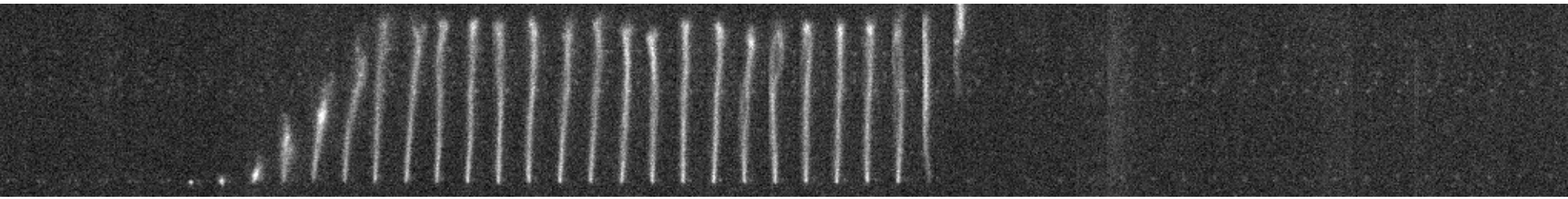
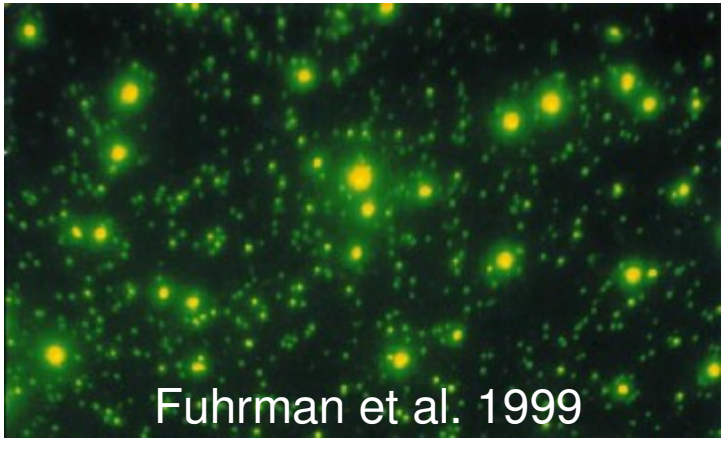
Watching phage λ ejection: towards a single-molecule Hershey-Chase experiment

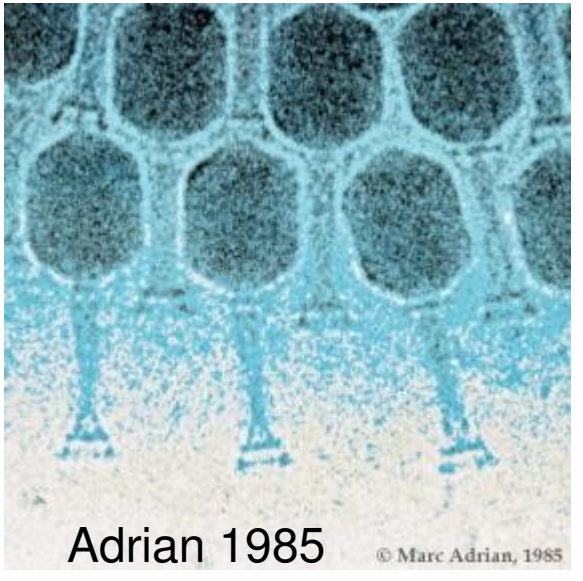
Paul Grayson
California Institute of Technology
March 14, 2007



Bacteriophages

- Most genomes are phage genomes (>10^{30})
- Found from polar ice to Sahara sand
- Similar to human viruses, *e.g.* HSV-1
- Direct causes of diseases, *e.g.* Cholera
- Challenge to models of evolution
- Tools for biotechnology


Fuhrman et al. 1999


Adrian 1985 © Marc Adrian, 1985

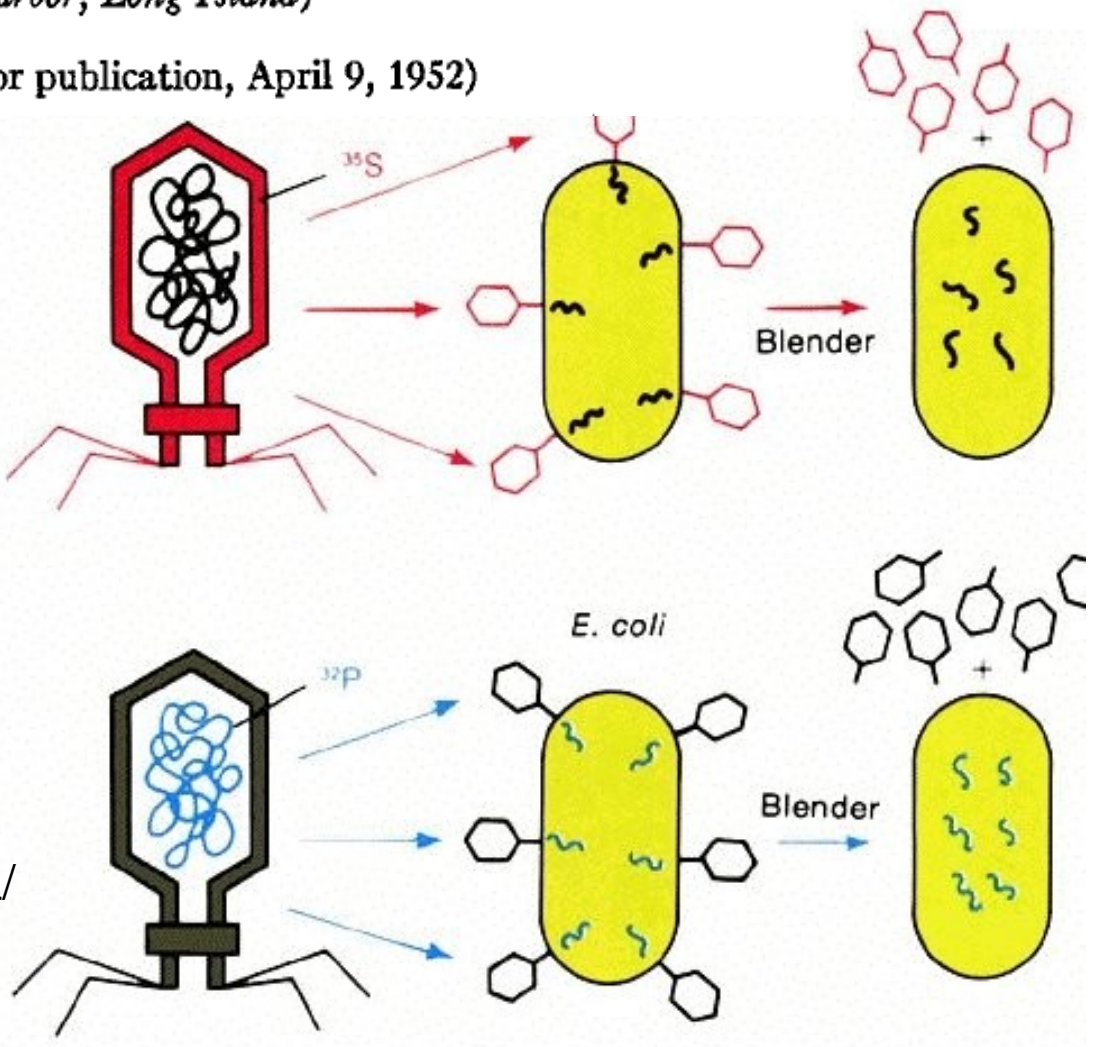
INDEPENDENT FUNCTIONS OF VIRAL PROTEIN AND NUCLEIC ACID IN GROWTH OF BACTERIOPHAGE*

By A. D. HERSHEY and MARTHA CHASE

(*From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island*)

(Received for publication, April 9, 1952)

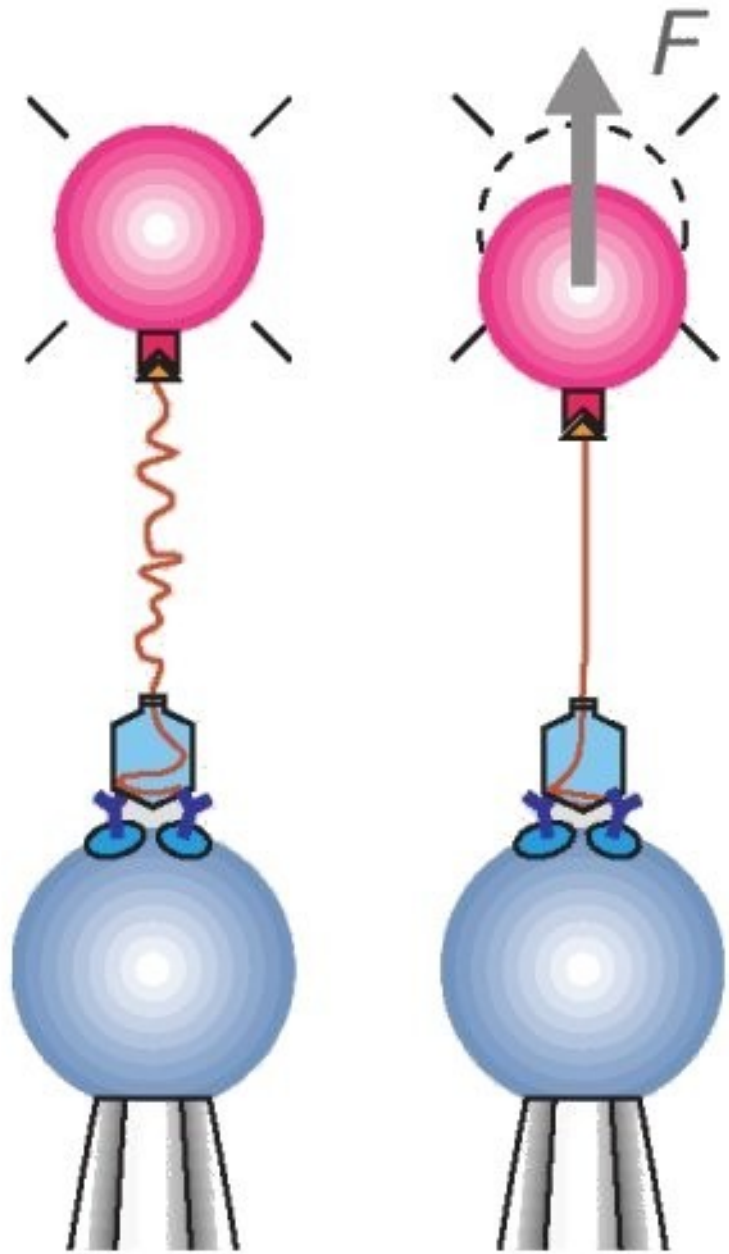
http://www.mun.ca/



- Hershey & Chase demonstrated DNA ejection (1952)

  ...Physics asks: *how* does ejection happen?

An inspiring experiment



Single-molecule measurements of phage packaging:

- Smith *et al.* (2001) – ϕ29 can exert ~60 pN

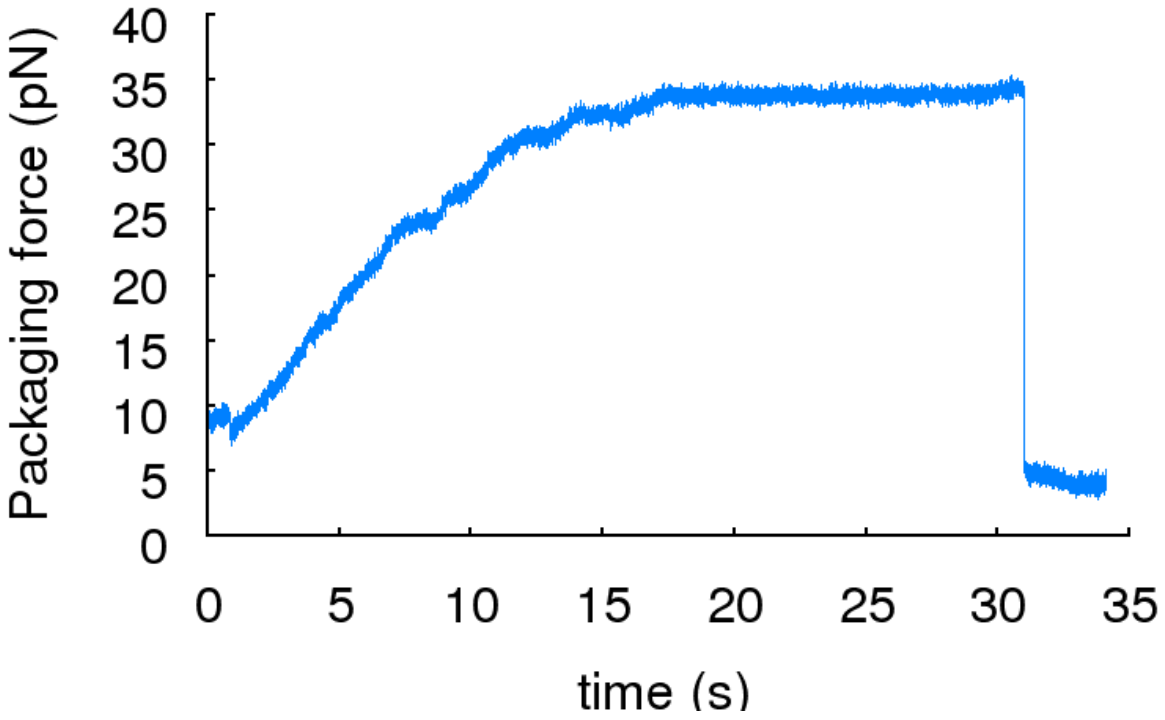
(At 65 pN, DNA is denatured!)

