BOULDER 2006: WILLIAM M. GELBART

PHYSICAL ASPECTS OF VIRAL INFECTIVITY

LECTURE 1: DNA PACKAGING IN VIRUSES

DNA is strongly confined; packaged by force

LECTURE 2: RNA PACKAGING IN VIRUSES

RNA is weakly confined; packaged spontaneously

LECTURE 3: MAKING VIRUSES "FROM SCRATCH"

Possible for both DNA and RNA cases

(The role of membranes)

QUESTIONS WE'LL BE ASKING:

WHAT *ARE* VIRUSES?

IN WHAT SENSE ARE VIRUSES *ALIVE*?

HOW IS IT POSSIBLE FOR THEM TO HAVE SO FEW GENES, AND HENCE SUCH A SMALL "PARTS LIST"?

WHAT ARE THEIR BASIC/GENERIC "LIFE CYCLES"?

AND HOW DO WE UNDERSTAND THEM IN TERMS OF BASIC PHYSICAL PRINCIPLES?

WHY ARE MOST VIRUSES SPHERICAL/ICOSAHEDRAL?

WHY -- HOW! -- IS IT POSSIBLE TO MAKE INFECTIOUS VIRUSES "FROM SCRATCH", I.E., FROM PURIFIED COMPONENTS?

This picture can't be displayed.

HOW BIG IS A VIRUS, AND WHY?

*MUST BE SMALL COMPARED TO CELL SIZE (i.e., << a micron)

*BUT MUST BE BIG ENOUGH TO ENCLOSE ITS GENOME

SO, HOW BIG IS A VIRAL *GENOME*?

How big is a gene ? 1000-base-pair length of DNA has a volume of
$1000 \times 0.34 \text{ nm} \times \pi\ (1.5 \text{ nm})^2 = 2400 \text{ nm}^3$

What about 10 genes? 24000 nm^3, suggesting a volume with

[VOLUMES SCALE AS CUBES OF LINEAR DIMENSIONS, IMPLYING FACTOR OF 10 RANGE IN GENOME VOLUME GIVES ONLY FACTOR OF TWO CHANGE IN VIRUS SIZE]

ALL VIRUSES ARE ABOUT 50 nm IN DIAMETER….!

This is why viruses (unlike bacteria and other infectious microbes):

***were *not visible* (i.e., were not detectable in the best microscopes available at the time -- one century ago), and**

***were *not filterable* in efforts to isolate them:**

INDEED, VIRUSES
-- IN PLANT, ANIMAL, AND BACTERIAL CASES --

WERE DISCOVERED BY INFECTING HOSTS WITH THE FLUIDS COLLECTED FROM FILTERING GROUND-UP LEAVES, BLOOD, AND FECES…FROM INFECTED HOSTS…

SOME HISTORY OF VIRUSES:

plant case

first to be discovered, early-1890's (TMV)
first to be crystallized, 1935 (TMV)
first to be reconstituted, 1955 (TMV)

animal case

discovered, mid-1890's (FMDV)
cell culture and plaque assays 1952
vaccines (polio), 1950's; gene delivery…

bacterial case

discovered, 1915 ("intestinal phage")
phage and the origin of
molecular biology, 1937 --
first EM pictures, 1941

d'Herelle's 1915 discovery of bacteriophage, simultaneous with that of Twort

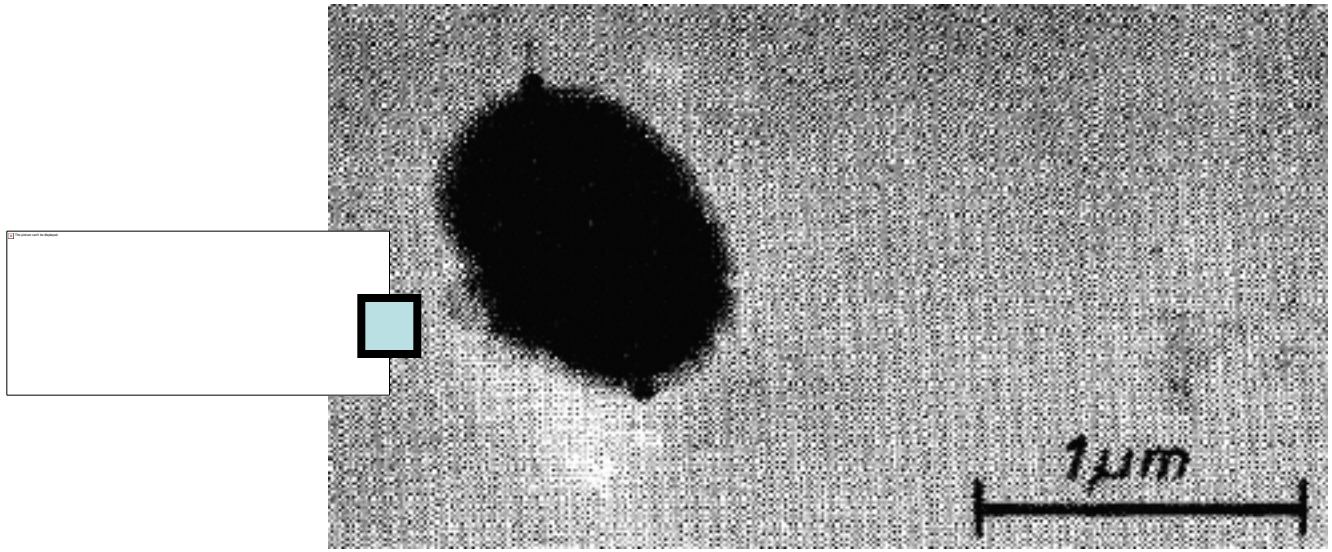
**"I made an emulsion of some still bloody stool *and filtered it*…;
to a broth culture of the dysentery bacillus isolated days earlier, I
added a drop of the filtrate and spread a drop of this mixture on agar."**

**"…The next morning, on opening the incubator, I
experienced one of those rare moments of intense emotion* which
reward the research worker for his pains:
at the first glance I saw that the broth culture, which the night before had
been very turbid, was perfectly clear -- *all the bacteria had vanished*."**

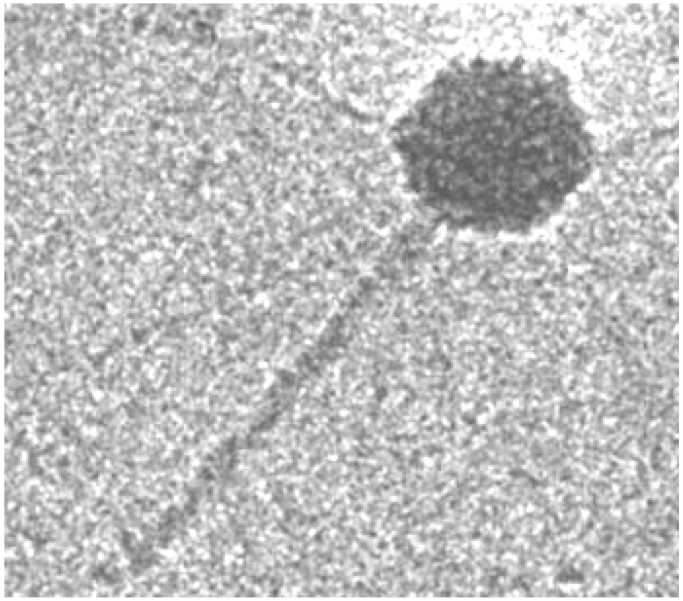
**"…What caused my emotion was that in a flash I had understood:
what caused my clear spots was in fact an invisible microbe --
a filterable virus, *a virus parasitic on bacteria*."**

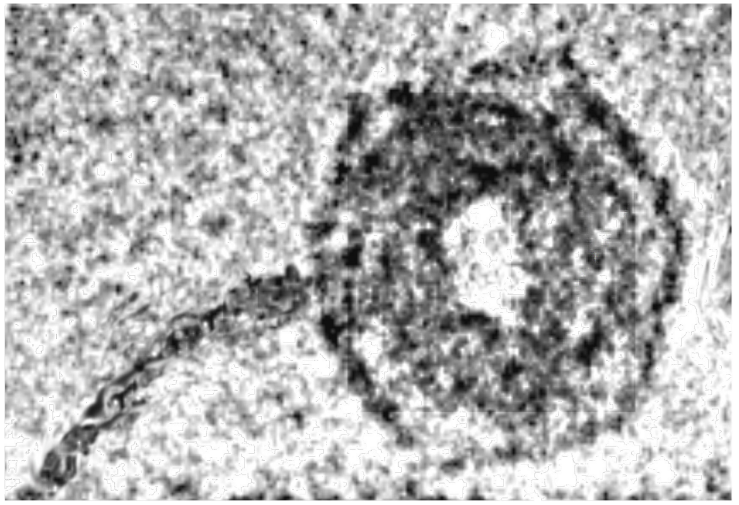
*** whilst examining feces in a morgue…!**

FIRST EM PICTURE (NOBEL-PRIZE-WINNING) OF PHAGE (1941, RUSKA)



