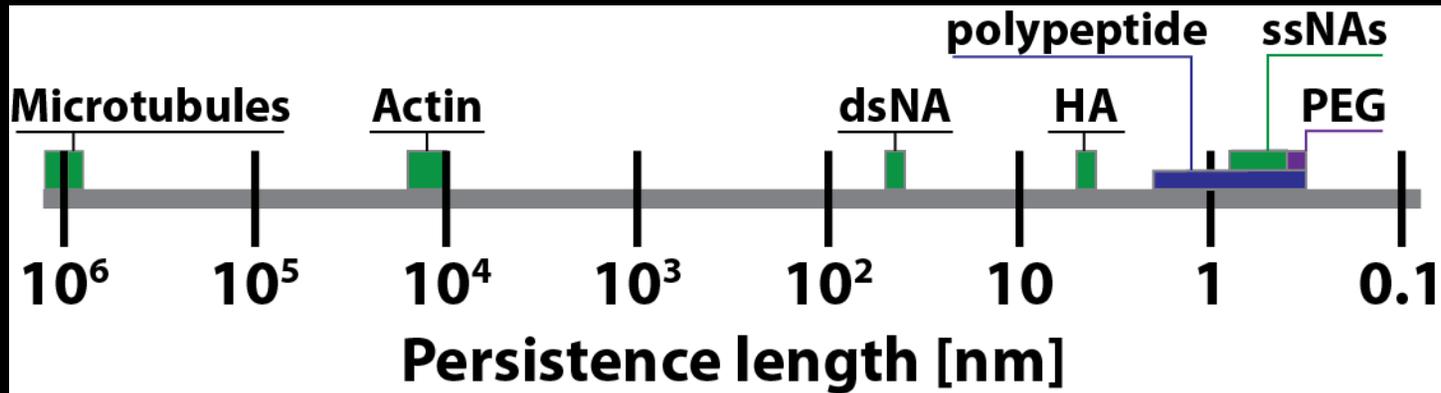
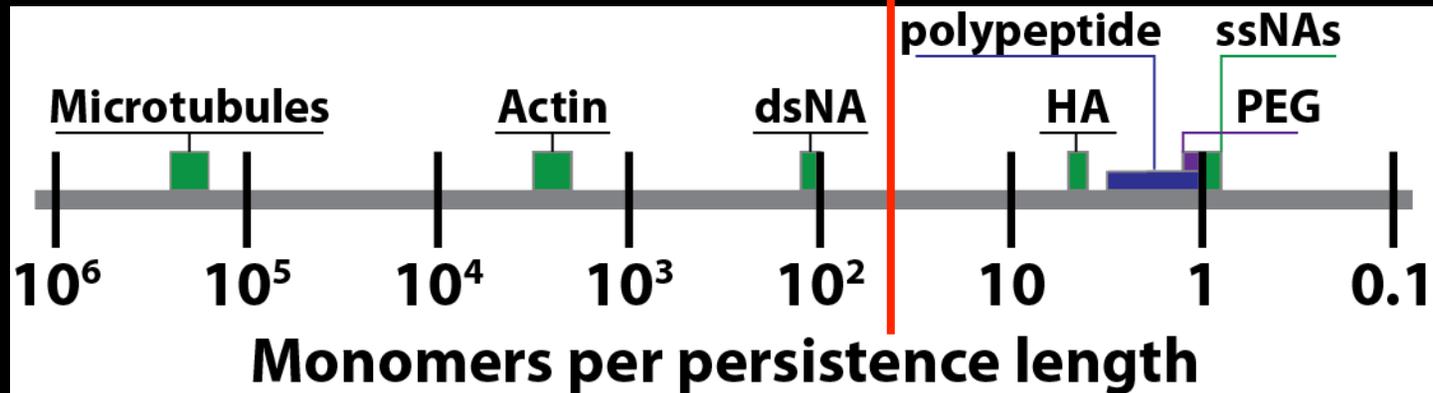


# Comparative mechanics of biopolymers (if neutral)

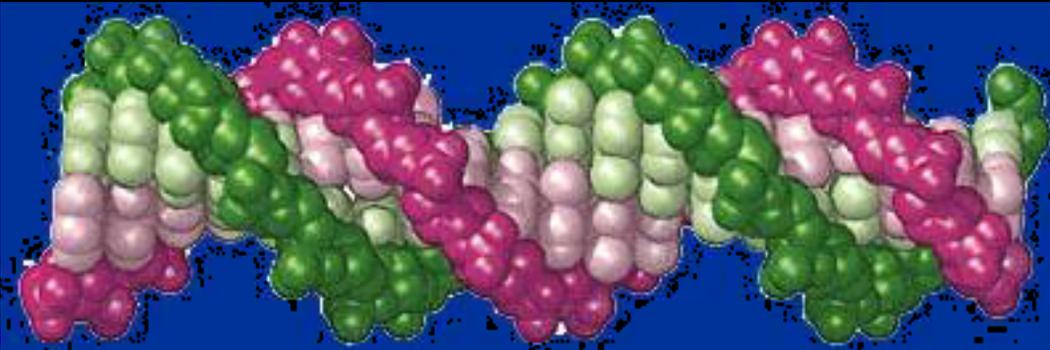


Continuum ← → Discrete

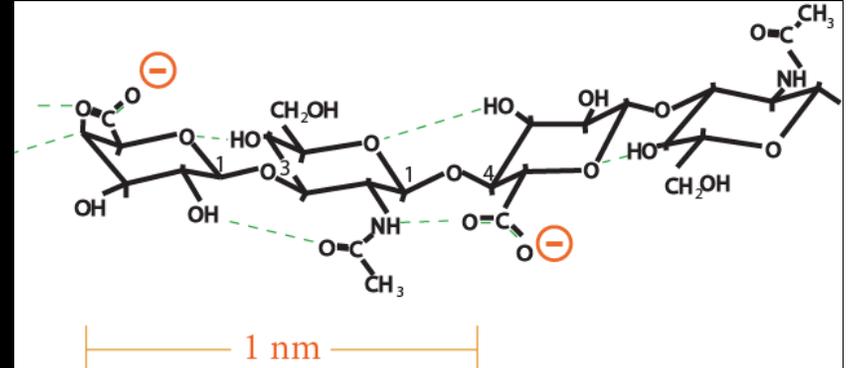


# Biology abounds with charged polymers (polyelectrolytes)

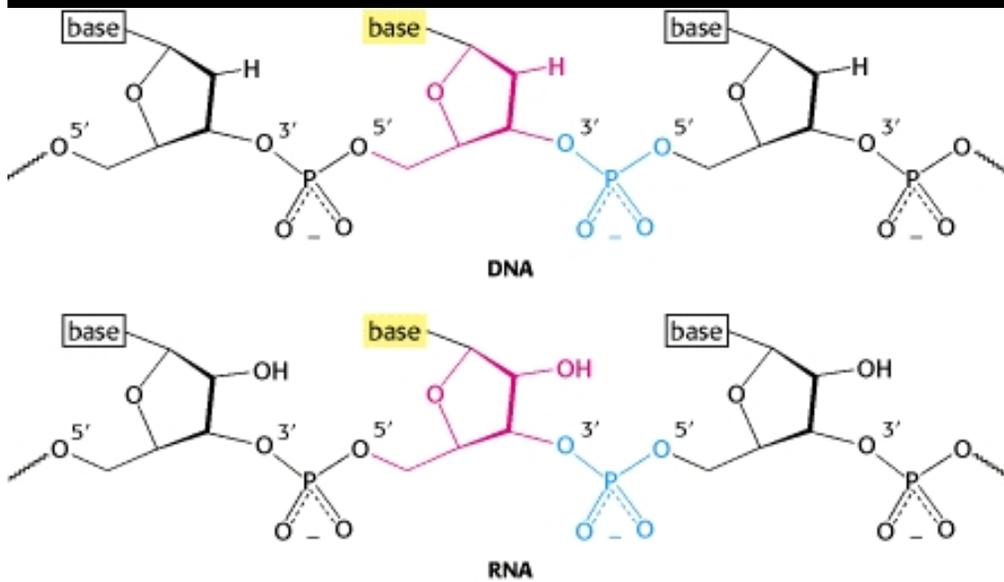
## Double-stranded DNA



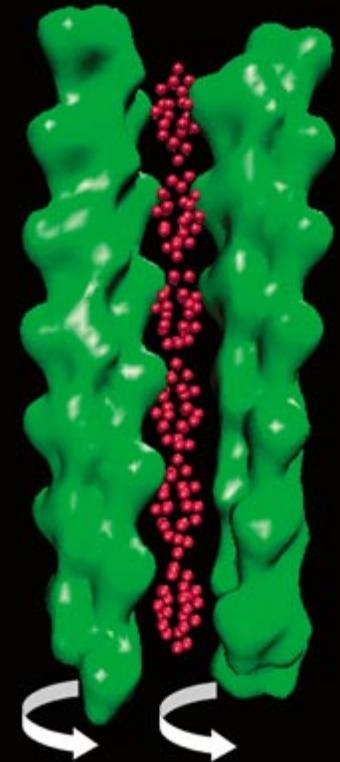
## Polysaccharides (e.g. HA)



## ssDNA/ssRNA

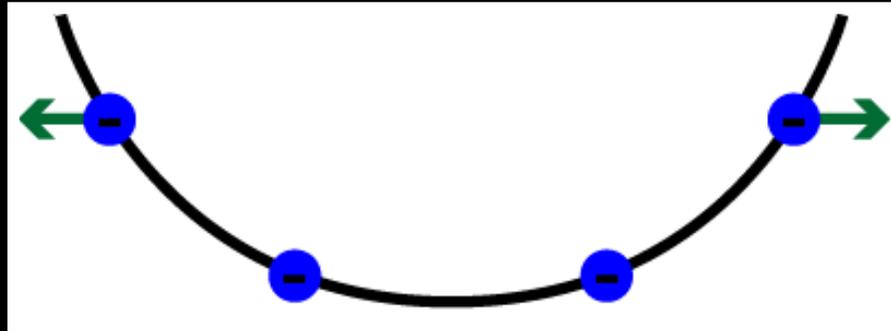


Bundles of charged  
**actin**  
(Angelini *et al.*,  
PNAS, 2003)

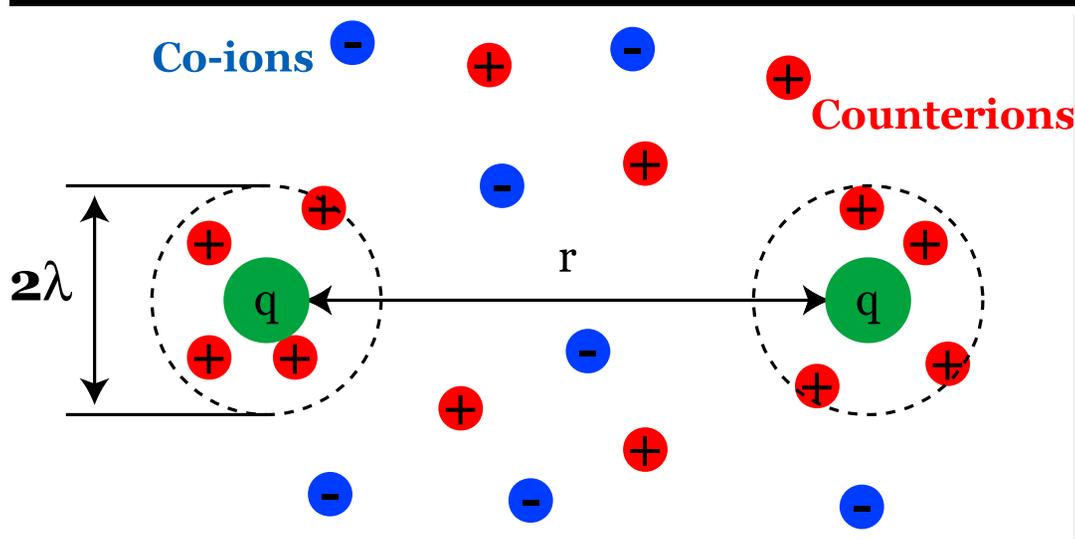


## Adding charge to biopolymers: Basics

1) Electrostatic repulsion favors straight configurations...



2) ...and the strength of electrostatics in sol'n is screened over a char. (Debye) length,  $r_D \sim c^{-0.5}$



$$\epsilon(r) \sim \frac{1}{r} e^{-r/r_D}$$

## Basic motivation of this lecture:

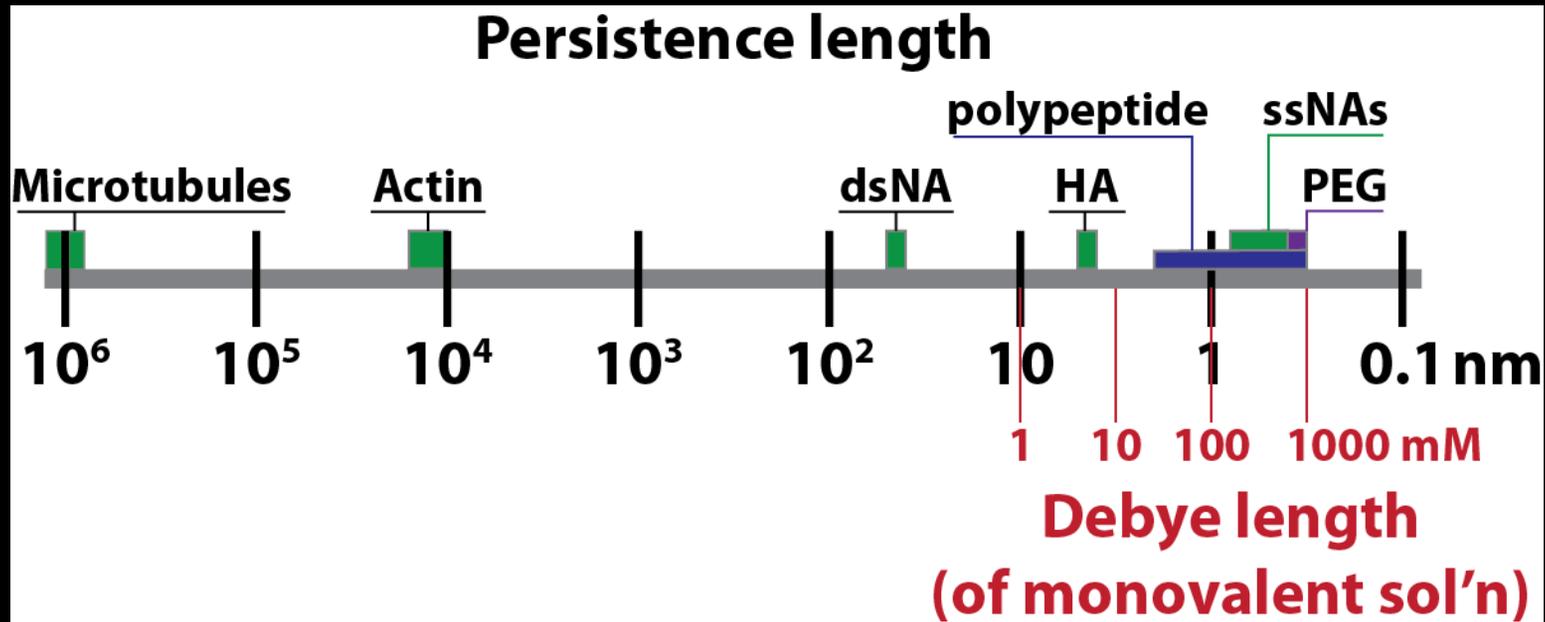
Physics indicates electrostatics should increase the stiffness of a charged polymer...

...how can we quantify this?

What are the key models, and is there data that supports or refutes those models?

An expectation (perhaps naïve) is that there should be a relation between the persistence length,  $l_p$ , and the Debye length,  $r_D$

# Comparing length scales: Debye length vs. Persistence length



## Conclusions:

- 1) The electrostatic effect is a **perturbation** for continuum-limit biopolymers (dsNAs, actin, microtubules)
- 2) The electrostatic effect is large, even **dominant**, for discrete-rotation biopolymers (if they are charged)

# Electrostatics as a perturbation: The Odijk-Skolnick-Fixman (**OSF**) Limit Odijk (1977); Skolnick and Fixman (1977)

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

Intrinsic

Electrostatic

The polymer has a persistence length which can be defined as the sum of an 'intrinsic',  $l_o$ , and 'electrostatic',  $l_e$ , stiffness.

Assume  $l_o \gg r_D$

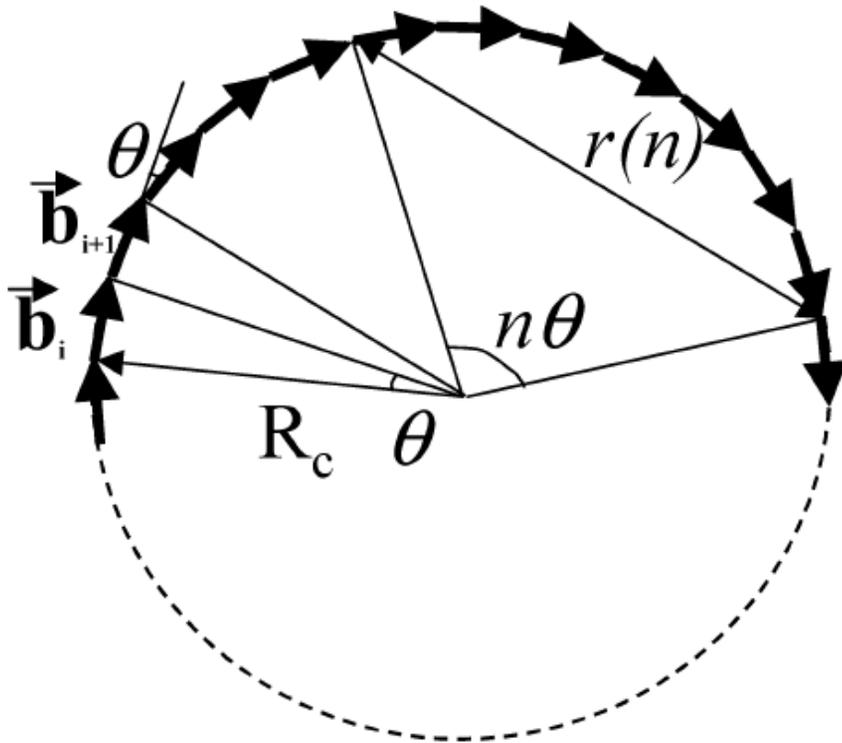
Electrostatic stiffness arises from the energy of electrostatic self-repulsion

Intrinsic stiffness arises from all other energies (bond deformation, steric interactions, ...)

# OSF Theory

## Assumption

The thermally bent configurations of a stiff polymer are well-approximated by a circular geometry

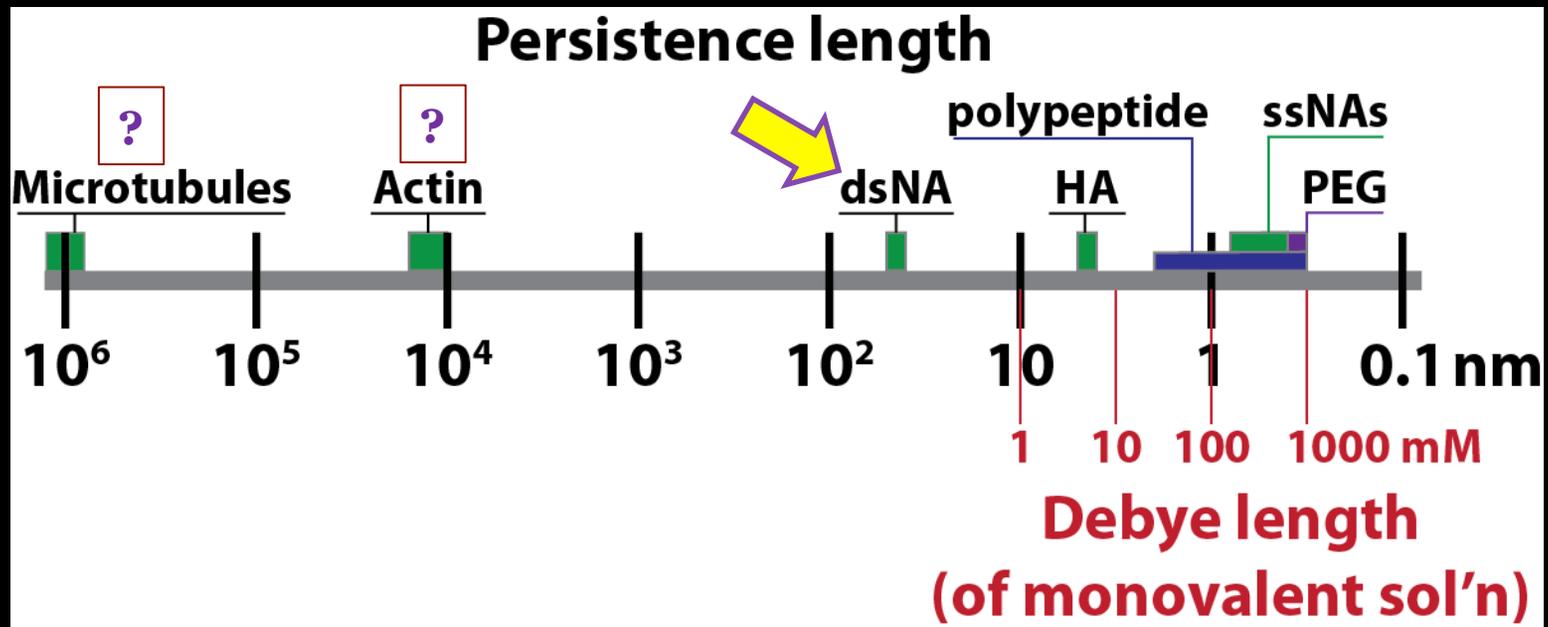


Then: Sum the Debye-Huckel electrostatic interactions around the circle to find an energy quadratic in  $\theta$ , giving