DNA packaged by a strong motor

Single-molecule measurements of phage packaging:

- 2007: The λ packaging motor can exert ~35 pN !

Optical tweezer measurement in Smith lab, 2007



Can we do *ejection* at the single-molecule level?

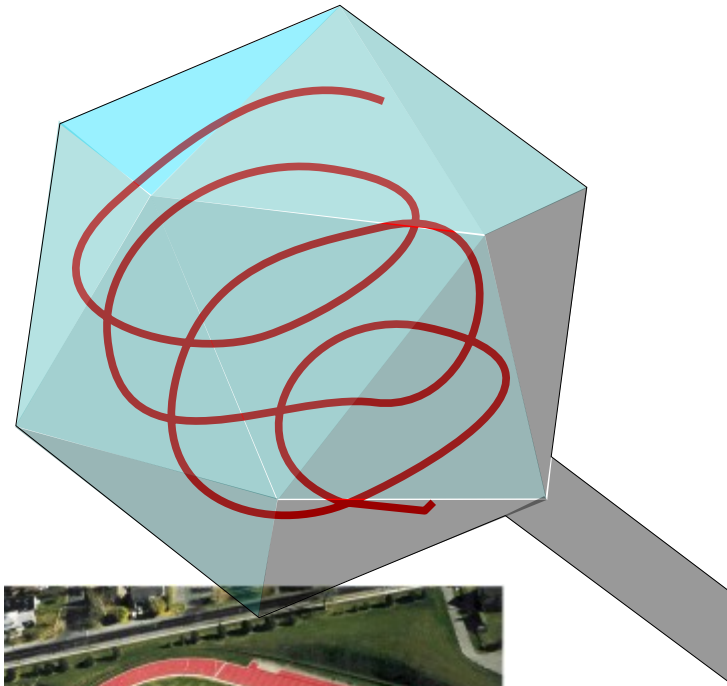
Why so much force?

- DNA density:

  16 um of DNA in a 57 nm capsid

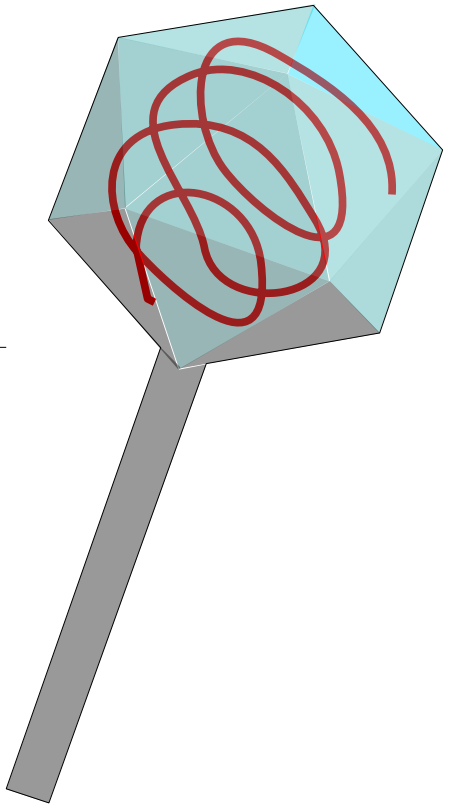
  My visualization:

  1 marathon in 150 m



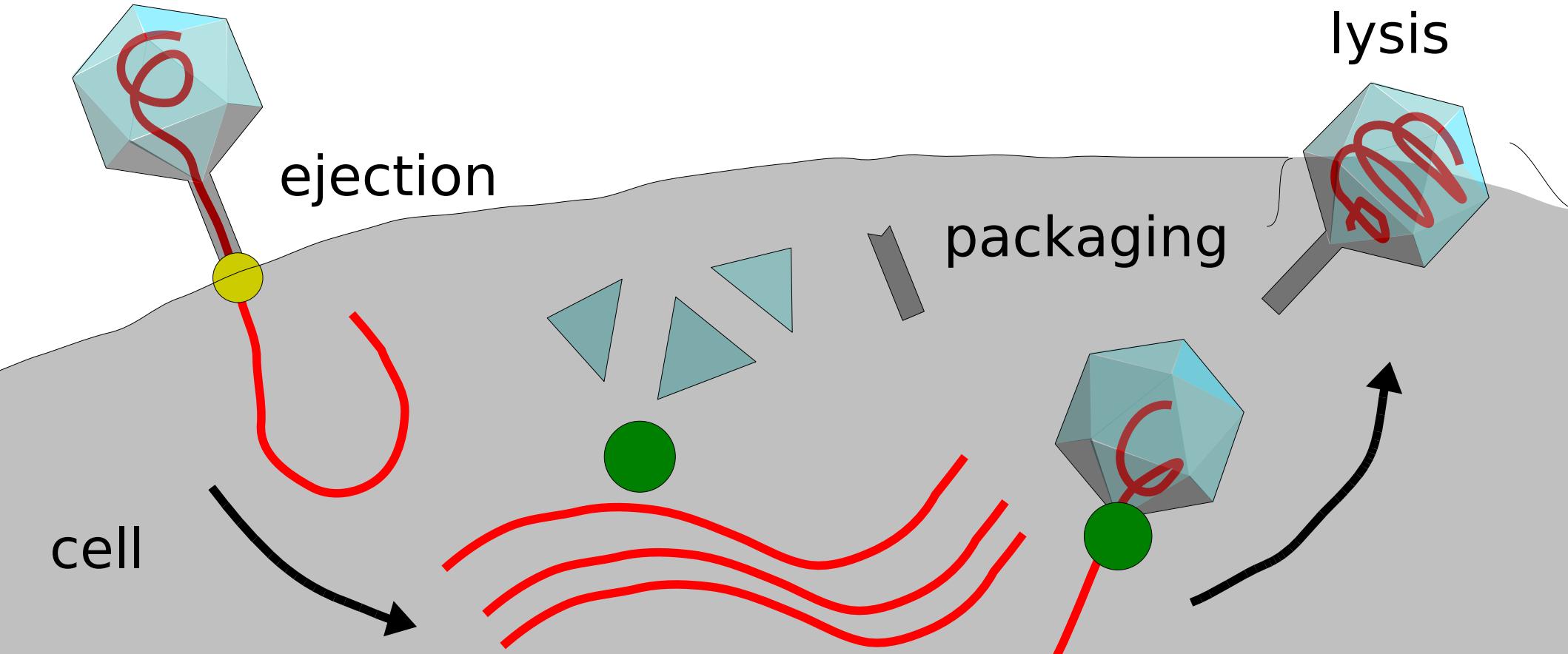
Genome density in viruses

Virus	Genome	Capsid size	Packing density
λ	48.5 kbp	57 nm	53.4%
T7	40 kbp	55	49.0
ϕ29	19.3 kbp	44.1	45.9
λb	38 kbp	57	41.9
polio	7.4 kb	27 nm	29.8%
HIV	18.4 kb	70	4.3
pox	186 kbp	220	3.6



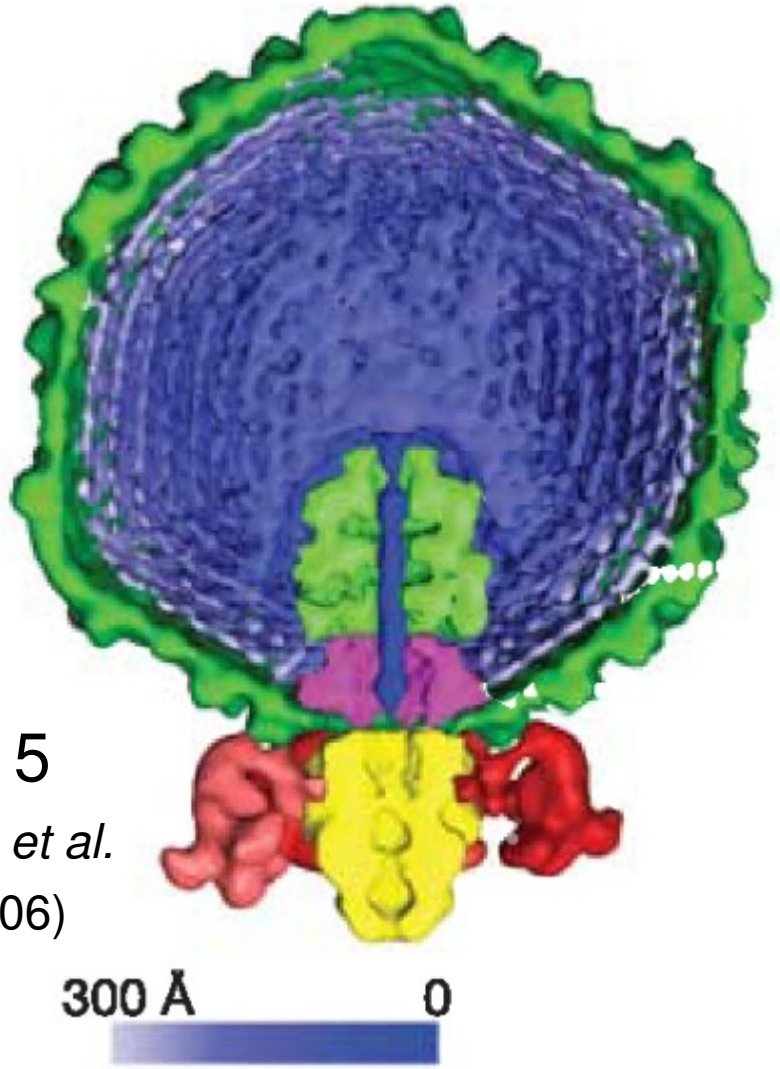
Force is needed for ejection

- >10 pN of force, used to get DNA into the host?
- See also Grayson and Molineux, *Is phage DNA "injected" into cells - biologists and physicists can agree.* (Curr Opin Microbiology, 2007)