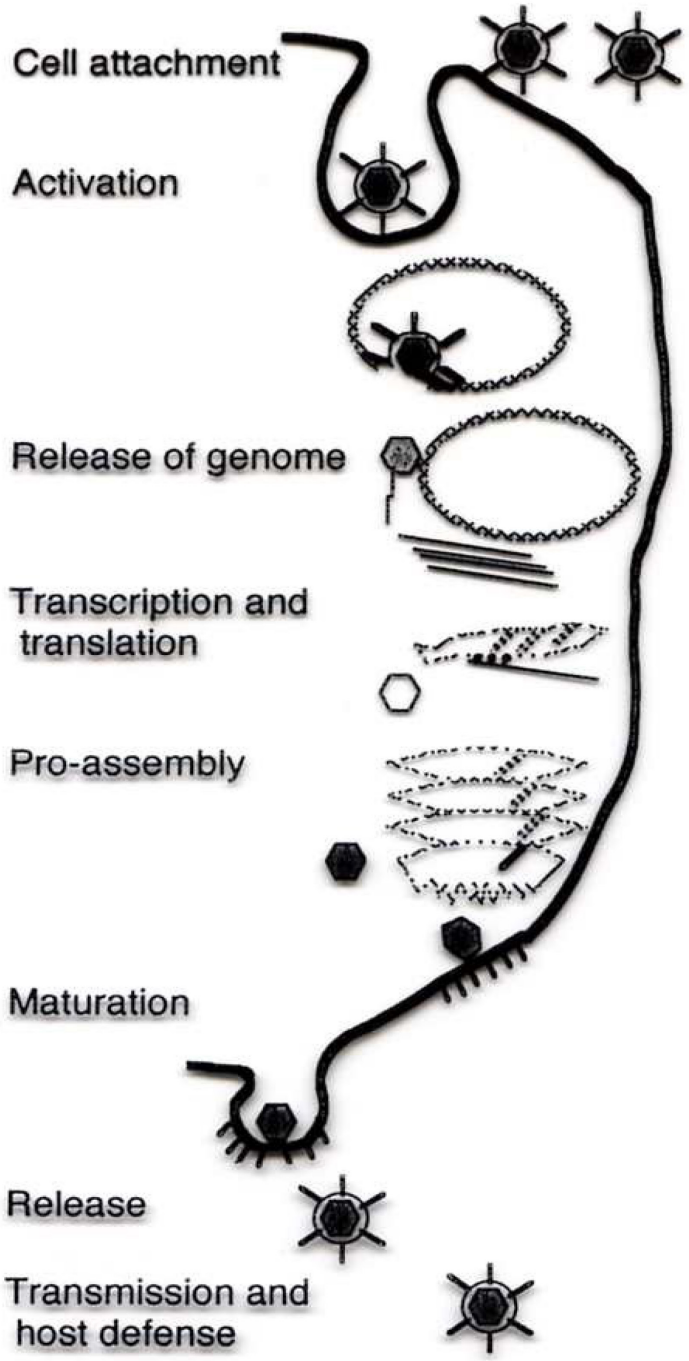
CURRENT EM PICTURES OF PHAGE (2005, EVILEVITCH)





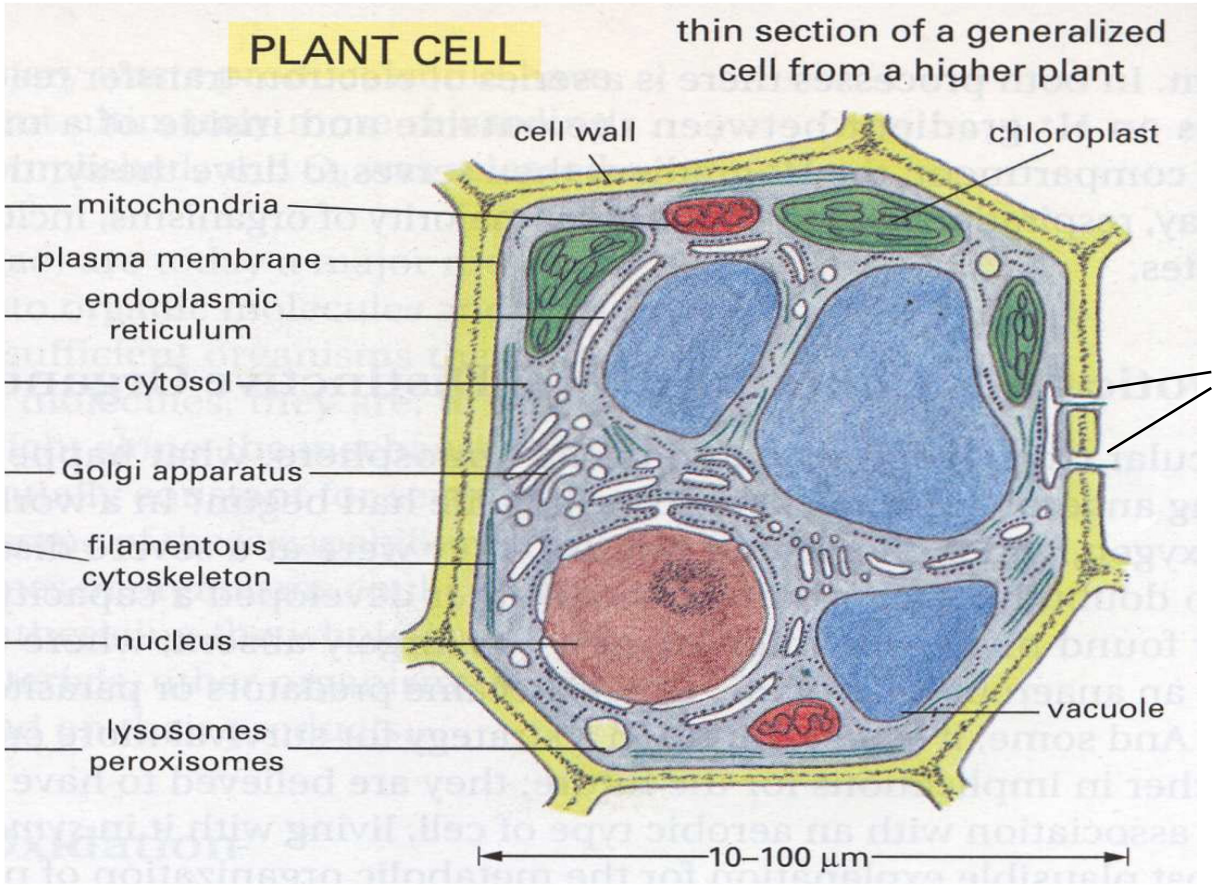
T5 bacteriophage "infecting" a lecithin vesicle reconstituted with a few receptor (FhuA) molecules, and full of spermine

Lambert, Letellier, Gelbart and Rigaud, PNAS 97, 7248 (2000)



ANIMAL CELL "LIFE CYCLE"

*Entry involves receptor-mediated binding/endocytosis or fusion
*Whole viral particle enters cell
*New virions leave via "budding"

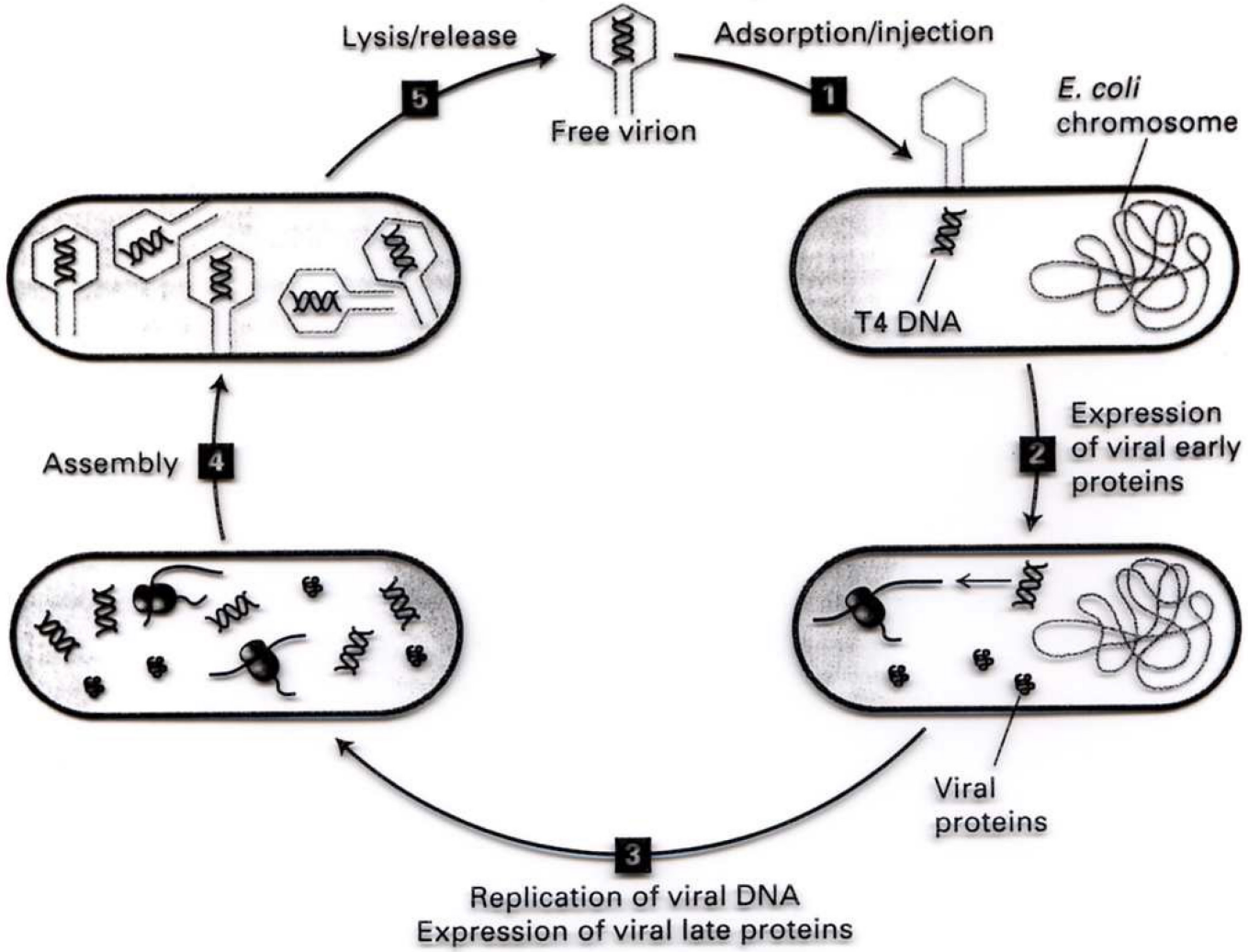


*Cells are each surrounded by a rigid (cellulose) wall, which must be "broken" (e.g., by abrasion) in order for viral particles to enter

*Consequently, ***a large number of viral particles enter the cell*** and without the need for a receptor-mediated process

*Replicated virions leave cell through plasmodesmata

"LIFE CYCLE" OF BACTERIAL VIRUS ("PHAGE)



LET'S PURSUE THE LIFE CYCLE OF BACTERIAL VIRUSES…

Q: WHAT IS (*PHYSICALLY*) RESPONSIBLE FOR INJECTION OF THE MANY-MICRON LONG DNA?

