1. Biopolymers under tension

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Tell me to talk slower!

Ask questions (no break until I get enough questions)!

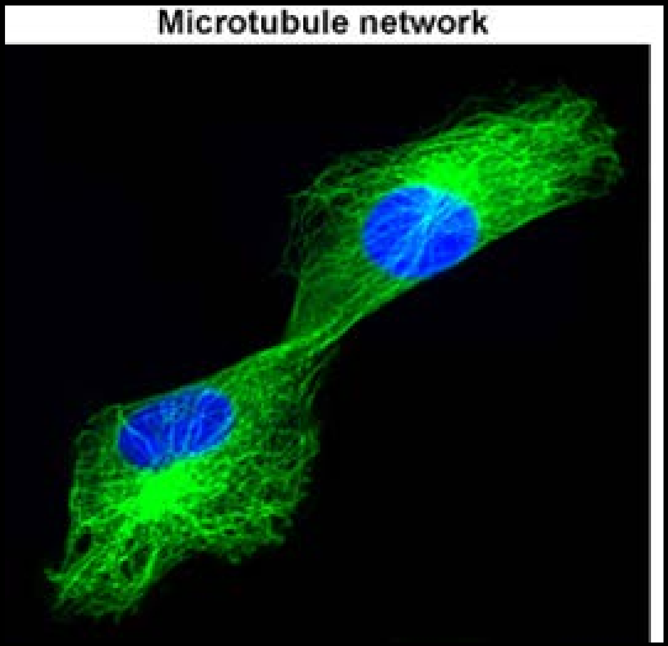
Why do we care about biopolymers under tension?

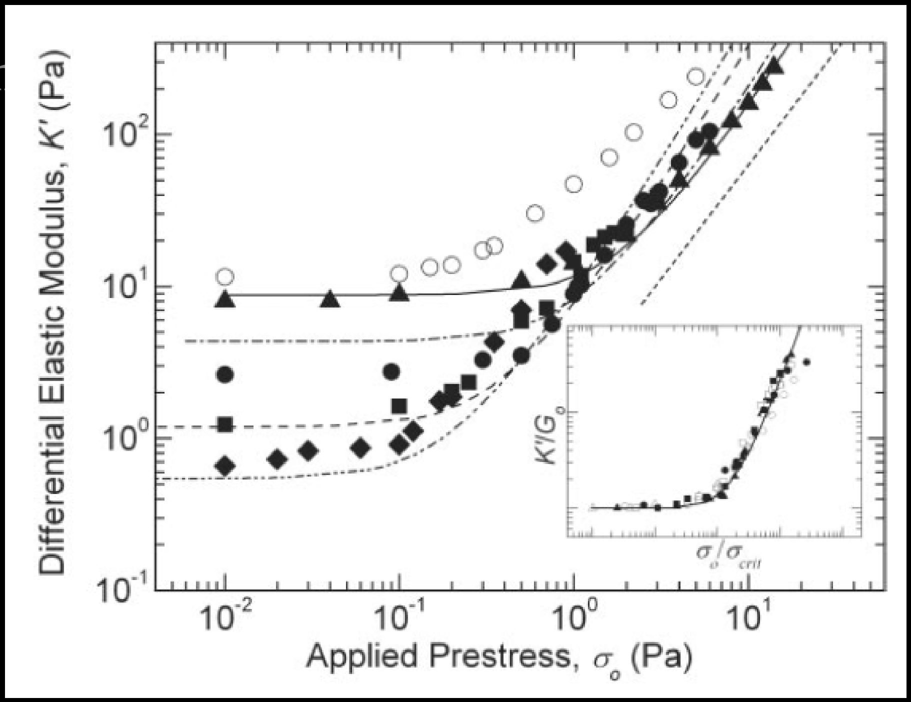
Biopolymer mechanics determines…

1. …polymeric material mechanics
2. …polymer film (brush) structure
3. …polymer relaxation

…and can be used to quantify…

1. …polymer structure
2. …polymer/ligand (e.g. DNA/protein
   interactions
1. …forces generated by cells





Gardel *et al.*, 2004

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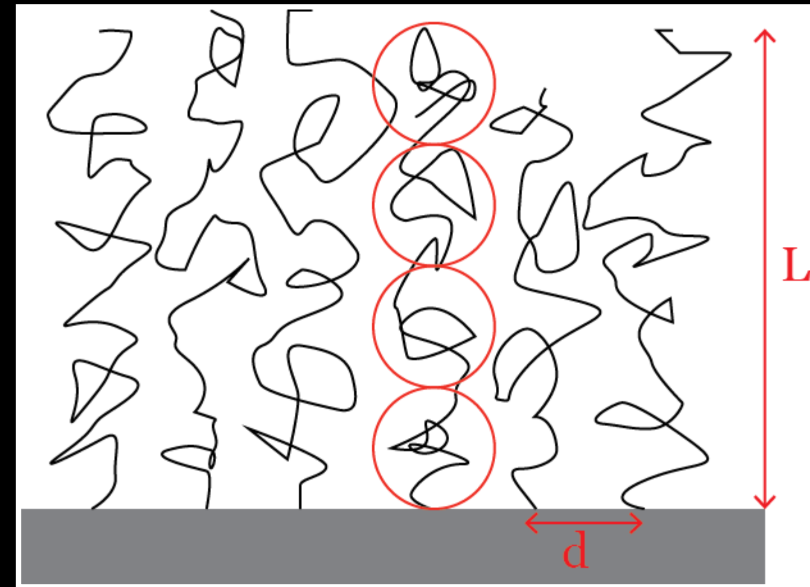

…and can be used to quantify…

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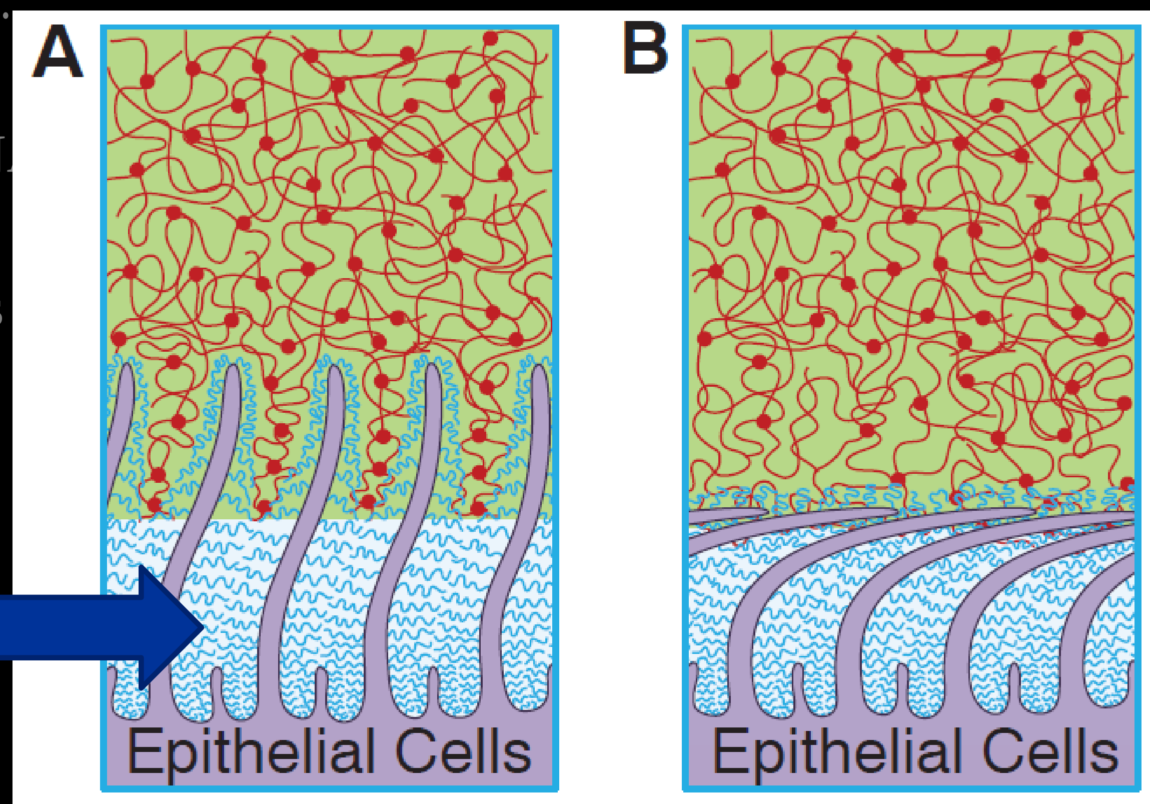

A polysaccharide brush surrounding cilia controls mucus clearance
Button *et al.*, 2012

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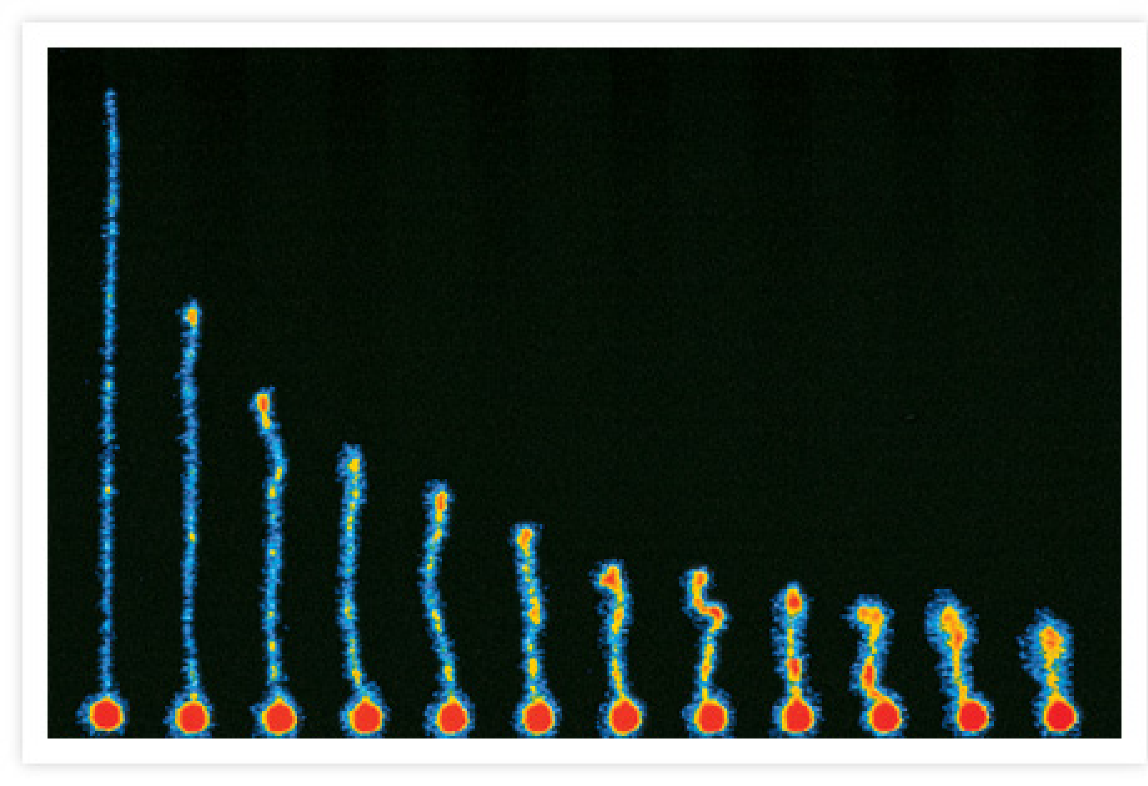

Perkins *et al.*, 1994

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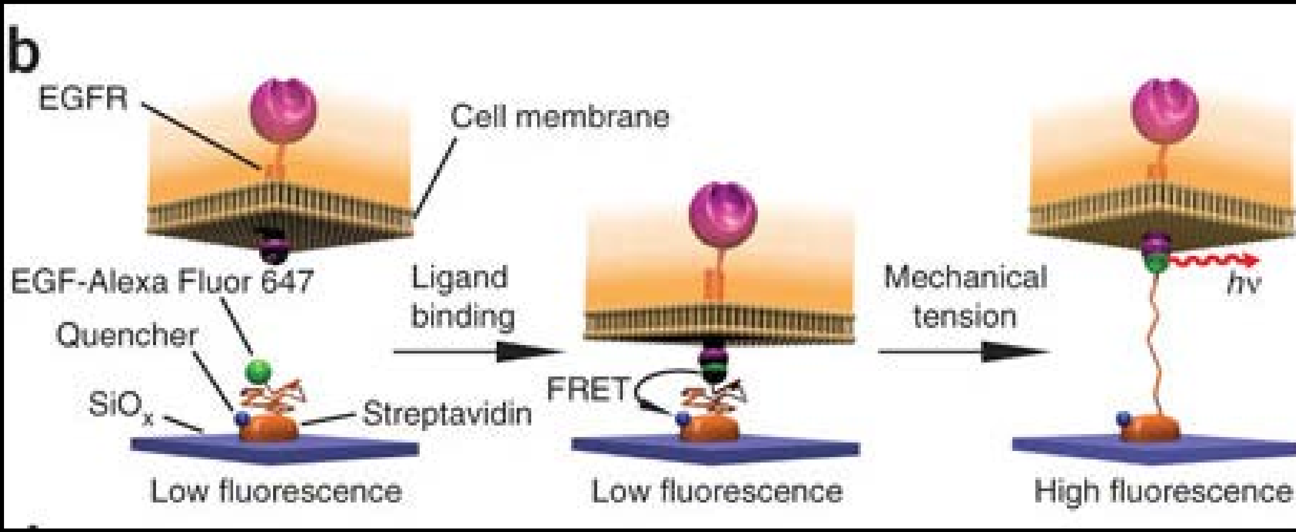
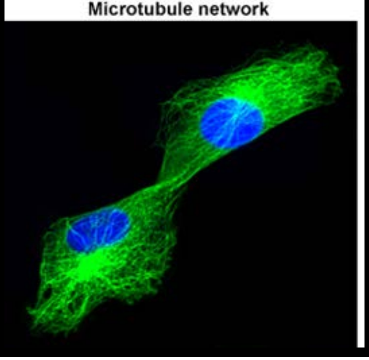
Stabley *et al.*, 2012



Why do we care about biopolymers under tension?



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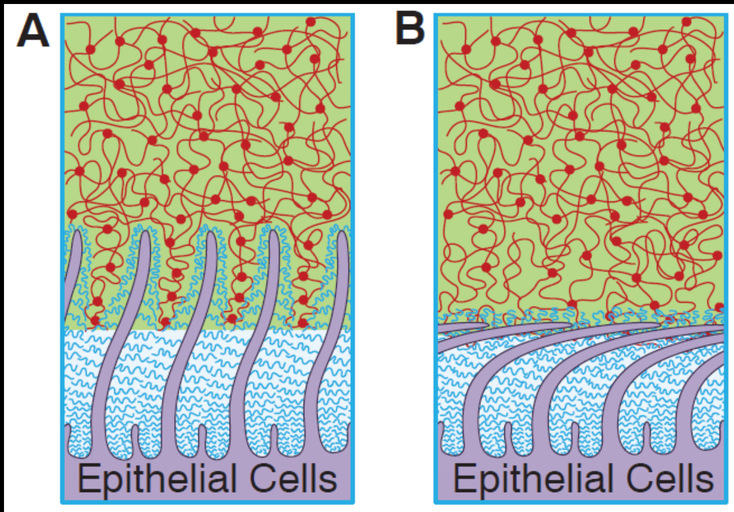

Button *et al.*, 2012

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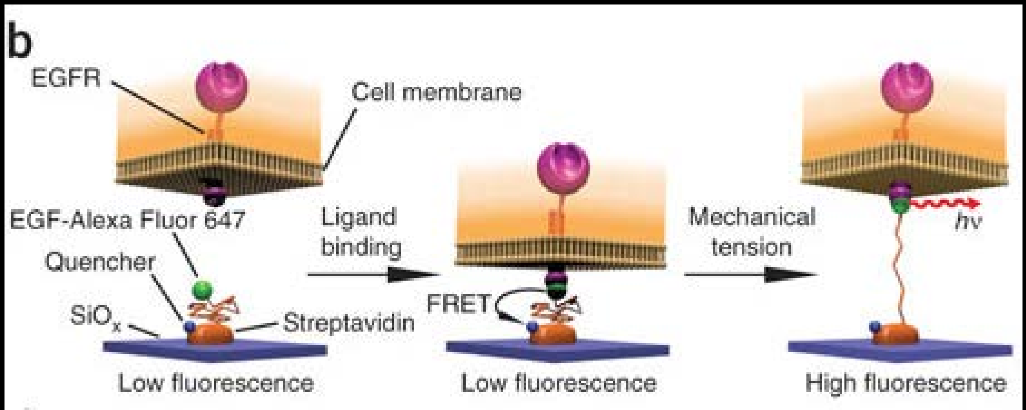
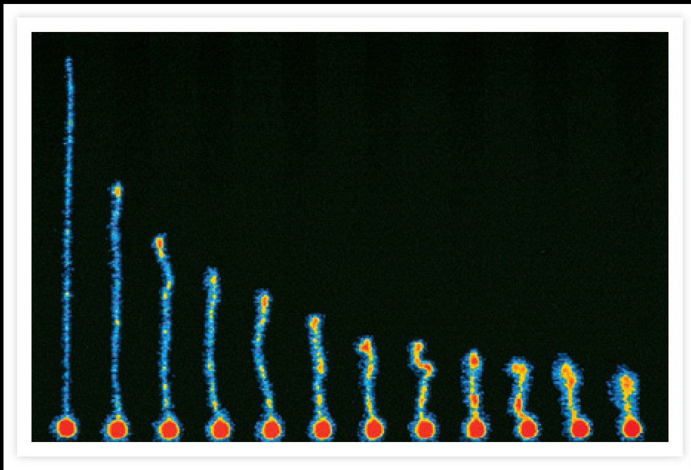
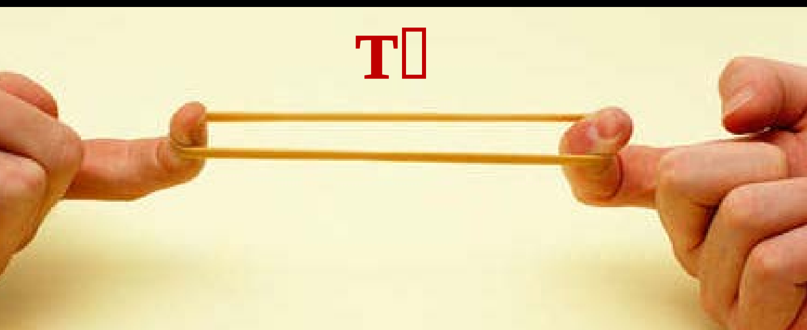
Perkins *et al.*, 1994