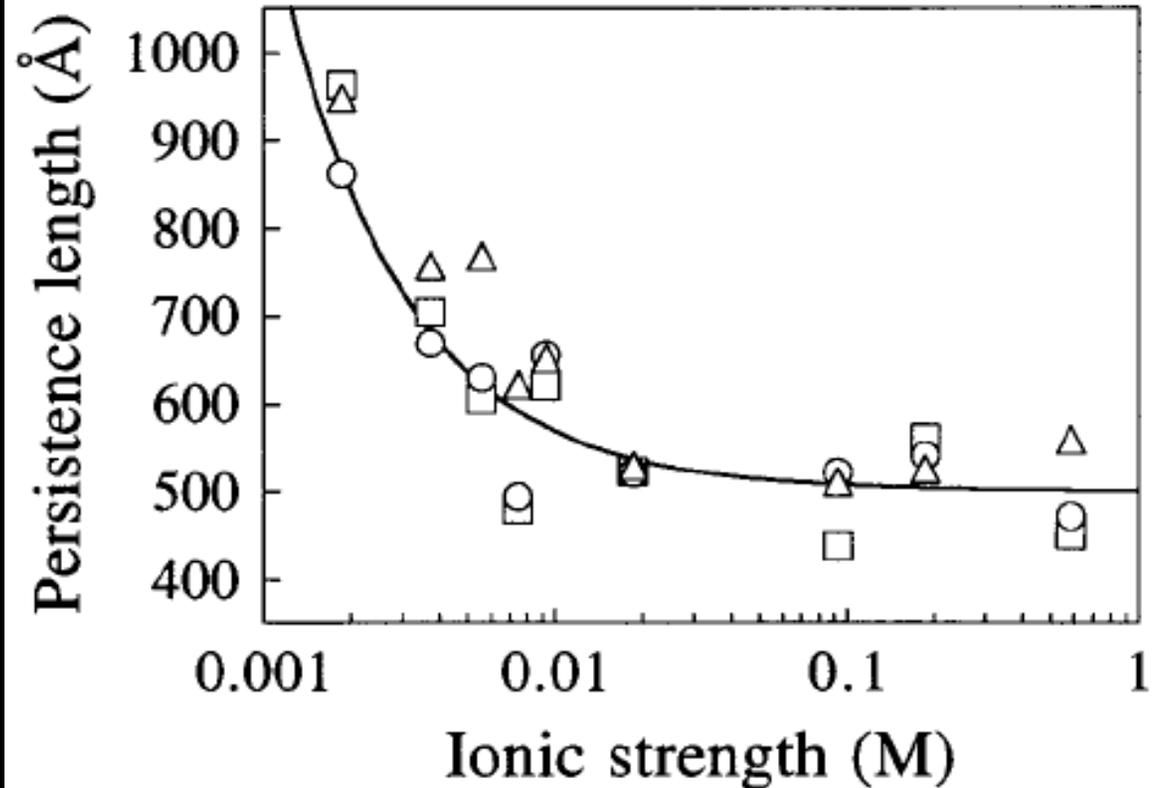
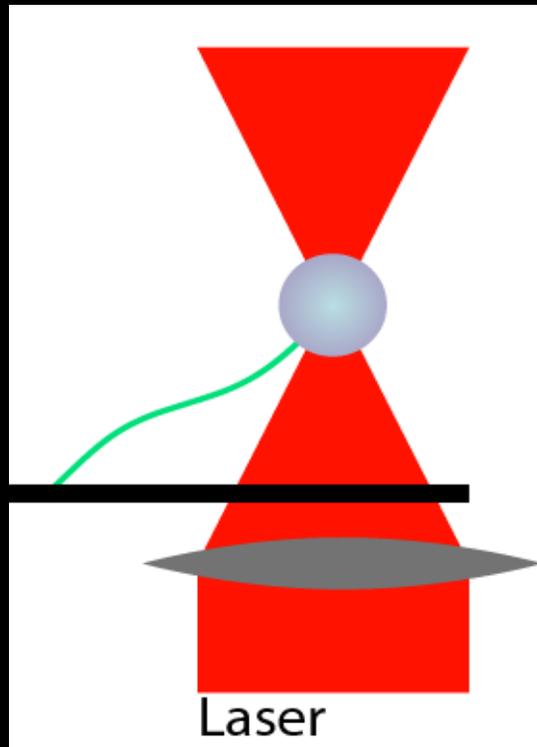
$$l_{e,OSF} \sim r_D^2 \sim 1/c$$

Image: Dobrynin, 2005

# Experiment in limit $l_o \gg r_D$ ?

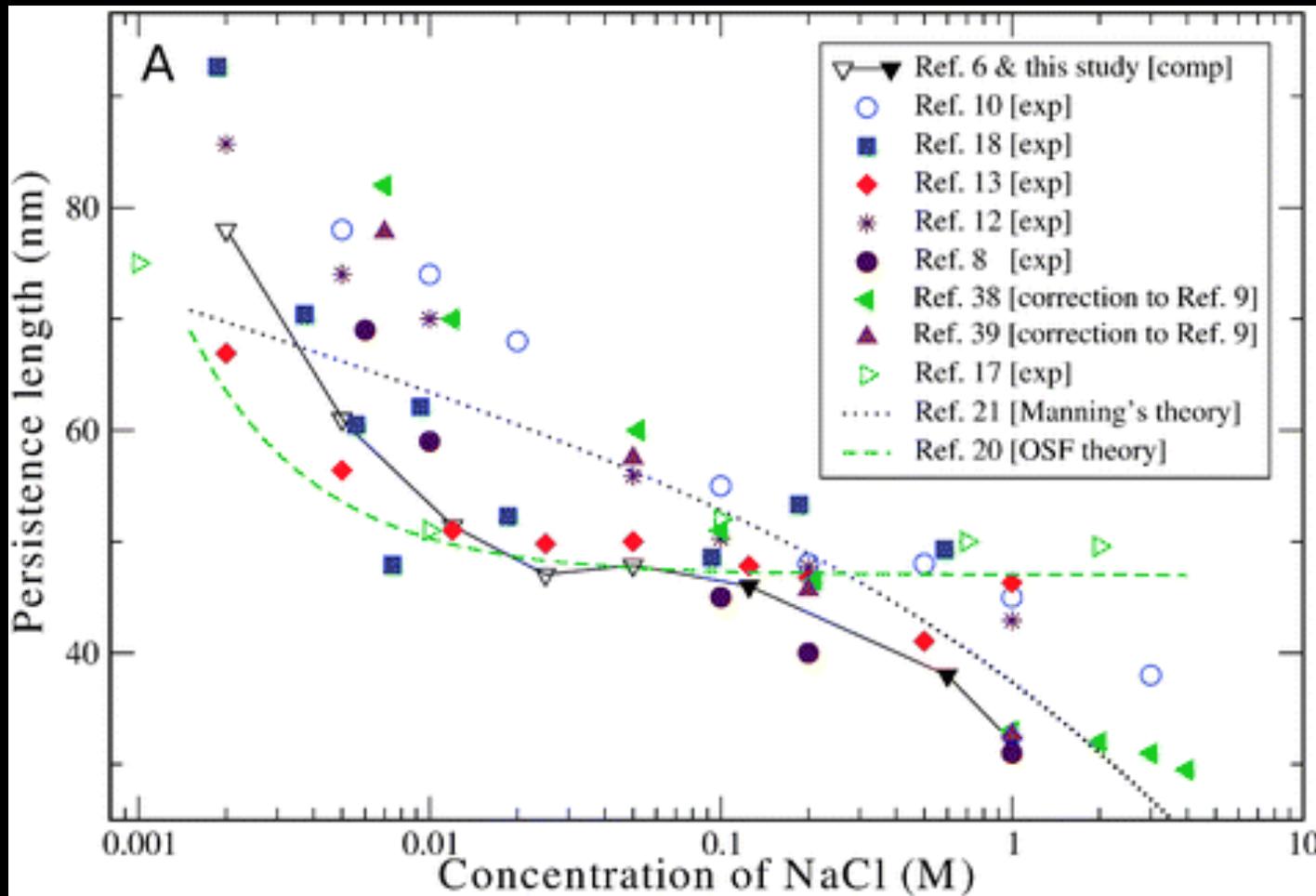


# 1-molecule stretching data on **dsDNA** ( $l_o \gg r_D$ ): Apparent agreement with OSF...

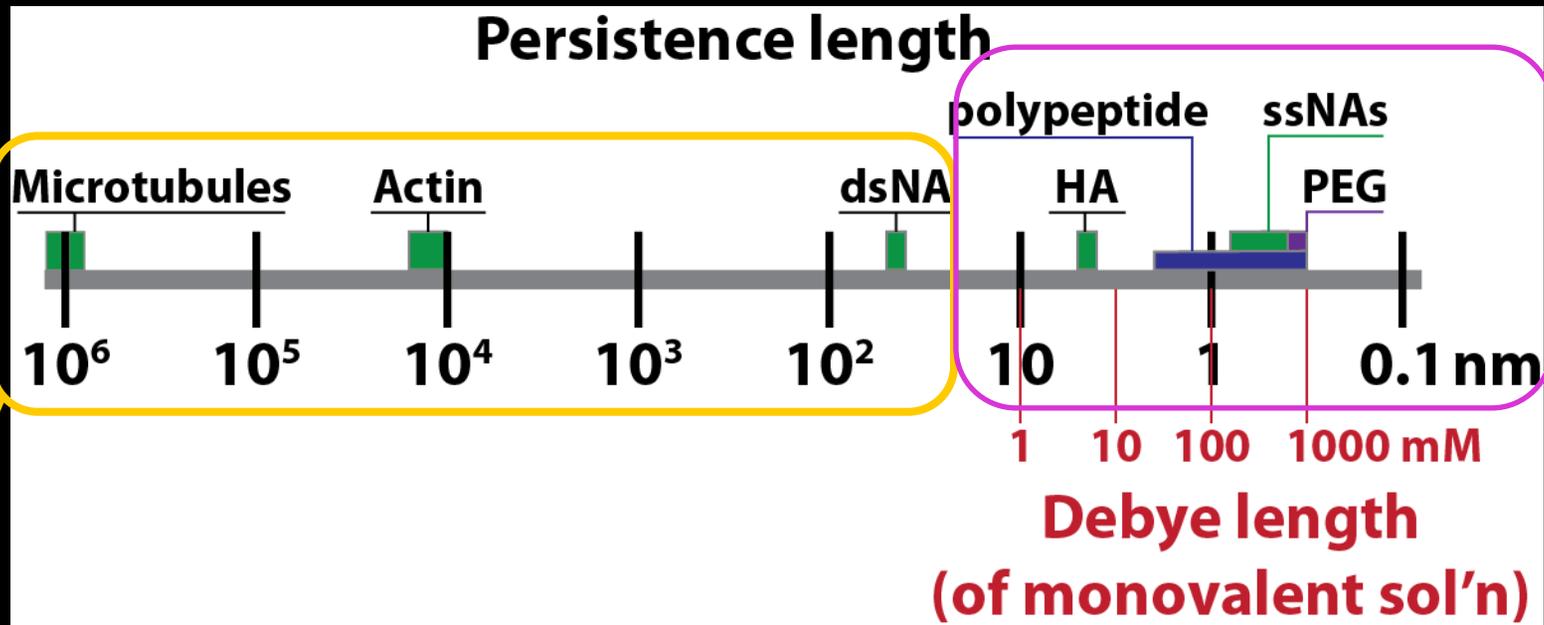


$l(c)$  estimated from Marko-Siggia fits to dsDNA  
(Baumann et al., 1997)

**..but a more recent compendium of results on dsDNA persistence vs. salt is less clear:**



**Savelyev, 2012**



The OSF picture offers a simple, robust, and logical estimate of the effects of salt on the stiffness of intrinsically-stiff polymers...

...but, 35 years later, it has still not been conclusively verified by experiment, and has been questioned by competing theories

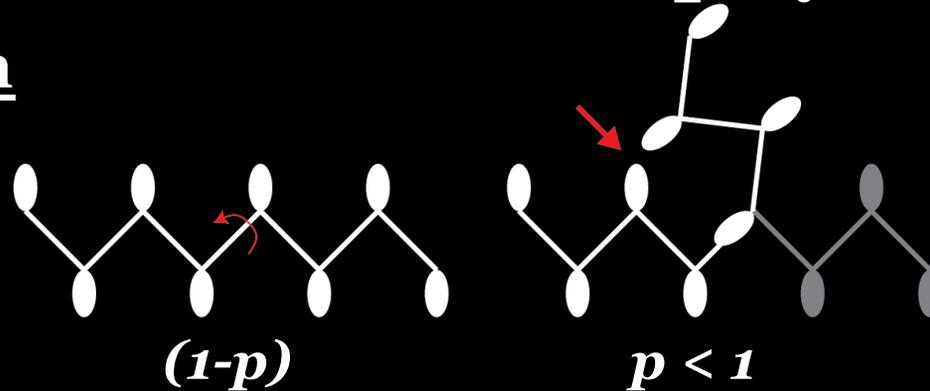
(Barrat and Joanny, 1993  
 Everaers *et al.*, 2002  
 Dobrynin, 2005)

OSF, by construction, does not apply down here...

**How does electrostatics affect flexible chain structure and mechanics?**

# The problem with flexible polyelectrolytes

## Neutral chain



Probability:

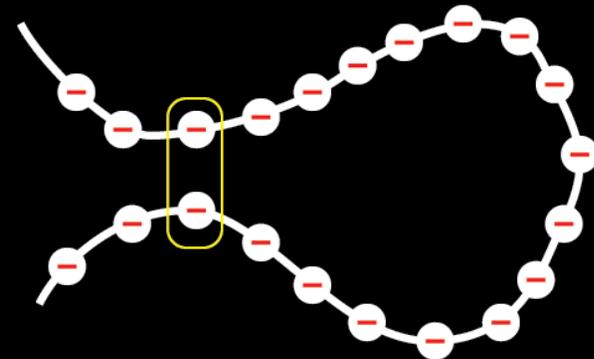
$$(1-p)$$

$$p < 1$$

**Only *local* interactions, transmitted sequentially along chain; gives geometric series and exponential correlations:**

Probability of  $n$  without rotation  $(1-p)^n$

## Polyelectrolytes



**Interactions are fundamentally non-local (long range);  
Conformation *not* transmitted along chain**

The argument for exponential correlations for flexible chains relies on *local* interactions, while electrostatics are clearly *long-range*.

Result: **A 35 year-old argument** (*de Gennes, Pincus, Odijk, Skolnick and Fixman, Khokhlov and Khachaturian, Barrat and Joanny, Thirumalai, Netz, Rubinstein, Dobrynin, Muthukumar,...*) with roughly three views:

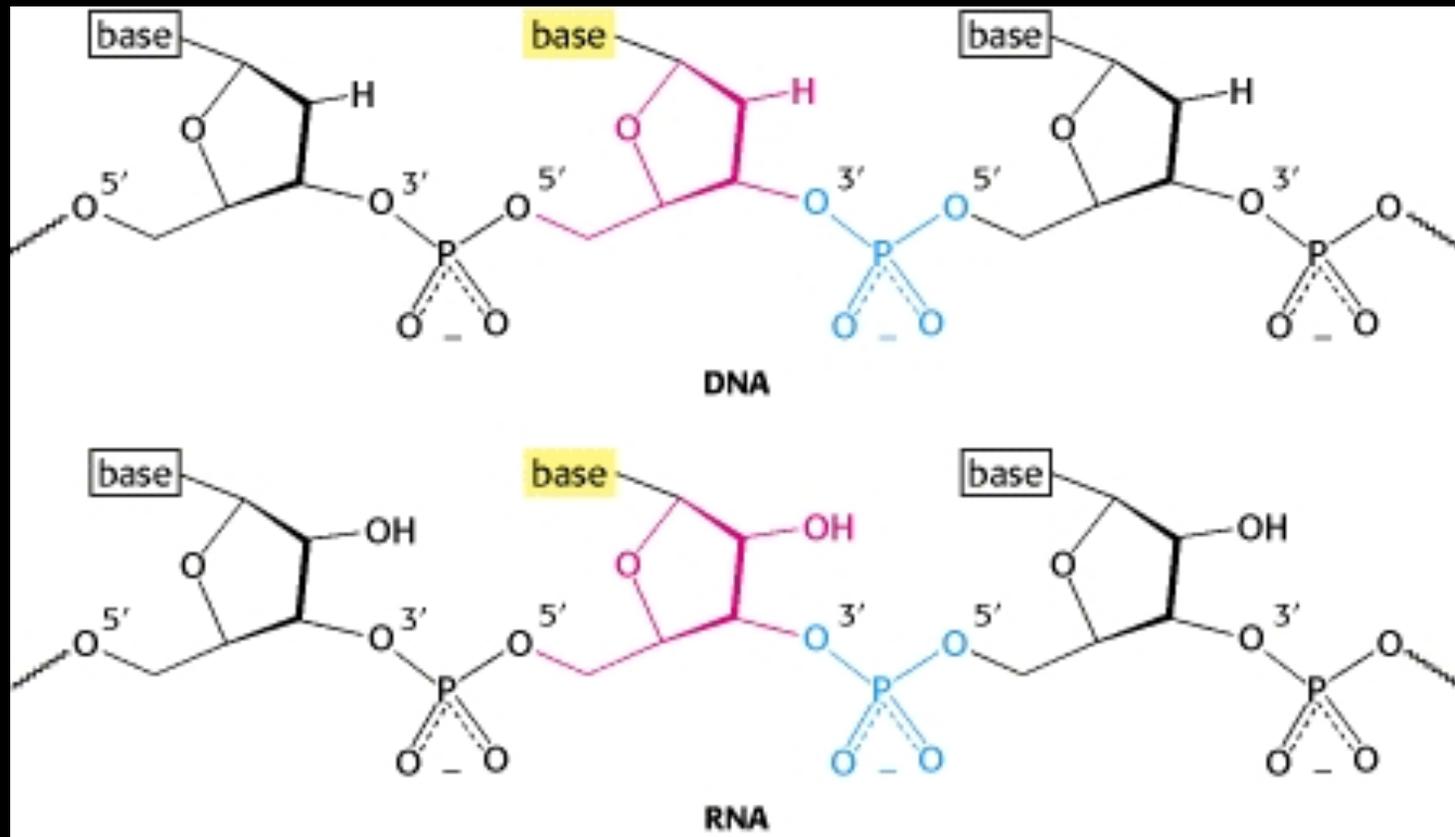
**View 1)** Don't worry about it! OSF should still hold:  $l_e \sim r_D^2$

**View 2)** Something different must be happening...probably:  $l_e \sim r_D$

**View 3)** You're both crazy...I don't think you can even define a persistence length in this situation

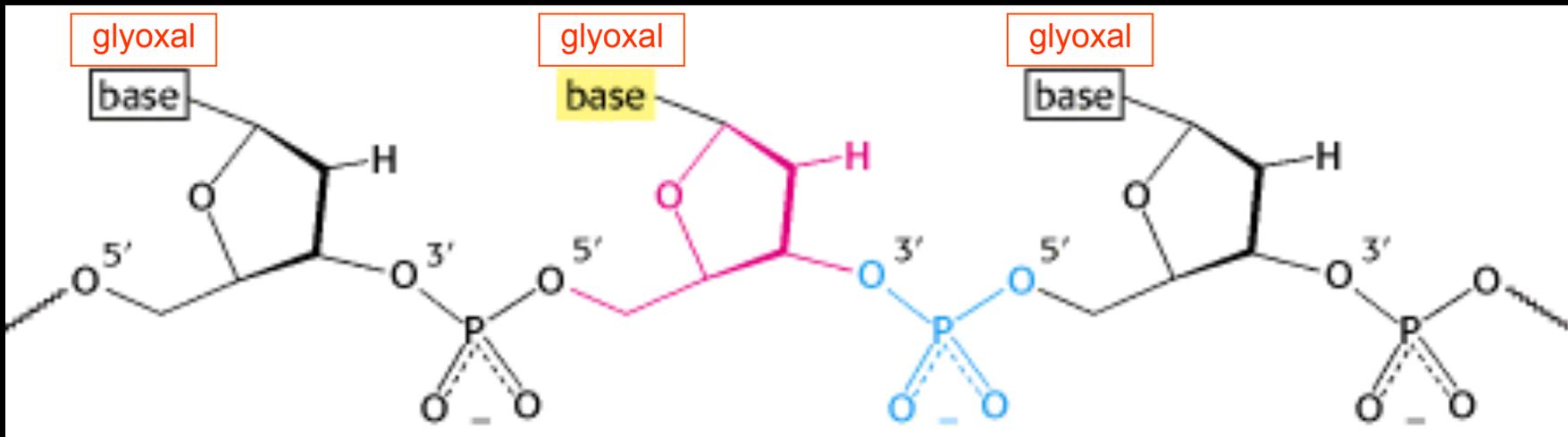
**Our experiments agree with these things**

# Model (flexible) polyelectrolyte #1: Single-stranded nucleic acids (ssNAs):



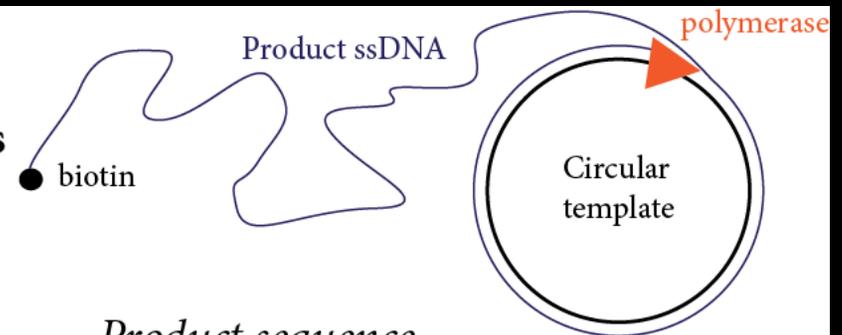
*Biochemistry* (Berg, Tymoczko, Stryer)

