Stretching flexible (bio)polymers

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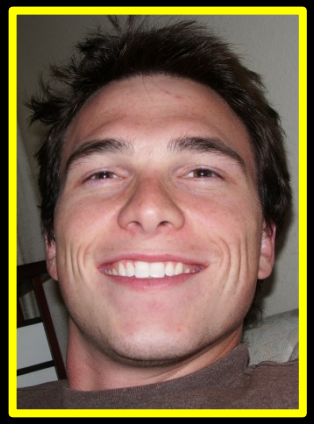
Acknowledgements

Dustin McIntosh

Andrew Dittmore



Philip Pincus

Publications

Saleh et al., PRL (2009)
McIntosh et al., PRE (2009)
McIntosh et al., Macromolecules (2011)
Dittmore et al., In submission

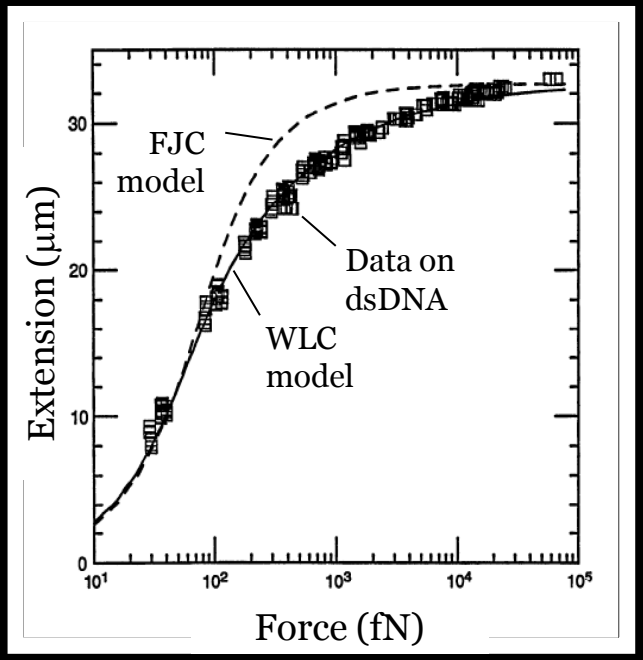
Funding

UCSB MRL
NSF
HFSP
NIH

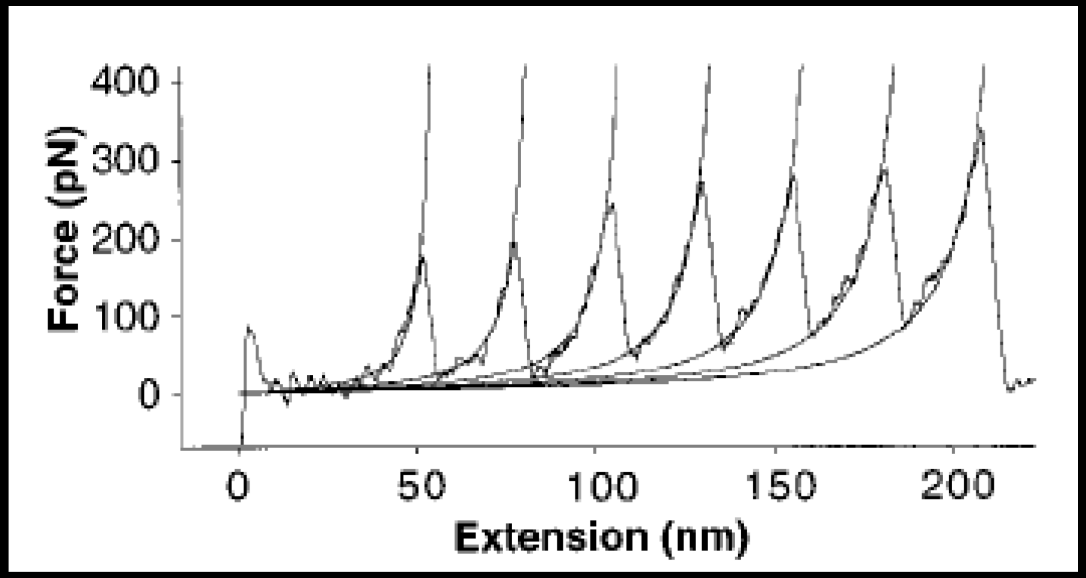
Stretching single flexible (bio)polymers: Unexplored Territory

Nearly all stretching experiments are done in the highly-aligned regime:

Force $f > f_c = k_B T/l$ Kuhn Length



dsDNA: l ~ 100 nm, f_c ~ 0.1 pN
[Bustamante, Marko, Siggia, et al., 1994]

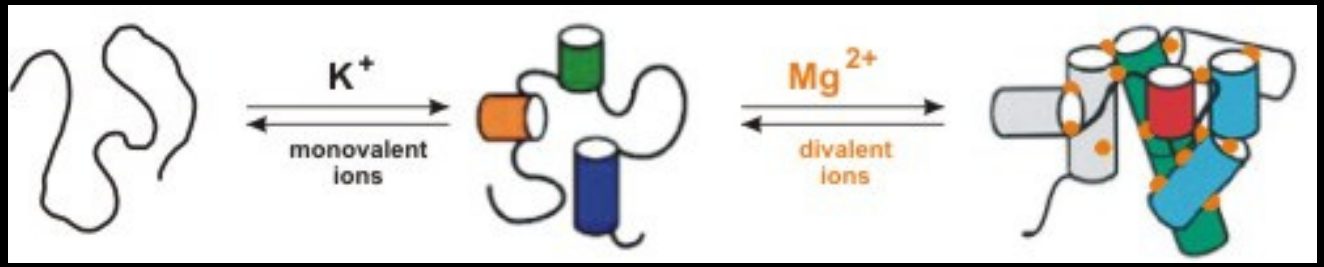


Proteins: l ~ 1 nm, f_c ~ 4 pN, but it folds!
[Rief et al., 1997]

$f < f_c$: Unexplored!

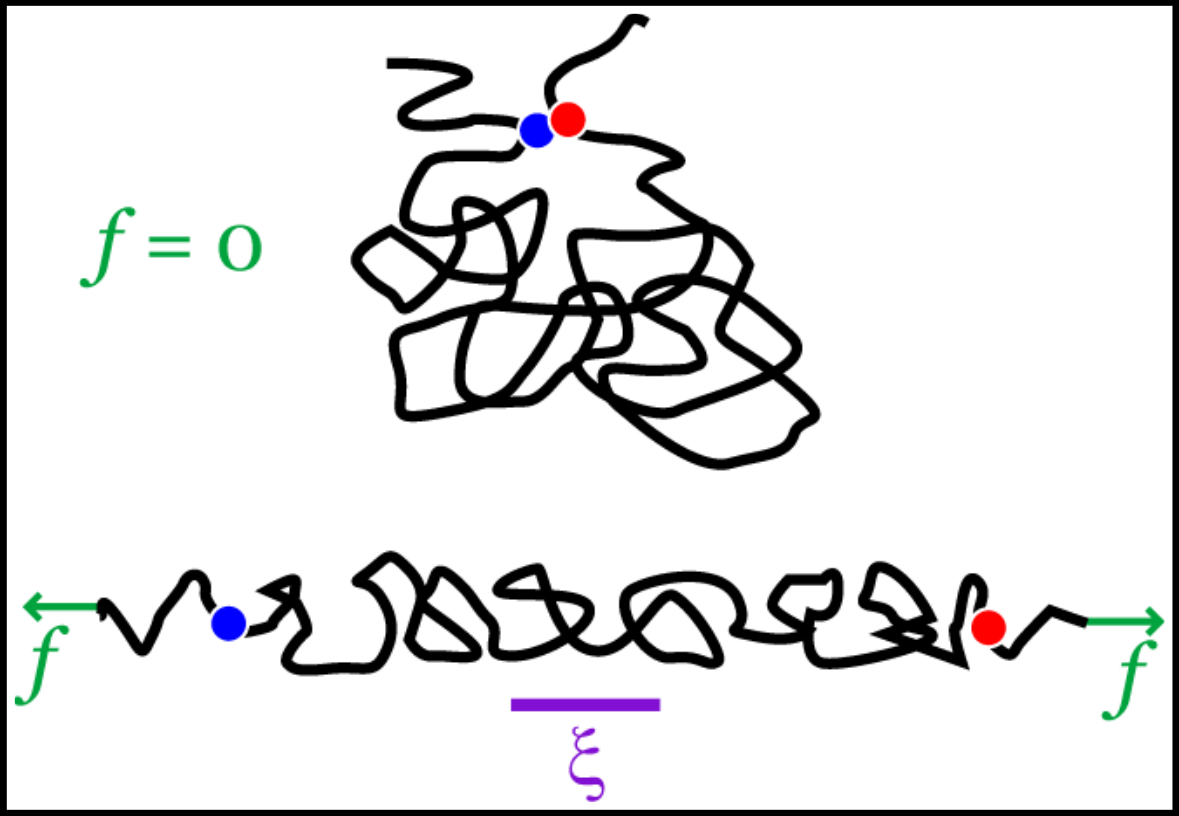
What is interesting about low forces?

- Low force is closer to zero force
  - Relevance to applications; swelling
- Low force is not high force
  - deformations
- Some new polymer physics
- Sensitivity to electrostatics



From Sigel lab; http://www.aci.uzh.ch/rna/index.php/News

The tensile screening length, $\xi \equiv kT/f$

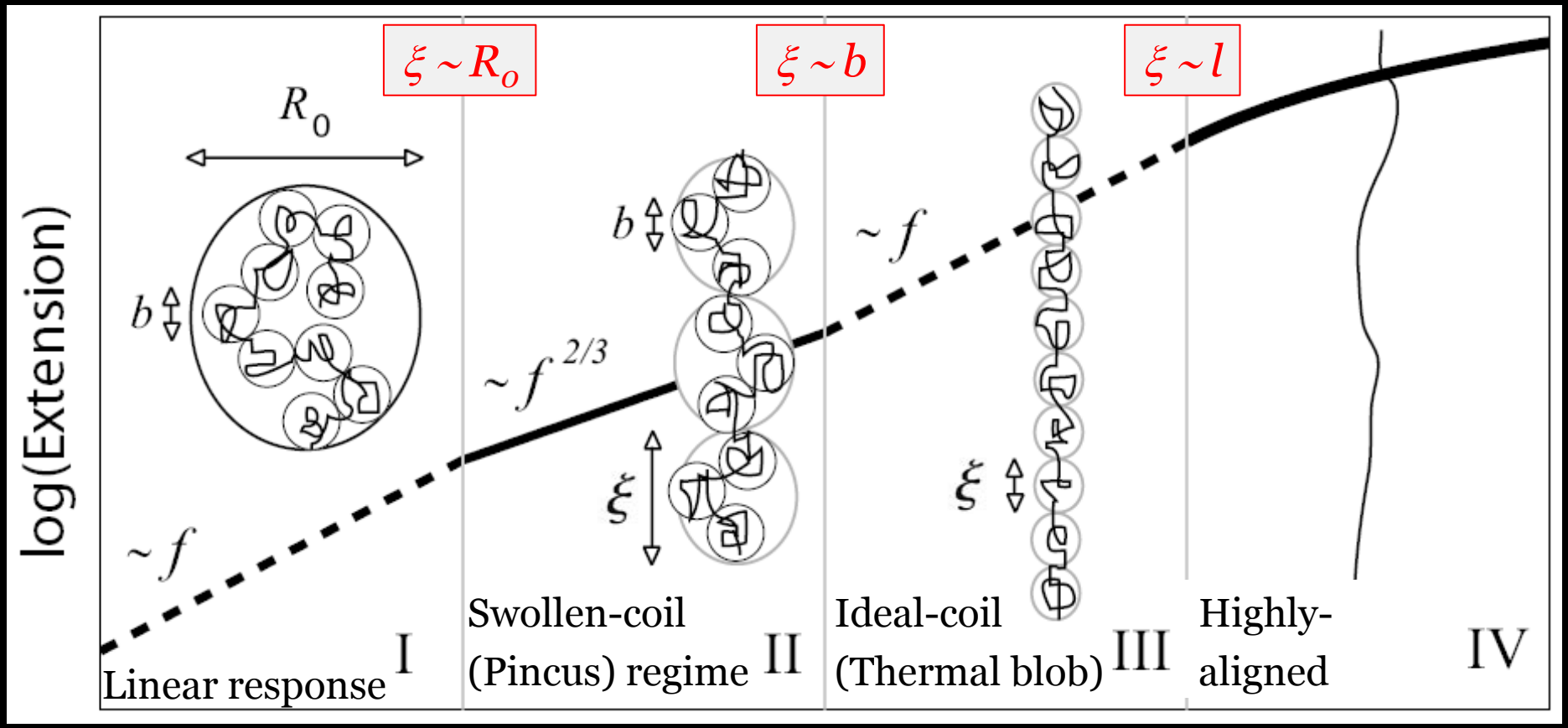


Highly aligned: $\xi < l$

Low-force regime: $\xi > l$

Low-force elasticity:
A transition whenever $\xi \sim$ (char. length)

R_o– polymer extent; b– thermal blob extent; l– Kuhn length

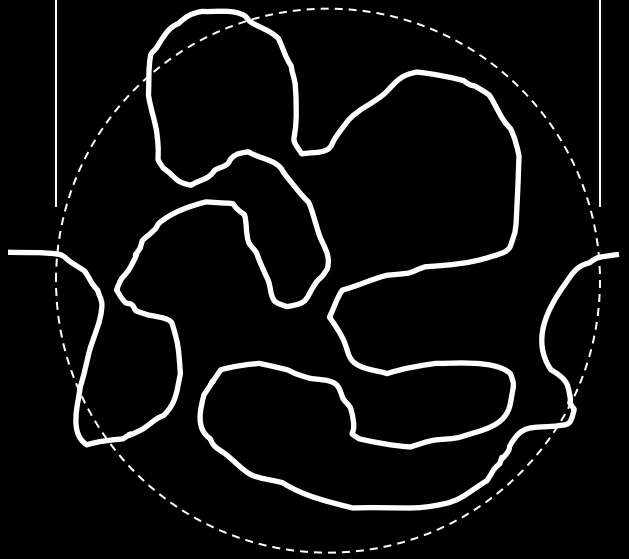


Netz, *Macromolecules* (2001)

McIntosh *et al.*, *PRE* (2009)

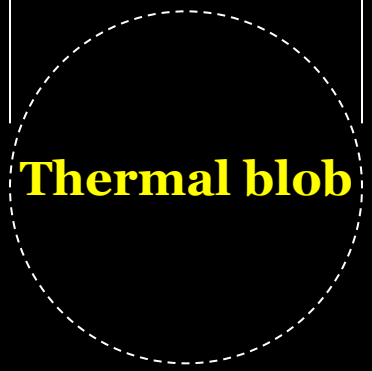
Long polymers are swollen

$N^{3/5} l^{2/5} v^{1/5}$



Crossover size:

$b \sim l^4/v$



Short polymers are ideal

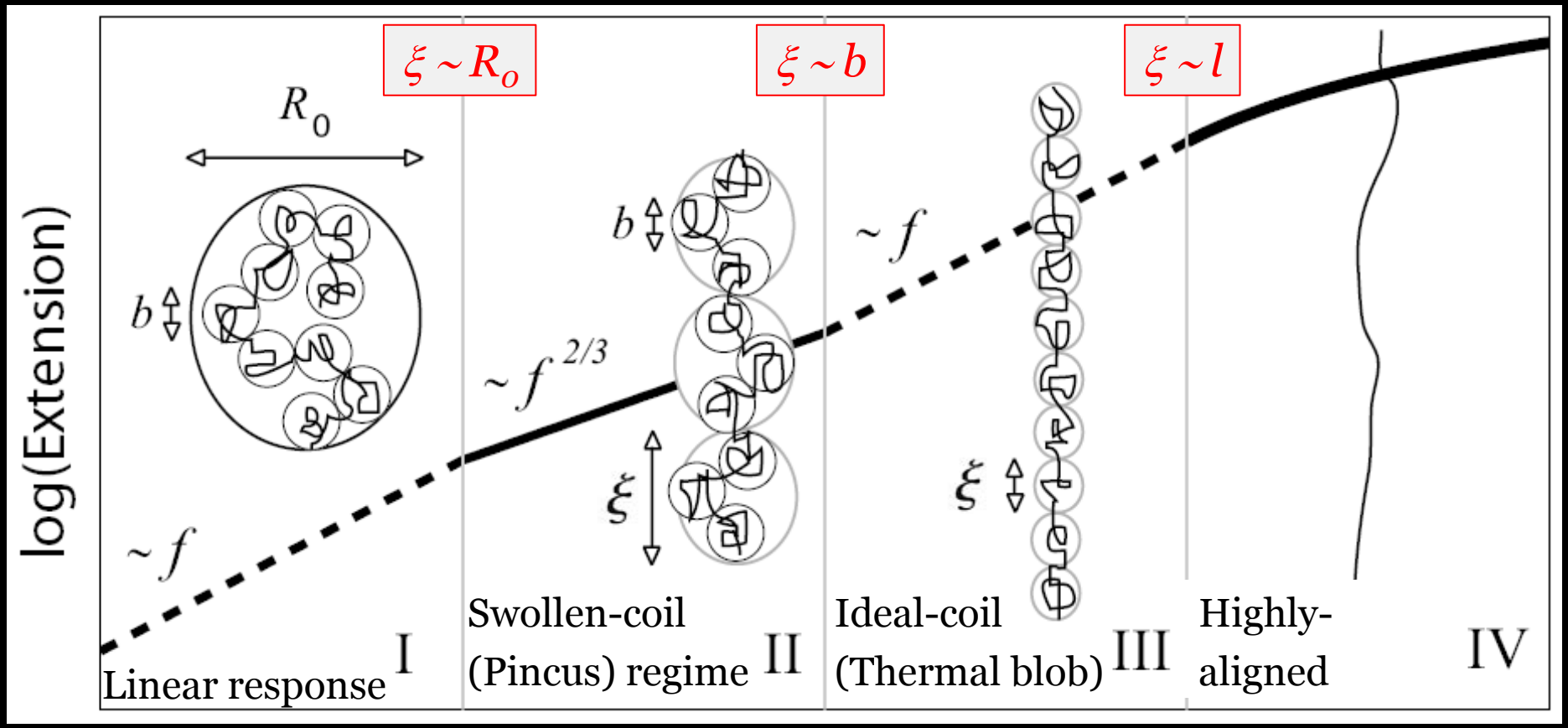
$N^{1/2} l$

Microscopic Parameters

- Kuhn length l
- Excluded volume v

Low-force elasticity:
A transition whenever $\xi \sim$ (char. length)