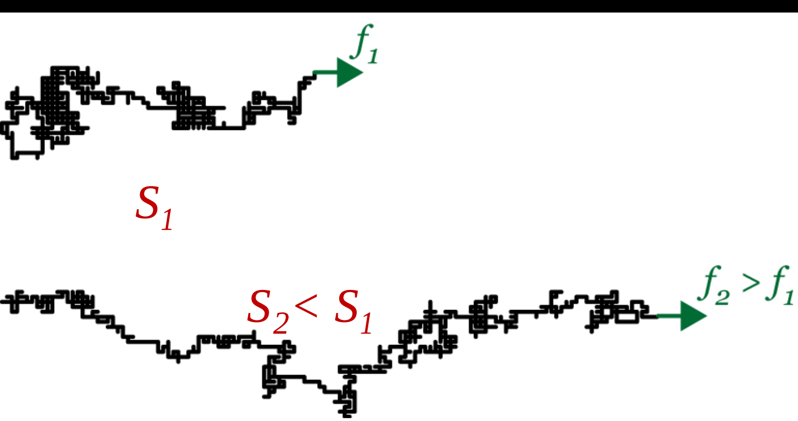
Stabley *et al.*, 2012





Stretching polymers: An old problem!
(the oldest biopolymer problem?)
□ Rubber elasticity





Gough (1805), Joule (1859):

Rubber heats when stretched

Kelvin (1857):

This has to do with entropy changes upon stretching

Staudinger (1920s):

Rubber is made of long chains

Meyer, Susich & Valko (1932):

Long chains lose entropy when stretched

Exact calculations of force/extension models for ideal chains (to the board!)

1. Linear response
2. FJC
3. WLC

Review of polymer elasticity models:
Saleh, JCP (2015)

Indirect experimental support for the FJC

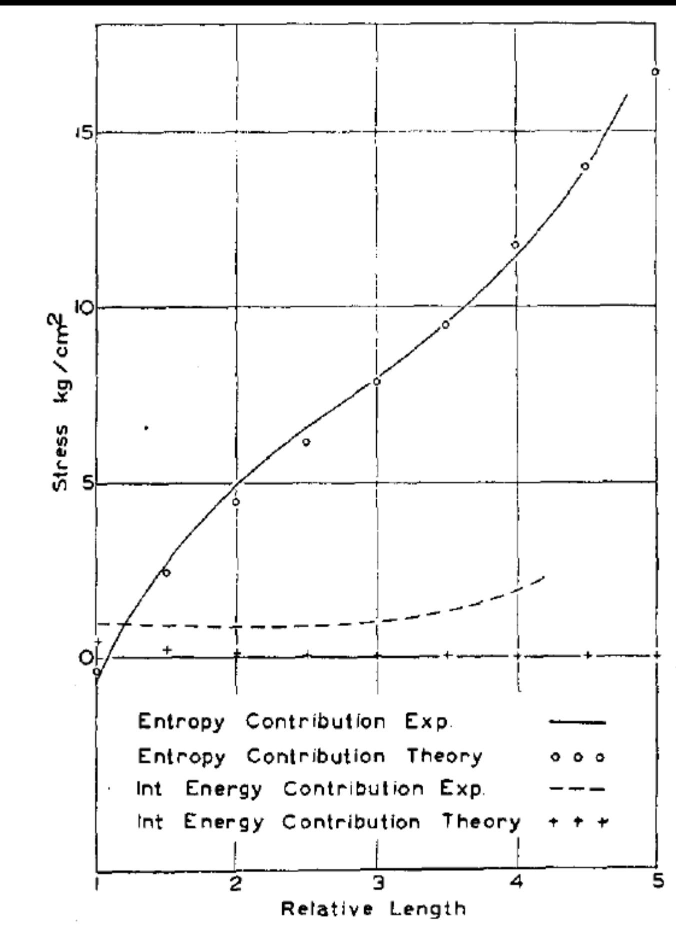


FIG. 3. Comparison of theory and experiment for natural rubber A.

James and Guth, 1943

Guth and Mark (1934); Kuhn (1936):

The Entropic spring:

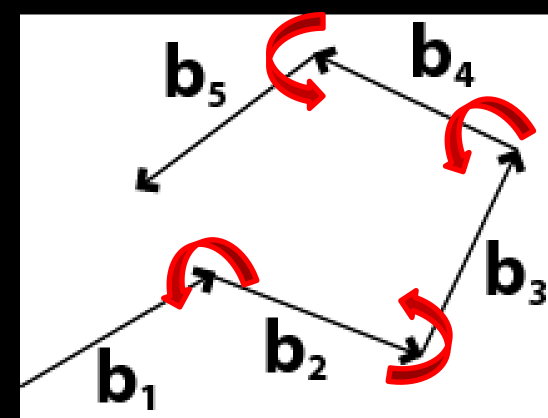
$$f = \frac{3k_B T}{< R^2 >} L$$

Kuhn (1942), James and Guth (1943):

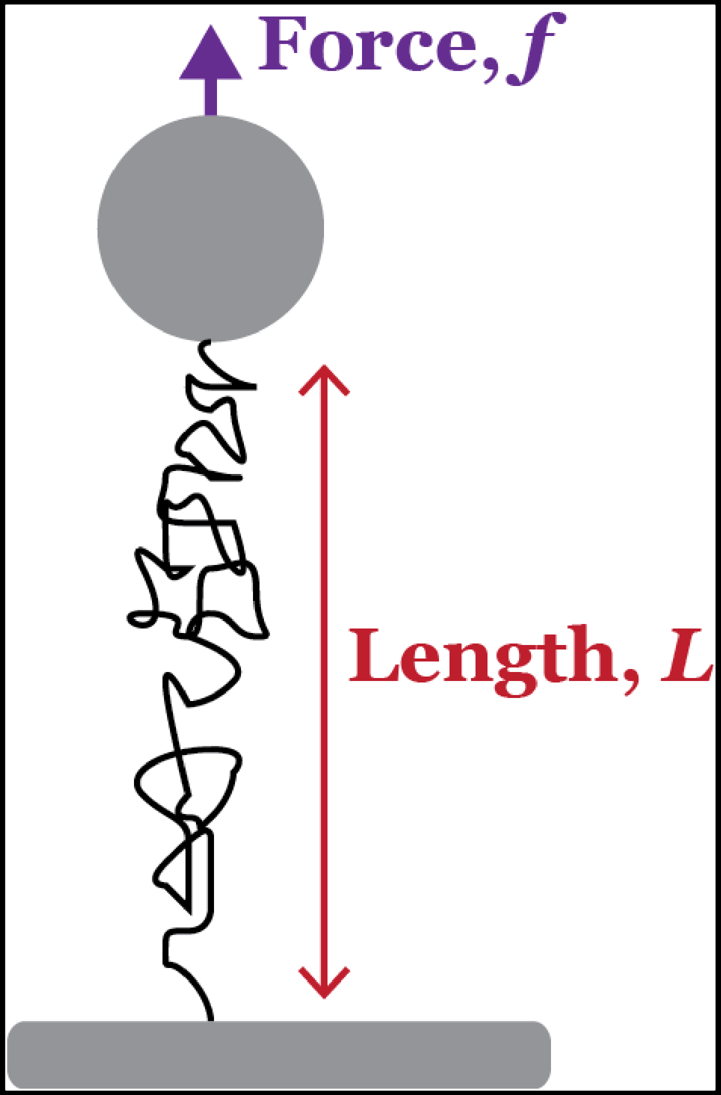
The Freely-Jointed Chain

$$L = L_0\left(\coth\frac{fl}{k_B T} - \frac{k_B T}{fl}\right)$$

$$\approx L_0\left(1 - \frac{k_B T}{fl}\right)$$



Breakthrough in the 90s: Micromanipulation permits *direct* testing of force/extension relations



Single-molecule manipulation (force spectroscopy) techniques permit direct control of force, and measurement of extension, on the scale relevant to single molecules.

Relevant energy scale:

$$k_B T \approx 4 \times 10^{-21}\, J \approx 4 \text{ pN nm}$$

Relevant length scale:

$$\approx 1 \text{ nm}$$

Relevant force scale:

$$k_B T / 1 \text{ nm} \approx 4 \text{ pN}$$

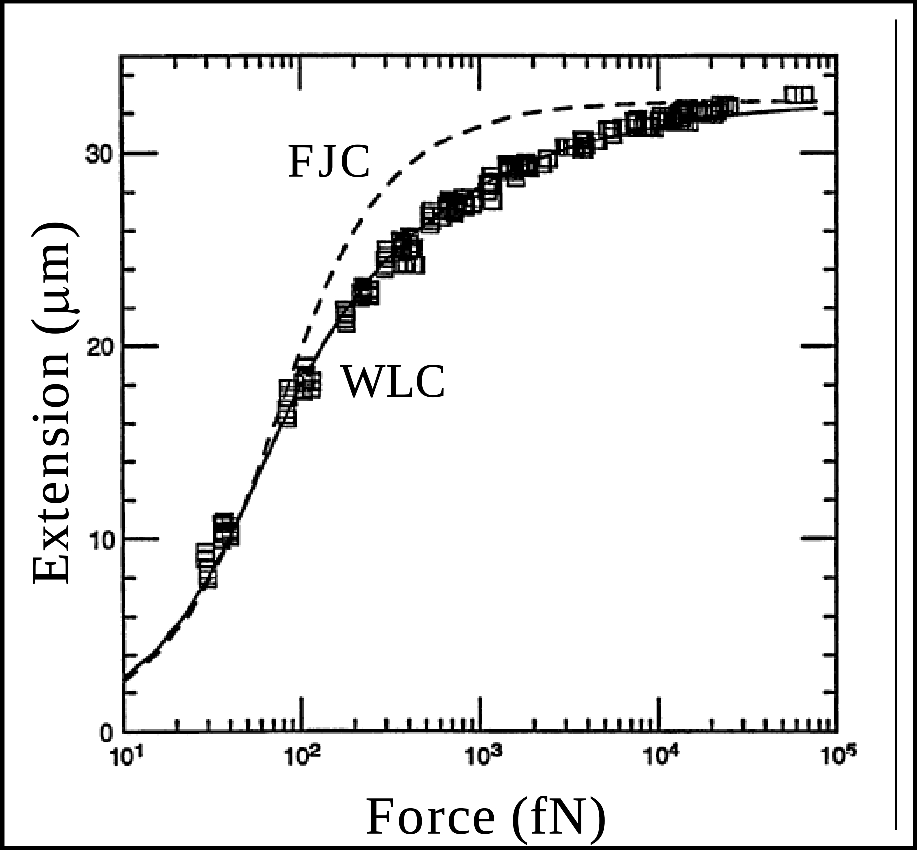
Experimental evidence refutes the FJC, supports the MS-WLC

Stretching double-stranded DNA (dsDNA), $l_p \approx 50$ nm

Some notes:
-DNA as a model system

-Nothing is a FJC

-Enthalpic (linear stretch) elasticity apparent, and quantifiable



Bustamante, Marko, Siggia, and Smith, *Science* (1994)

Application of Marko-Siggia: The modulus of gels of filamentous biopolymers

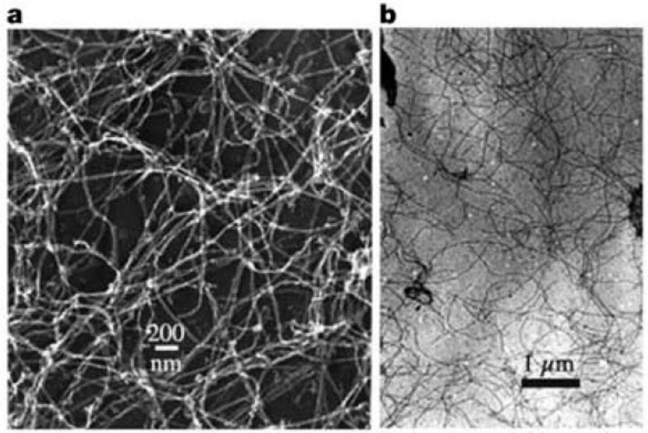
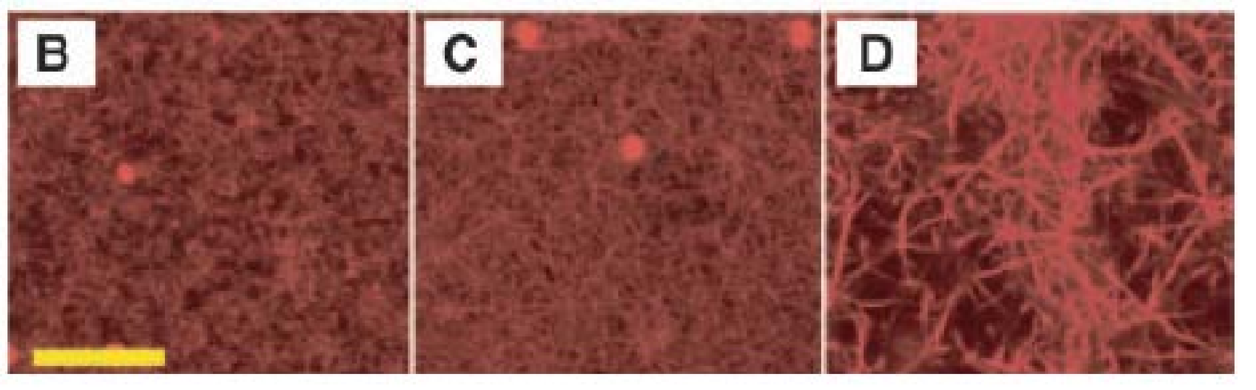
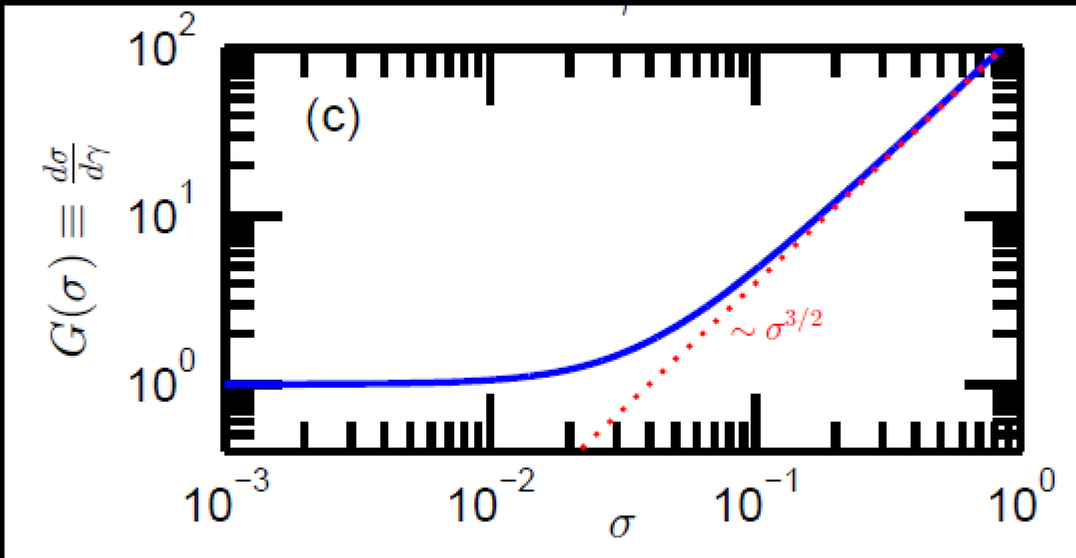


Figure 1 Neurofilament and fibrin protofibril networks. These TEM images show the finite excess of filament contour length between crosslinks and overlap points. **a**, Metal-shadowed neurofilaments, and **b**, uranyl acetate-stained fibrin protofibrils, prepared as described in refs 25 and 26, respectively.

