Charge inversion accompanies DNA condensation by multivalent ions

DNA-based nanotechnology:
Construction, mechanics, and electronics
11 May 2008

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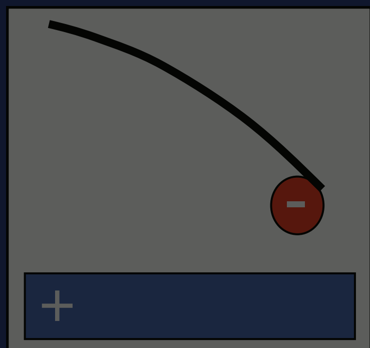
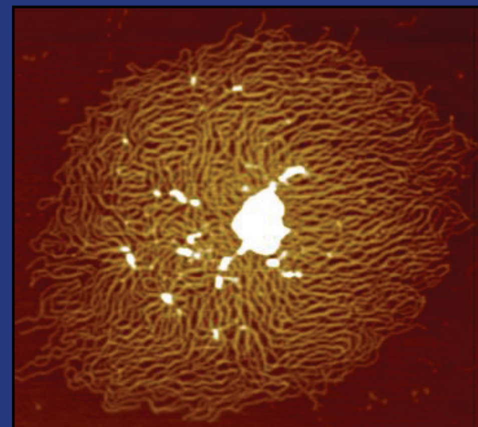
Overview: research lines

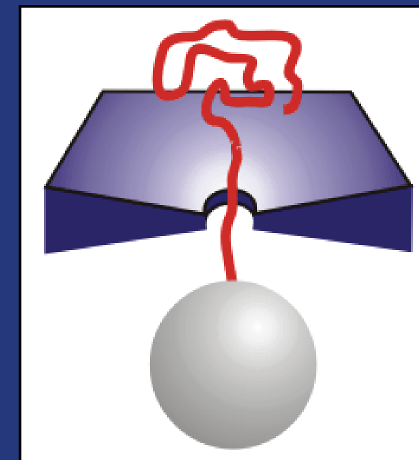
Electrostatics in liquid



Charge inversion

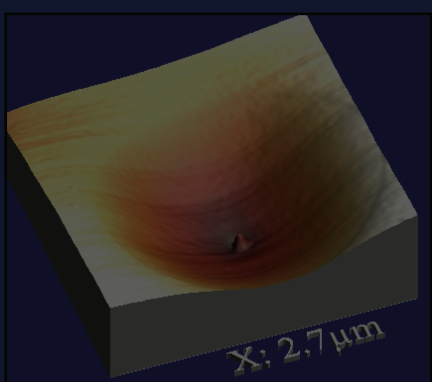


DNA condensation

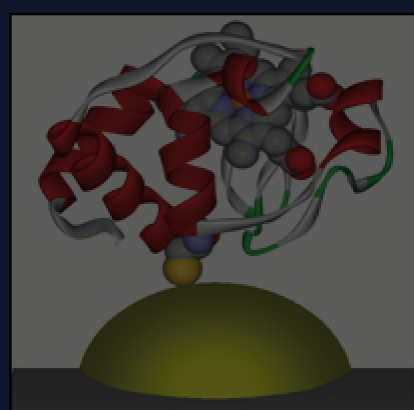


Electrophoresis

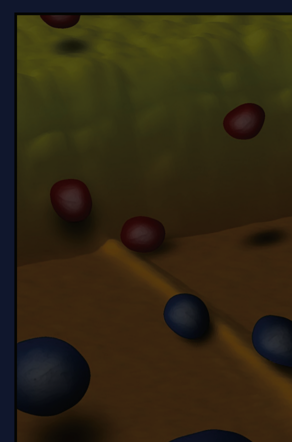
Nano-ionics



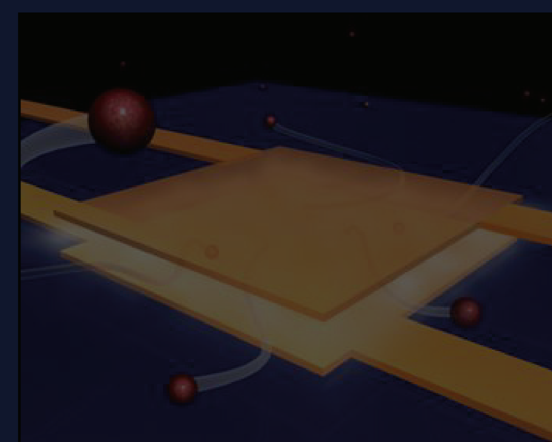
Nanopores



Voltammetry



Nanotubes



Single-molecule detection

Outline

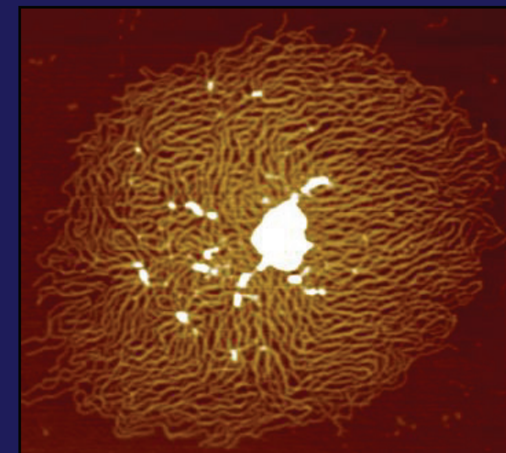
Introduction

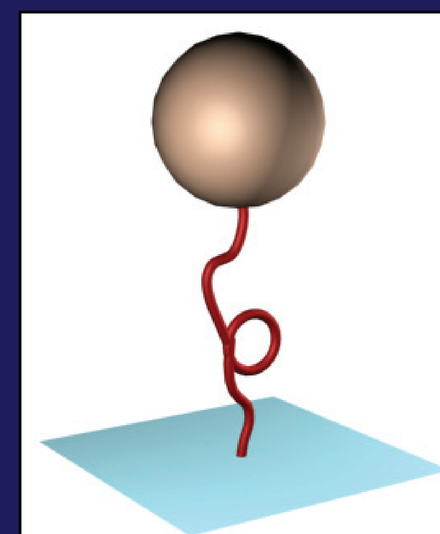
1. Atomic Force Microscopy

2. Magnetic tweezers

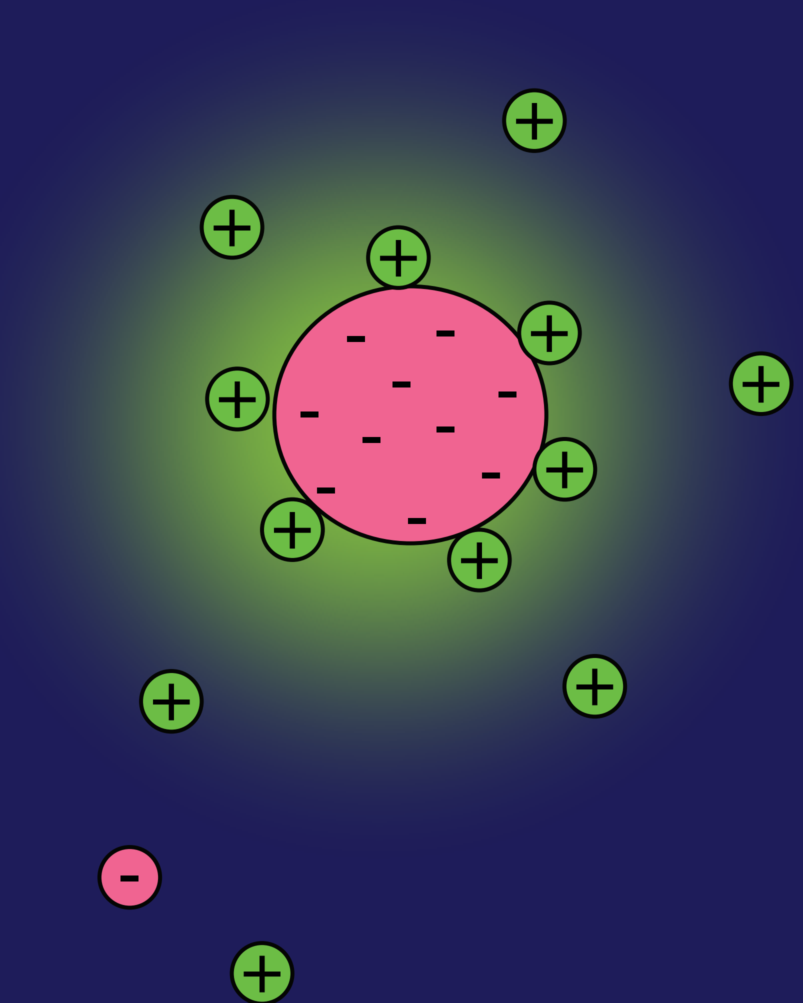
3. Electrophoresis

Summary





Basics of ionic screening



Poisson-Boltzmann equation:

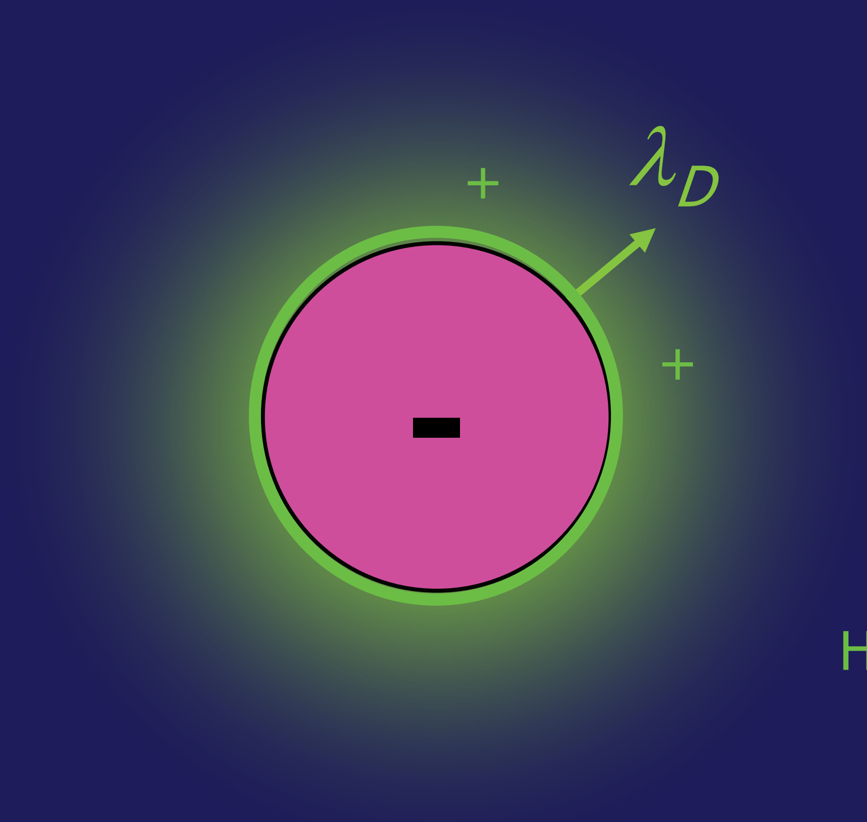
$$\underbrace{\frac{\partial^2 \phi}{\partial x^2}}_{\text{Electrostatic potential}} = -\frac{e}{\varepsilon} \sum_i z^i c_\infty^i \exp(-z^i e\phi / kT)$$

Ionic concentrations:

$$c^i(x) = c_\infty^i \exp(-ze\phi / kT)$$

Debye, Gouy & Chapman (1920's)

Basics of ionic screening

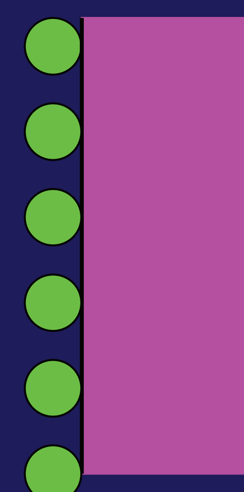


Debye length

$$\lambda_D = \sqrt{\frac{kT\varepsilon}{\sum_i z_i^2 e^2 c_i^\infty}}$$

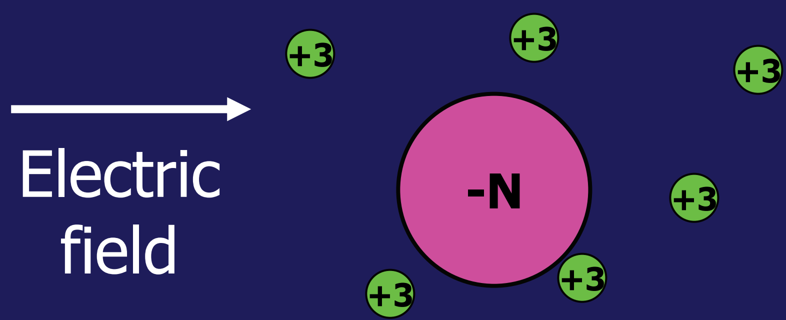
High surface charge:

"Compact layer"
"Helmholtz layer"
"Stern layer"
"Manning condensate"



Some odd observations…

1. Charge inversion



Electrophoretic mobility reverses sign at high concentration of multivalent counterions

James & Healey, J. Coll. Int. Sci. **40**, 42 (1972)
Quesada-Perez et al., ChemPhysChem **4**, 234 (2003)

Force-distance apparatus

Pashley, J. Coll. Int. Sci. **102**, 23 (1984)
Petrov, Miklavic & Nylander, J. Phys. Chem. **98** 2602 (1994)

Atomic force microscopy

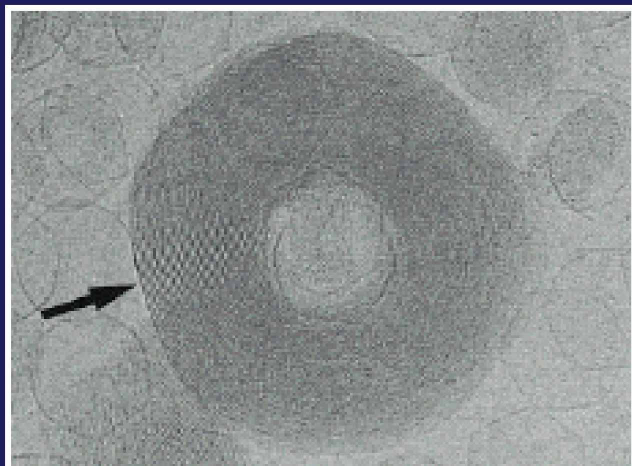
Vithayaveroj, Yiacoumi & Tsouris, J. Disp. Sci. Techn. **24**, 517 (2003)
Besteman et al, PRL 93, 170802 (2004)

Streaming currents

van der Heyden et al, PRL 96, 224502 (2006)