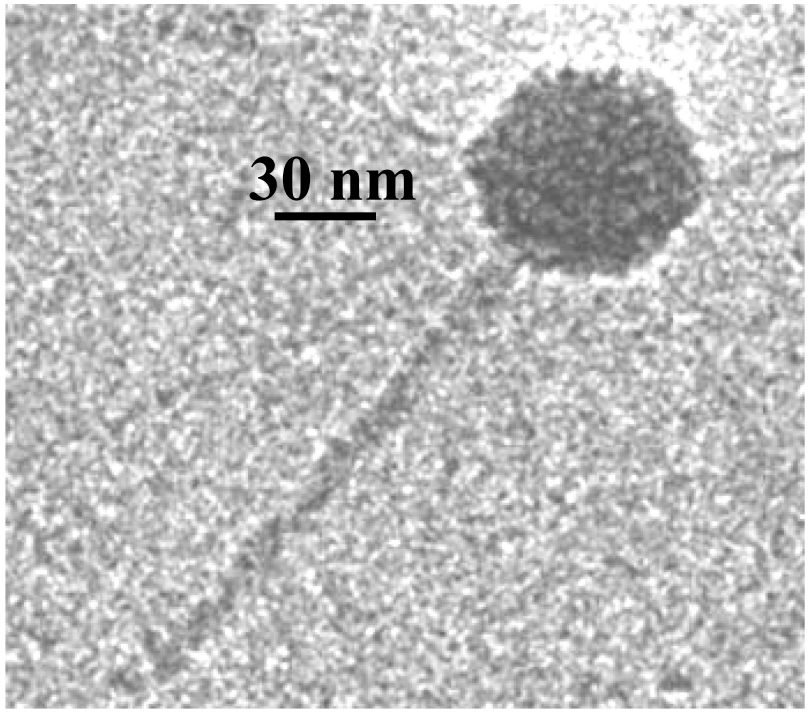
A: THE PRESSURE -- ENERGY DENSITY -- INSIDE THE VIRAL CAPSID

WHAT IS THE *ORIGIN* OF THIS STRESS?

HOW CAN ONE *CALCULATE* AND *MEASURE* FORCES AND PRESSURES OF THIS KIND?

STRESS IMPLIES THAT (FREE) ENERGY HAS BEEN *STORED*: WHAT PERFORMS THE WORK OF ACCOMPLISHING THIS TASK?

WHY DO PHAGE EVOLVE IN THIS "UNLIKELY" WAY?



Bacteriophage λ

Its dsDNA genome, 17000 nm long, is *highly stressed* in its capsid (30 nm radius), due to:

Electrostatic Repulsion

DNA is packed at crystalline density and is highly crowded

Bending Energy

Persistence length (ξ), 50 nm, implies DNA is strongly bent

CAN *CALCULATE* THESE ENERGIES AS FUNCTION OF PACKAGED LENGTH

Kindt, Tzlil, Ben-Shaul, and Gelbart, Proc. Nat. Acad. Sci. (USA) 98, 13671 (2001)

See, also:

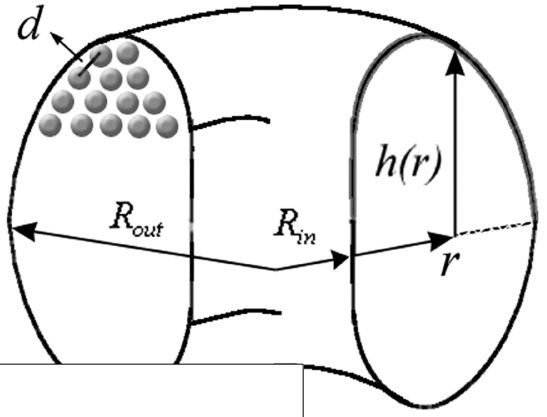
Tzlil, Kindt, Gelbart and Ben-Shaul, Biophys. J. 84, 1616 (2003) *and*
Purohit, Kondev and Phillips, Proc. Nat. Acad. Sci. (USA) 100, 3173 (2003)

Tzlil, Kindt, Gelbart, and Ben-Shaul, Biophys. J. 84, 1616 (2003)

ε(d) is the interaction energy per unit length of neighboring portions of DNA separated by distance d

κ ($=\xi k_B T$) is the 1D bending modulus of DNA

R(s) is the radius of curvature at arc length s of the L-length DNA



Π d

Rau and Parsegian (1992) measurement of pressure vs interaxial spacing

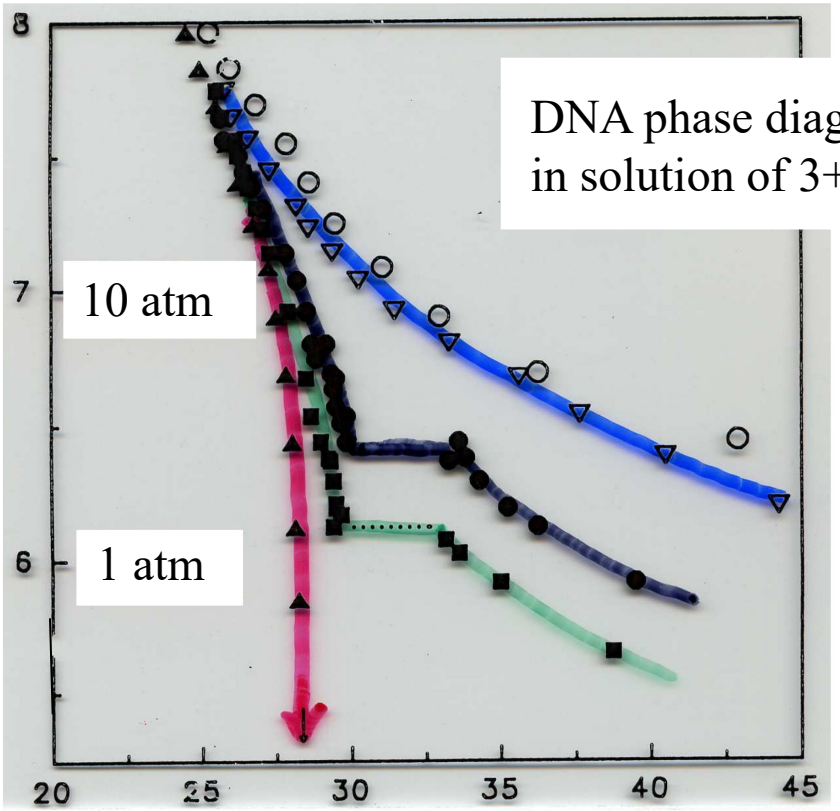
(10 mM Tris buffer; 250 mM NaCl)