# Folding is avoided using covalent modification or special sequences



McMaster and Carmichael, 1977

## Rolling Circle Amplification for ssDNA synthesis

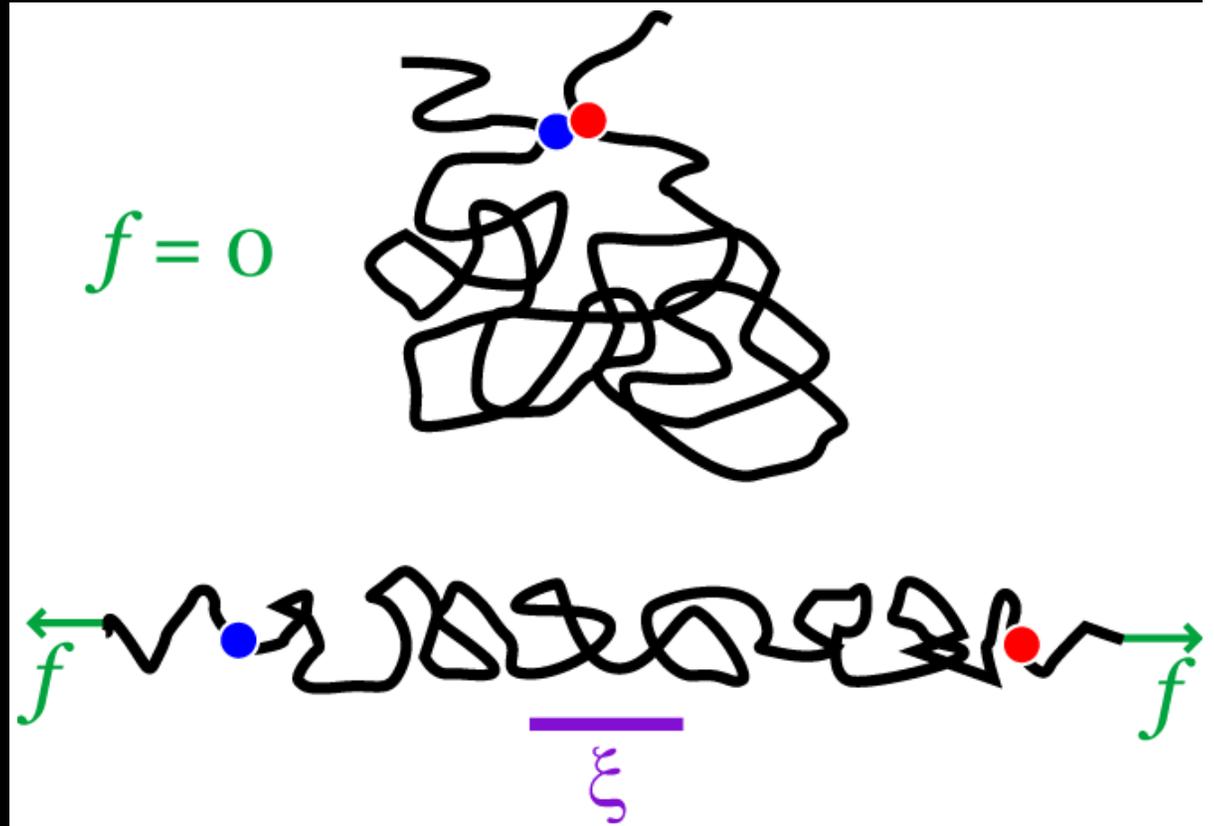
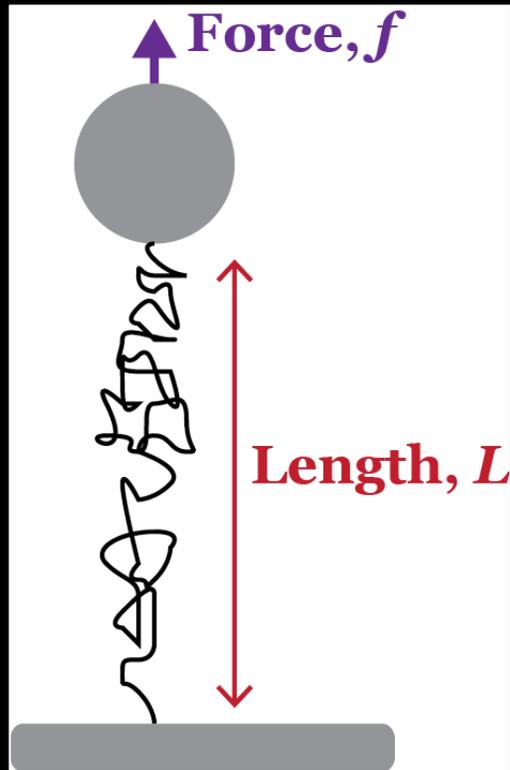


Name	Template sequence	Product sequence
poly(dA)	(T) <sub>50</sub>	(A) <sub>n</sub>
polypyrimidine <sup>3</sup>	AGGAGA <sub>5</sub> GA <sub>7</sub> GA <sub>4</sub> GAAGG	(CCTTCT <sub>4</sub> CT <sub>7</sub> CT <sub>5</sub> CTCCT) <sub>n</sub>

Brockman *et al.* (2011)

**RNA:** poly(dU) synthesized using polynucleotide phosphorylase

# Experimental approach: Low-force single-molecule manipulation



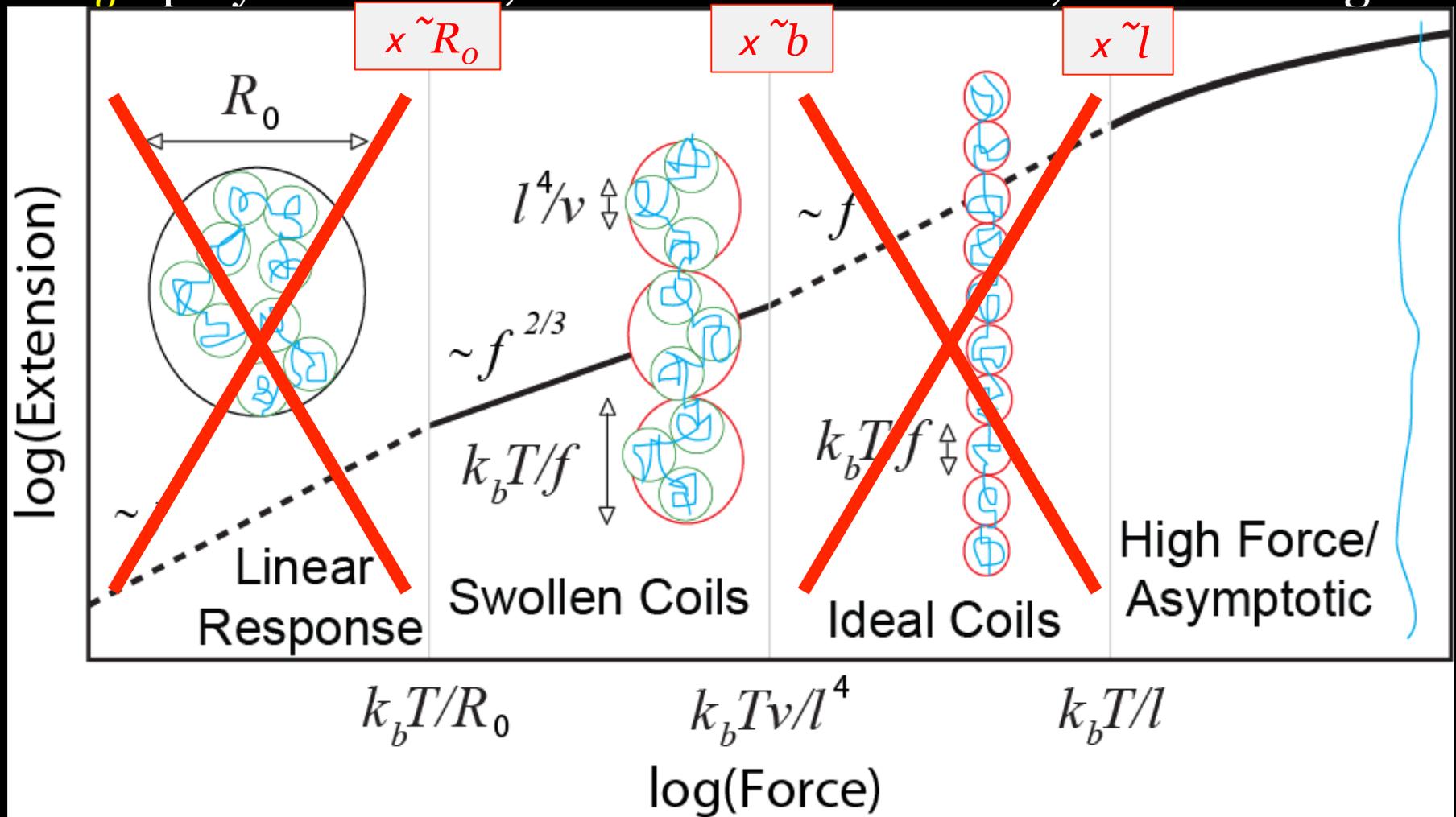
A tension  $f$  creates a  
*tensile screening length,  $x$ :*

$$x = kT/f$$

# Scaling of tensile elasticity:

A transition whenever  $x \sim$  (char. length)

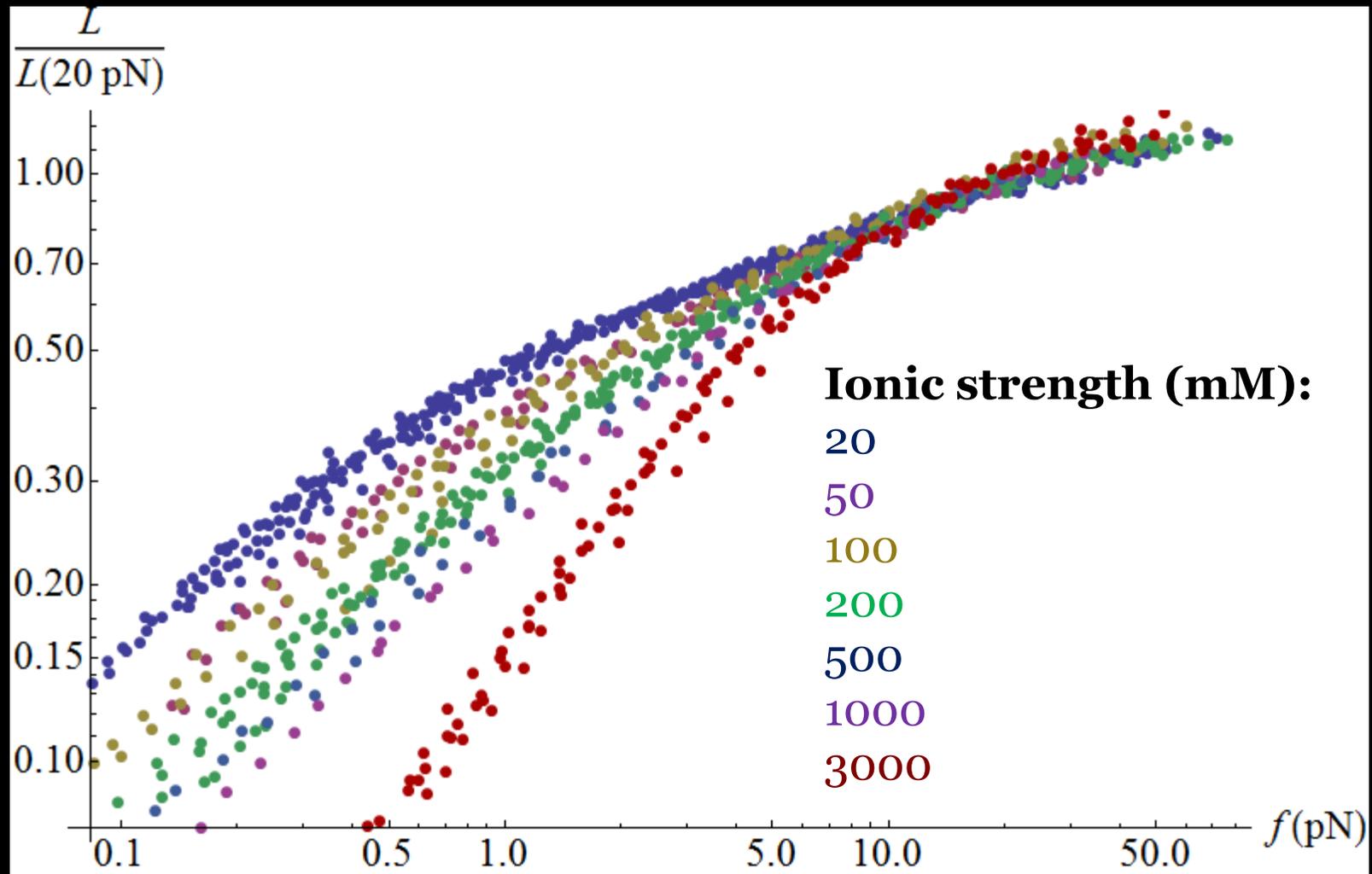
$R_0$  – polymer extent;  $b$  – thermal blob extent;  $l$  – Kuhn length



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

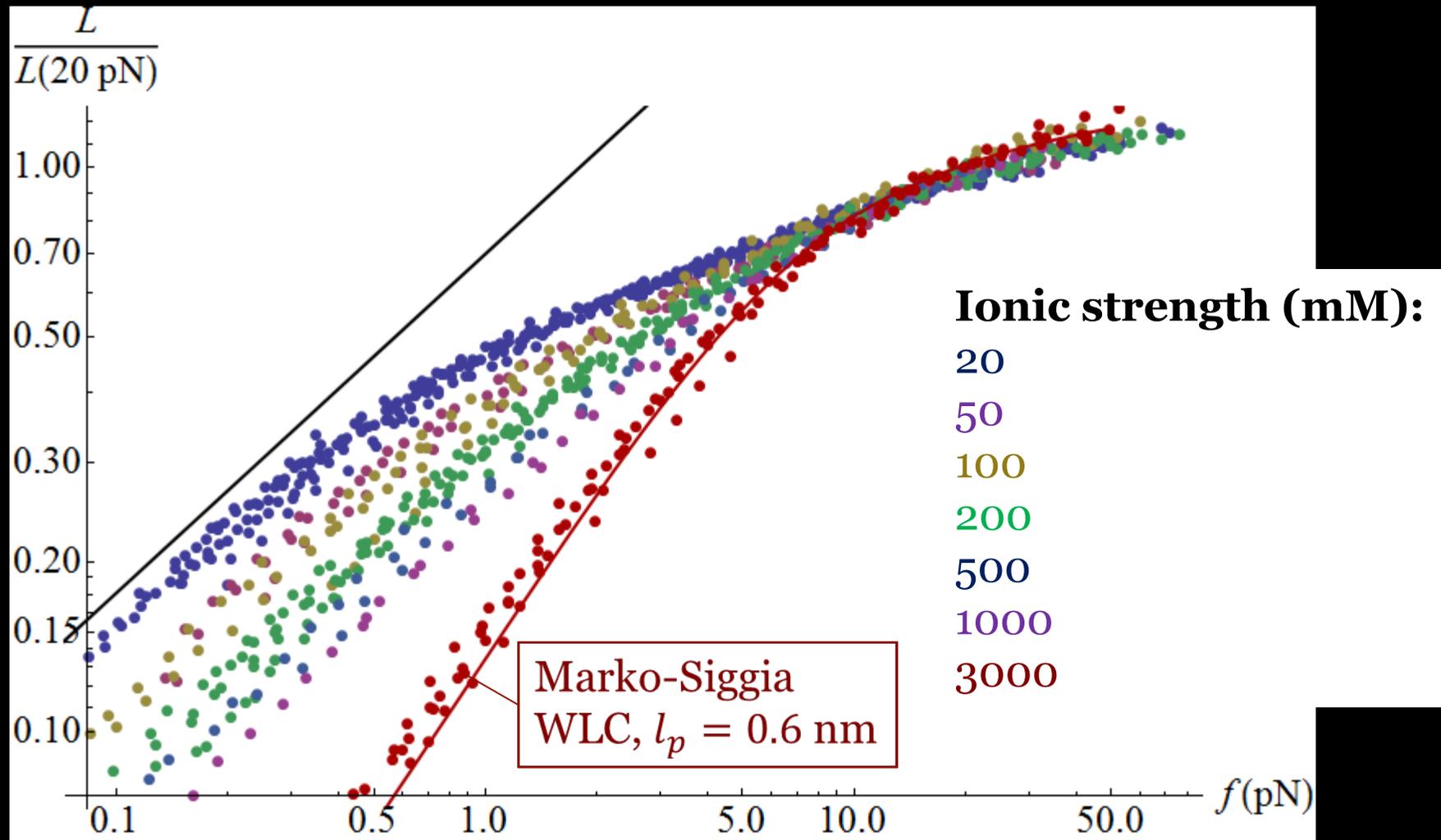
McIntosh, Ribeck and Saleh *PRE* (2009)

# ssDNA elasticity vs. salt



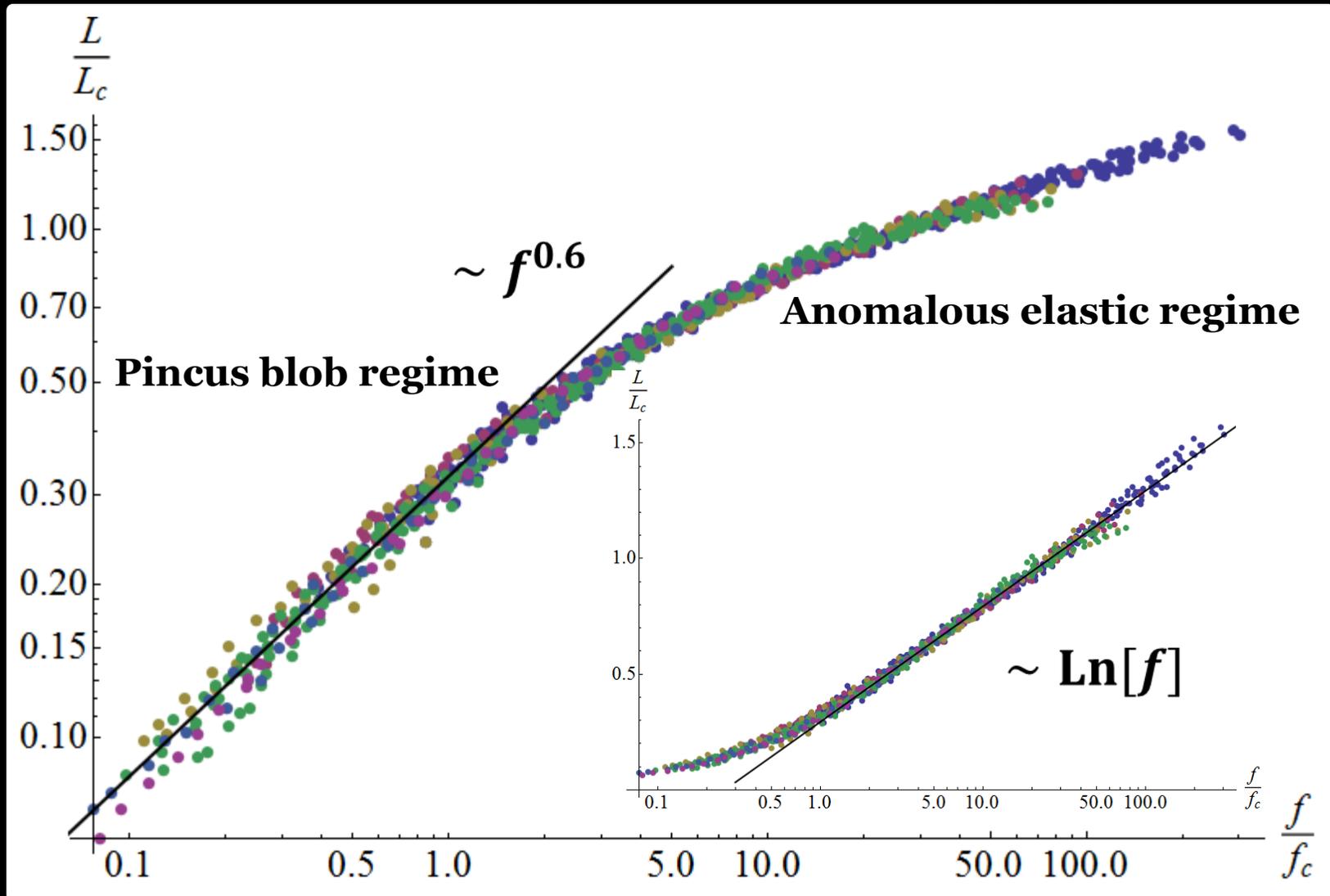
Saleh, McIntosh, Pincus, and Ribeck *PRL* (2009)

# ssDNA elasticity vs. salt



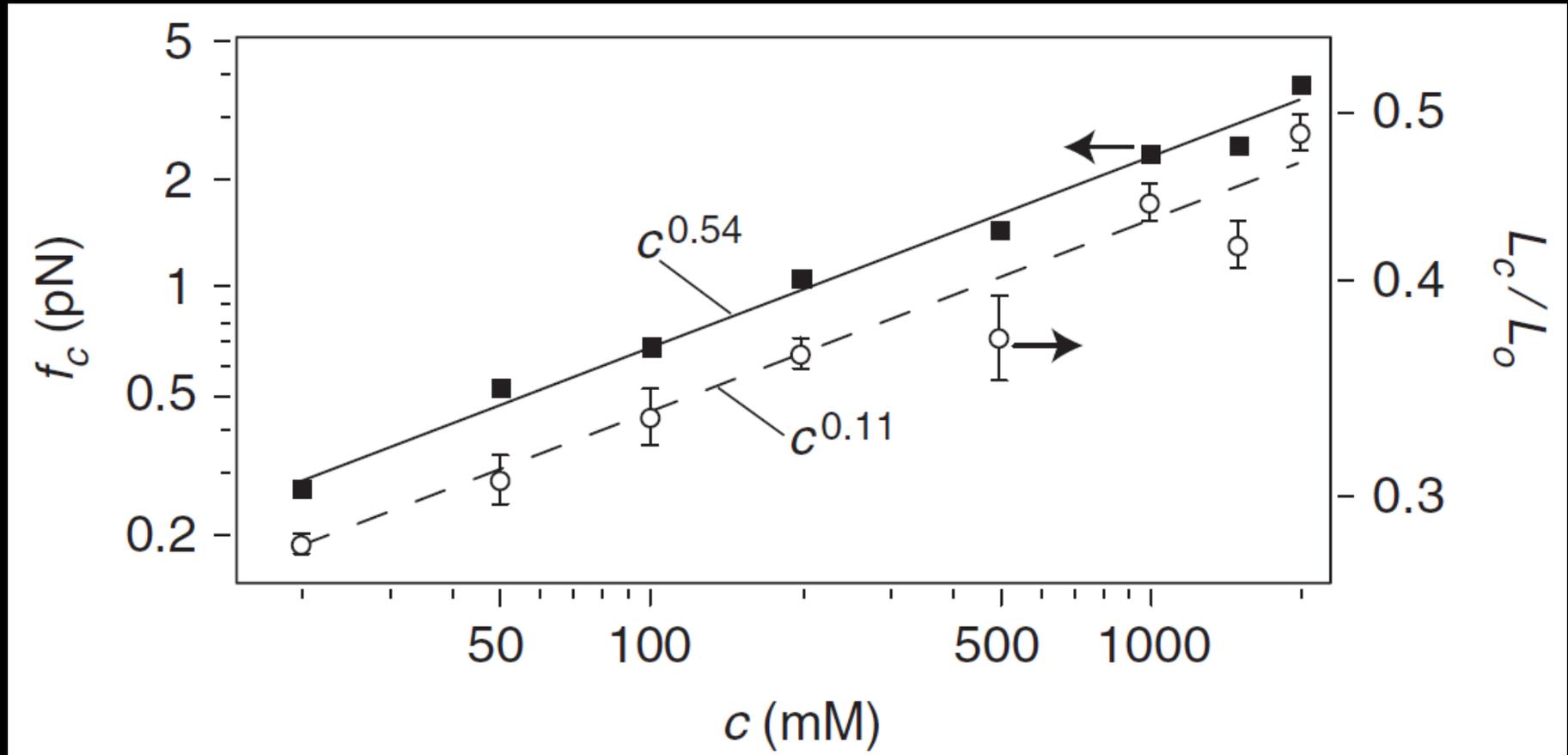
Saleh, McIntosh, Pincus, and Ribbeck *PRL* (2009)