Storm *et al.*, 2005



Actin networks
Gardel *et al.*, 2004



Observation:

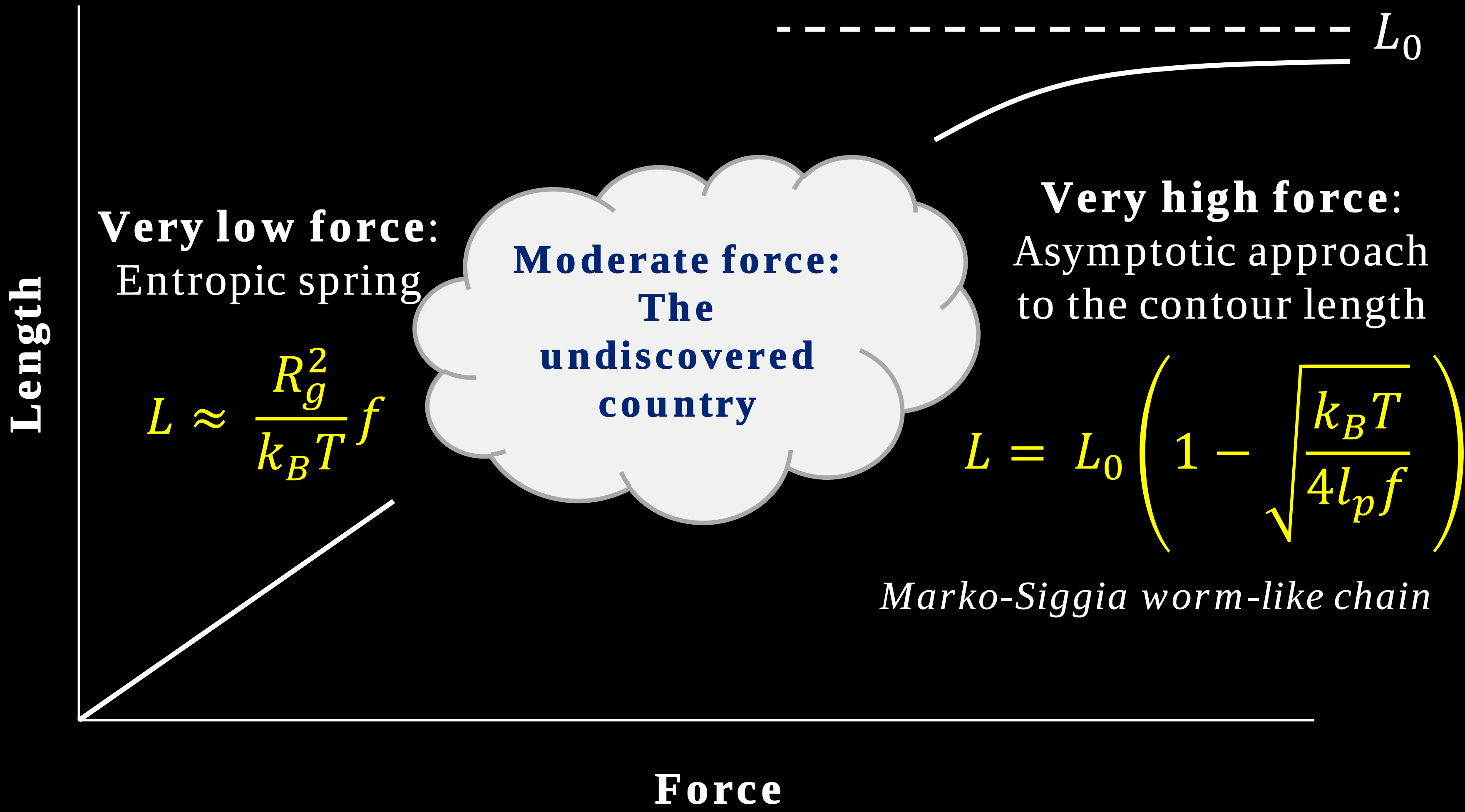
At high stress, The differential modulus of certain filamentous gels grows as strain to the 3/2 power

Problem: Why?

Key references:

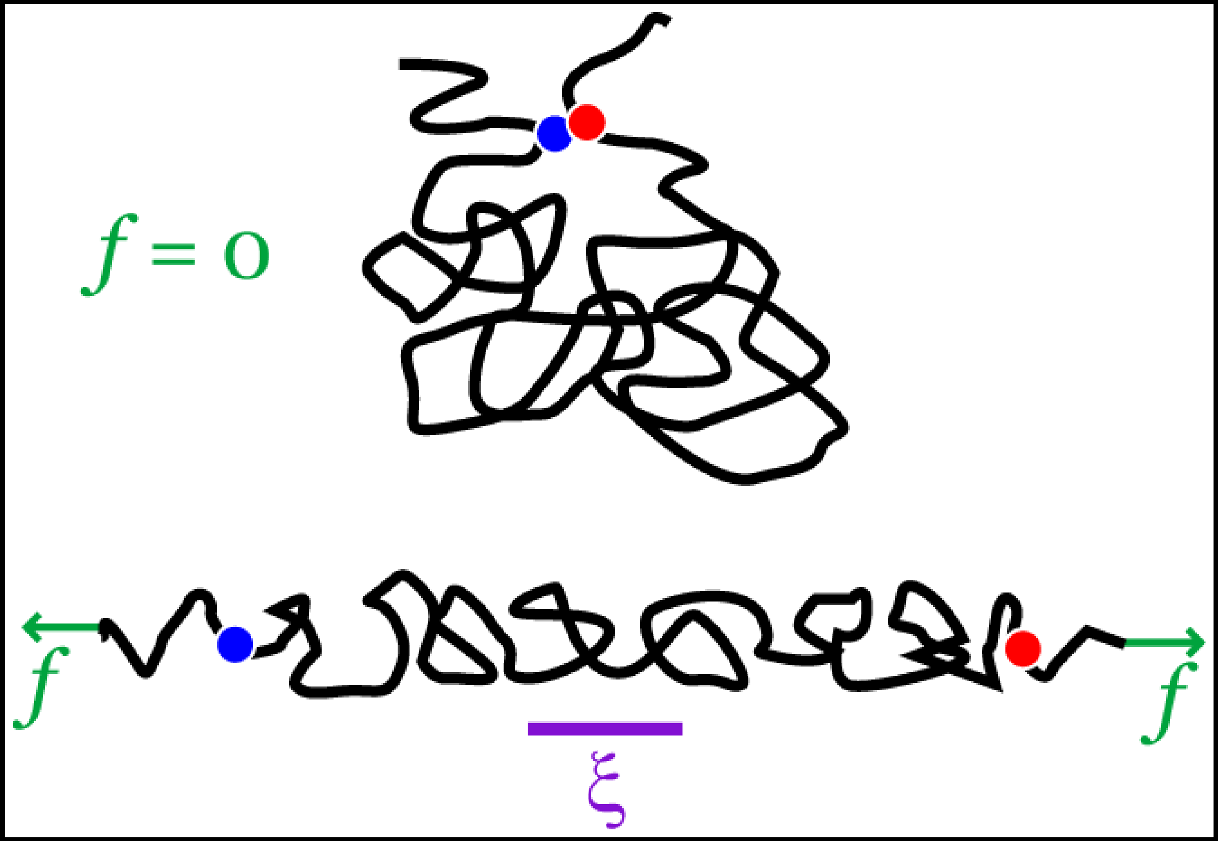
1. Marko and Siggia (1995)
2. Gardel *et al.*, Science (2004)
3. Storm *et al.*, Nature (2005)
4. D. Vader *et al.*, PloS ONE (2009)

???



Very low force:
Entropic spring
$L \approx \frac{R_g^2}{k_B T} f$
Moderate force:
The undiscovered country
Very high force:
Asymptotic approach to the contour length
$L = L_0\left(1 - \sqrt{\frac{k_B T}{4 l_p f}}\right)$
Marko-Siggia worm-like chain
L_0
Length
Force

An alternate approach to elasticity: Scaling



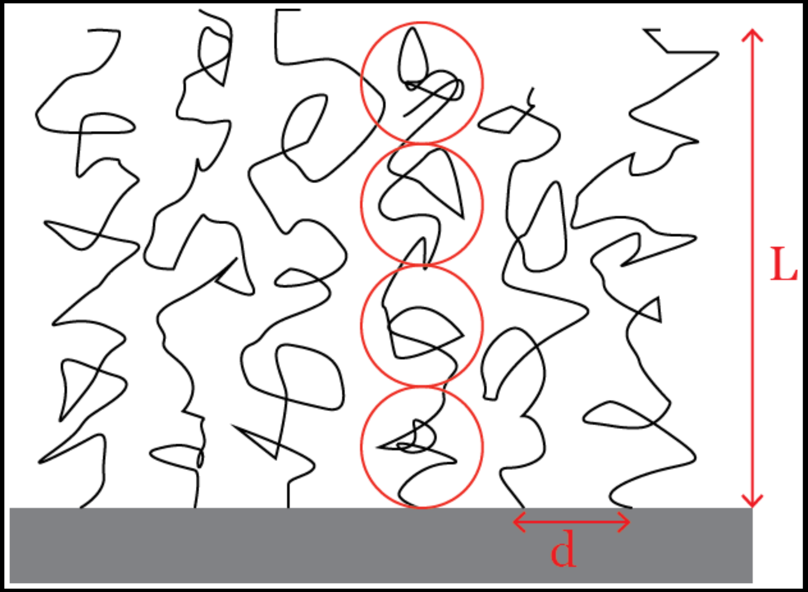
A tension f creates a
tensile screening length, ξ:

$$\xi \equiv k_B T / f$$

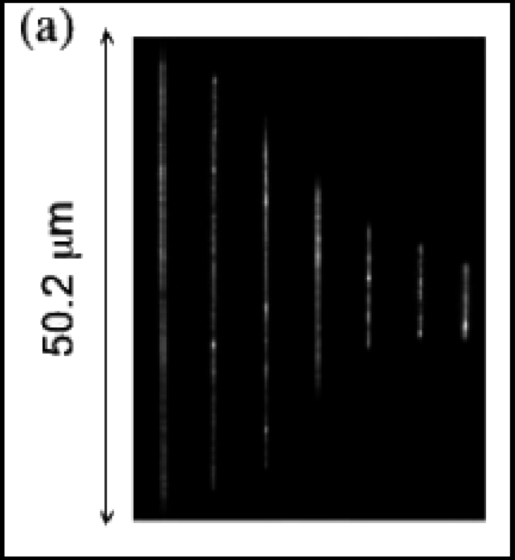
Elasticity of real polymers: Calculations, and the blob picture (to the board)

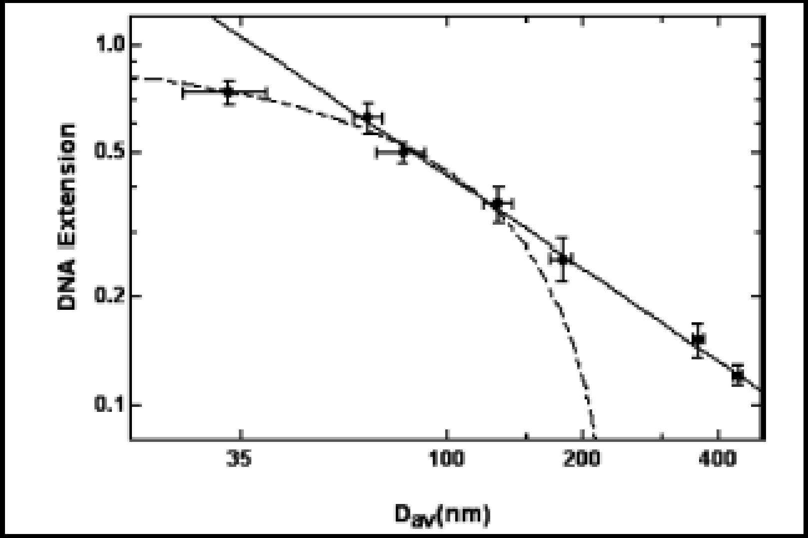
Blob models useful for multiple types of confinement

Confinement from other polymers
e.g. polymer brush:



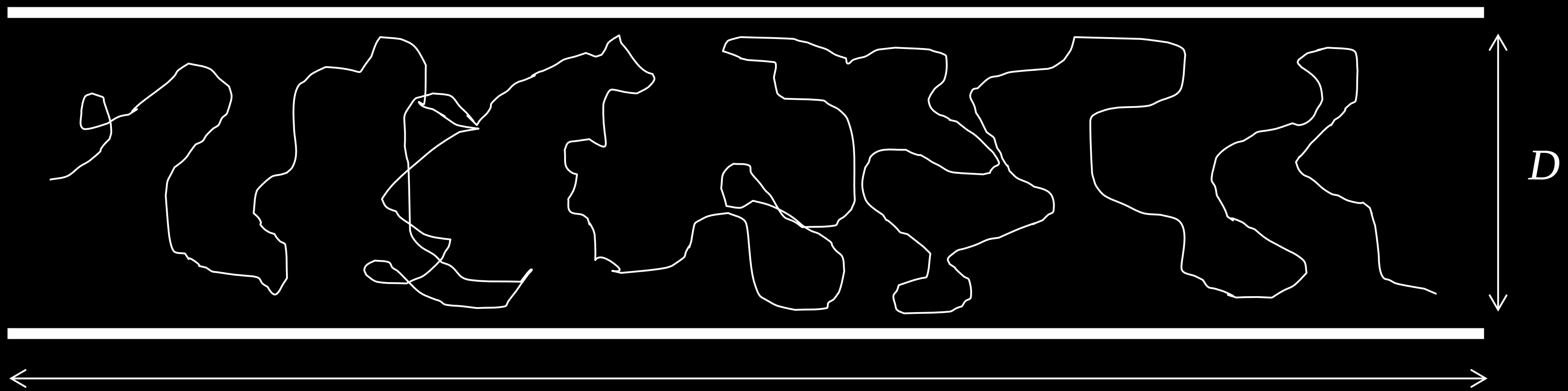
Reisner *et al.* (PRL, 2005) : Confining DNA within nanochannels





Problem: Consider a polymer confined to a tube of diameter D. How does the polymer extension depend on D? What if it is a 2-D slit?

Assume $R_g >> D >> l_p$



Extension: X
Contour length: L

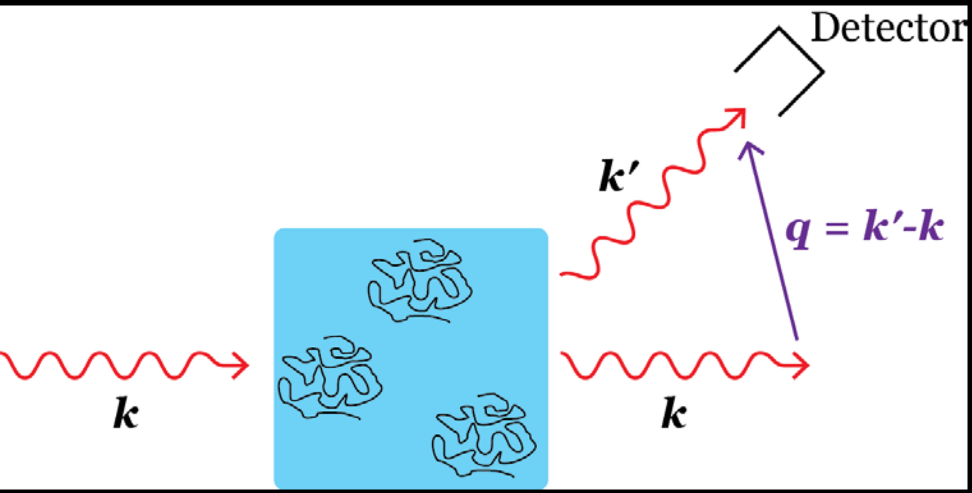
How to reconcile the various force/extension regimes?