Blue: 0 mM 3+

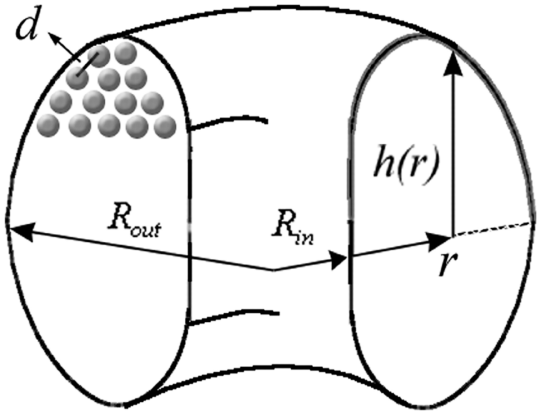
Black: 8 mM 3+

Green: 12 mM 3+

Red: 20 mM 3+



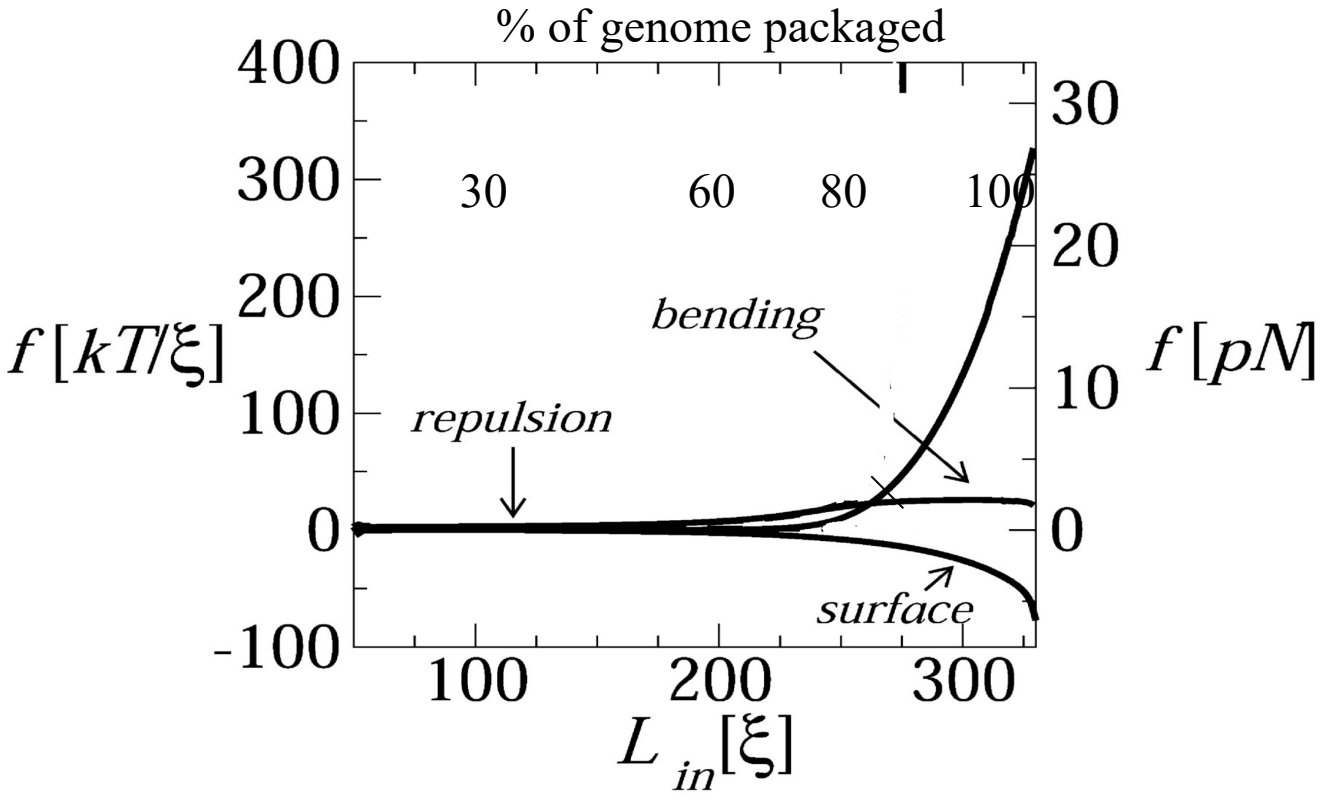
ENERGY OF PACKAGED DNA:



Minimize E[h(r), d;L] for each L,

under constraint of capsid confinement, $4\pi\gamma\int_{R_{\text{in}}}^{R_{\text{out}}} h(r)rdr = L\pi\left(\frac{d}{2}\right)^2 = V_{\text{DNA}}(d)$,

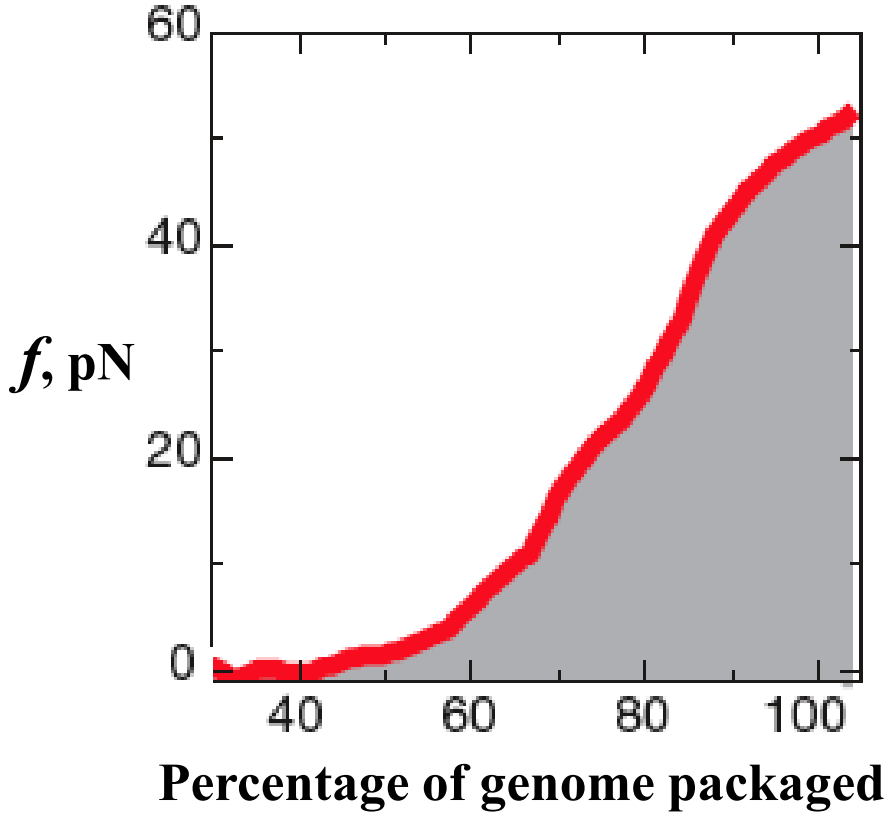
giving E(L) and hence the force, -dE/dL, acting along the DNA



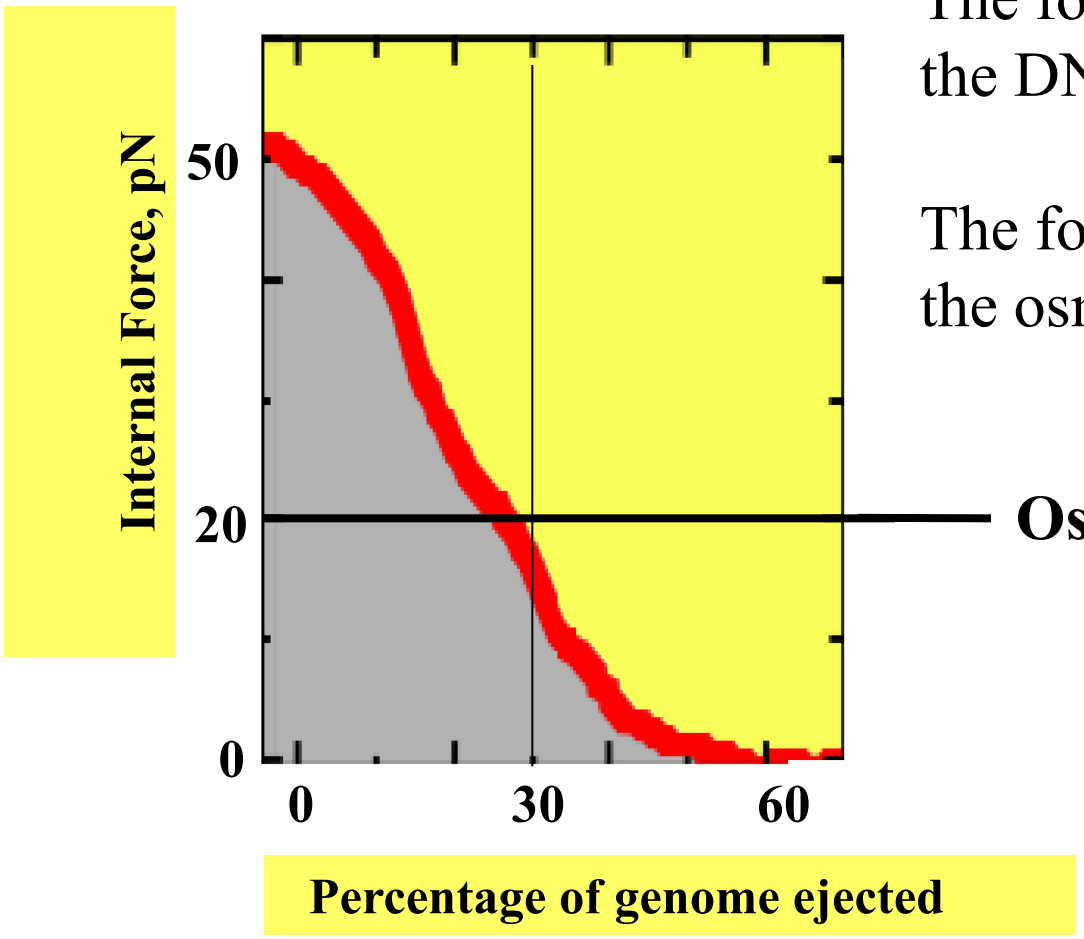
DNA-DNA repulsion is dominant energy contribution; and force increases dramatically only at end of packaging

Packaging Force

Smith, Tans, Smith, Grimes, Anderson and Bustamante, Nature 413, 748 (2001)



What about *ejection*, in presence of "external" (osmotic) resisting force?

