolution

Kinesin

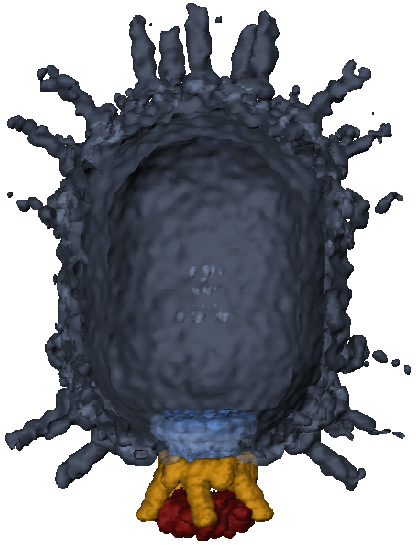


"Large" step size: 8nm

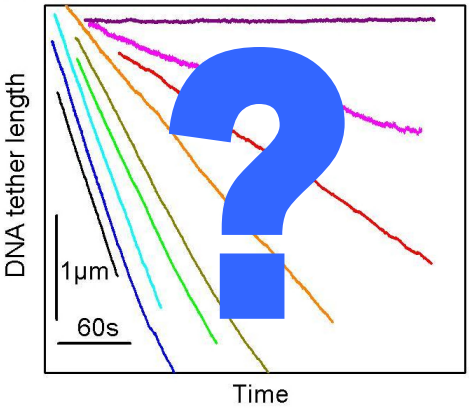


Asbury, et al. *Science* (2003)

DNA motor

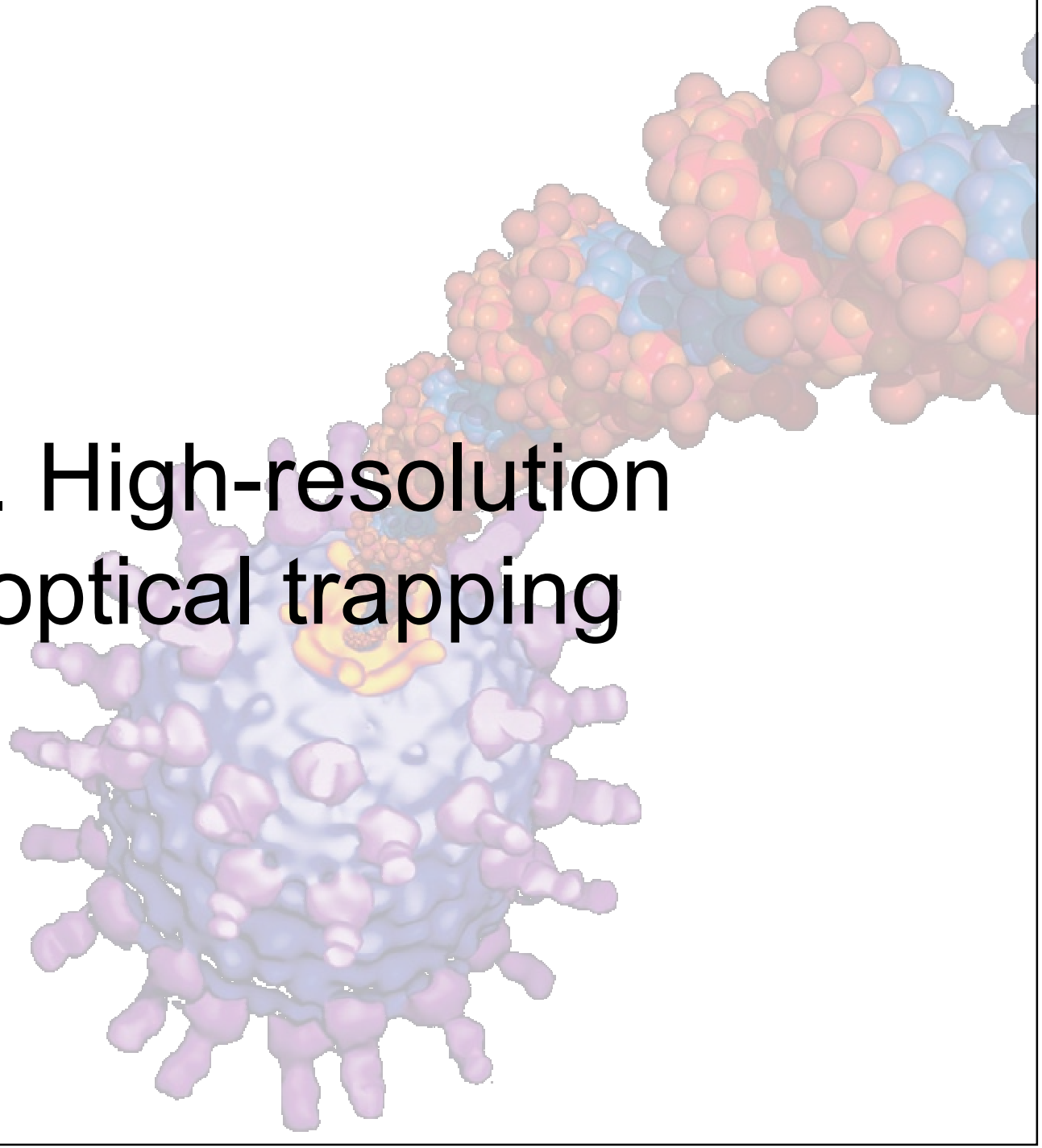


Small step size: ~3.4Å (1bp)



Chemla et al. *Cell* (2005)

II. High-resolution optical trapping



Achieving high resolution

Goal:

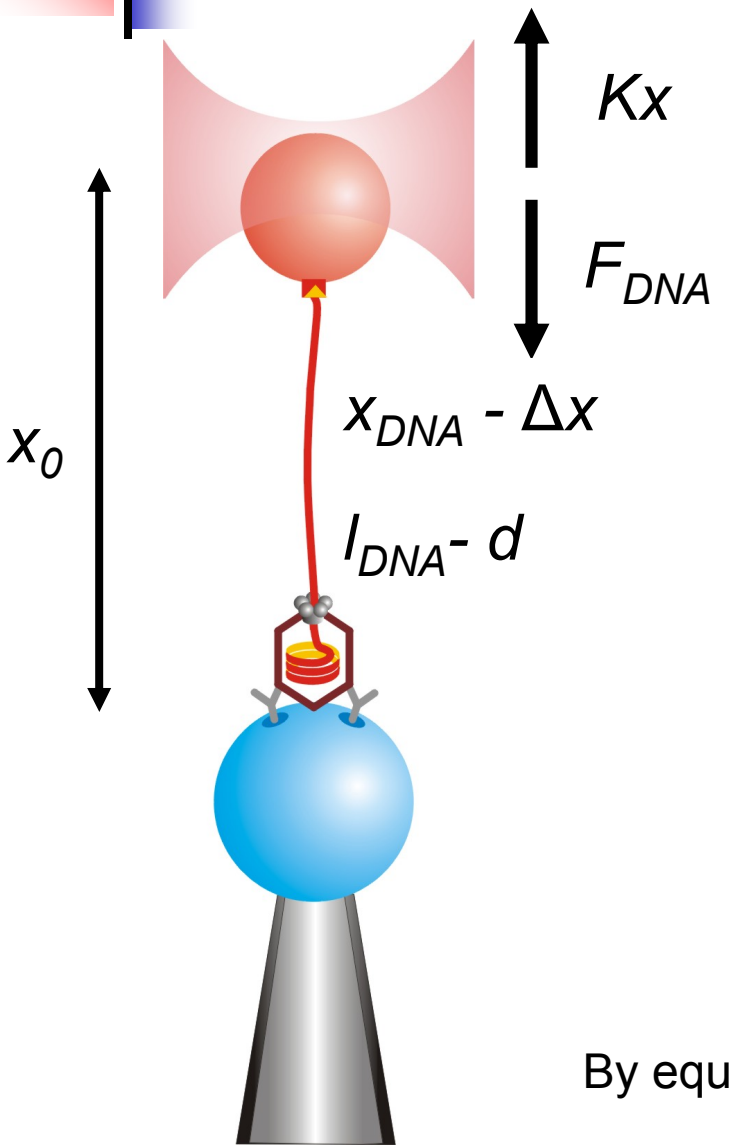
– Directly observe movements on the scale of 1bp (3.4Å)

Issues: Managing noise

– Environmental noise
– Instrumental noise
– Brownian noise

Bustamante et al., in *Single Molecules: A Laboratory Manual* (ed. Selvin, Ha), in press

Optical trap resolution



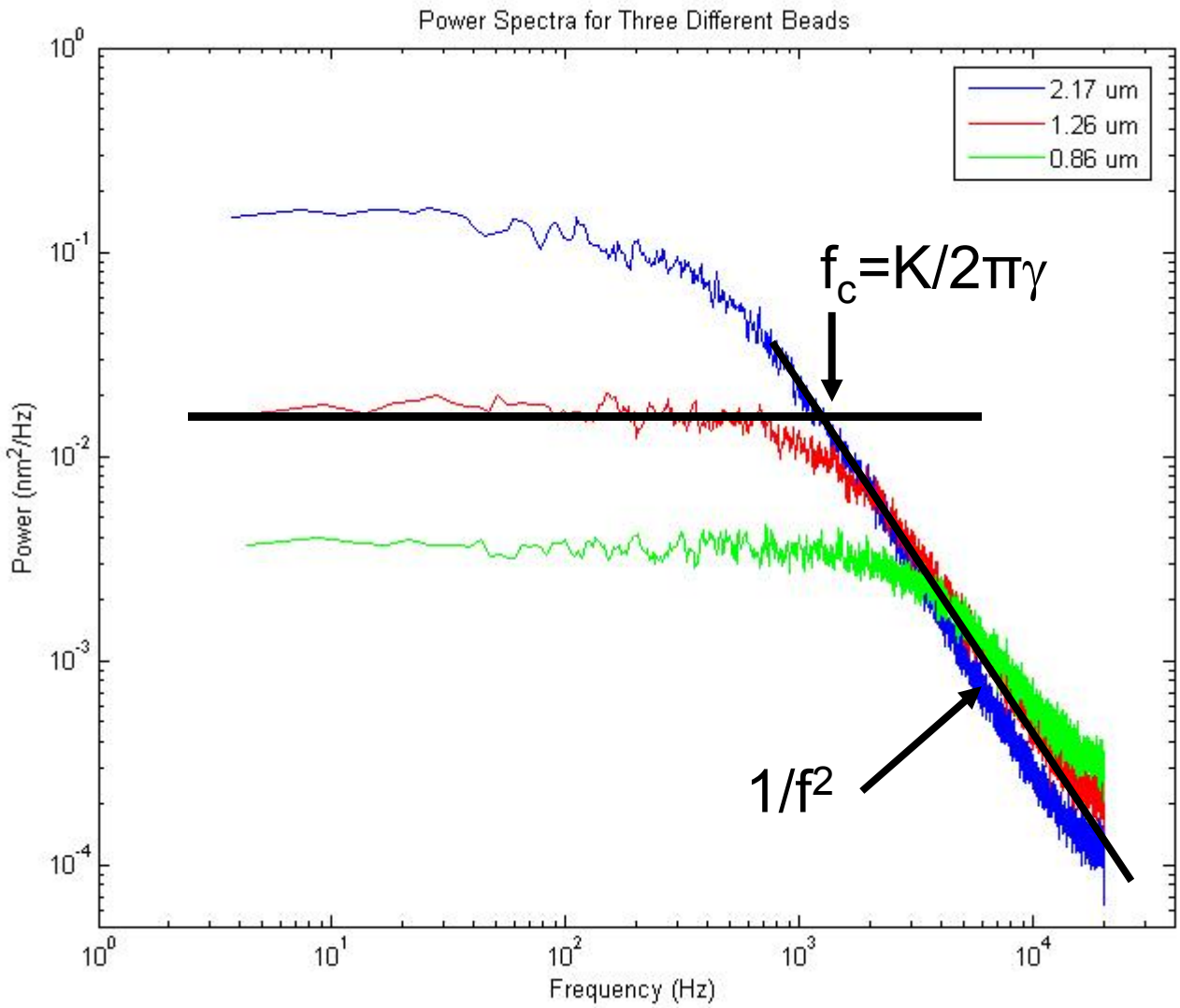
Bead in trap moves in response to motor step