R_o– polymer extent; b– thermal blob extent; l– Kuhn length



Netz, *Macromolecules* (2001)

McIntosh *et al.*, *PRE* (2009)

Experiments:

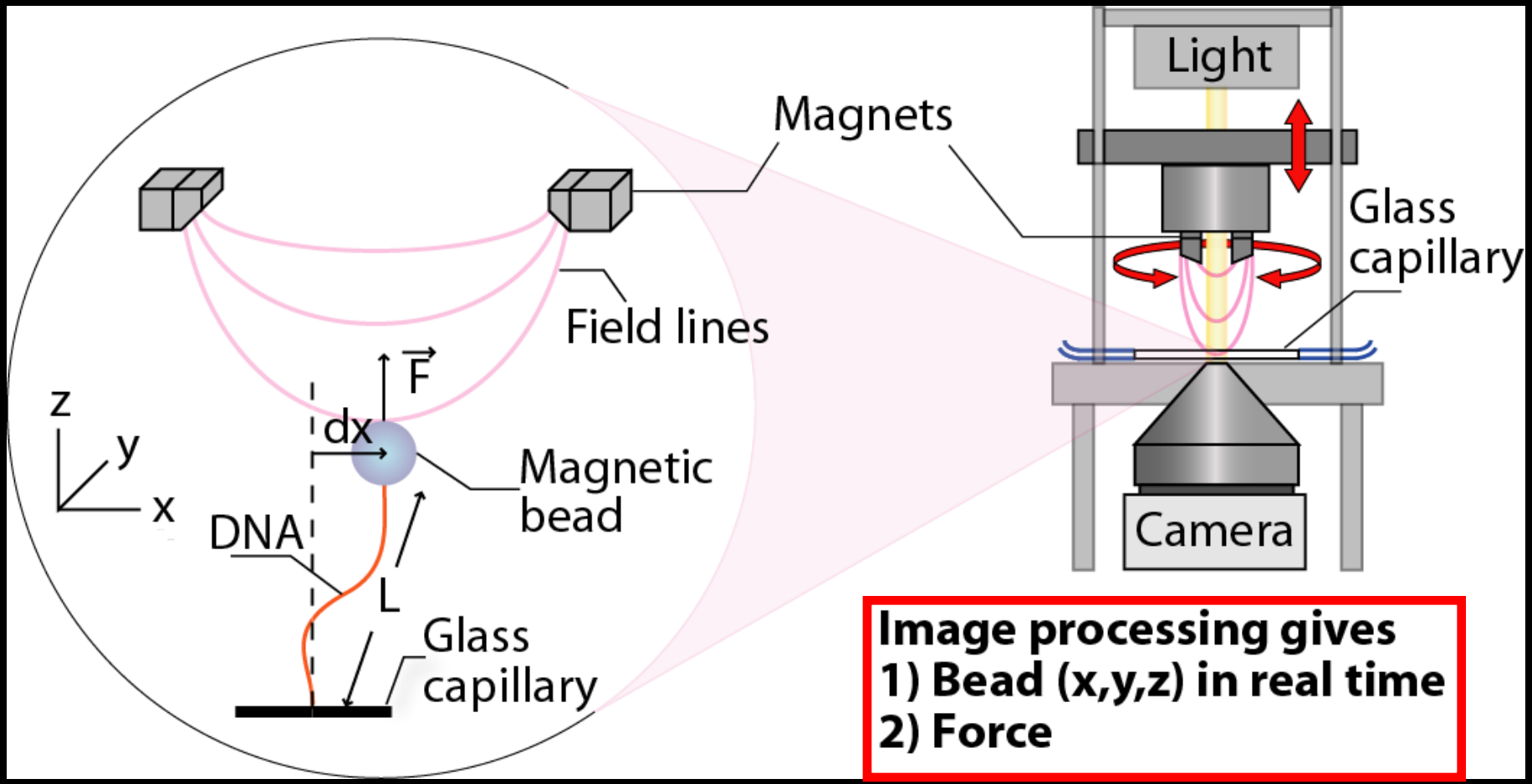
Accessing the real polymer regime ($\xi > l$) requires:

1. Low forces (large ξ)

   ►The Magnetic Tweezer

2. A highly flexible polymer (small l)

The magnetic tweezer



Key: The MT is unsurpassed in the ability to stably apply small forces

Accessing the real polymer regime ($\xi > l$) requires:

1. Low forces (large ξ)

   ► The Magnetic Tweezer

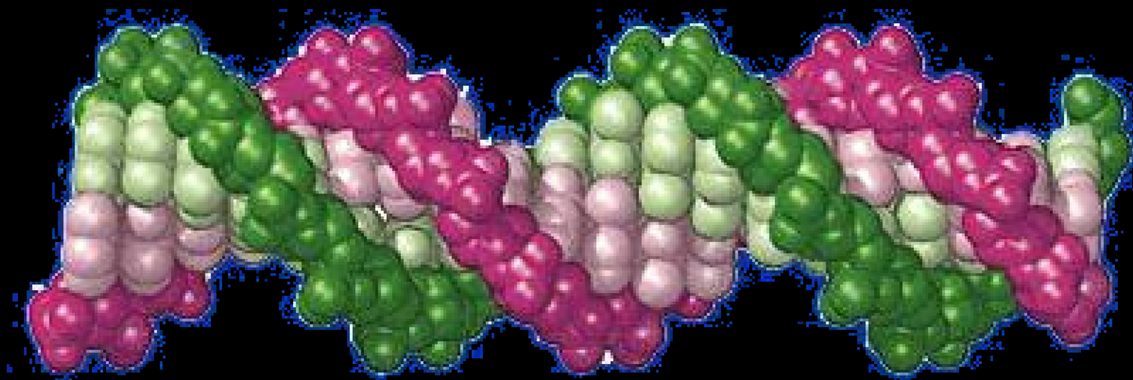
2. A highly flexible polymer (small l)

   ► Single-stranded DNA

   ► PEG

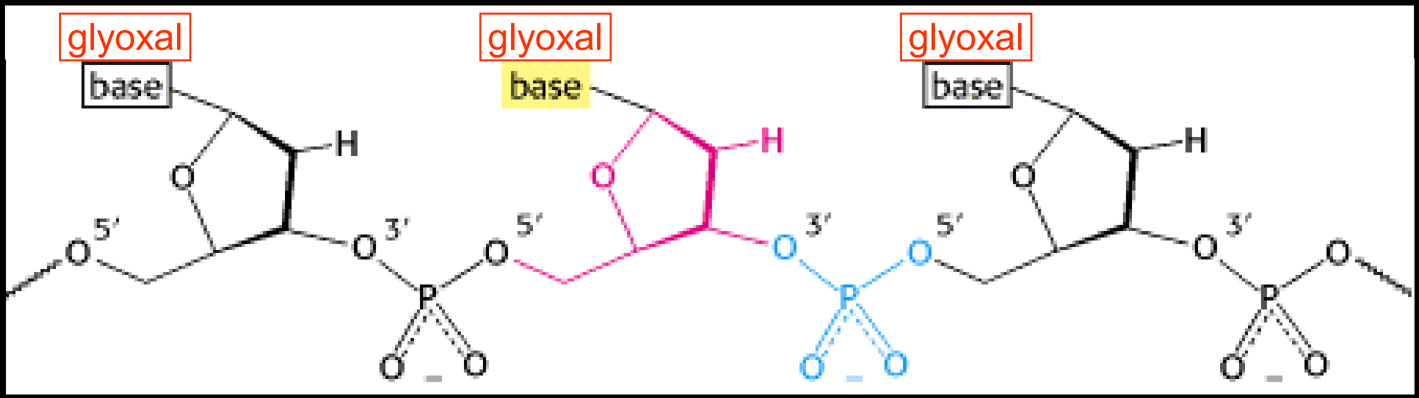
Nucleic acid structure

dsDNA



Highly-charged and rigid ($l \approx 100$ nm)

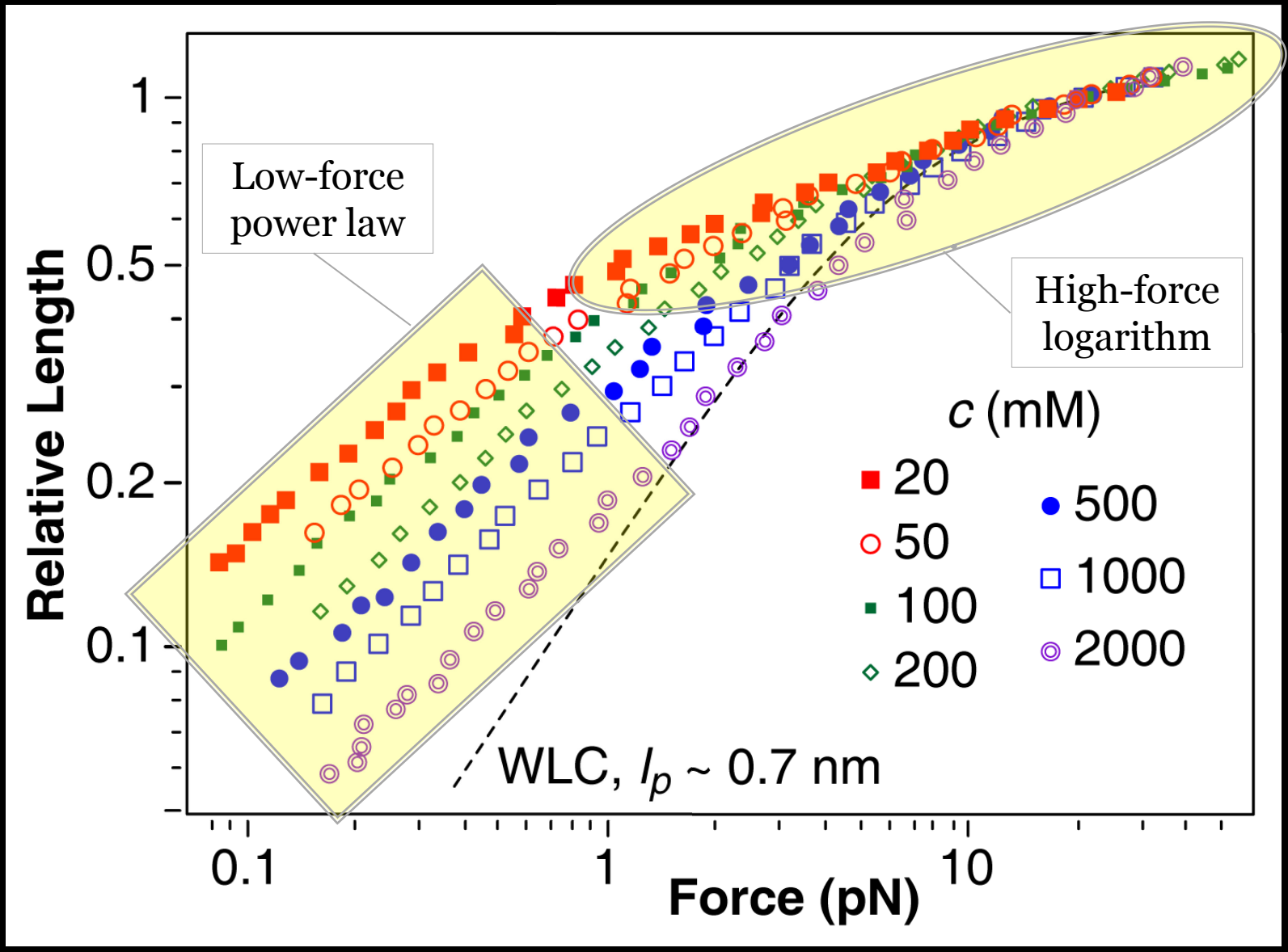
ssDNA



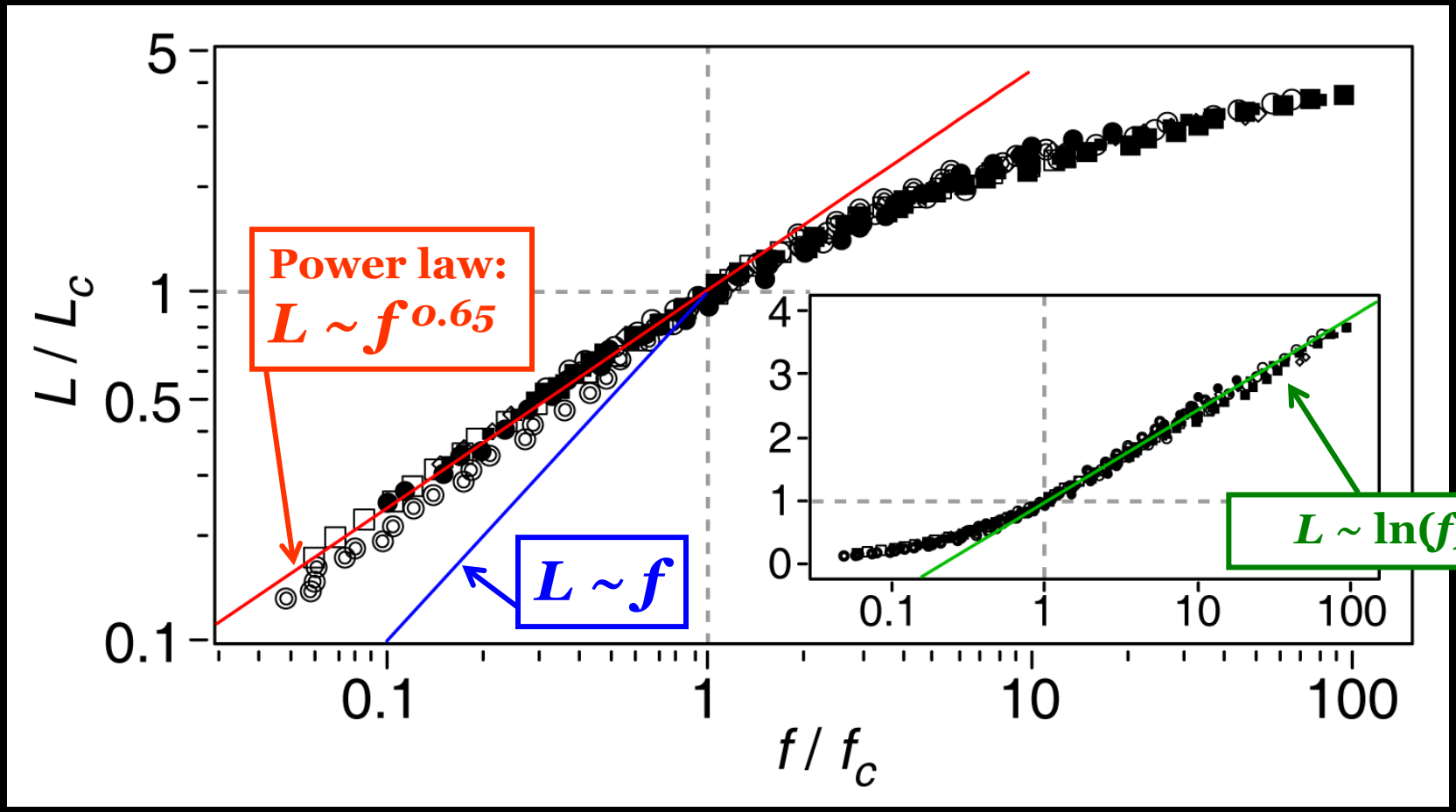
Highly-charged and flexible ($l \approx$ a few nm)