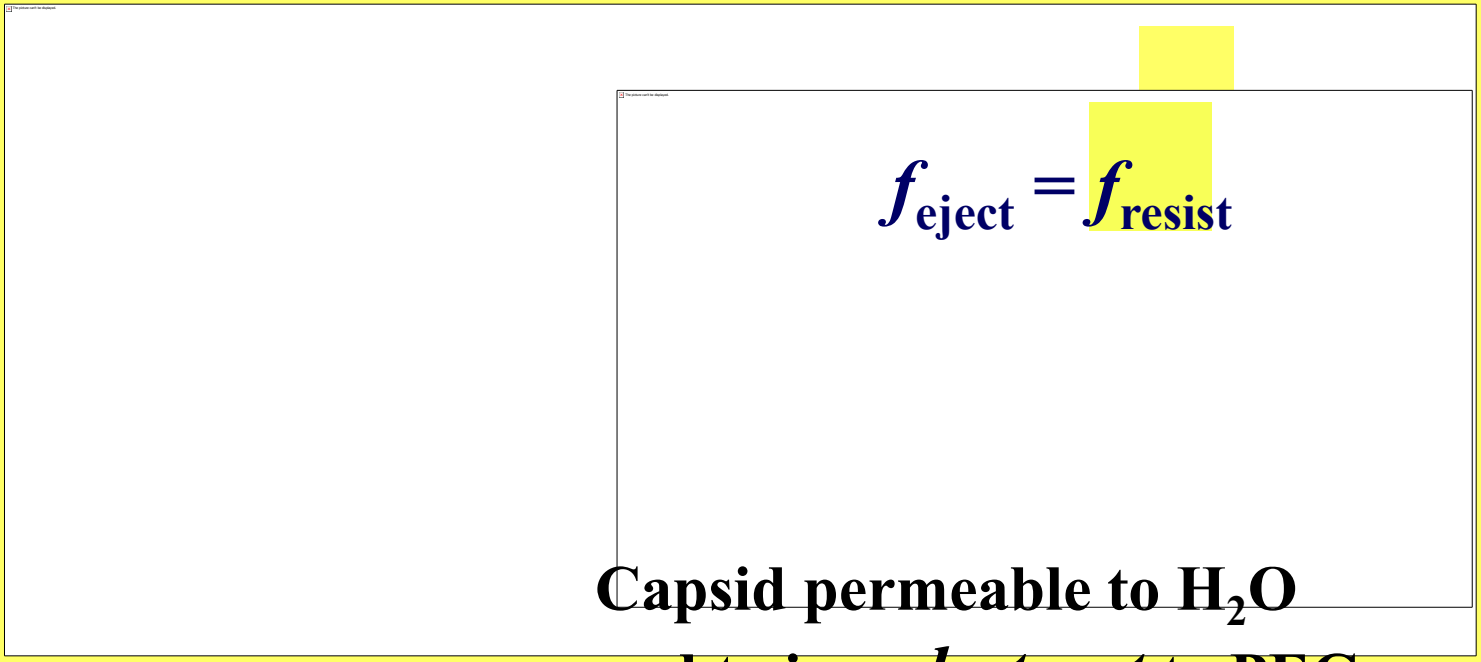


The force ***driving*** ejection is due to the DNA crowding and bending

The force ***resisting*** ejection is due to the osmotic pressure difference

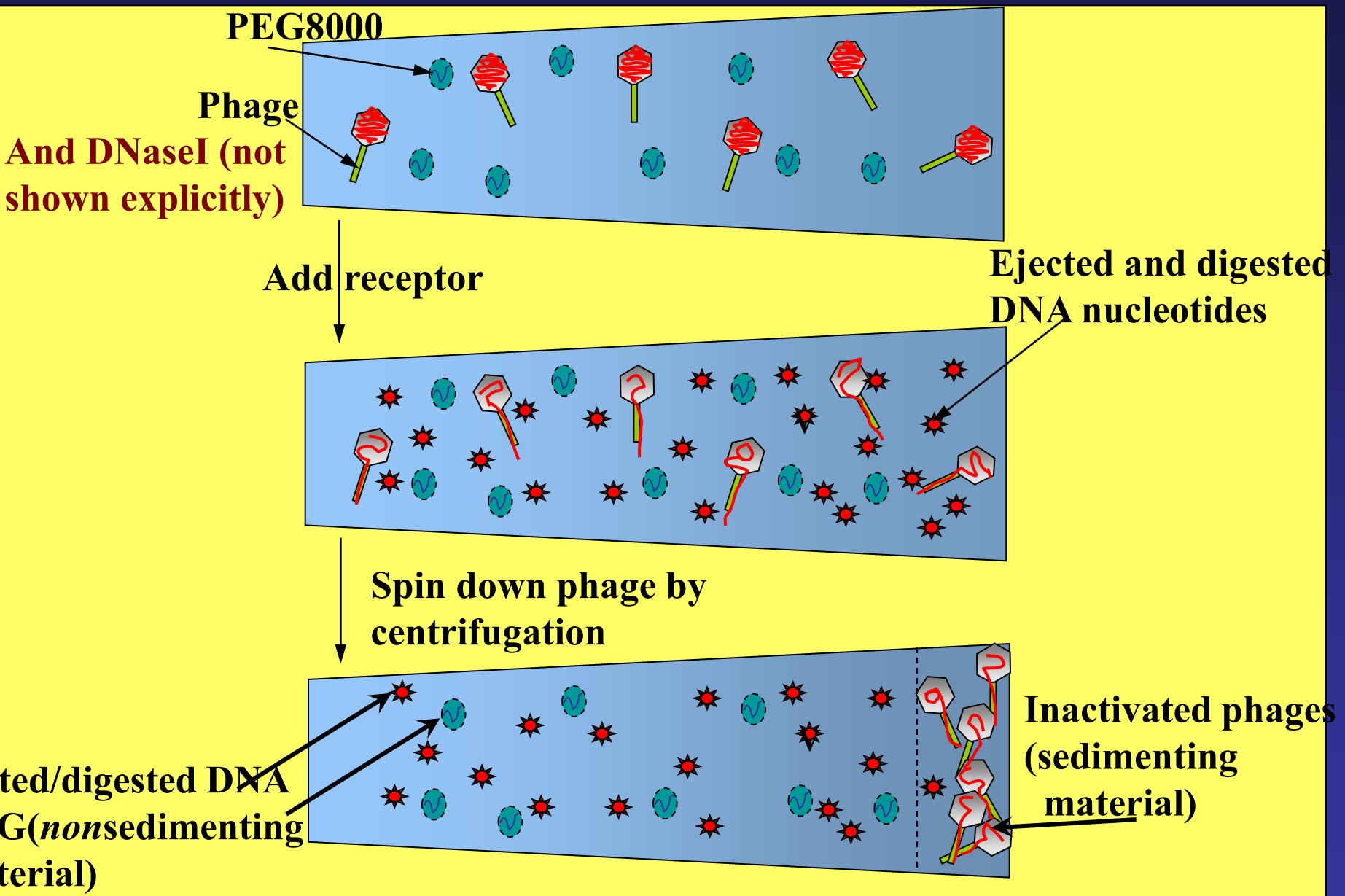
Osmotic force:

EXPERIMENT: COUNTERBALANCE EJECTION FORCE BY ESTABLISHING AN EXTERNAL OSMOTIC PRESSURE



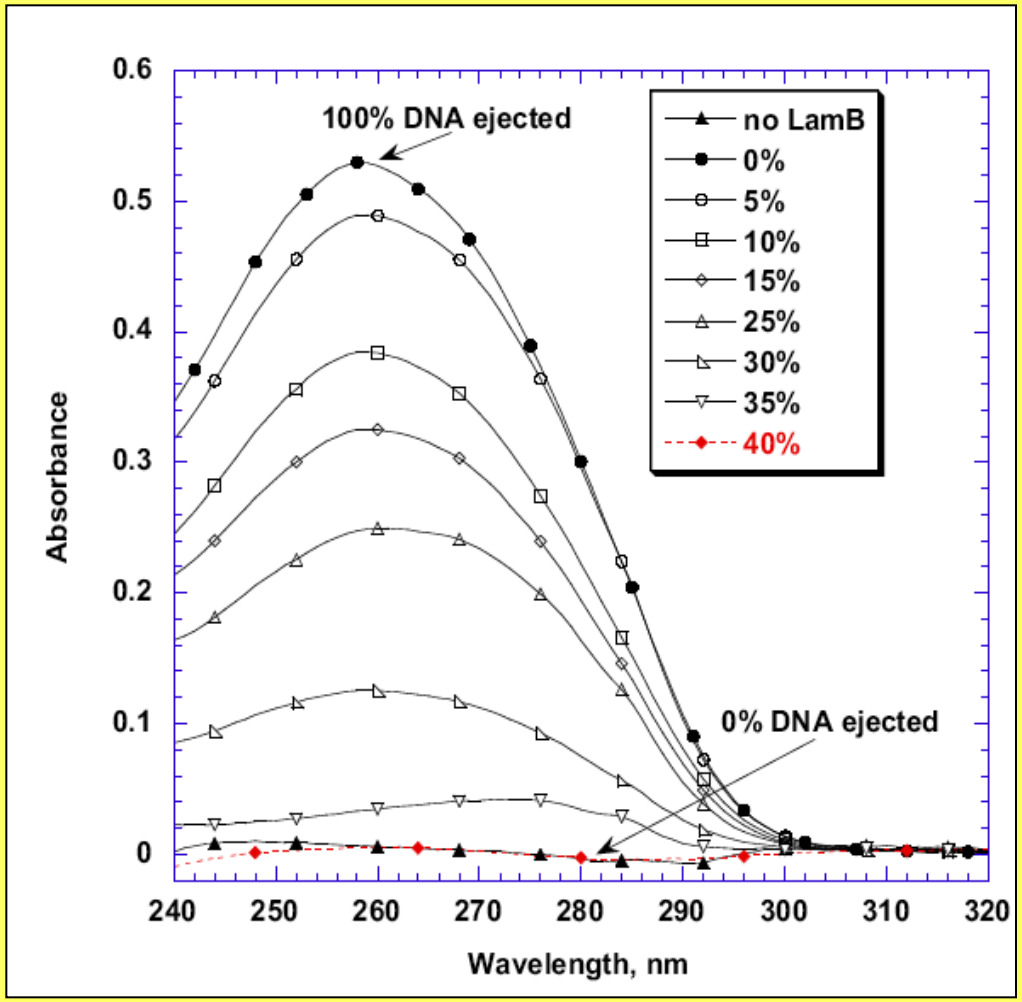
Measure DNA concentration by 260-nm absorption -- but must distinguish DNA ejected from that remaining in capsid