$$\Delta x = \frac{dK_{DNA}}{K_{DNA} + K_{Trap}} < d$$

Bead in trap undergoes Brownian motion

By equipartition: $\Delta x_{rms} = \sqrt{\langle \delta x^2 \rangle_\infty} = \sqrt{\frac{k_B T}{K_{DNA} + K_{Trap}}}$

Power spectrum



Brownian Noise - Simulation

Large bd - 2um Small bd - 1um

Not to scale

$$\Delta x_{rms} = \sqrt{\langle \delta x^2 \rangle_\infty} = \sqrt{\frac{k_B T}{K}} \sim 6\text{nm !}$$

Brownian Noise - Simulation

Average down 100X

Large bd - 2um Small bd - 1um

Not to scale

$$\Delta x_{rms} = \sqrt{\left\langle \delta x^2 \right\rangle_B} = \frac{\sqrt{4 k_B T \gamma B}}{K}$$

Signal-to-noise ratio

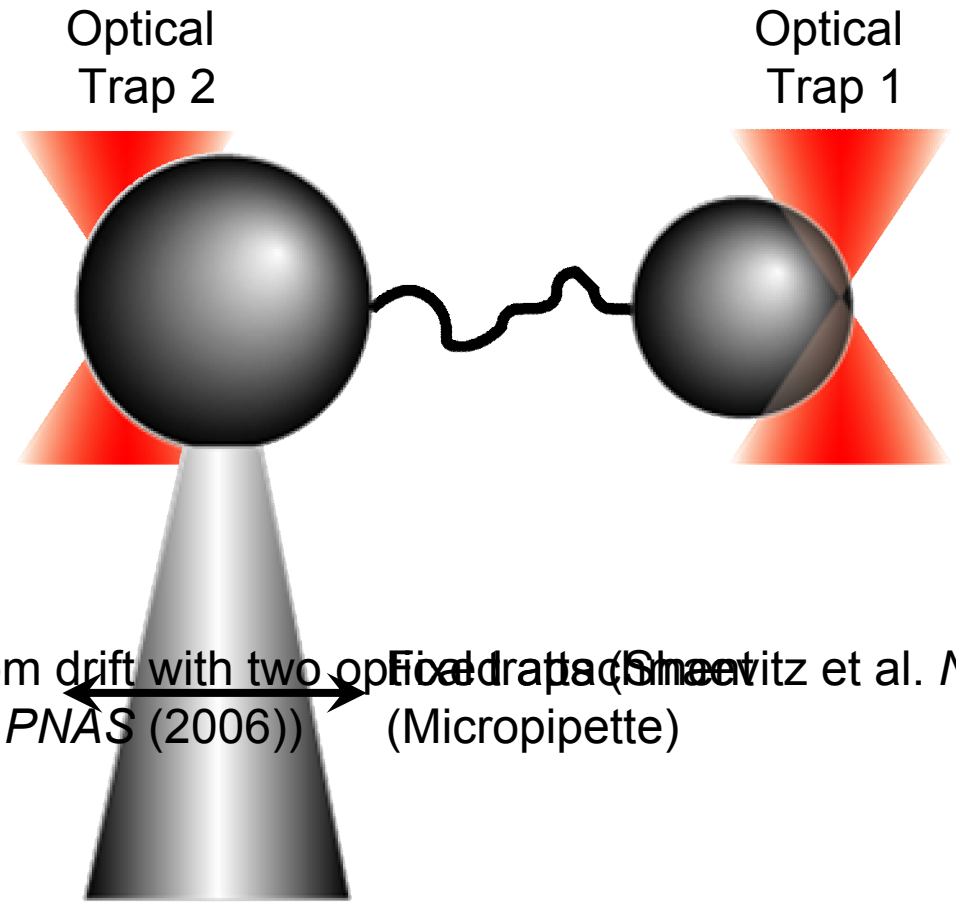
$$SNR = \frac{\Delta x}{\Delta x_{rms}} = \frac{dK_{DNA}}{\sqrt{4k_B T \gamma B}}$$

Independent of K_{Trap}!

Instrumental noise

Stage introduces drift

– Monitor & subtract drift (Nugent-Glandorf & Perkins, *Opt. Lett.* (2004))



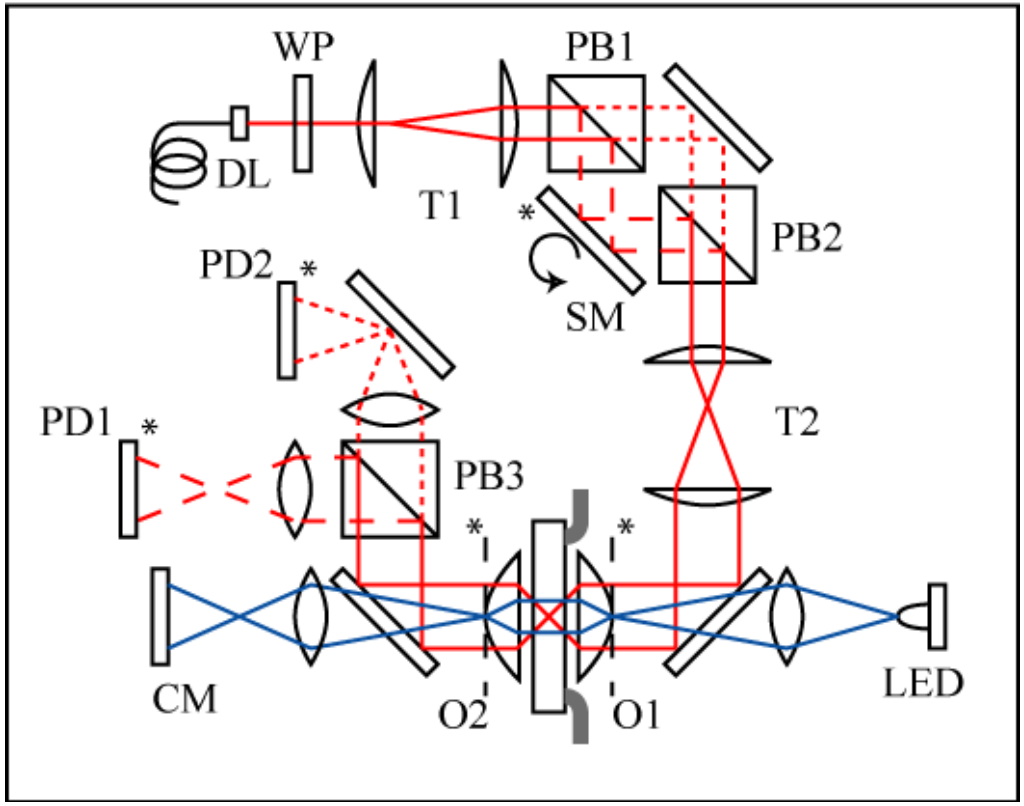
– Decouple from drift with two optical traps (Smith et al. *Nature* (2003), Moffitt et al. *PNAS* (2006))

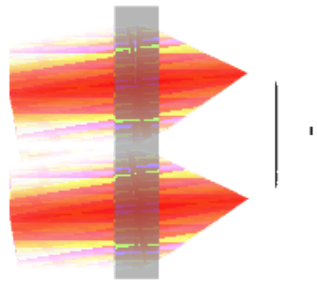
Fixed trap (Micropipette)