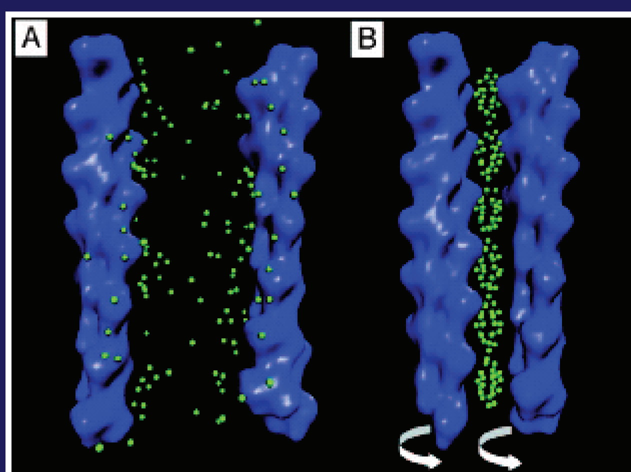
Some odd observations…

2. Like-charge attraction



DNA condensation
by spermine^{4+}

Lambert et al., PNAS 97, 7248 (2000)



Actin filaments condensed by Ba^{2+}

Angelini et al, PNAS 100, 8634 (2003)

Proposed mechanisms

W. M. Gelbart *et al*, Physics Today **53**, 38 (2000) [overview]

F. Oosawa, Biopolymers **6**, 1633 (1968)

Rouzina & Bloomfield, JPC **100**, 9977 (1996)

Kornyshev & Leikin, PRL **82**, 4138 (1999)

Golestanian, Kardar & Liverpool,
PRL **82**, 4456 (1999), PRE **66**, 051802 (2002)

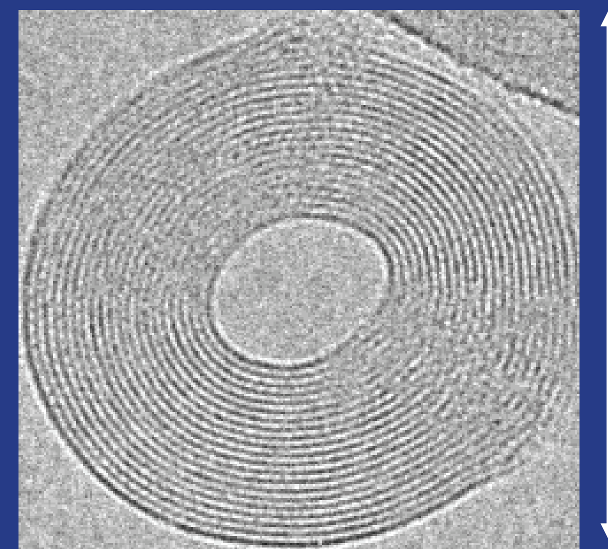
Zhang & Shklovskii, Physica A **349**, 563 (2005)

… and many others

But no smoking gun experiment!!!

Why study DNA condensation?

- Prototypical system for studying electrostatic effects (high charge density)
- High level of control
- Biological relevance (DNA packaging, chromatin structure)
- Potential application to gene therapy
- Because it's fun!

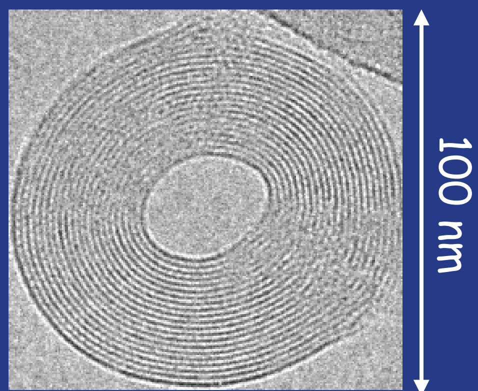


DNA condensed by spermine^{4+}

Hud *et al*, PNAS **98** (2001)

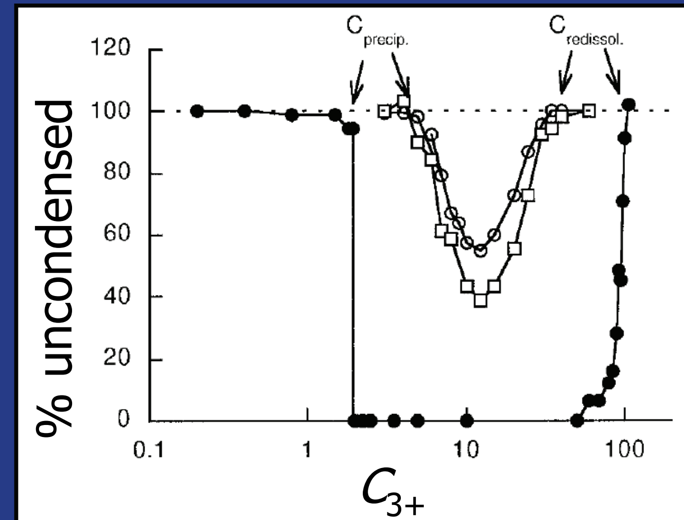
Previous experimental work

TEM, AFM



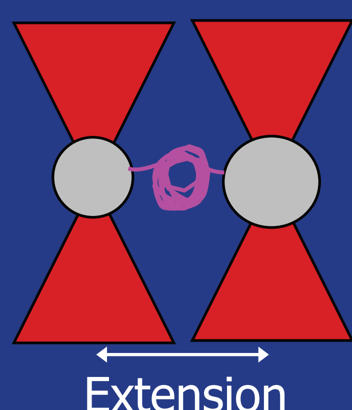
Hud *et al*, PNAS **98** (2001)

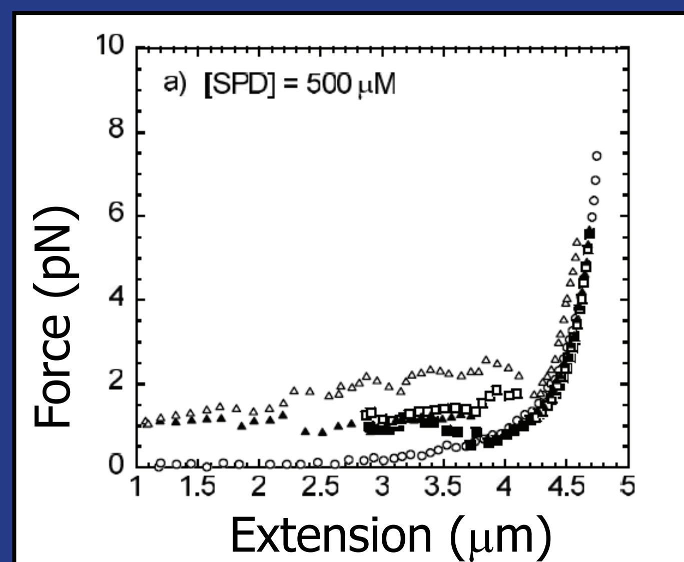
Sedimentation



Raspaud et al, Biophys. J. **74**, 381 (1998)
Pelta, Livolant & Sikorav, J. Biol. Chem. 271, 5656 (1996)

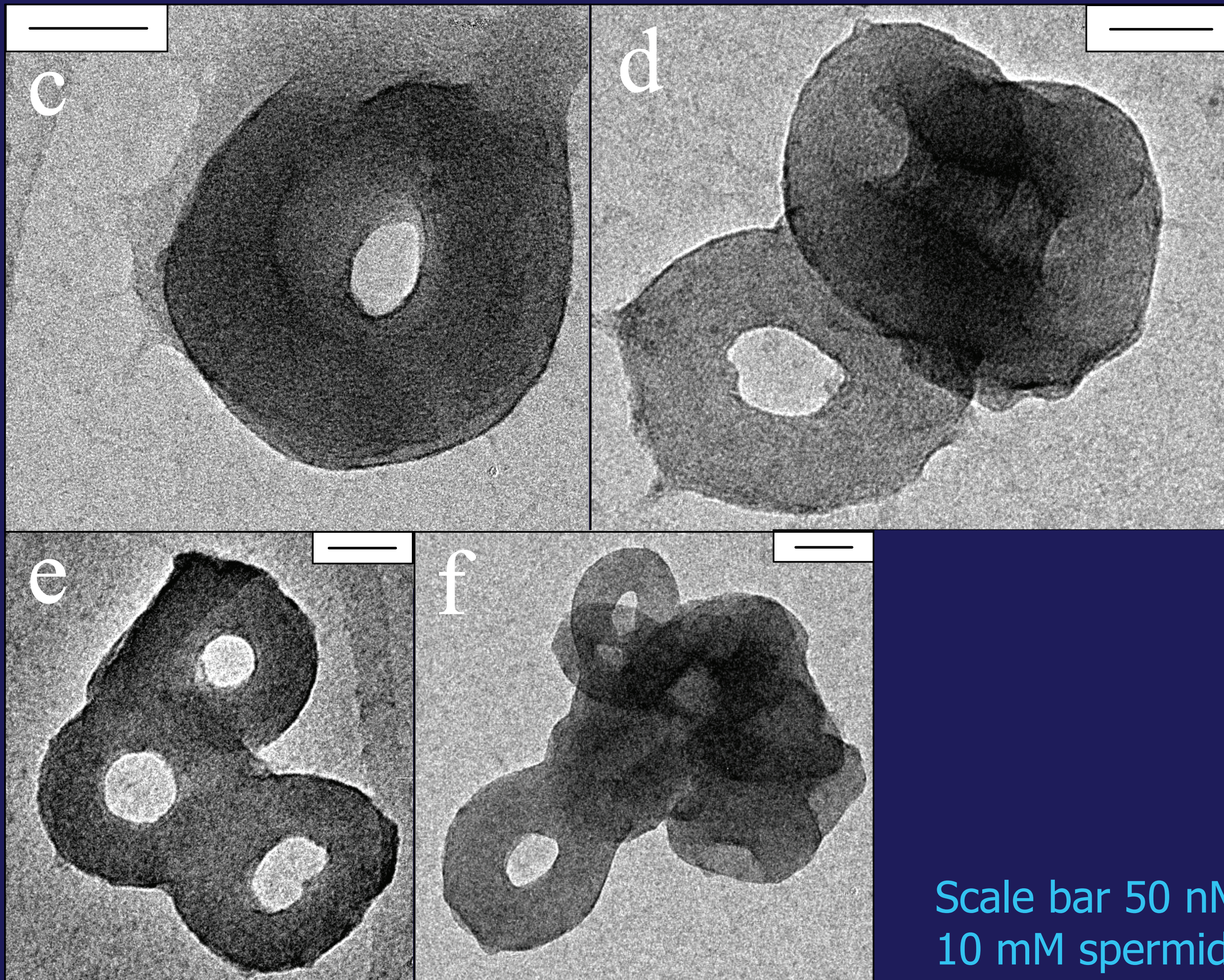
Optical tweezers





Murayama et al, PRL **90**, 018102 (2003)

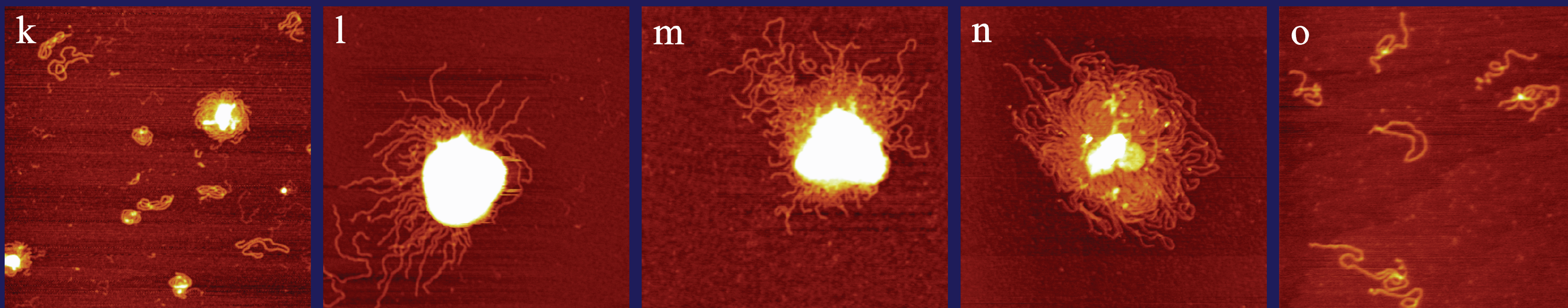
TEM images



Scale bar 50 nM
10 mM spermidine (3+)