Experimental Design

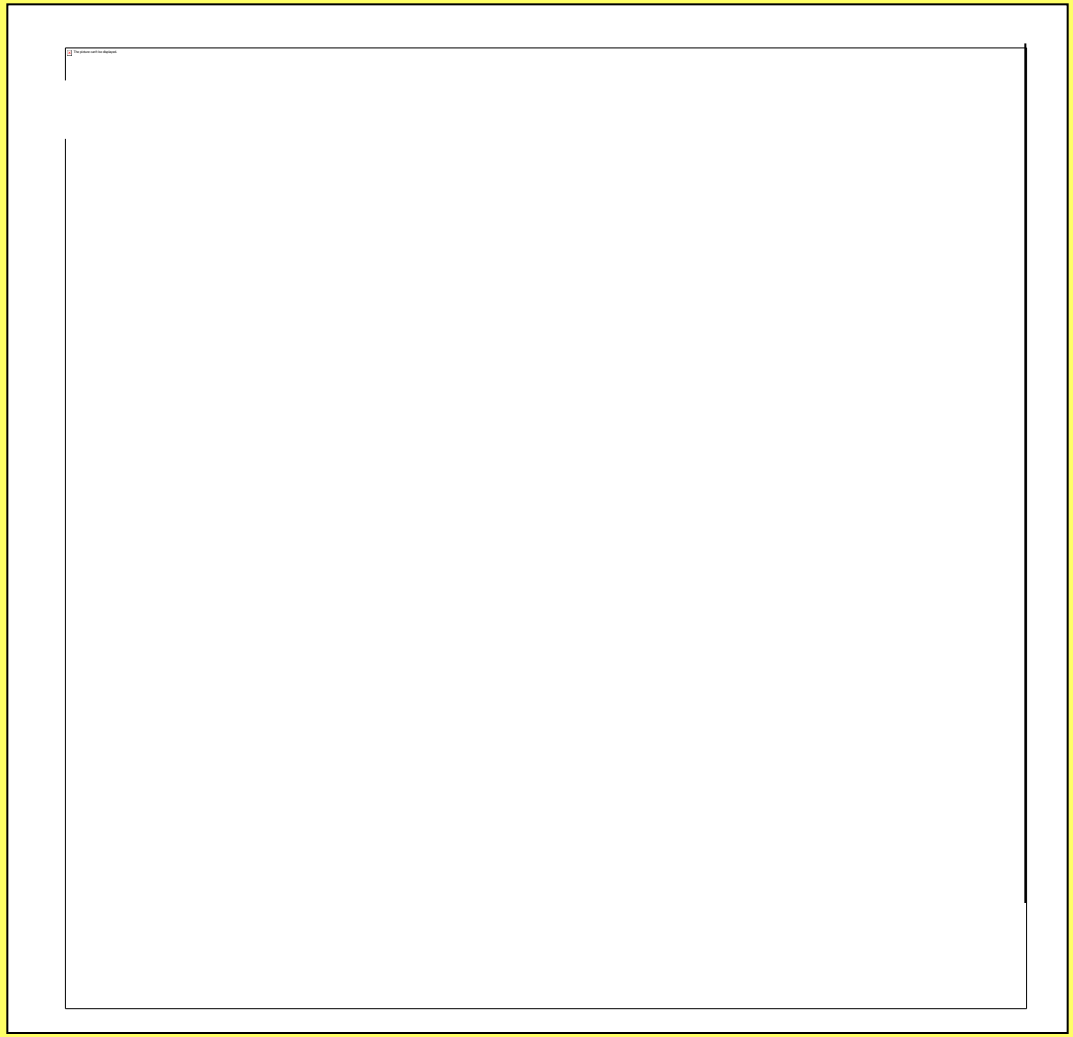


Evilevitch, Lavelle, Raspaud, Knobler and Gelbart Proc. Nat. Acad. Sci. (USA) 100, 9292 (2003).

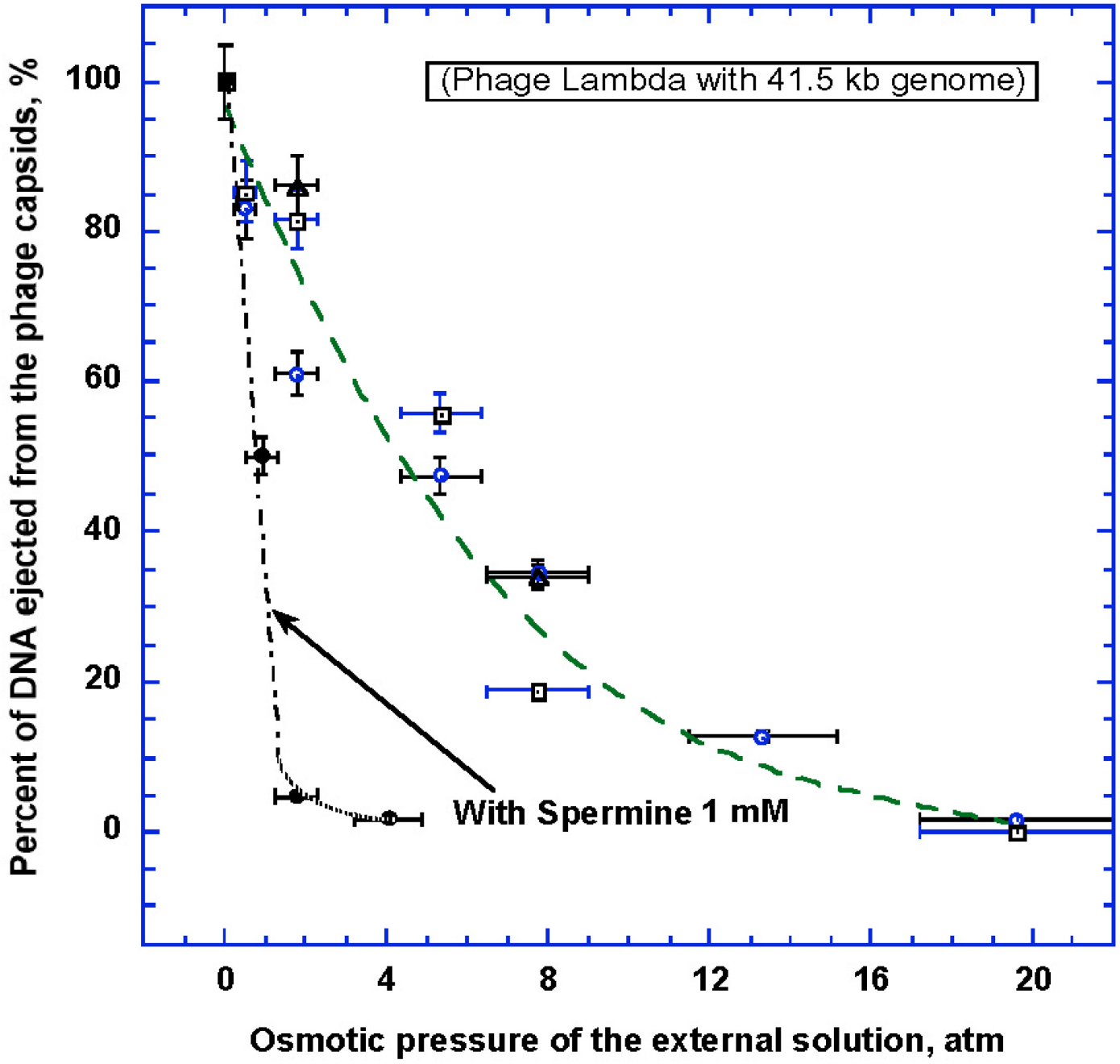
UV absorbance of DNA ejected from phage as a function of PEG8000 concentration.



Extent of Ejected DNA *vs* Osmotic Pressure in Solution



Evilevitch, Gober, Phillips, Knobler and Gelbart, Biophys. J. 88, 751 (2005)


(Phage Lambda with 41.5 kb genome)
Percent of DNA ejected from the phage capsids, %
100
80
60
40
20
0
With Spermine 1 mM
0
4
8
12
16
20
Osmotic pressure of the external solution, atm