# Universal behavior for $I \leq 1M$ after scaling by shift factors ( $f_c, L_c$ )



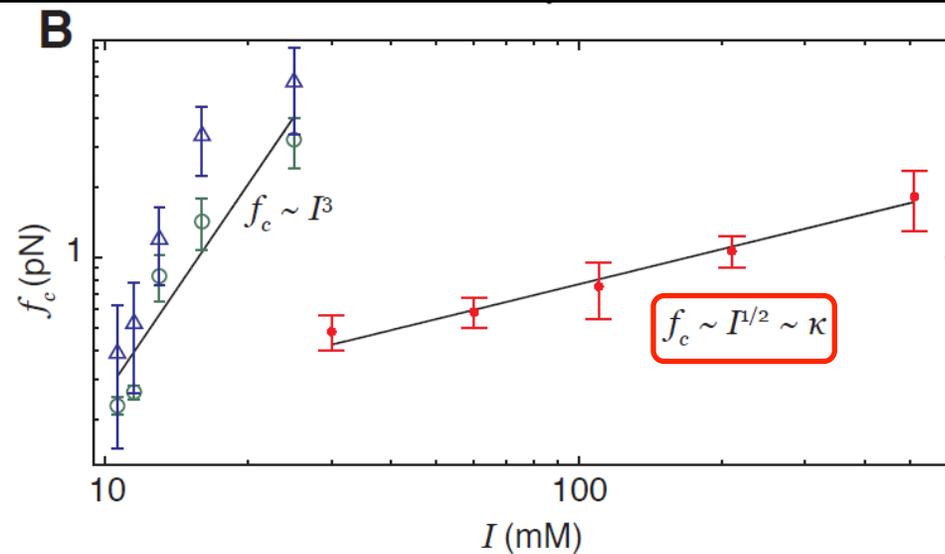
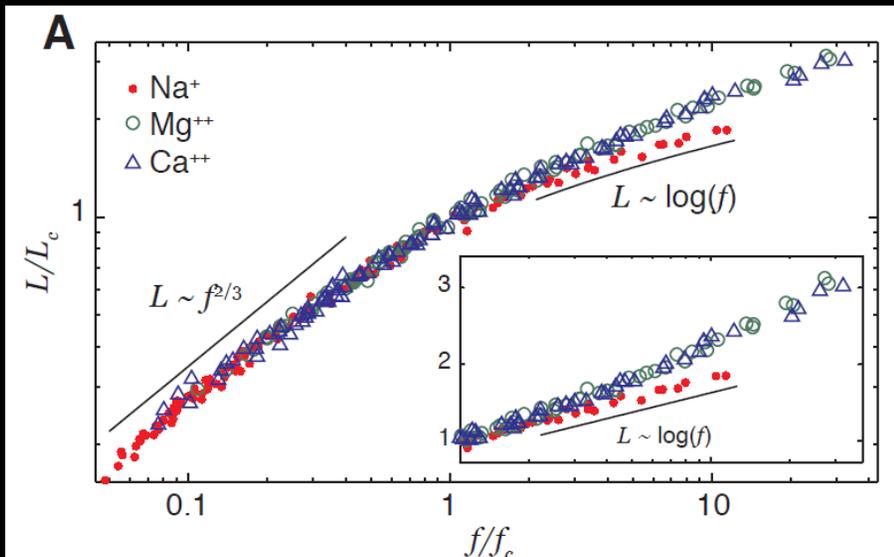
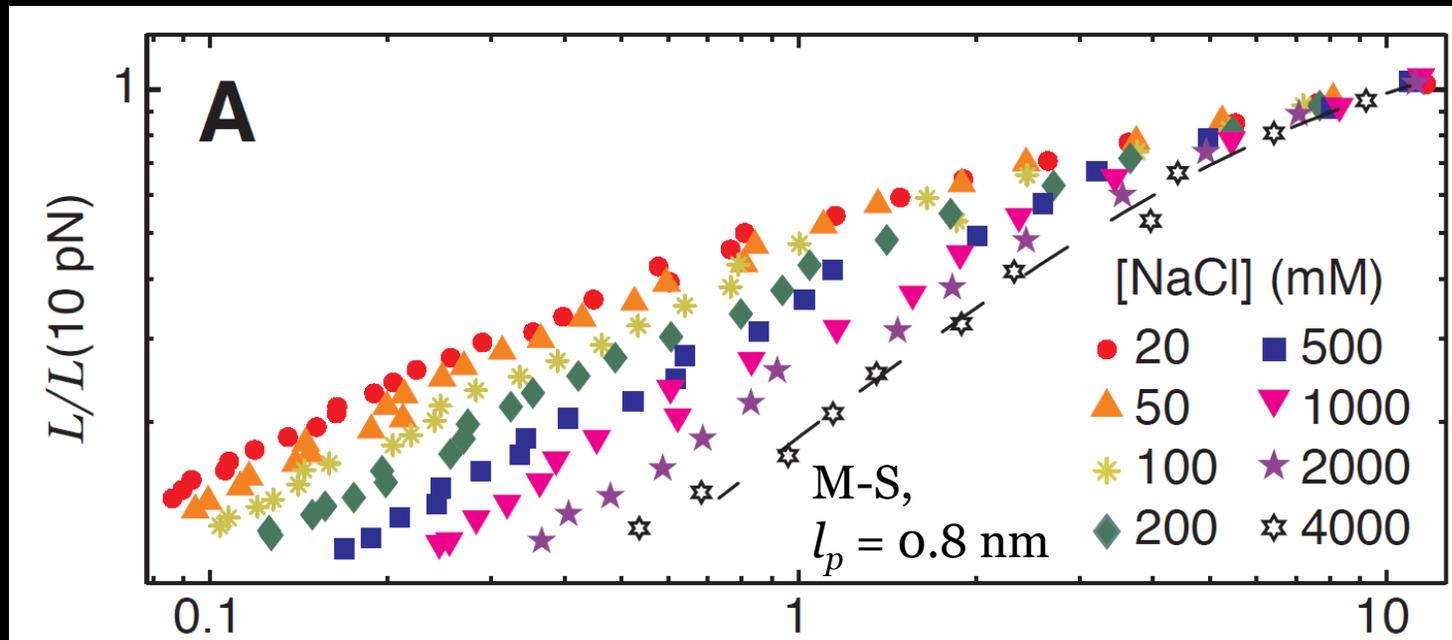
# Shift factor scales inversely with Debye length:

$$f_c \sim 1/r_D$$

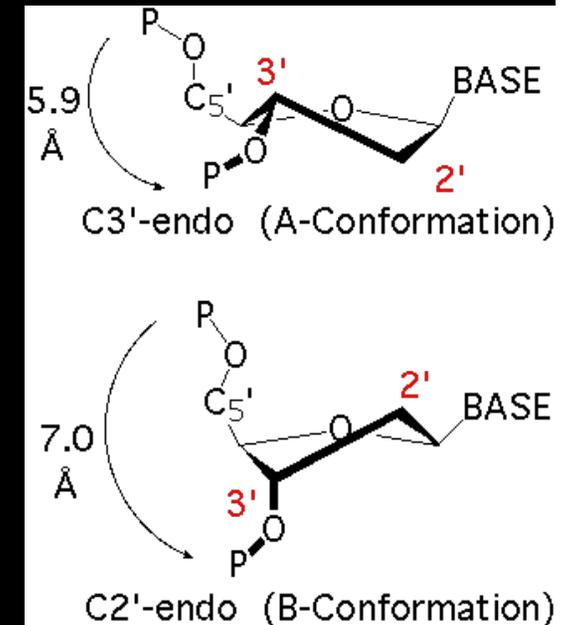
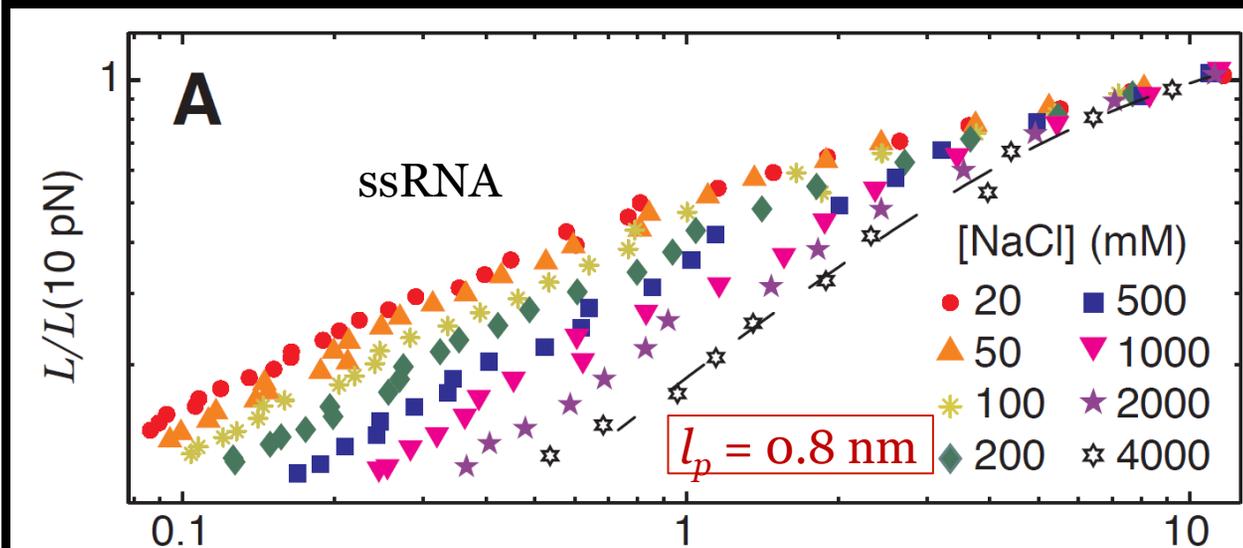
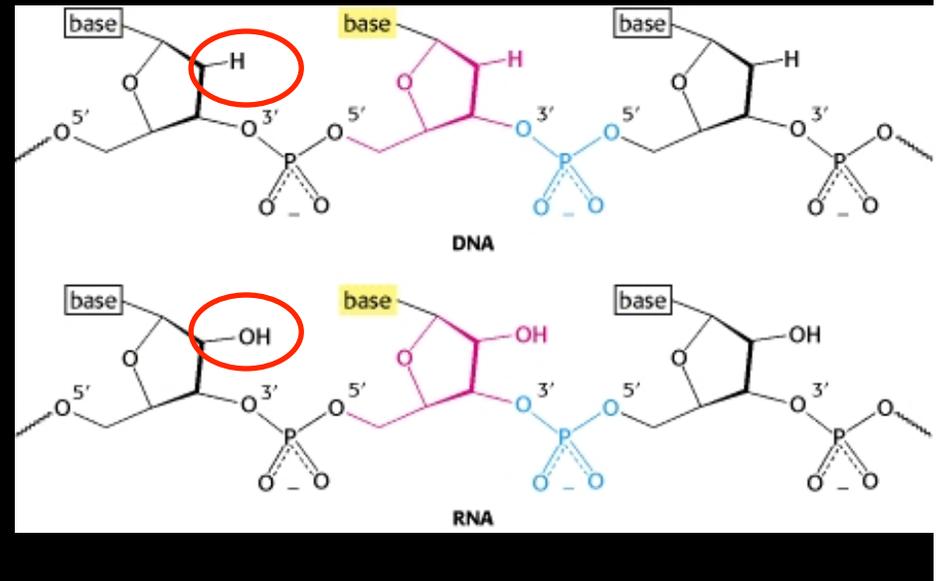
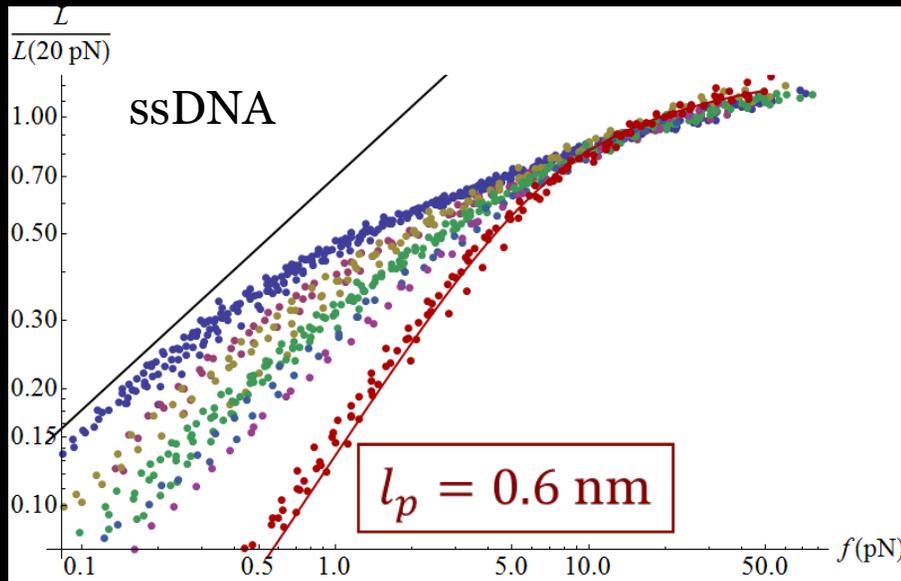


Recall:  $r_D \sim 1/\sqrt{c}$

# Poly(U) ssRNA: Results nearly identical



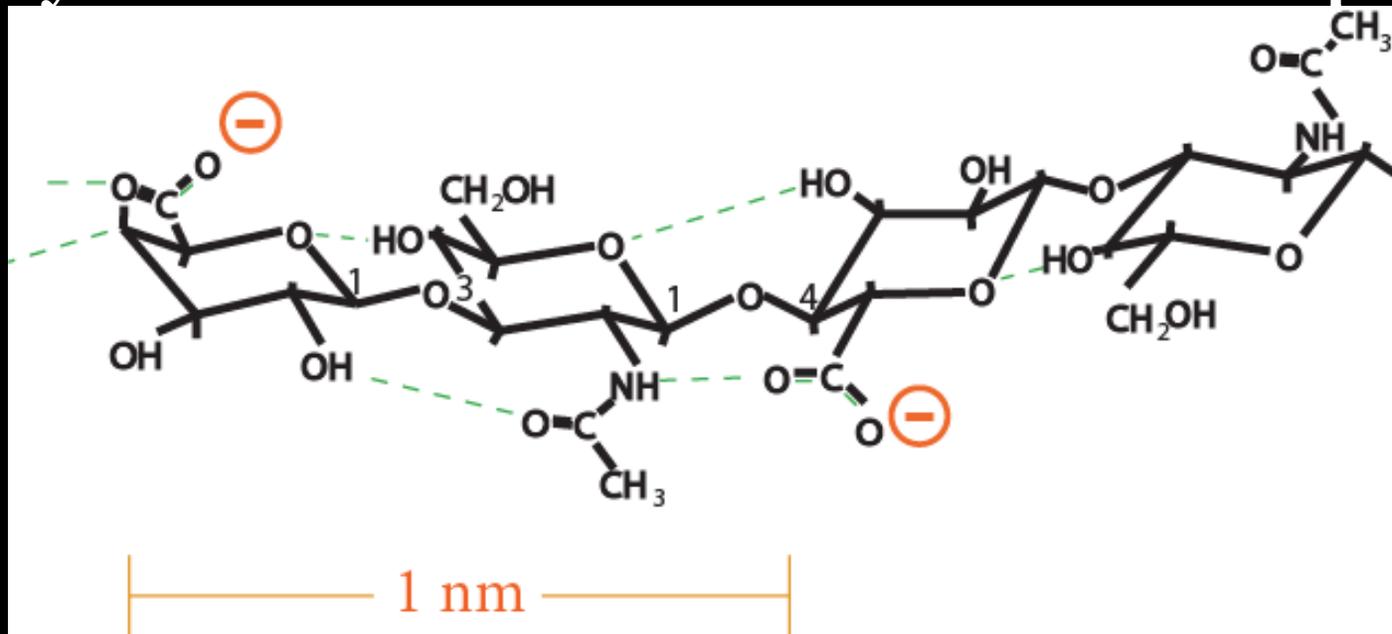
# Salient difference in ssRNA/ssDNA: ssRNA slightly stiffer in theta conditions



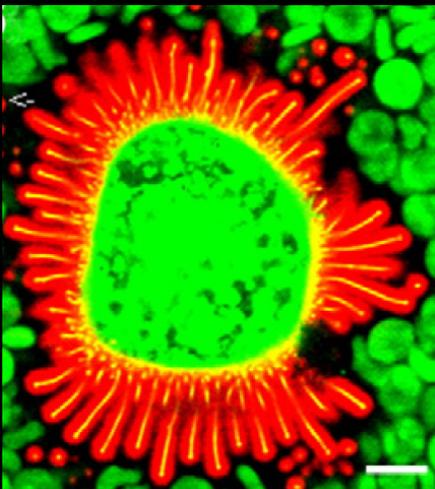
**There is a fundamental length scale in  
ssNAs that scales as  $\frac{k_B T}{f_c} \sim r_D$**

**What about other charged polymers?**

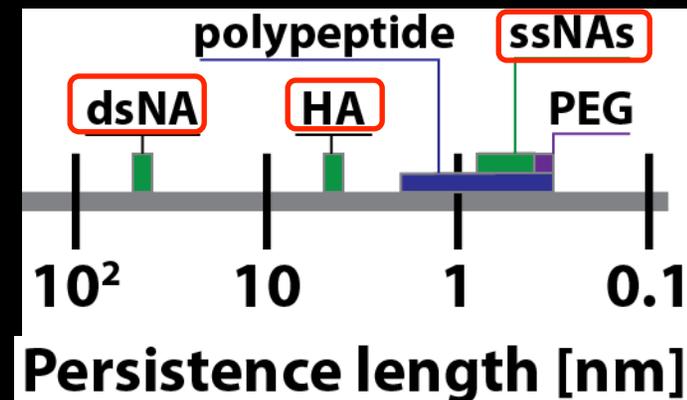
# Hyaluronic acid: A polysaccharide common to extra-cellular spaces



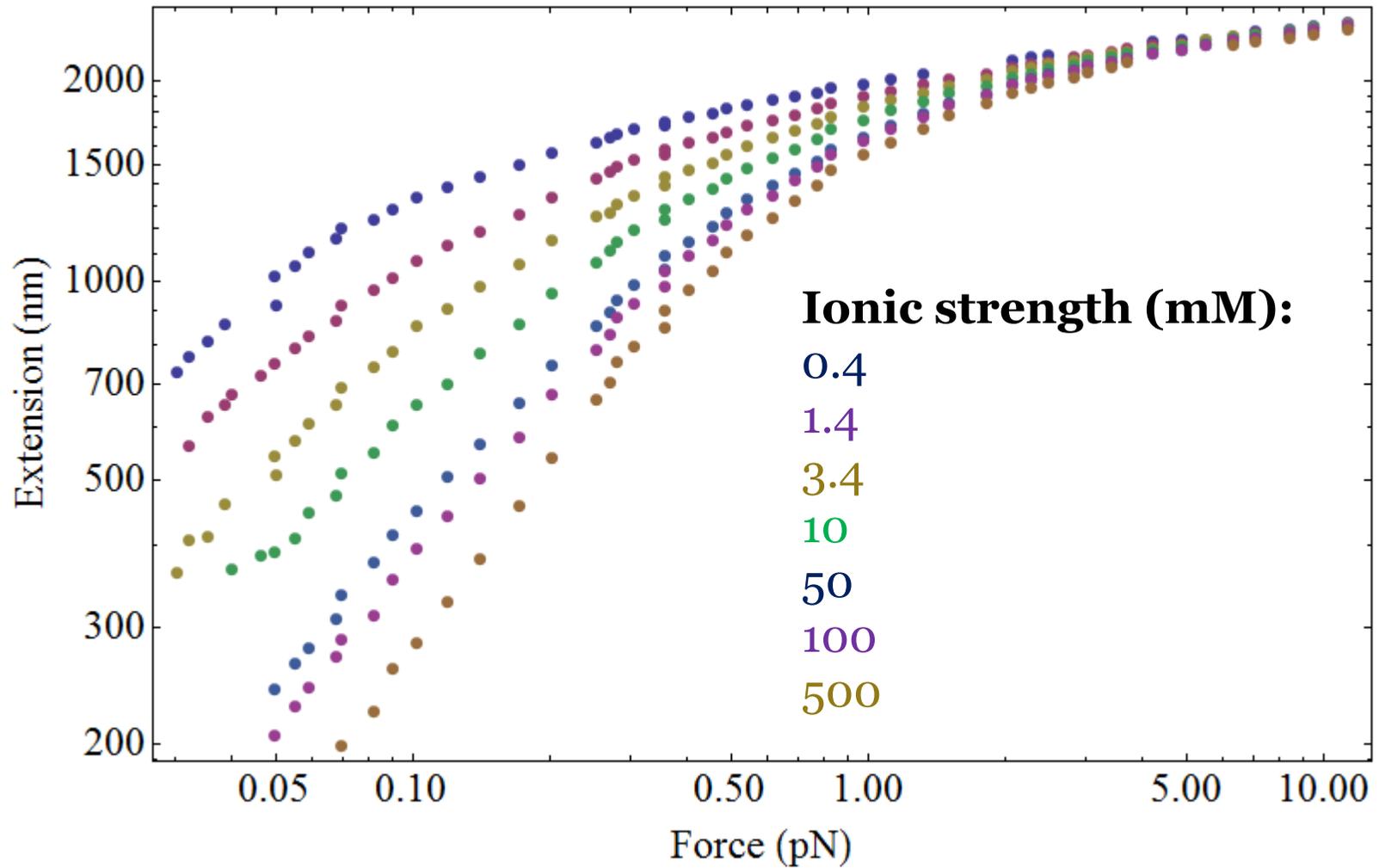
**2<sup>ary</sup> structure:** Intra-strand H-bonds lead to stiffening,  $l_{p,0} \approx 6 \text{ nm}$