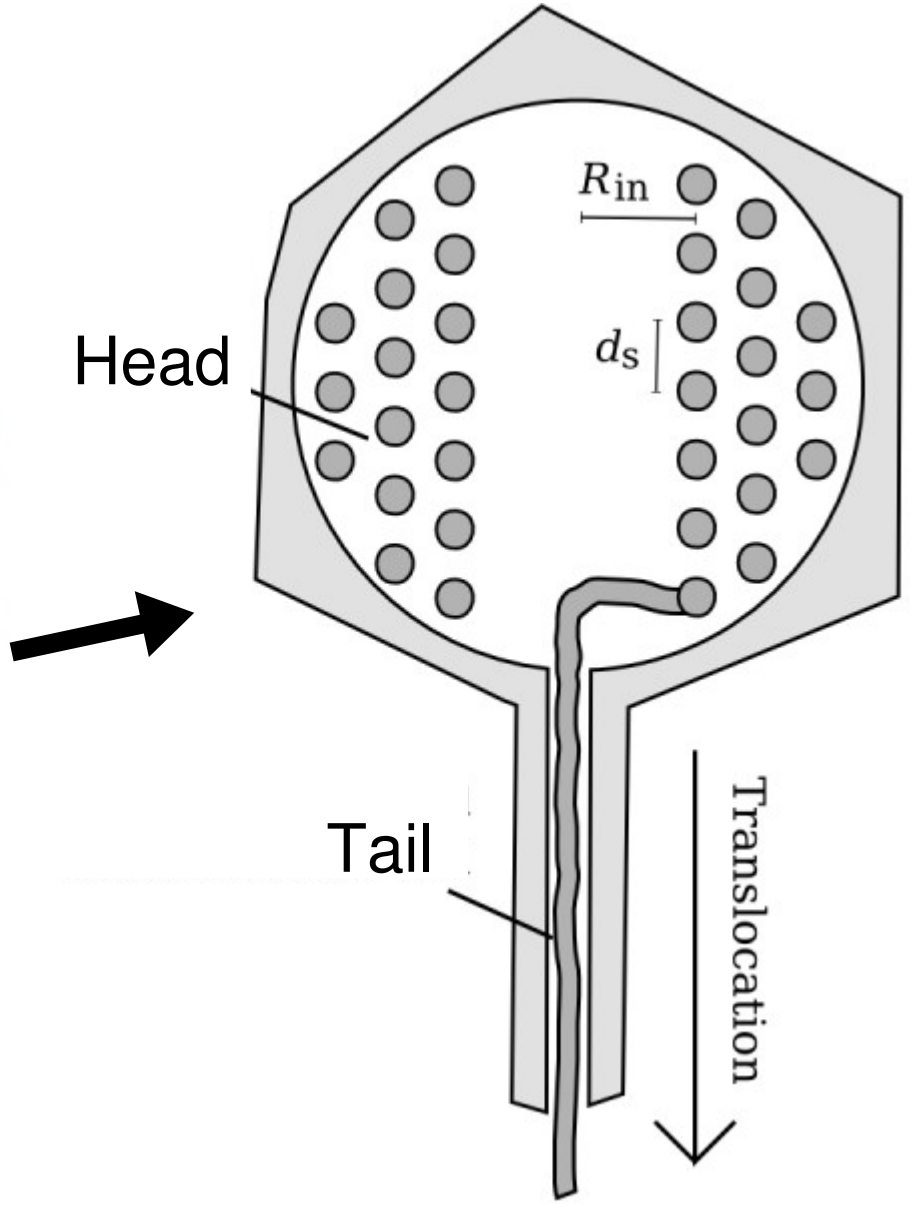
Theorizing about phages

Inverse-spool model:



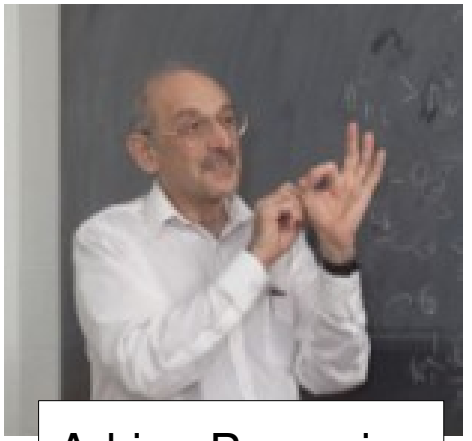
ε15

Jiang *et al.* (2006)



Force depends on DNA density


Don Rau


Adrian Parsegian

- X-ray measurements on bulk DNA: pressure ➔ force
- Bending stiffness relatively insignificant for the force

↓

Theory for the ejection force with **no fitting!**

Purohit *et al., Forces during bacteriophage DNA packaging...* (Biophys J, 2005).
Garcia *et al., Biological consequences of tightly bent DNA...* (Biopolymers, 2007).

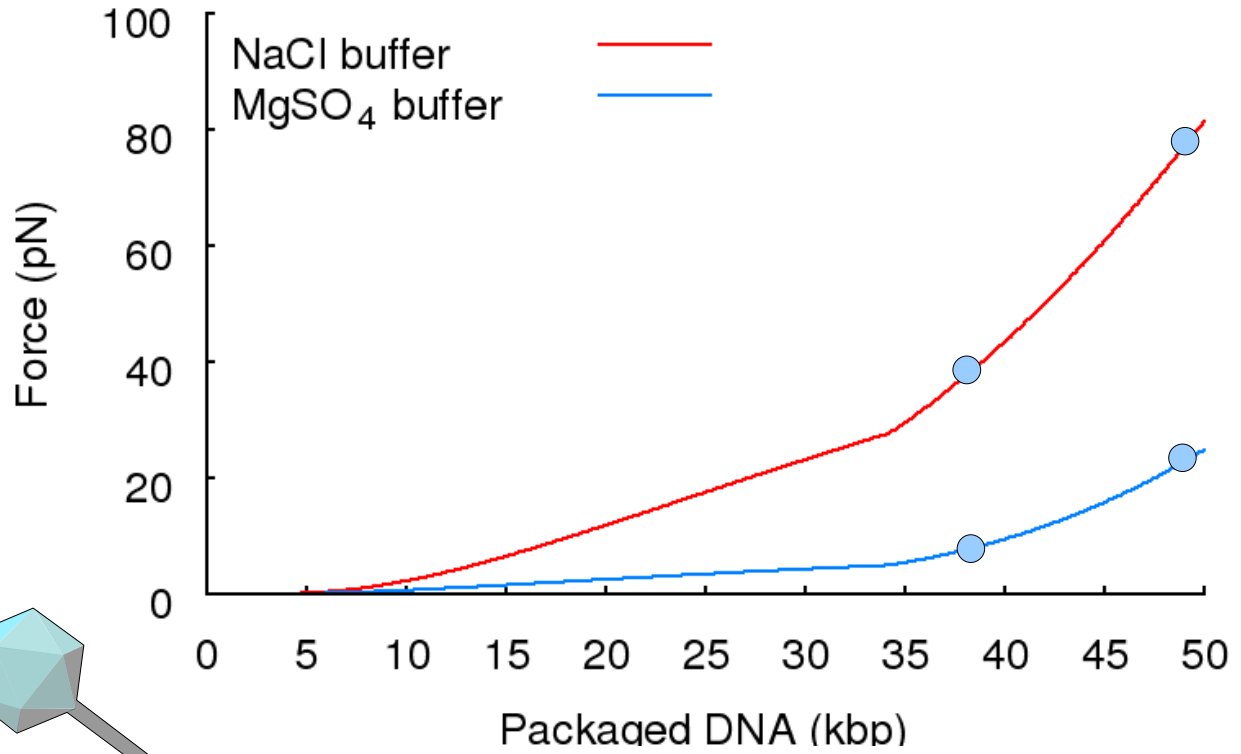
Force as a function of...

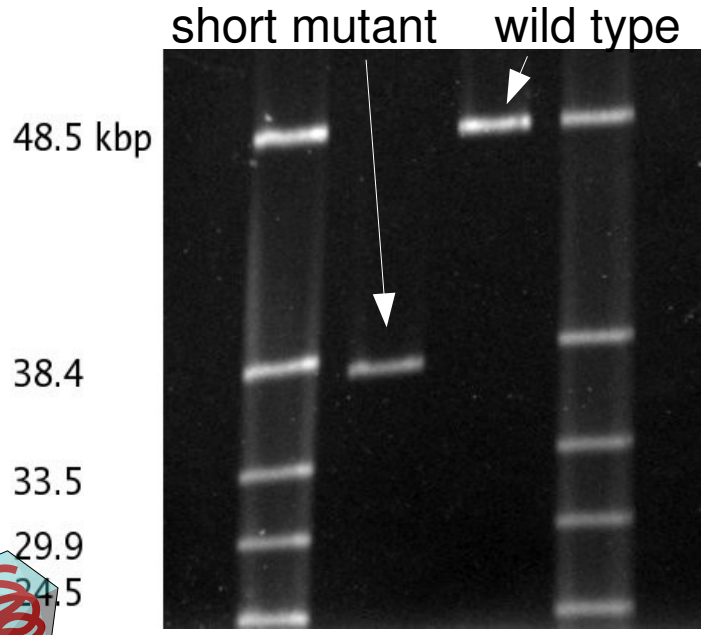
Force depends on:

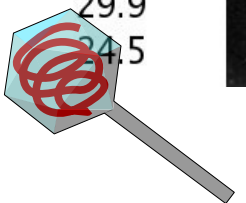
- Genome size (38/48 kbp)
- Ions (Mg^{2+} / Na^{+})


Prashant Purohit


Mandar Inamdar







What about dynamics?

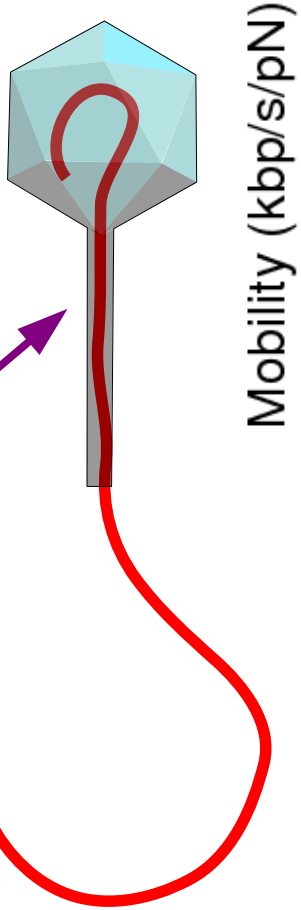
- Define **mobility** = v/F .

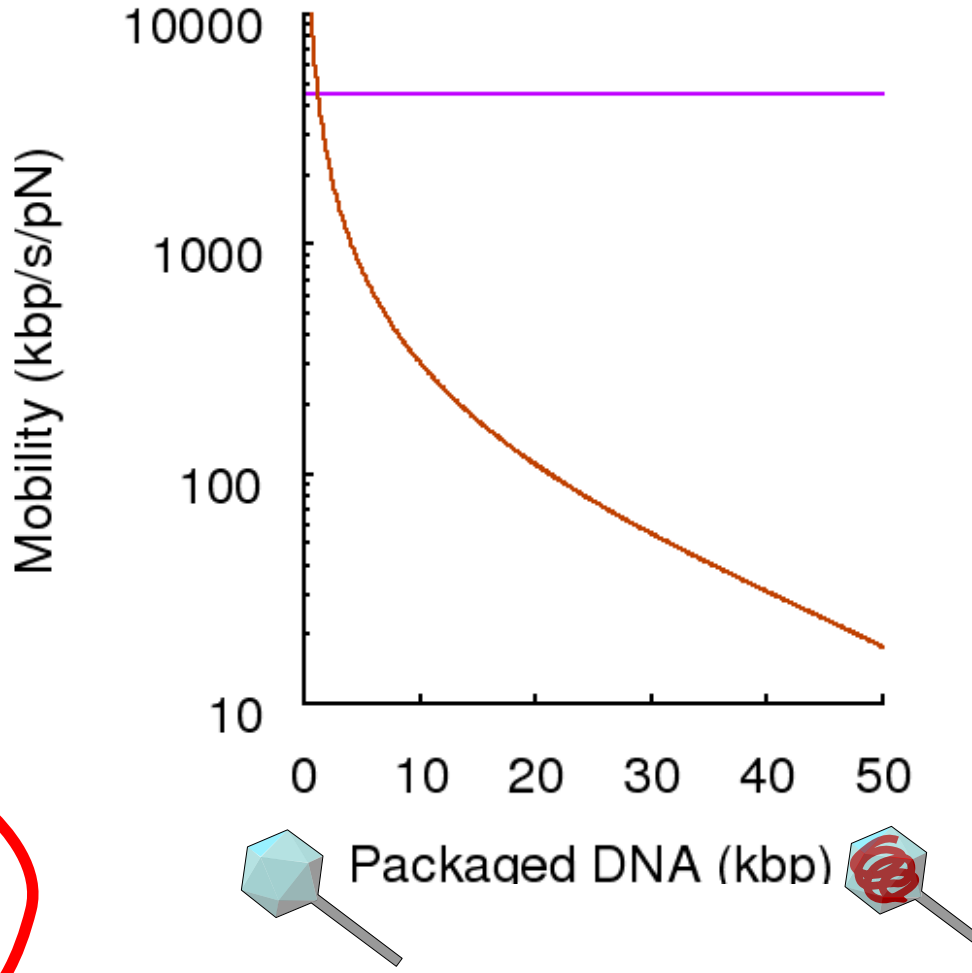
DNA-DNA interactions?

Mobility depends on DNA density.

DNA-tail interactions?

Constant mobility.