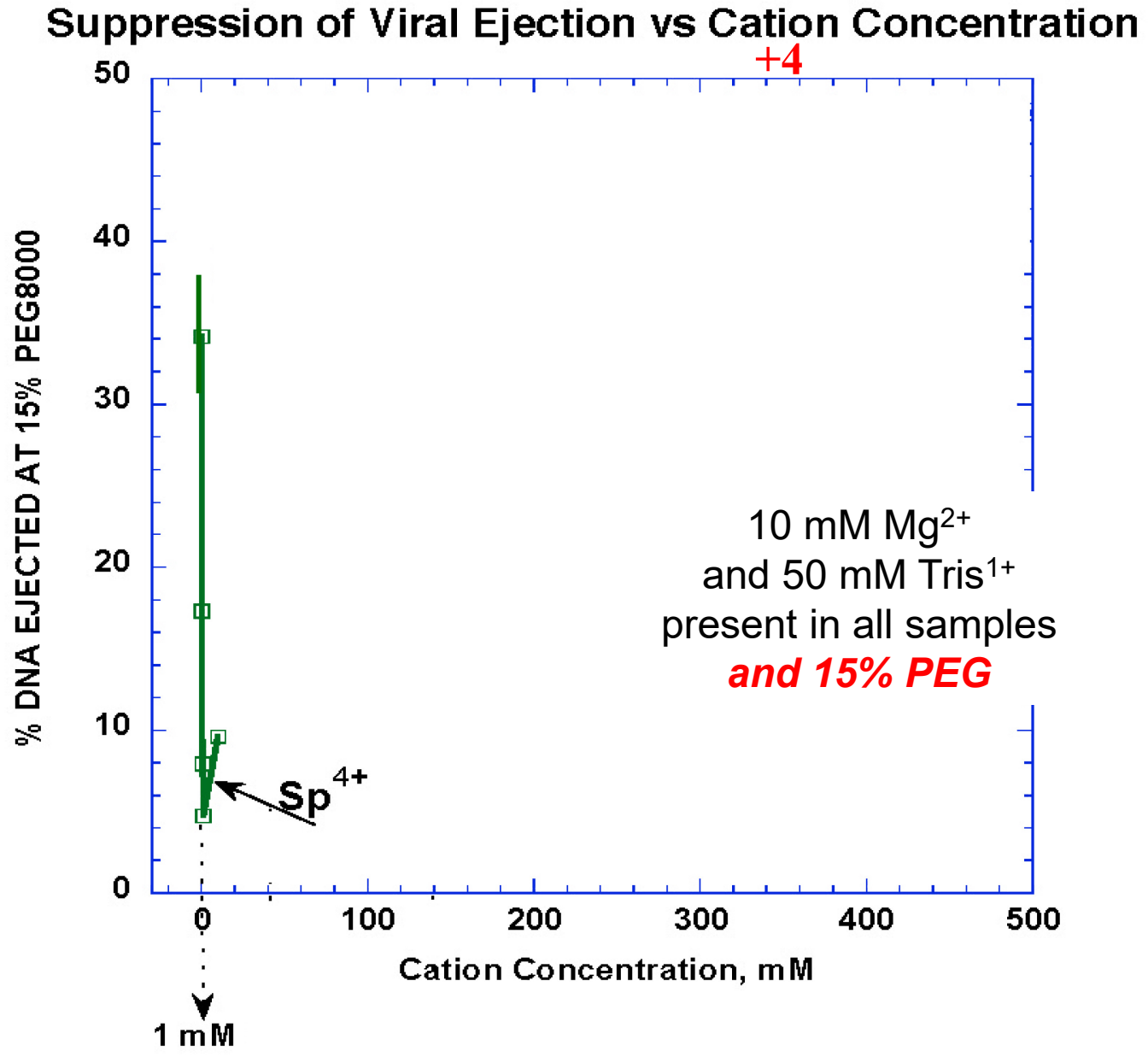
It is possible to alter -- *control* -- the ejection force by:

* Modifying the electrostatic interactions between neighboring portions of DNA by the addition of mono- and multi- valent salts (since capsids are permeable to salts)

* Changing the length of the genome

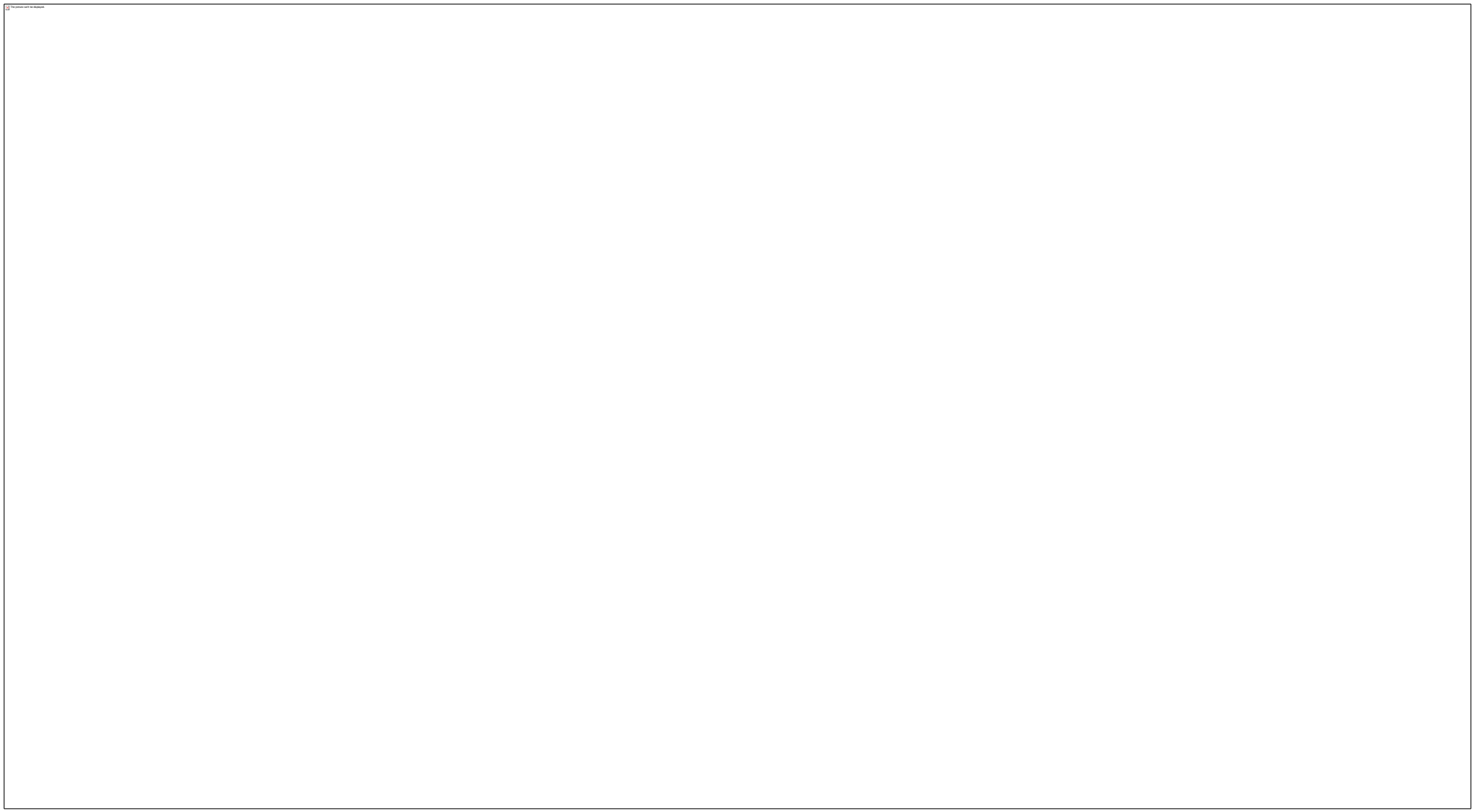
Effective Strength of

DNA-DNA Interactions

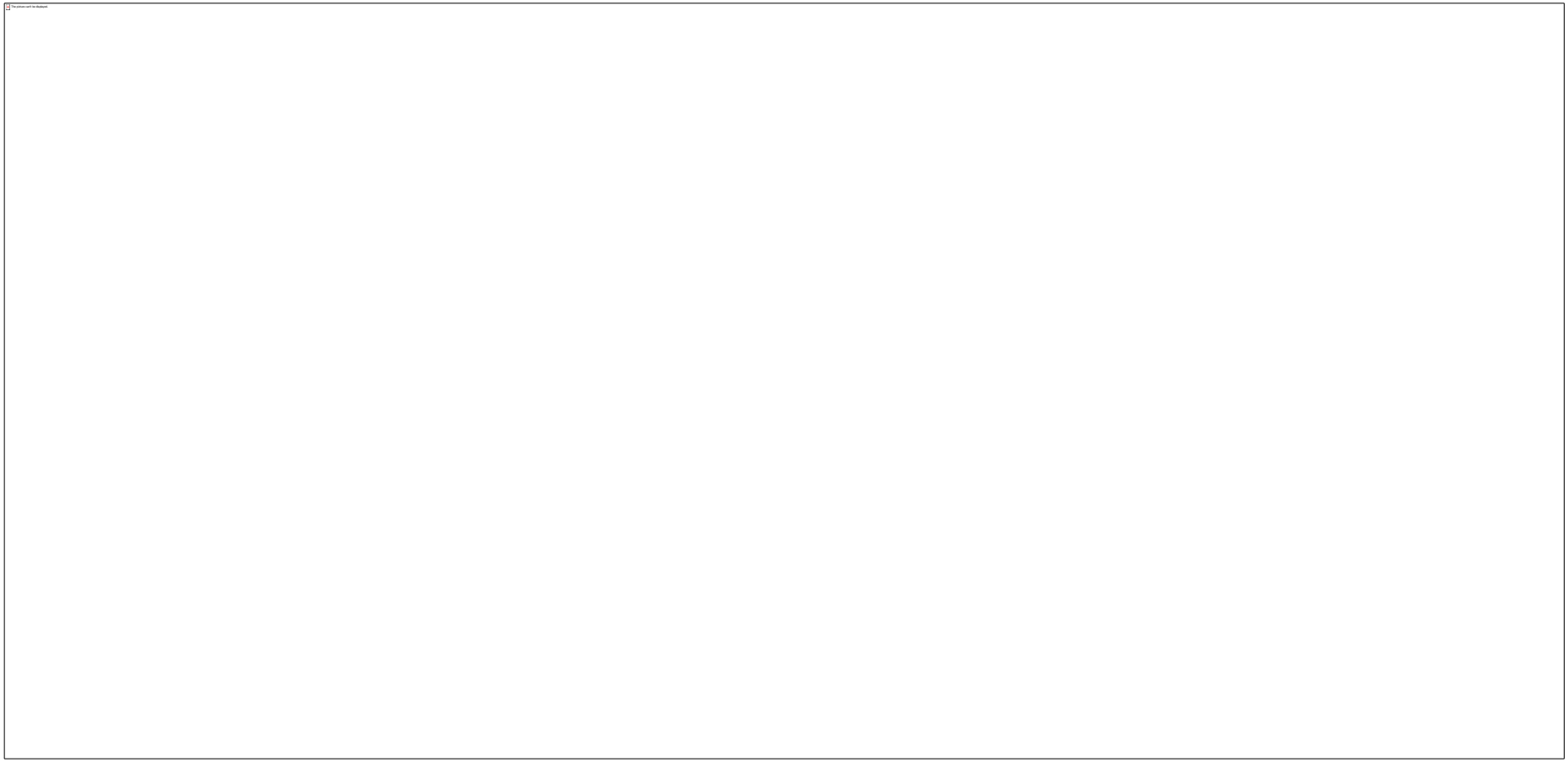


Evilevitch, Fang, Castelnovo, Rau, Parsegian, Knobler and Gelbart

Monovalent cation (+1) has relatively small effect on ejection…



But divalent (+2) case is more interesting….