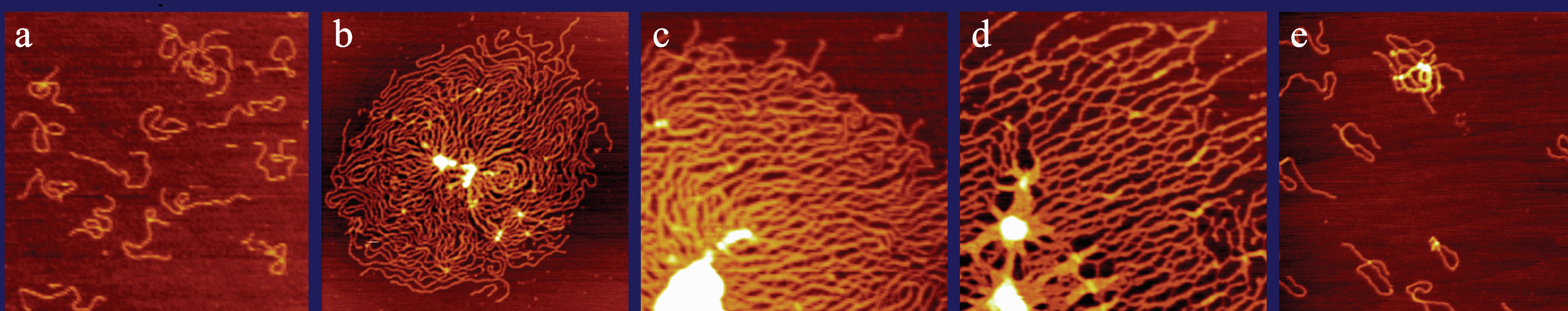
Highly-charged surfaces

POSITIVE CHARGE: Polylysine-coated mica



100 μM | 1 mM | 10 mM | 100 mM | 900 mM

NEGATIVE SURFACE: bare mica



100 μM | 1 mM | 10 mM | 100 mM | 900 mM

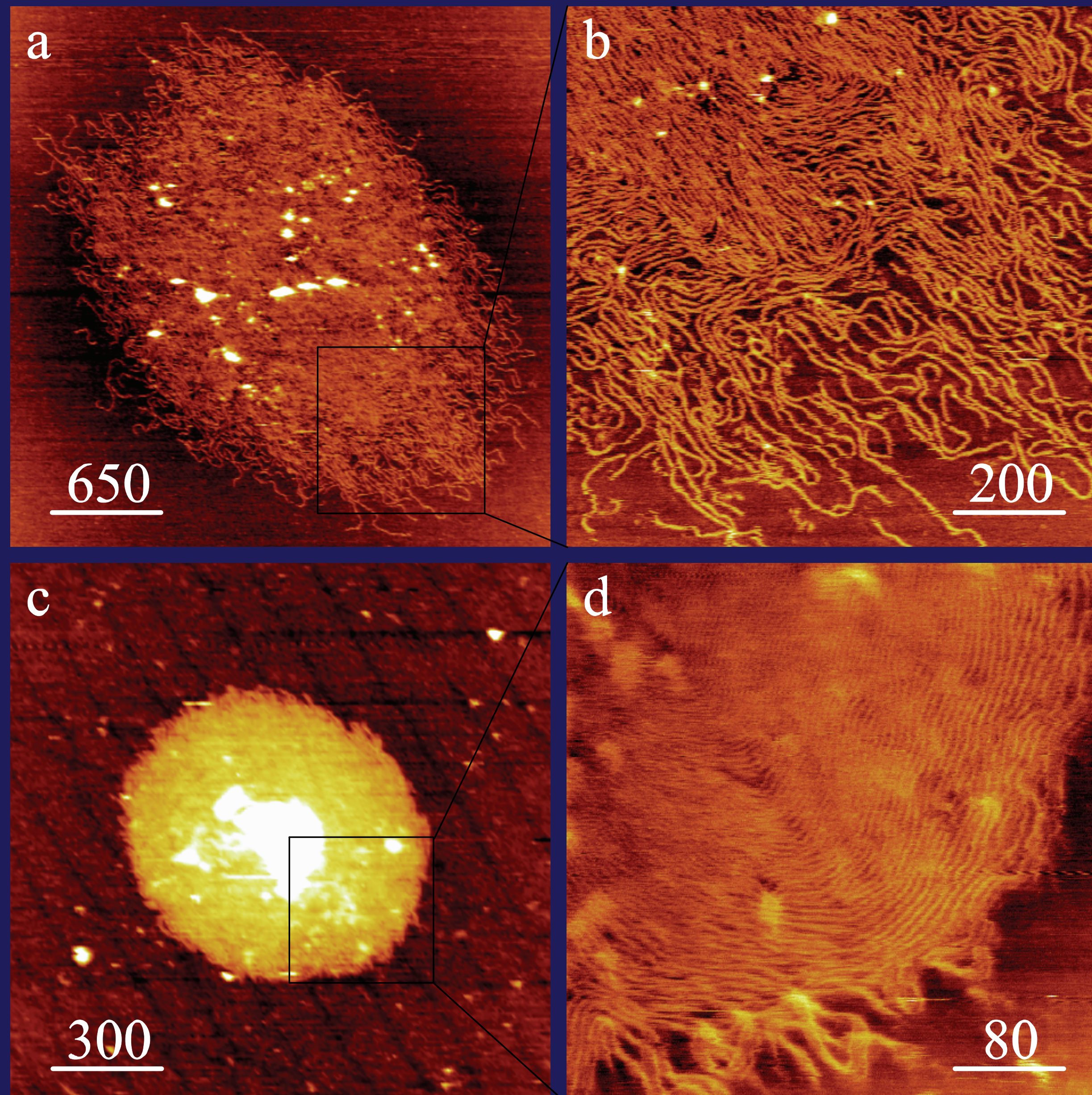
1 μm × 1 μm

Spermidine (3+)

Imaging in liquid

Bare mica

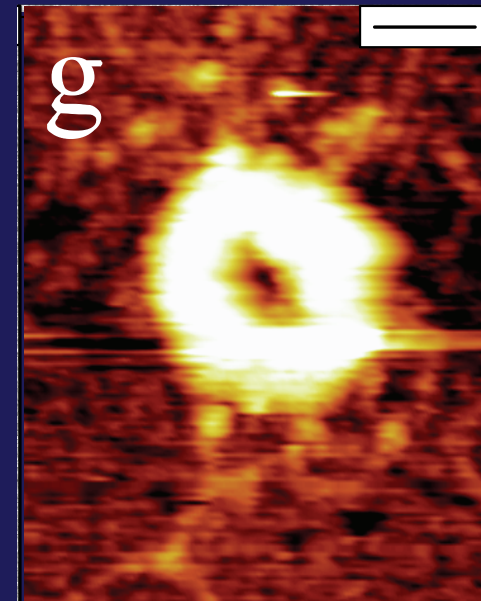
PL-coated mica



Graphite

1 mM spermidine (3+)

Scale bar 50 nm



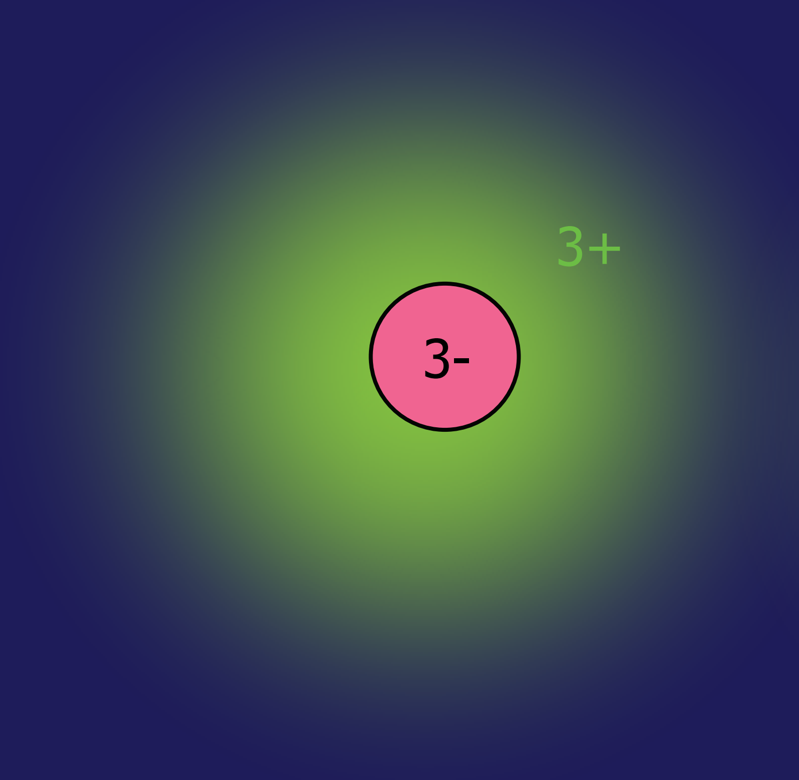
Take-home messages

Don’t use AFM to learn about condensate morphology!

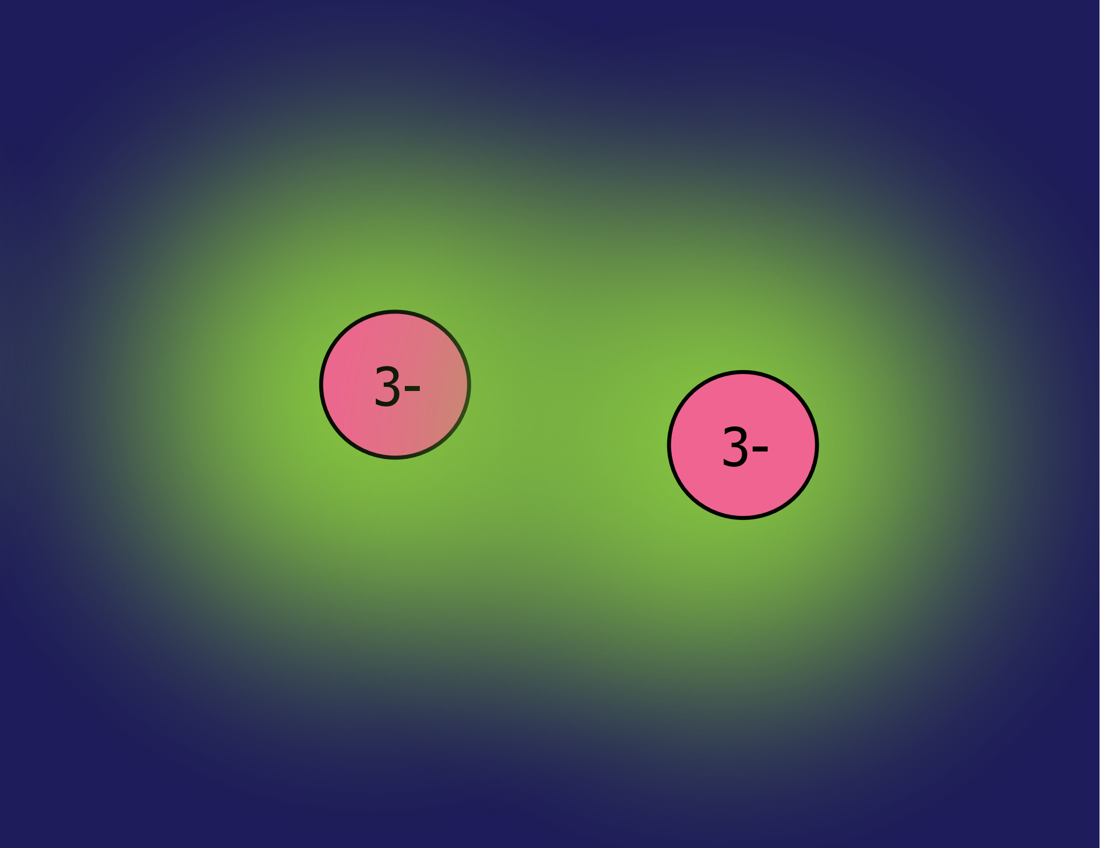
At mM concentrations of trivalent ions, highly-charged objects stick together

What’s wrong with mean-field theories?

No charge inversion

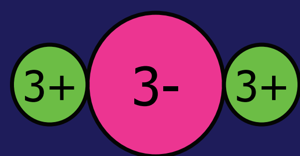


No like-charge attraction

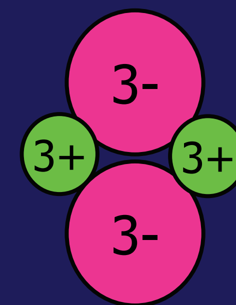


Coulomb's law

Charge inversion possible



Like-charge attraction possible



New elements:

- discreteness of charge
- spatial correlations
- electrostatic energies must be > kT

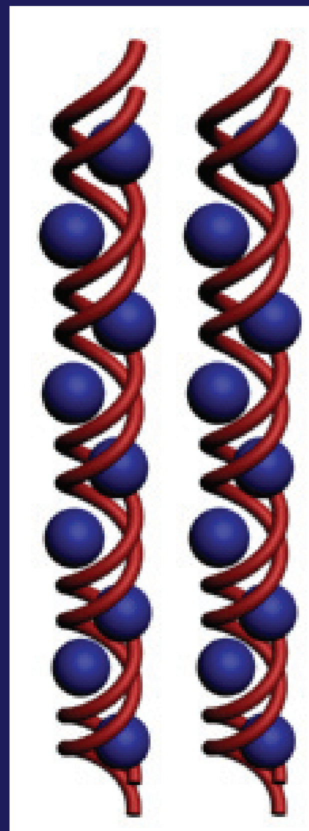
In general, minimize free energy

$$G = U - TS$$

U dominates for low T, high Z

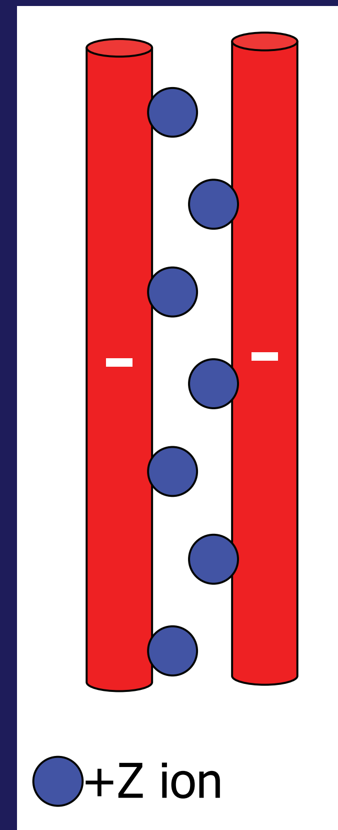
S " " high T, low Z

Proposed electrostatic mechanisms



"Electrostatic zipper"

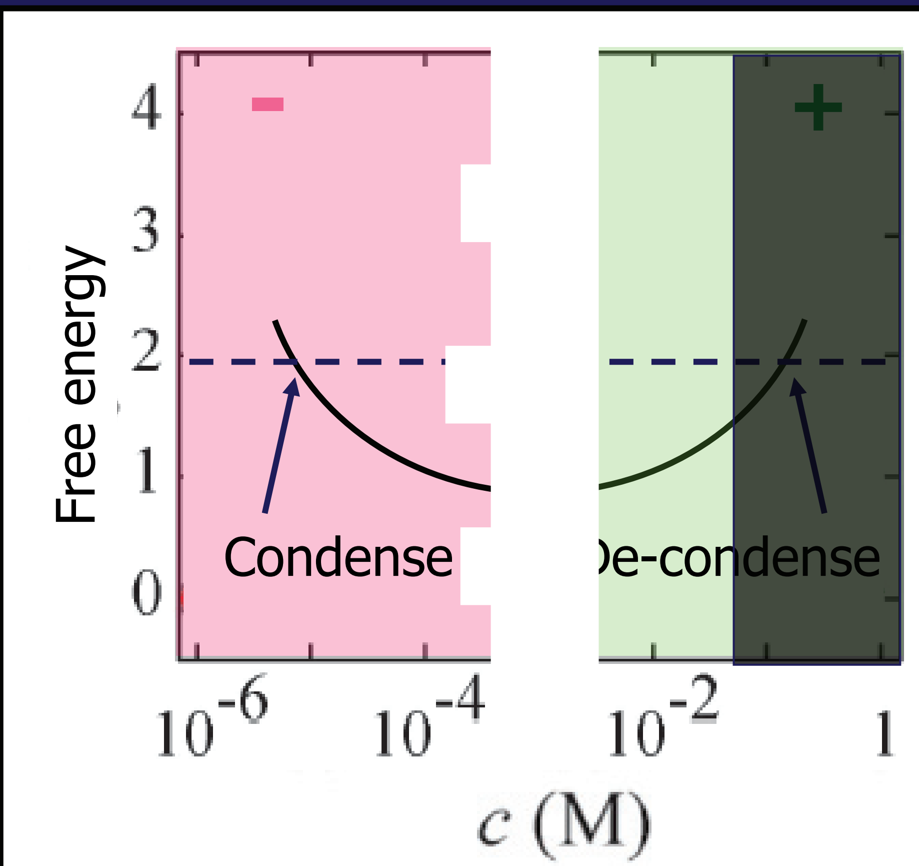
Kornyshev & Leikin, PRL **82**, 4138 (1999)



Correlated liquid

Rouzina & Bloomfield, JPC **100**, 9977 (1996)
Zhang & Shklovskii, Physica A **349**, 563 (2005)