Caveat: must remove intrastrand basepairing w/ special sequences or chemistry (glyoxal; Mcmaster and Carmichael, 1977)

Force/extension behavior of ssDNA at various [Na]

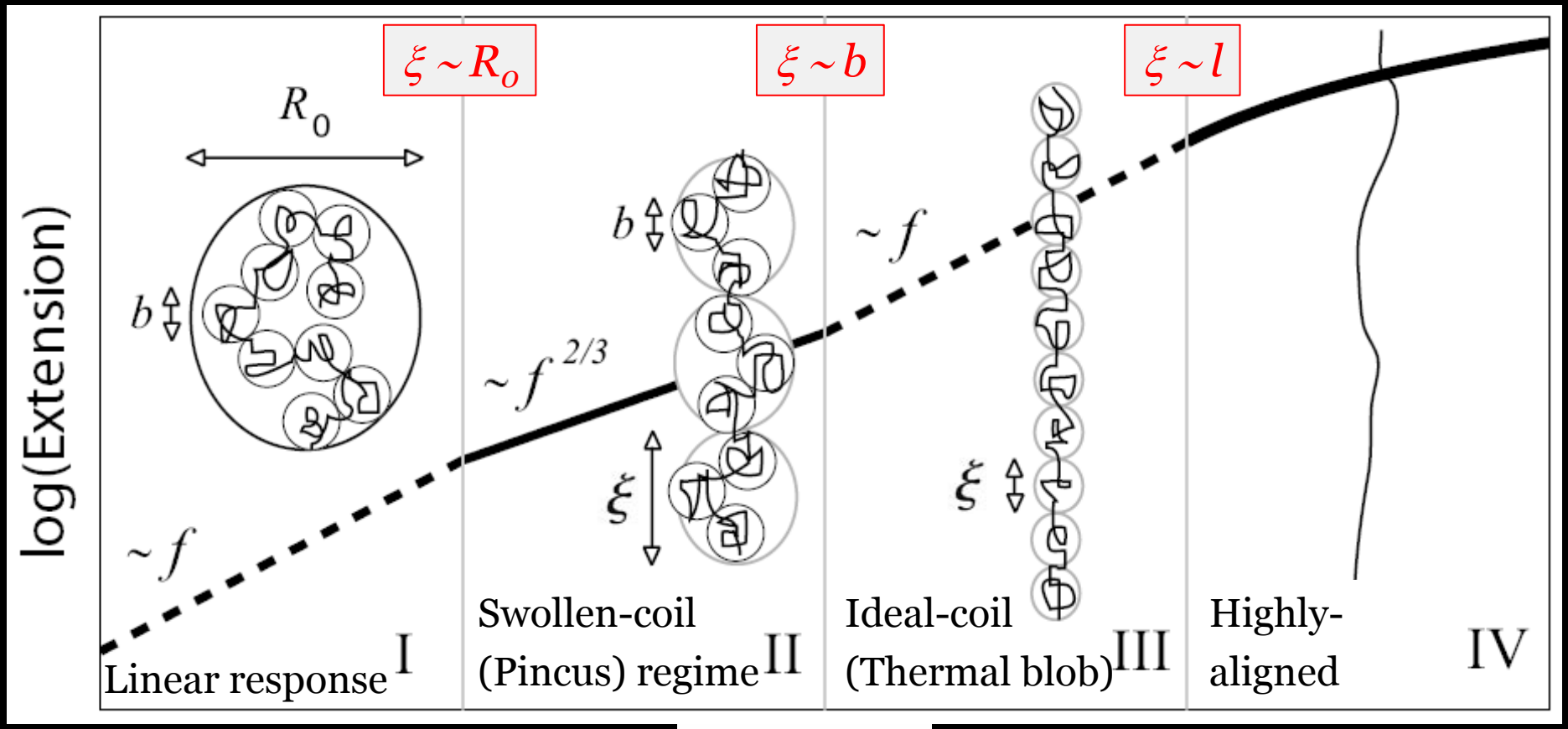


Scale f/ext data by crossover point (f_c , L_c): Universal behavior for 20-2000 mM



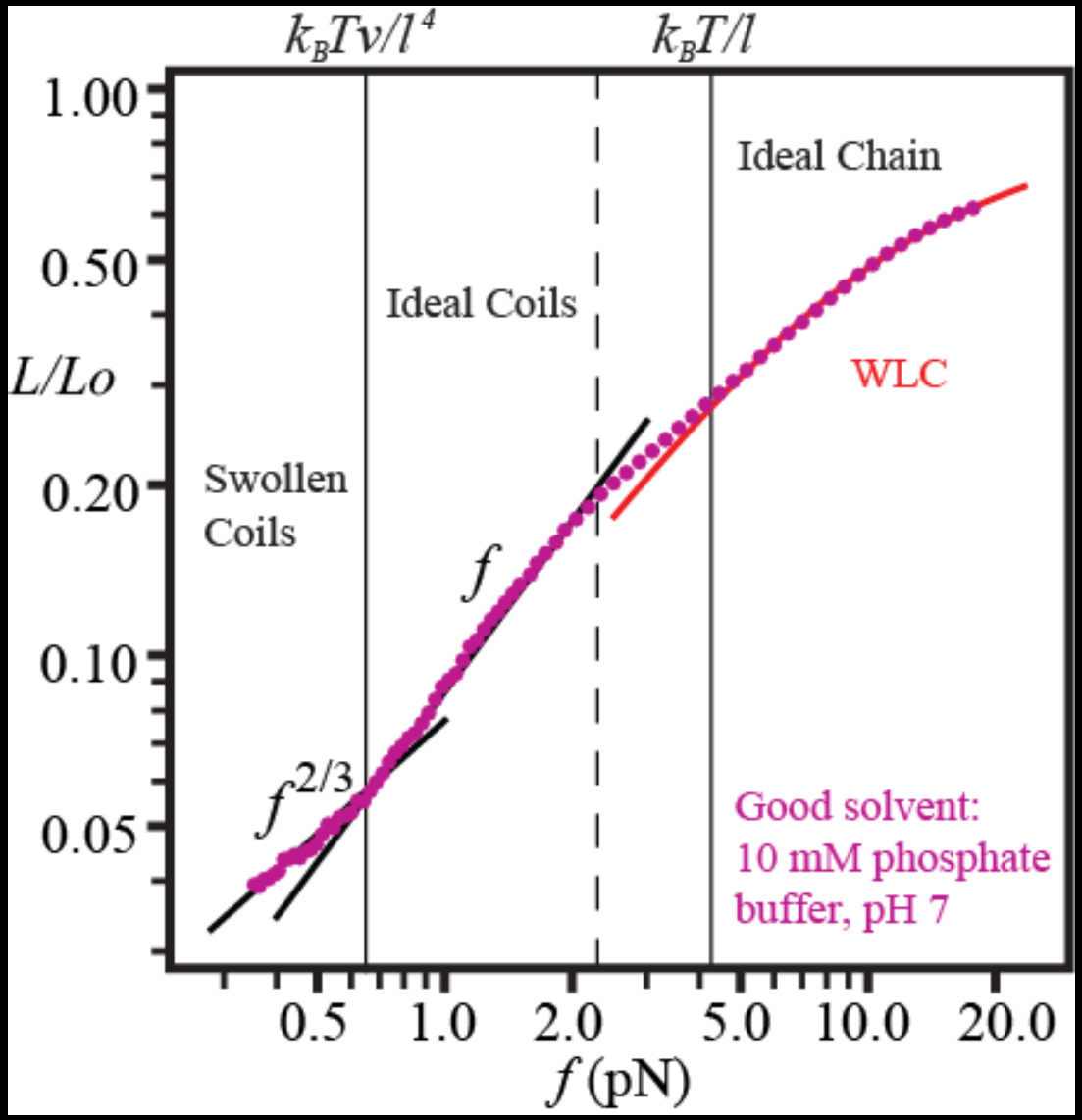
1. All salt dependence is captured in (f_c , L_c)
2. Clear observation of Pincus regime, $L \sim f^{2/3}$
3. Direct transition from Pincus to sub-linear regime

...what happened to regime III (ideal coils)?



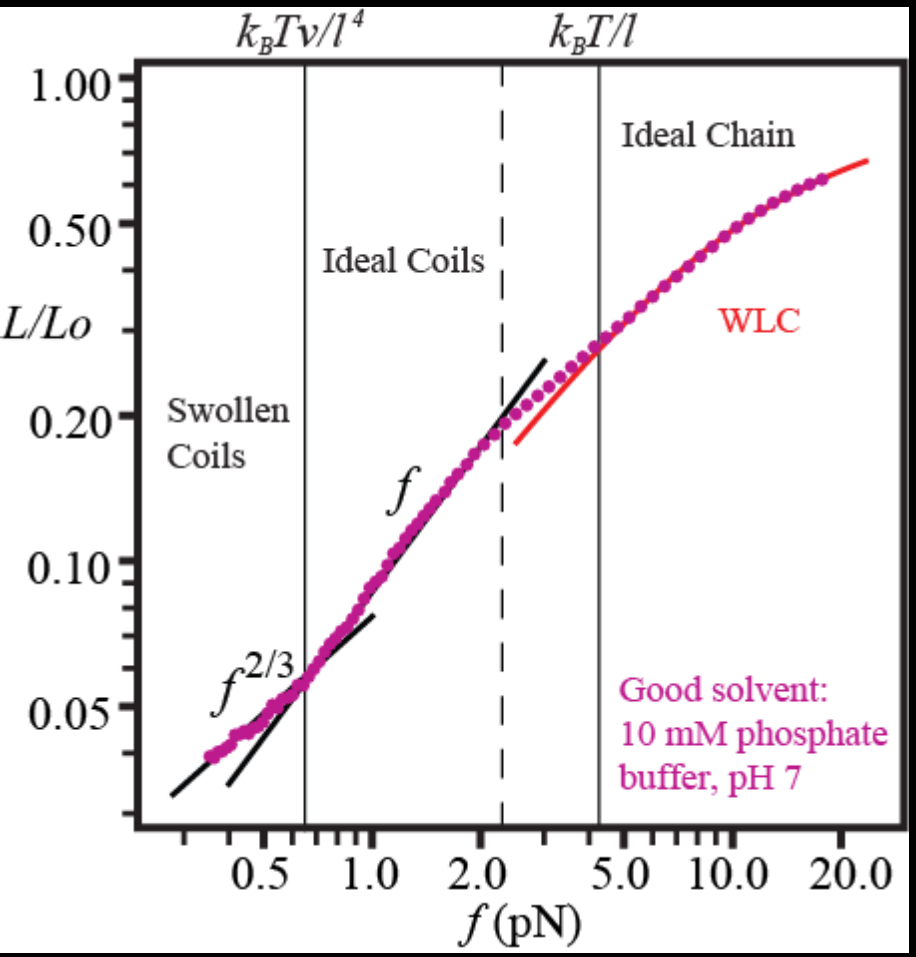
Let's look at another polymer…

Low-force stretching of PEG



1. Regime III appears!
2. Transition at 2 pN?
3. MS-WLC fits highly-aligned regime

An aside: Stretching makes a polymer flexible



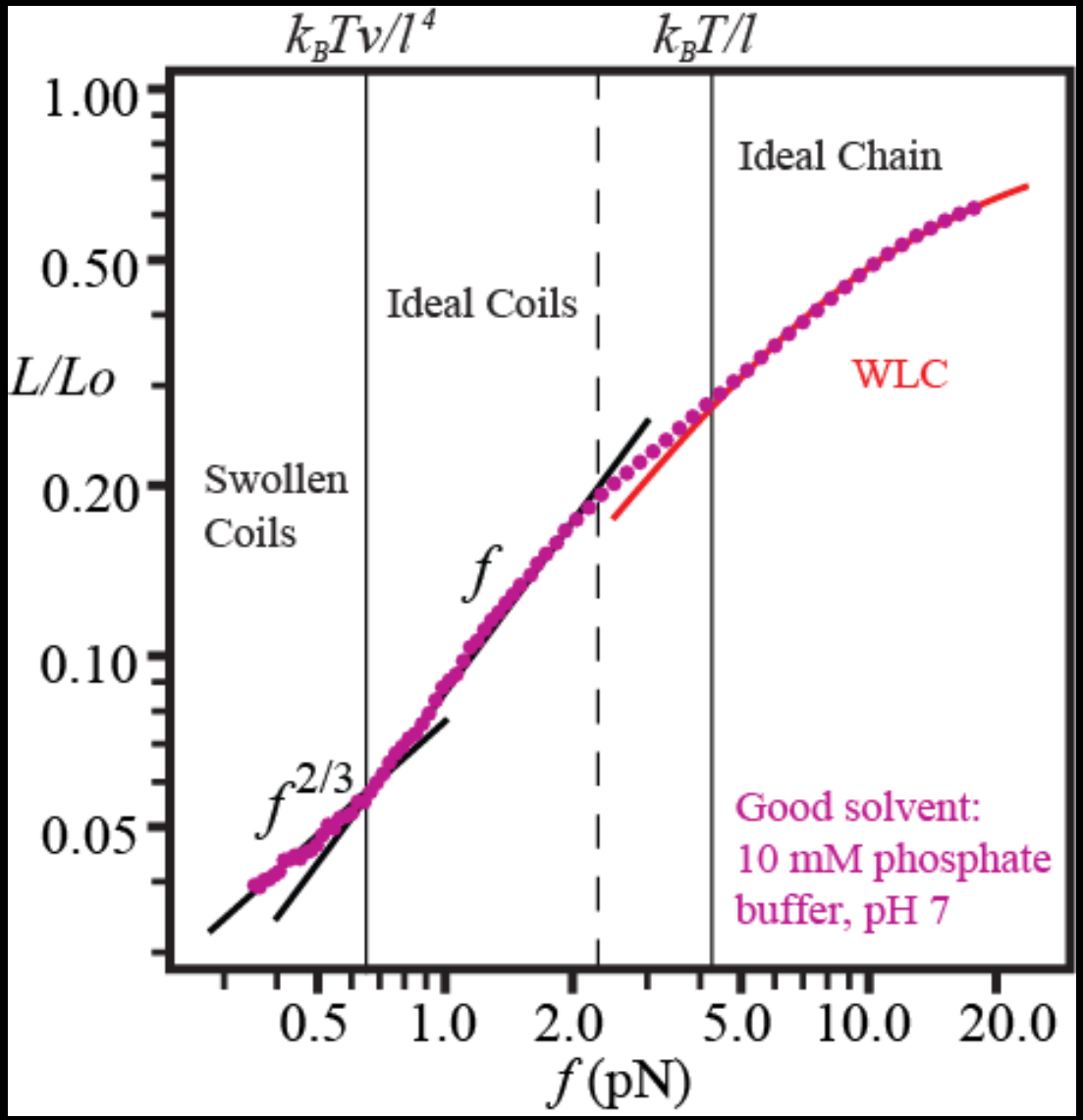
0.7—2 pN: l ~ 1.1 nm
(from slope of ideal regime)

4—15 pN: l~0.9 nm
(from MS-WLC fit)

>25 pN: l~0.76 nm
Kienberger *et al.* (2000)

High force □ Small ξ □
Screening of neighboring monomers
□ Reduced stiffness

Livadaru, Netz and Kreuzer (2003)
Toan and Thirumalai (2010)
Dobrynin, Carrillo and Rubinstein (2010)



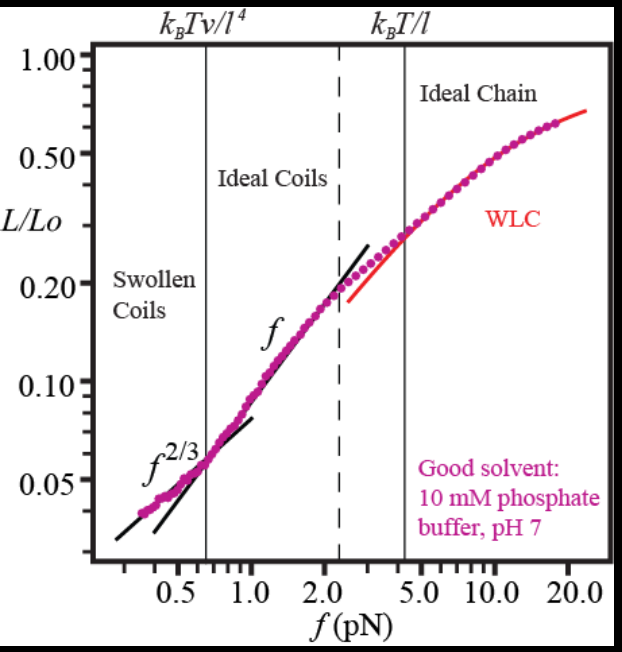
1. Regime III appears!
2. Transition at 2 pN?
3. MS-WLC fits highly-aligned regime

Regime II kind of small; do we believe it?