HA forms a coating layer (red) around certain cells

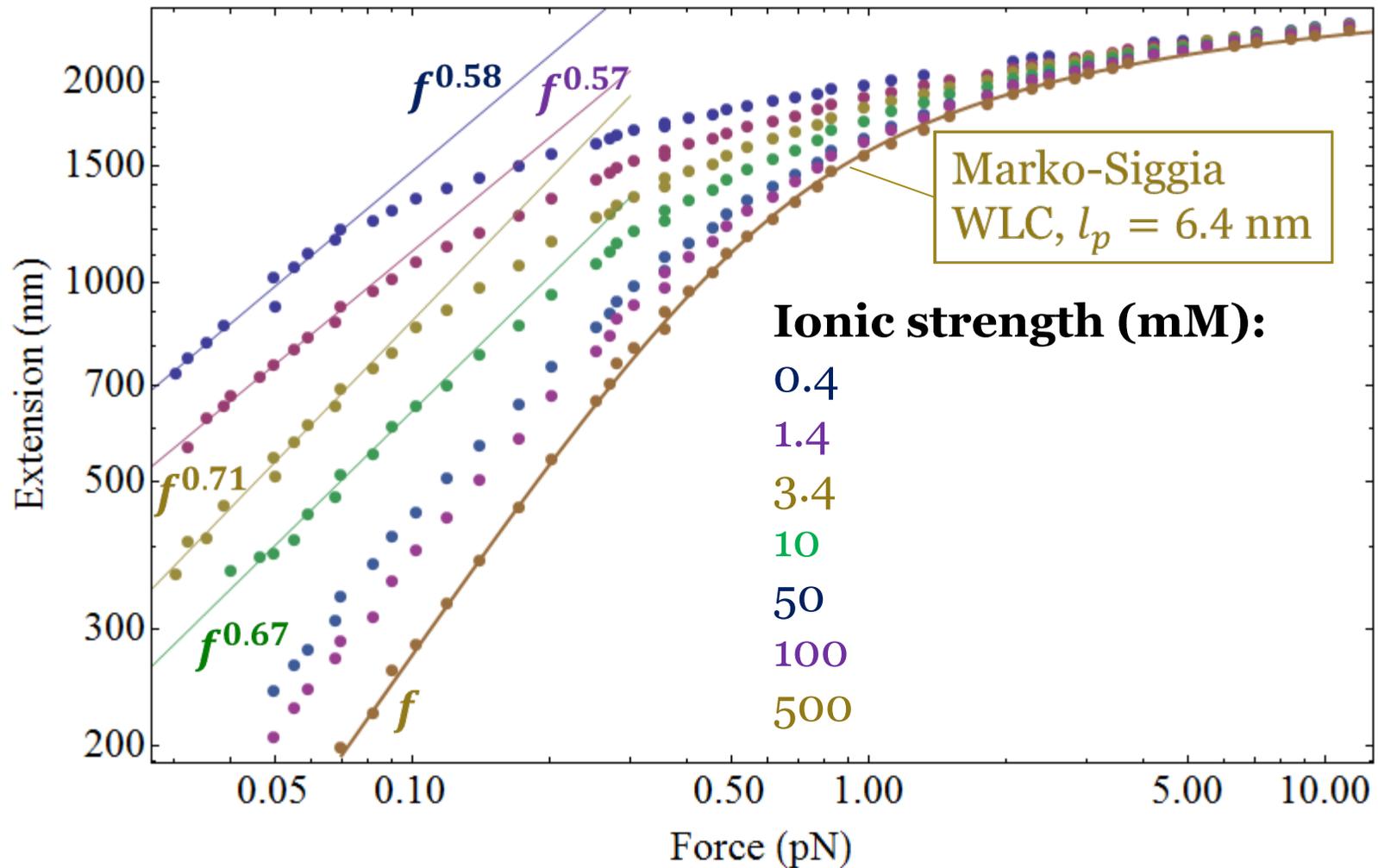


# Hyaluronic acid elasticity vs. salt



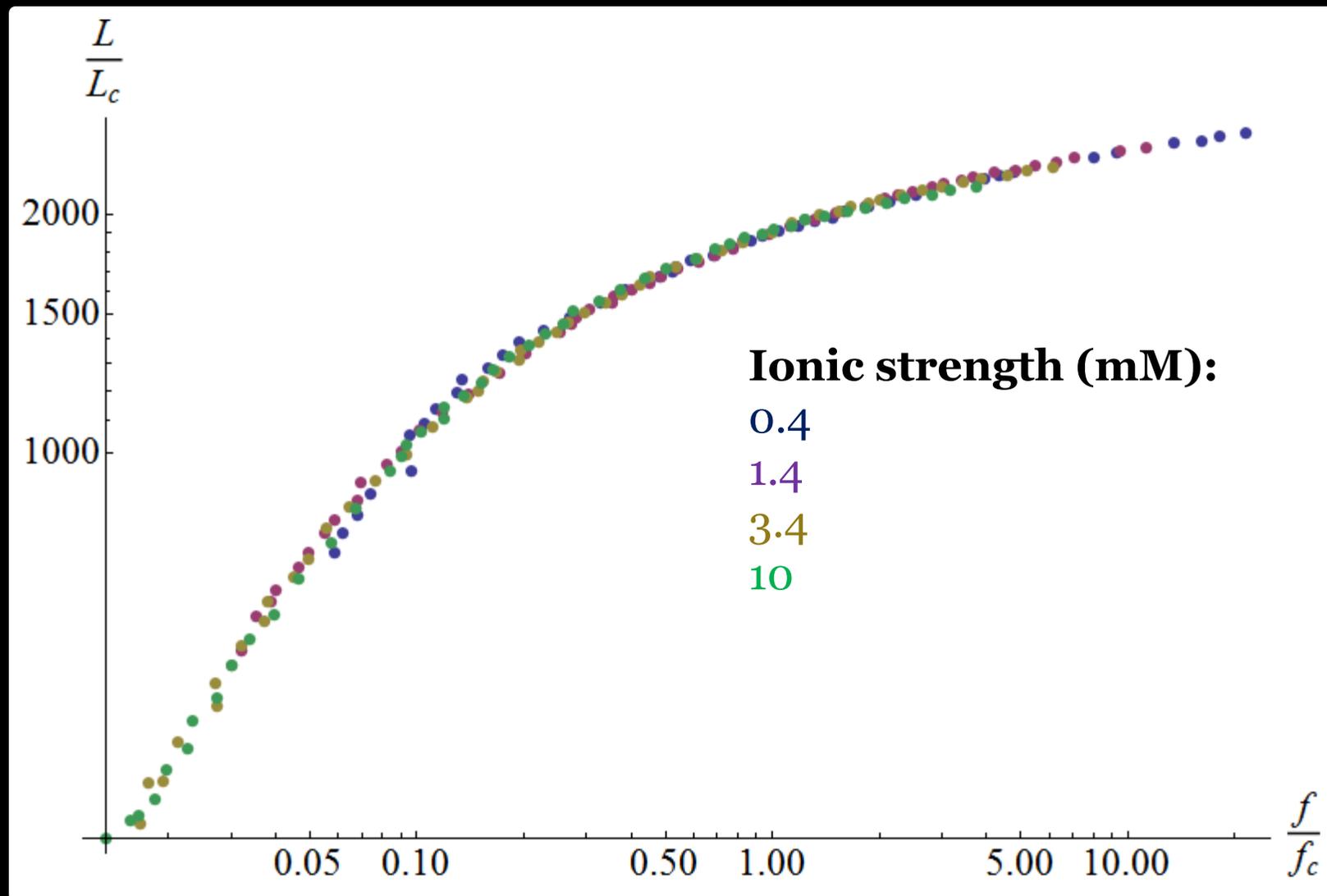
Berezney and Saleh, unpublished data

# Hyaluronic acid elasticity vs. salt

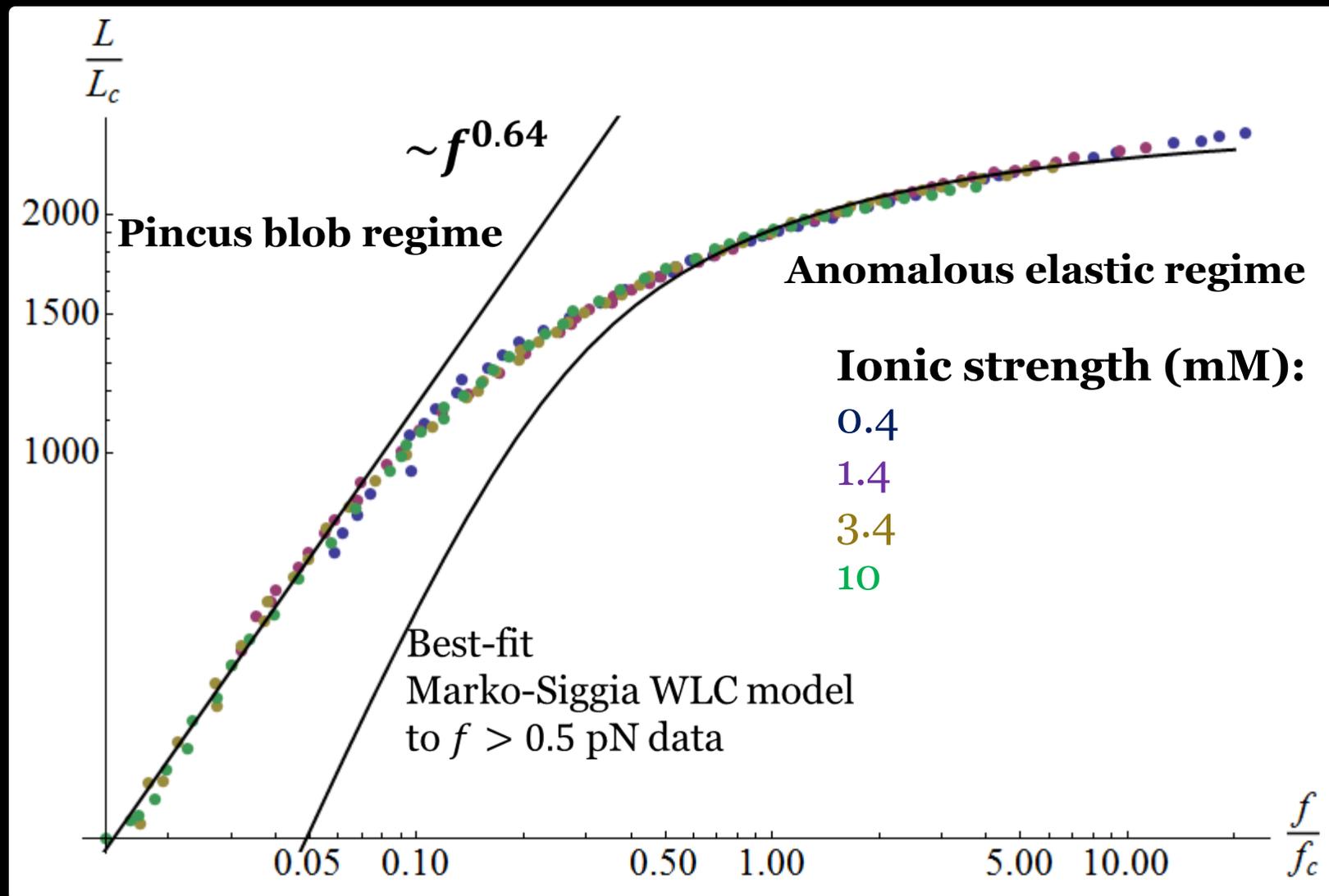


Starting at 50 mM, we see a transition to a theta condition occurring at  $\sim 500$  mM

**For  $I \leq 10$  mM,  
Universal behavior after scaling by shift factors ( $f_c, L_c$ )**

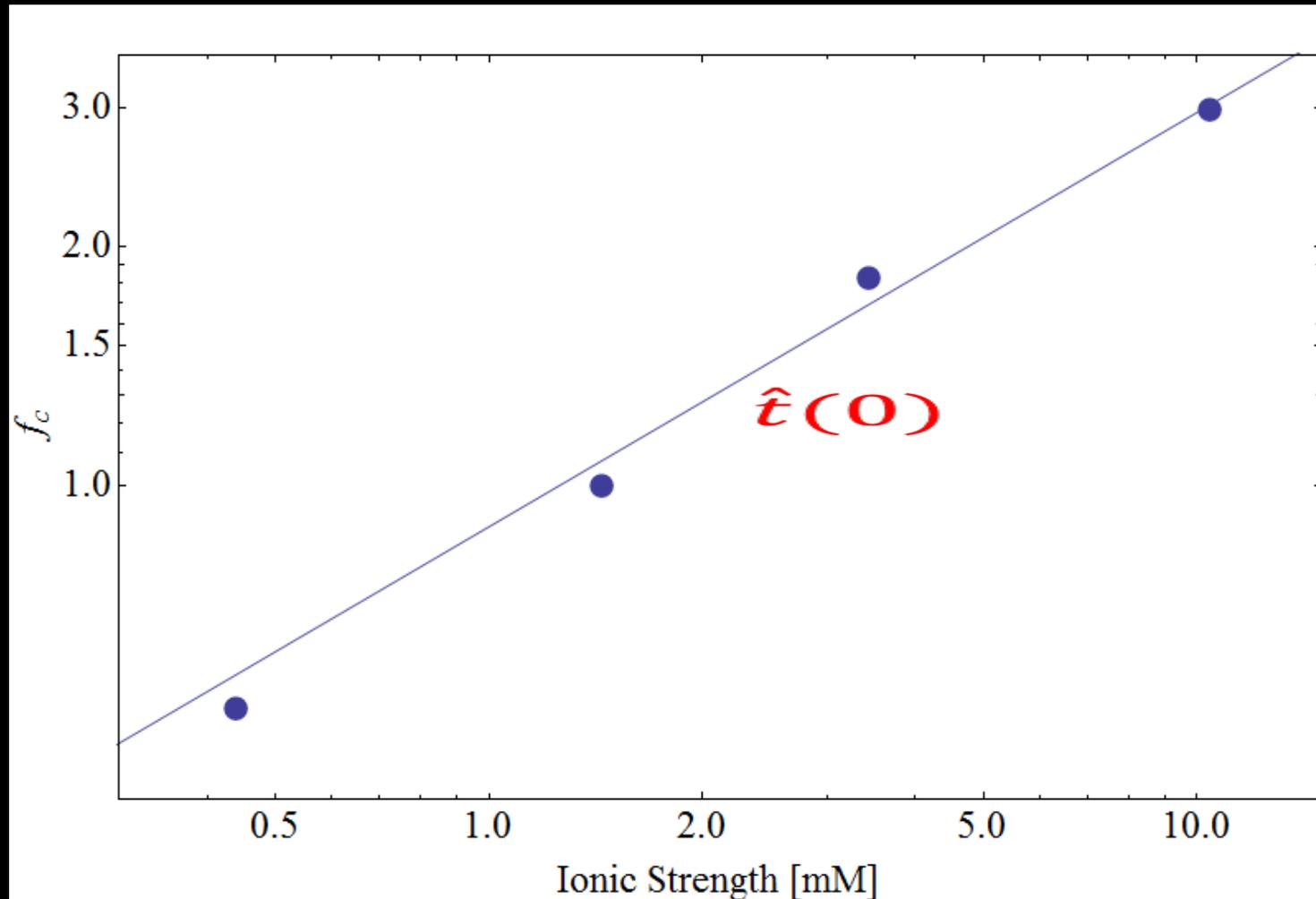


**For  $I \leq 10$  mM,**  
**Universal behavior after scaling by shift factors ( $f_c, L_c$ )**



Shift factor scales inversely with Debye length:

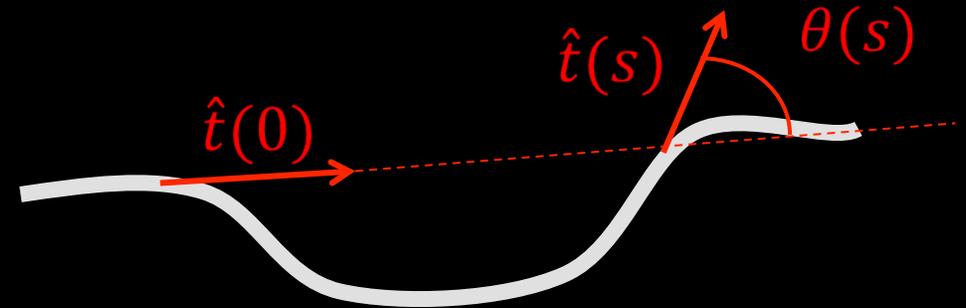
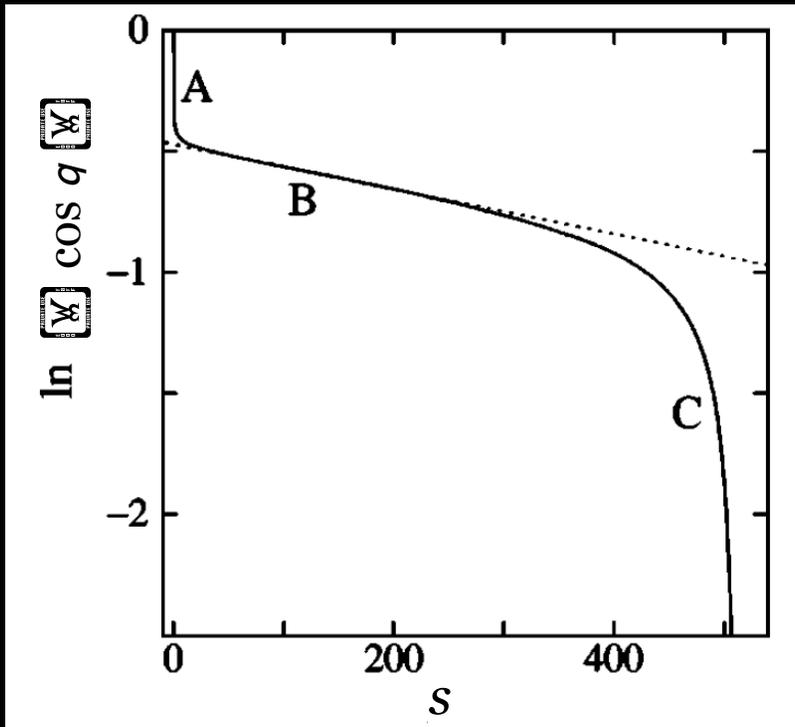
$$f_c \sim 1/r_D$$



Recall:  $r_D \sim 1/\sqrt{I}$

*t*(*s*)

# High-force anomalous elasticity appears related to short-range non-WLC behavior



WLC:

$$\langle \hat{t}(0) \cdot \hat{t}(s) \rangle = \langle \cos \theta(s) \rangle = e^{-s/l_p}$$

Toan and Thirumalai, *JCP* (2012):

$$\langle \hat{t}(0) \cdot \hat{t}(s) \rangle = \langle \cos \theta(s) \rangle = s^{-g}$$

## *Prior simulations of SLC behavior*

Name: Ullner (2005)

Nguyen and Shklovskii (2002)

Everaers, Milchev, Yamakov (2002)

Ullner and Woodward (2002)

Carrillo and Dobrynin (2011)

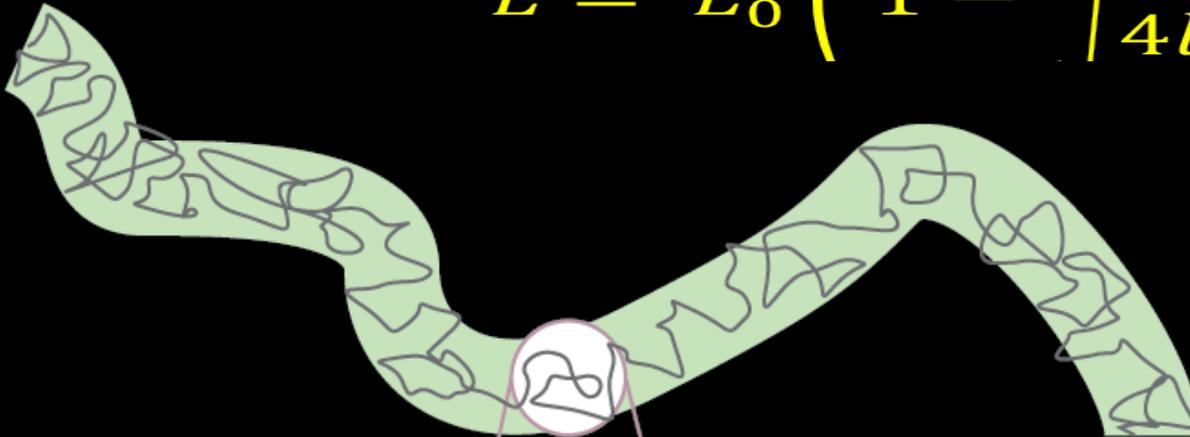
## *Direct connection of SLC structure to elasticity*