Design principles

Design instrument to be insensitive to drift

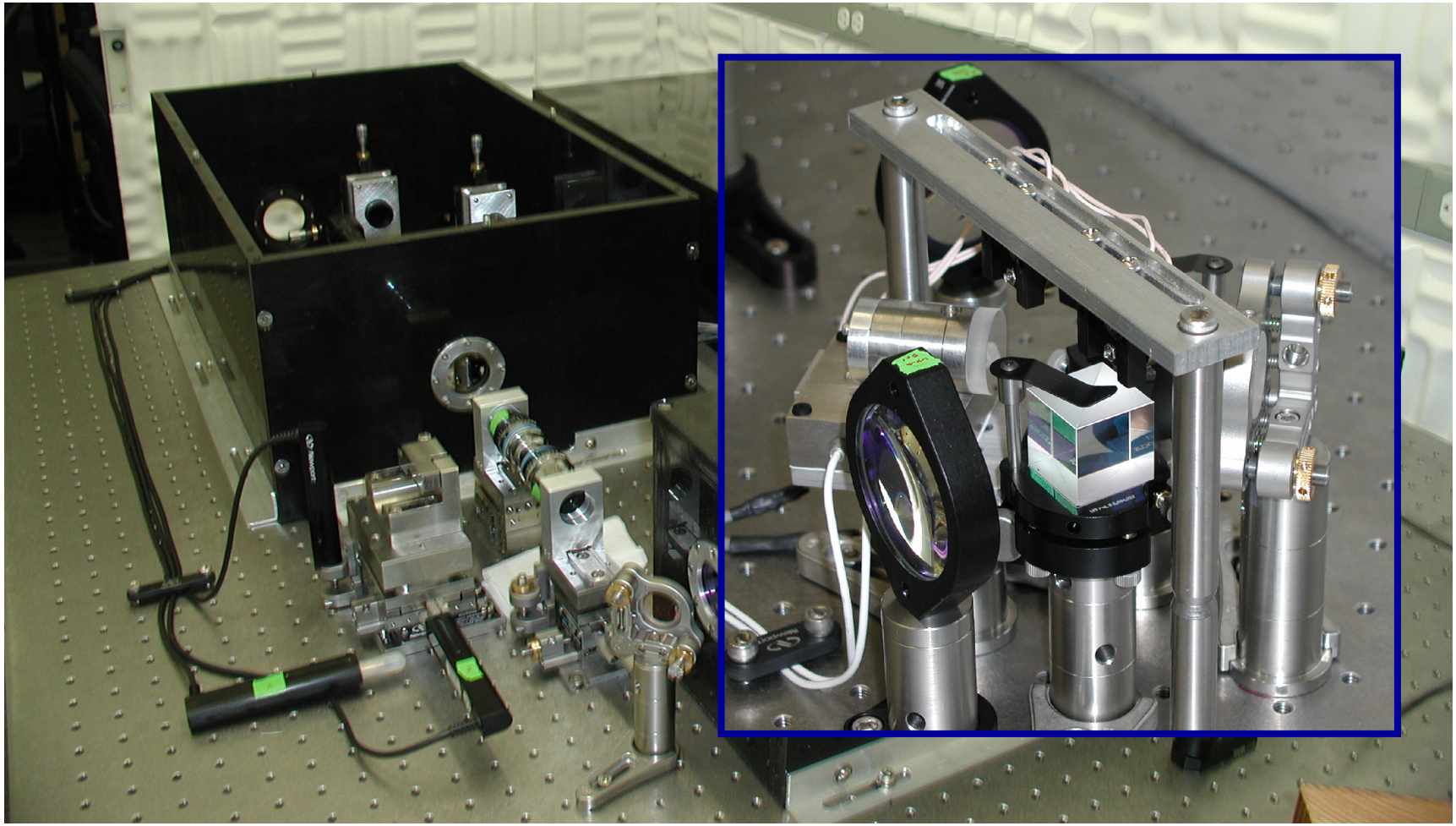
Laser pointing instabilities
– Two optical traps formed from one laser split by polarization





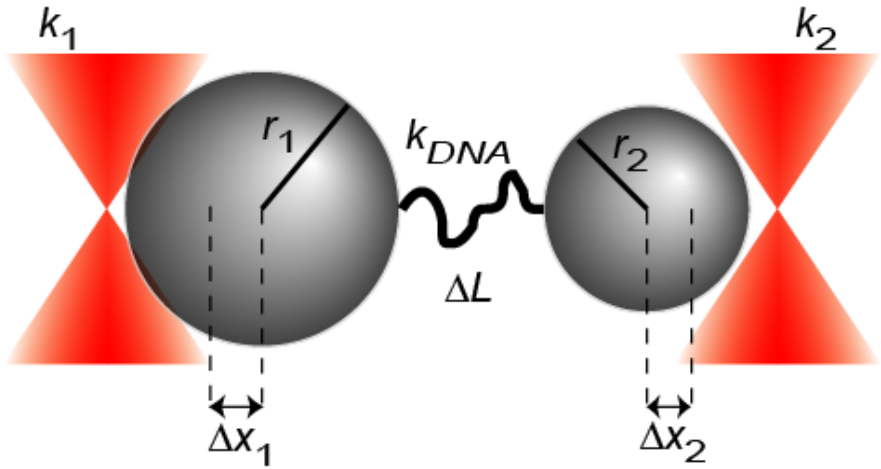
Moffitt, et al. *PNAS* (2006)

Instrument layout



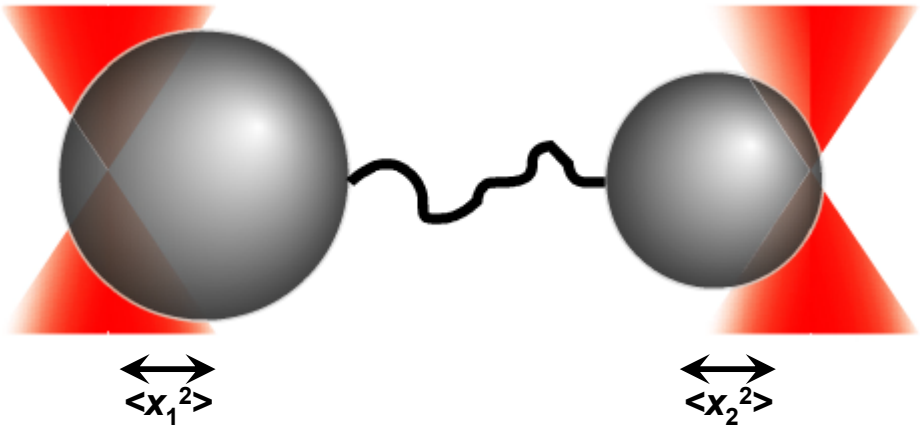
Brownian noise

Action of molecular motor deflects beads from initial position



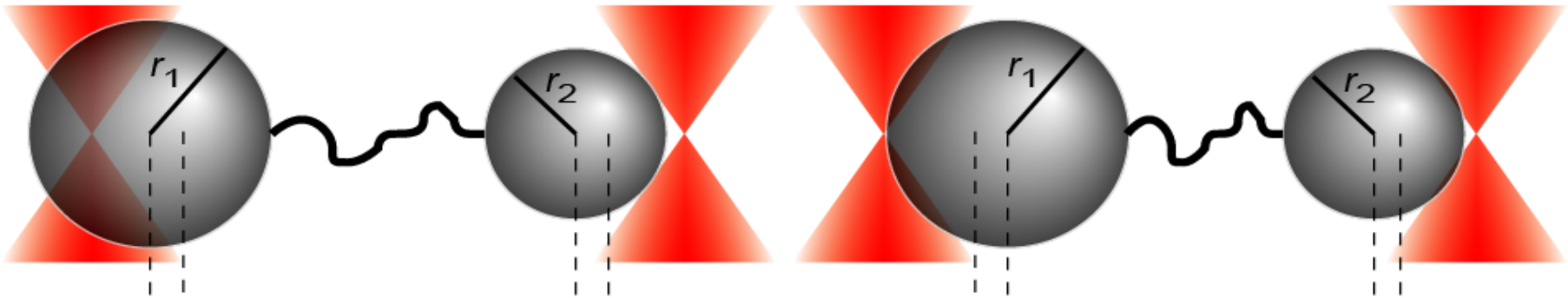
Brownian fluctuations of beads limit ability to resolve deflections

– Signal-to-noise ratio (SNR) = $\Delta x/\sqrt{\langle x^2\rangle_B}$



Brownian motion - exploiting correlations

Brownian fluctuations from 2 microspheres



Correlated motion

Anticorrelated motion

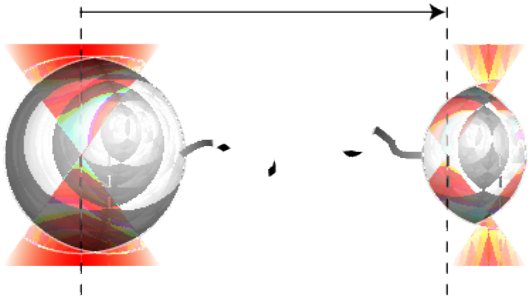
Only anticorrelated motion is relevant to detection of signal

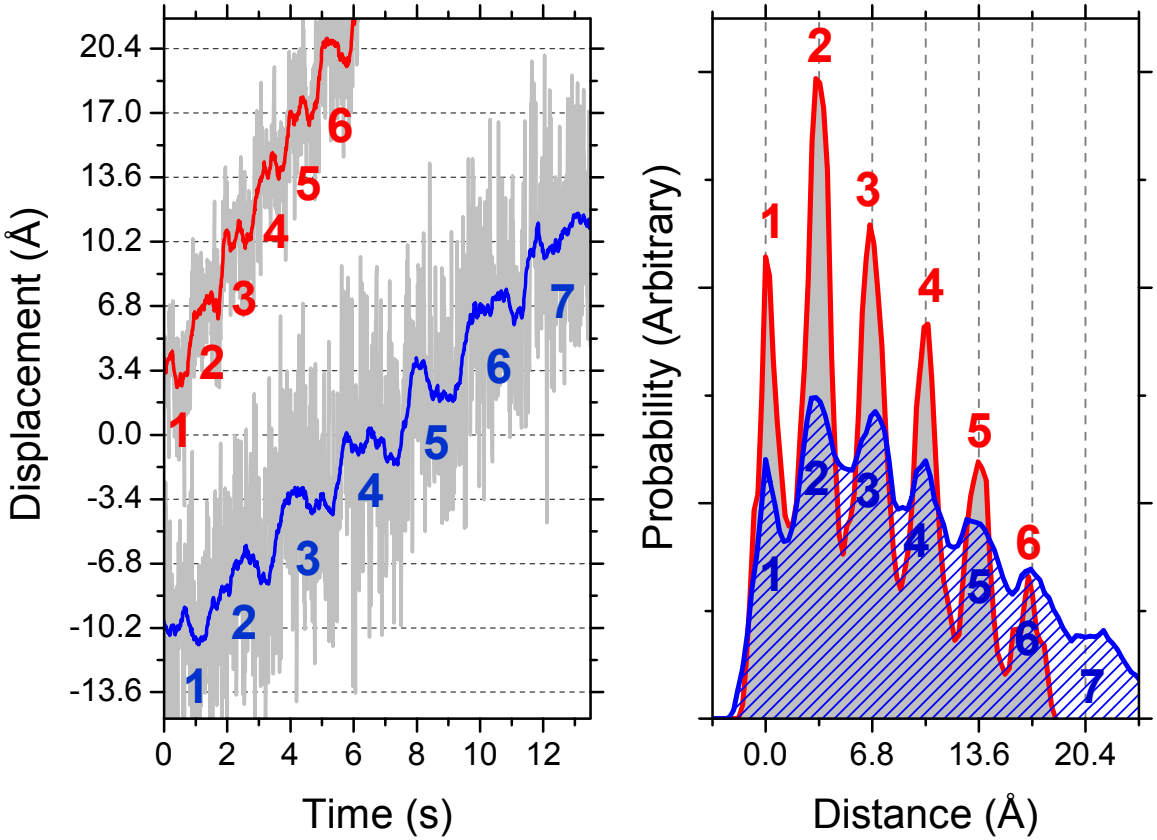
Form differential coordinate $x \equiv x_1 - \alpha x_2$ which optimizes resolution:

$$SNR_{opt} = \frac{k_{DNA}\Delta L}{\sqrt{4k_B TB\gamma_{eff}}} \qquad \gamma_{eff} = \frac{\gamma_1\gamma_2}{\gamma_1 + \gamma_2}$$