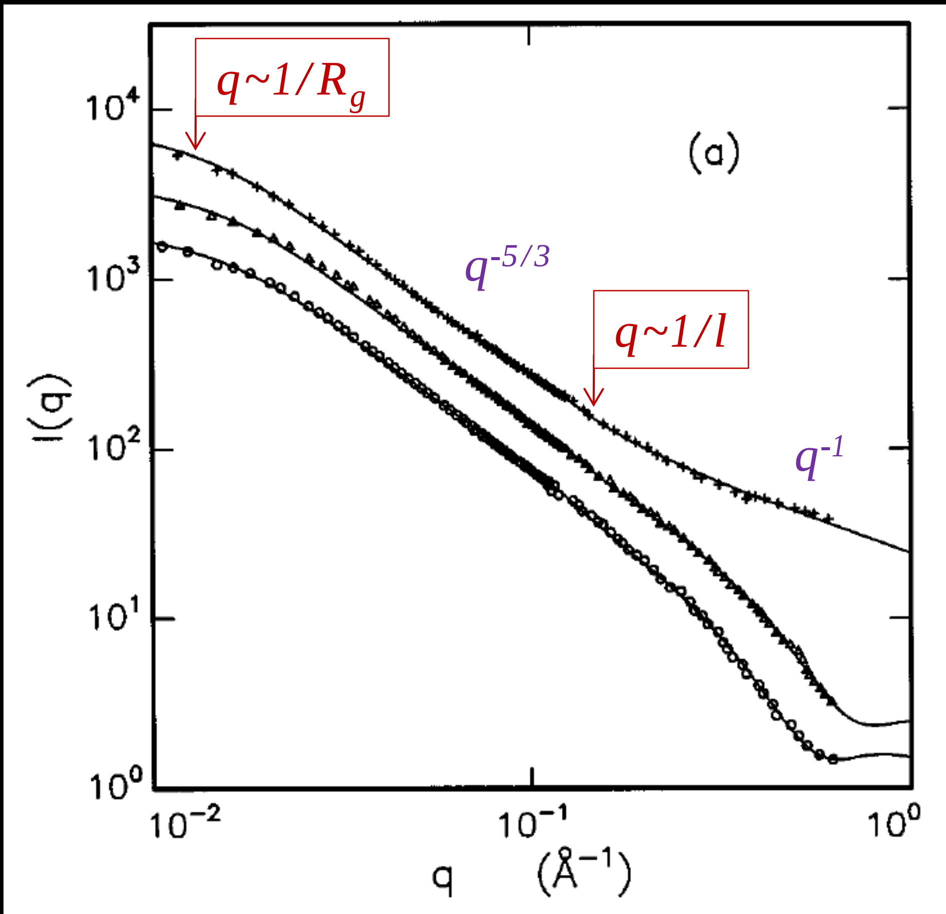
A scaling view:

An elastic transition will occur whenever

$\xi \sim$ (characteristic length scale of the polymer)

An analogy: Scattering measures various scales of structure by varying *q*

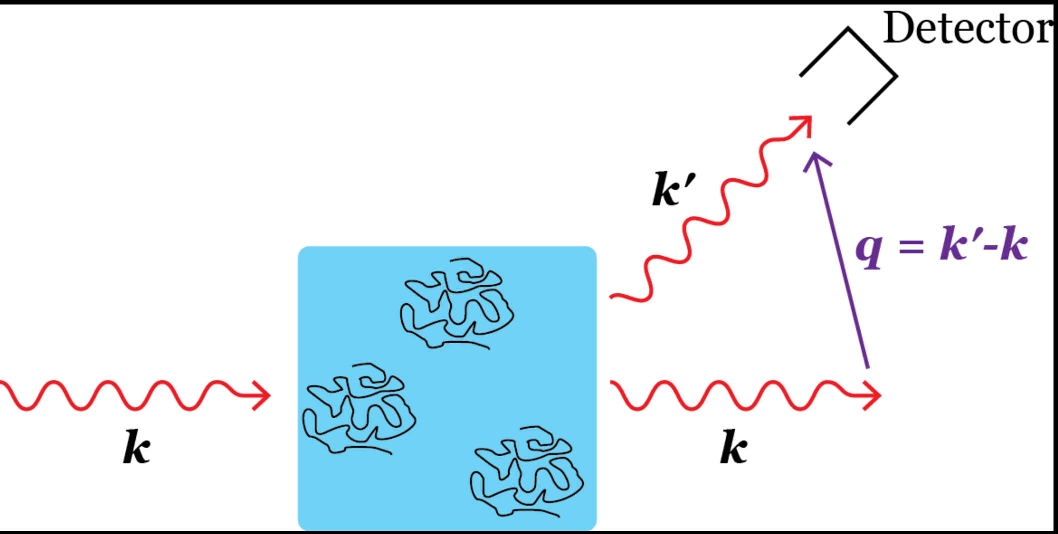


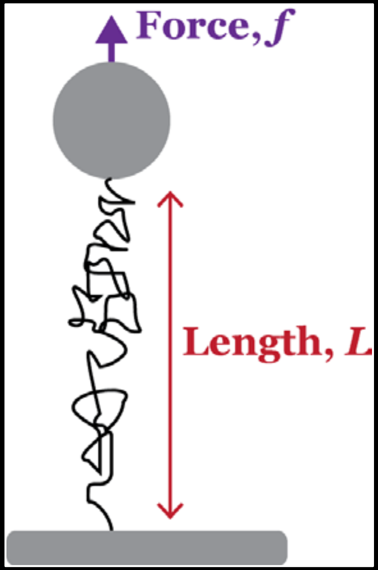


- Key length scales (R_g, l) identified from **transitions** in $S(q)$
- Type of structure (swollen random walk, rigid rod) identified from S vs q relationship

Polystyrene in carbon disulfide
Pedersen and Schurtenberger, 1996
Rawiso et al., 1987

Elasticity vs. scattering: Both control a length scale, but elasticity offers a superior single-polymer signal



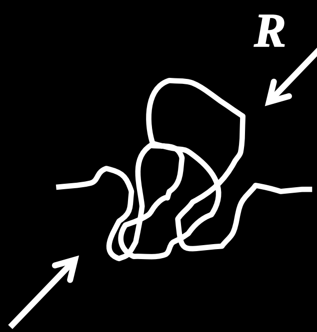


Measured behavior: $I(q)$ ⇒ $L(f)$

Length scale: $1/q$ ⇒ kT/f

The power of elasticity at studying single-chain structure will be a main subject of my next lecture.

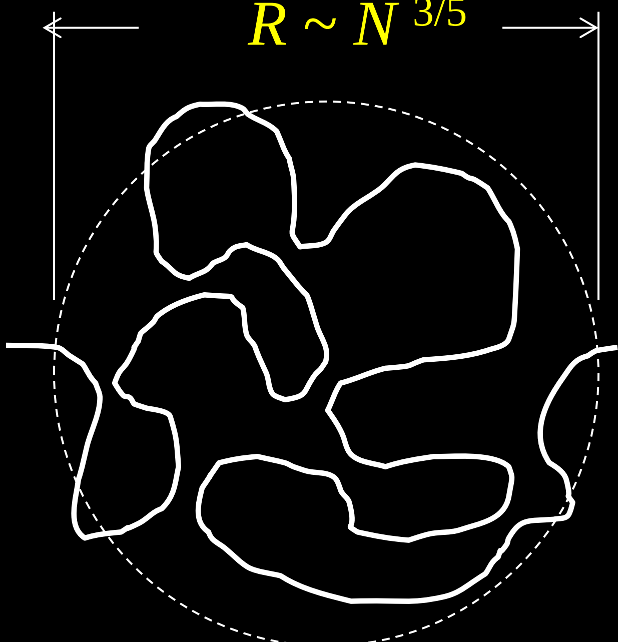
Length scales of a neutral polymer



Kuhn length, l (random-walk step size)
Thermal blob size, b (crossover extent)
RMS extent, R

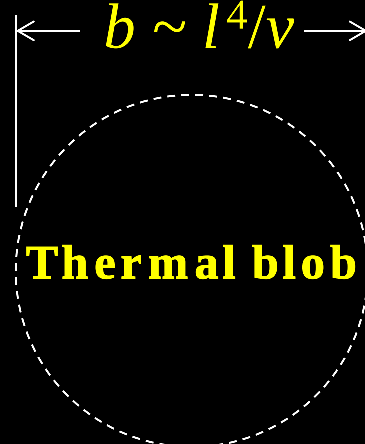
Long polymers are swollen

$R \sim N^{3/5}$



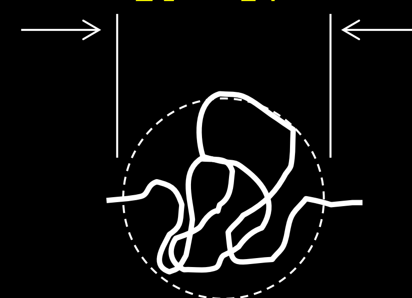
Crossover size:

$b \sim l^4/v$



Short polymers are ideal

$R \sim N^{1/2}$

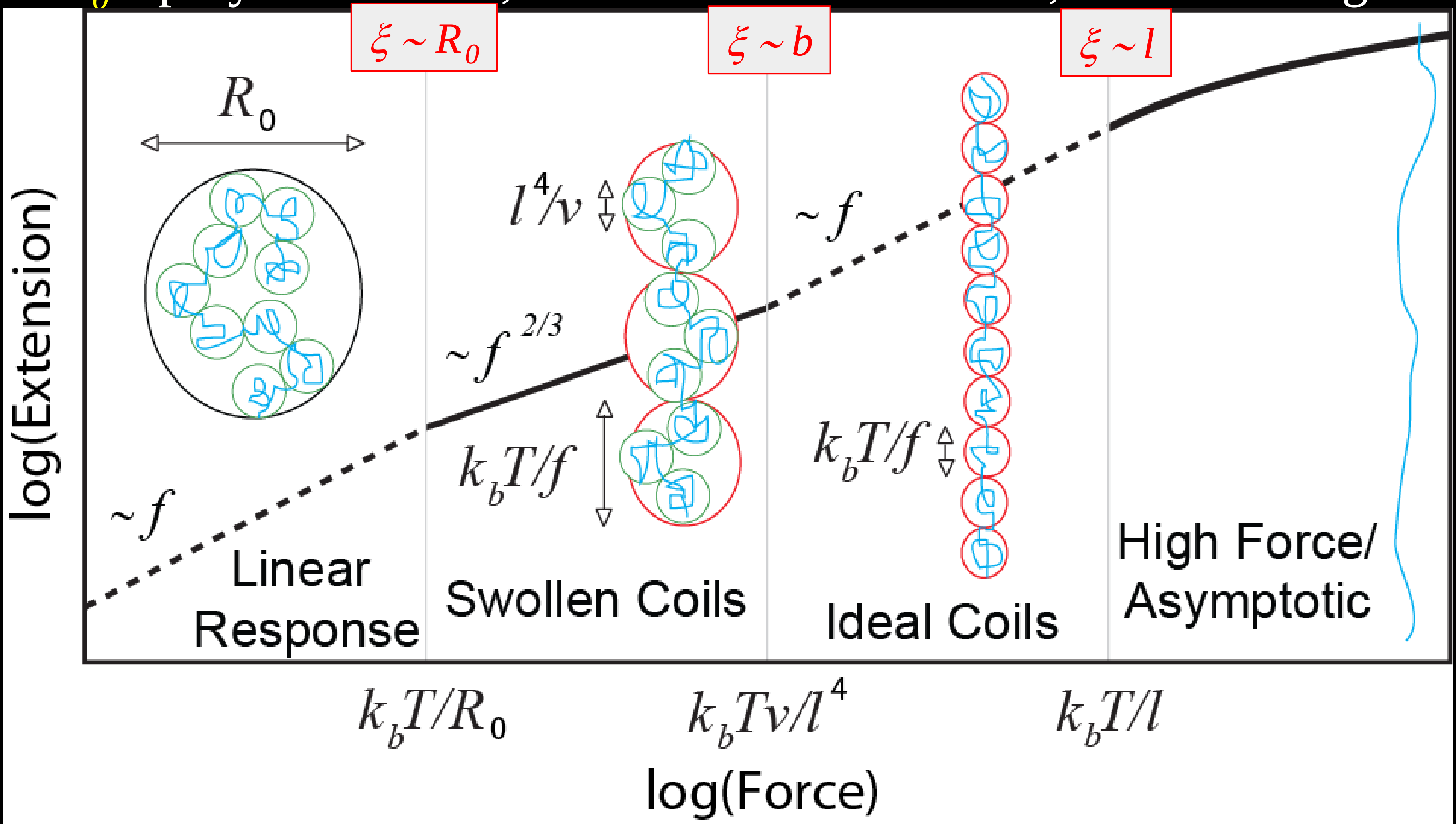


v: Excluded volume parameter

Thermal blob: the crossover scale below which a polymer acts ideally and above which it is swollen

Scaling of tensile elasticity:
A transition whenever $\xi \sim$ (char. length)

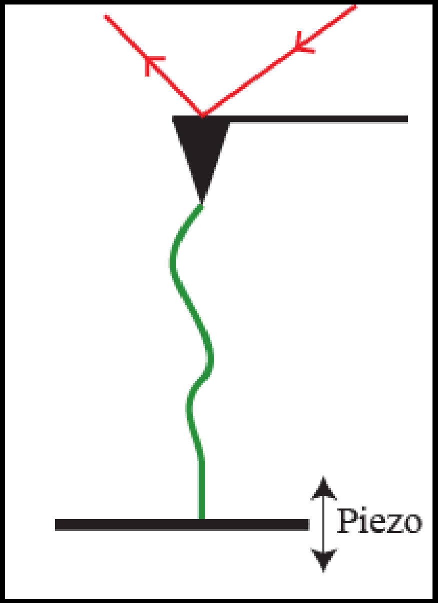
R_0– polymer extent; b– thermal blob extent; l– Kuhn length



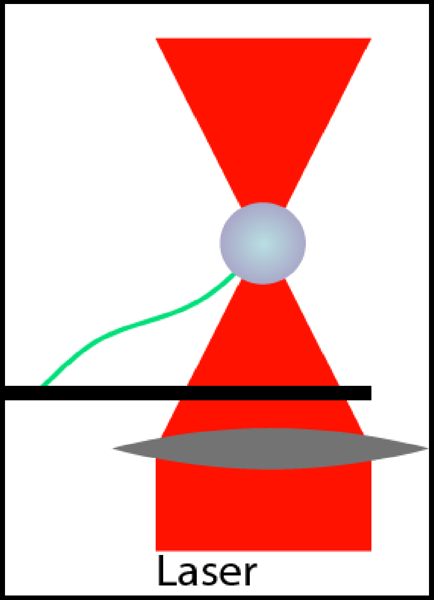
Pincus, *Macromolecules* (1976); Netz, *Macromolecules* (2001)

McIntosh, Ribeck and Saleh *PRE* (2009)

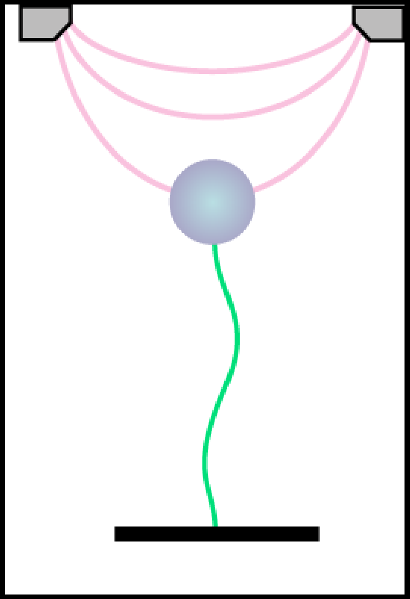
Measurements of polymer elasticity



The Atomic Force Microscope

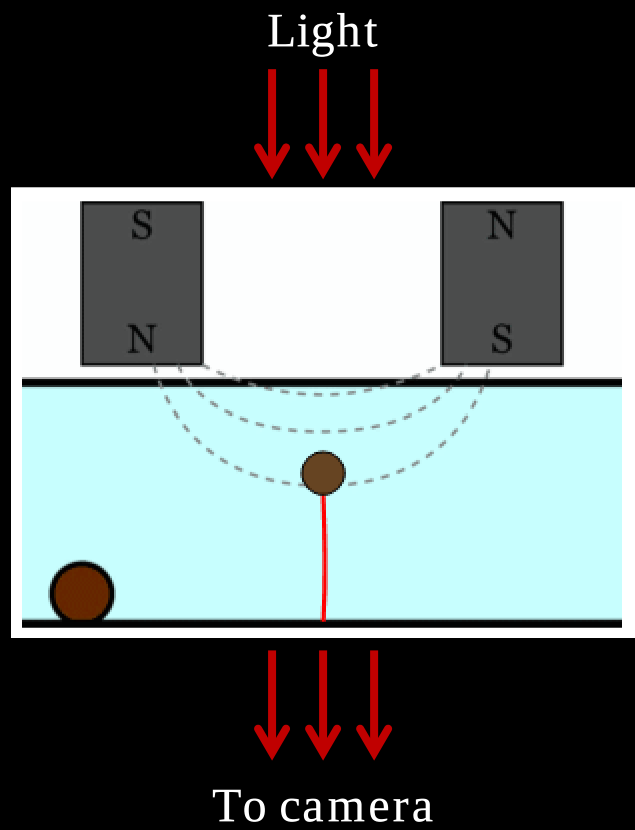


The Optical Tweezer



The Magnetic Tweezer

The Magnetic Tweezer



Polymer extension, *L*, from 3D bead tracking

Gosse and Croquette (2002)