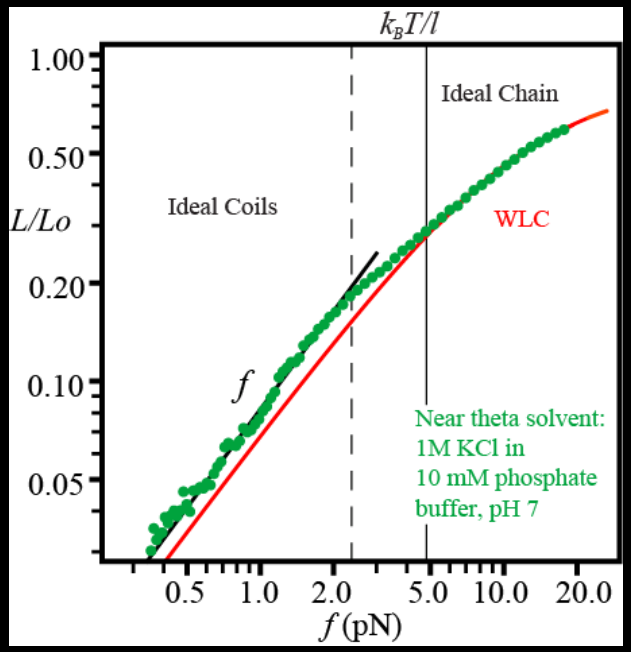
Support for msmt of onset of excluded volume:

Modulating solvent quality through water structure
causes first regime II, then III, to disappear

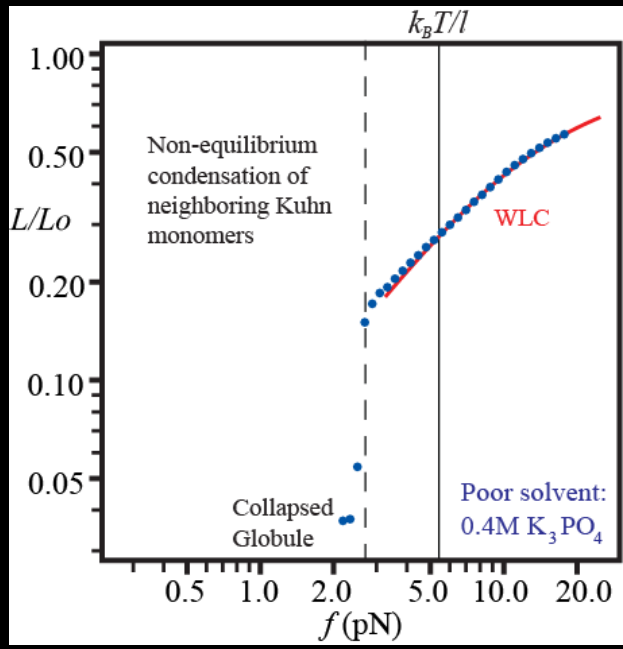
Good solvent



Theta solvent

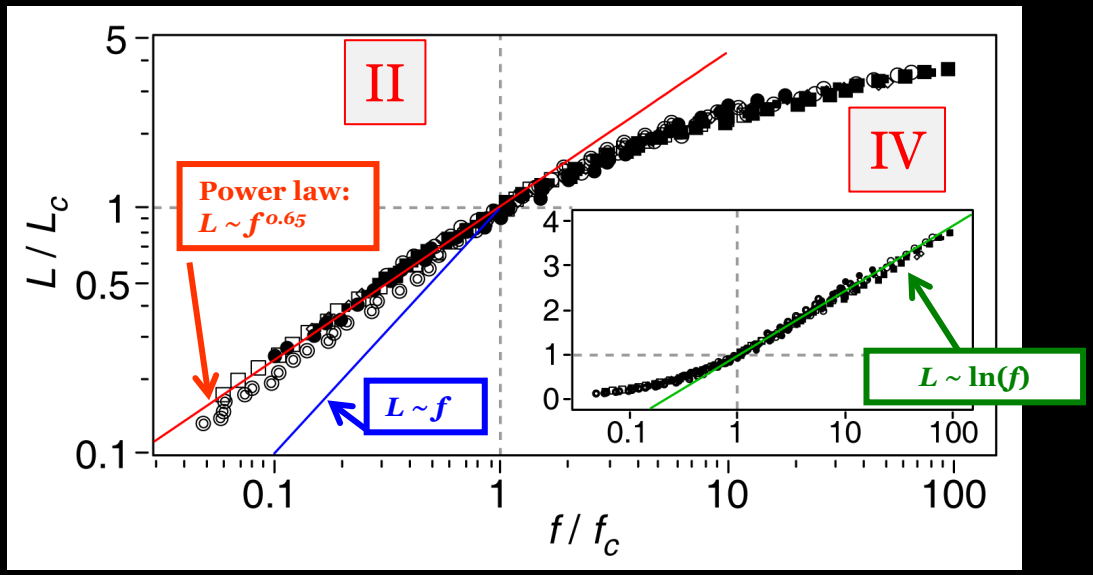


Poor solvent

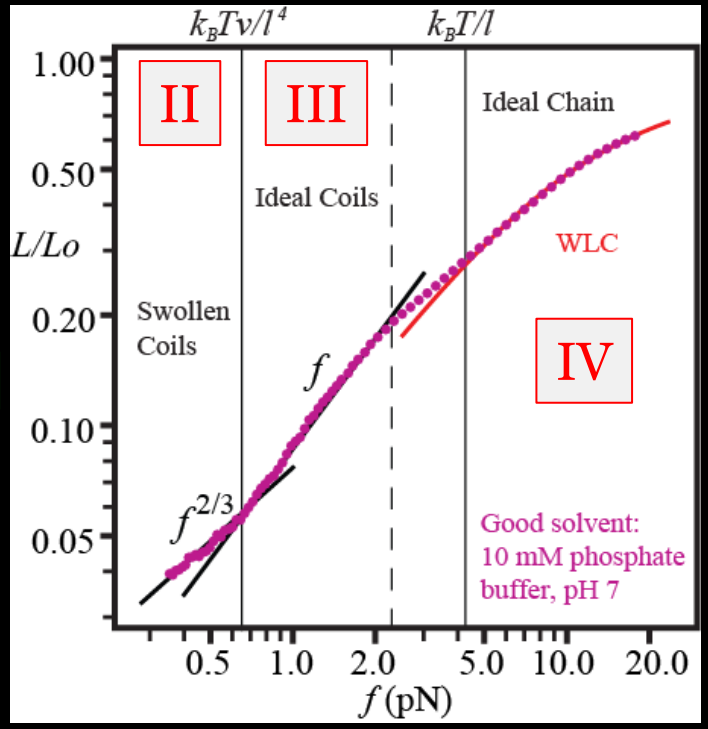


Why the difference?

ssDNA



PEG



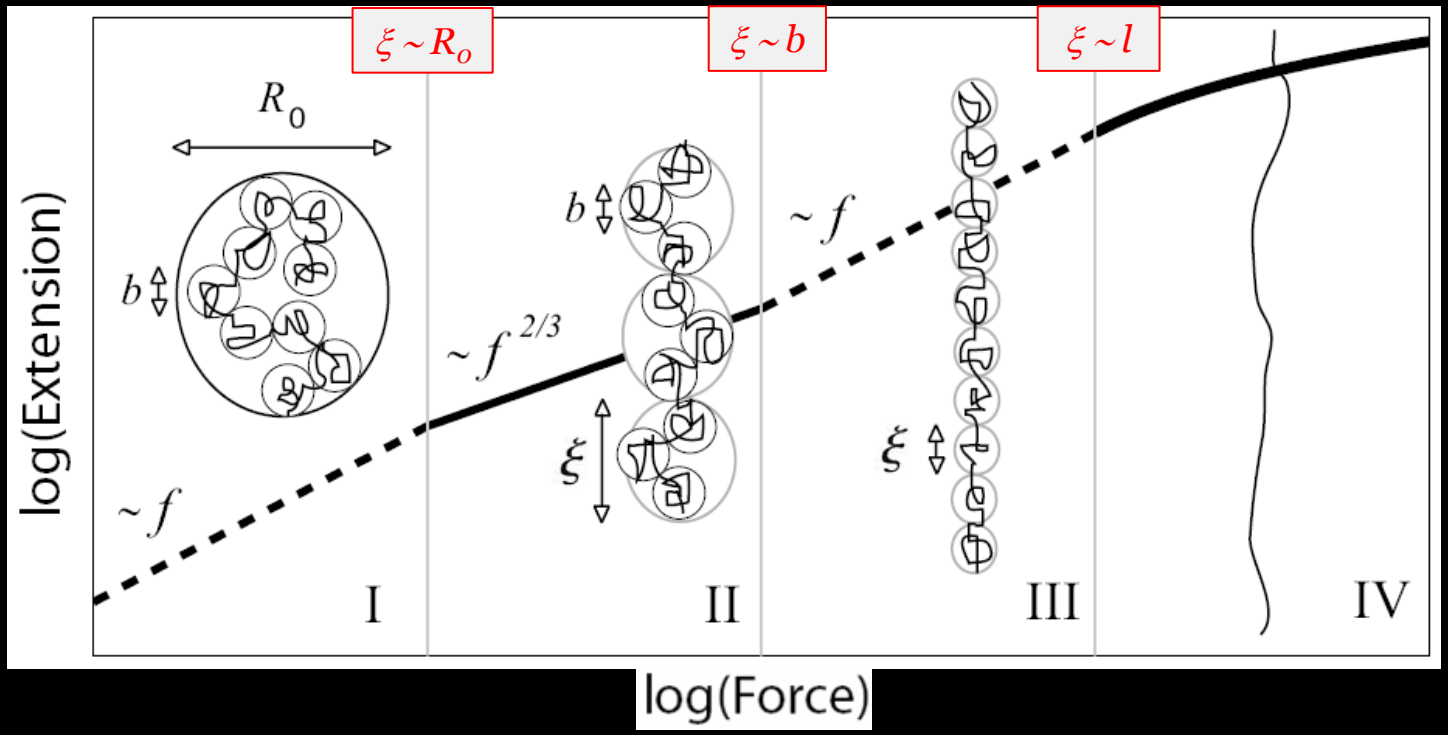
- Presence/lack of regime III consistent with aspect ratio of monomers

Thermal blob size: $b \sim l^4/v$

If the statistical monomers are spherical:

$v \sim l^3 \Rightarrow b_{spherical} \sim l$

So $\xi \sim b$ coincides with $\xi \sim l$, and the ideal-coil regime (III) disappears



Polymers with spherical monomers

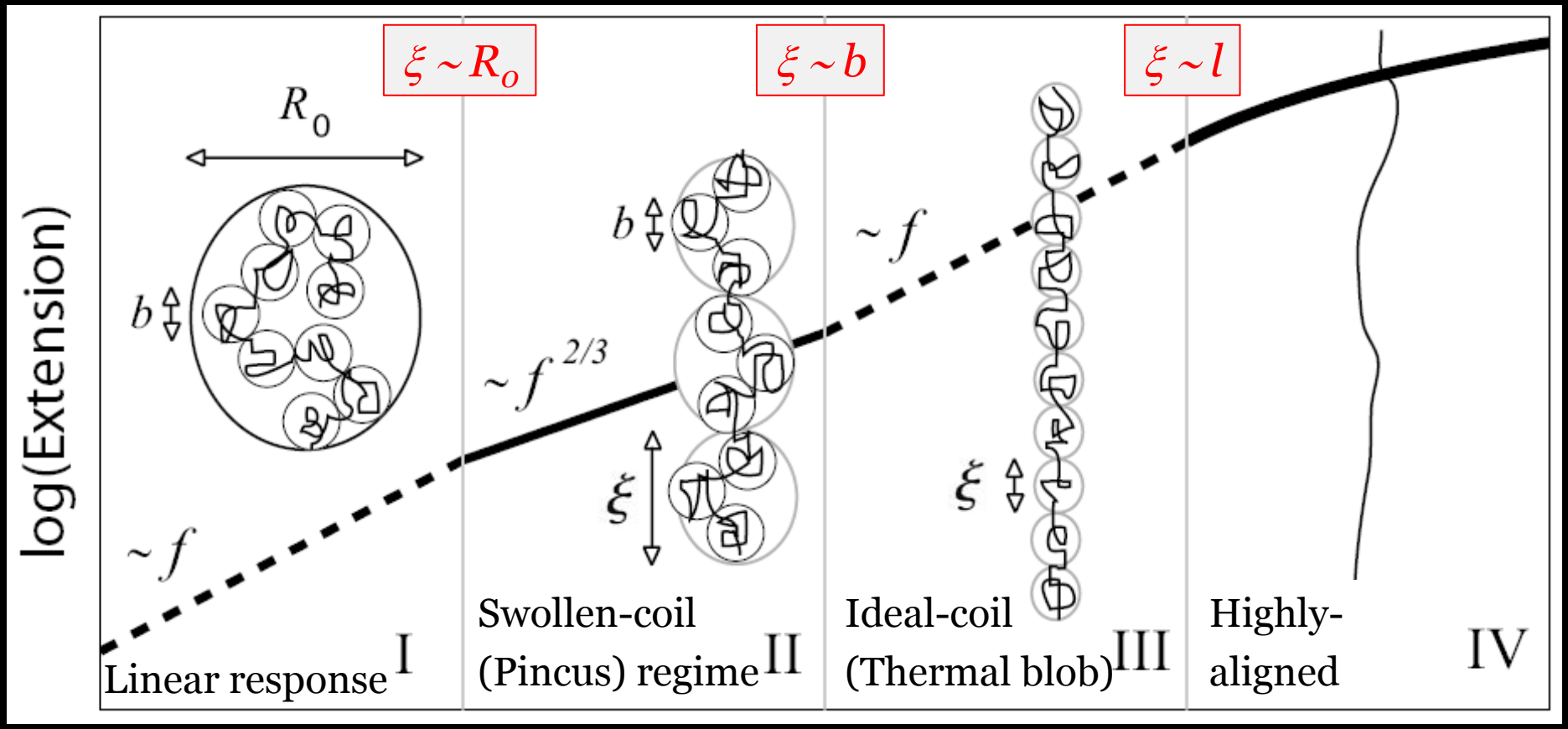
Mixed-base ssDNA: dominated by (isotropic) e-statics

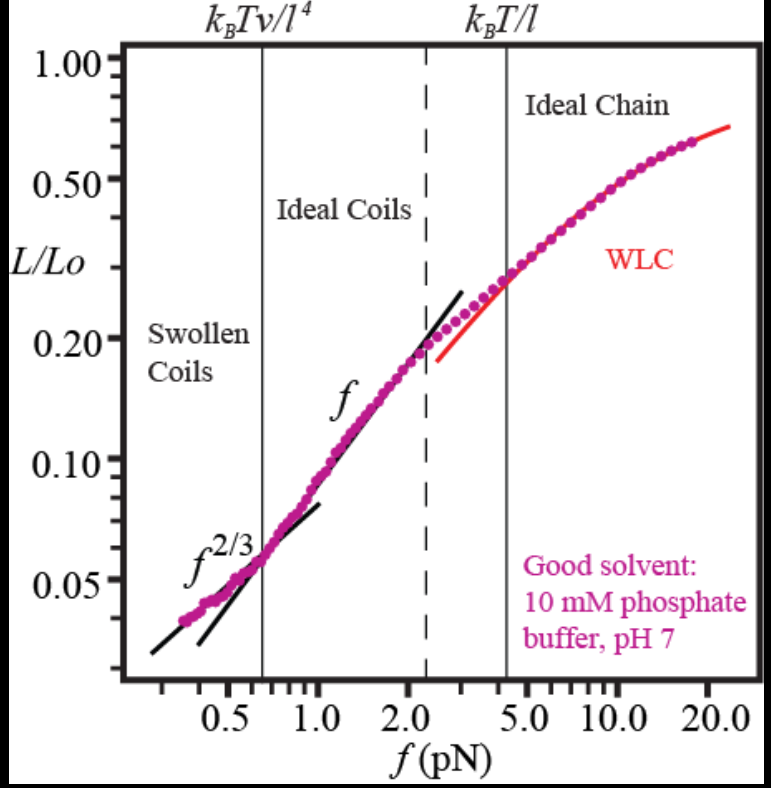
Polymers with rod-like monomers

PEG: dominated by n.n. interactions (bond rotations)

Part 2: Electrostatics

Char. length scales lead to elastic transitions,
□ Measured transitions give estimates of length scales