Moffitt, et al. *PNAS* (2006)

Resolution of 1-bp steps





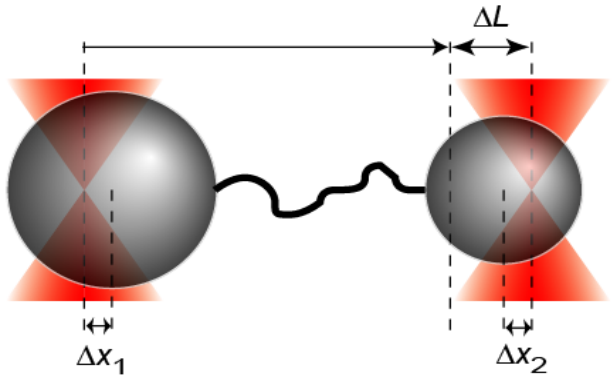
3.4 kb DNA

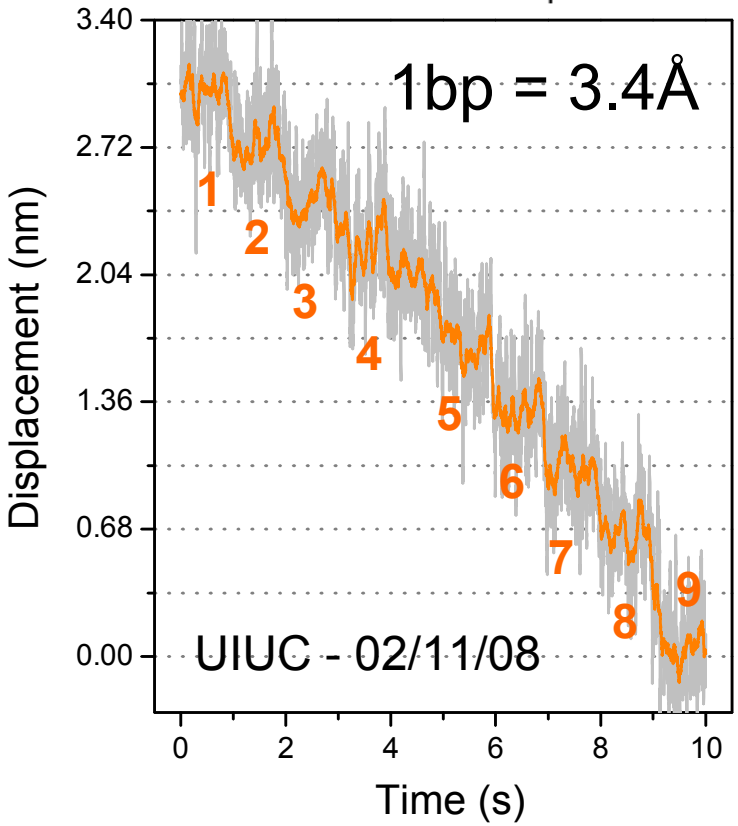
F ~ 7, 27 pN

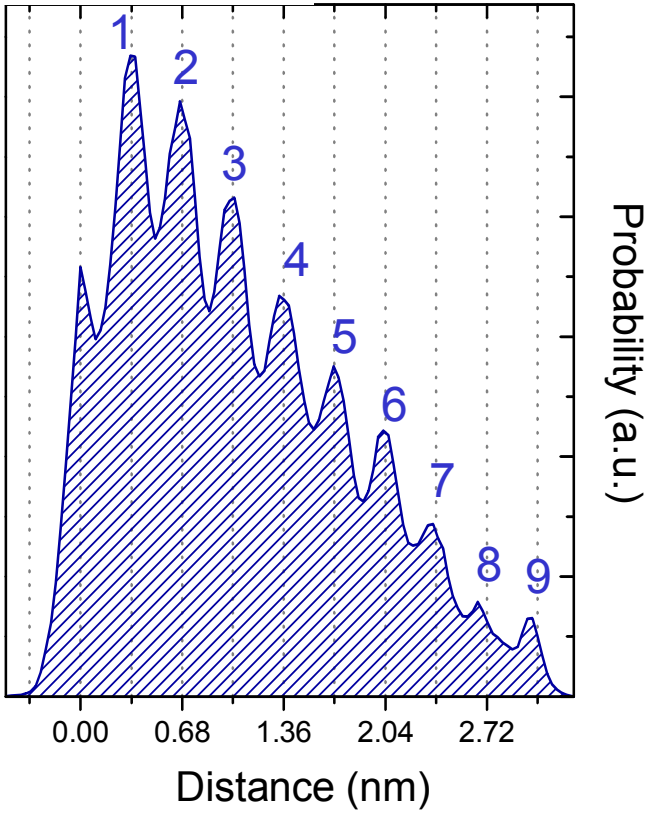
f = 50, 5, 5Hz

Moffitt, et al. *PNAS* (2006)