Stevens, McIntosh, and Saleh, *Macromolecules* (2012)

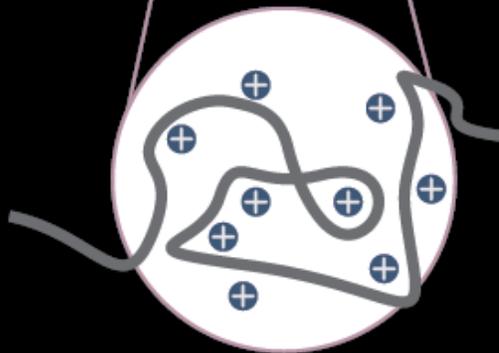


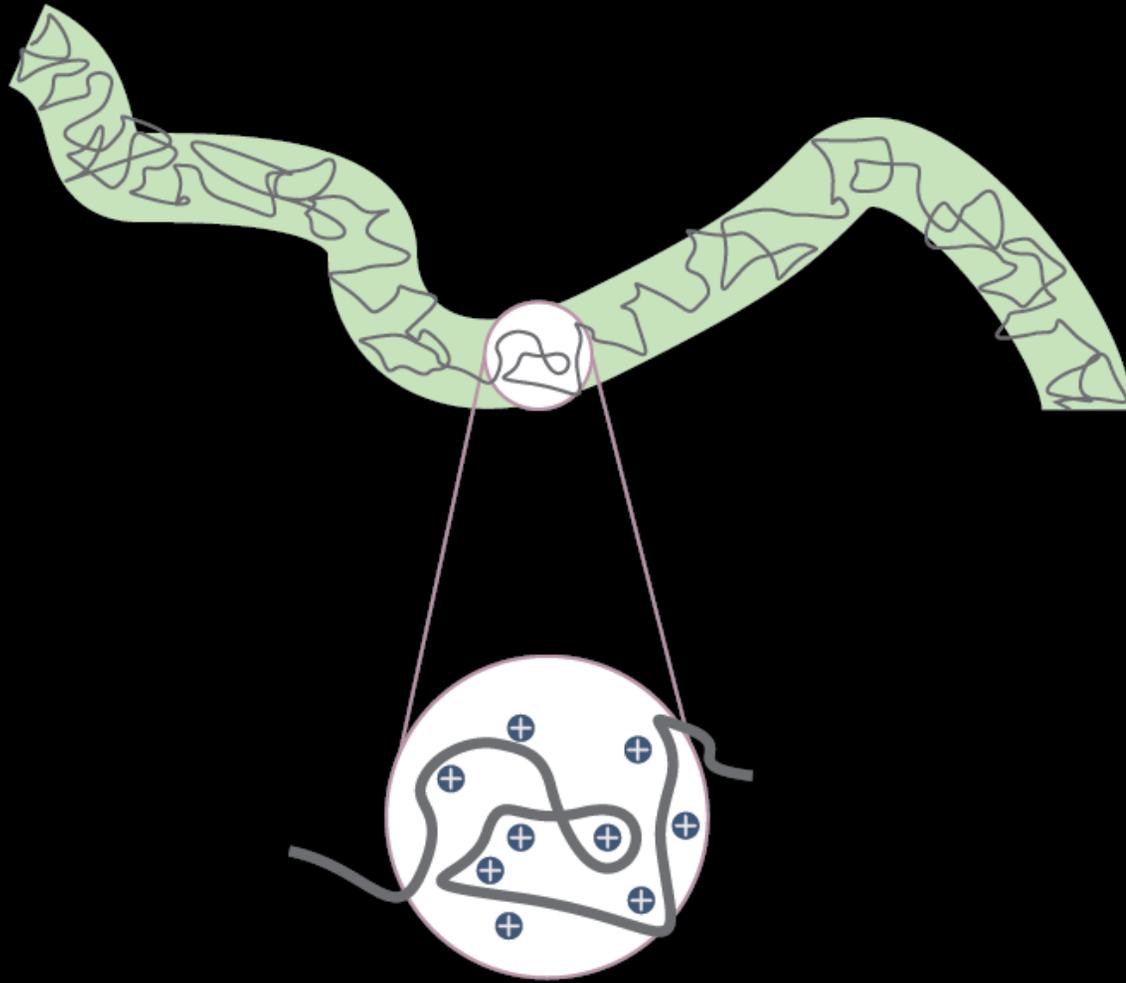
Leads to elastic prediction (Marko/Siggia):

$$L = L_0 \left( 1 - \sqrt{\frac{k_B T}{4l_n f}} \right)$$



Name– Ullner (2005)





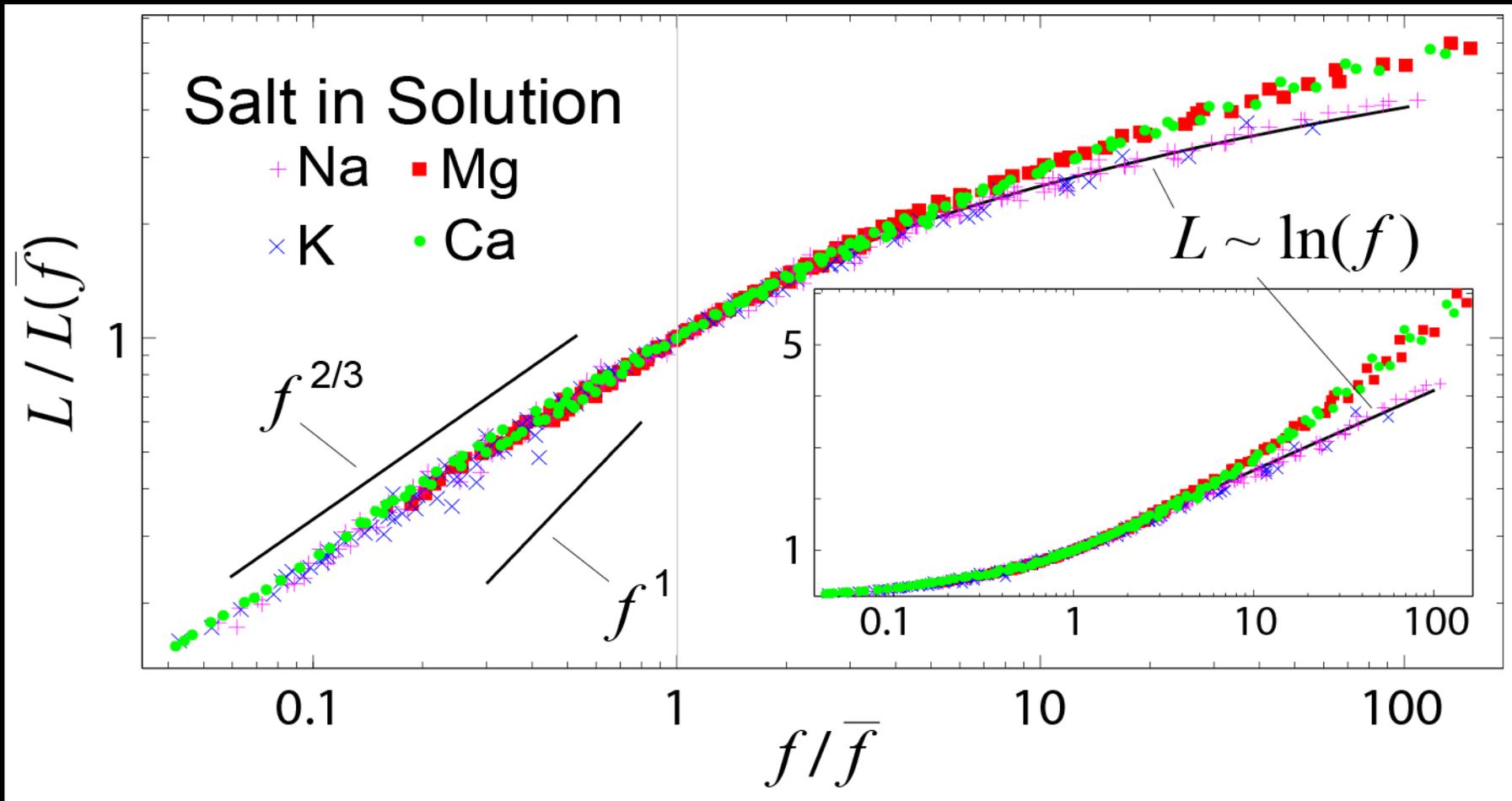
Scales greater than  $r_D$ :  
A swollen random-walk,  
 $\langle \cos(\theta) \rangle = e^{-s/l}$   
 $R \sim N^{3/5}$   
 $L \sim f^{2/3}$

Scales less than  $r_D$ :  
An electrostatic crumple,  
 $\langle \cos(\theta) \rangle = s^{-g}$   
 $R \sim N^{0.75}$   
 $L \sim \ln(f)$

### Testable implications

- 1) **Divalents** have stronger electrostatics, should crumple the chain more on short length scales, and affect the anomalous elastic regime (but not the Pincus regime)

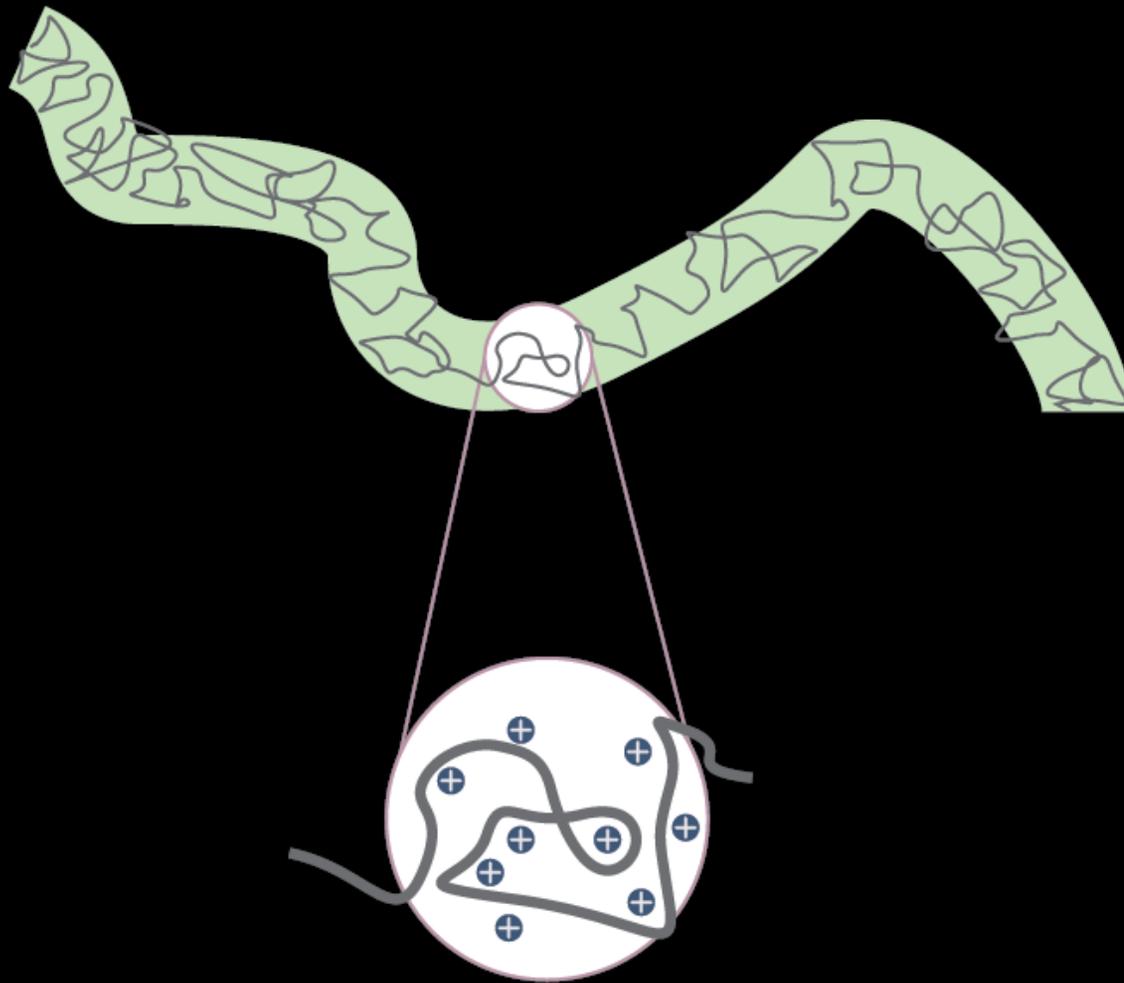
# Divalent elasticity shows increased compliance in high force regime, consistent with more crumpling



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

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

McIntosh and Saleh, *Macromolecules* (2011)



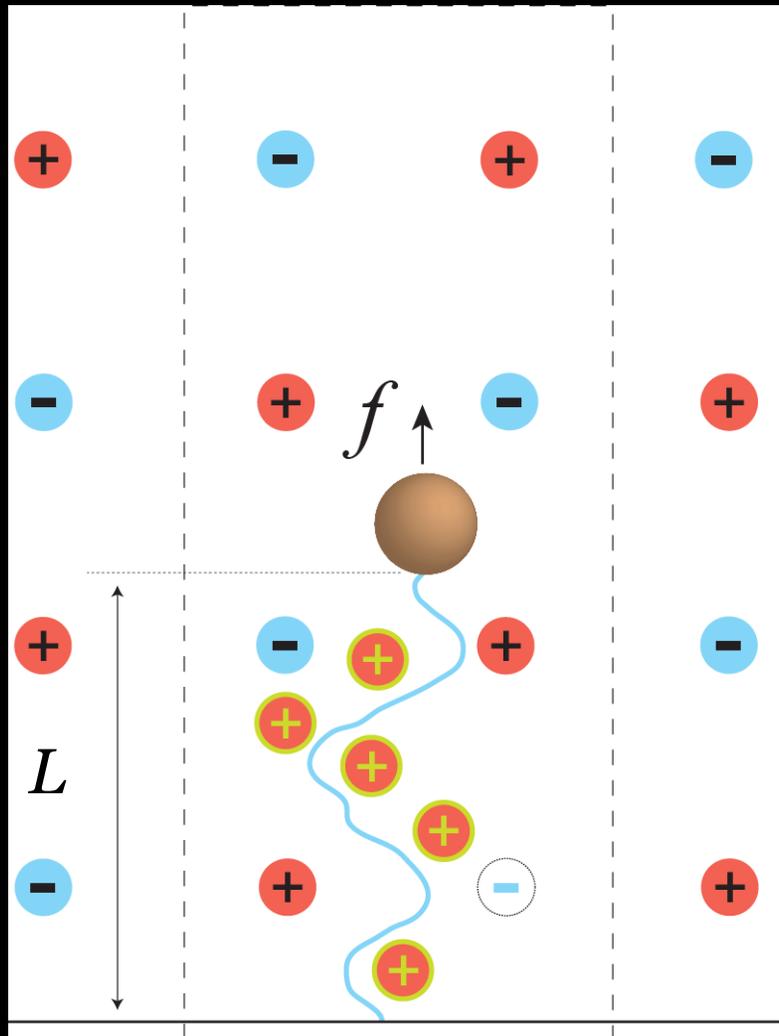
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 $L \sim \ln(f)$

### Testable implications

- 1) Divalents have stronger electrostatics, should have more crumpling
- 2) Crumples must be **highly ion-associated**, so stretching should **drive away many ions**

# Thermodynamics of ion-counting



**Independent variables:**

Force,  $f$ , and chemical potential,  $\mu_i(c_i)$

**Conjugate dependent variables:**

extension,  $L$ , and ion number,  $N_i$

Associated Maxwell relation:

$$\frac{\partial L}{\partial \mu_i} = \frac{\partial n_i}{\partial f}$$

$n_i$  : Ion excess

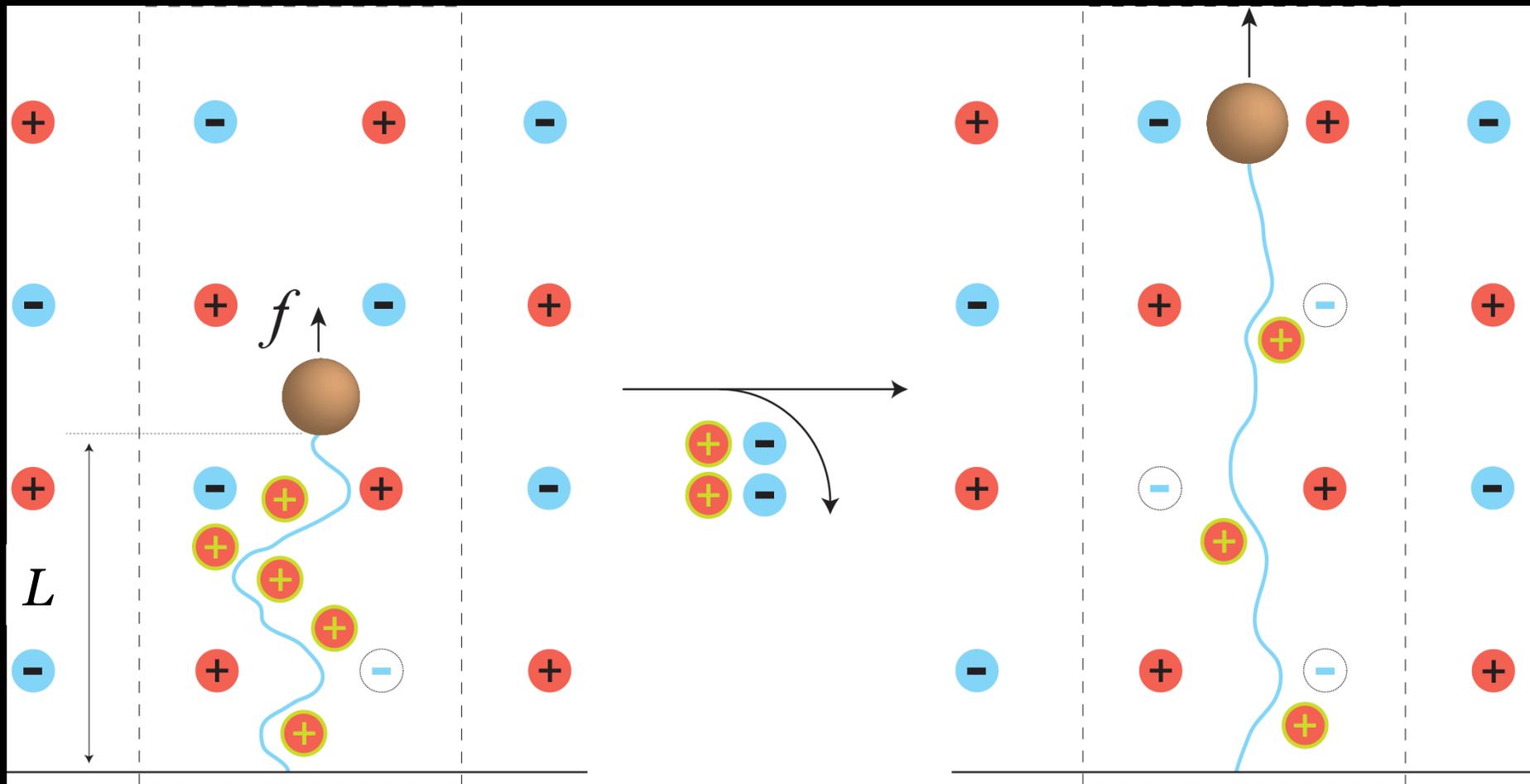
$$\mu_i = k_B T \ln c_i$$

Parsegian, Rand and Rau, *PNAS*, (2000)

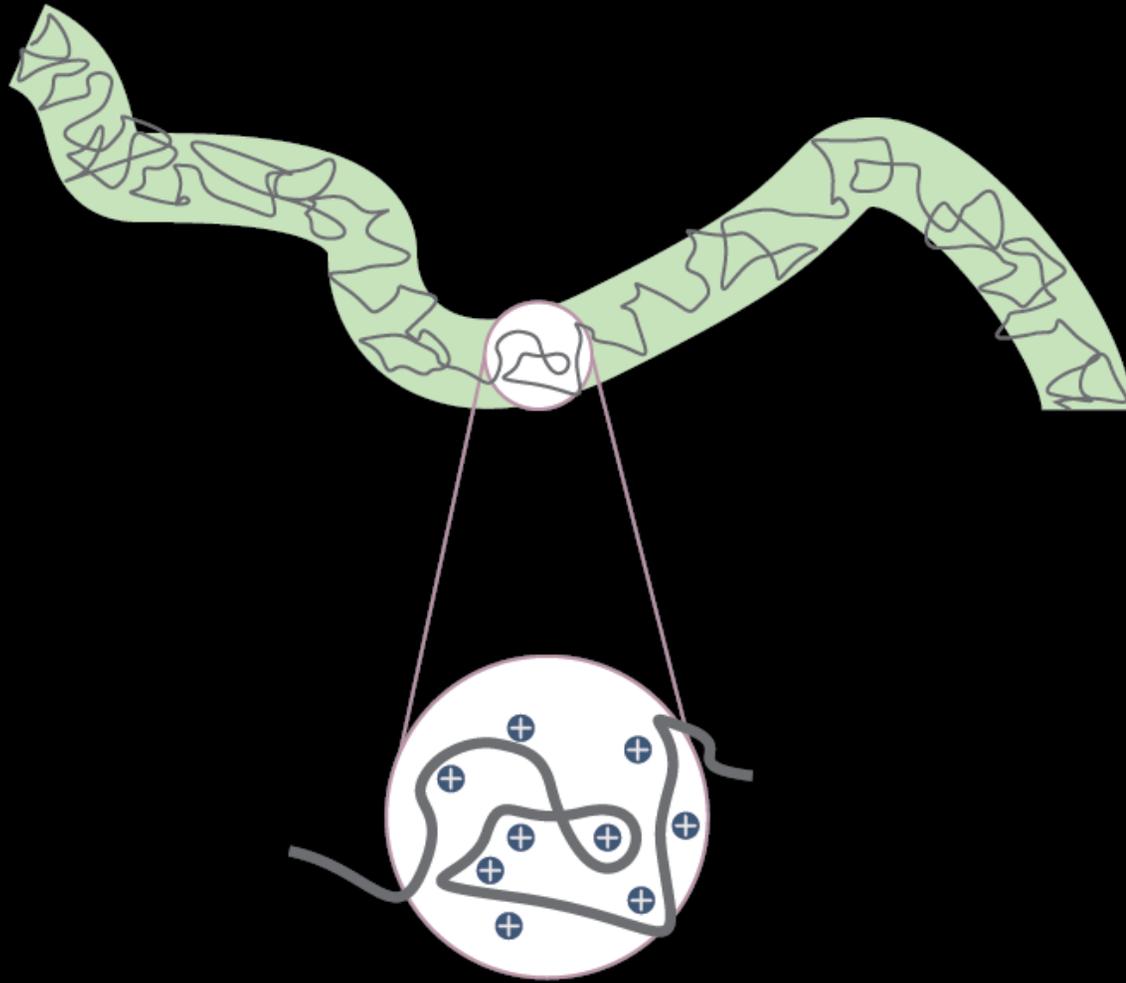
Zhang and Marko, *PRE* (2008)