Strongly correlated liquid mechanism



Charge inversion

Effective DNA potential

$$\phi = (kT/Ze)\ln(c/c_0)$$

c_0 = charge inversion concentration
DNA positive when $c > c_0$

Like-charge attraction

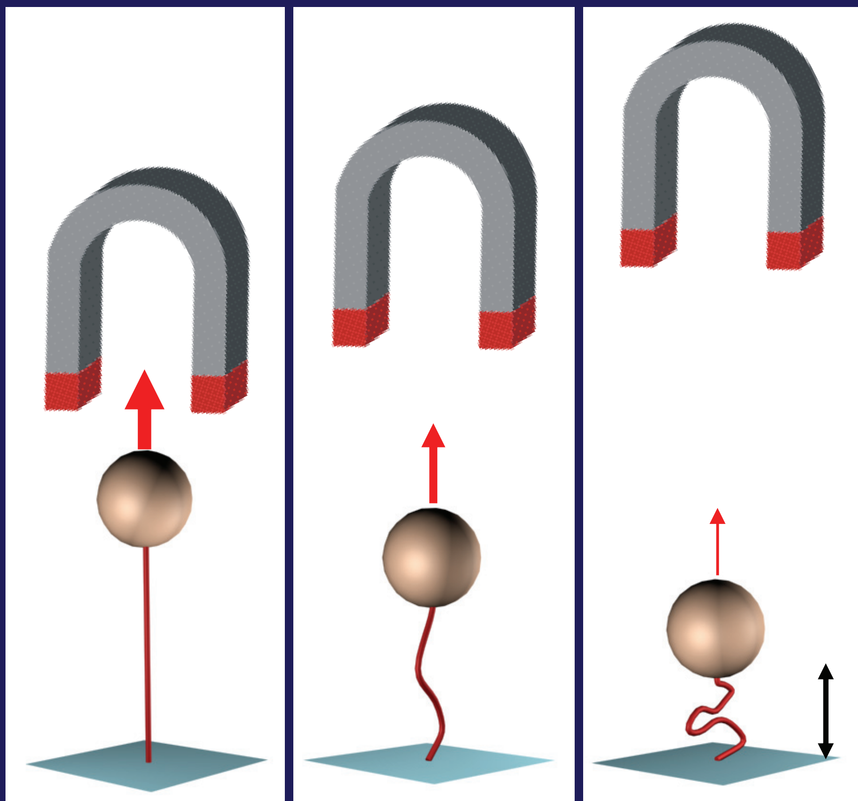
$$\text{Free energy} = \underbrace{-G_{\text{SCL}}(n)}_{\text{Short-range attraction}} + \underbrace{\frac{1}{2} C_{\text{cond}} \phi^2}_{\text{Repulsion due to net charge}}$$

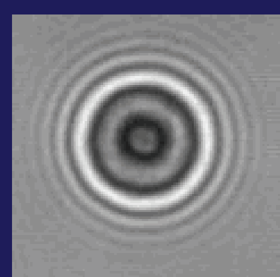
Experimental test: Measure as function of concentration

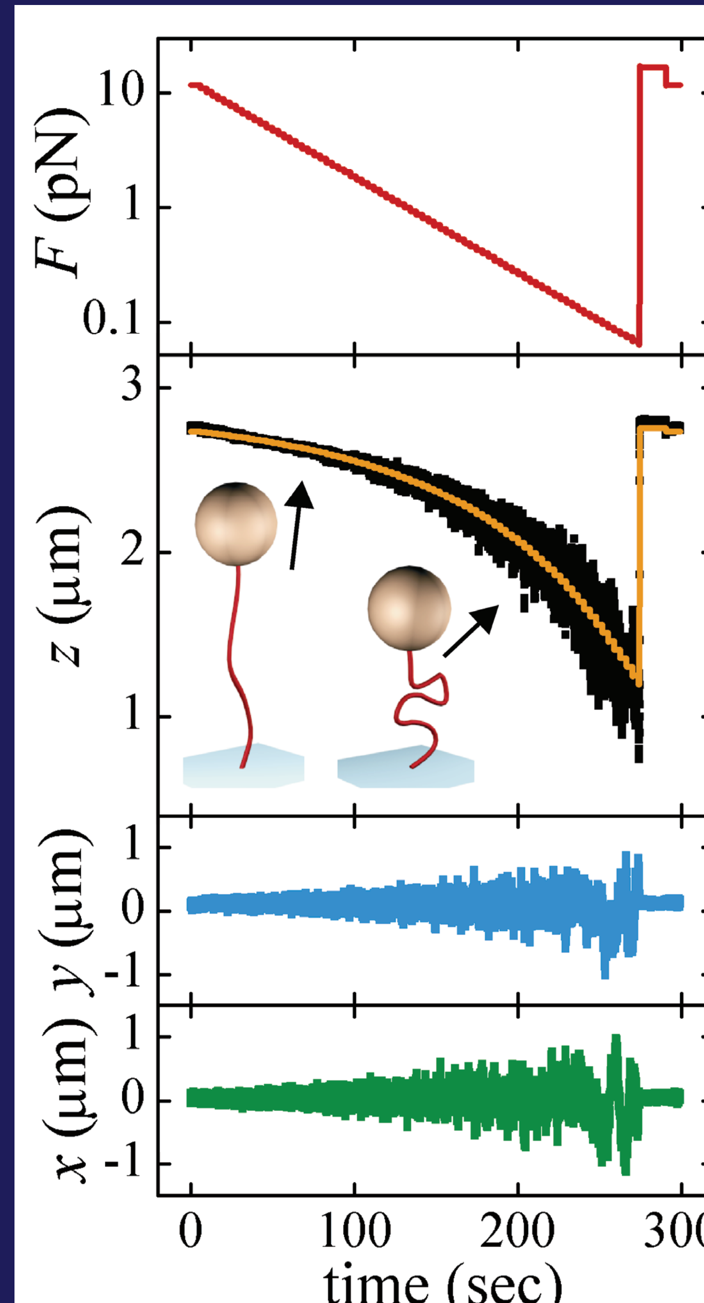
1. Free energy of condensation
2. Net charge of condensates

Yang & Rau, Biophys. J. 89, 1932 (2005)

Magnetic tweezers



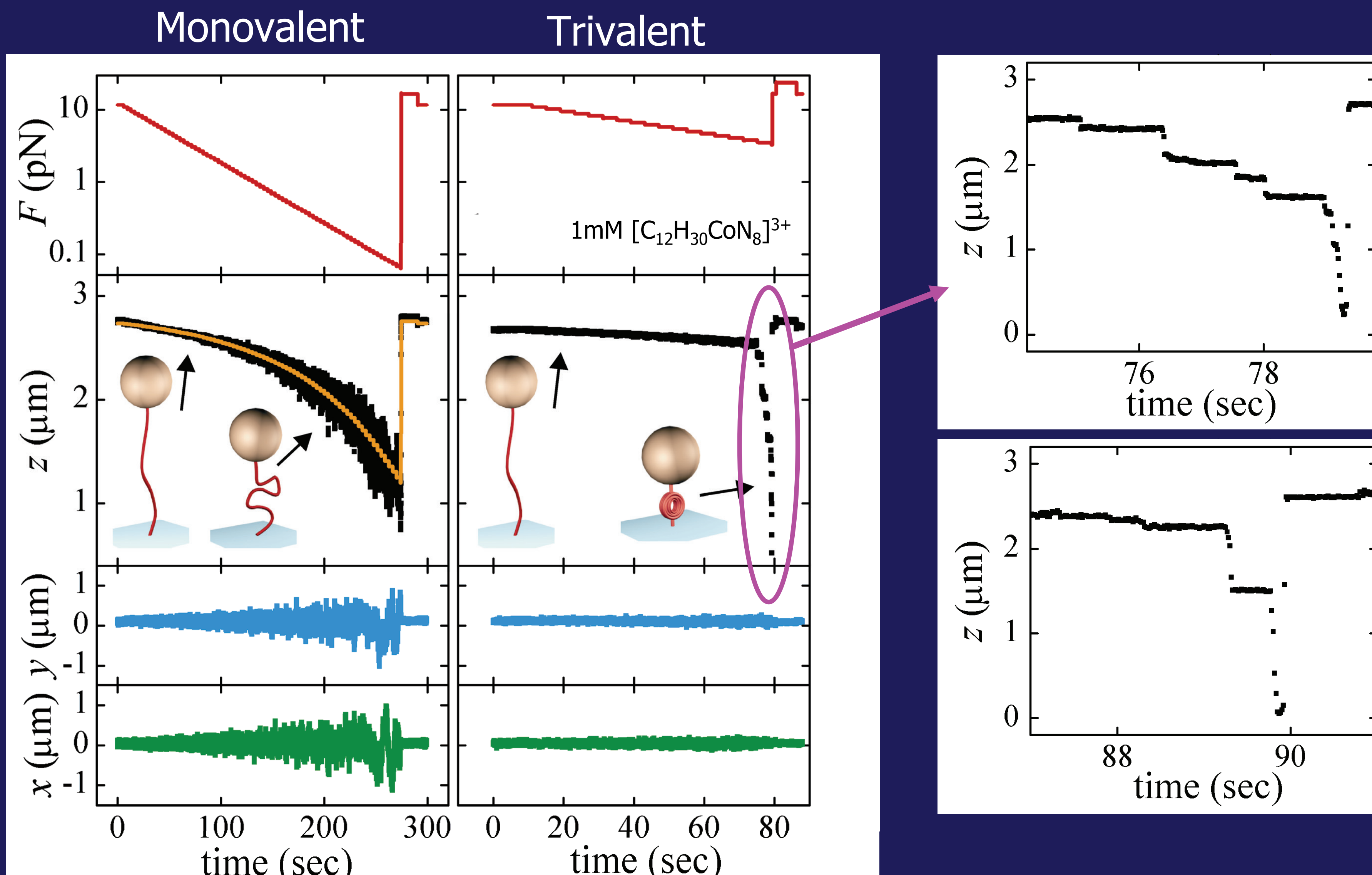




8kbp nicked DNA, 10mM TRIS, pH7.5

Worm-like-chain fit L = 2.85 μm, p = 51.6 nm

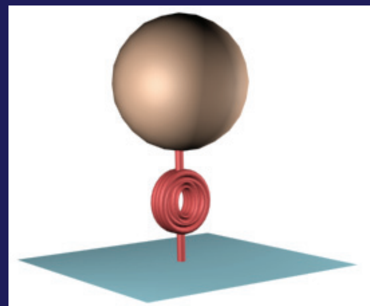
DNA condensation



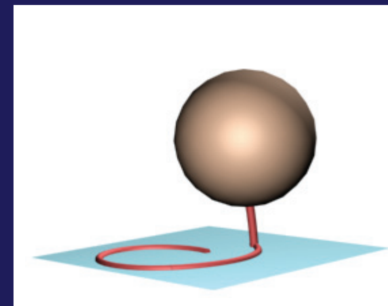
Condensation is a first-order, nucleated process

Ruling out DNA-surface interactions (1/3)

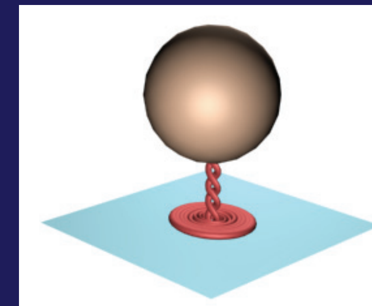
Potential problem



DNA-DNA

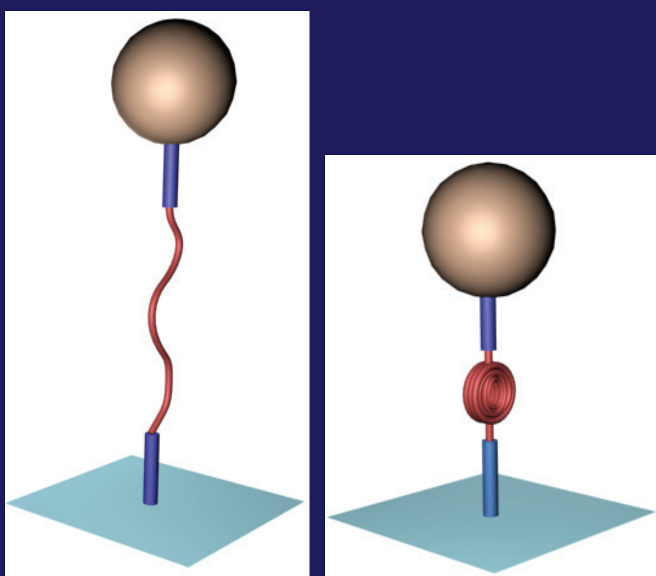


DNA-surface



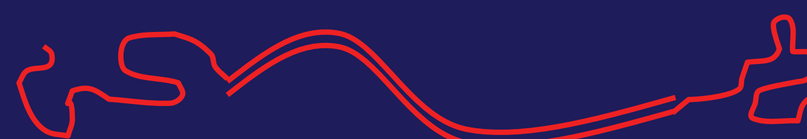
Both

Solution

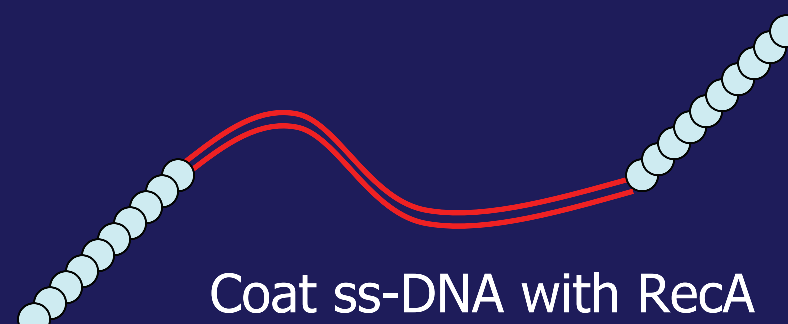


Stiff spacers

Implementation



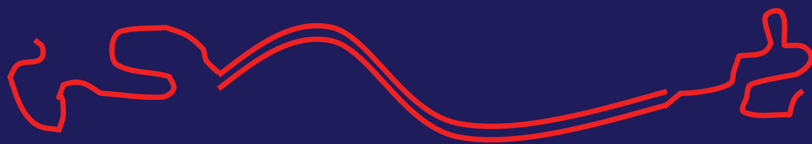
ss-ds-ss DNA

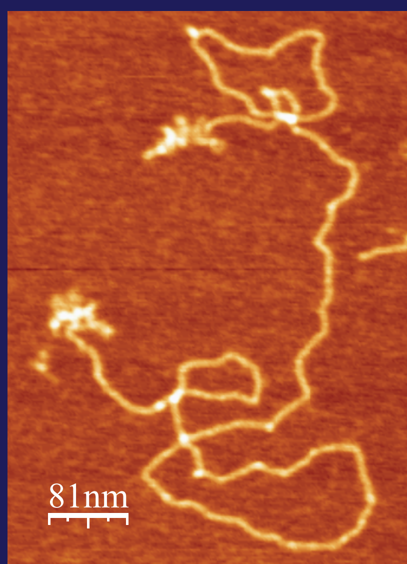


Coat ss-DNA with RecA

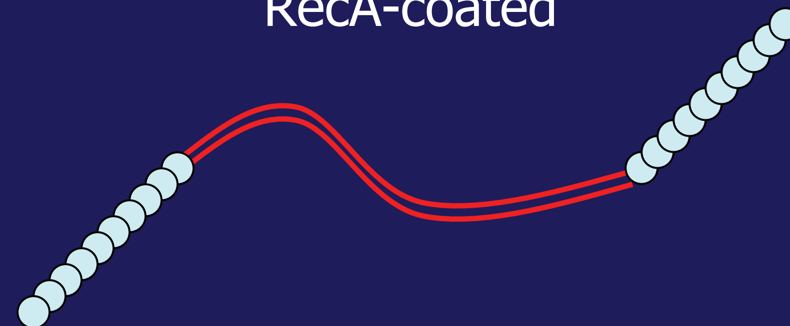
Ruling out DNA-surface interactions (2/3)