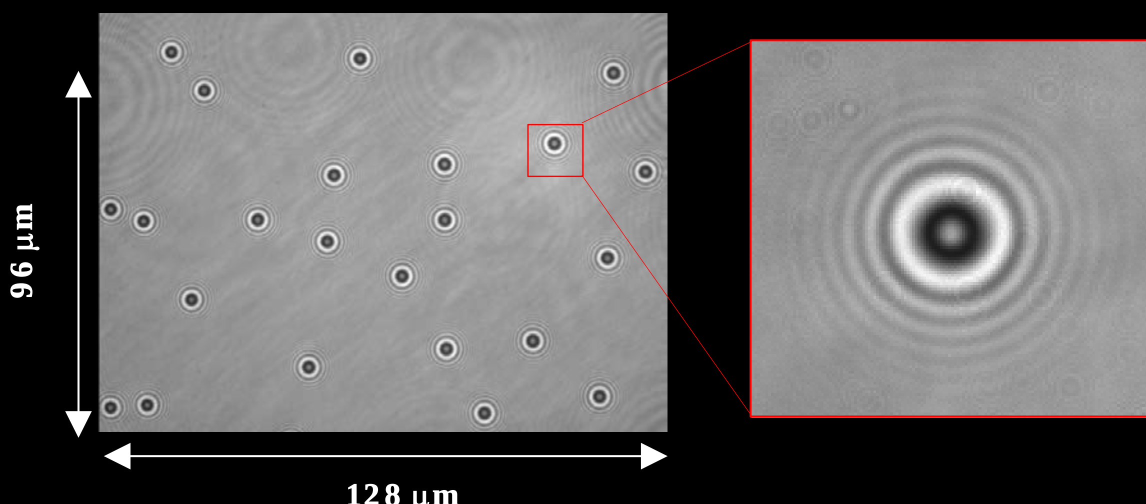
Ribeck and Saleh (2008)

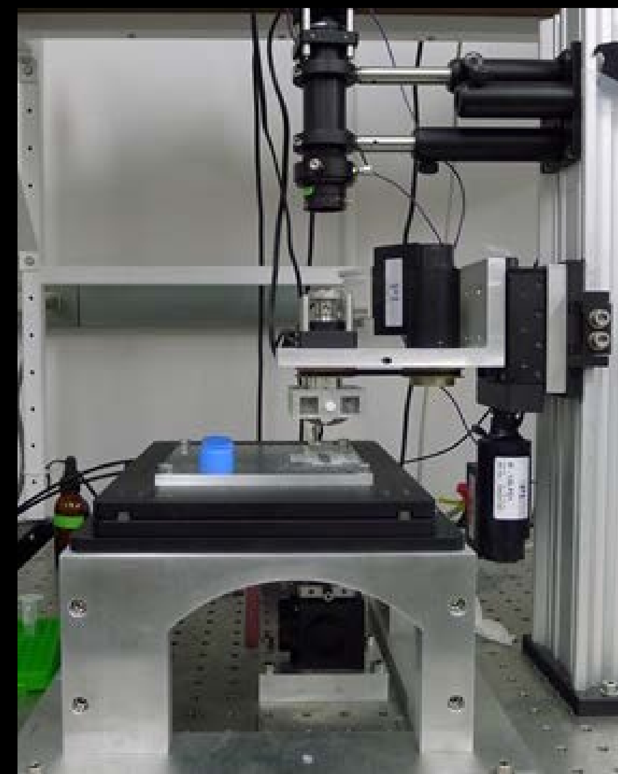
Force, *f*, from measured bead fluctuations

Lansdorp and Saleh, RSI (2012)

Low force: Stability of permanent magnets + ability to move them far away

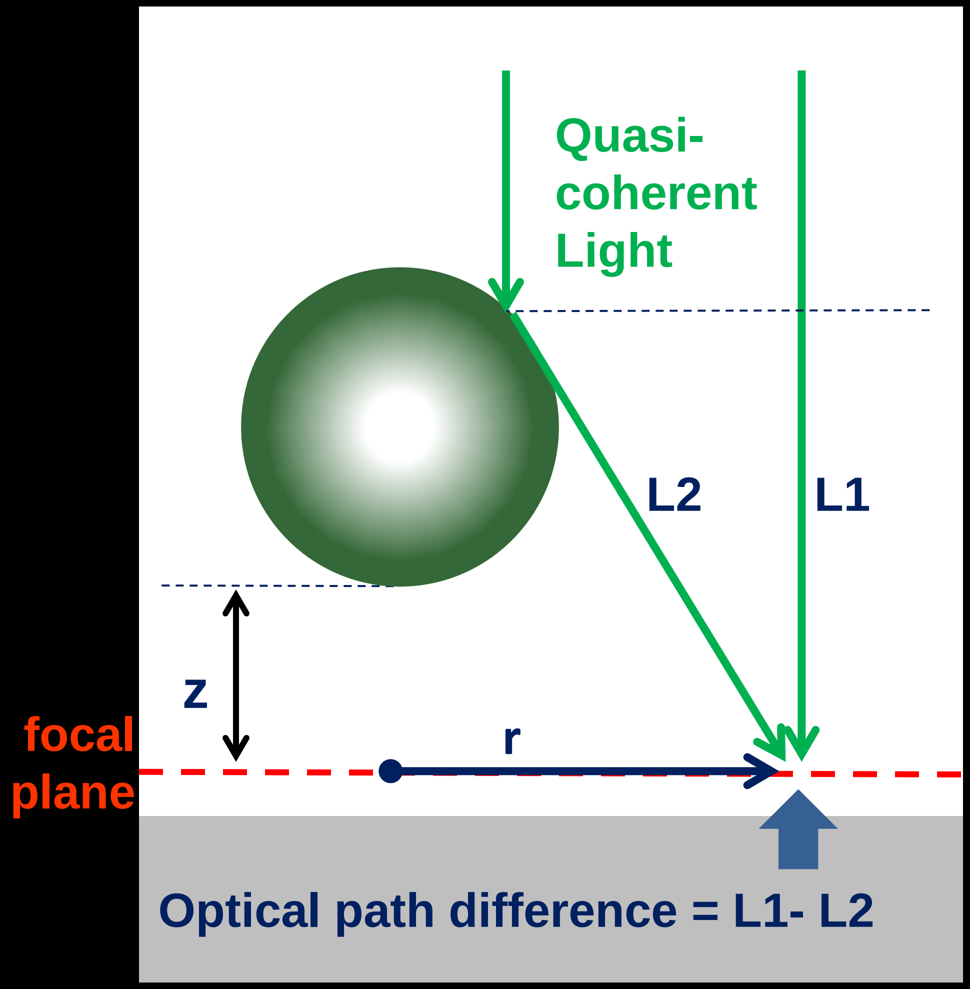
Long chains are needed!

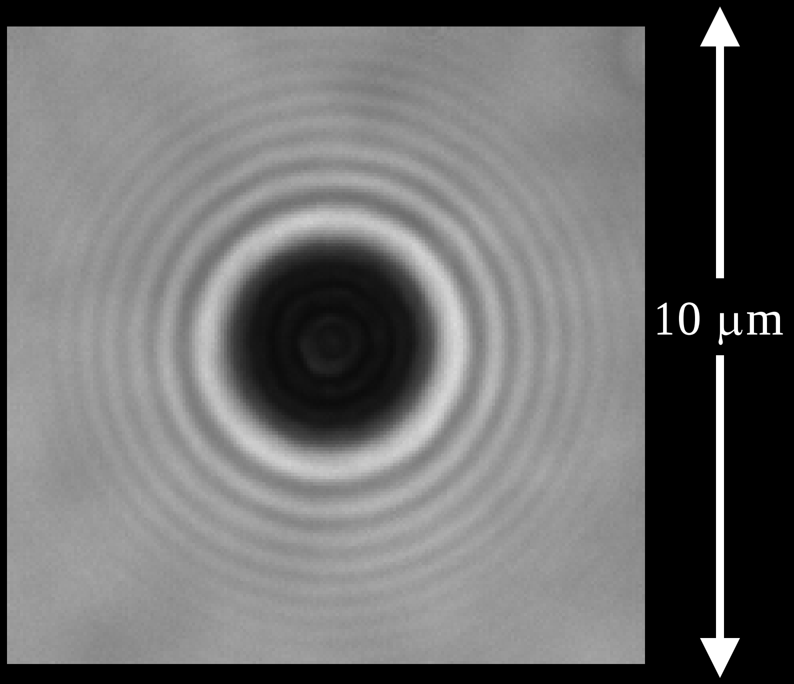




Imaging-based particle tracking

The measured diffraction ring radius, *r*, depends on bead height *z*



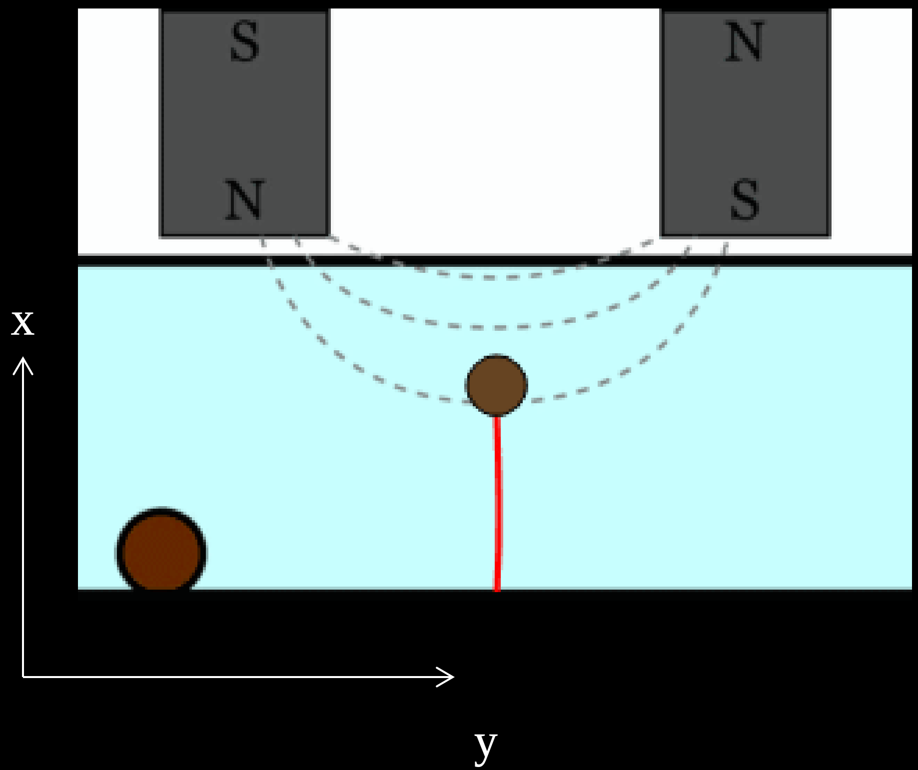


(x,y) : Found from autocorrelation algorithm

z : Found from diff. rings

Gosse and Croquette, 2002

Force estimation in a magnetic tweezer



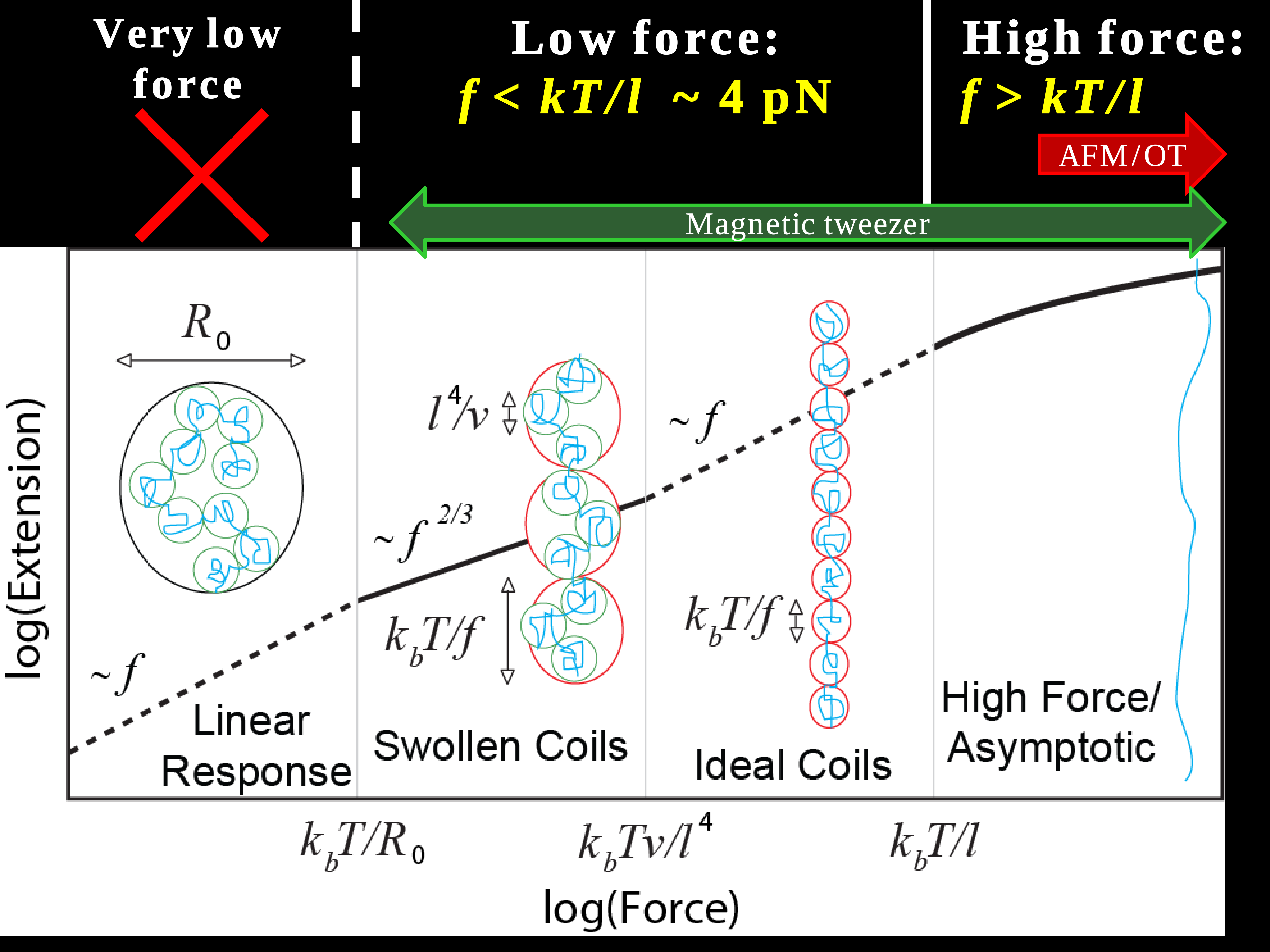
Simple version: Equipartition!

$$k = \frac{k_B T}{< y^2 >} = f/< x >$$

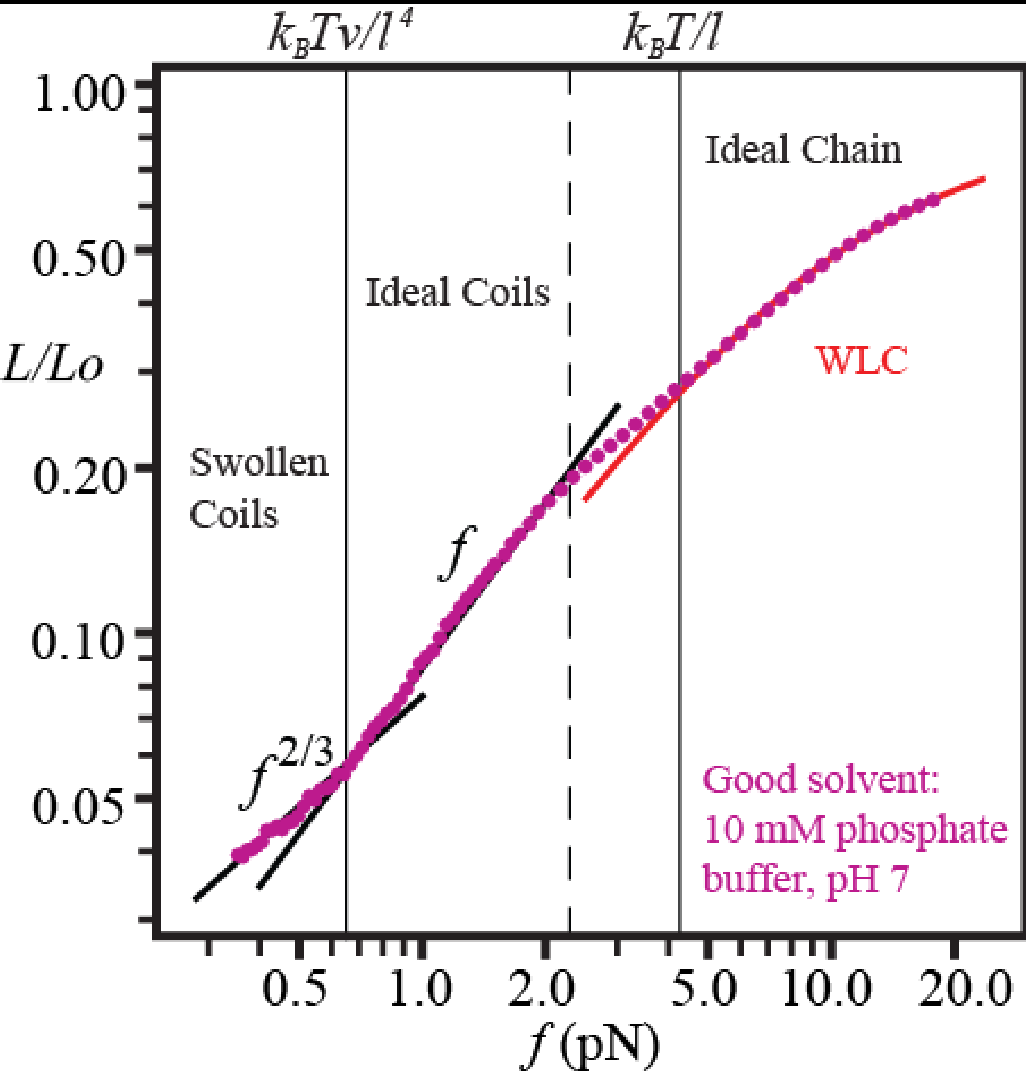
More precise, but complex:

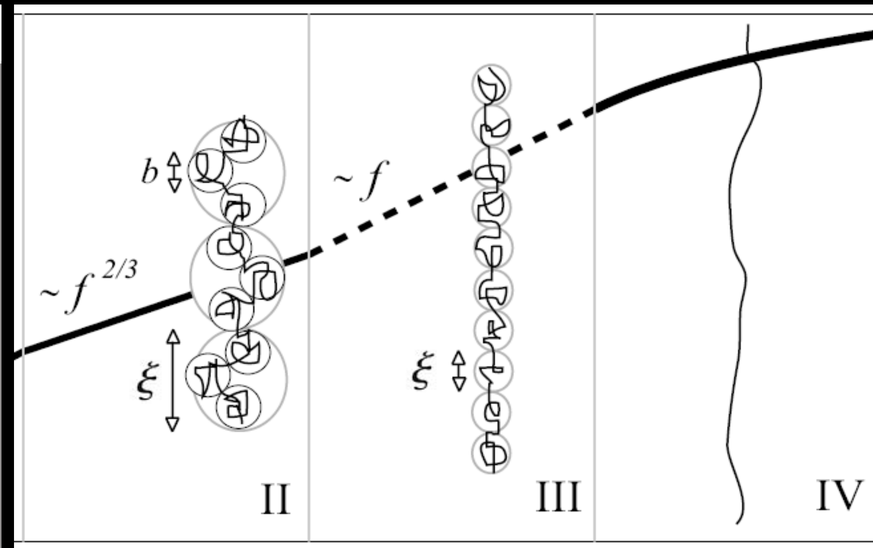
Power spectra, Allan deviation...