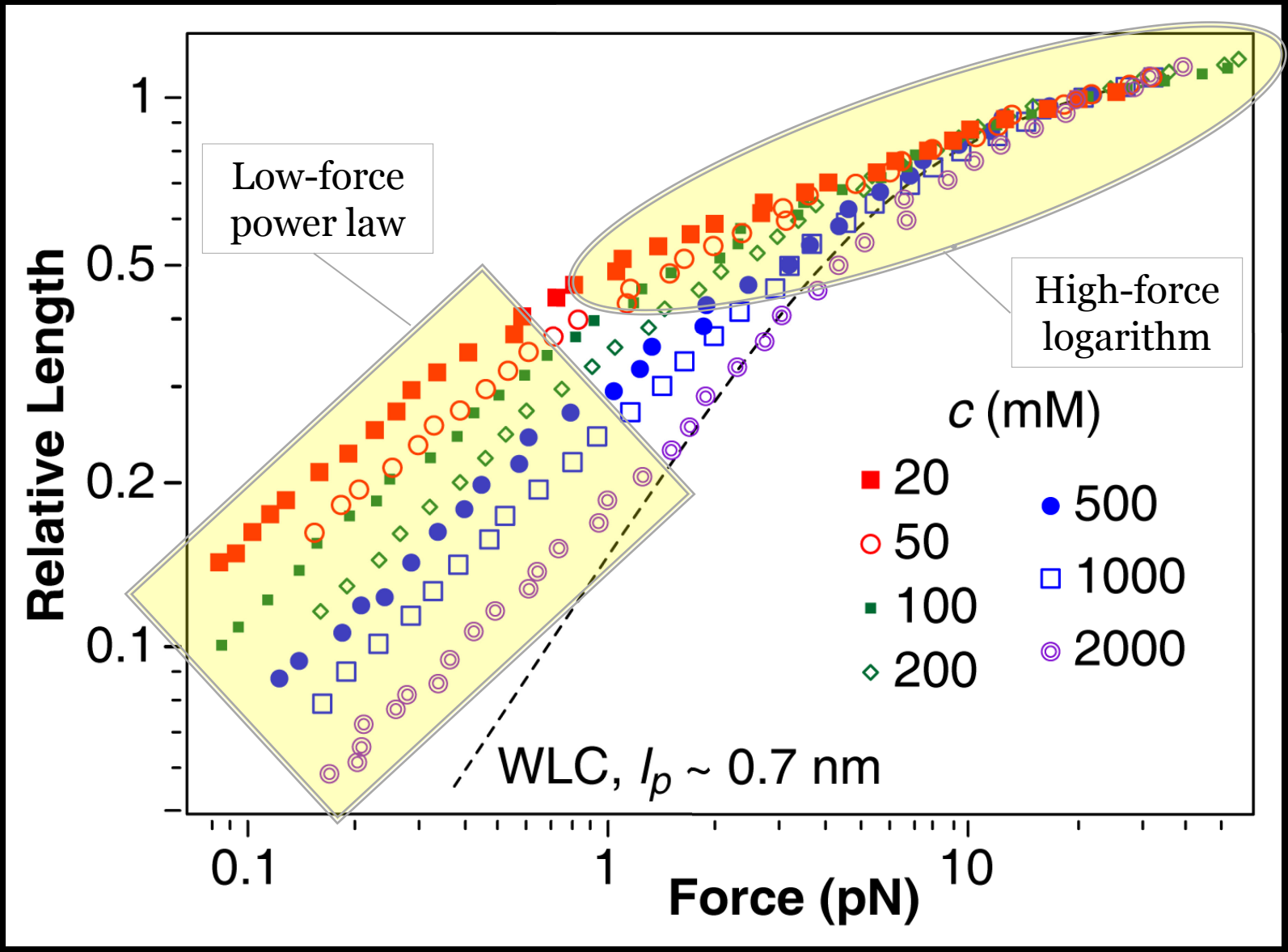




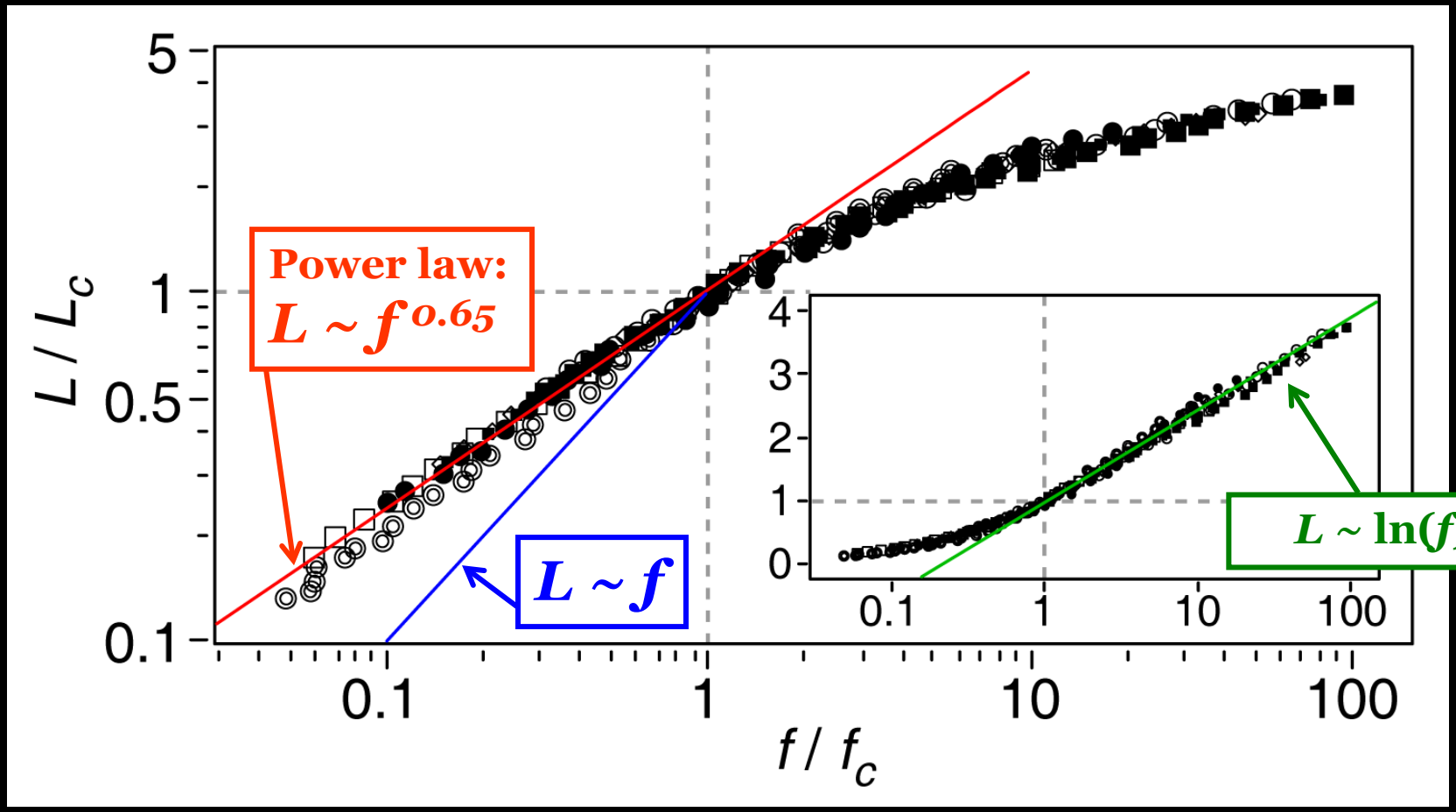
Many parameters from one f/ext curve:
PEG in 25°C water

Contour length (per monomer)	Persistence length	Thermal blob size	Kuhn length	Excluded volume
$L = 0.31$ nm	$p = 0.47$ nm	$b = 0.6$ nm	$l = 1.1$ nm	$v = 0.2$ nm^3
From high-force fit to the Marko-Siggia wormlike chain model		From $b \sim k_BT / f^*$	From linear elastic regime slope	From $v \sim l^4/b$

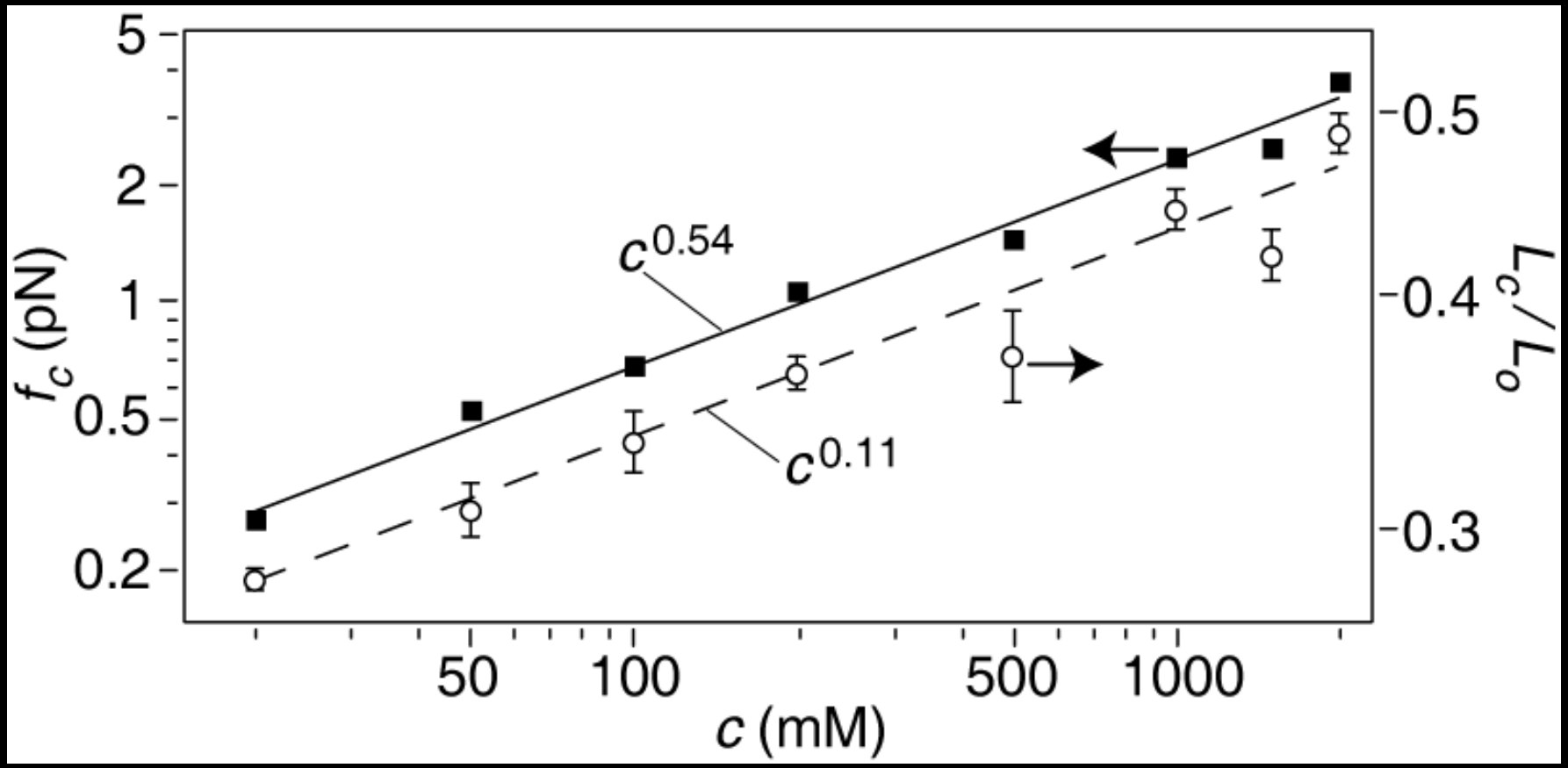
Force/extension behavior of ssDNA at various [Na]



Re-scaled by f_c , L_c: Universal behavior
Scaling picture: $l([\mathrm{NaCl}]) \sim kT/f_c$



Crossover force/length gives scaling of *l* with salt



$$l_e \sim c^{-0.51 \pm 0.04} \sim \kappa^{-1}$$

Some history

$$l = l_o + l_e(c)$$

Intrinsic

Electrostatic

Behavior	Theory	Experiment
$l_e \sim \kappa^{-2} \sim c^{-1}$	**OSF (1977):** $l_o >> \kappa^{-1}$ **KK (1982):** all l_o **BJ (1993):** $l_o >> \kappa^{-1}$ **Everaers *et al.* (2002)**: all l_o	**Baumann (1997)**: 1-mol, dsDNA ↑ Proper excl. vol. considerations
$l_e \sim \kappa^{-1} \sim c^{-0.5}$	**BJ (1993):** $l_o < \kappa^{-1}$ **Dobrynin (2005):** all l_o, torsionally-free	**Reed (1991)**: scattering, flexible chain **Beer (1997)** scattering, flexible chain **Tinland (1997)**: diffusion, ssDNA

Our work on ssDNA:

Unlike scattering, we rely on a simple scaling model that *directly accounts for excluded volume* (thus swelling cannot rectify the discrepancy with OSF)

Our current conclusion: either

1) OSF does not apply to flexible polymers
2) Our assumption of $f_c \sim kT/l$ is wrong

So far: only up to [NaCl] = 2 M…
What happens at higher salt?

Expectation:

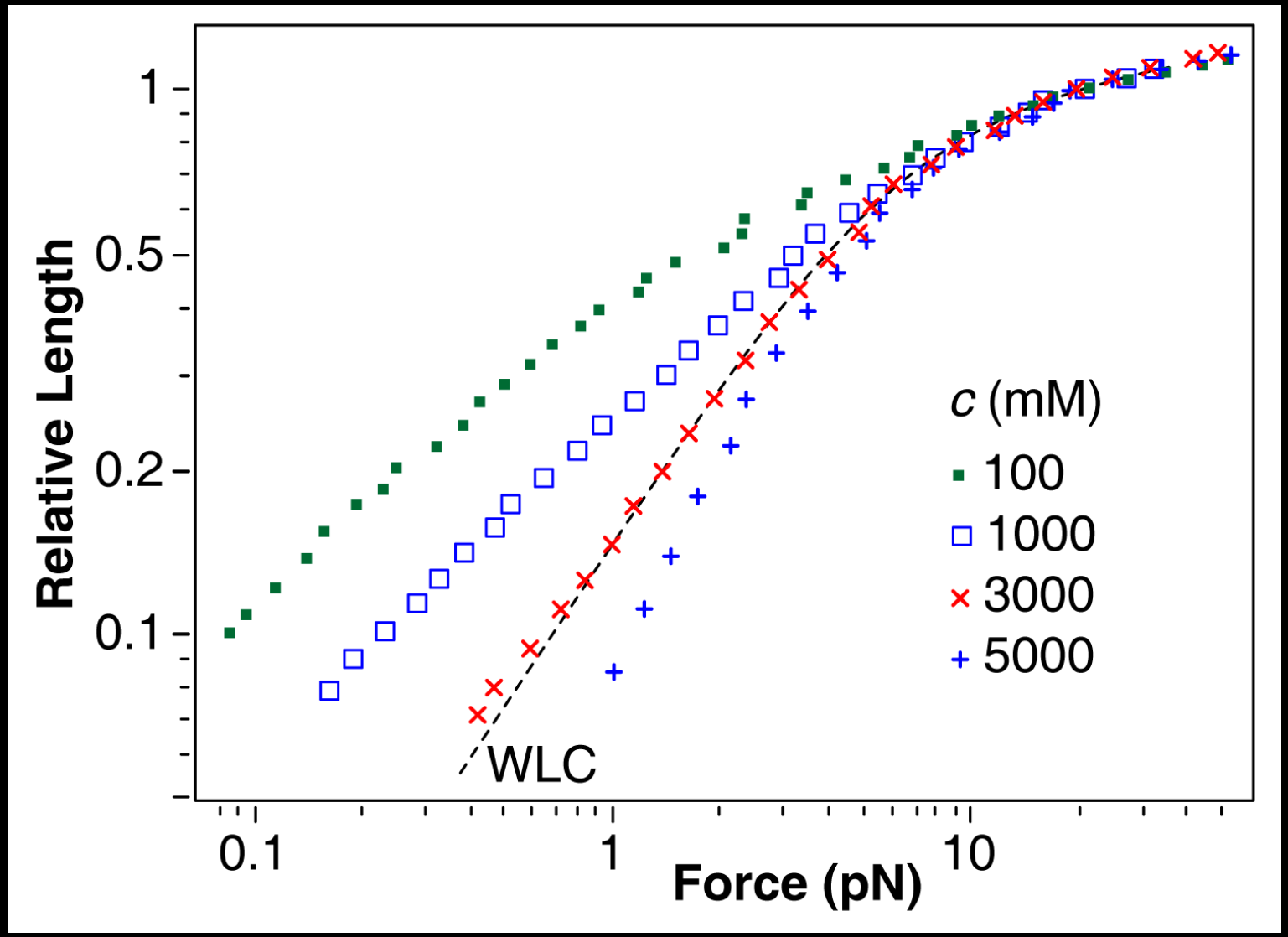
At high salt, monomer repulsions/attractions cancel, leading to a theta condition **($v = 0$)**