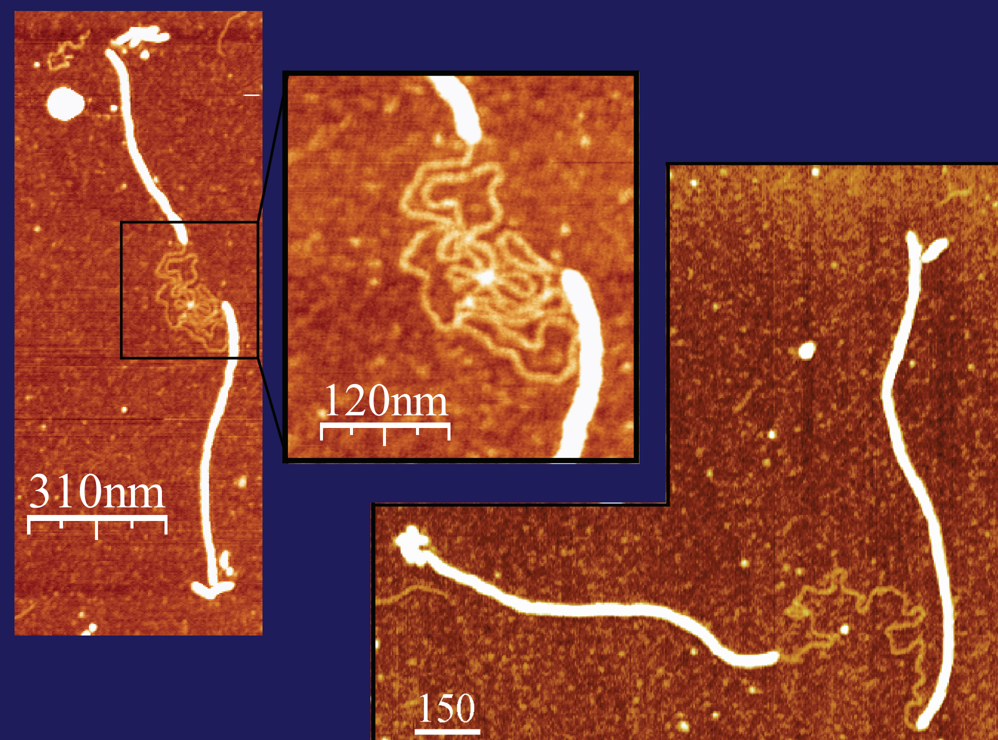
ss-ds-ss-DNA



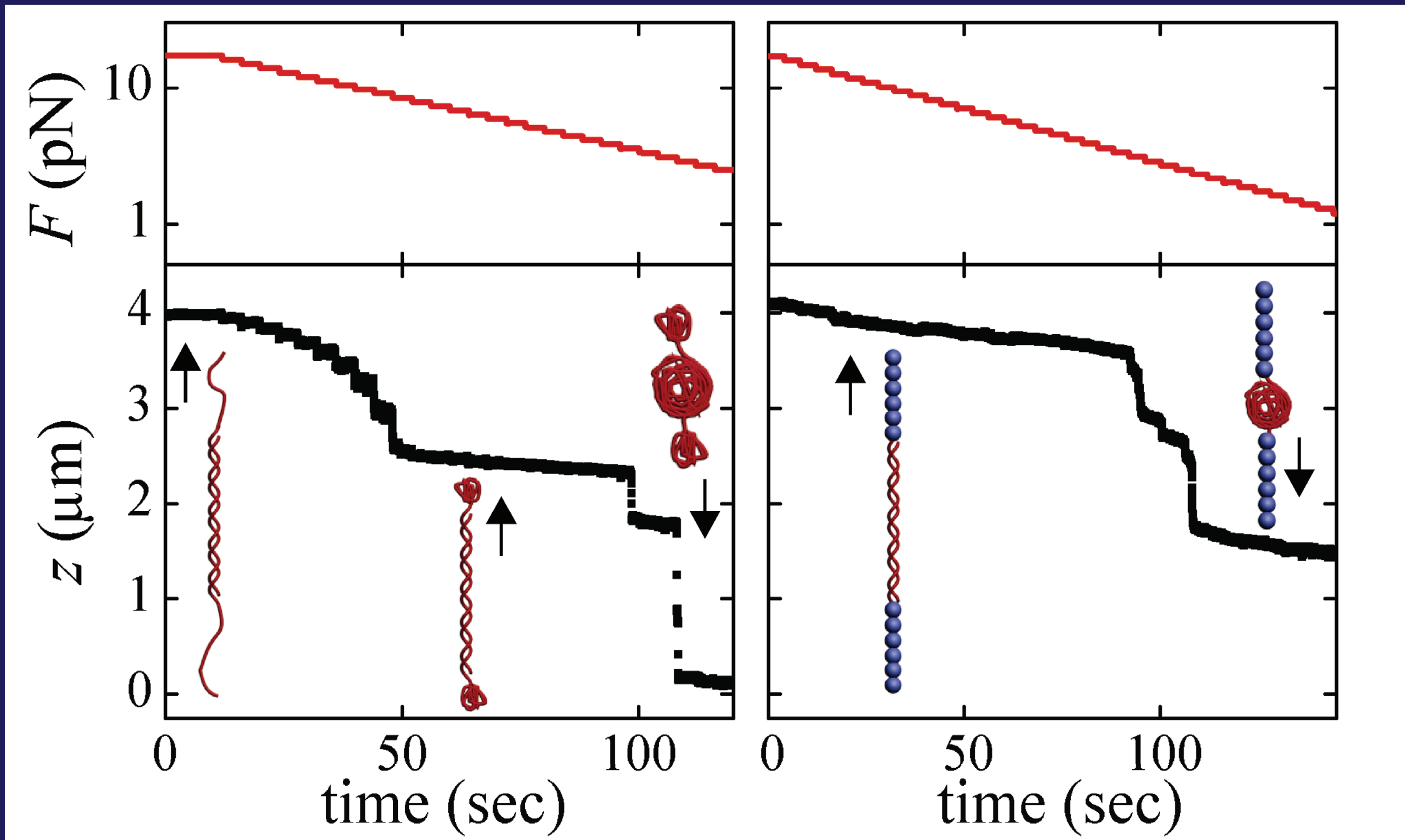


RecA-coated



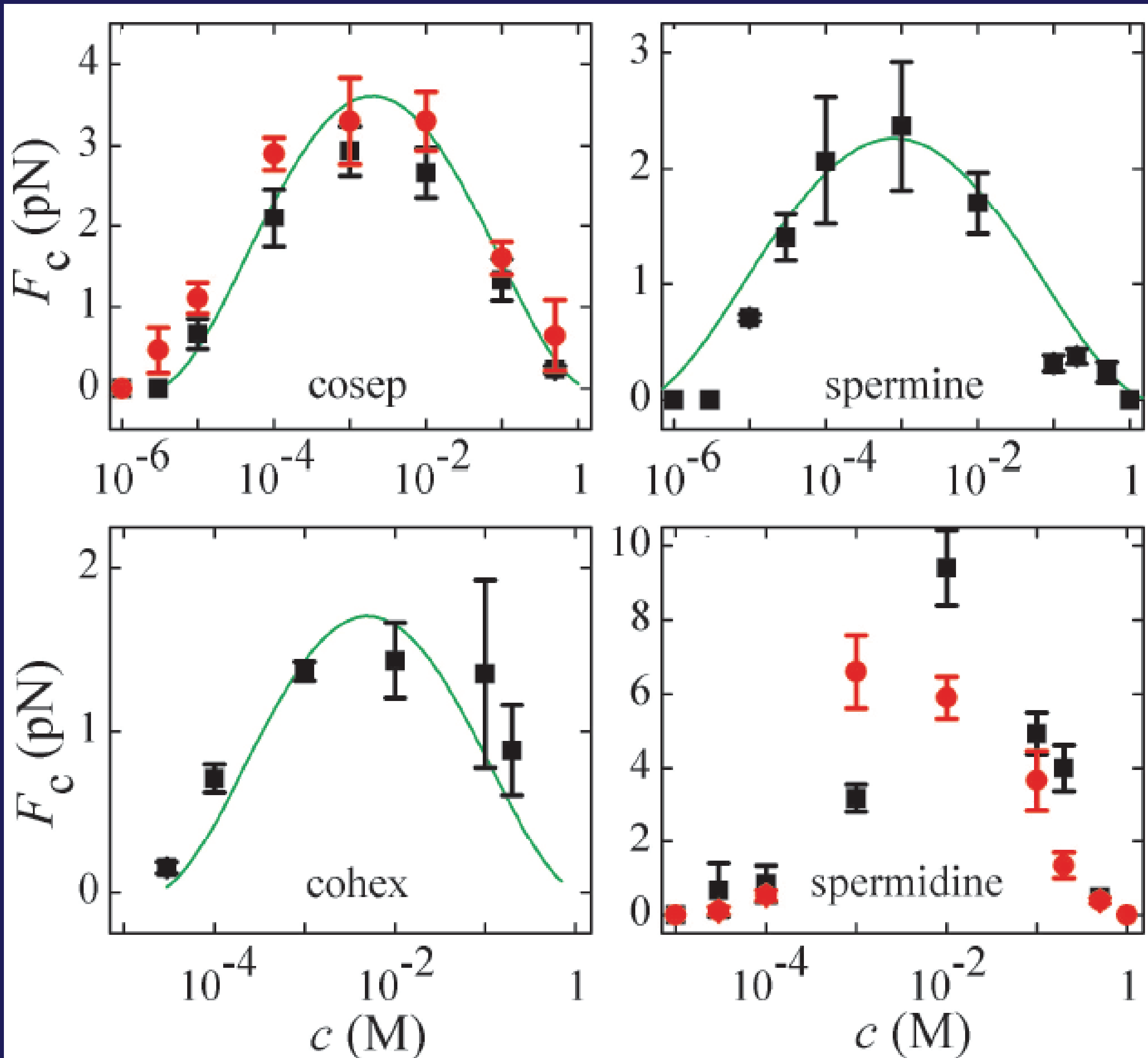


Ruling out DNA-surface interactions (3/3)



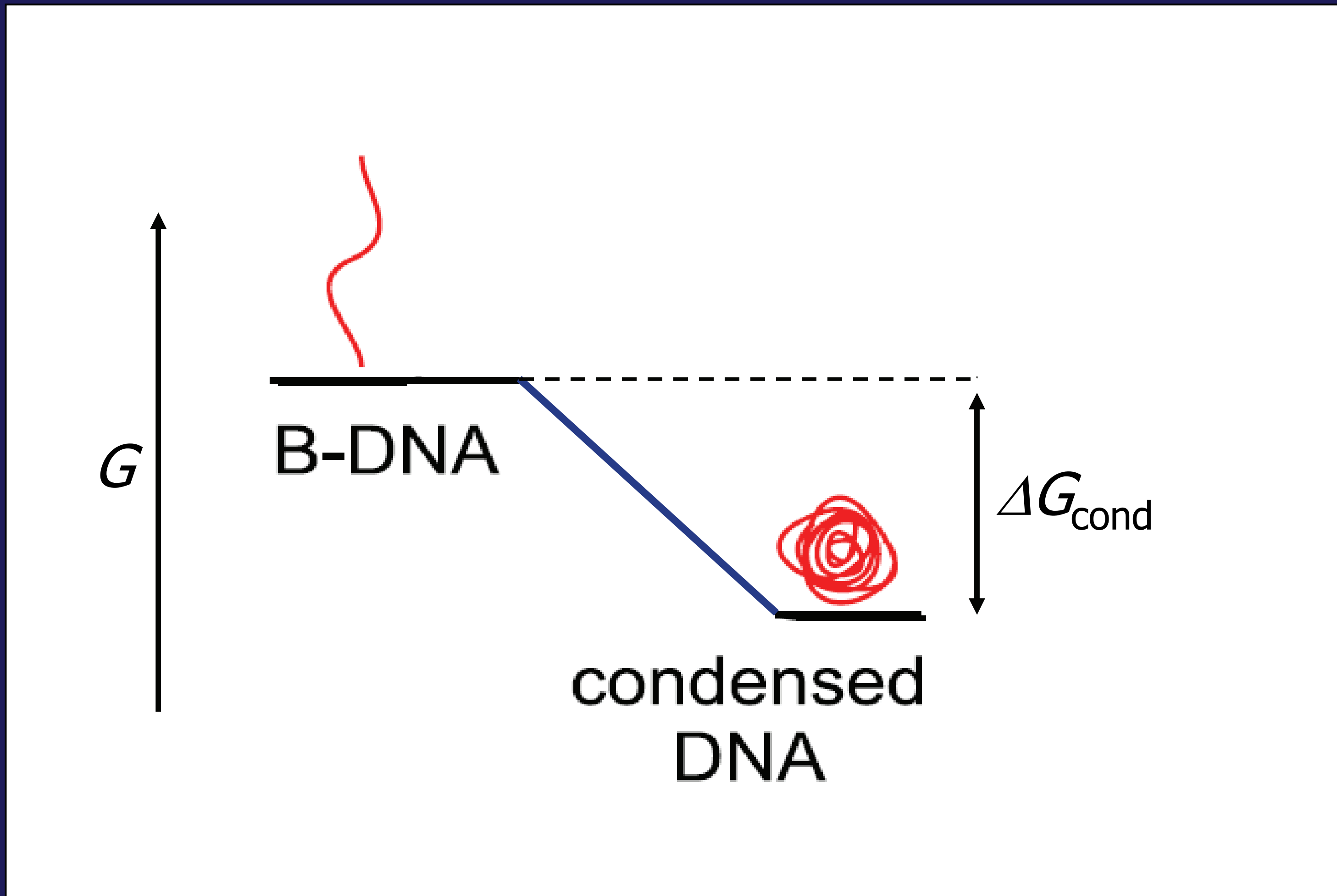
- RecA-coated ss-DNA behaves as stiff rod
- Condensation concentration and dynamics unaffected by surfaces

Condensation force F_c



- Reentrant behavior manifest at single-molecule level
- Some ion specificity (especially spermidine)
- Approximately quadratic in ln (c/c_0) "as expected"