Gabashvili and Grosberg (1992)

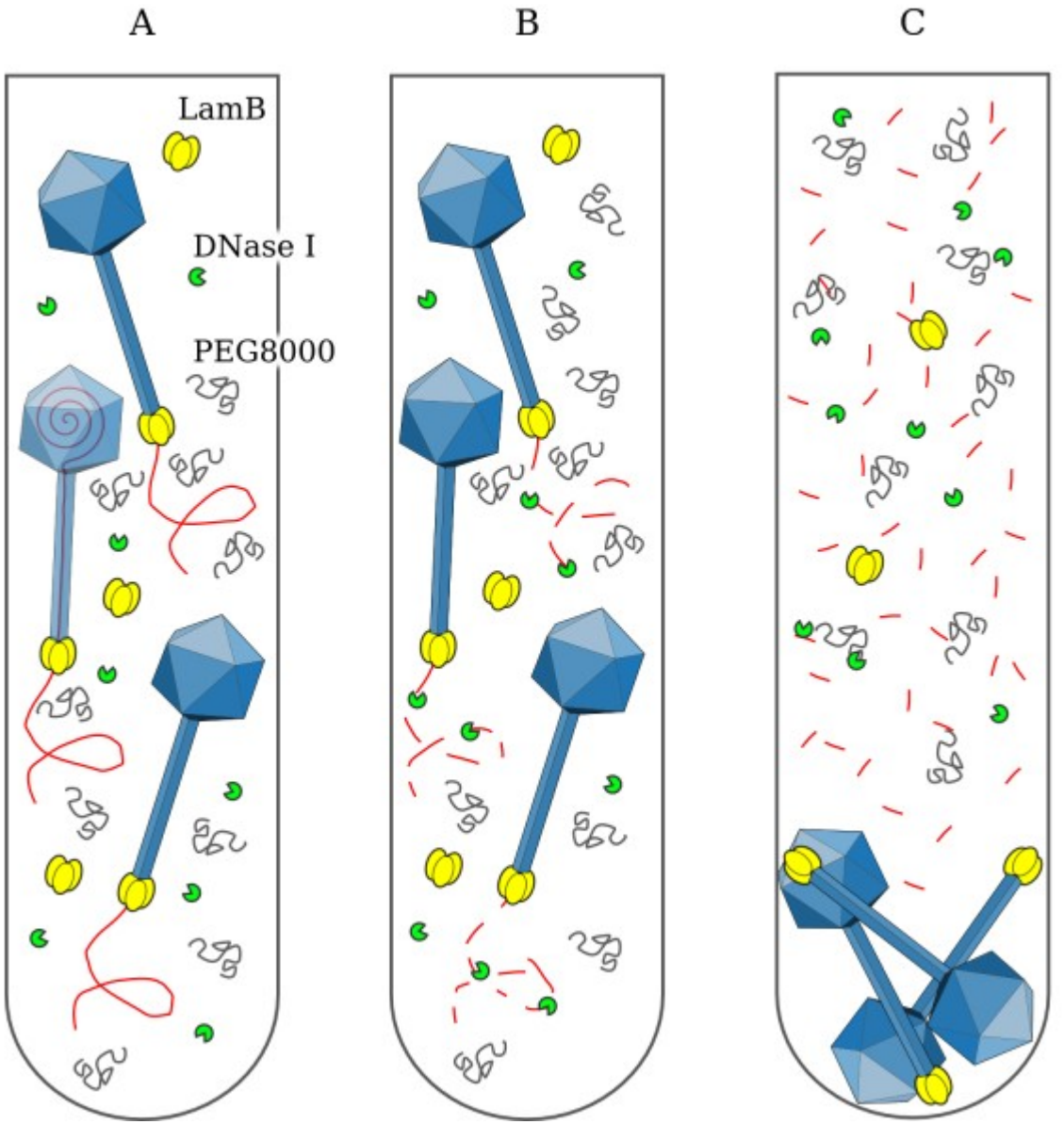
Testing theory with λ: static force

Osmotic force from PEG8000 balanced with internal force.

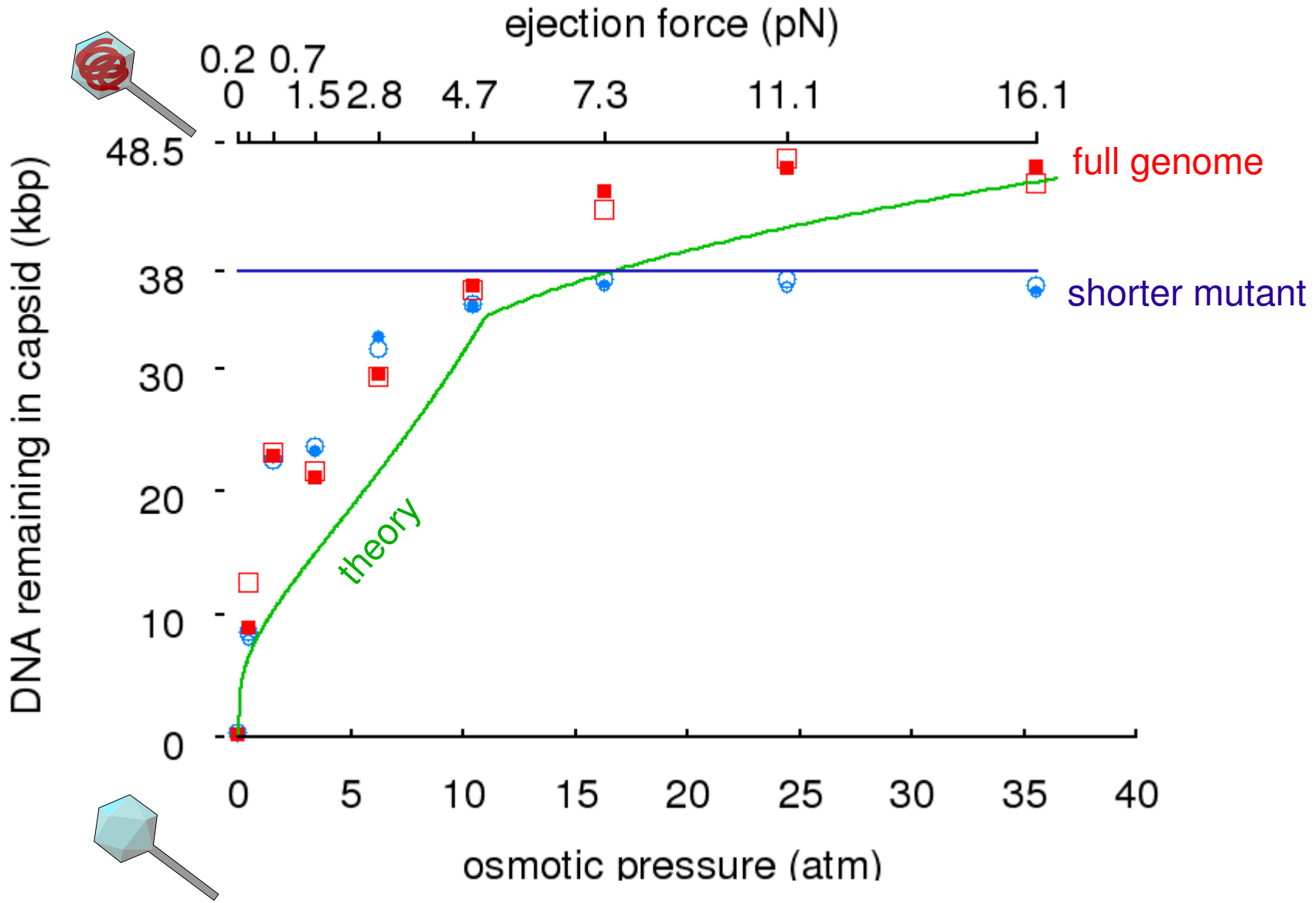
Bill Gelbart sa

Chuck Knobler

Alex Evilevitch



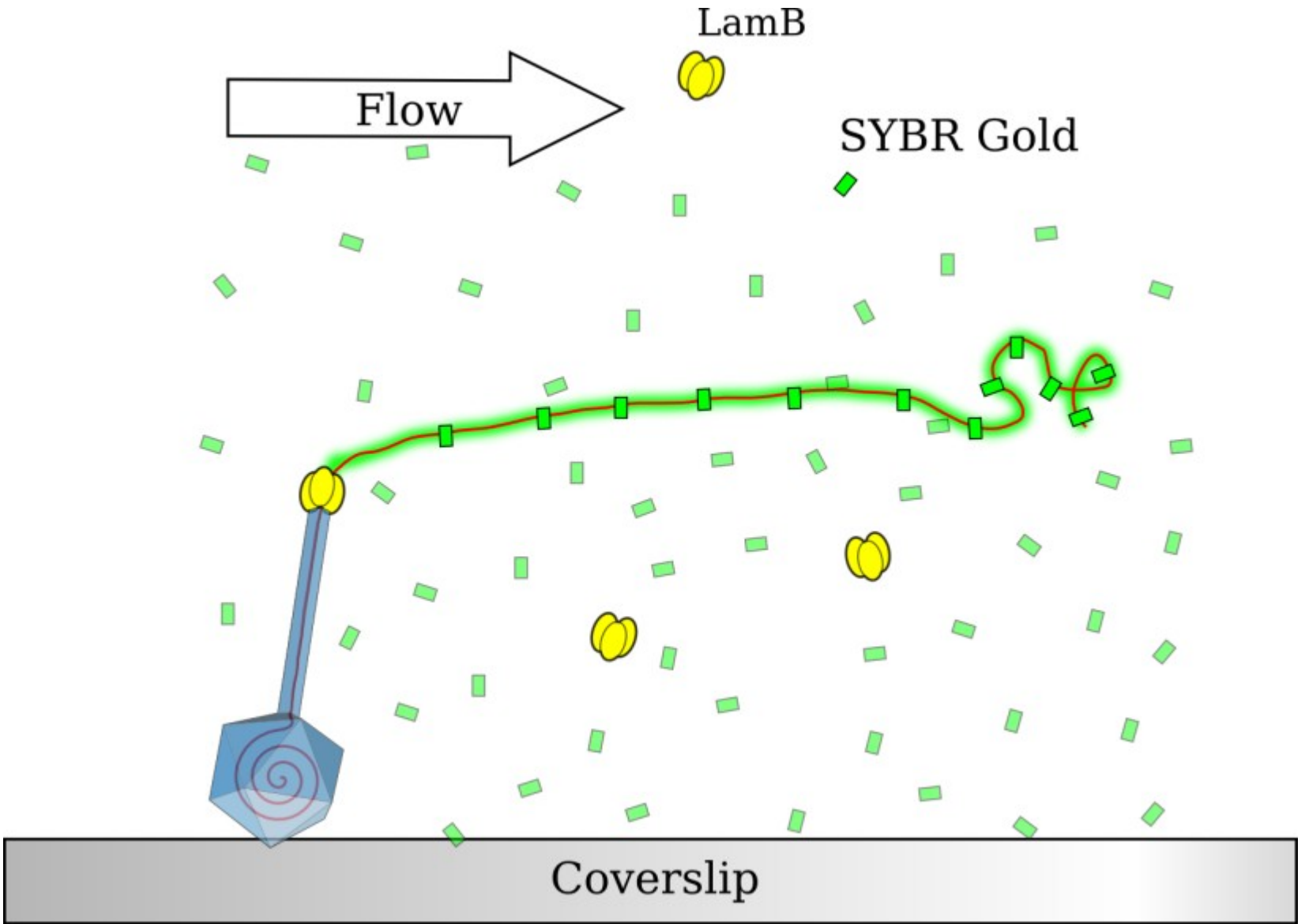
Testing theory with λ: static force



Grayson *et al., The effect of genome length on forces in bacteriophage λ* (Virology, 2006).