Basepair resolution at UIUC





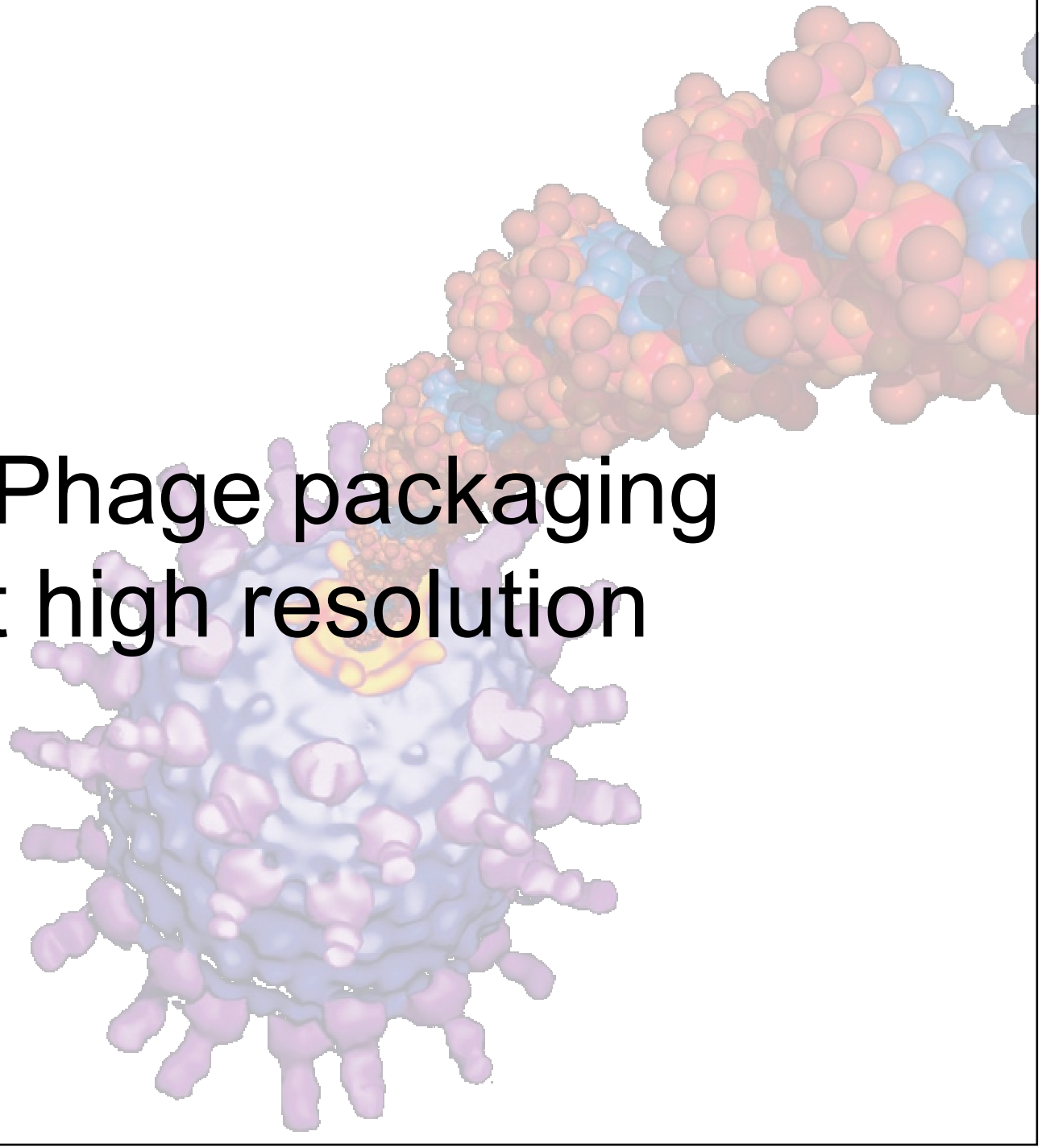


3.4 kb DNA

F ~ 20 pN

f = 100Hz, 10Hz

III. Phage packaging at high resolution



Acknowledgements

Jeff Moffitt[1]
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Sander Tans[3] (AMOLF)
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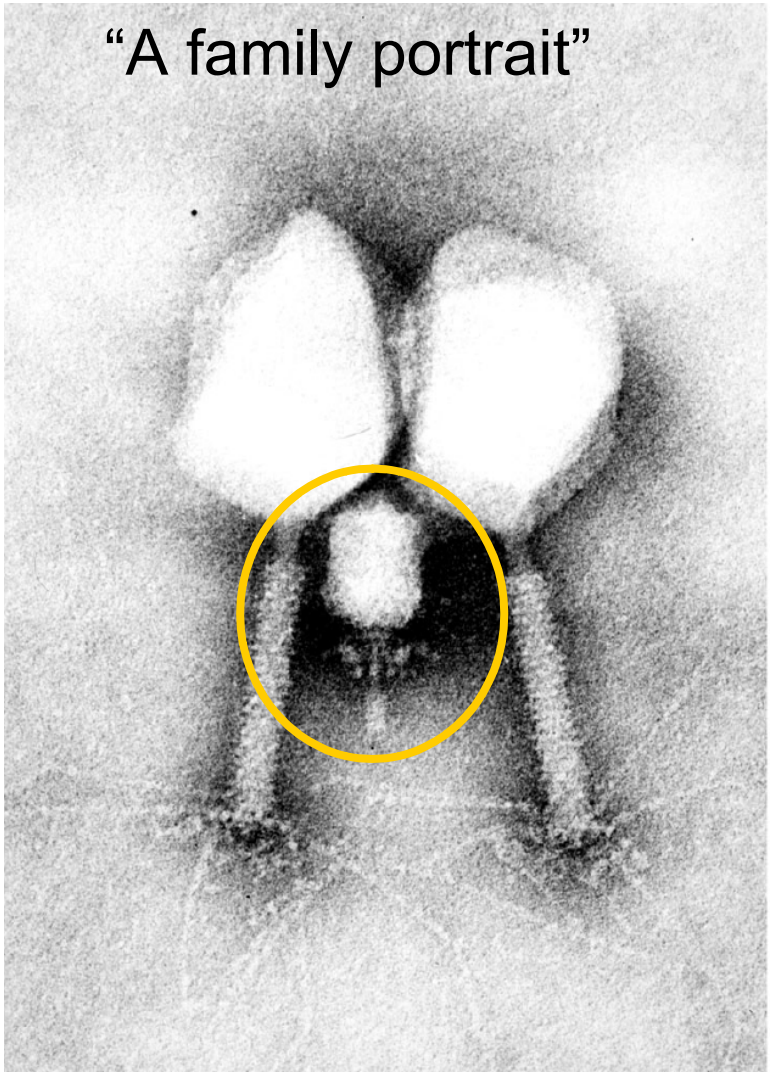
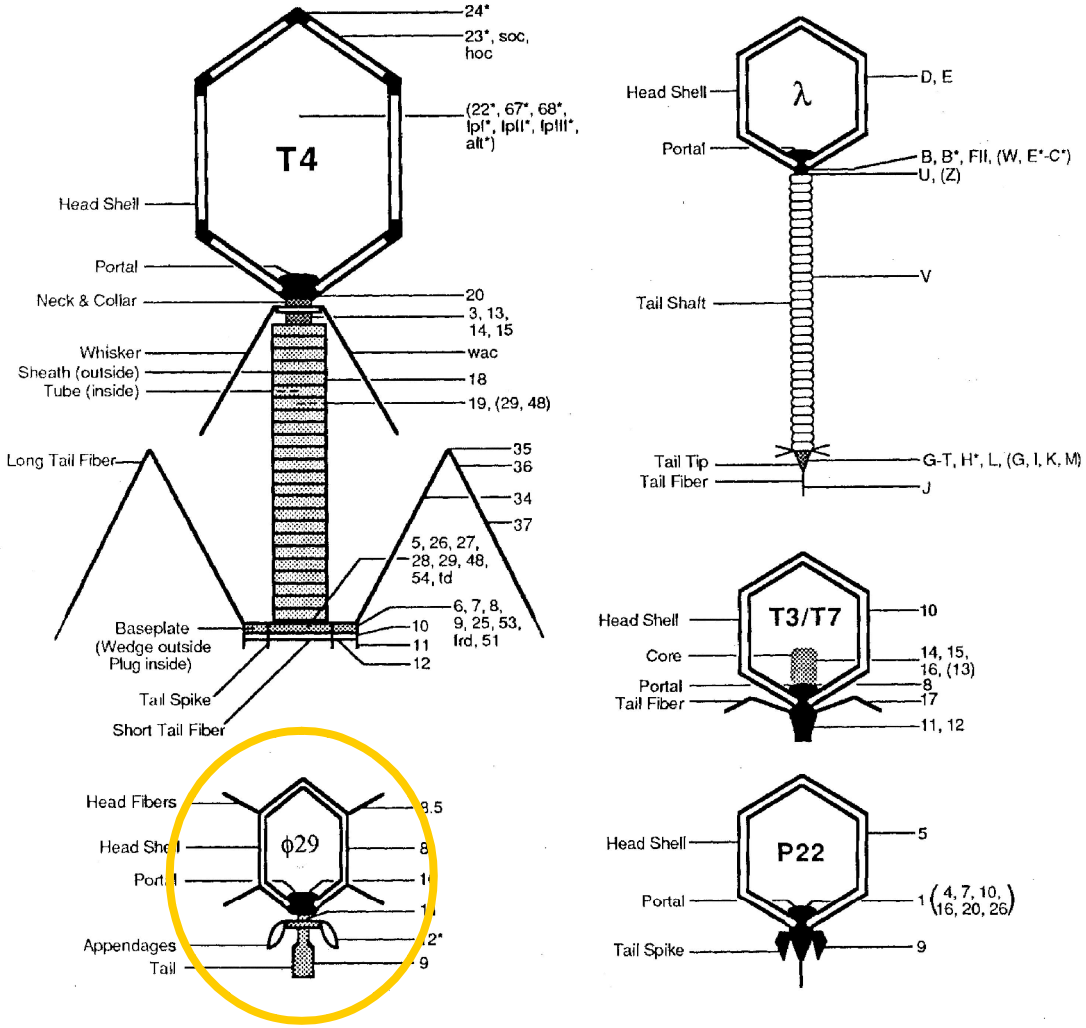
Shelley Grimes[2,3]
Paul Jardine[2]
Dwight Anderson[2,3]

[1]Moffitt et al., *PNAS* (2006)
[2]Chemla et al., *Cell* (2005)
[3]Smith et al., *Nature* (2001)

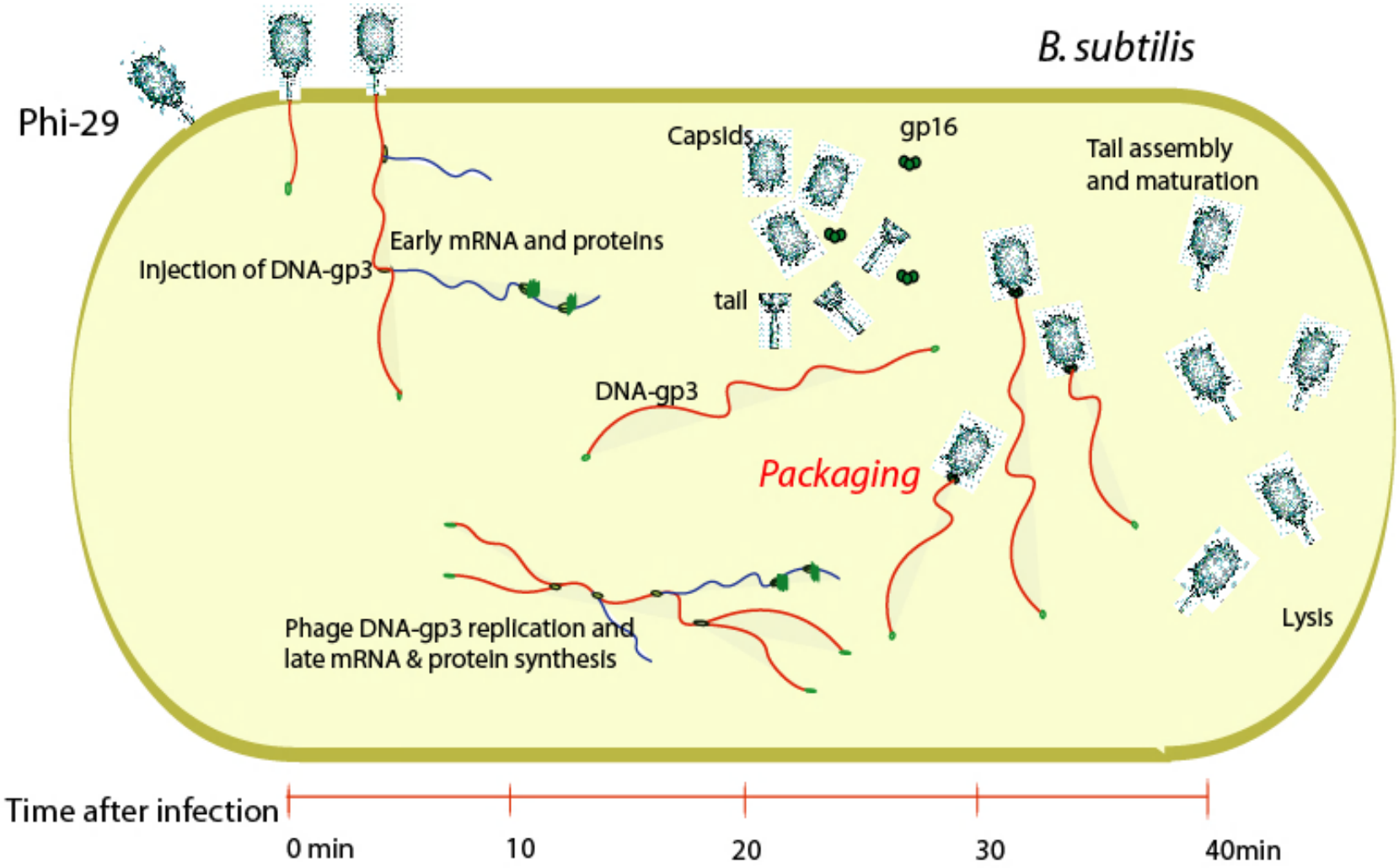
NIH National Service Research Award
Burroughs Wellcome Fund – Career Awards at the Scientific Interface