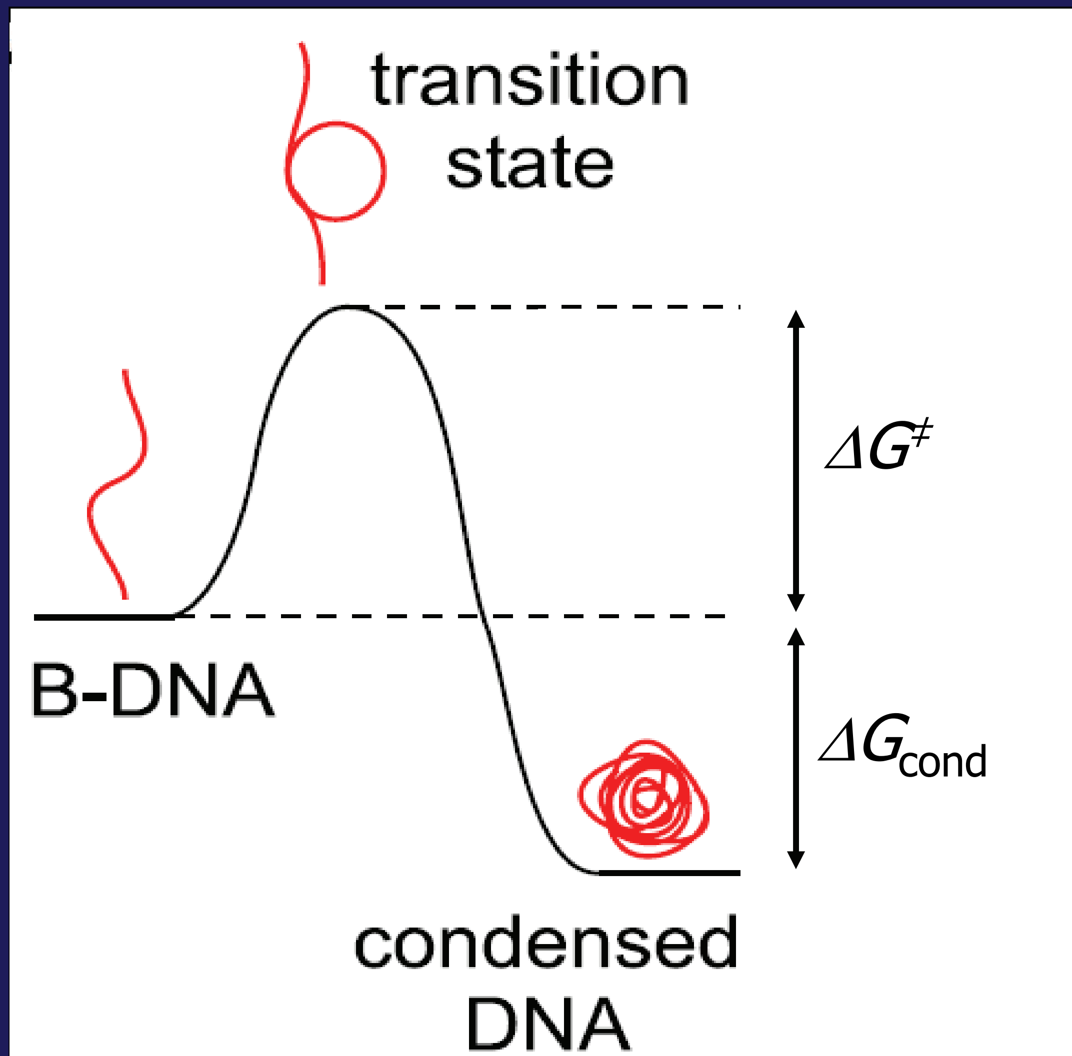
What are we probing?



F_c = free energy of condensation per unit length

Murayama et al, PRL **90**, 018102 (2003) (exp't)
Zhang et al, Physica A **349**, 563 (2005) (theory)

But we observe nucleation kinetics!



Hypothesis: transition state is a loop

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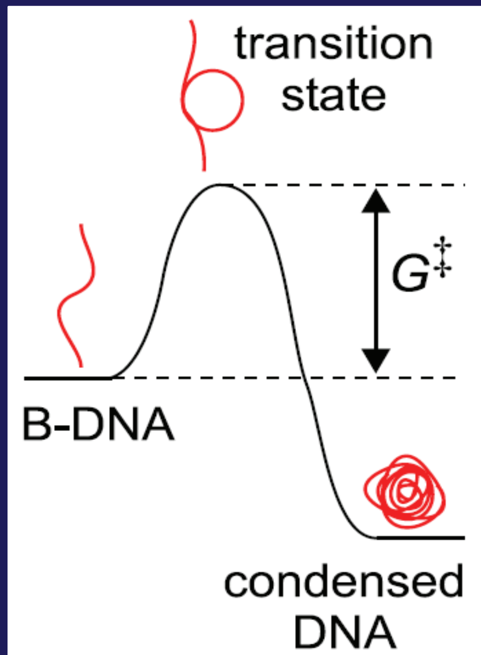
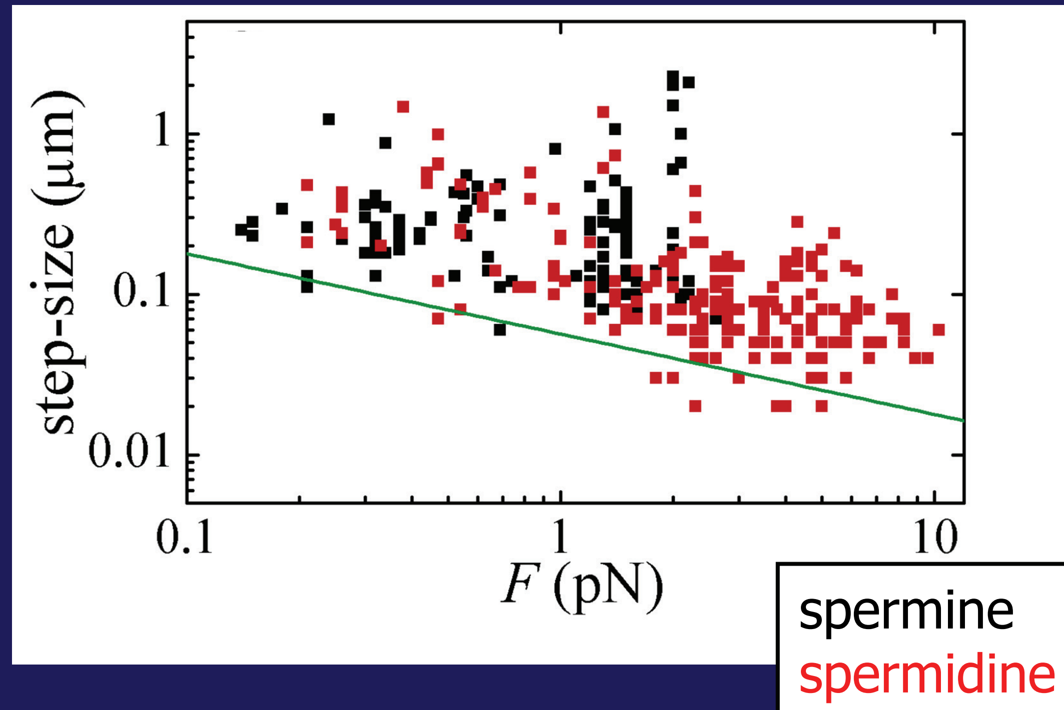




$$U_{loop} = \underbrace{\frac{\pi k_b T p}{R}}_{\text{bending energy}} + \underbrace{2\pi R F}_{\text{work against force}}$$

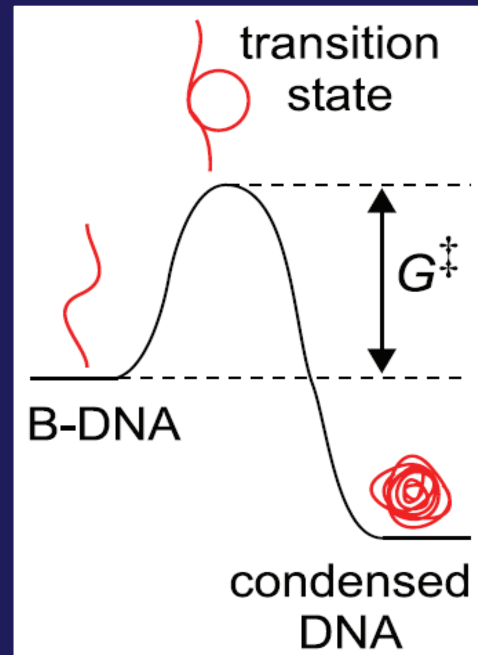
Lowest-energy loop:

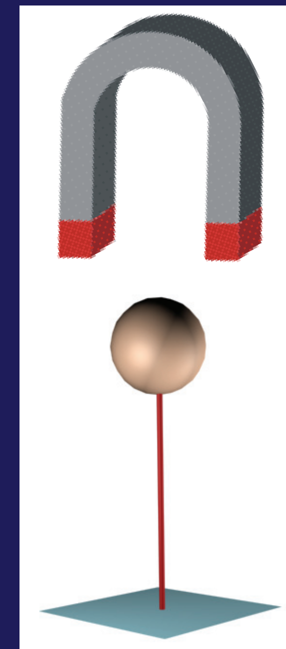
$$R = \sqrt{\frac{k_b T p}{2F}}$$

T. R. Strick et al., Prog. Biophys. Molec. Biol. 74, 115 (2000)

Testing the loop hypothesis

Winding the spring:
Pre-twisting a torsionally constrained molecule lowers the energy for forming a loop





$$\Delta G^{\ddagger} = U_{\text{bend}}(F) + U_{\text{twist}}(n) + G_{\text{elec}} + \ldots = G_{\text{crit}}$$

Loop energy

Torsional energy per turn

$$\delta U_{\text{twist}} + \delta U_{\text{bend}} = 0$$

$$U_{\text{bend}}(F) = \sqrt{8\pi^2 k_b T p F}$$

$$U_{\text{twist}}(n) = -\frac{4\pi^2 C|n|}{L}$$

T. R. Strick et al., Prog. Biophys. Molec. Biol. 74, 115 (2000)

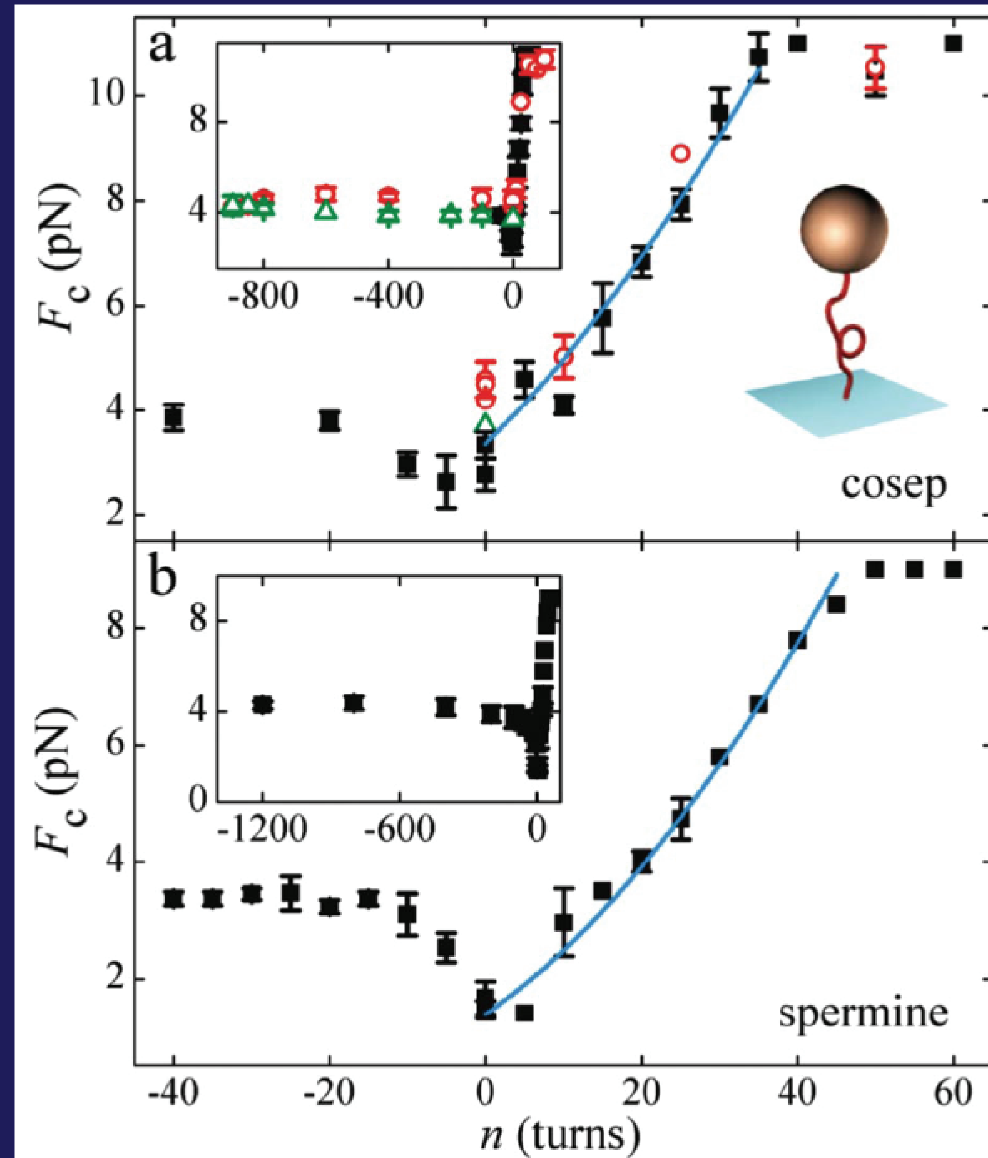
Testing the loop hypothesis

$$F_c(n) = F_c(0)\left(1 + \frac{2\pi C}{L\sqrt{2k_b T p F_c(n=0)}}\, n\right)^2$$

$F_c(0)$ = fit parameter

C = 86 nm$k_b T$

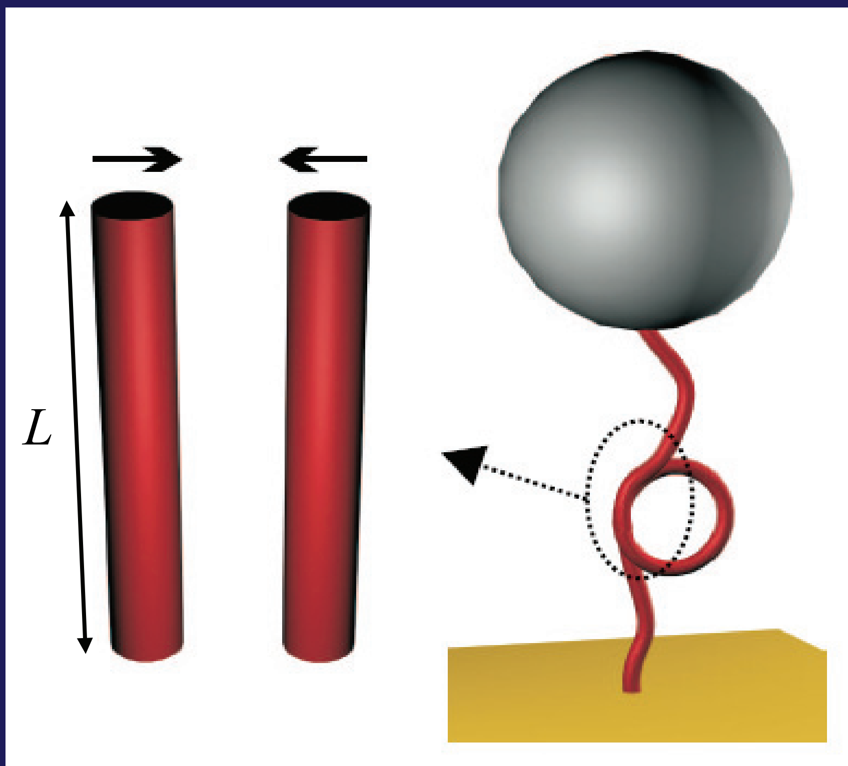
p = 50 nm



Nucleation state contains a DNA loop

So what causes the concentration dependence?