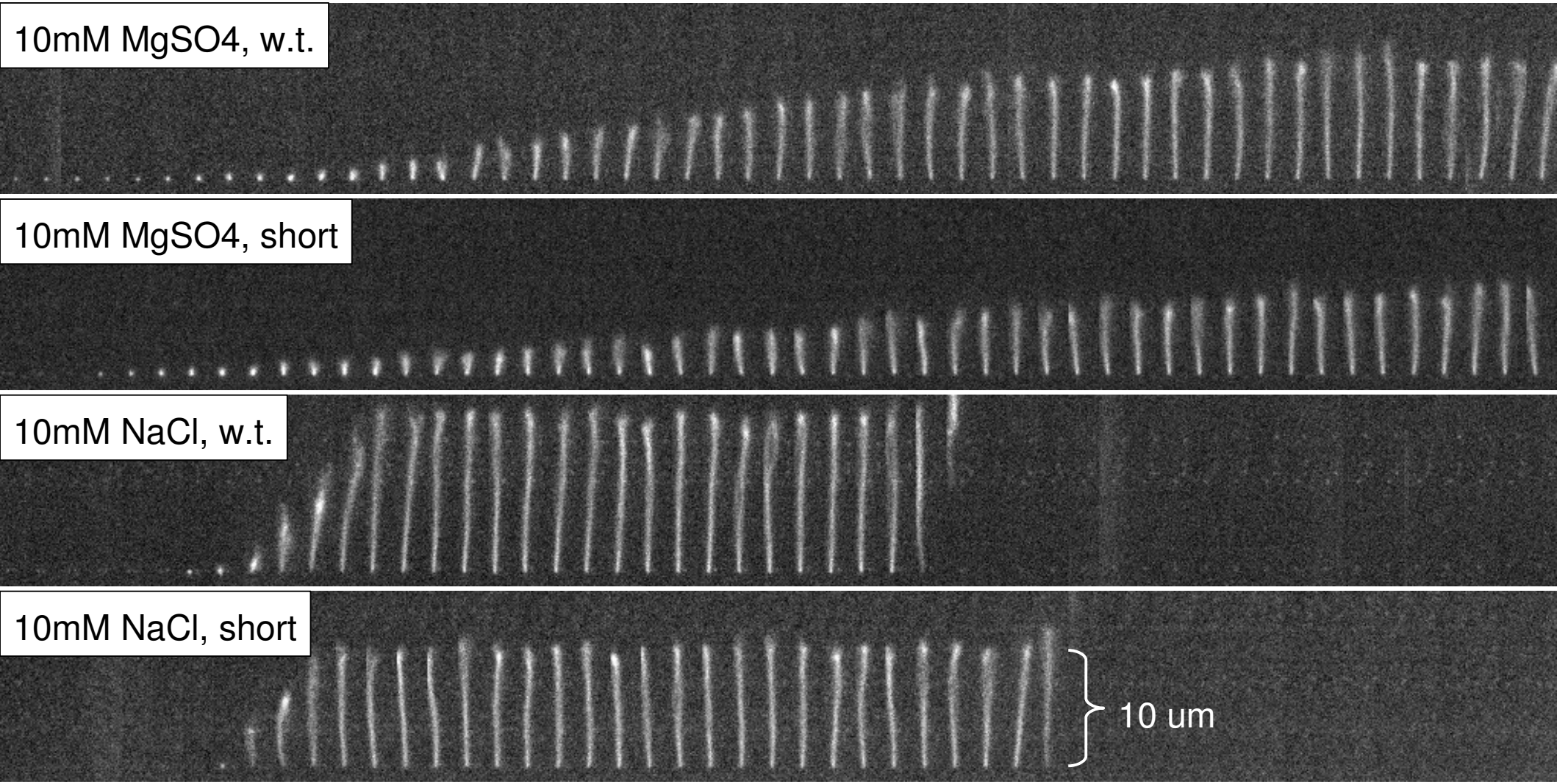
Testing theory with λ: dynamics

Combines methods of Mangenot *et al.* (2005) and Novick & Baldeschwieler (1988).

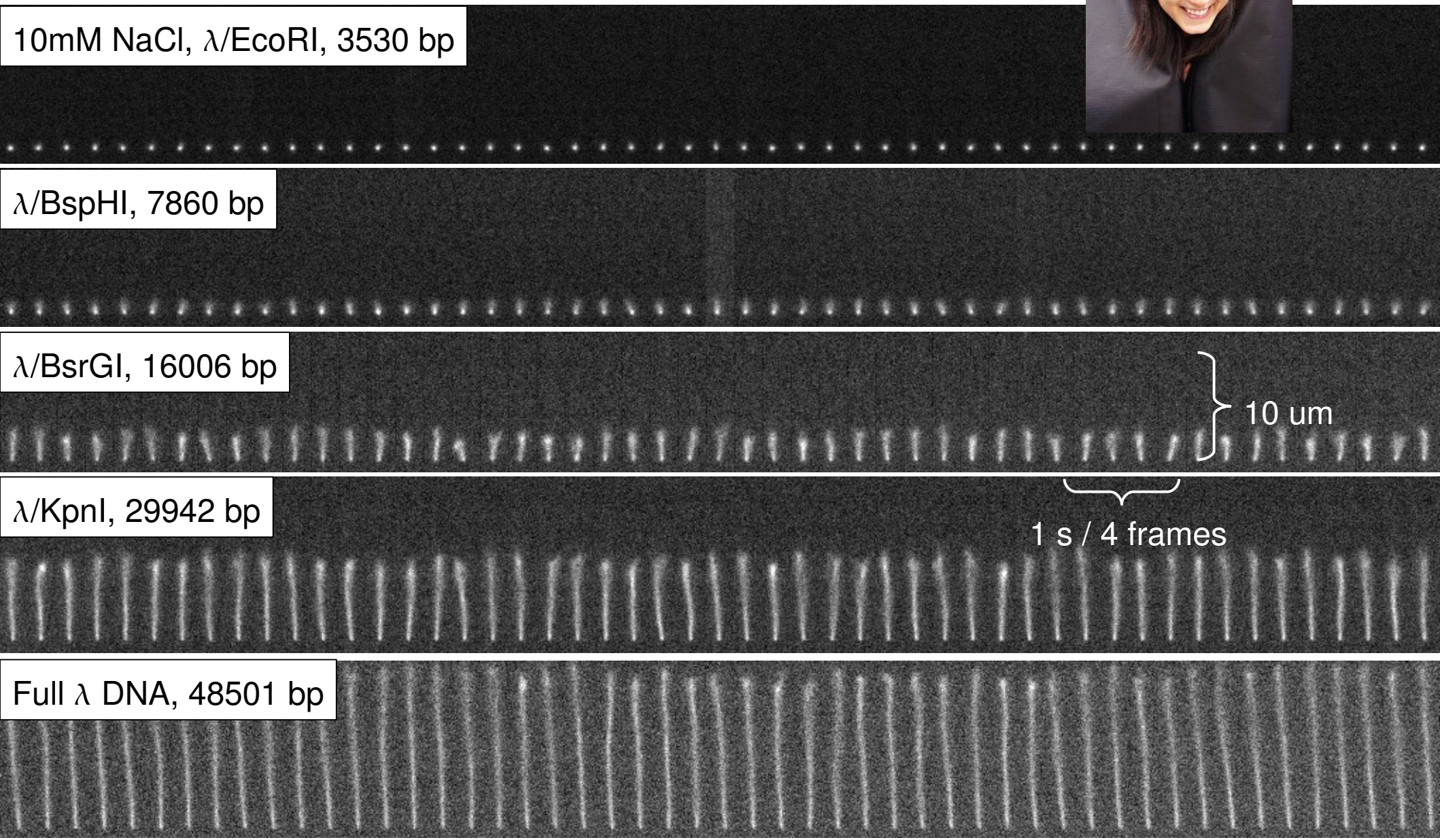


(show movie)