dsDNA bacteriophages





Bacteriophage life cycle



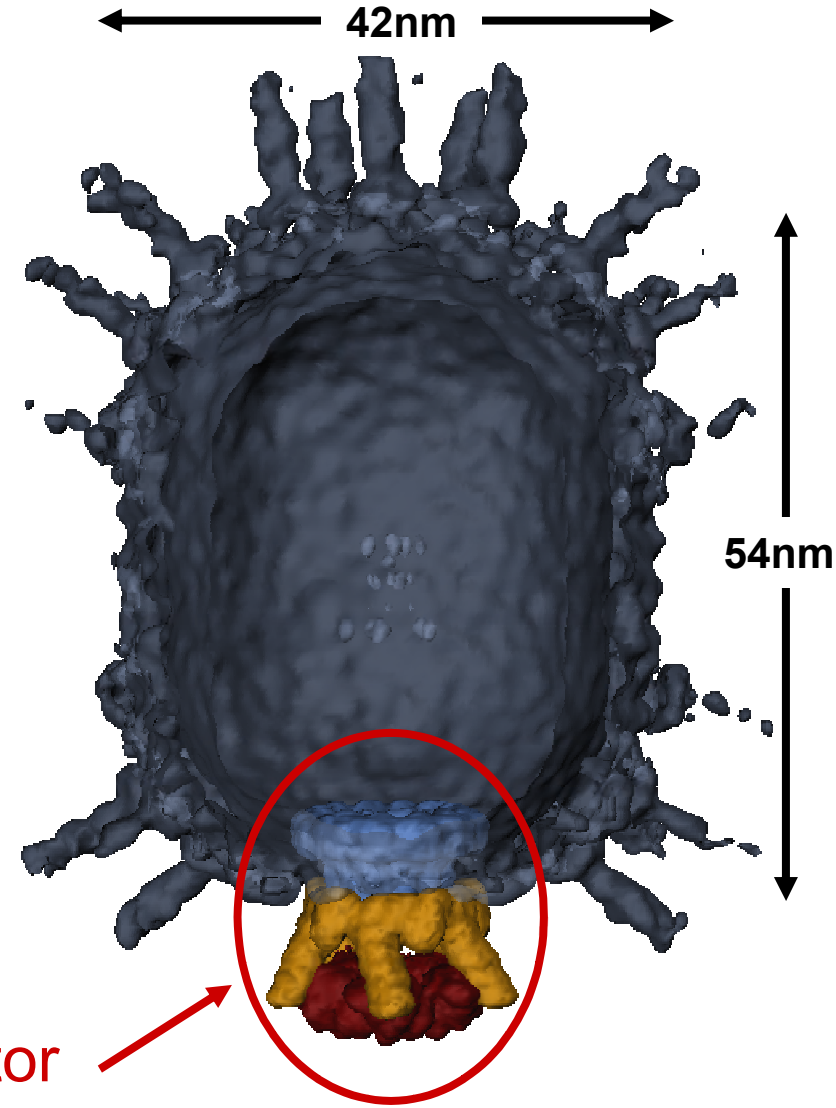
The bacteriophage φ29

φ29 packages a dsDNA genome 19.3Kbp (~6.6μm) in length

Volume of capsid: ~50×10^3 nm^3
Volume of DNA: ~21×10^3 nm^3

DNA density inside the capsid is ~400 mg/ml!

DNA charged, stiff (persistence length ~50nm)

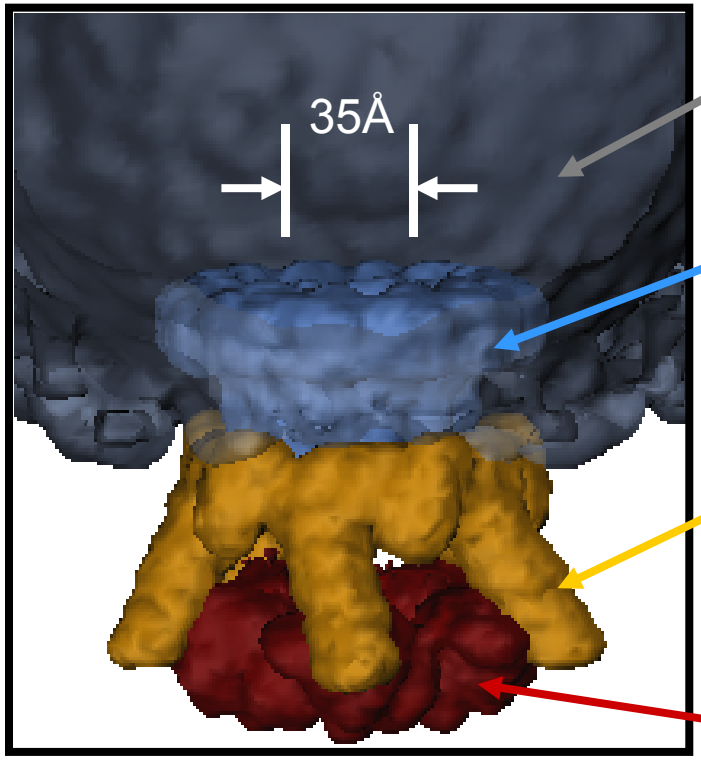


DNA packaging motor
1ATP/2bp (6.8 Å)*

*Guo et al. *J. Mol. Biol.* (1987)

φ29 Portal Motor Components

Portal motor - connector, prohead RNA, gp16-ATPase

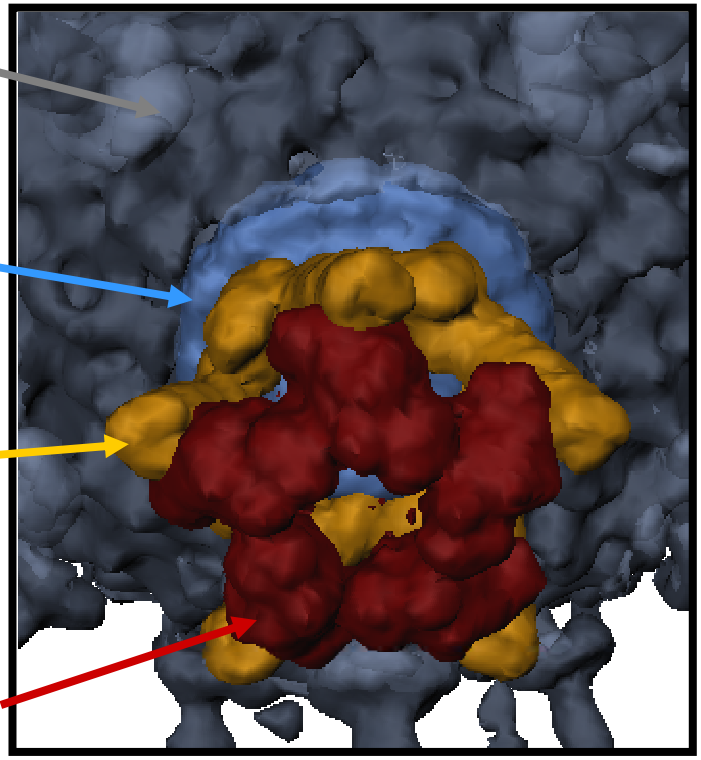


capsid
(5-mer vertex)

connector*
(12-mer)

pRNA
(5-mer)**

gp16-ATPase
(5-mer)†‡¶



Thanks: M. Morais

2bp/ATP

*Simpson et al. *Nature* (2000)
**Shu et al. *EMBO* (2007)

† Mitchell et al. *Nucl. Acids Res.* (2002)
‡ Iyer et al. *Nucl. Acids Res.* (2004)
¶ Draper and Rao, *JMB* (2007)

A single-molecule experiment

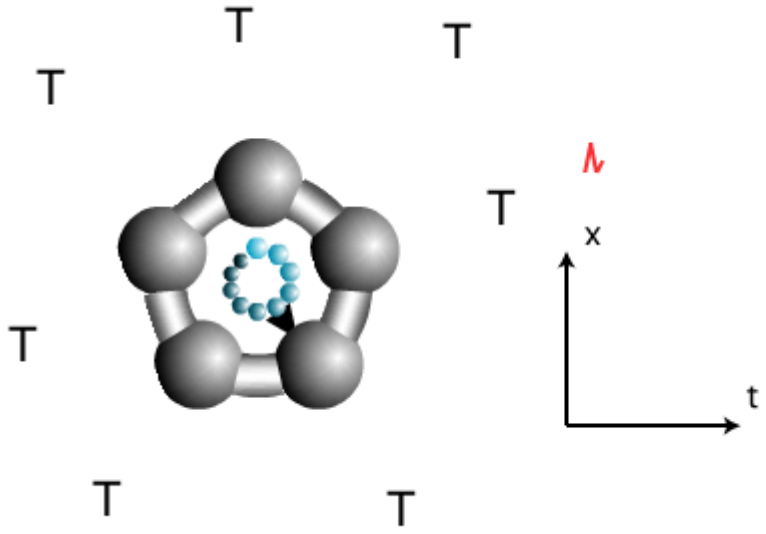
Model of packaging (c. 2005)

Observation:

– 1 ATP hydrolyzed / 2 bp DNA packaged*‡

Model:

– Assumes 1 ATP = 1 step = 2 bp DNA packaged†‡



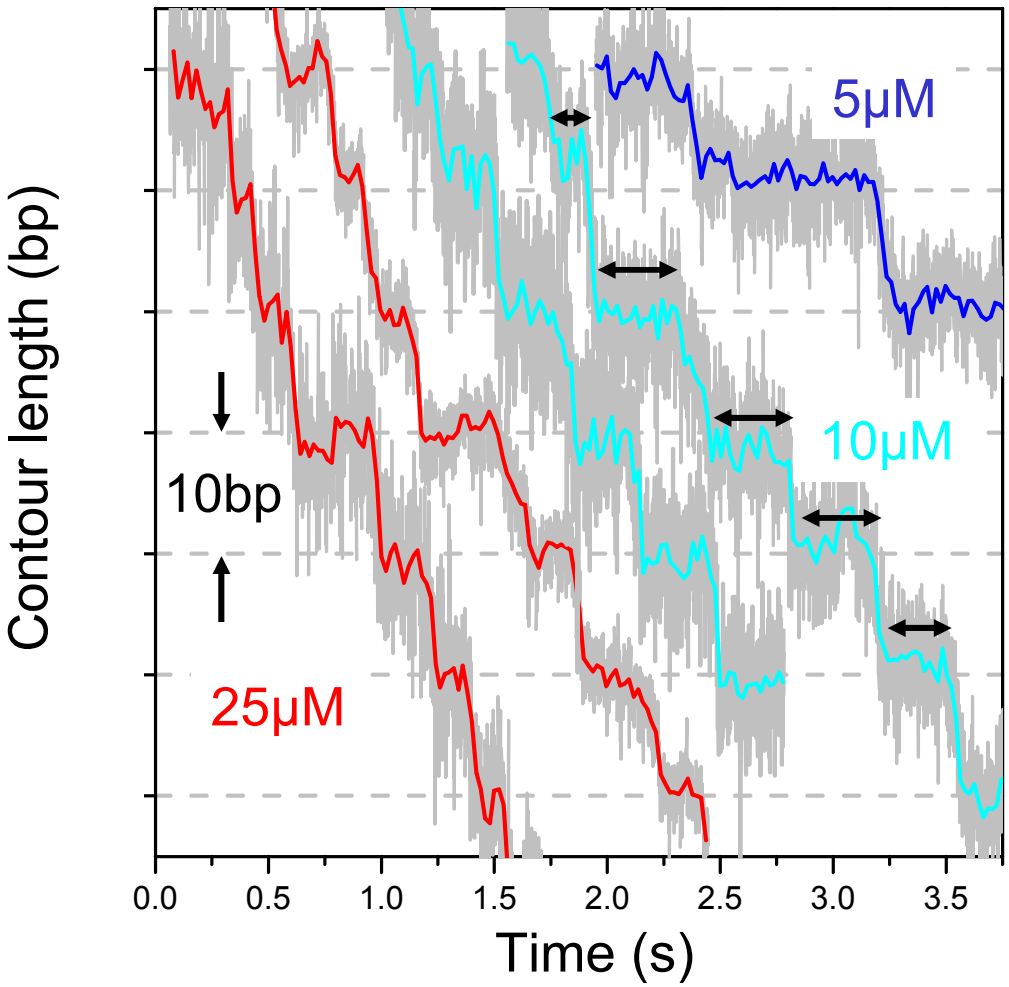
*Guo et al. *J. Mol. Biol.* (1987)
†Simpson et al. *Nature* (2000)
‡Chemla et al. *Cell* (2005)