Ideal and real behavior are easily distinguished:

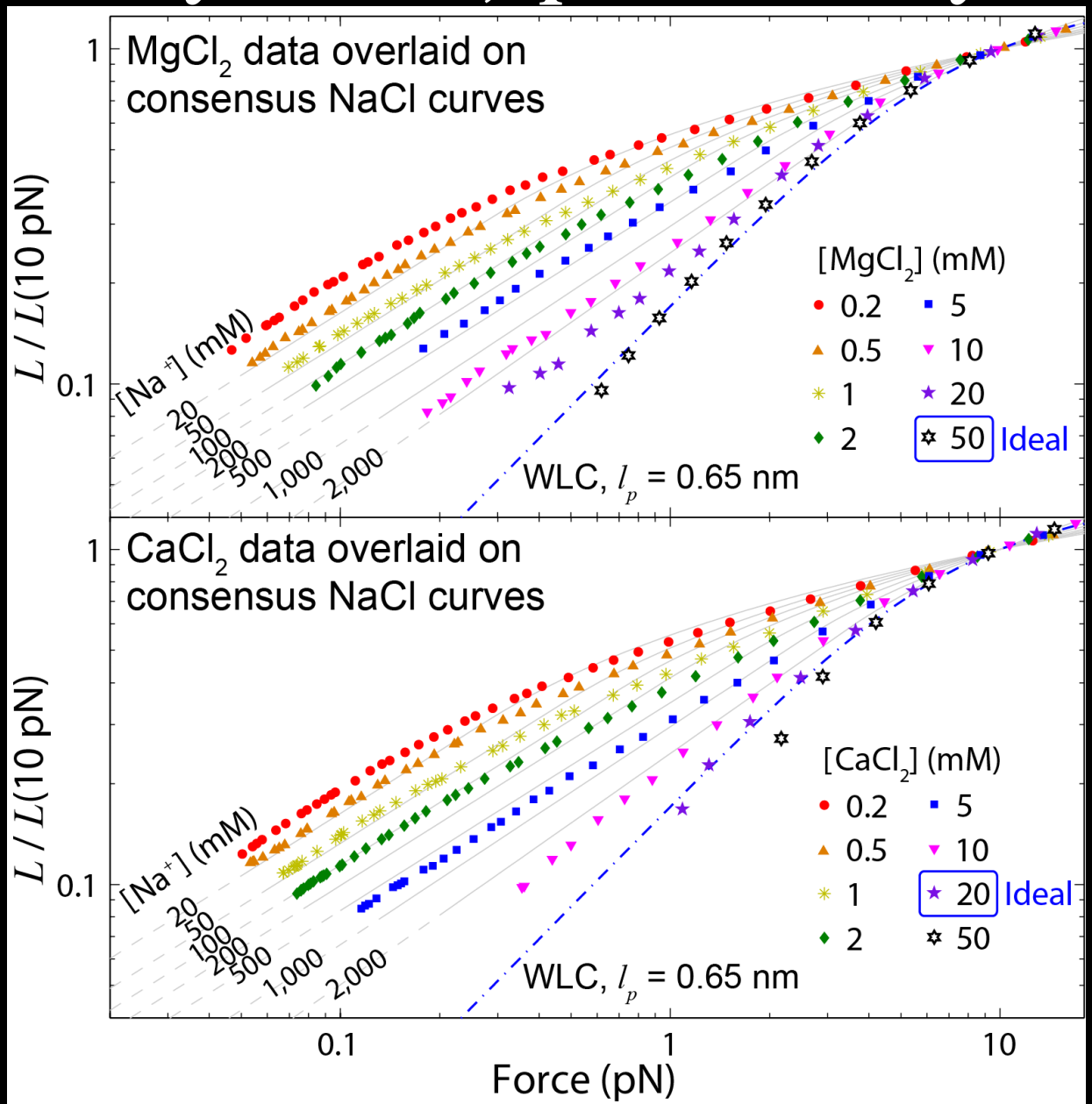
	Linear response ($\xi > R$)	Intermediate force ($R > \xi > b$)
Real	$L \sim f$	$L \sim f^{2/3}$
Ideal	$L \sim f$	$L \sim f$

Ideal f/ext models work at the θ-point:

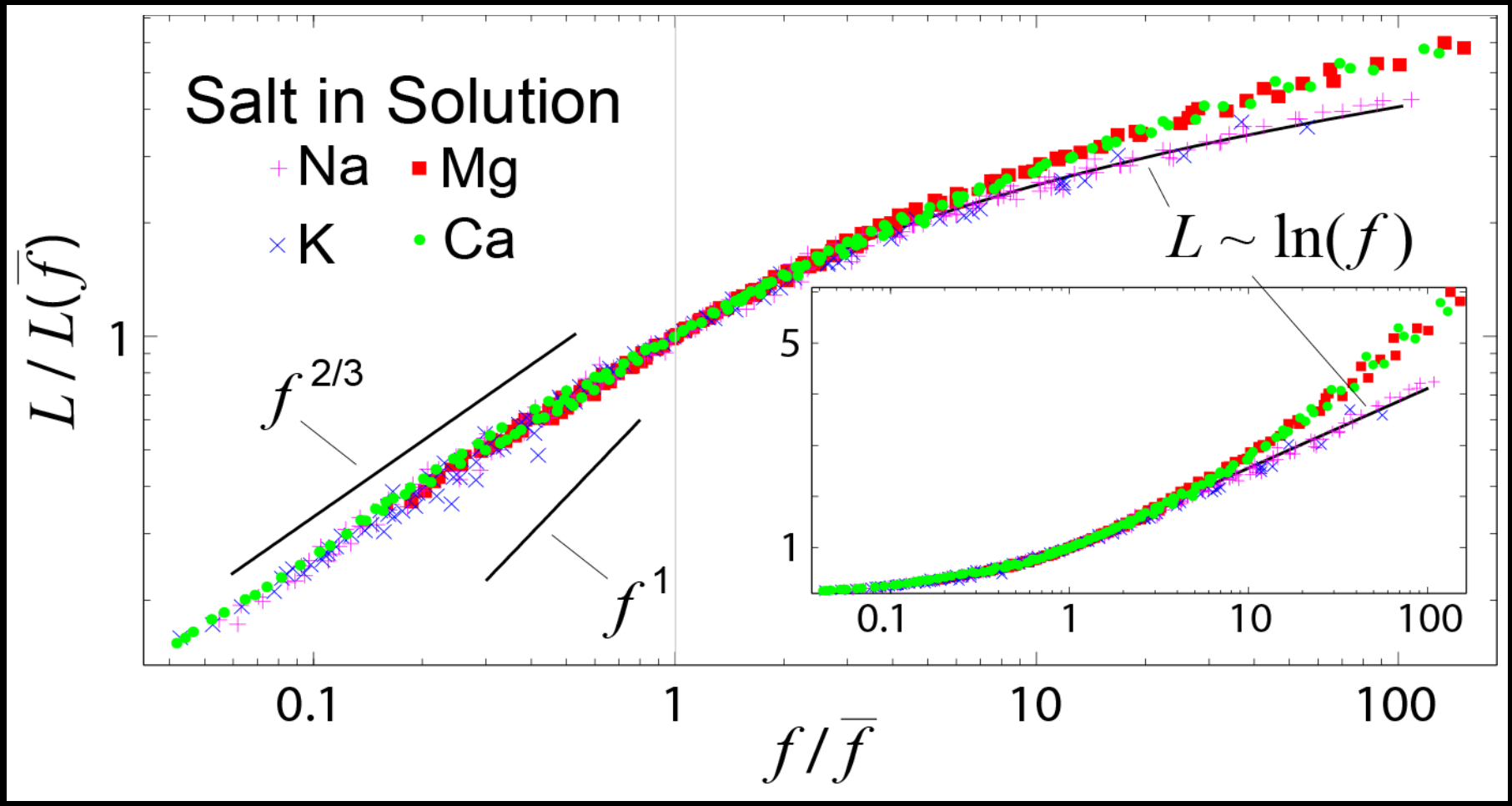
WLC model fits, with $l_{p,o}$ = 0.65 nm



Divalent cations:
Qualitatively similar, quantitatively different



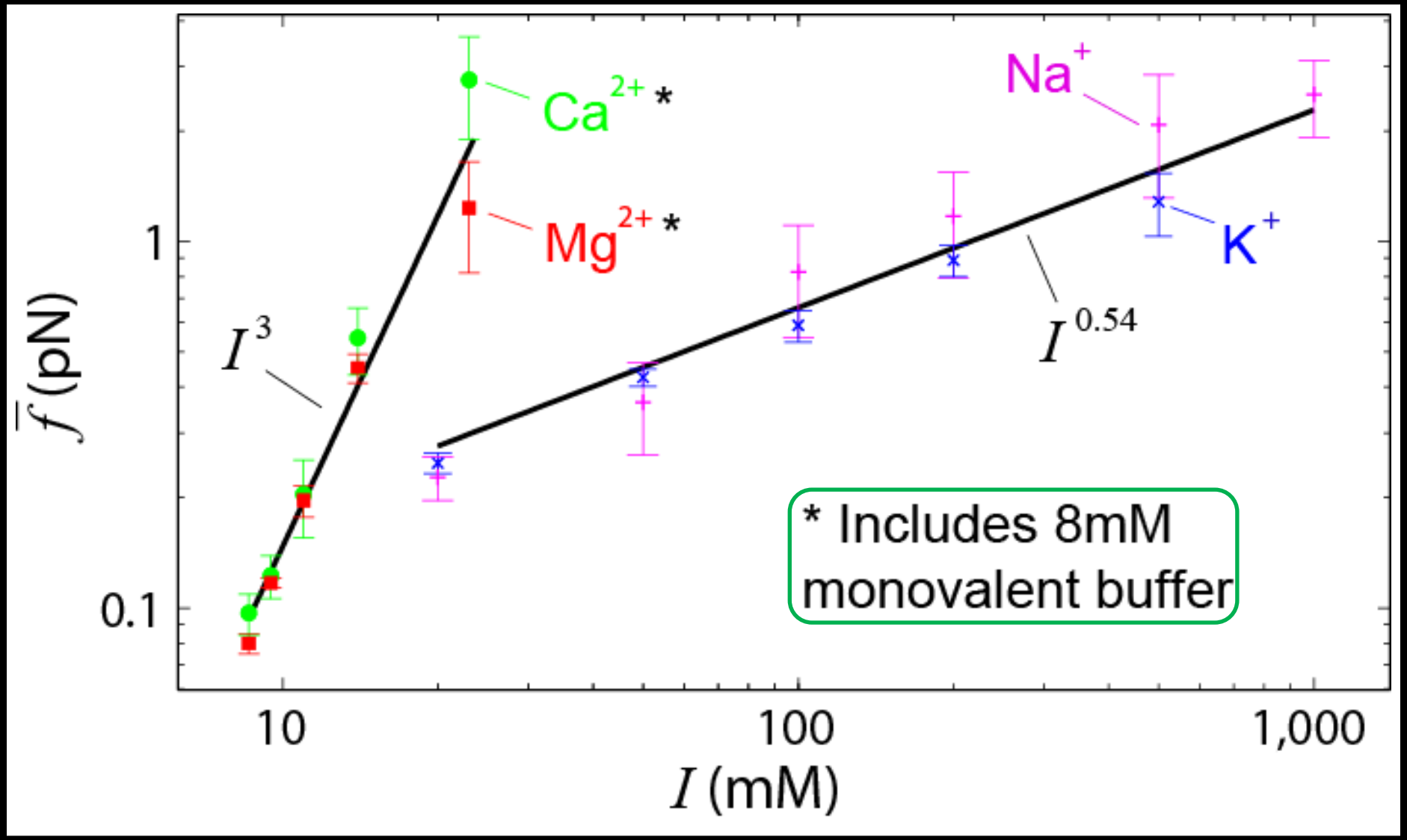
Upon rescaling:
Universality among, but not between, valencies



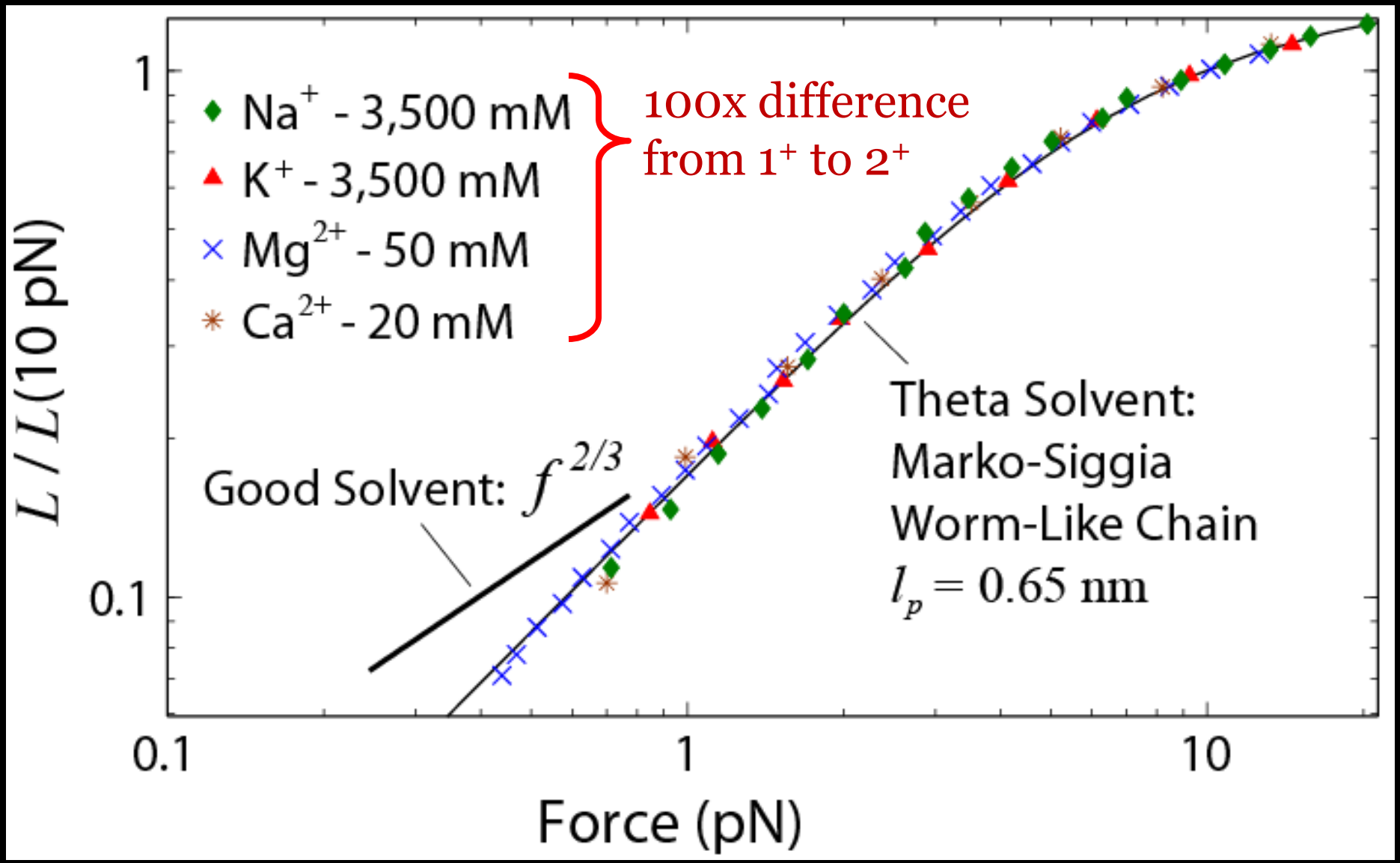
20-1,000 mM monovalent salts
0.2-5 mM divalent salts