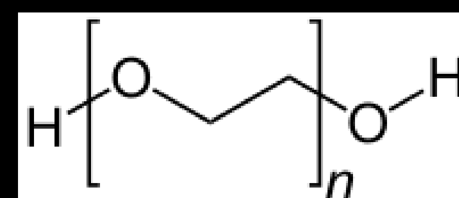
see Lansdorp and Saleh, 2012, and references therein

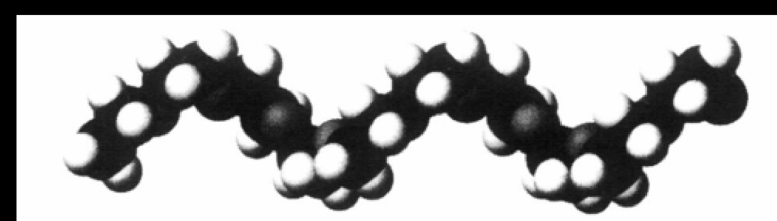

Very low force
Low force:
f < kT/l ~ 4 pN
High force:
f > kT/l
AFM/OT
Magnetic tweezer
R0
~f
Linear Response
l4/v
~f 2/3
kbT/f
Swollen Coils
~f
kbT/f
Ideal Coils
High Force/ Asymptotic
log(Extension)
kbT/R0
kbTv/l4
kbT/l
log(Force)

The force-extension behavior of PEG shows all three accessible elastic regines







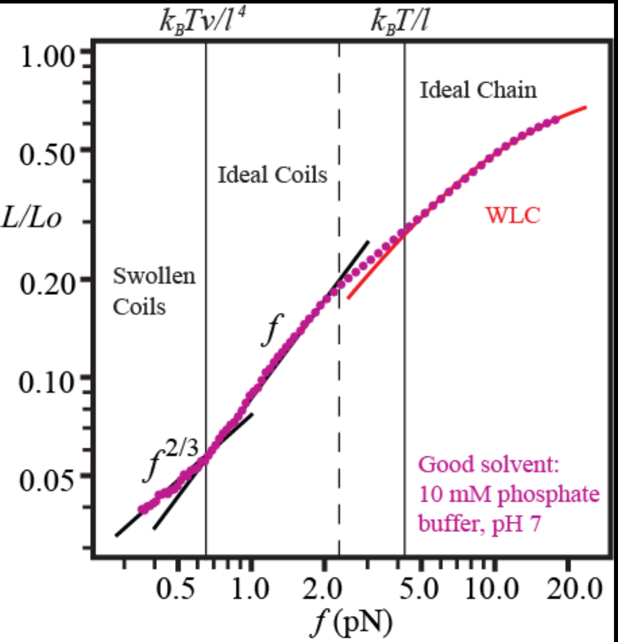


Helical PEG structure;
from Bruce and Vincent (1993)

Dittmore, McIntosh, Halliday, and Saleh *PRL* (2011)

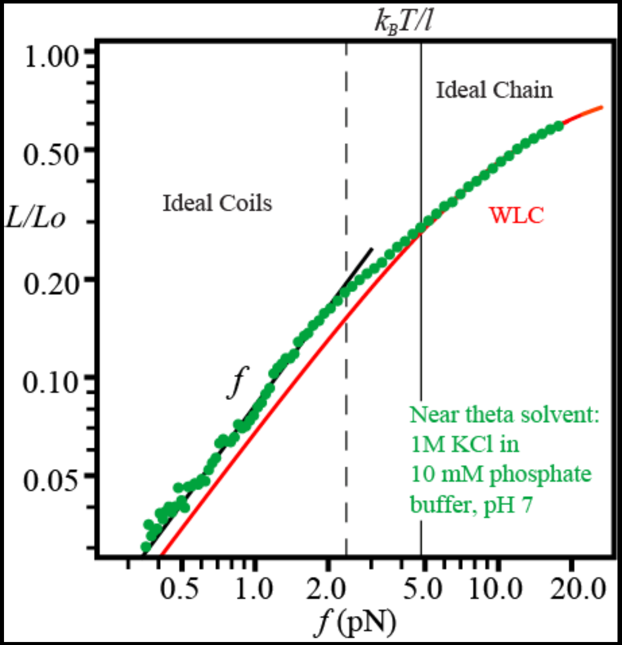
Solvent quality modulation removes swollen regime, then ideal regime

Good solvent



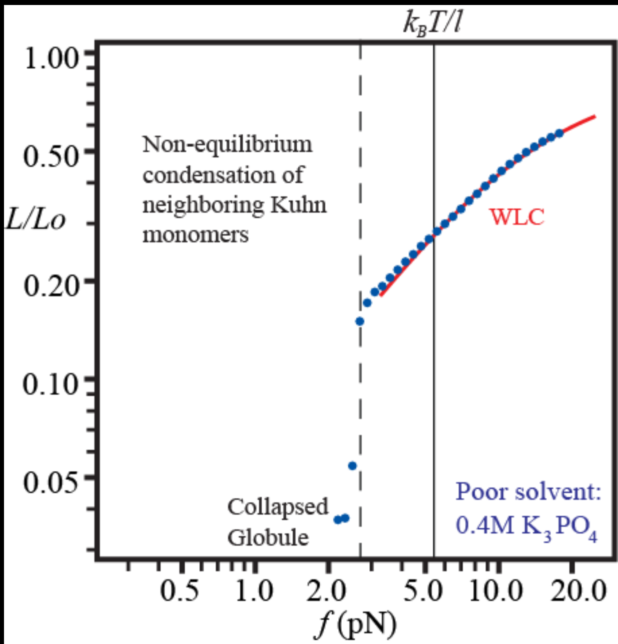
10 mM Phosphate buffer, pH 7

Theta solvent



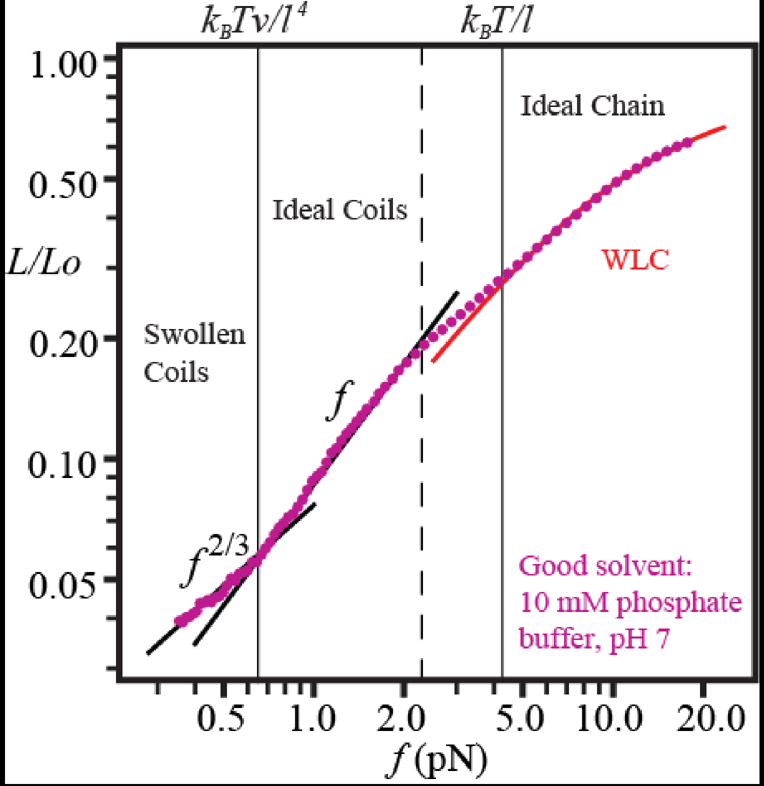
1 M KCl in 10 mM PB, pH 7

Poor solvent



0.4 M K_3PO_4, unbuffered

Dittmore, McIntosh, Halliday, and Saleh *PRL* (2011)

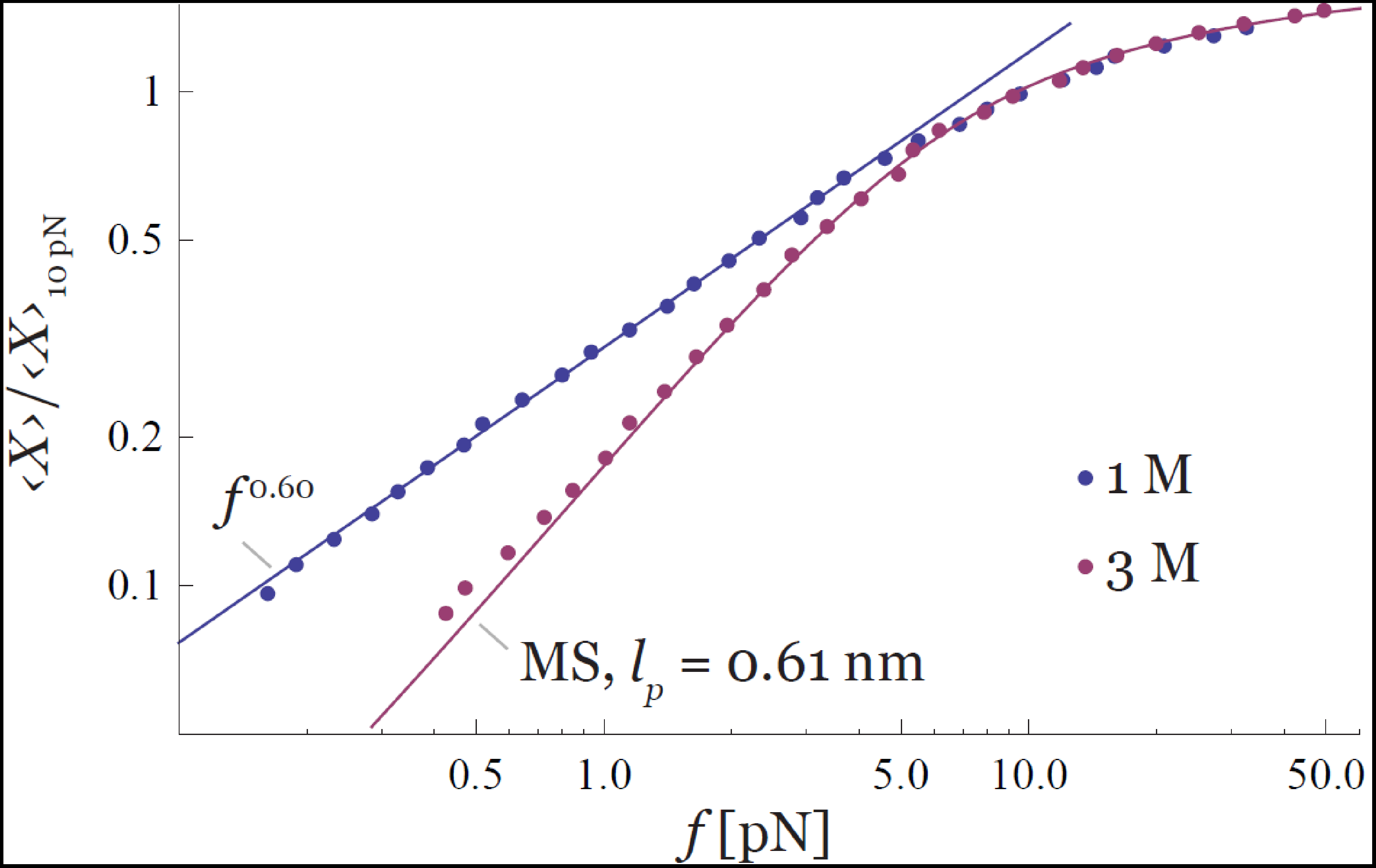


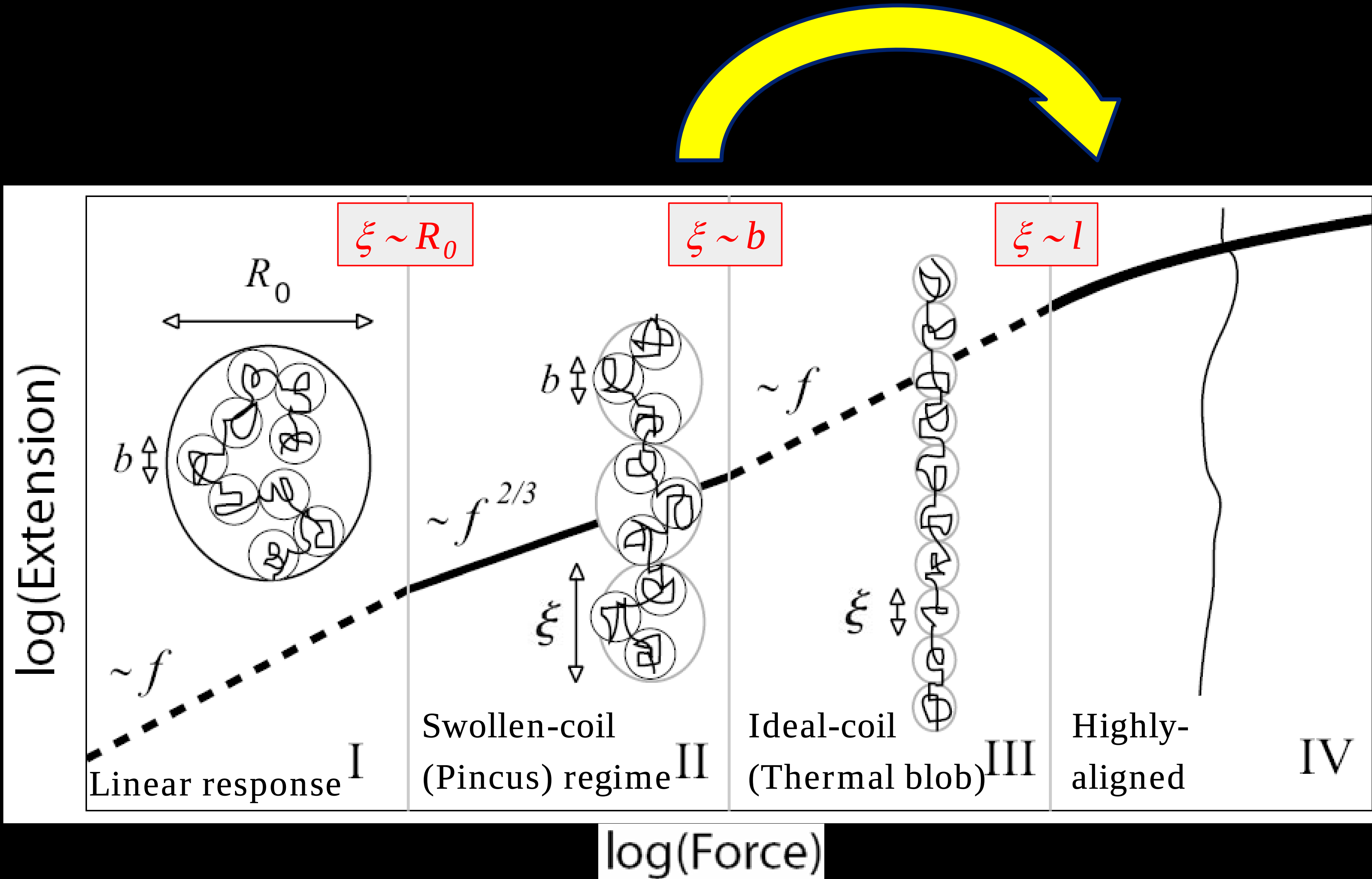
Direct measurement of the onset of swelling permits a quick estimate of many structural parameters