Landy, McIntosh and Saleh, *PRL* (2012)

$$\frac{\partial L}{\partial \mu} < 0 \text{ implies } \frac{\partial n}{\partial f} < 0$$







Scales greater than  $r_D$ :  
A swollen random-walk,  
 $\langle \cos(\theta) \rangle = e^{-s/l}$   
 $R \sim N^{3/5}$   
 $L \sim f^{2/3}$

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An electrostatic crumple,  
 $\langle \cos(\theta) \rangle = s^{-g}$   
 $R \sim N^{0.75}$   
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### Testable implications

- ✓ 1) **Divalents** have stronger electrostatics, should have more crumpling
- ✓ 2) Crumples must be **highly ion-associated**, so stretching should **drive away many ions**

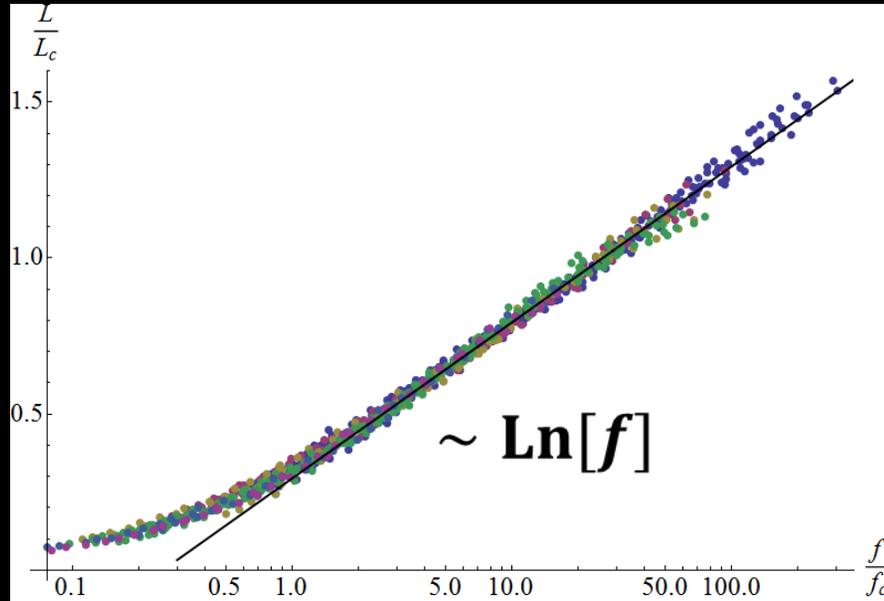
## Conclusion:

Charged, flexible polymers seem to act like 'snake-like chains', comprising:

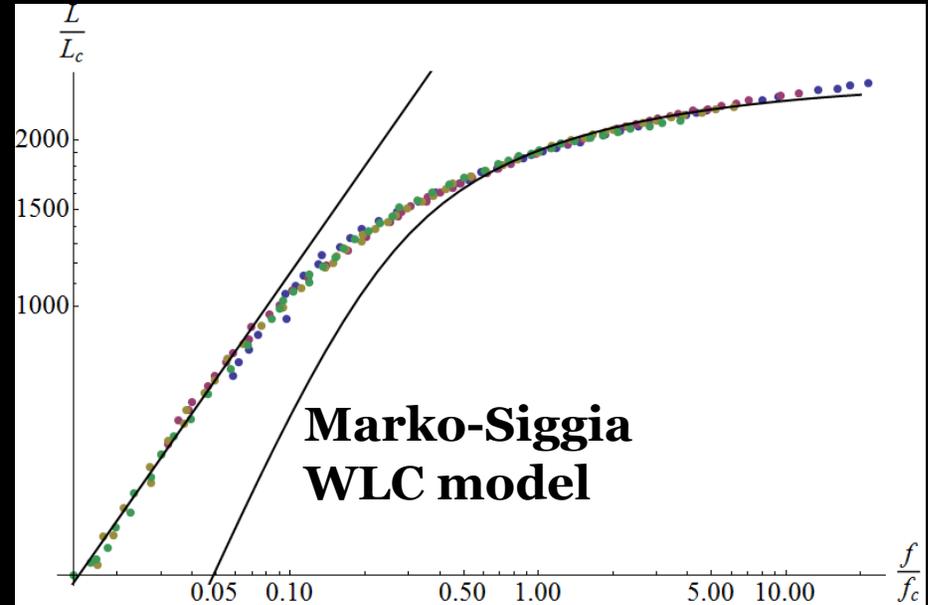
- 1) A large length scale regime which is a non-OSF persistent chain of blobs ( $l_p \sim r_D$ )
- 2) A short length-scale regime in which the concept of persistence length is not well defined, due to the long-range nature of electrostatics

# What microscopic models can explain the high-force elasticity/short-length structure?

Lin-Log plot of ssDNA stretching



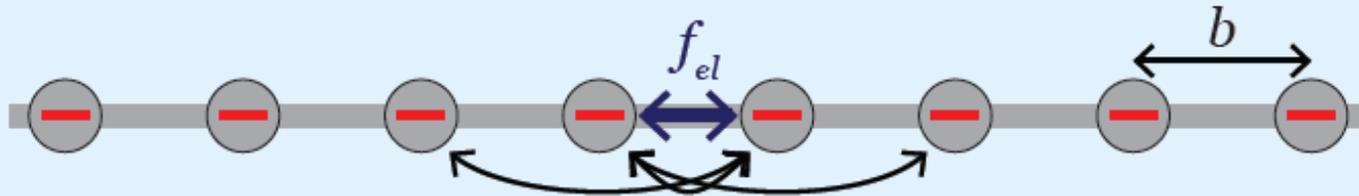
Log-Log plot of HA stretching



Hypothesis:

Look at the interplay between external force and **internal electrostatic tension**

# Electrostatic tension: $f_{el}$



Along a charged chain, such as ssDNA or ssRNA, inter-monomer repulsion creates an intrinsic stretching force,  $f_{el}$

$$u(r) = k_B T e^{-\kappa r} \frac{l_B}{r}$$

In monovalent salt, monomer repulsion is well-modeled by a **screened Coulomb interaction**... ( $\kappa^{-1}$ : Debye length;  $l_B \approx 0.7$  nm: Bjerrum length)

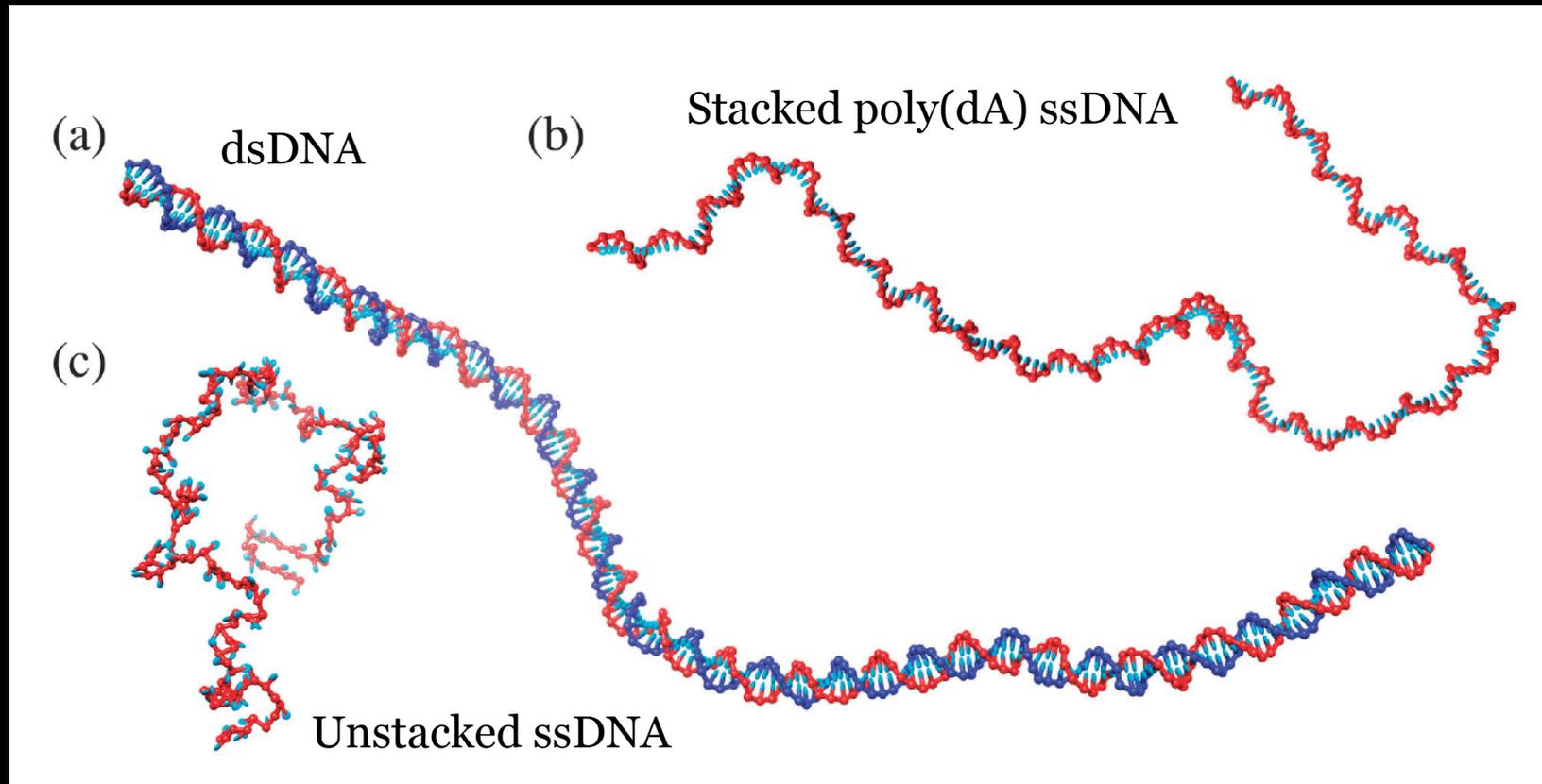
...which, summed along a line of charges, yields the **Netz model**<sup>1</sup> for  $f_{el}$  ( $b$ : charge spacing)

$$f_{el} = \frac{k_B T l_B}{b^2} \left( \kappa b \frac{e^{-\kappa b}}{1 - e^{-\kappa b}} - \ln \left( 1 - e^{-\kappa b} \right) \right)$$

Netz (2001)

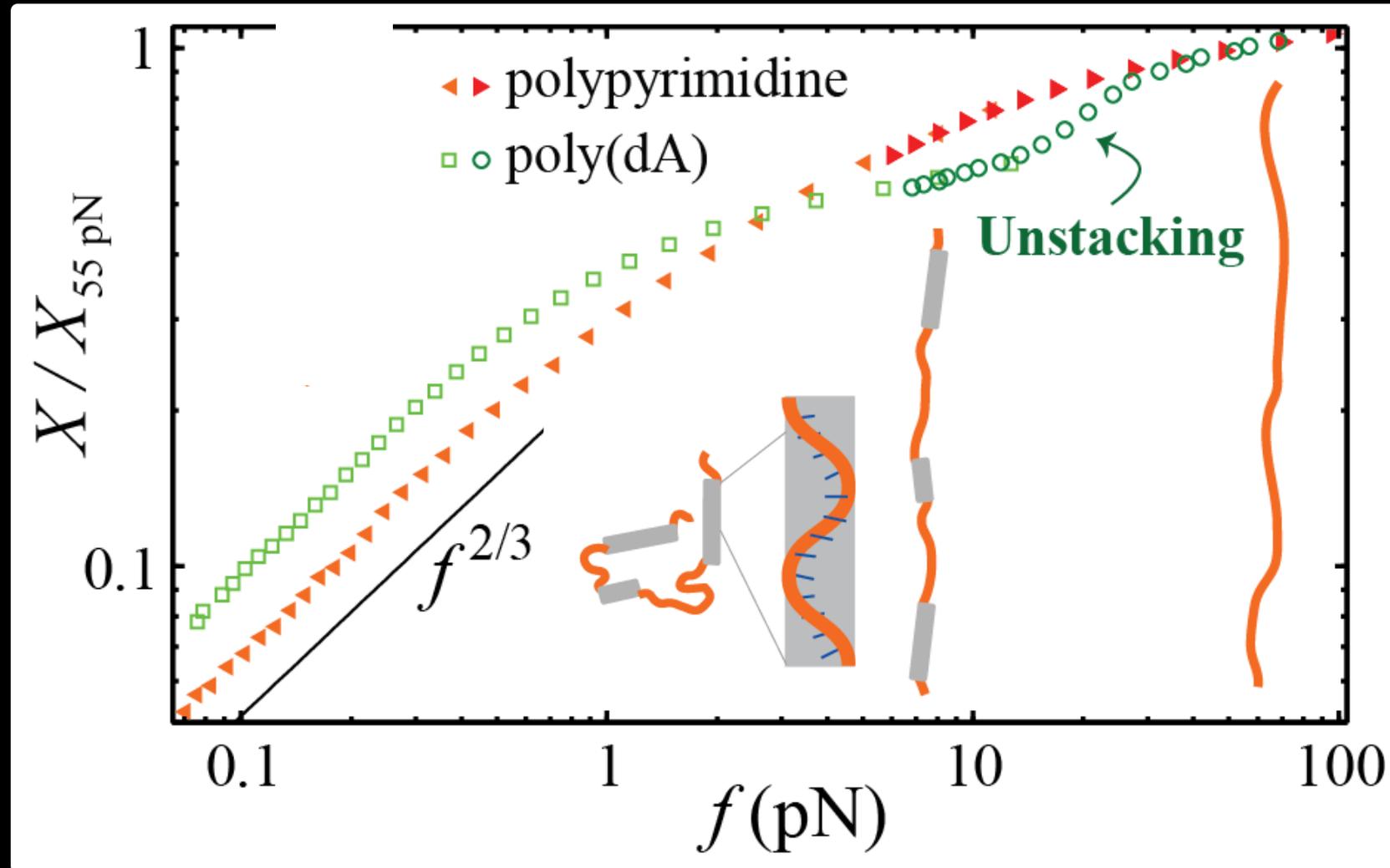
Manning (2006) offers an alternate formula that accounts for counterion entropy loss

# Mechanical unstacking can be used to measure electrostatic tension

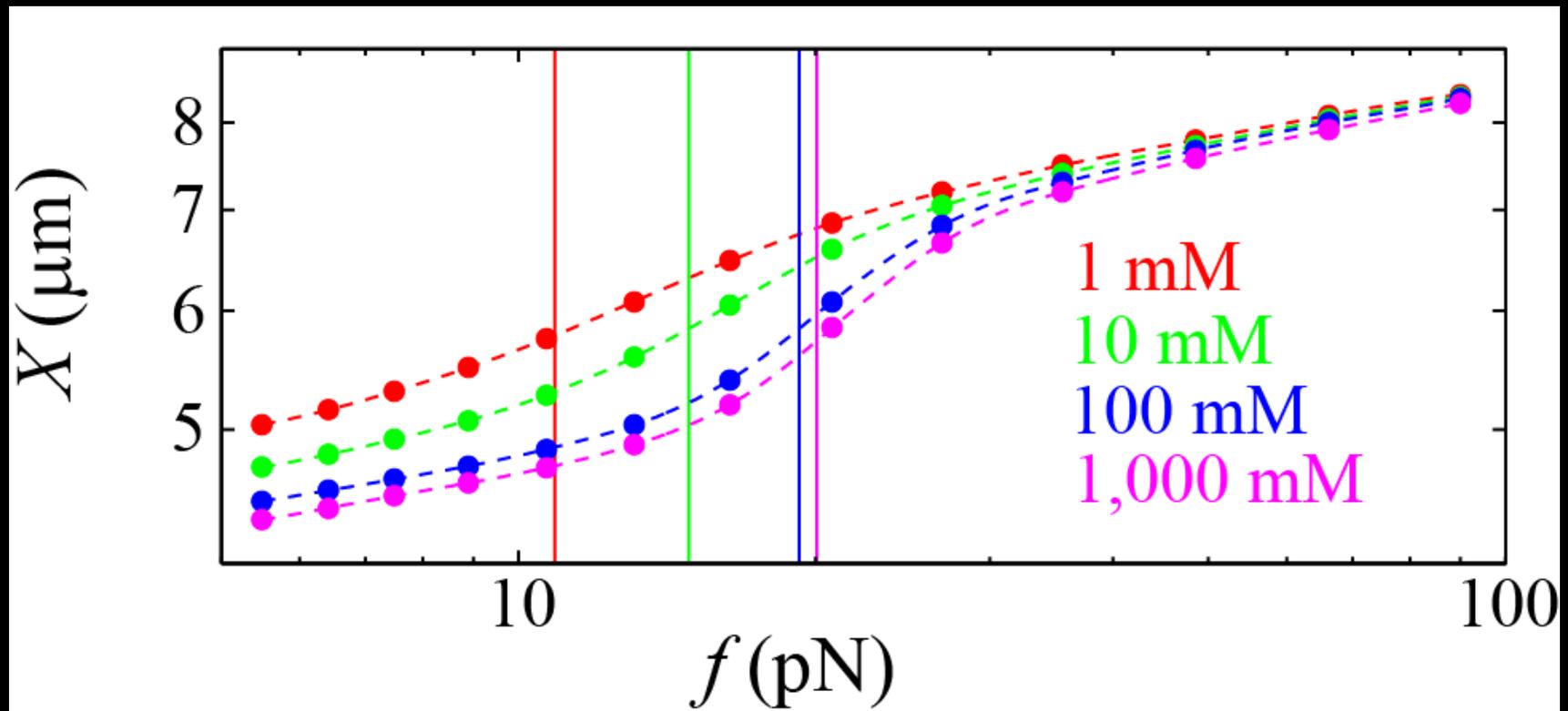


Doye *et al.* (2013)

# Poly(dA) shows a sigmoidal unstacking transition at high force that is missing in unstacked ssDNA



# Salt dependence: Higher salt conditions require larger forces to achieve unstacking



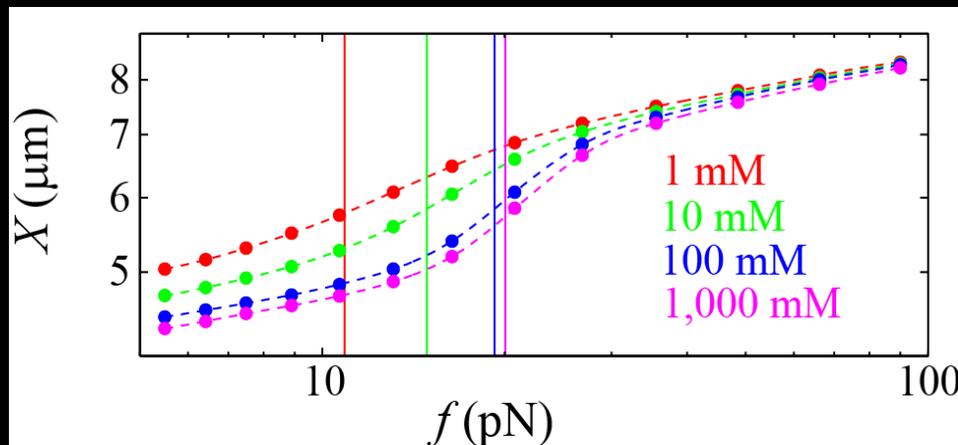
# Using base-unstacking to measure electrostatic tension vs. salt