$\bar{f}$: Transition force out of Pincus regime

ssDNA becomes flexible extraordinarily quickly in divalent salt

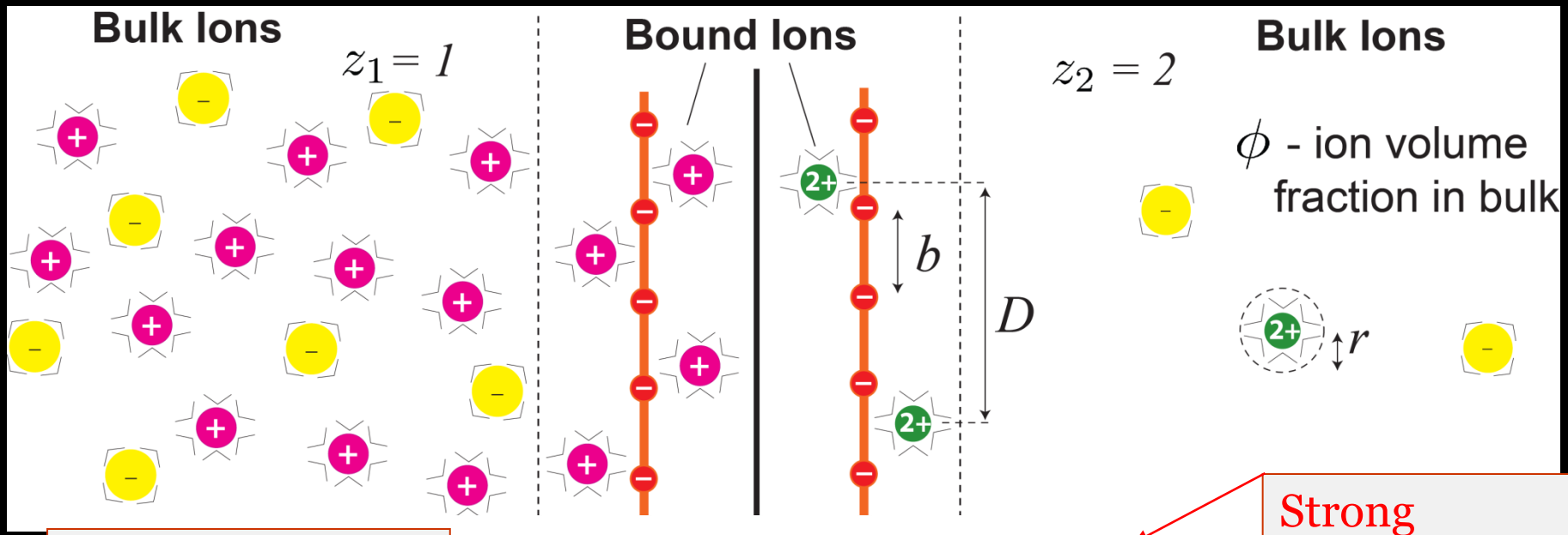


Theta point shifts 100-fold in divalents



Similar effects seen in RNA folding studies (Heilman-Miller *et al.*, 2001) and dsDNA SAXS studies (Qiu *et al.*, 2006, 2007)

Difference between 1^+ and 2^+ at Θ:
Hydrated, condensed ion thermodynamics



Weak dependence on salt conc. c

Strong dependence on ion valence

$$\underbrace{k_b T \ln(\phi)}_{\text{Ion entropy}} = -\underbrace{\frac{1}{4\pi\epsilon}\frac{zDe^2}{b\,r}}_{\text{electrostatics}}$$

The condition for constant charge density (w/ hydrated ions)

$$\longrightarrow c_1/c_2 \sim 50$$

Summary:
There's plenty of room at the bottom

PEG: rod-like monomers; identifiable t-blob regime

ssDNA: spherical monomers; no t-blobs

Monovalent salt: $l \sim I^{-1/2}$

Divalent salt: $l \sim I^{-3}$, Theta 100x lower

Open questions:

- OSF?
- ssDNA in monovalent at high forces: $L \sim \ln(F)$; why?
- ssDNA in divalent: $l \sim I^{-3}$; why?

Publications

Saleh et al., PRL (2009)

McIntosh et al., PRE (2009)

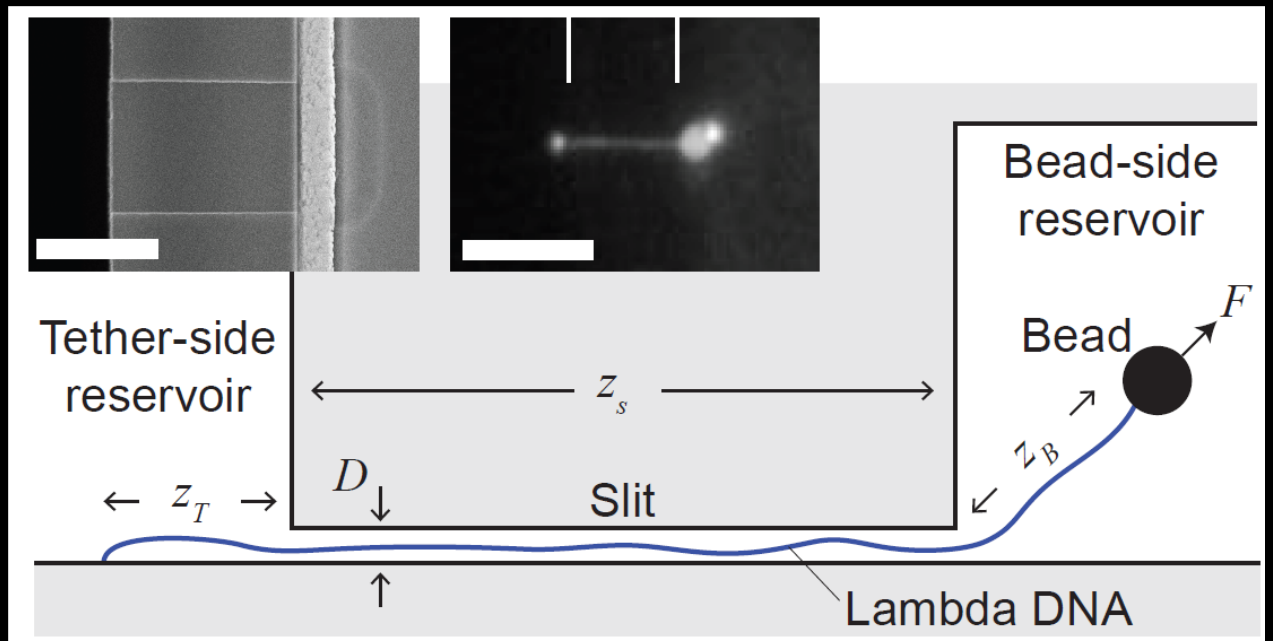
McIntosh et al., Macromolecules (2011)

Dittmore et al., In submission

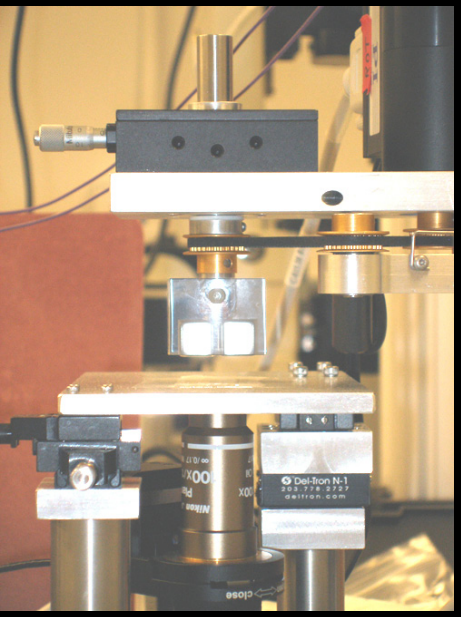
A quick plug: Conference presentations

Dustin McIntosh will present aspects of this work, with more on poly(dA)

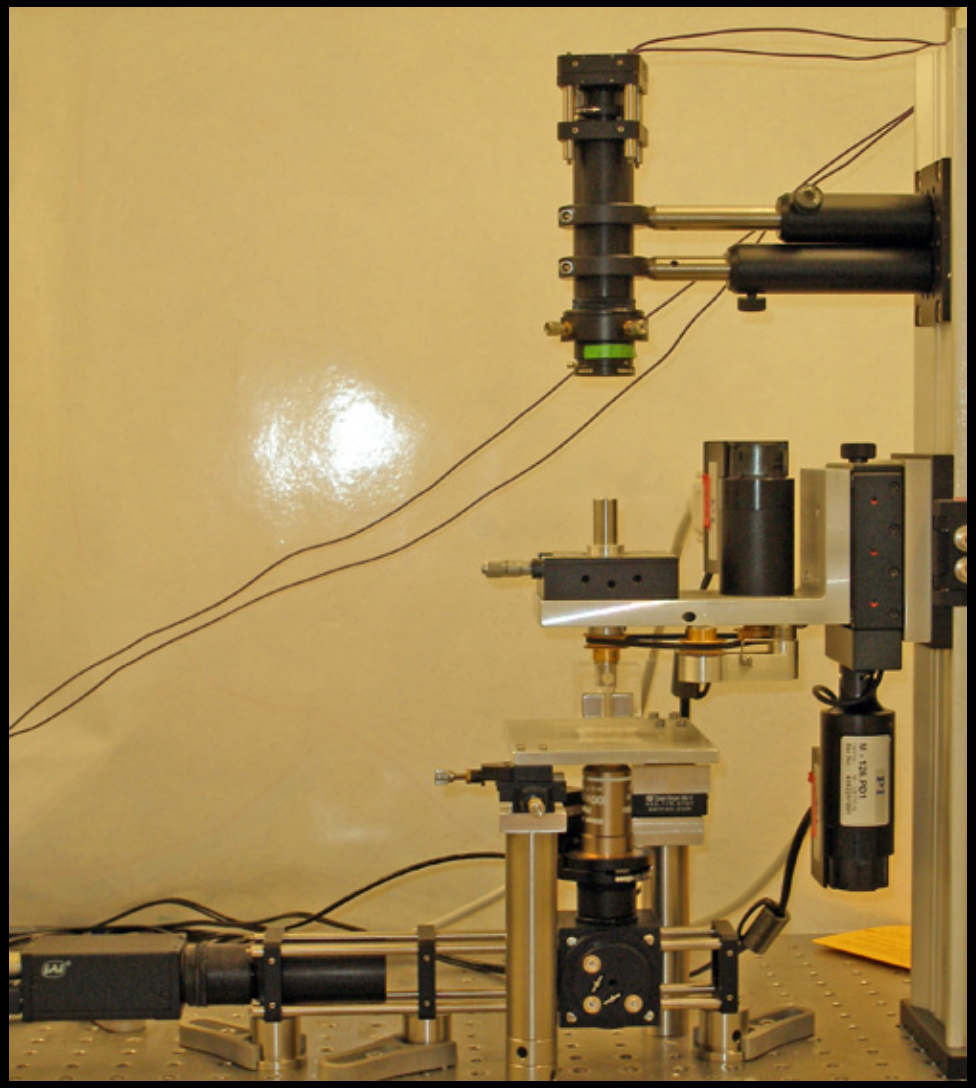
Jun Lin will present work on stretching dsDNA confined in nanochannels

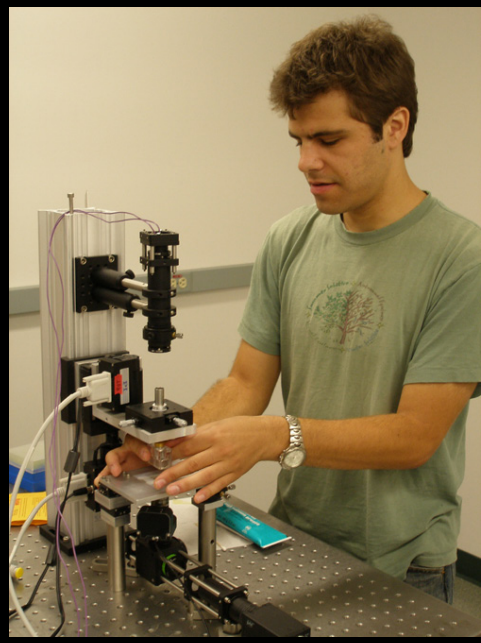


An actual device



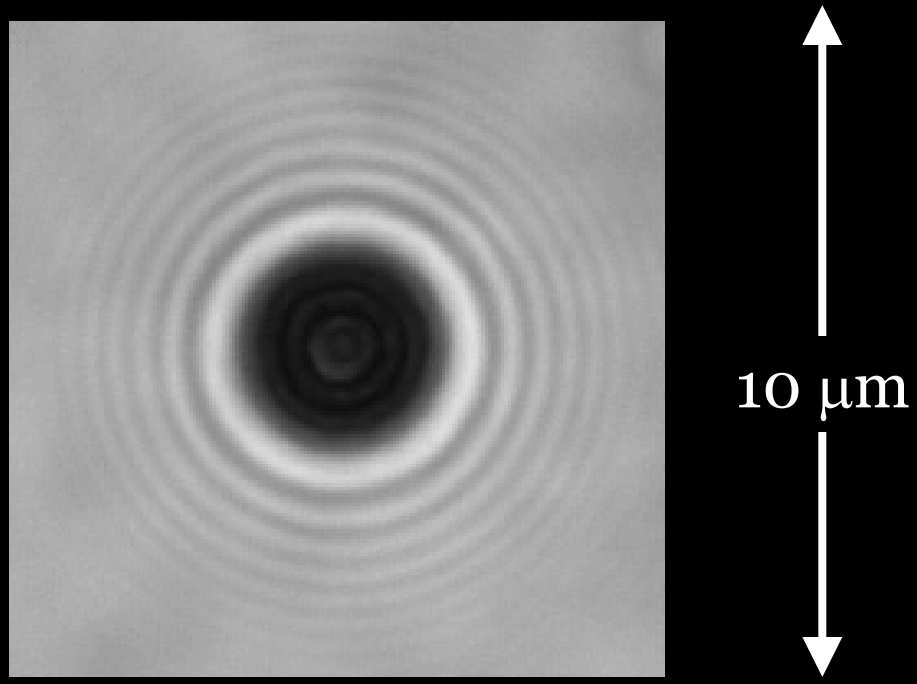
Close-up of the stage





An actual student doing work

Image analysis gives 3D Bead position with ~ 1 nm resolution in real-time at 60 Hz



Lateral *(x,y)* position:

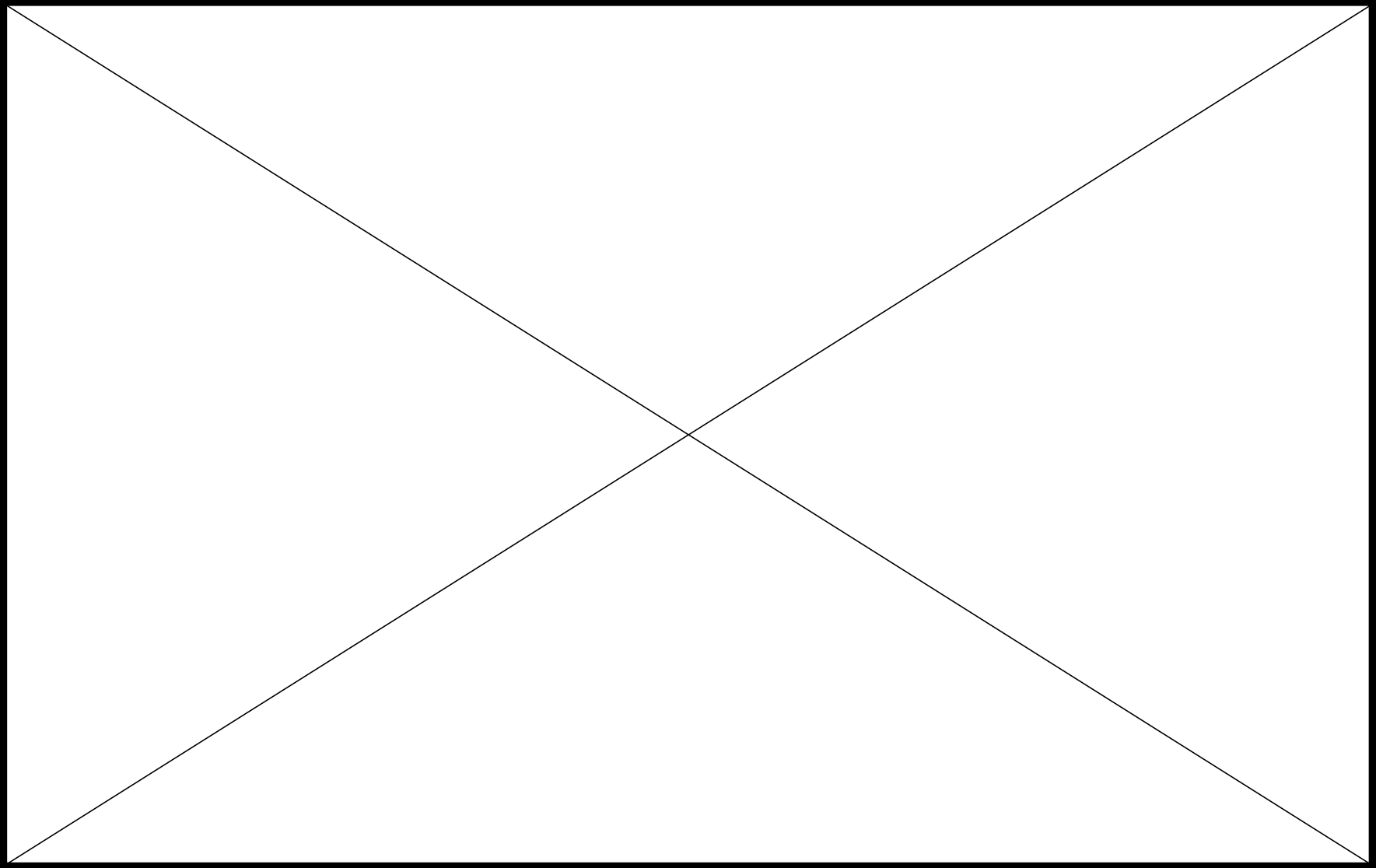
Found from FFT-based correlation algorithm