Contour length (per monomer)	Persistence length	Thermal blob size	Kuhn length	Excluded volume
$L = 0.31$ nm	$l_p = 0.47$ nm	$b = 6$ 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 $v \sim l^4/b$

Dittmore, McIntosh, Halliday, and Saleh *PRL* (2011)

Single-stranded DNA elasticity, at high salt

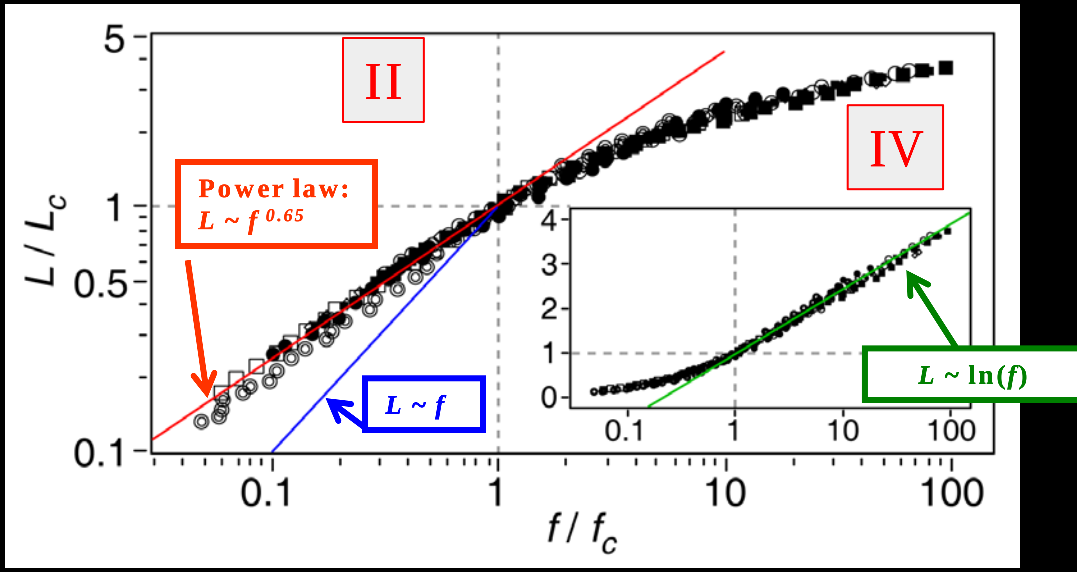




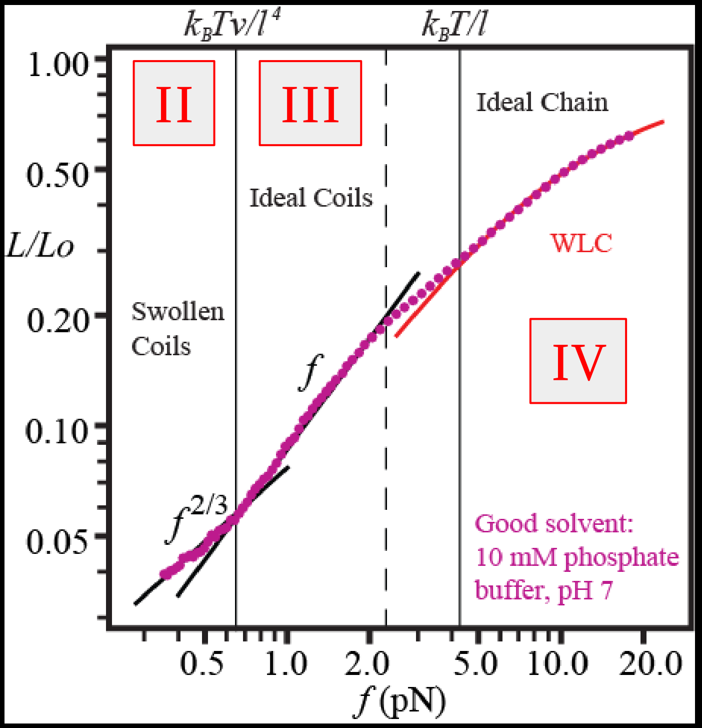
No thermal blob regime in ssDNA

Why the difference?

ssDNA



PEG



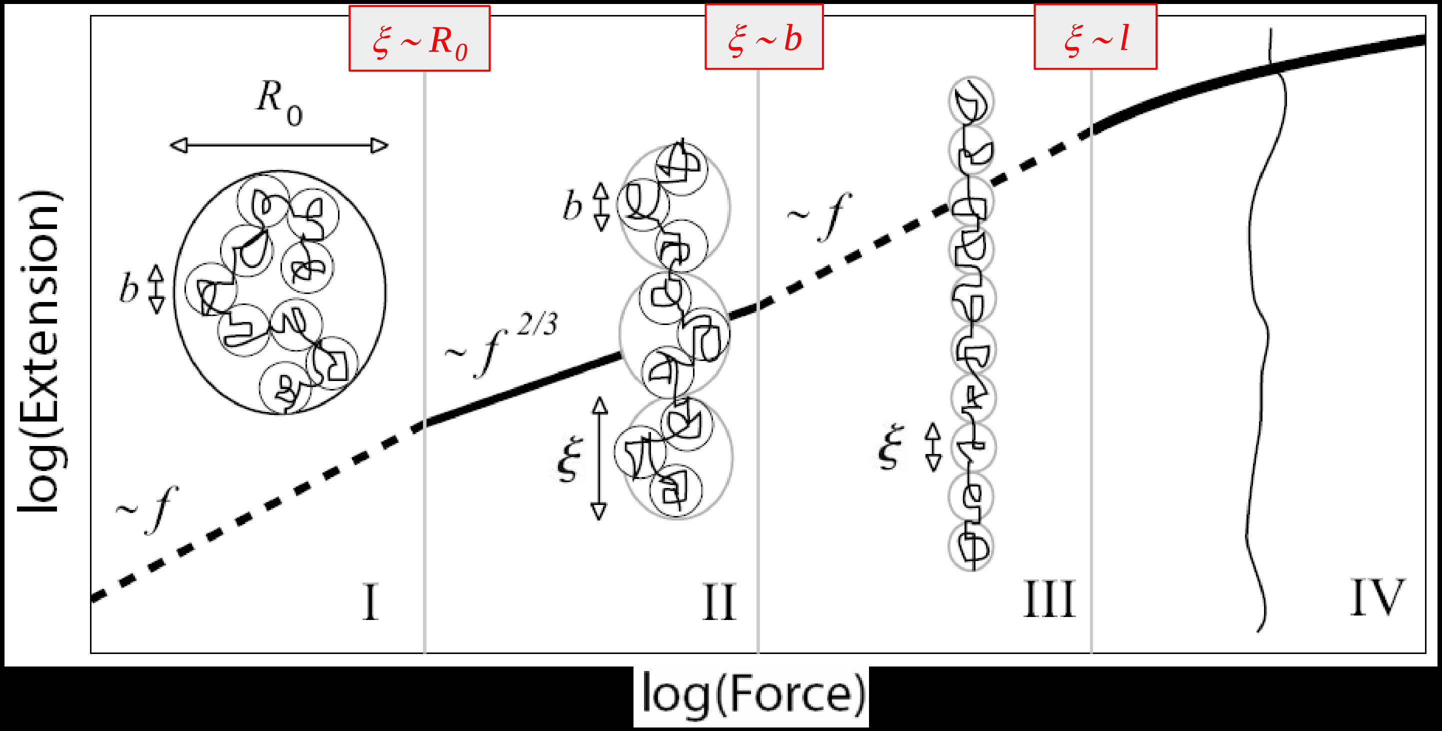
- Presence/lack of regime III consistent with aspect ratio of monomers

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

If the statistical monomers are spherical:

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

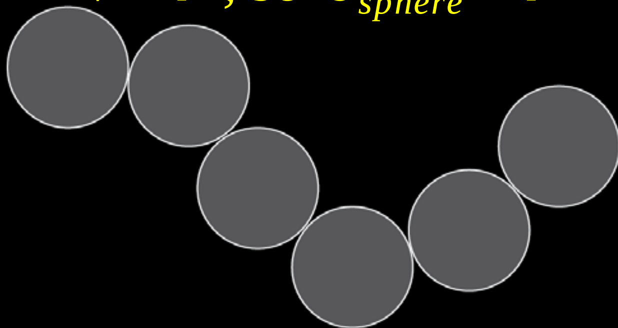
So $\xi \sim b$ coincides with $\xi \sim l$, and the thermal blob regime (III) disappears



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

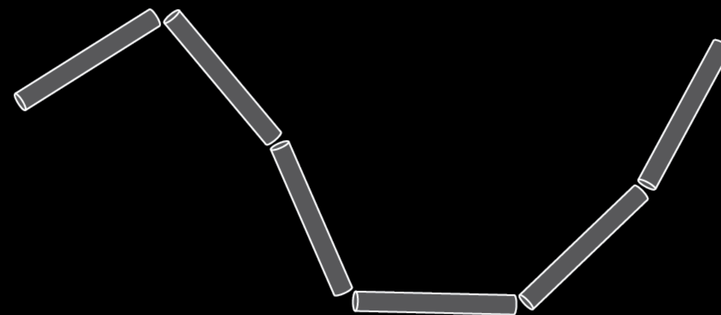
ssDNA: Electrostatic-dominated spherical monomers:

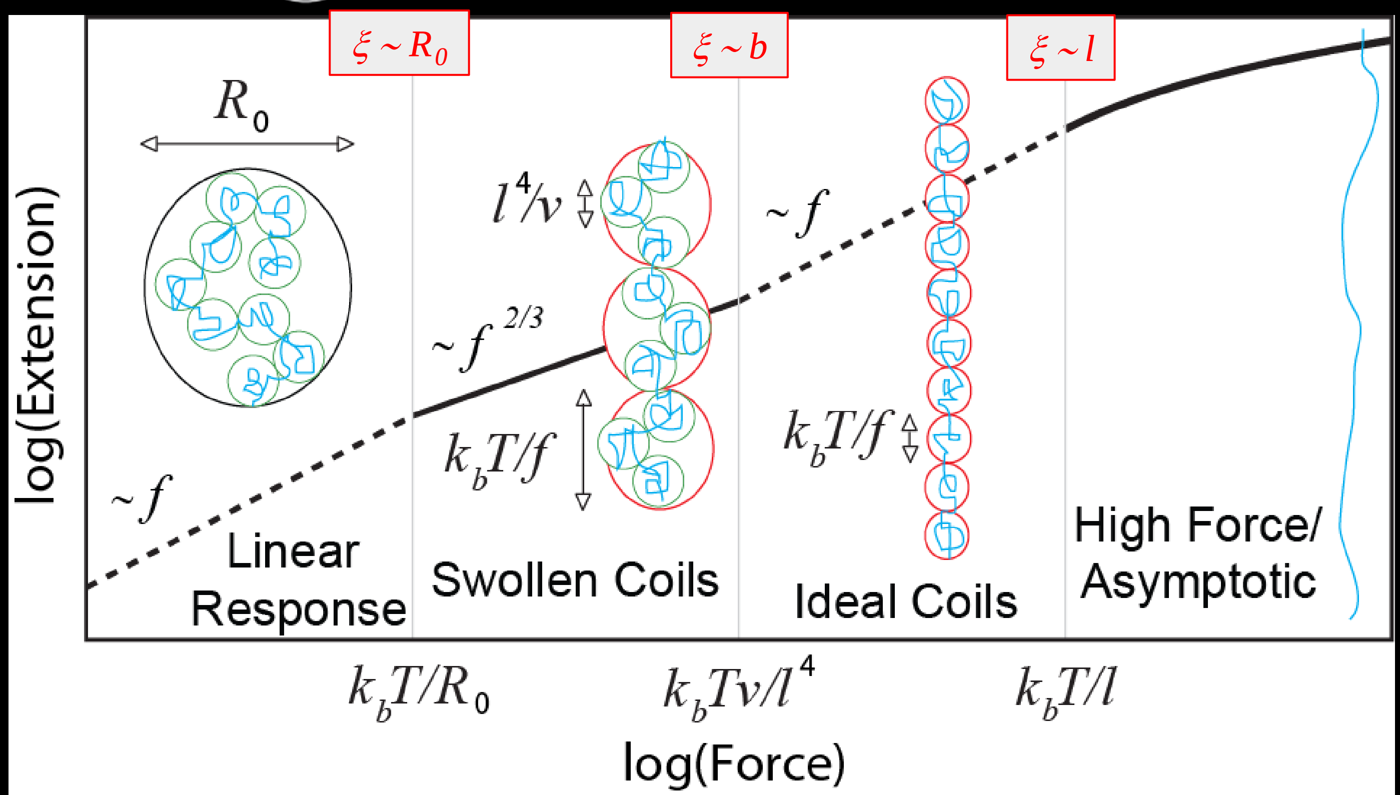
$v \sim l^3$, so $b_{sphere} \sim l$



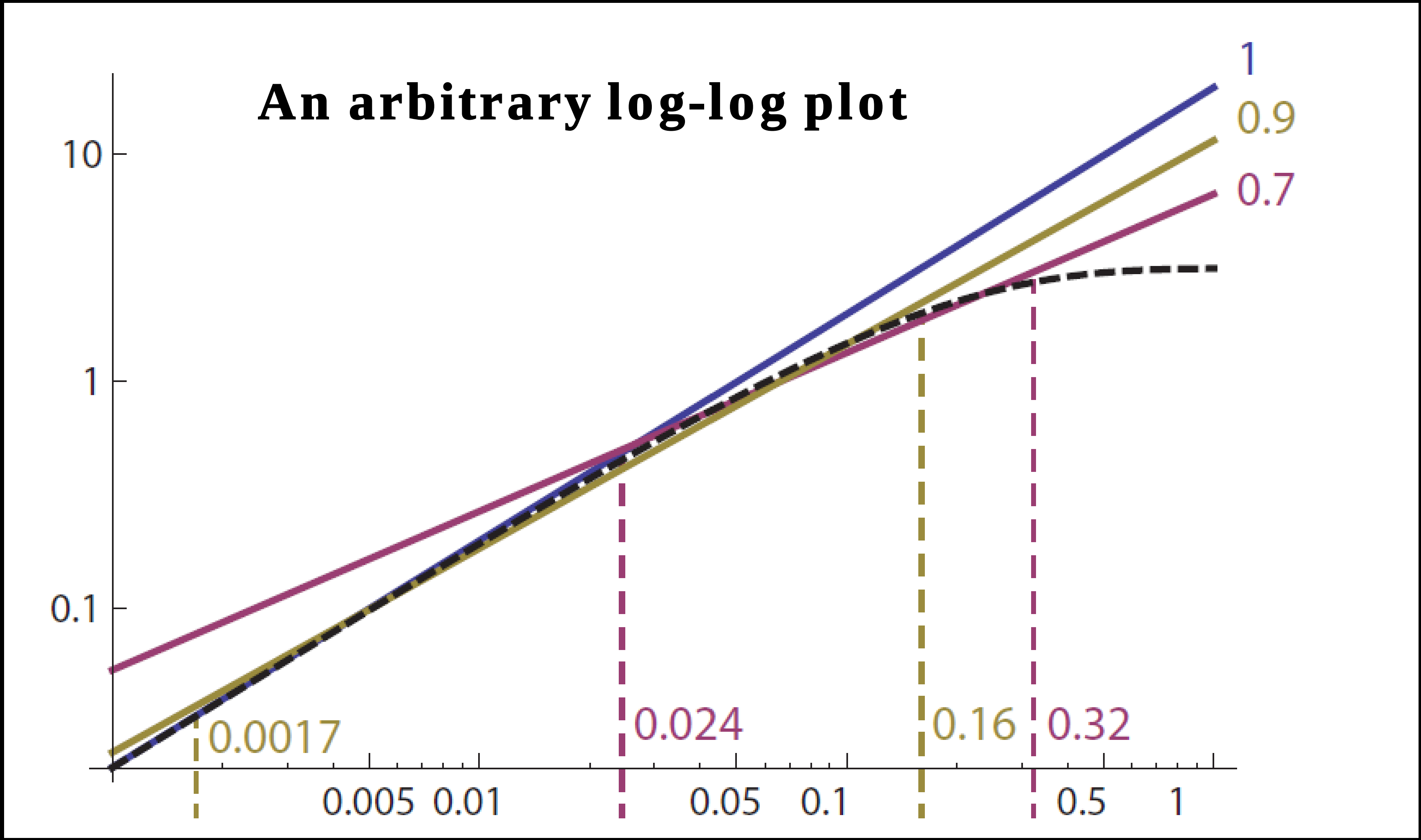
PEG: Chain-mediated rod-like monomers:

$v \sim l^2 d < l^3$, so $b_{rod} > l$





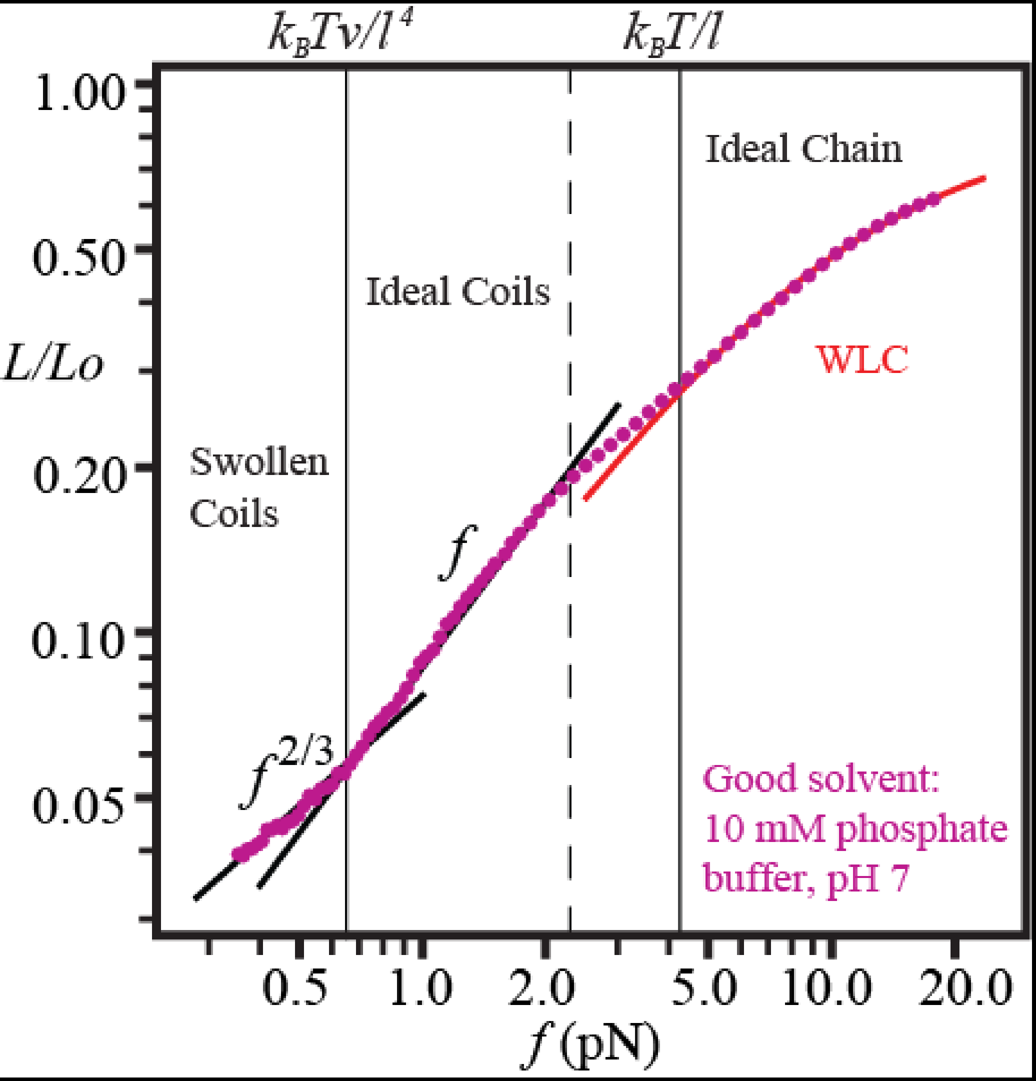
A word about data interpretation:
Beware of power laws!



Black line: linear to plateau w/ increasing *x*

Colored lines: power-laws (exponent noted) that fall within 10% of black line, over at least a decade in *x*

Problem:
It is difficult to quantify exponents from power-laws of limited duration



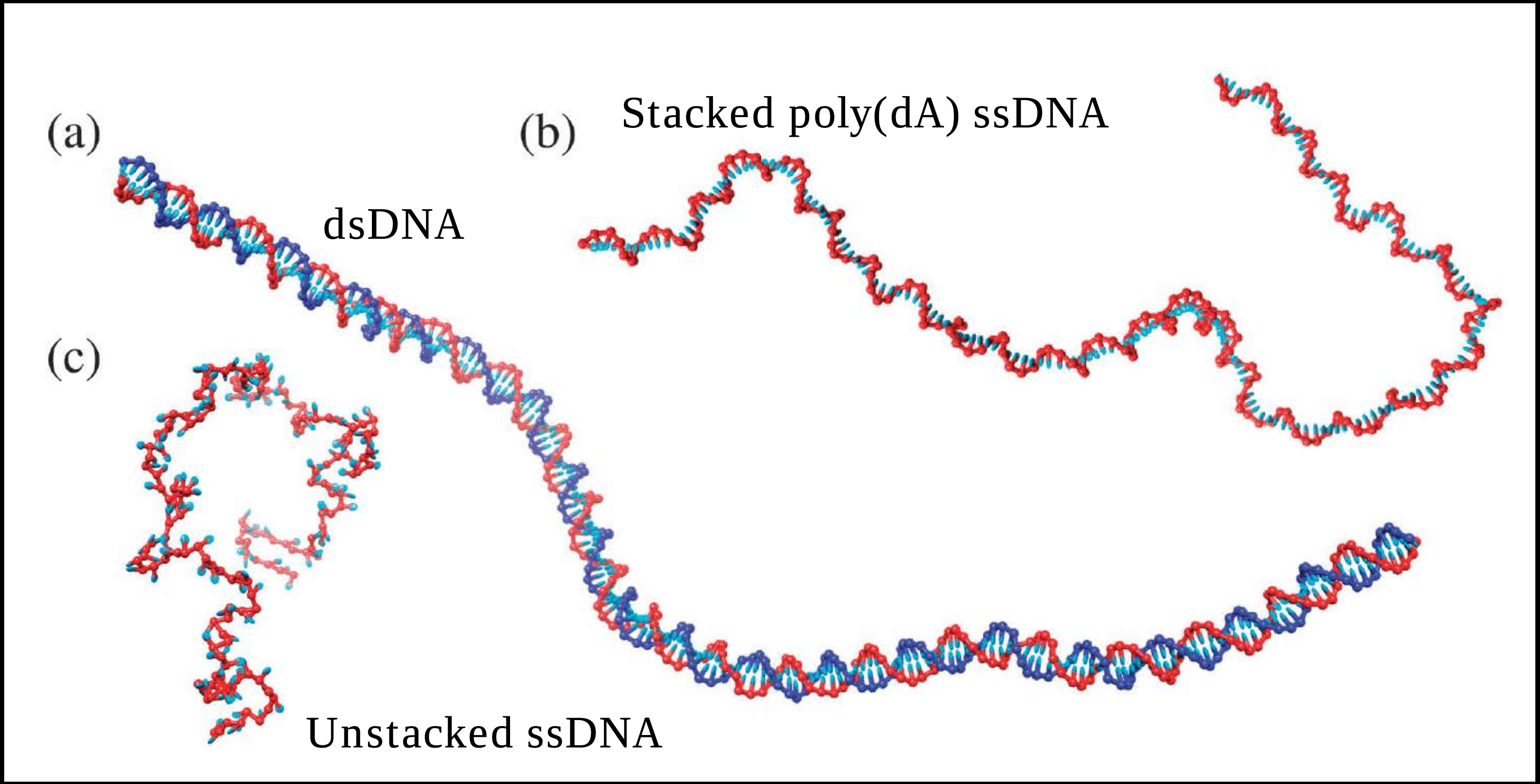
Must guess, by hand, where the regime starts and finishes, which biases exponent estimate

(Also, systematic error (e.g. in L) can disturb things)

Motivates the need for an alternate approach that measures the exponent *without* needing to guess the regime

A solution: linear-response based fluctuation analysis!
(to the board)

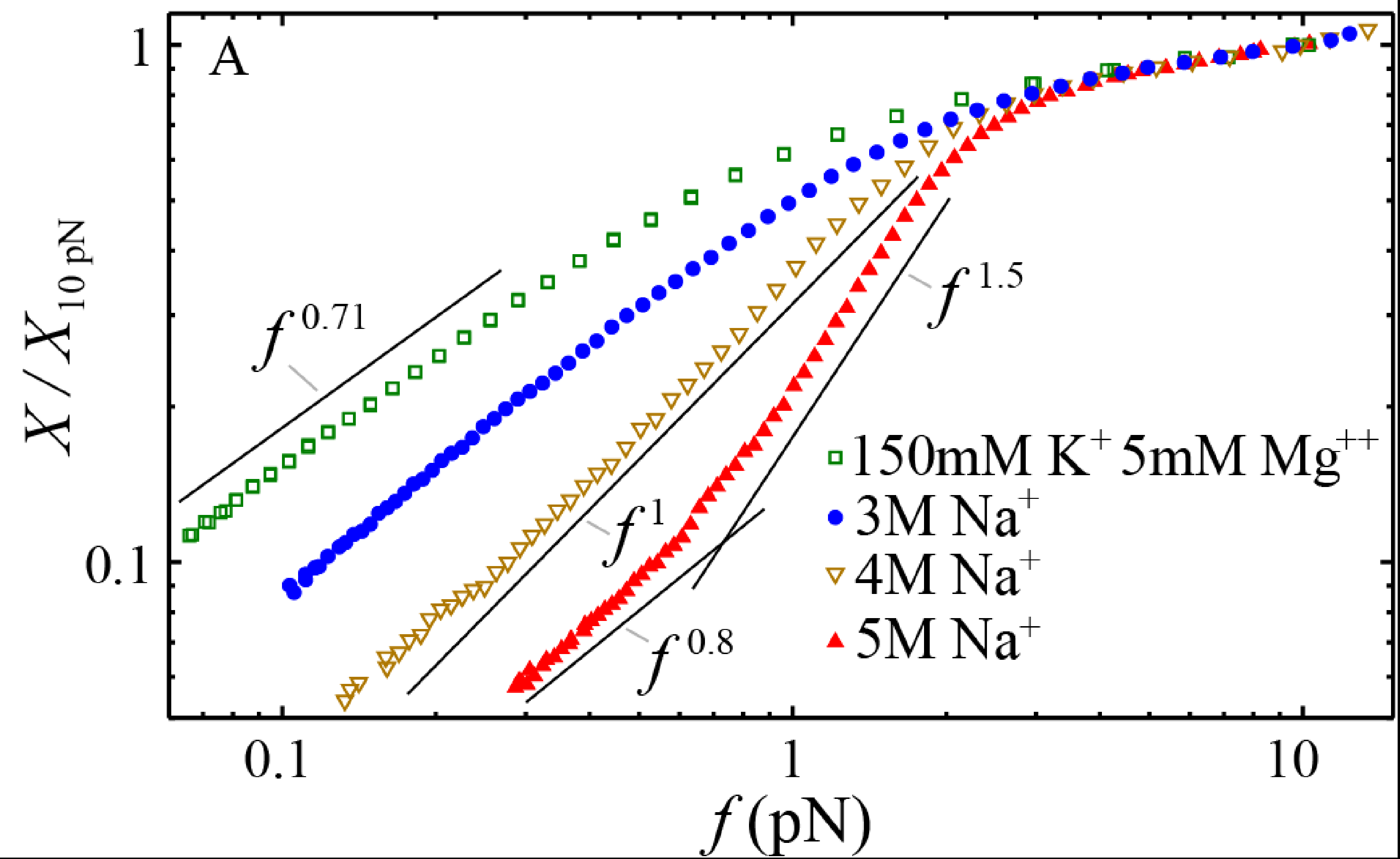
Model system: stiff, stacked ssDNA

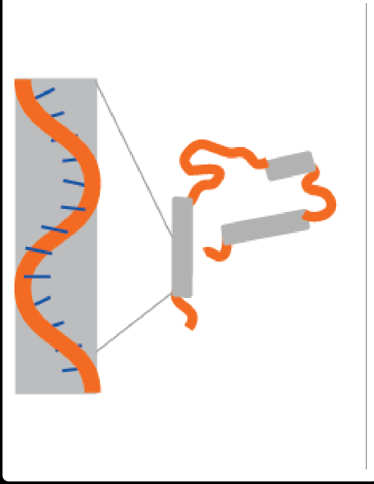


Doye *et al.* (2013)

poly(dA):

Base-stacking leads to rod-like monomers,
High-salt emergence of the thermal blob regime





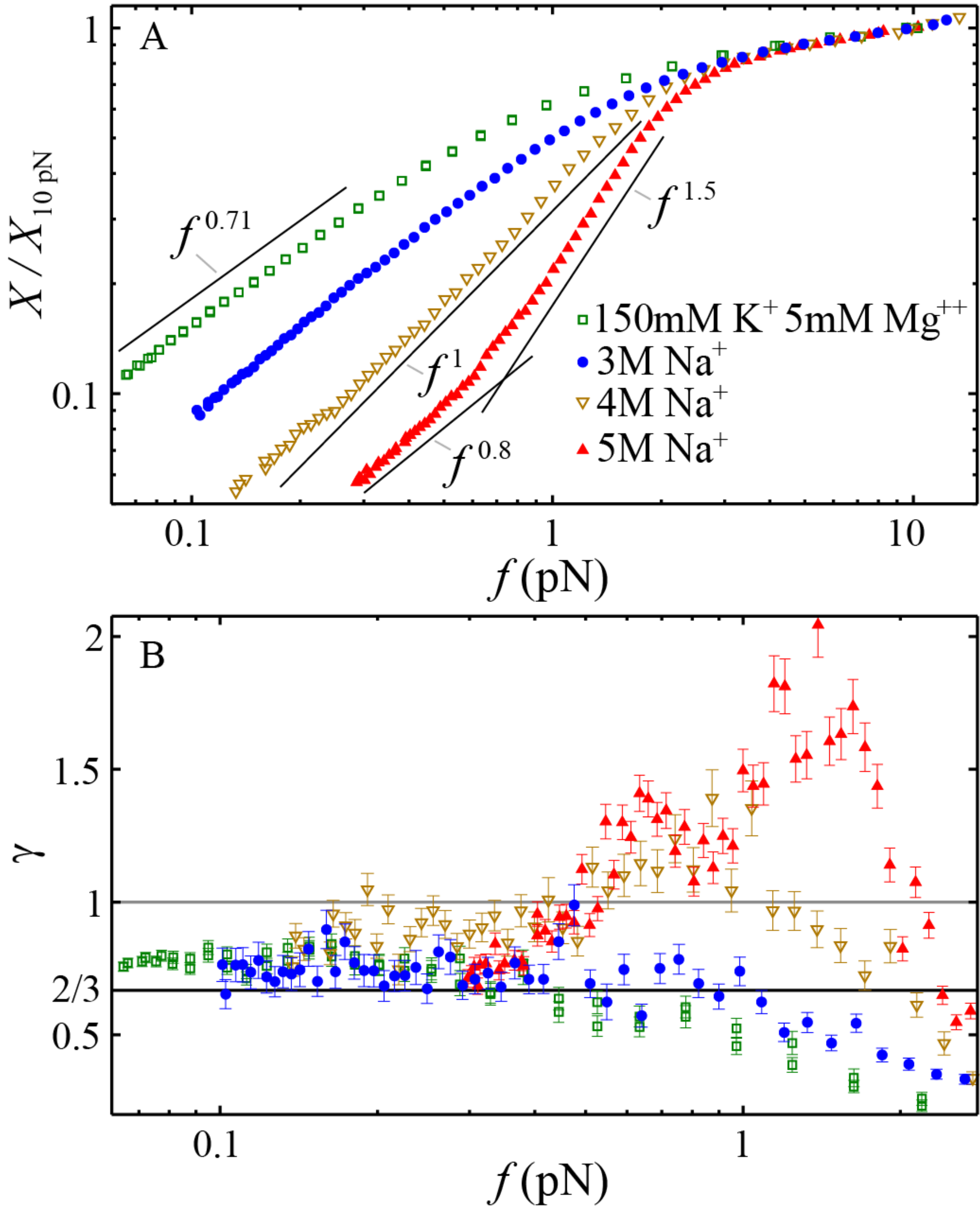
McIntosh *et al.*, *Biophys. J.* (2014)

poly(dA):

Base-stacking leads to rod-like monomers, emergence of the thermal blob regime at high salt:

Confirmation using fluctuations

McIntosh *et al.*, *Biophys. J.* (2014)



Take home messages

1) There are three well-established exactly solvable tensile elasticity models (linear response at low force, FJC at all forces, MS-WLC at high forces). FJC is not experimentally supported, WLC is supported, linear response must be true
2) Exponential persistence is expected for a WLC, based on considerations of bending an elastic rod
3) At low forces, swelling interactions affect elasticity, leading to a variety of potential elastic regimes, depending on a chain's precise microscopic structure (l, v)
4) Elastic information can be obtained simply by analyzing fluctuations