λ ejection trajectories

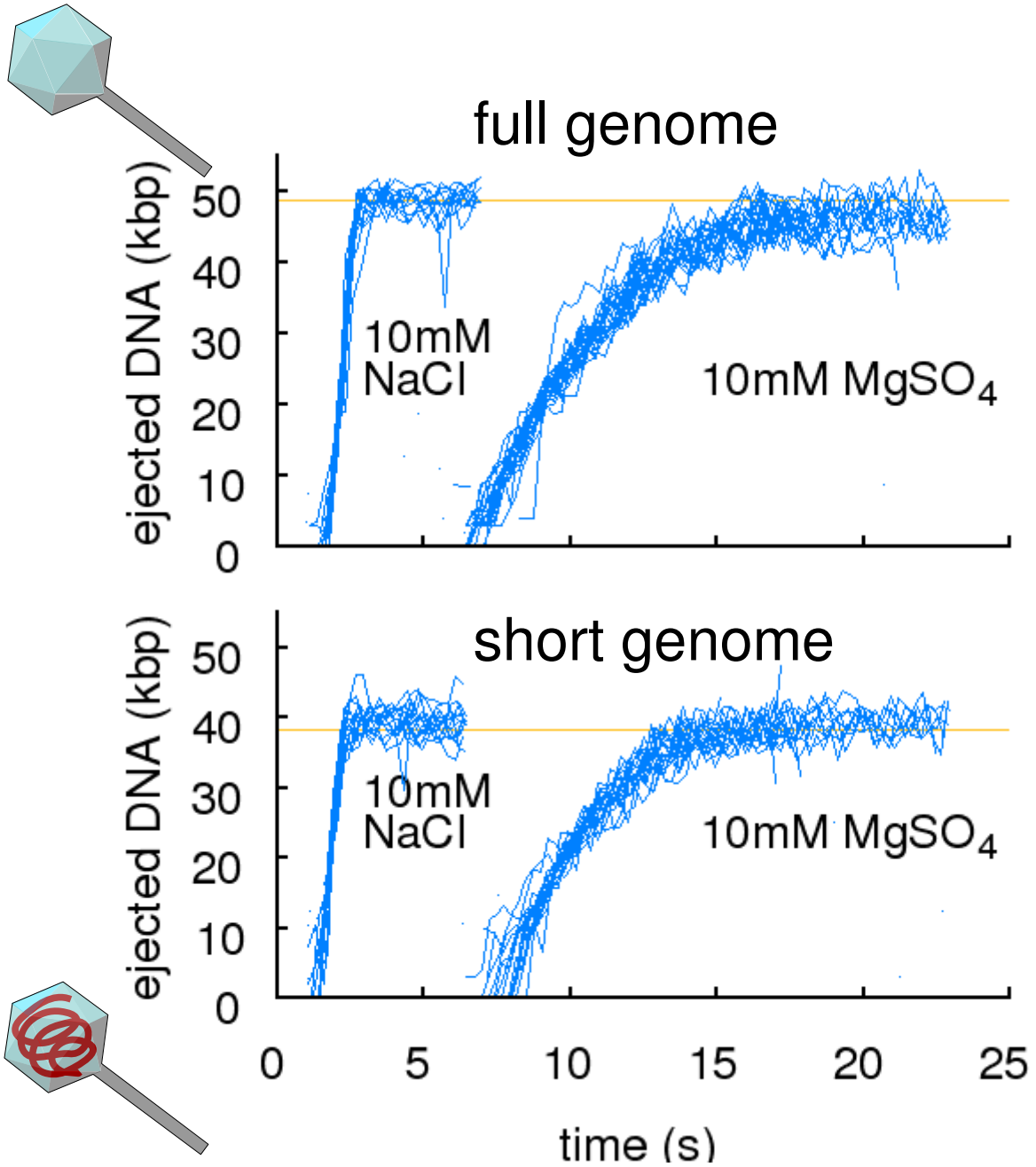


Calibration with λ fragments

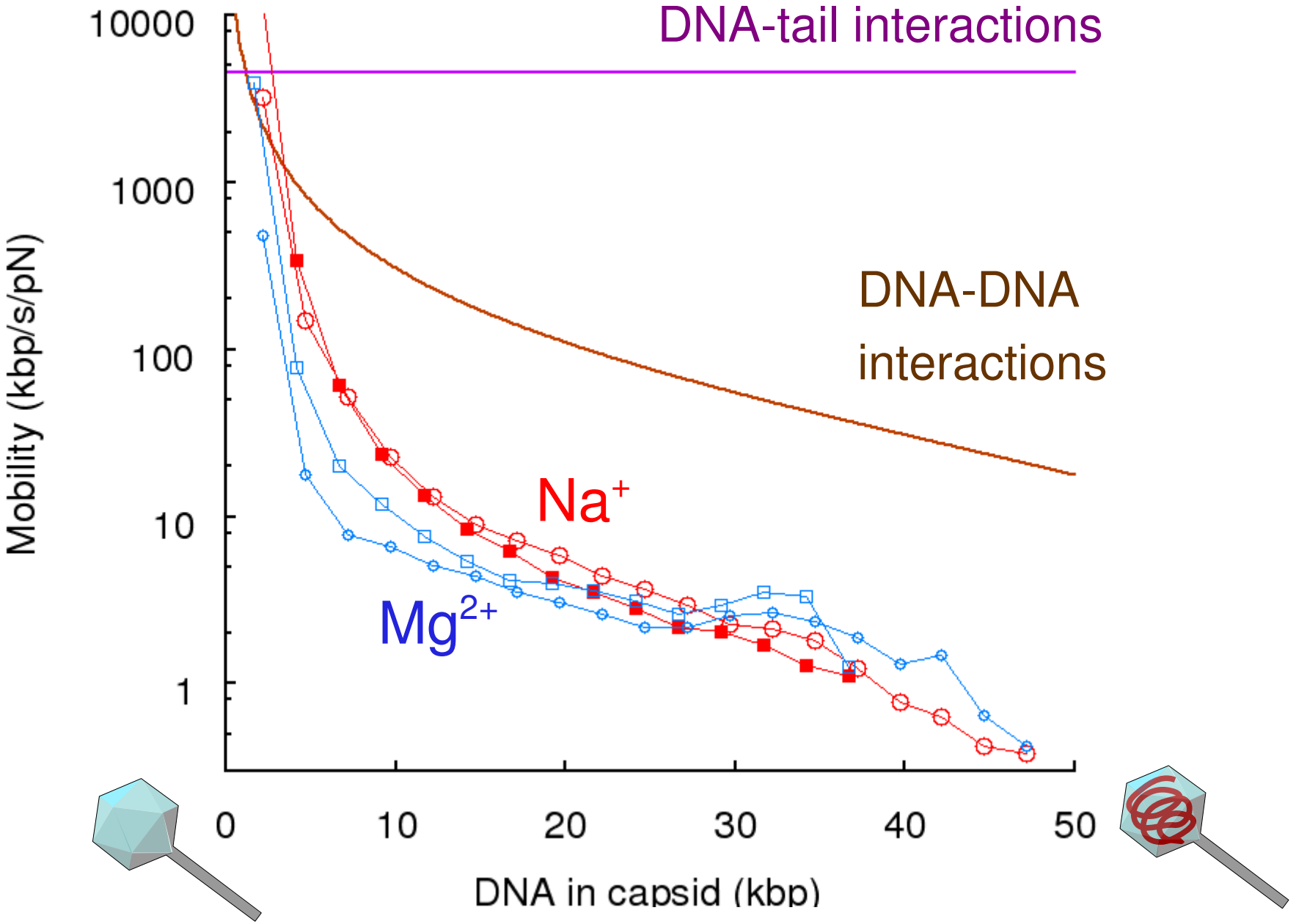


Trajectories

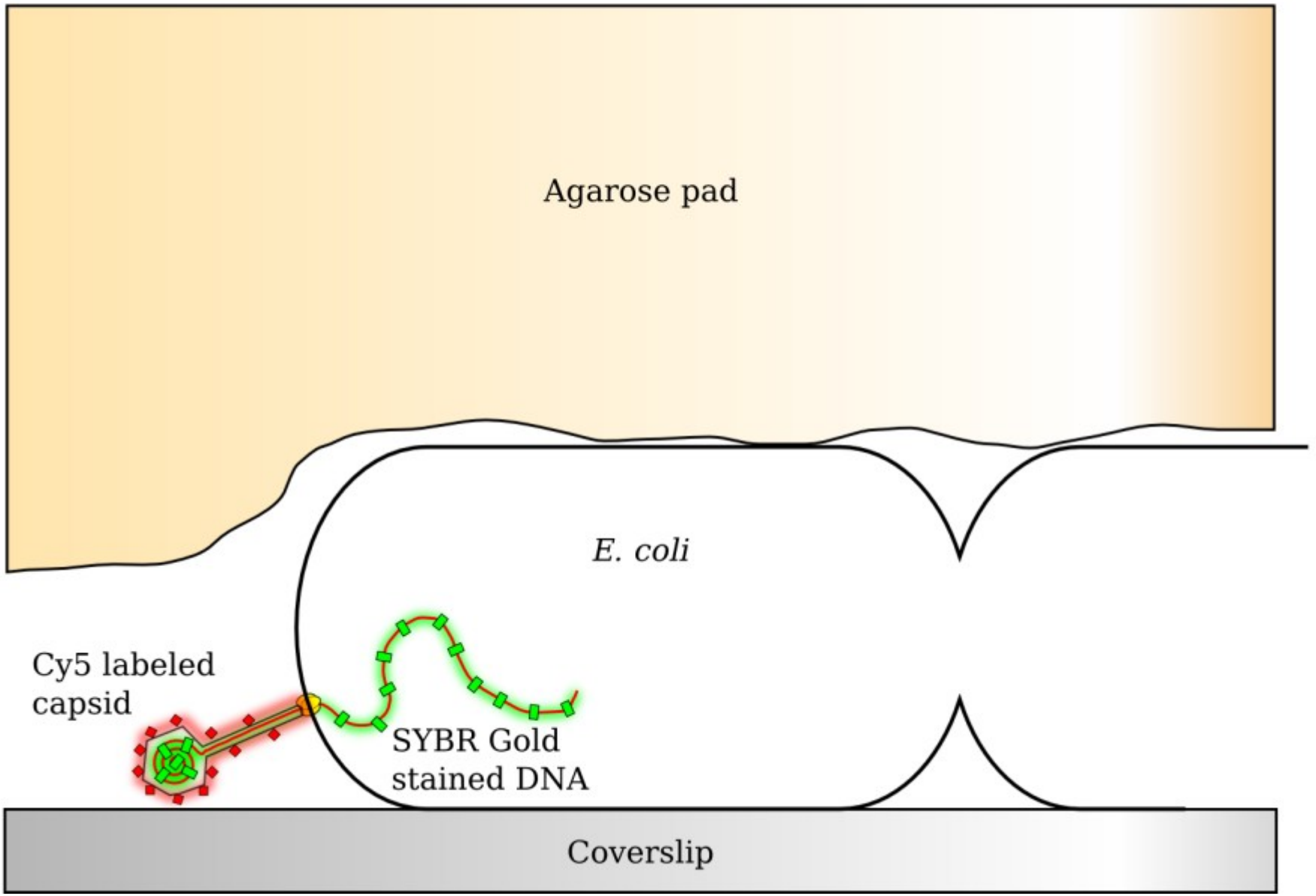
- All identical within exp. error
- Smooth motion
- Max velocity depends on ions
- Shorter phage slightly faster
- Long time at max. extension



Mobility = *v/F* depends on internal DNA

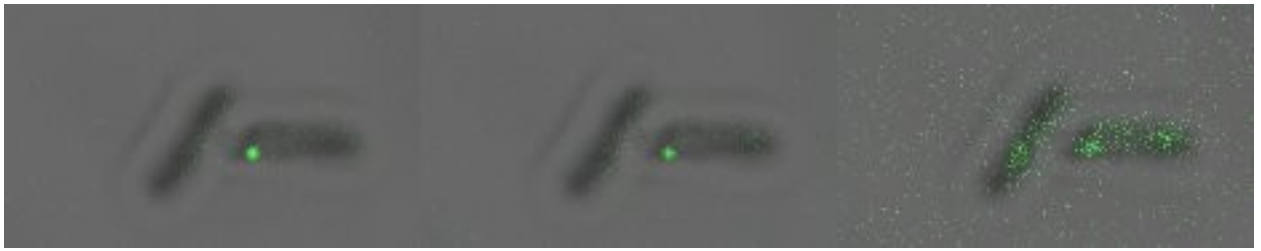


Hershey-Chase on single phages

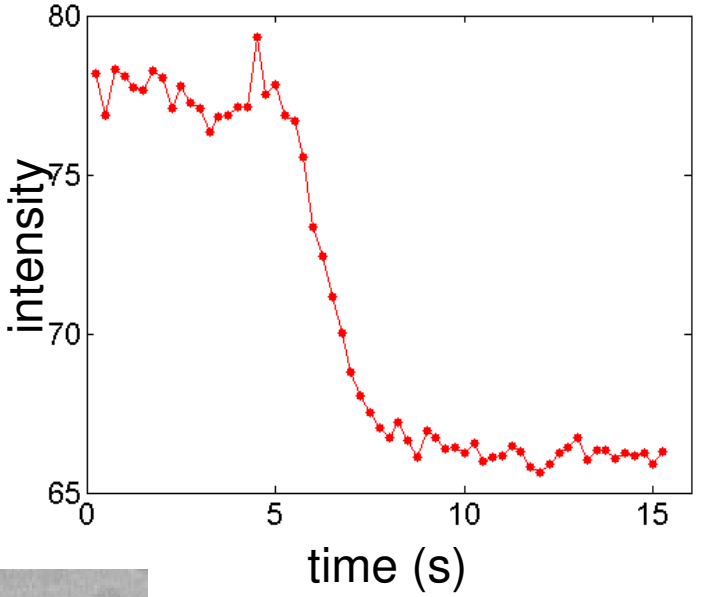


Ejection *in vivo*

Label internally with SYBR Gold:



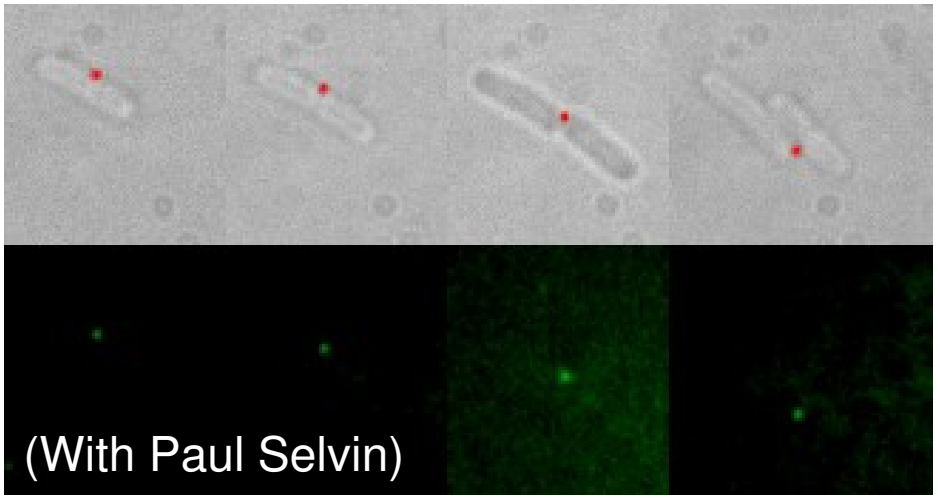
...ejection takes ~3s.



Label both DNA and protein:

Brightfield/Cy5 (Protein)

SYBR Gold (DNA)



...no ejection in ~2h. Work in progress!

Conclusions + questions

- Ejection powered by internal pressure *in vitro*.
- Friction caused by DNA-DNA interactions.
- How to quantitatively estimate friction?
- What motor completes ejection *in vivo*?

Acknowledgments

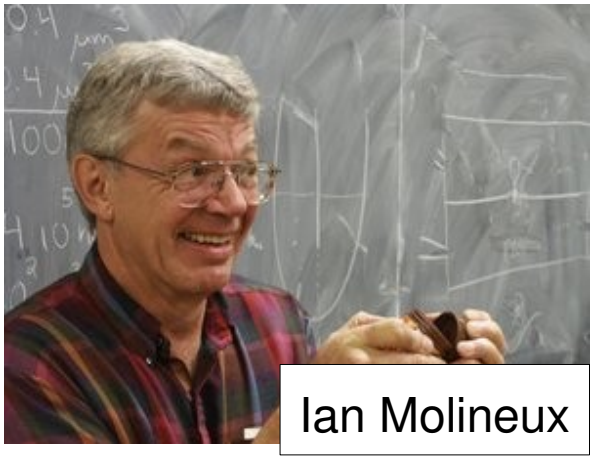
- Collaborators: Alex Evilevitch, Lin Han, Mandar Inamdar, Bill Gelbart, Chuck Knobler, Joey Koehler, Jané Kondev, Corinne Ladous, Catie Lichten, Ian Molineux, Kelsey Nelson-James, Rob Phillips, Prashant Purohit, Erdal Toprak, Zach Travis, and Tabita Winther.s
- Fraser, Jensen, Mayo, Rees, Selvin, and Quake labs. Keck Foundation, NIH, NSF.
- Lots of help from many others!! Thanks!!!