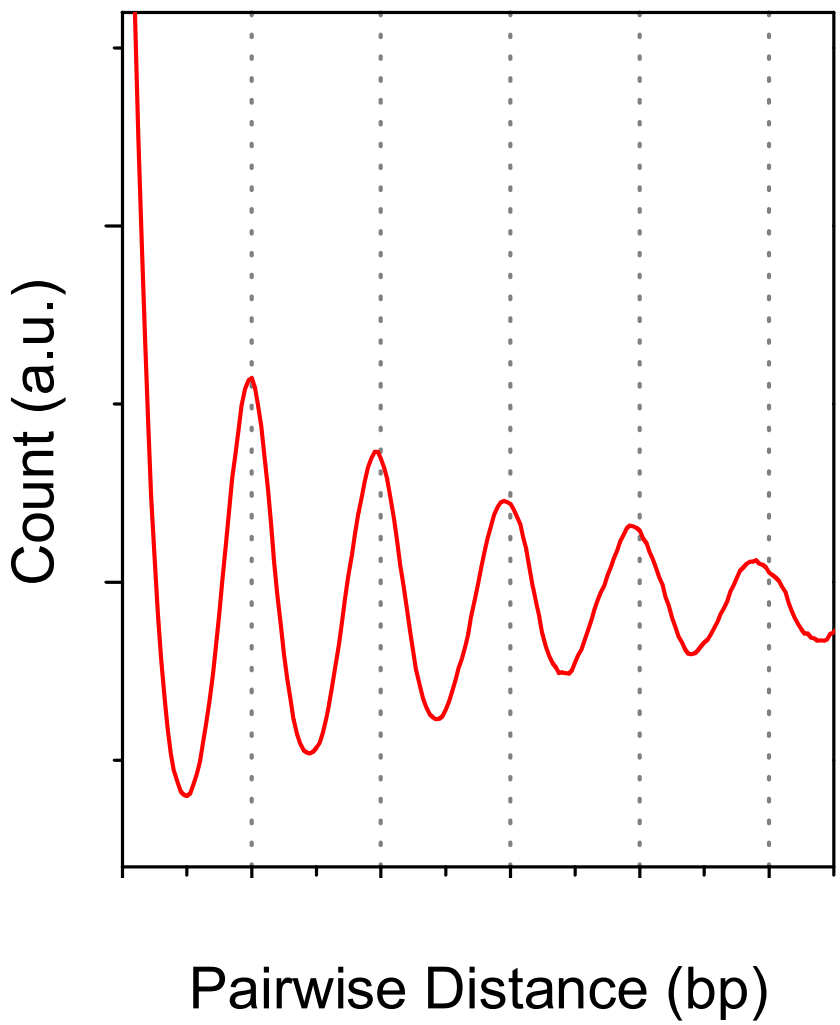
Goal:

– Directly observe putative packaging steps of 2bp (6.8Å)

First observation of phage steps

??? news: steps of 10 bp?

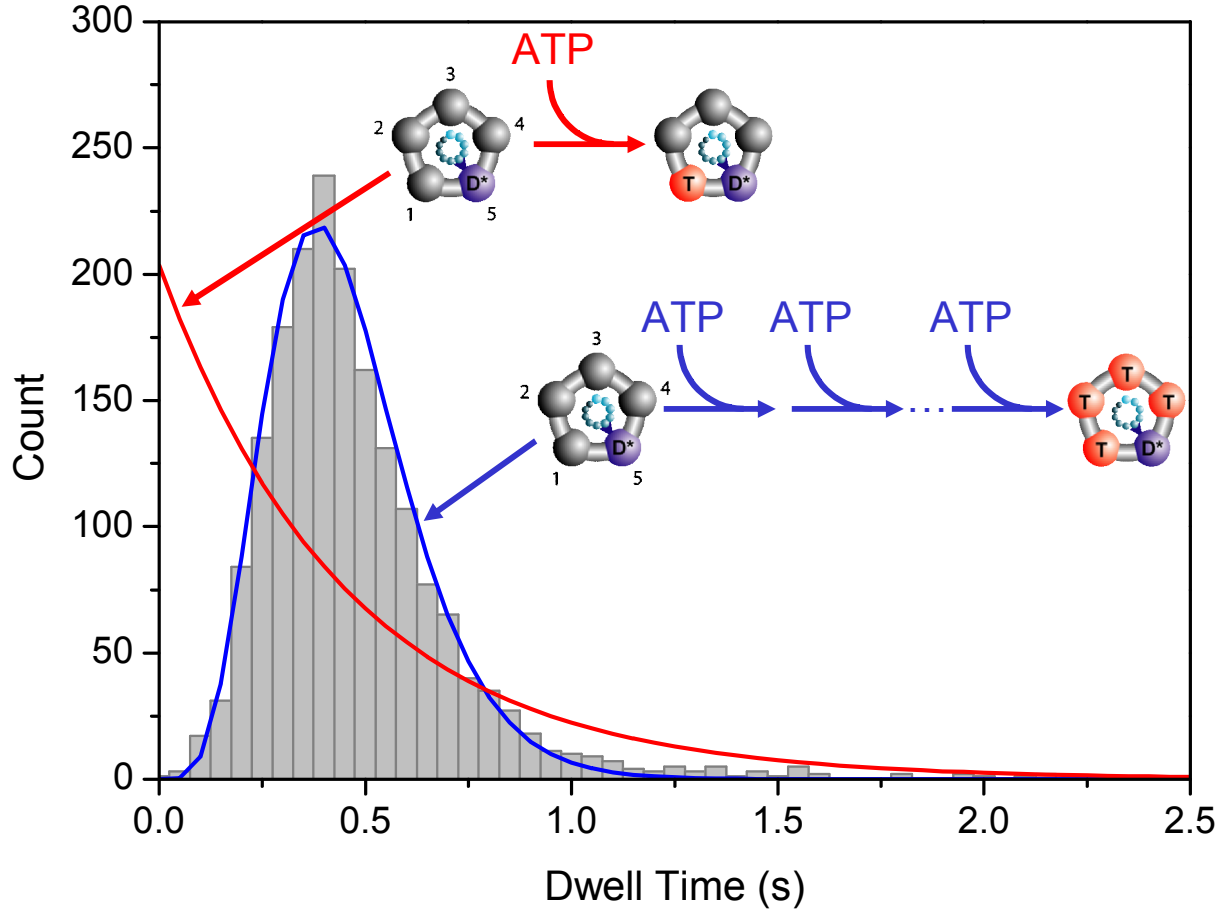




[ATP] = 5, 10, 25 μM < K_M F = 5-10pN f = 50Hz, 1kHz

Dwell time distribution

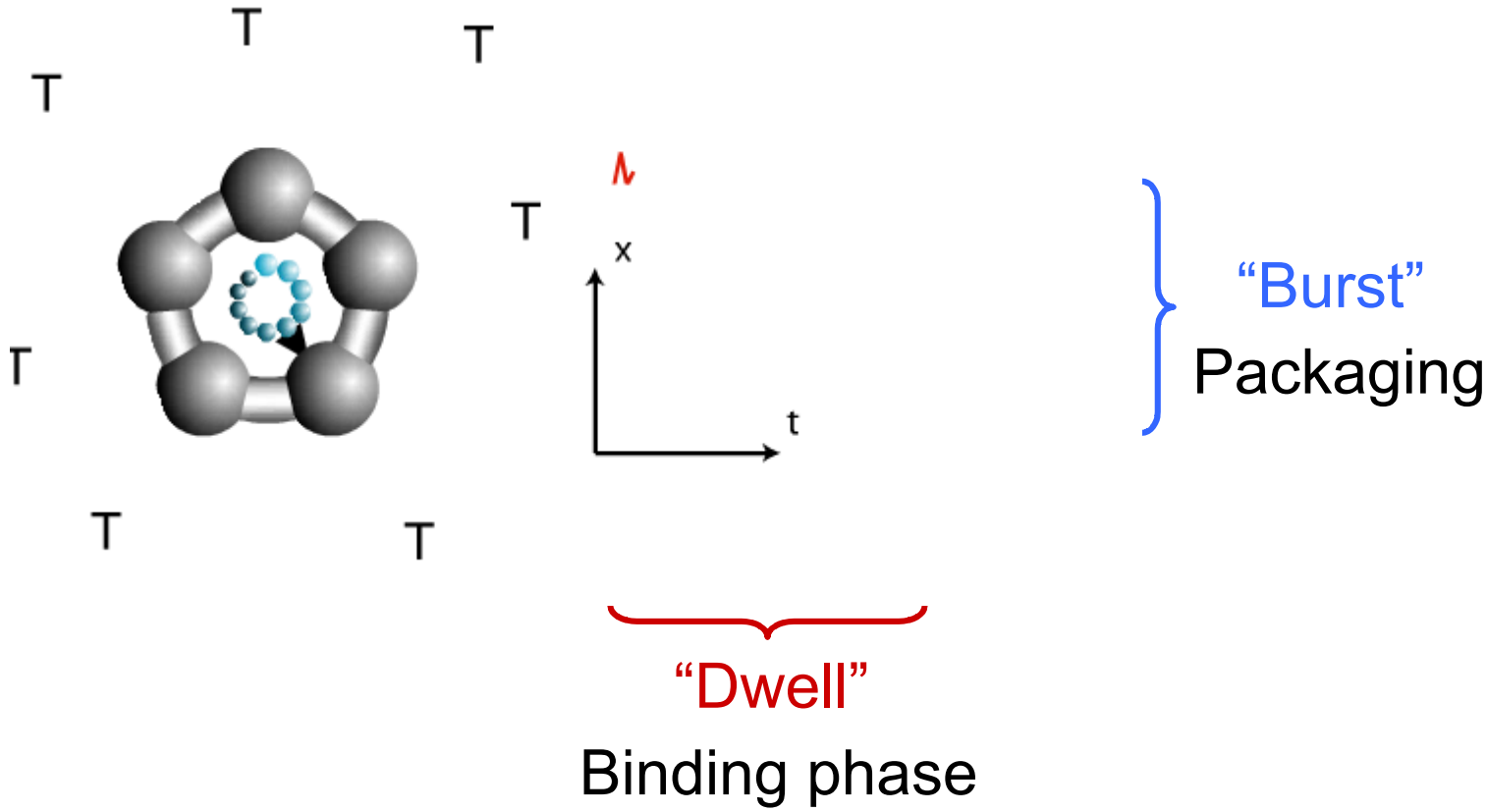
Dwell time *inconsistent* with 1 step = 1 ATP = **10**bp