Vertical (*z*) position:

Found by analysis of diffraction rings

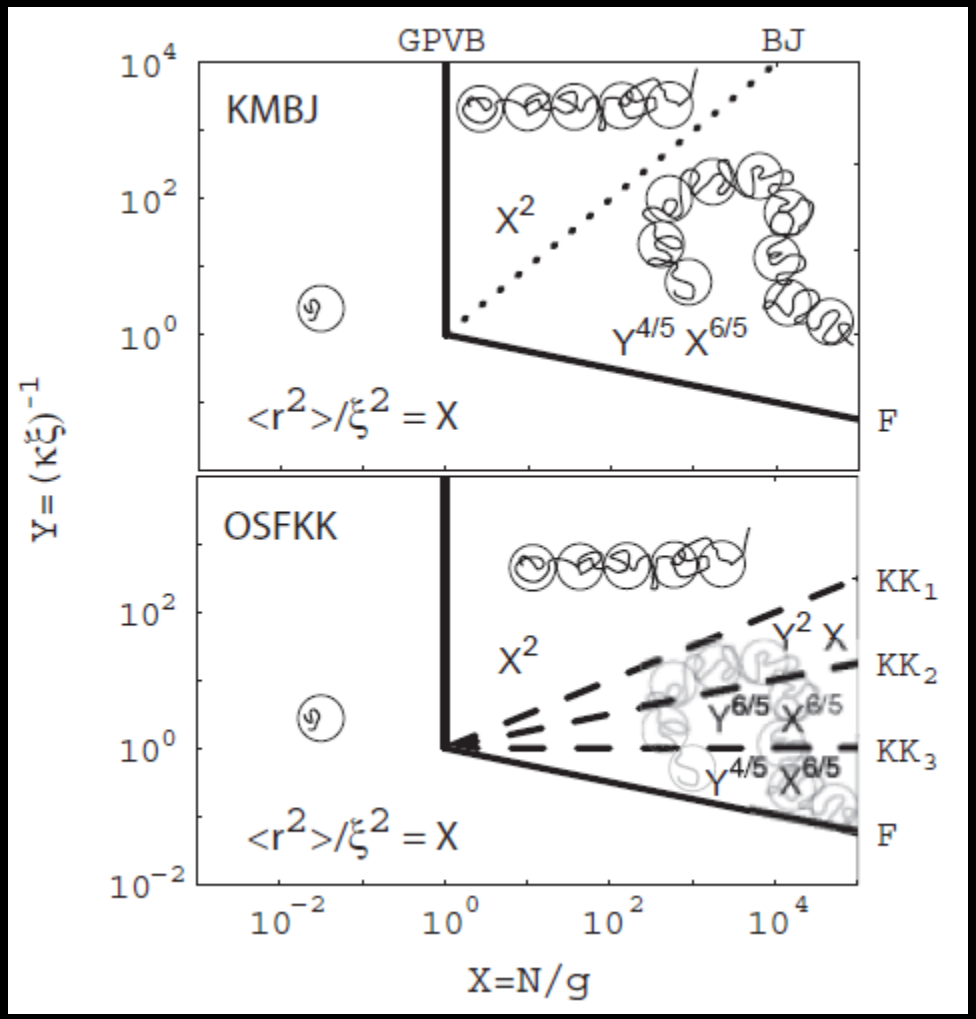
Gosse and Croquette, *RSI* (2002)

Measuring the force using fluctuations



Animation by G. Charvin

Fig. 1 from Everaers *et al.* (2002)



Normalized Debye Length

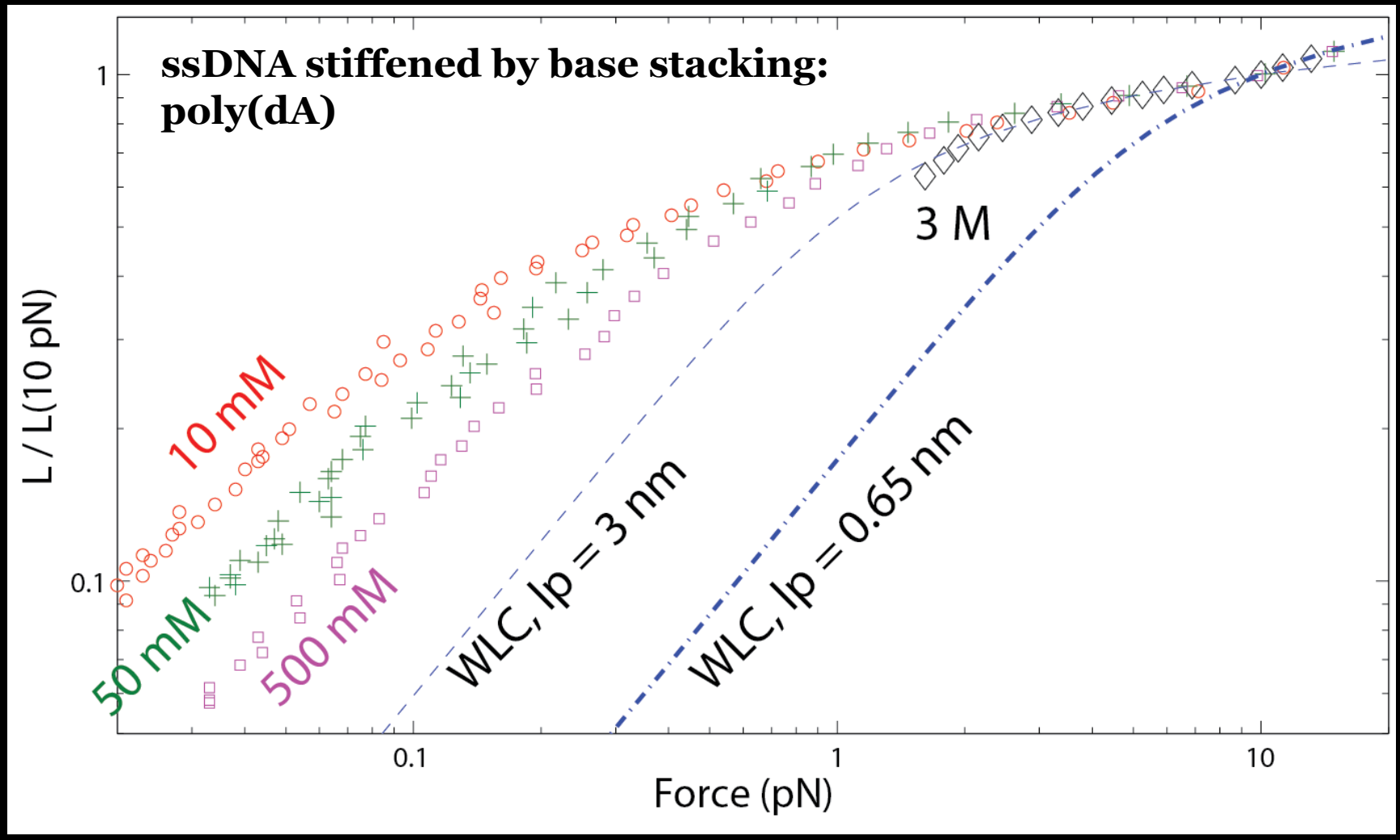
High salt

Low salt

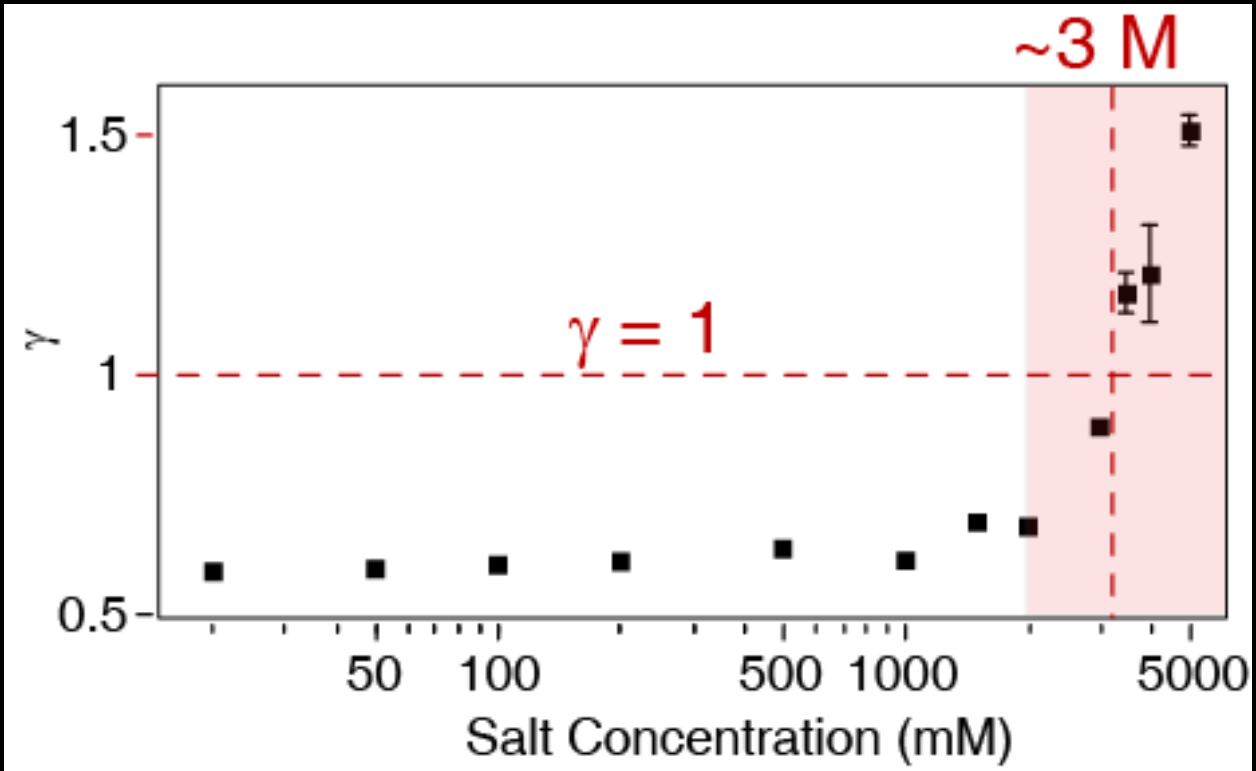
Normalized polymer length

High force

Low force


ssDNA stiffened by base stacking:
poly(dA)
1
0.1
L / L(10 pN)
10 mM
50 mM
500 mM
3 M
WLC, lp = 3 nm
WLC, lp = 0.65 nm
0.1
1
10
Force (pN)

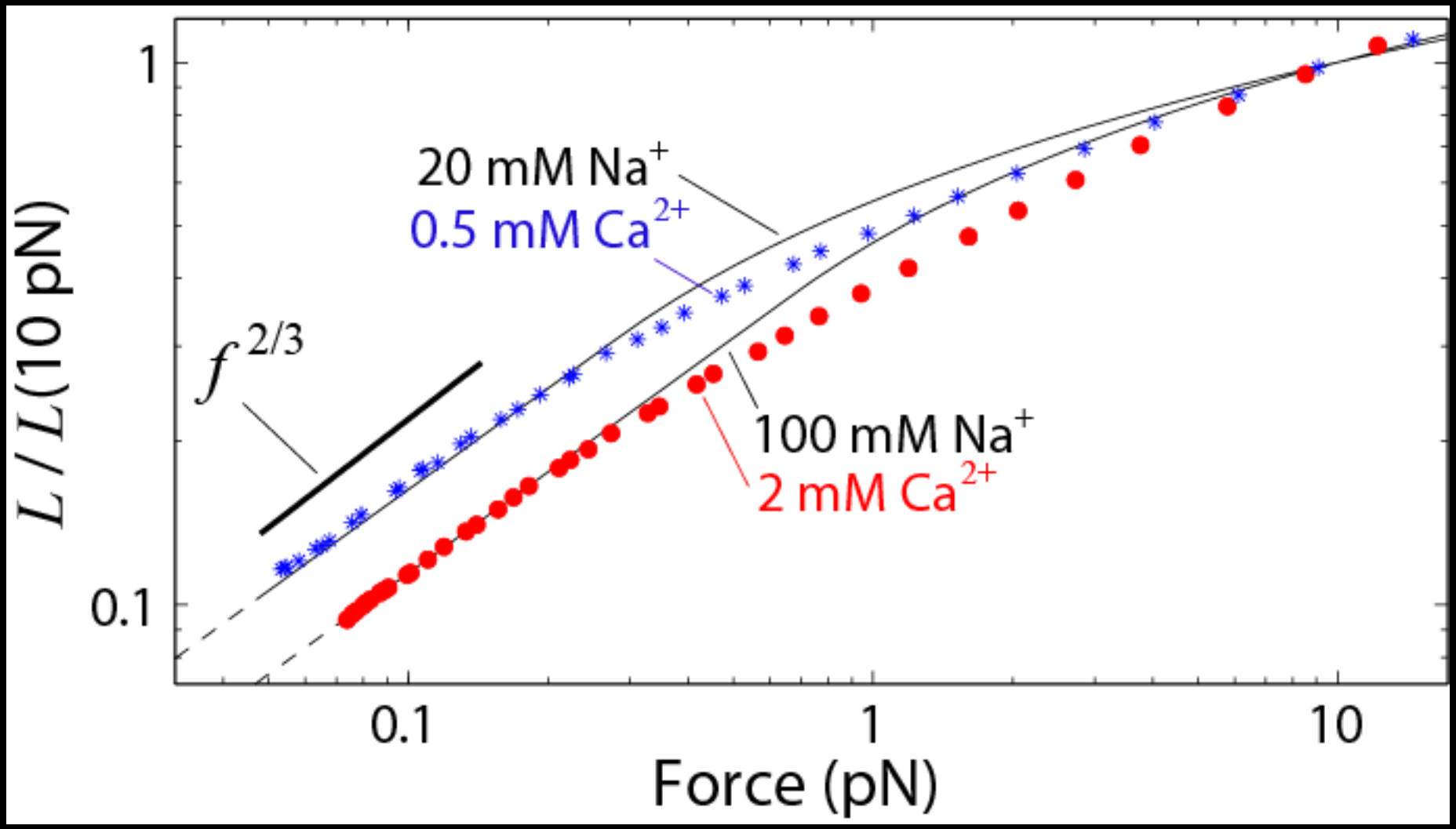
The low-force power-law becomes linear at [NaCl] ≈ 3 M



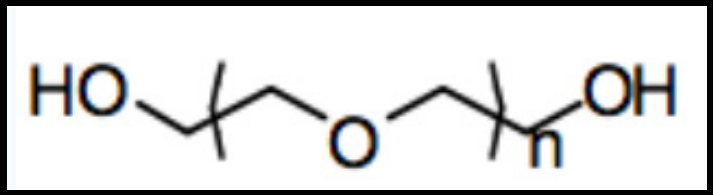
Beyond 3 M: $\gamma > 1$, corresponding to aggregation:

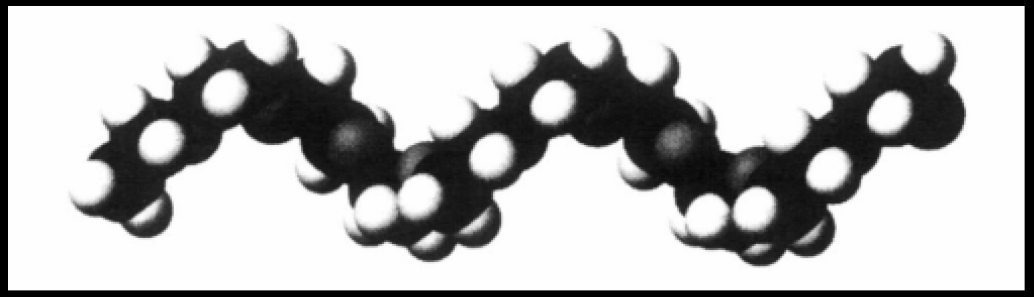
In poor solvent, $R \sim N^{1/3}$, giving $L \sim f^2$; but surface tension will cause $\gamma > 2$ (Morrison *et al.*, 2007)

Much smaller concentrations of divalent salt give similar elasticity



PEG structure





Proposed helical structure in water