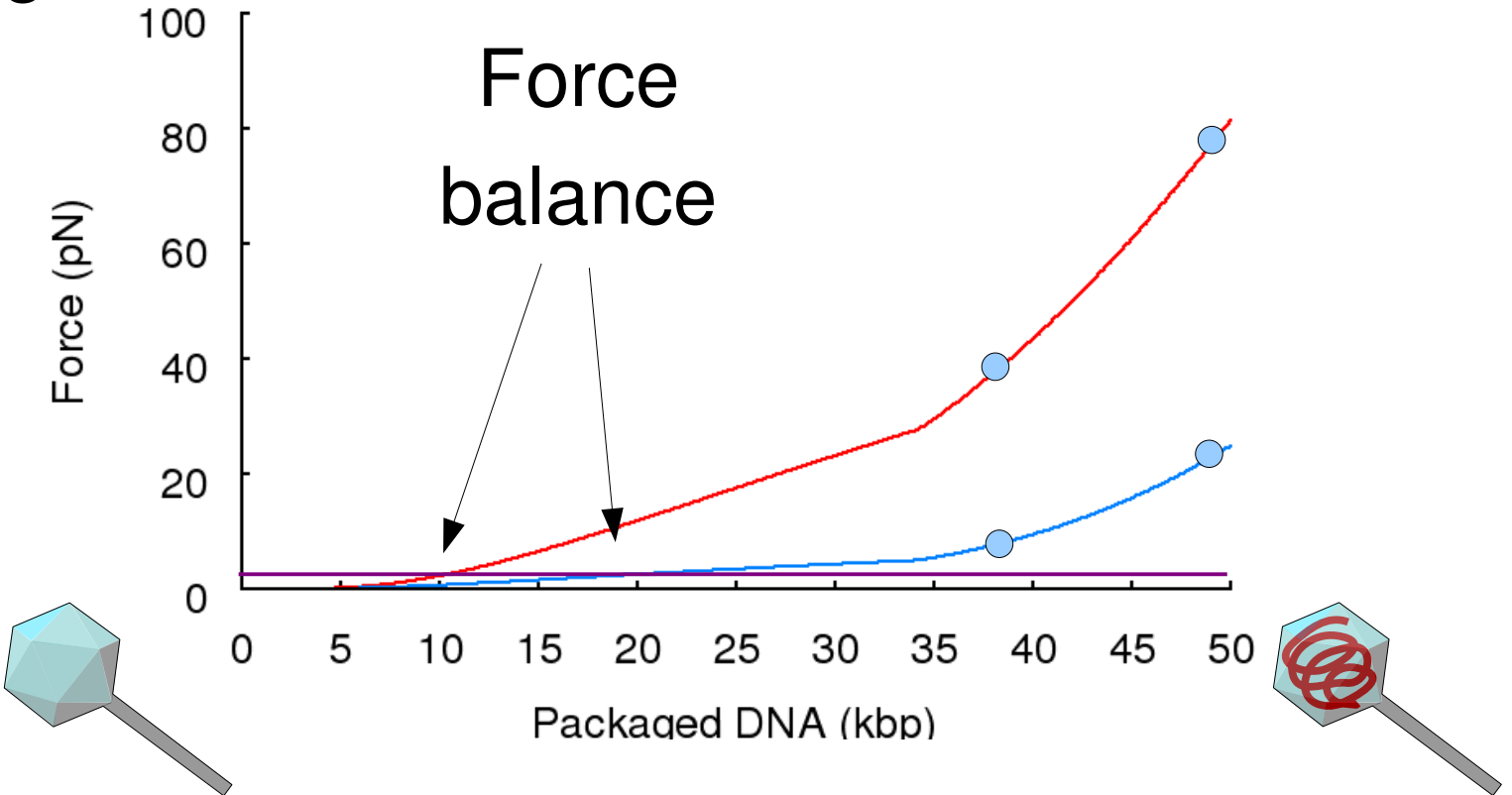
Is pressure enough?

E. coli internal osmotic pressure = ~3 atm.

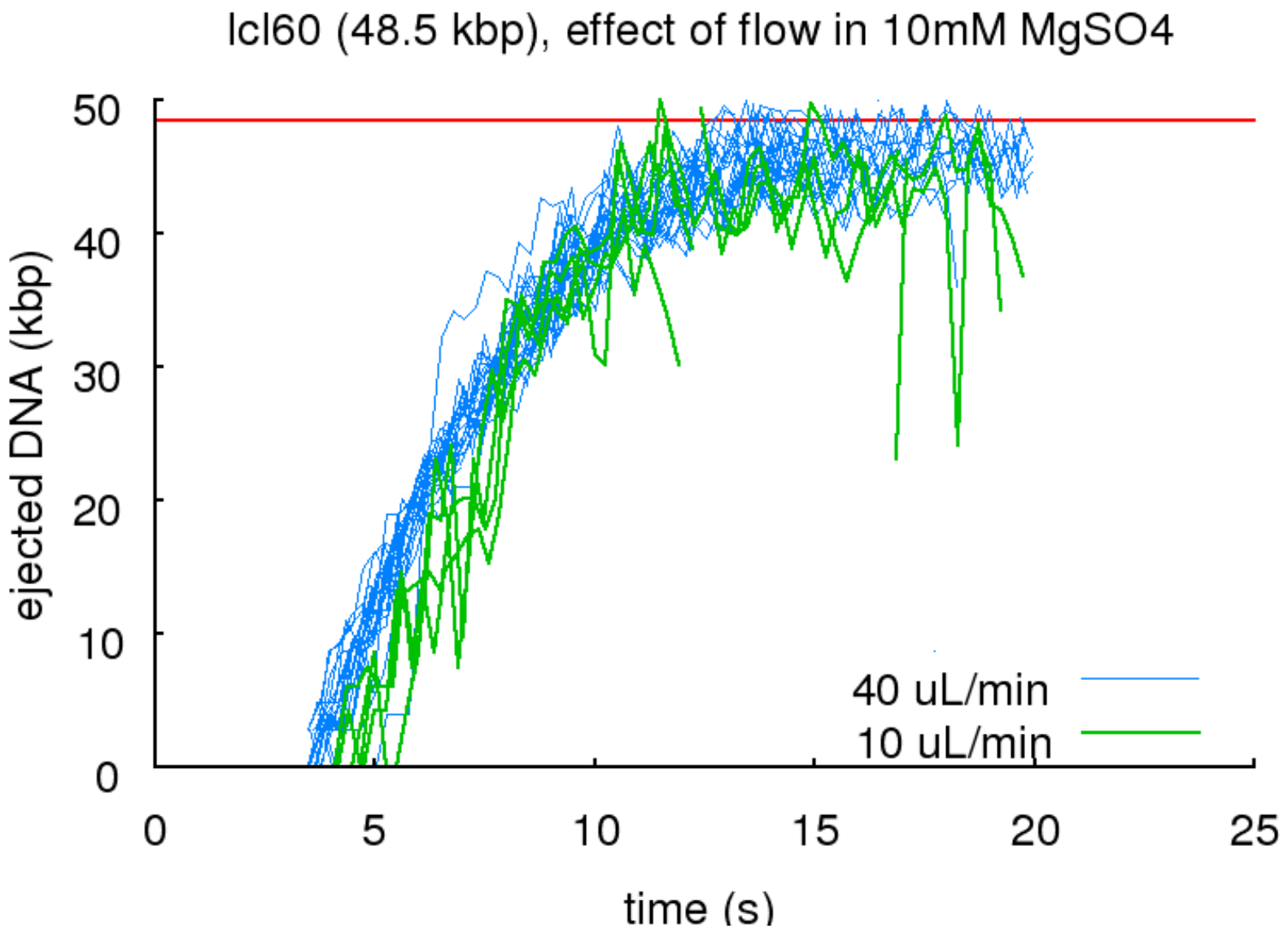
Internal motor needed!

In phage T7: RNAP.

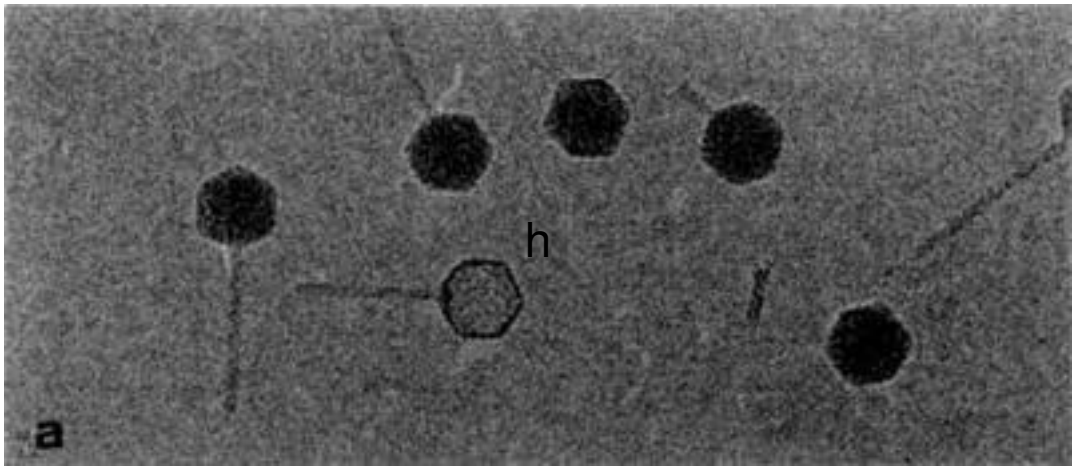

Ian Molineux



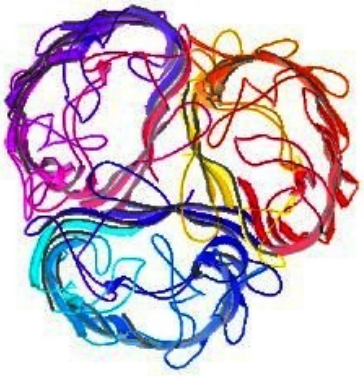
Effect of fluid flow on ejection



Bacteriophage λ

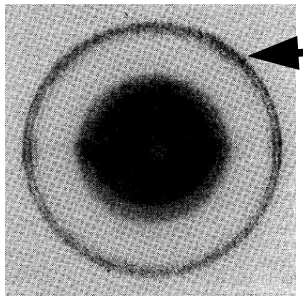


λ capsids(Dokland & Murialdo)



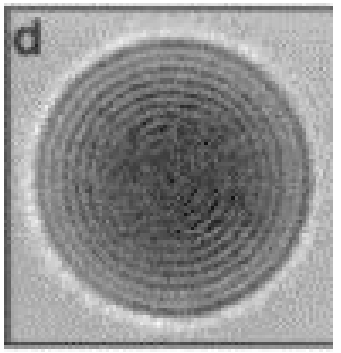
λ receptor (LamB, maltoporin)

Bacteriophage DNA packing

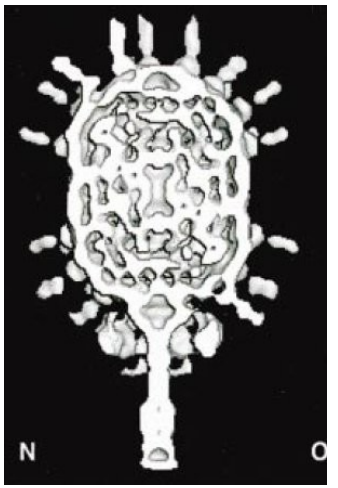


2.5 nm
spacing

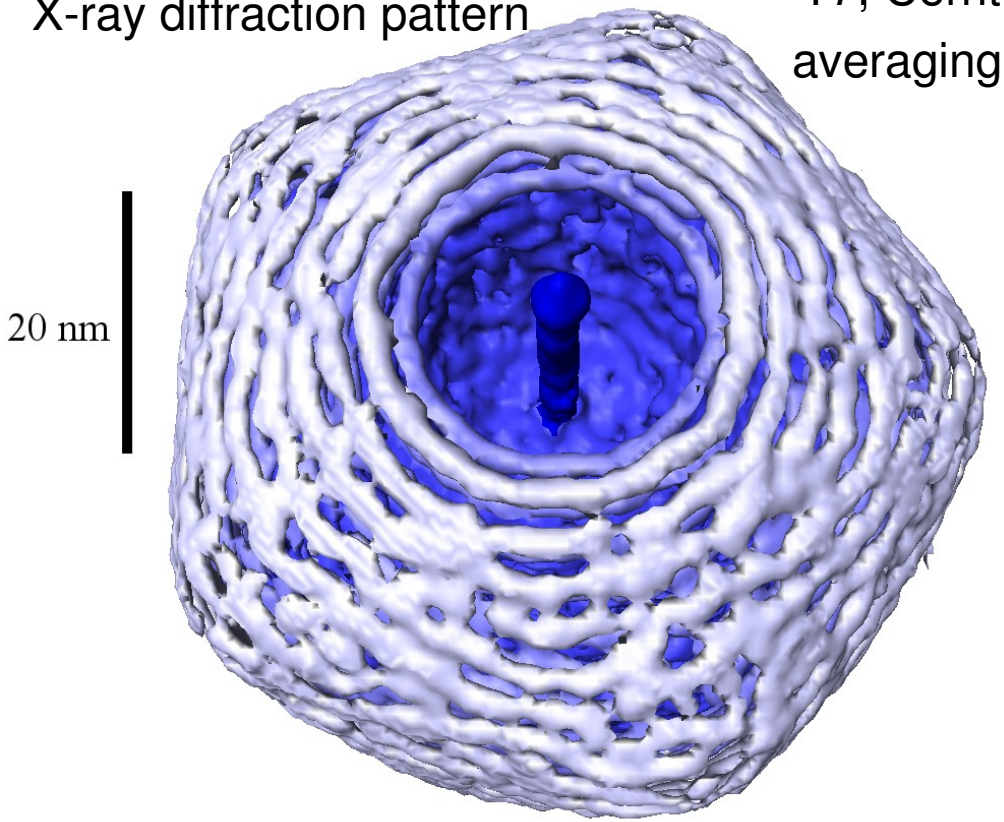
λ, Earnshaw & Harrison (1977)
X-ray diffraction pattern