$$\Delta G^{\ddagger} = U_{\text{loop}} + G_{\text{elec}} + \ldots = G_{\text{crit}}$$



Approximate as two cylinders

$$G_{\text{elec}} = \frac{\pi \varepsilon L}{\ln\left(1 + \lambda_D / R_c\right)} \phi^2$$

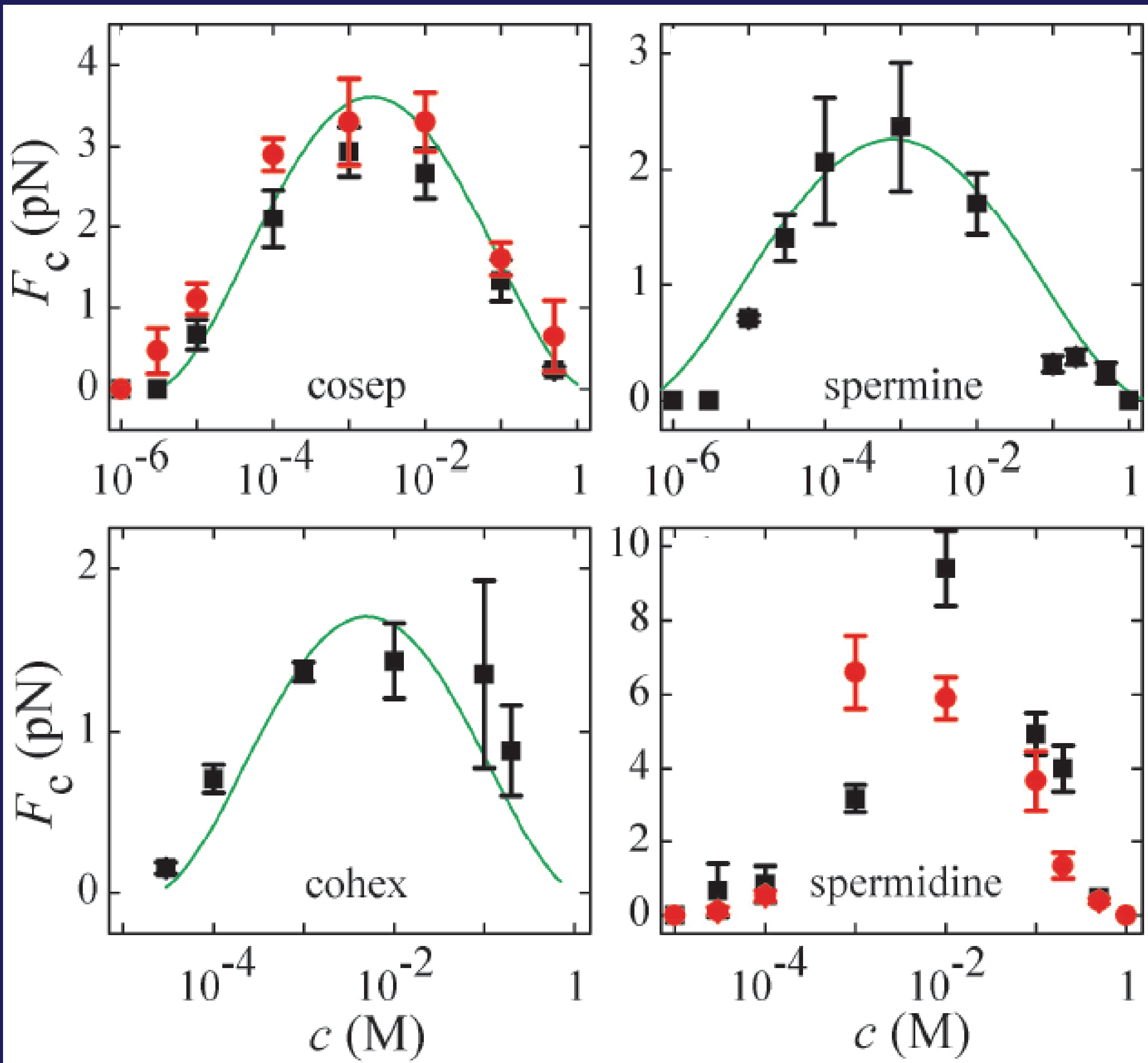
$$\phi = (kT / Ze) \ln (c / c_0)$$

$$\delta U_{\text{loop}} + \delta G_{\text{elec}} = 0$$

$$F_c(c) = F_c(c_0)\left(1 - \frac{L}{a} \ln\left(\frac{c}{c_0}\right)\right)^2$$

a = known constant

Fit to our simple model

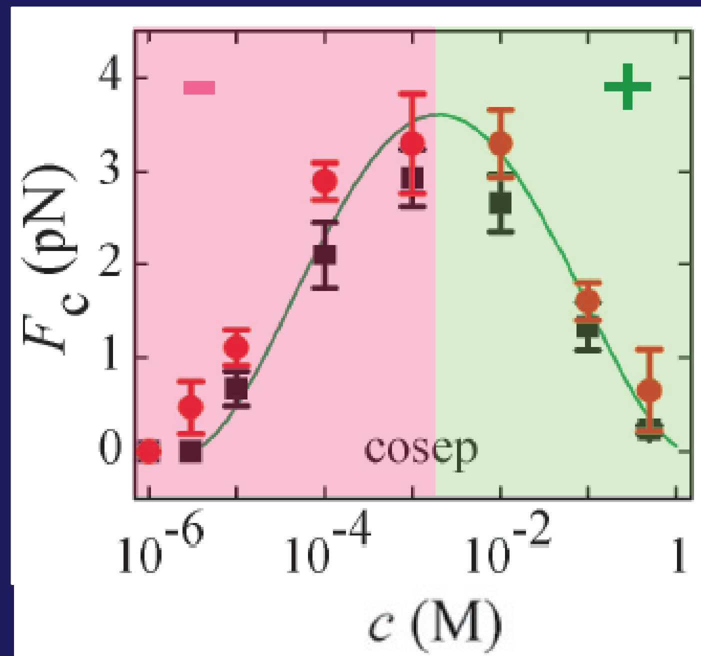


$$F_c = F_c(c_0)\left(1 - \frac{L}{a}\ln\left(\frac{c}{c_0}\right)\right)^2$$

- Qualitatively good fit (except spermidine)
- Fits yield
  L = 40 nm
  (compare to loop perimeter >34 nm)

Acid test

Prediction: For $c>c_0$, the DNA should be positively charged



Problem:

Nobody had ever reported positive DNA in electrophoresis!

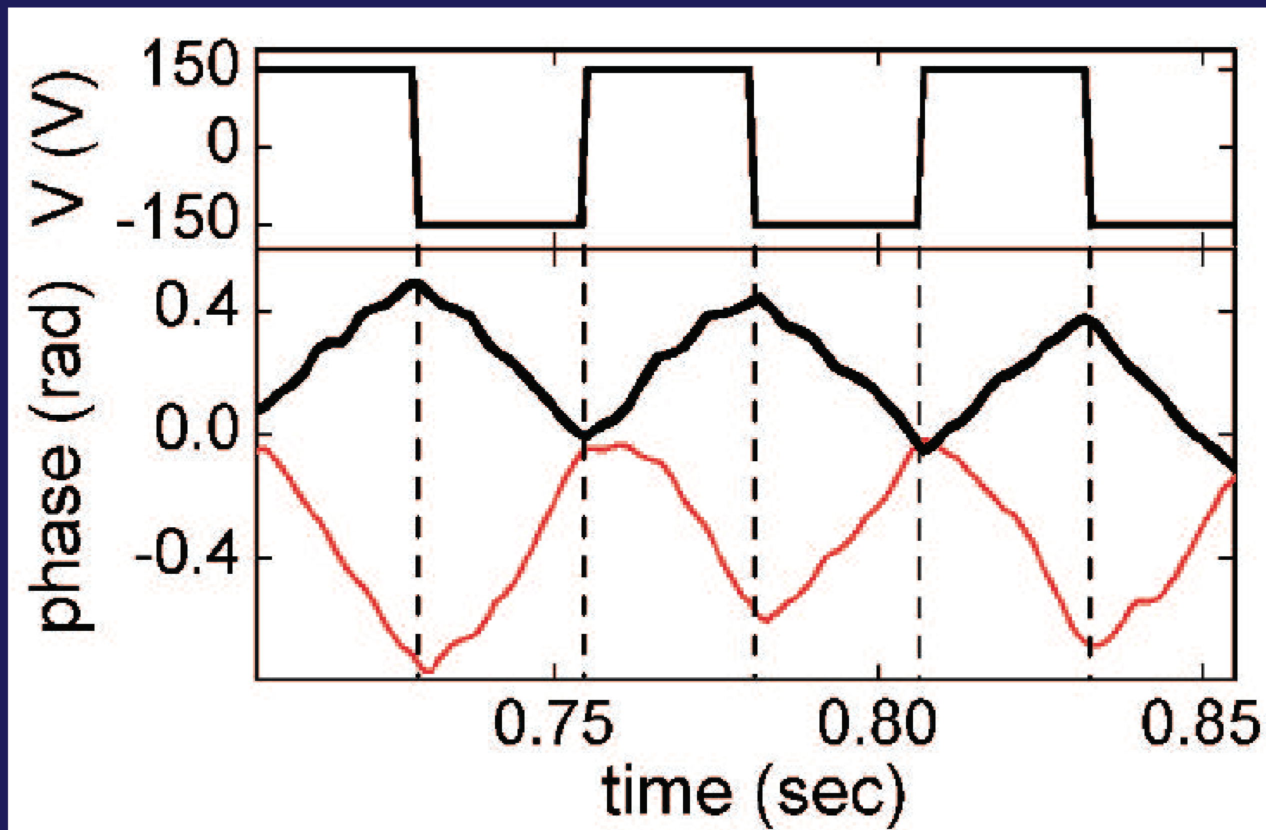
But not a straightforward experiment:

- Condensates get stuck in the gel
- Mobility is very low
- Electroosmotic flows

Dynamic light scattering

Raw data

Slope $\propto$ electrophoretic mobility



Spermine^{4+}

3 mM

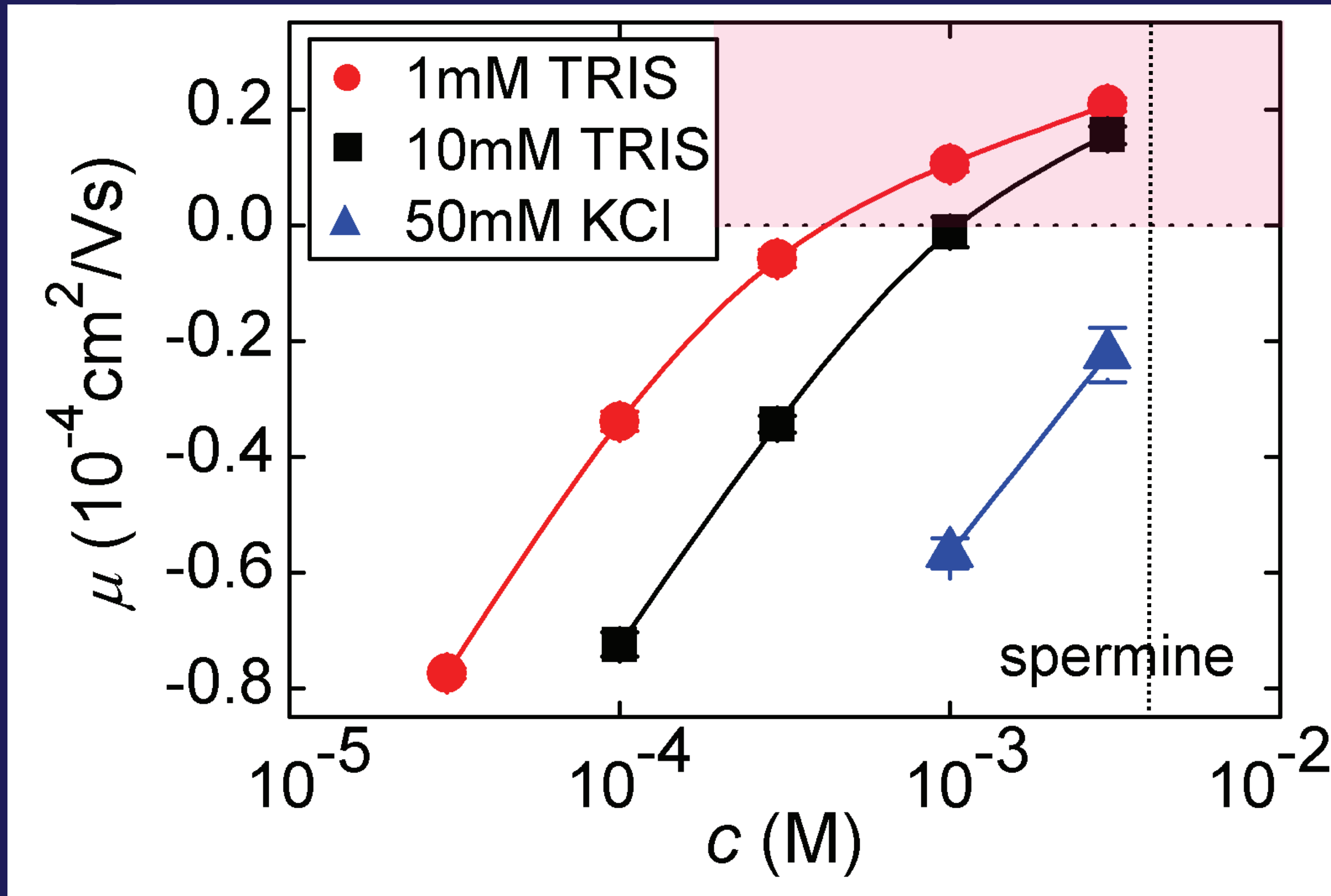
0.1 mM

Malvern zeta-sizer ZS (M3-PALS mode)