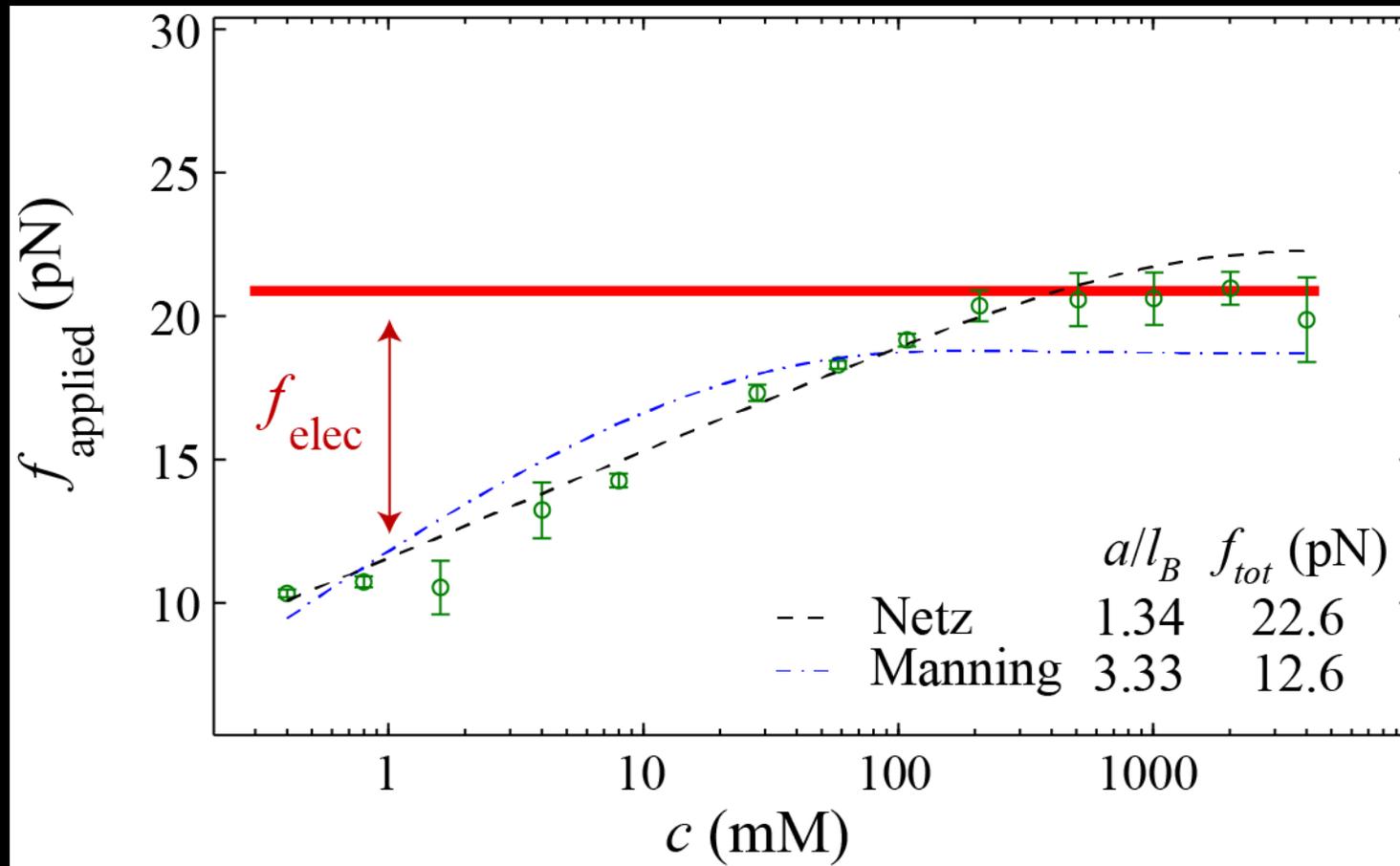
Charge repulsion leads to intrinsic, salt-dependent tension:  $f_{el}(c)$

Total tension is sum of applied and intrinsic:  $f_{tot} = f_{el}(c) + f_{appl}$

If we assume base-unstacking happens at constant  $f_{tot}$ ,  
we can measure  $f_{el}(c)$



$f_{el}$  is large ( $> \frac{kT}{l} \approx 4$  pN), and  
 $f_{el} \propto -\log(c)$ , following Debye-Huckel models



Netz, *Macromolecules* (2001)

Manning, *Biophys. J.* (2006)

# Can we model high-force polyelectrolyte stretching by modifying a standard force/extension formula?

$$L_{MS-WIC}(f) \rightarrow L_{MS-WIC}(f + f_{el})$$

$$L = L_c \left( 1 - \sqrt{\frac{k_B T}{4l_p(f_{ext} + f_{el})}} \right)$$

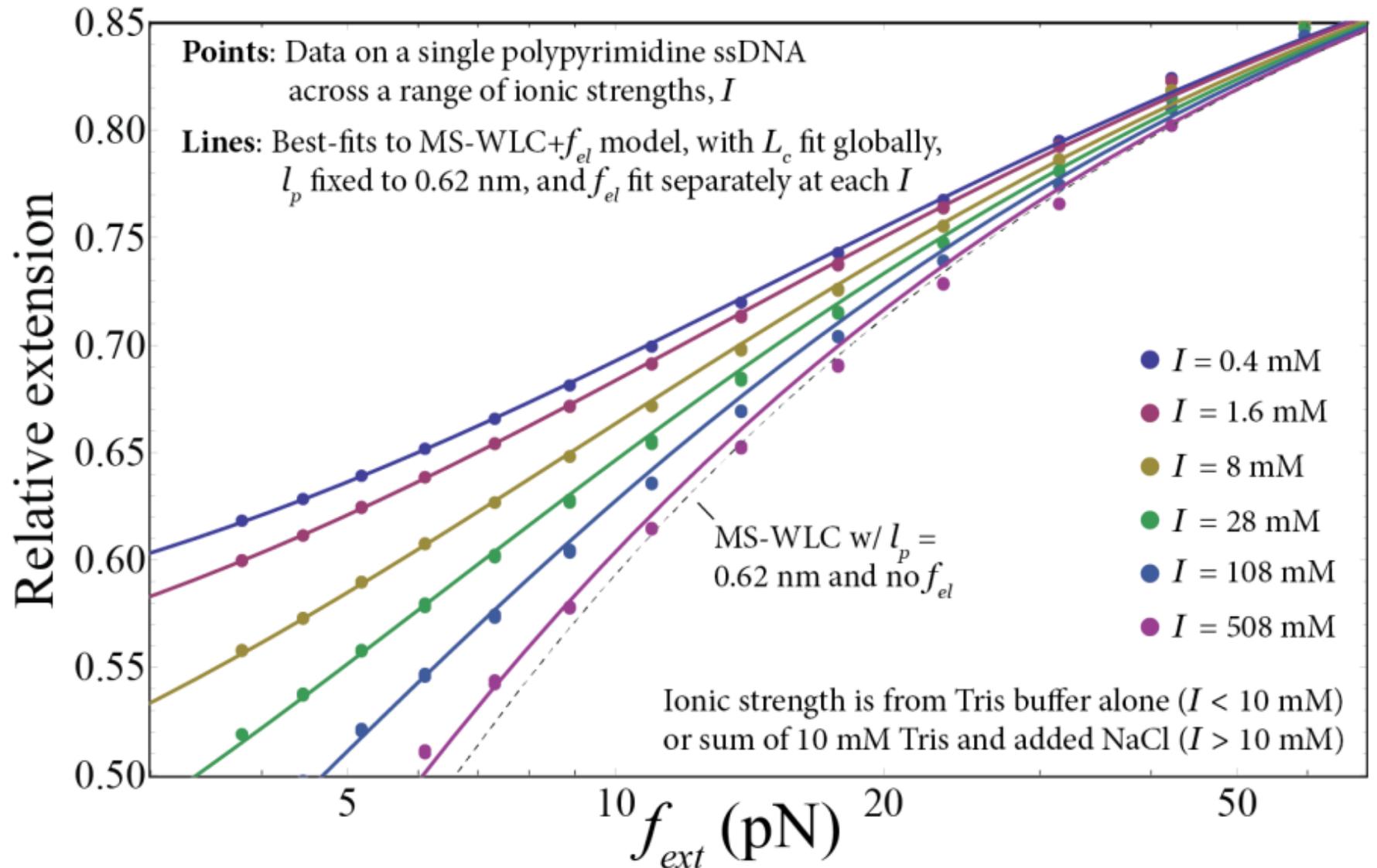
$L$ : extension

$L_c$ : contour length

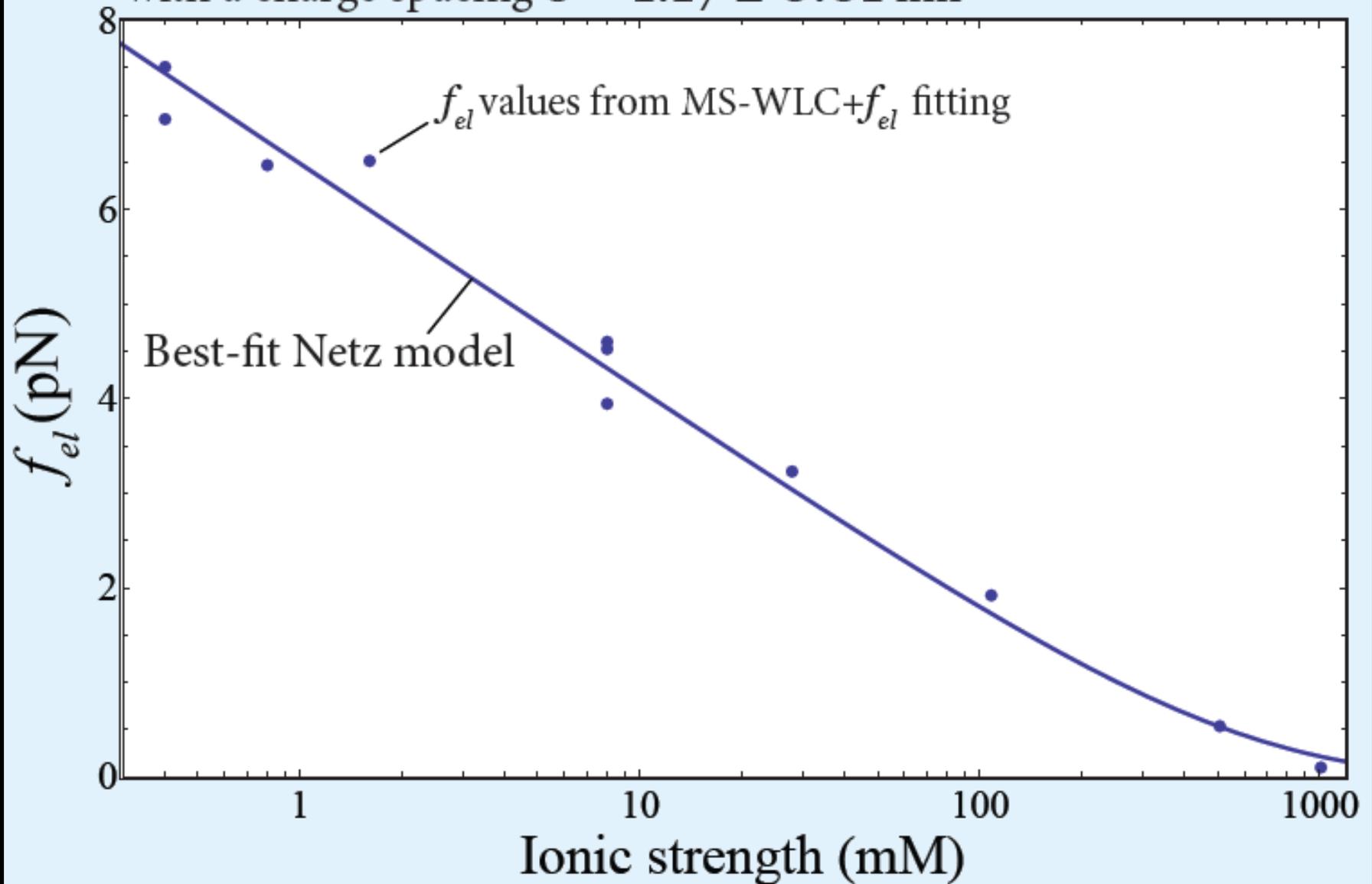
$l_p$ : persistence length of neutral chain

We fix  $l_p = 0.62$  nm based on prior results<sup>9</sup>.

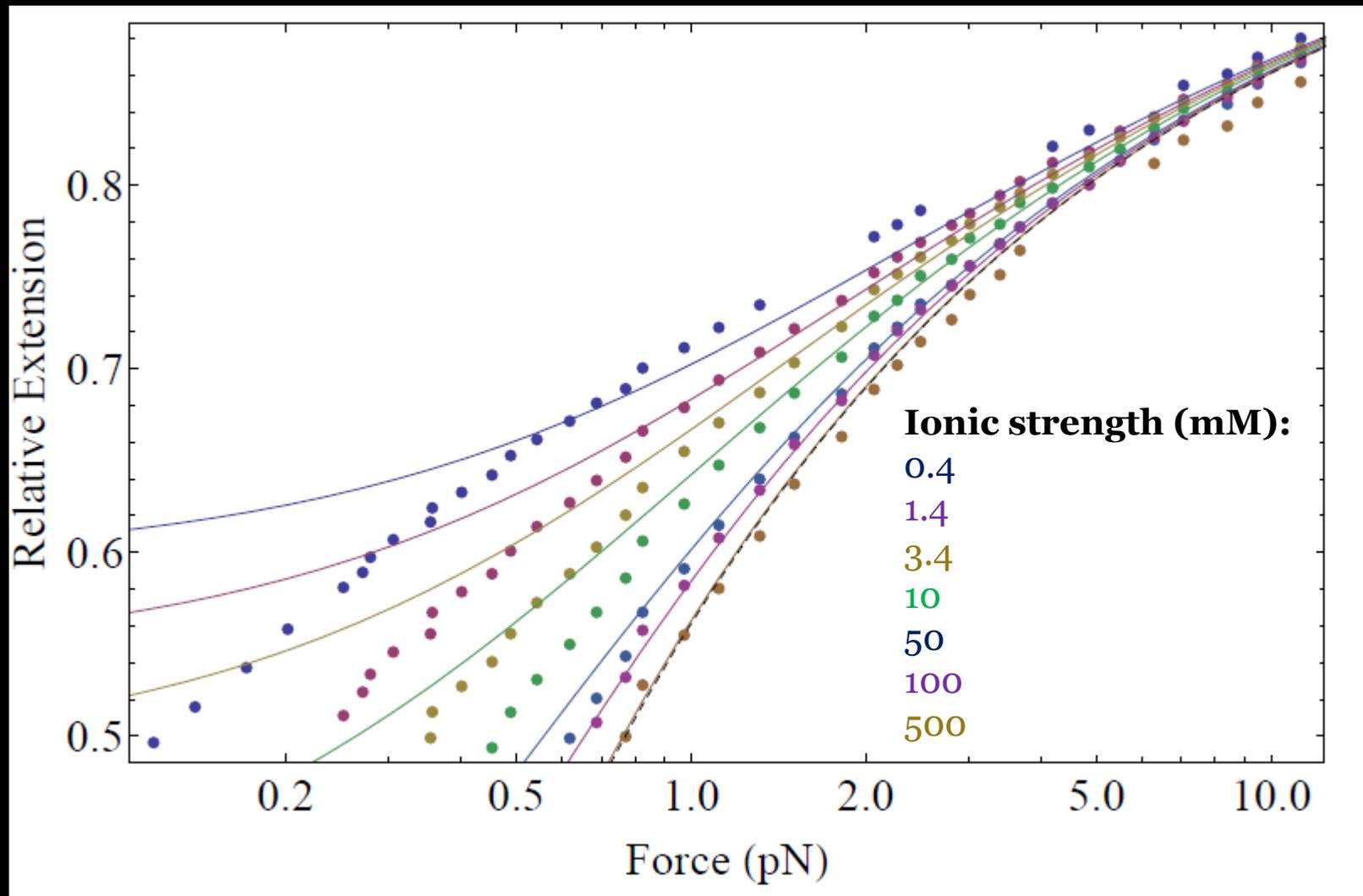
# Additive electrostatic tension works for ssDNA elasticity!



The extracted  $f_{el}$  values are themselves well-fit by the Netz model with a charge spacing  $b = 1.17 \pm 0.01$  nm



...but not so well for HA elasticity.



Conformation dependence of  $f_{el}$ ?

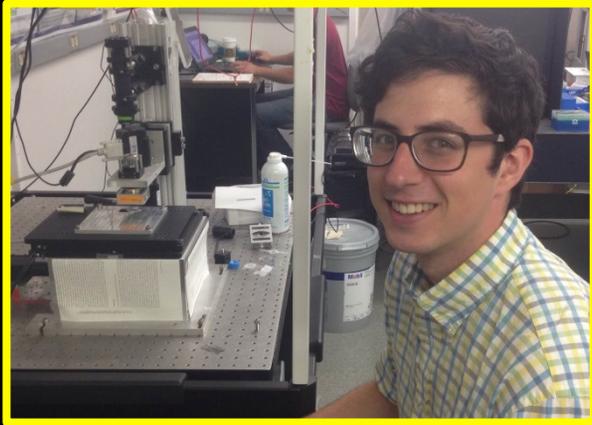
## Conclusion

- Electrostatic tension offers a simple and intuitive concept, as well as an analytically-tractable model, of the effect of electrostatics on short-range structure and elasticity of charged flexible biopolymers....
- ....yet it is too simplistic; more nuanced models capturing configurational fluctuations are likely needed (and could well be present in the literature already– e.g. Barrat and Joanny, 1993)

# Acknowledgements

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UCSB MRL  
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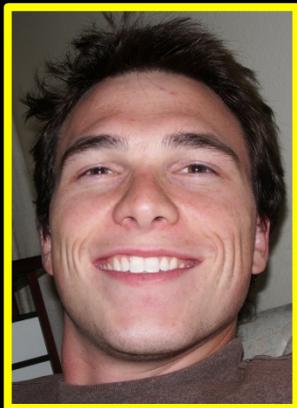
Dustin  
McIntosh



David  
Jacobson



Andrew Dittmore



Mark Stevens

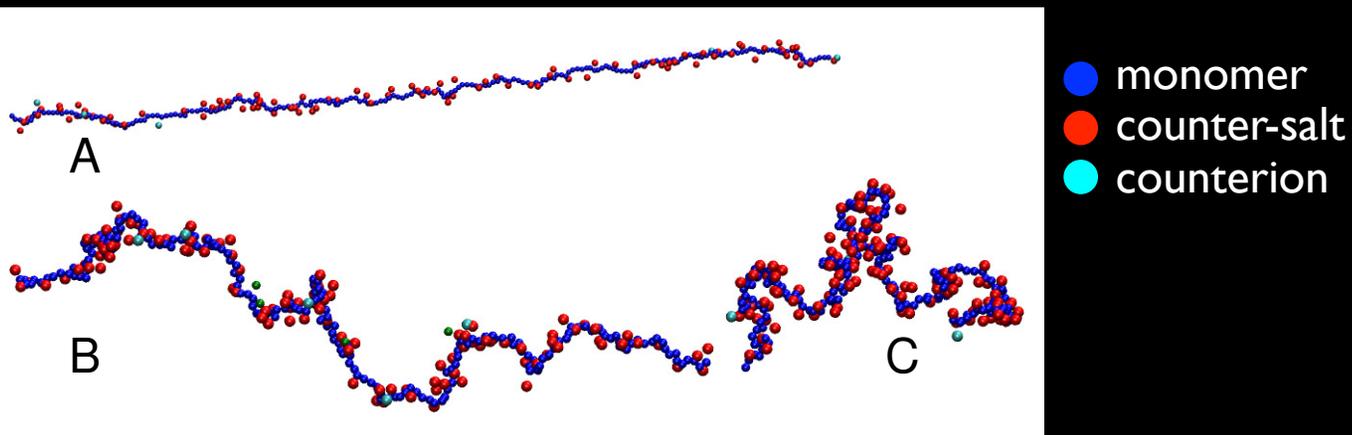


Philip Pincus

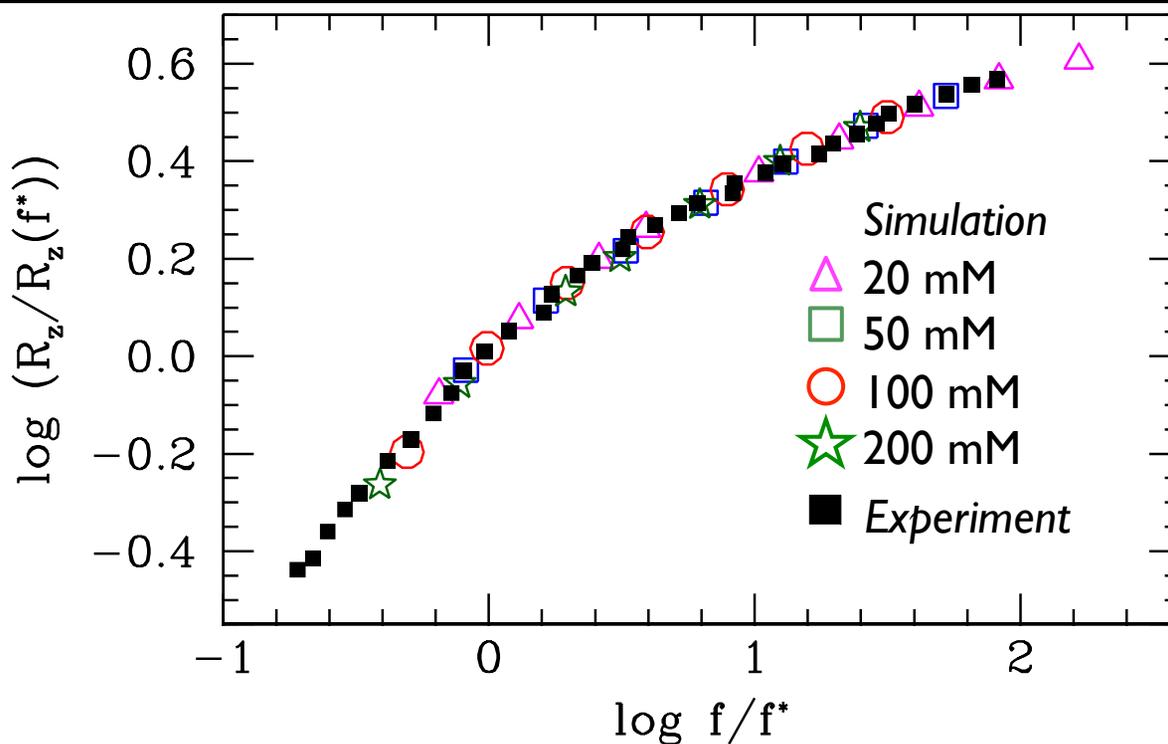


# Simulations of model polyelectrolyte match logarithmic elasticity

Stevens, McIntosh, and Saleh *Macromolecules* (2012)

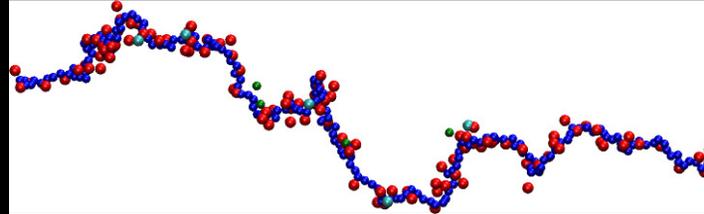


Mark Stevens,  
Sandia



*Renormalized extension v. force from expt and sim*

# Structure factor analysis: Log elasticity due to short length-scale 'crumples' unique to charged polymers



Stevens, McIntosh, and Saleh. *Macromolecules* (2012)