T7, Cerritelli et al. (1997)
averaging 10s of particles

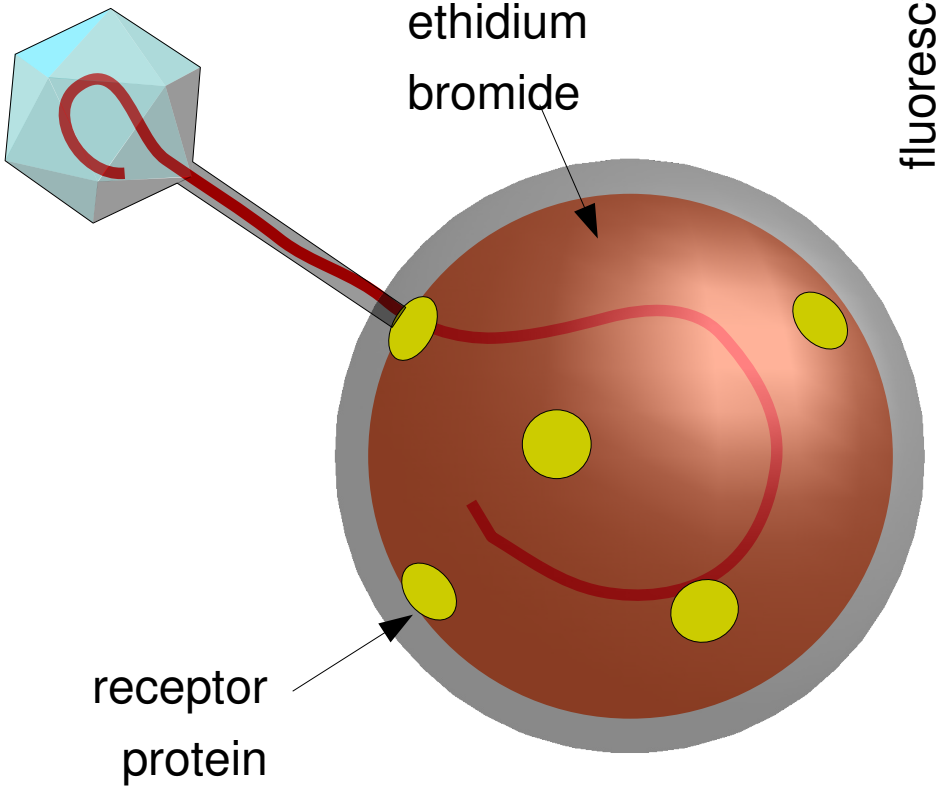


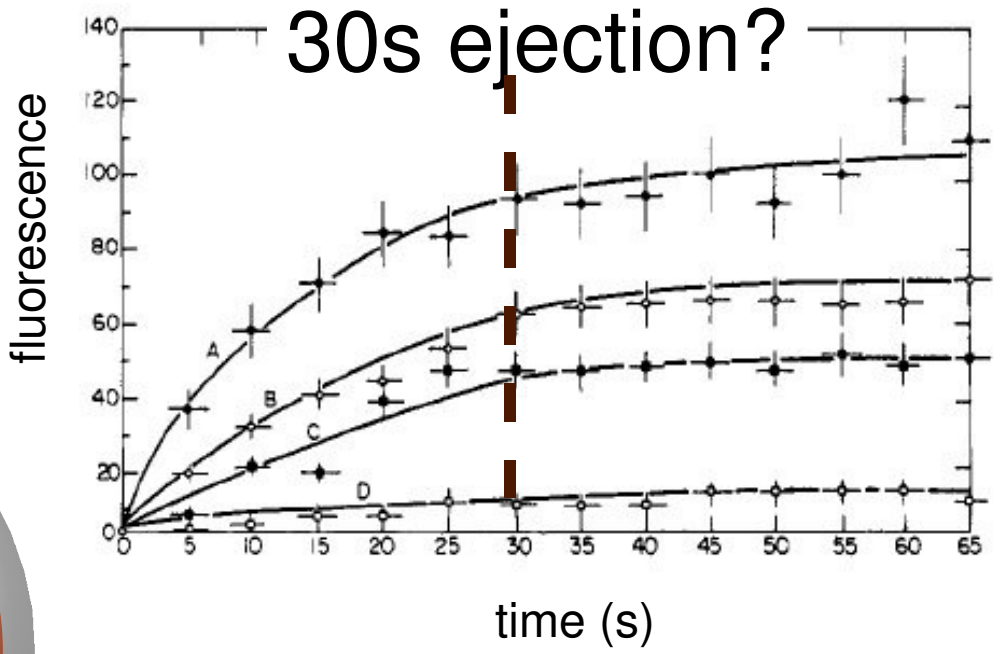
ϕ29, Tao *et al.* (1998)
averaging 100s of particles



ε15, Jiang et al. (2006)
averaging 1000s of particles

Original rate measurements by Novick & Baldeschwieler, 1988

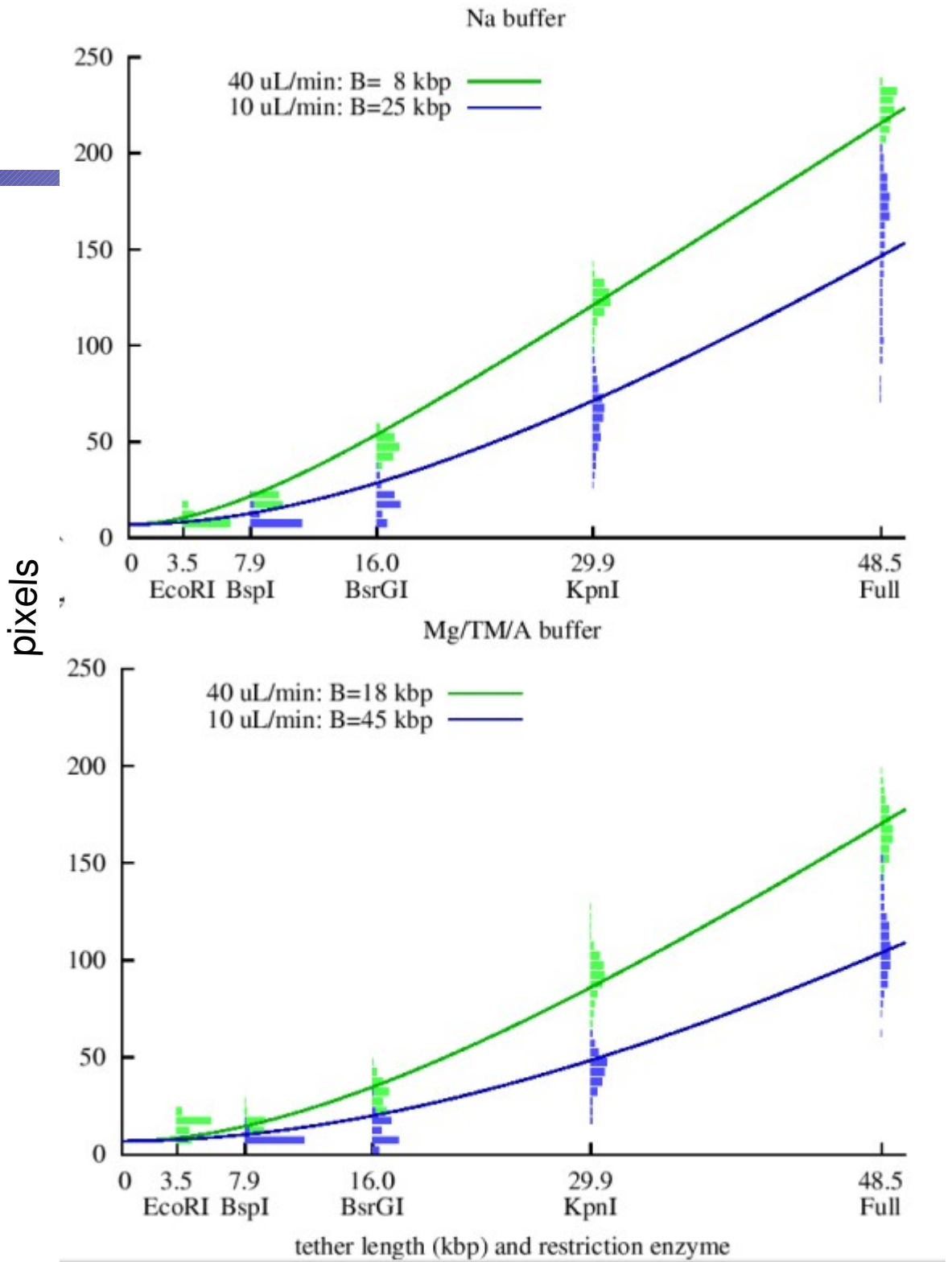




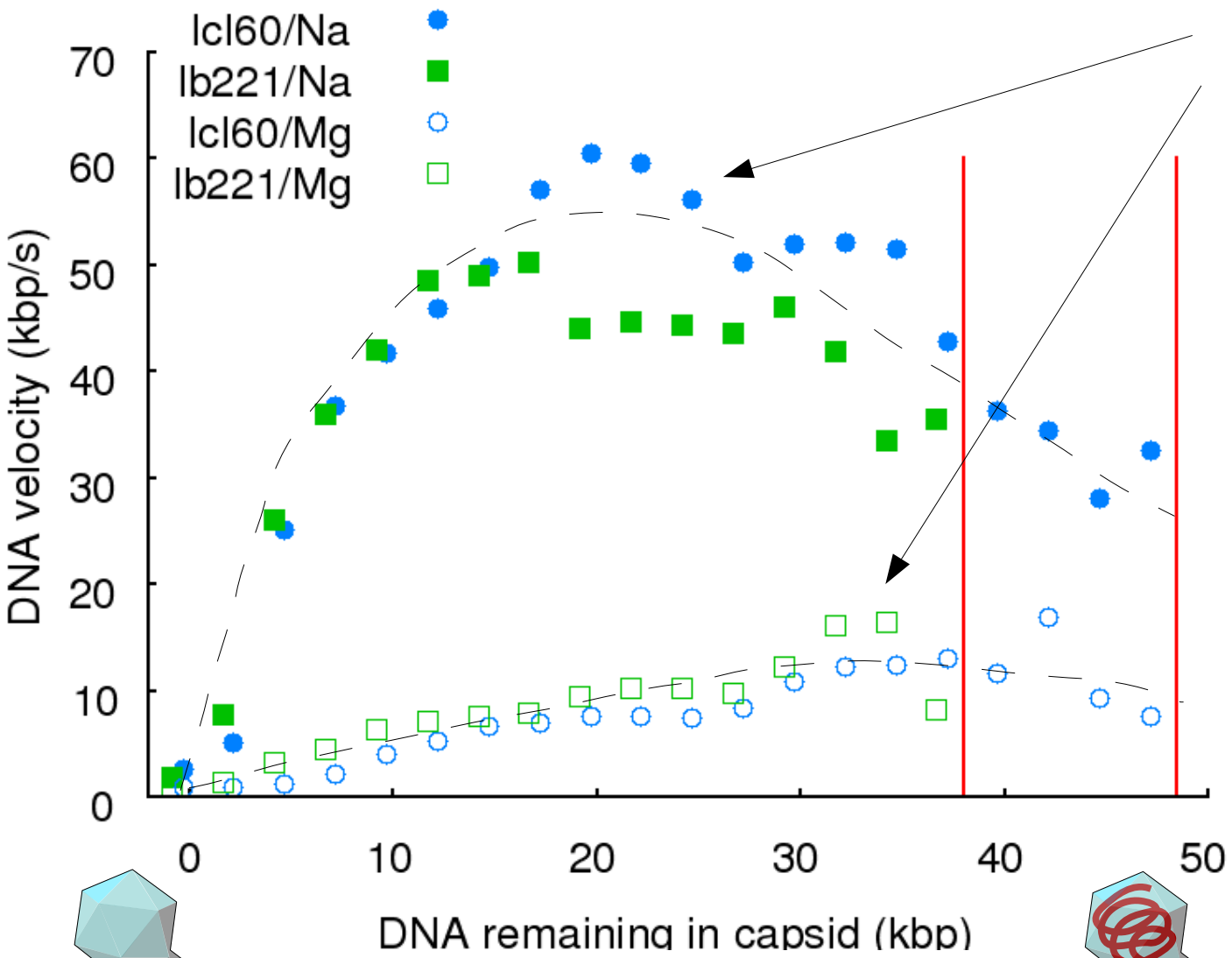
...but only 1000 molecules of dye in each vesicle!

This is *initiation* time.

Calibration



Measure velocity vs. position



Max velocity depends on buffer

Velocity independent of genome length