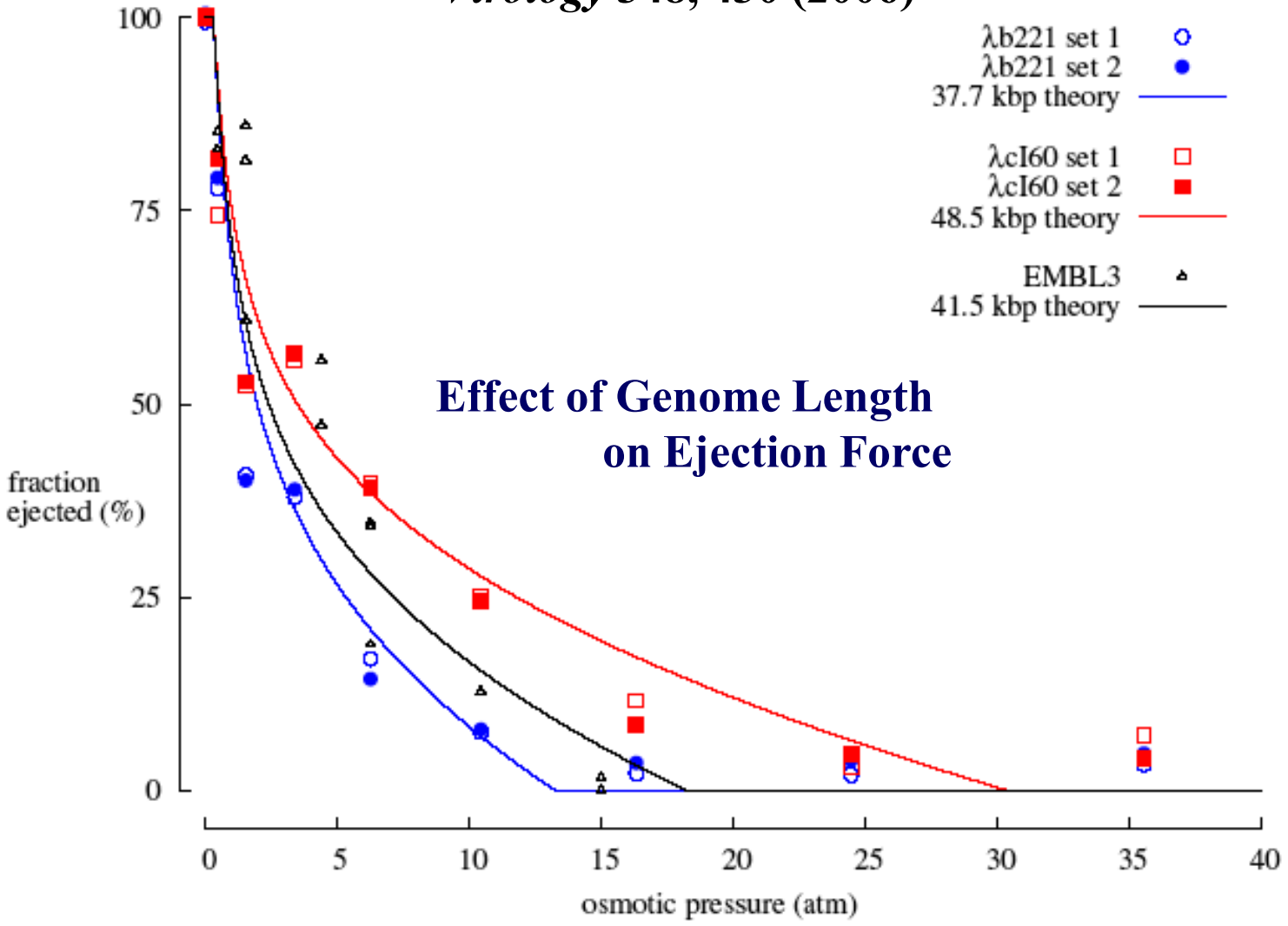


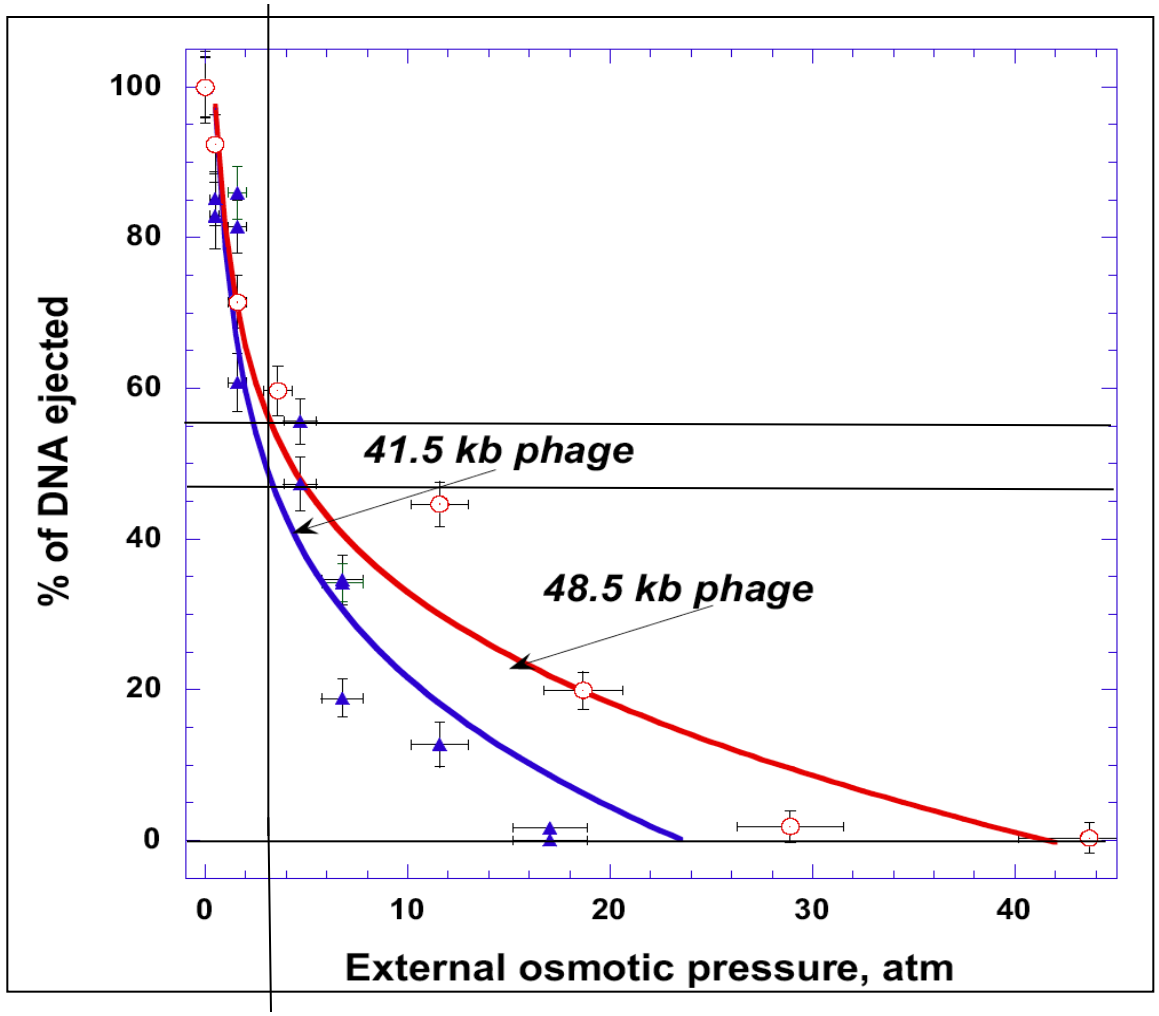
(Consistent with

Lee, Borukhov, Gelbart, Liu and Stevens, Phys. Rev. Lett. 93, 128101 (2004) and recent simulations by Kun-Chun Lee and Andrea Liu)

EJECTION FORCES ALSO DEPEND ON GENOME LENGTH…

Grayson, Inamdar, Purohit, Phillips, Evilevitch, Knobler and Gelbart,
***Virology* 348, 430 (2006)**





3-4 atms (in bacterial cytoplasm)

WHAT HAVE WE LEARNED?

- **The extent of DNA ejection from phage involves a balance between capsid stress and osmotic force**

- ***In vivo* genome delivery driven by capsid pressure is necessarily *incomplete***

  (What brings in the rest of the genome?)

- **Capsid stress is dominated by DNA-DNA repulsions**
- **Permeability of capsid allows us (and Nature) to control internal stresses by changing salt conditions**
- **The stresses inside viral capsids also depend strongly on genome lengths**

ACKNOWLEDGEMENTS

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Li Tai FANG (UCLA)