Dwell time consistent with 1 step = N ATPs = **10**bp

New model of packaging

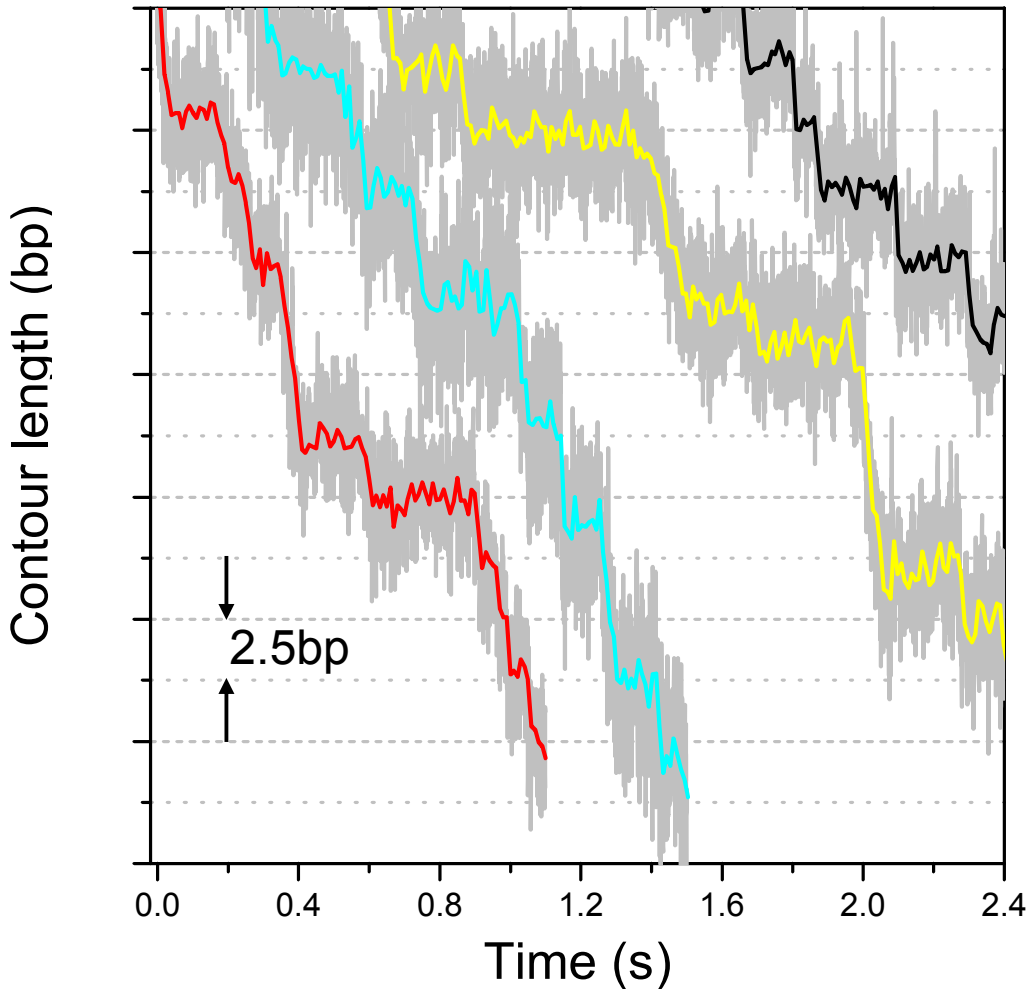
Old model: 1 ATP = 1 step = 2 bp is *incorrect*
Suggests new model: 5 ATP = 5 steps = 5 x 2 bp

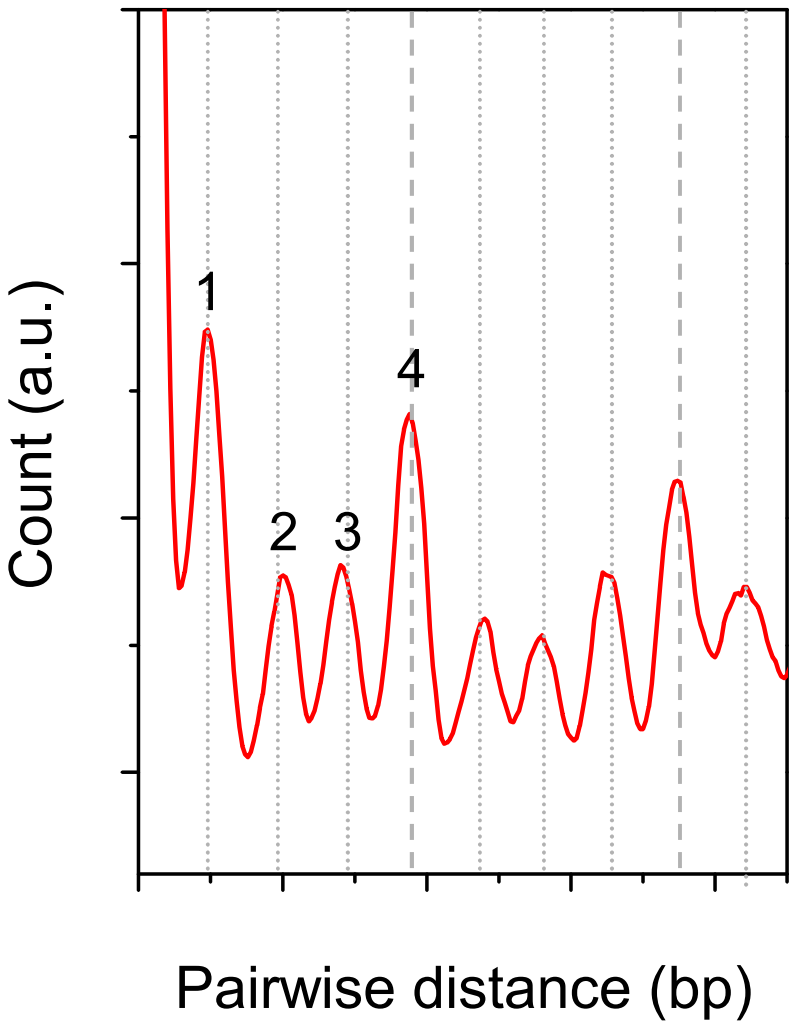


Use tension to observe steps in burst

Packaging at high force

10bp bursts interrupted by 2.5bp steps



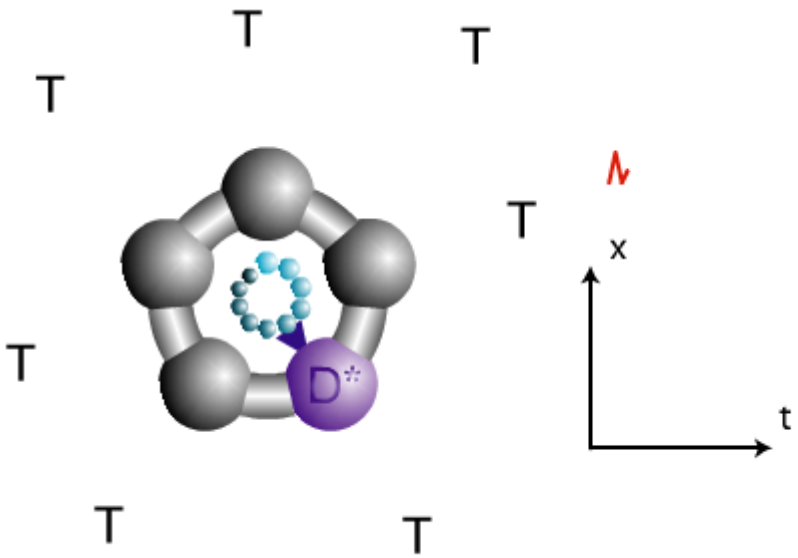


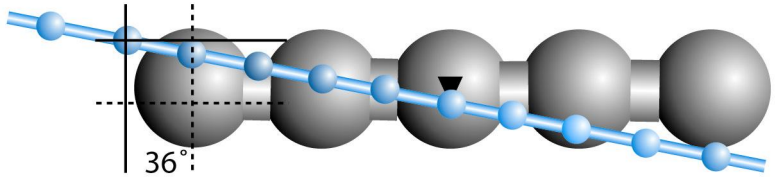
F = 35-45 pN f = 100Hz, 1kHz [ATP] = 250 μM

New(er) model of packaging 1

4 ATP = 4 steps = 4 x 2.5 bp = 10 bp

Closed ring model



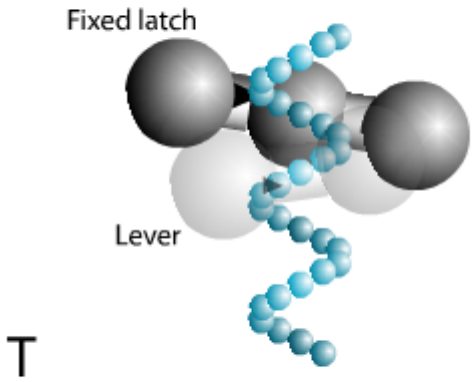


gp16-DNA contacts: 2 or 3bp? steric drive?
DNA rotation necessary 18˚/2.5bp step

New(er) model of packaging 2

4 ATP = 4 steps = 4 x 2.5 bp = 10 bp

Open ring model



Only two gp16-DNA contacts – package modified DNA?
No DNA rotation necessary

Conclusions

~~1 ATP binding = 1 step~~

~~1 step = 2bp~~

4 ATP = 4 steps = 4 x 2.5 bp = 10 bp


Thank you