5 ng/μL DNA

10 min incubation

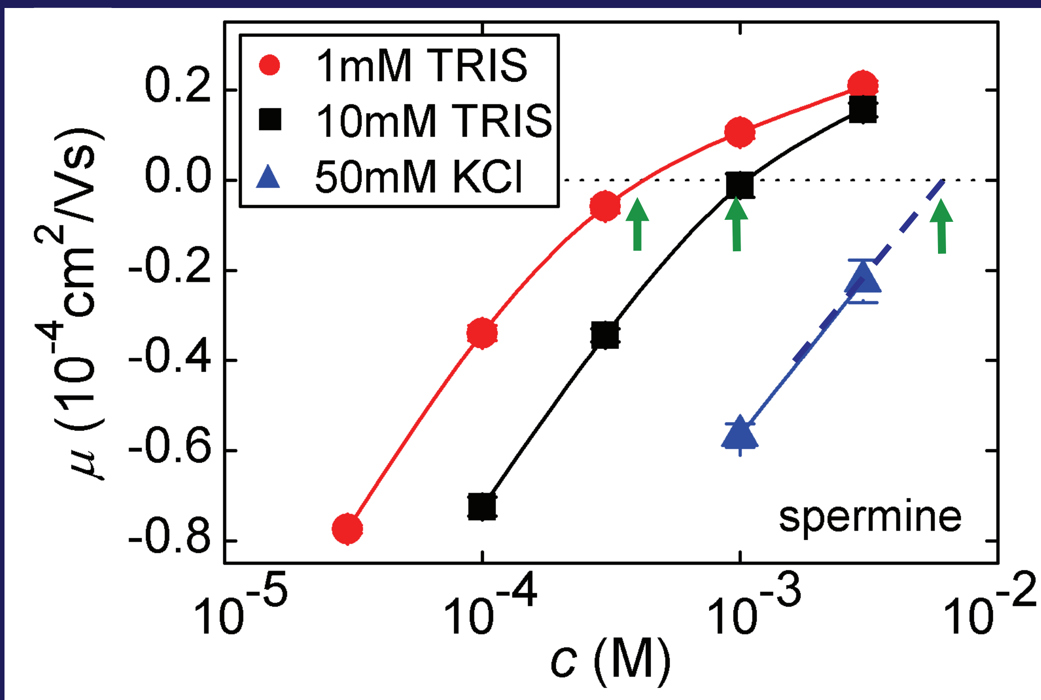
Electrophoretic mobility

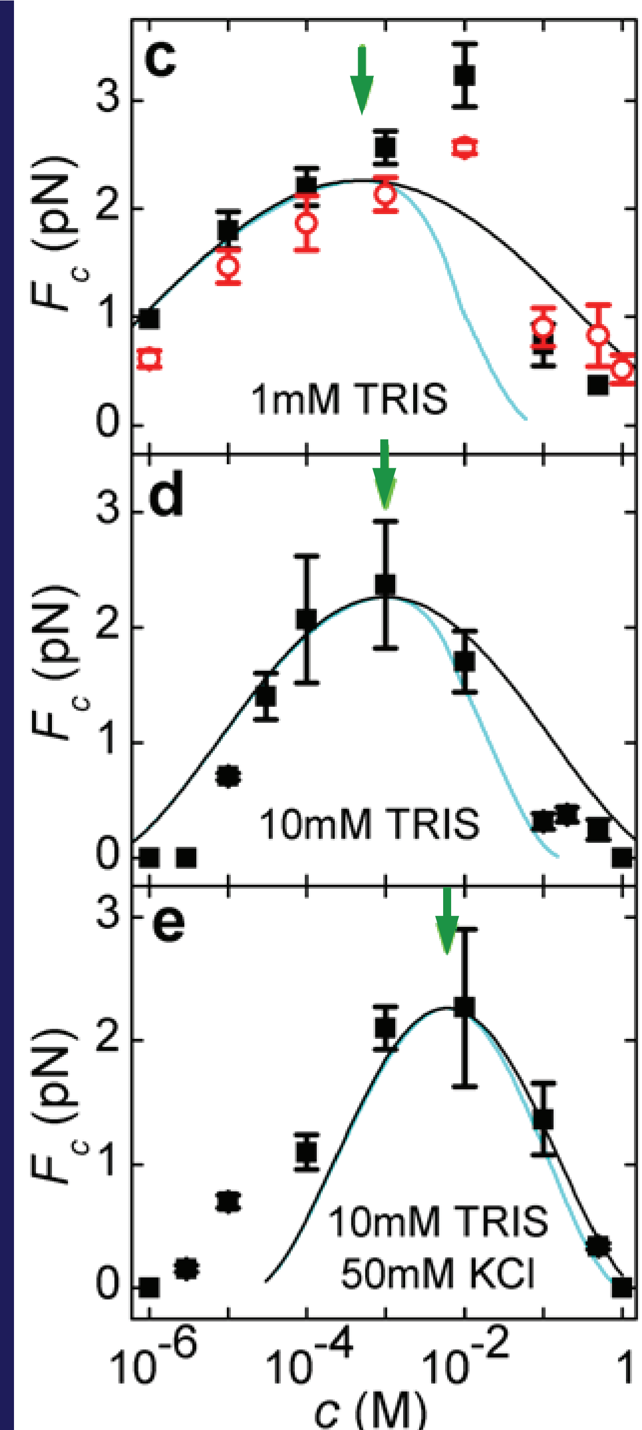


- Spermine <u>can</u> charge-invert DNA
- Charge inversion inhibited by monovalent salt (probably why it was not observed before)

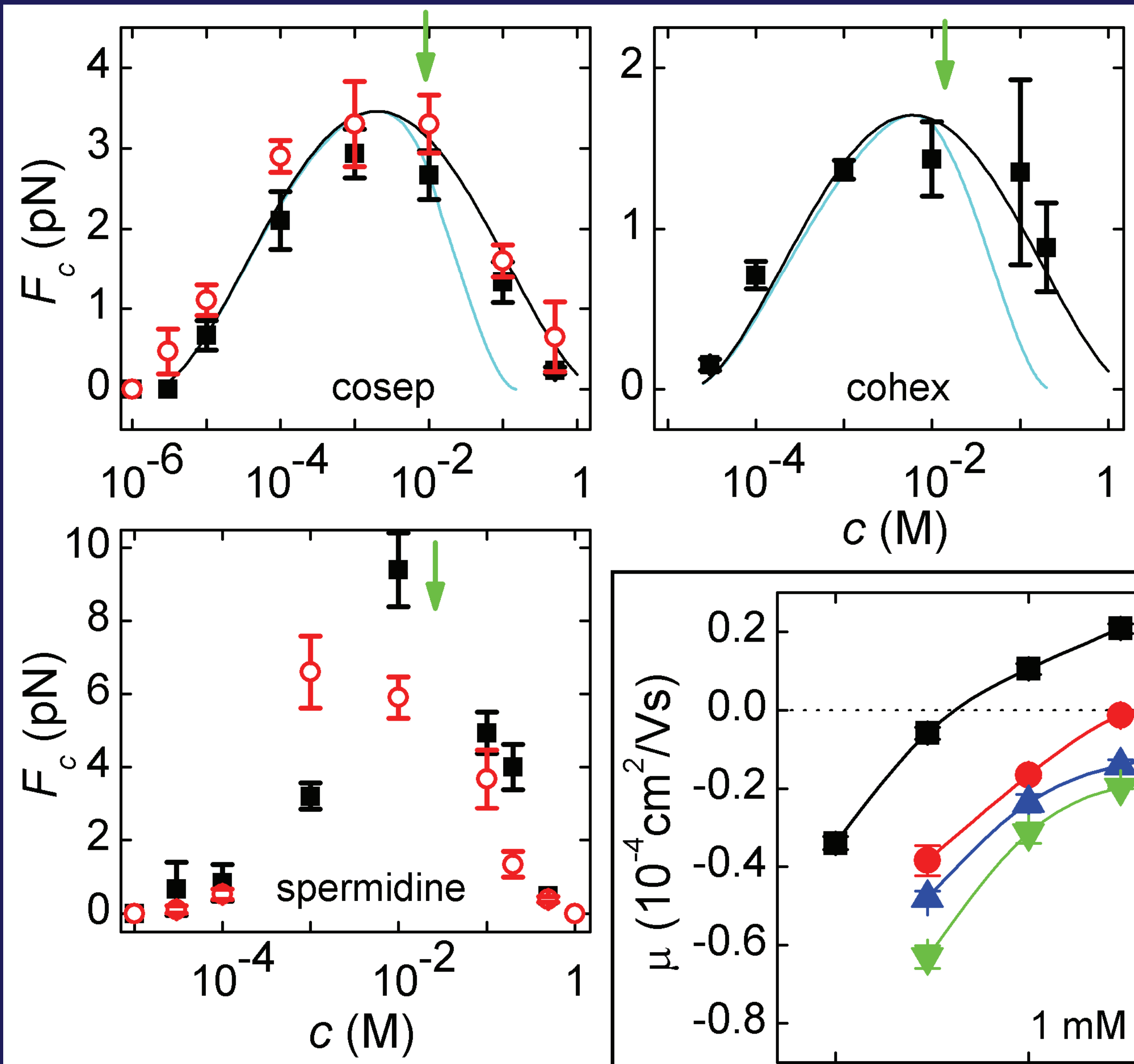
We observe a correlation between

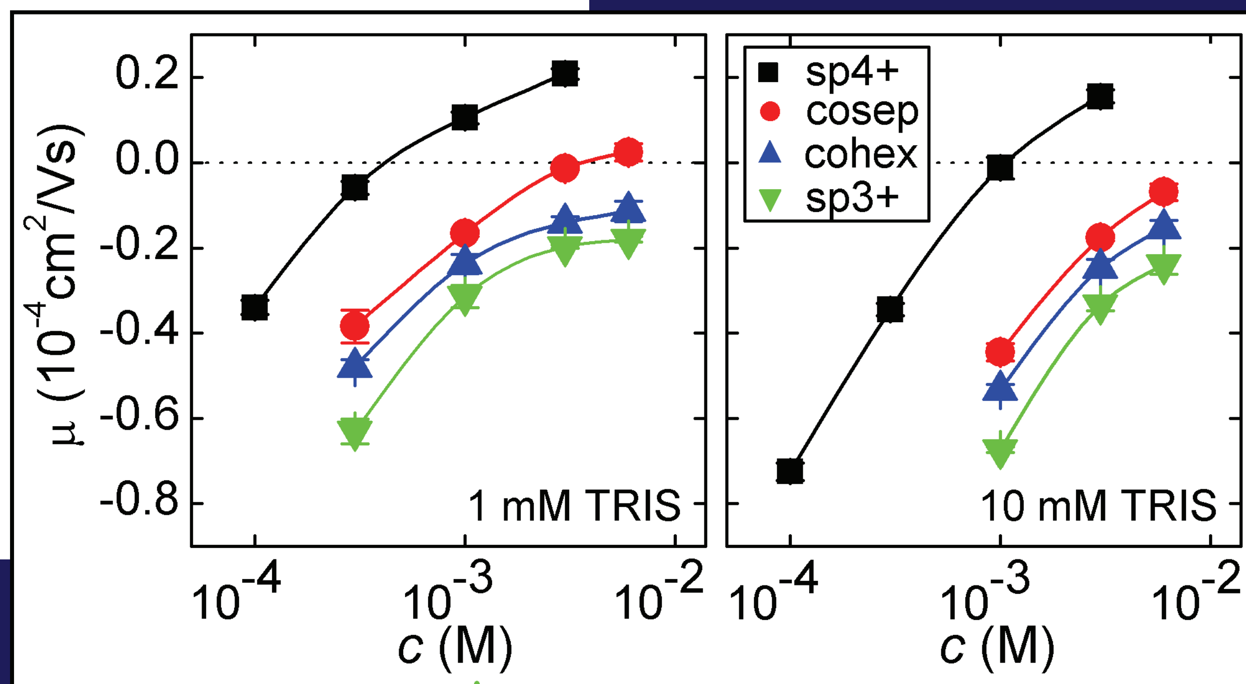
- the maximum force concentration
- the charge inversion concentration



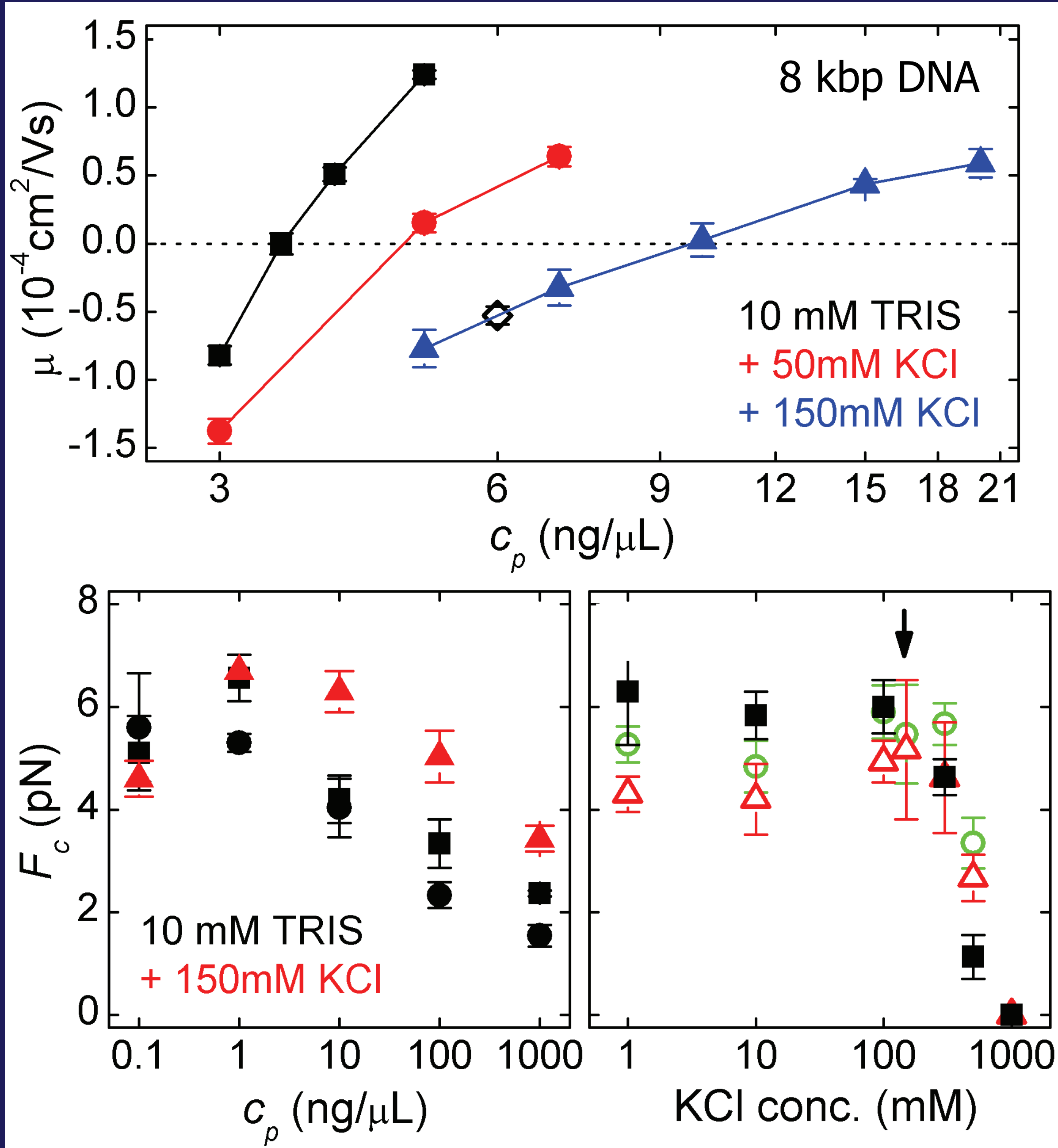


Trivalent cations





Salmon protamine (salmine)



- Small cationic protein (21 arginines out of 32 amino acids) that replaces histones in spermiogenesis
- Charge inversion clearly observed *(see also Raspaud et al., PRL 2006, for 150 bp DNA)*
- Force decreases at high protamine conc)
- Robust up to 300 mM

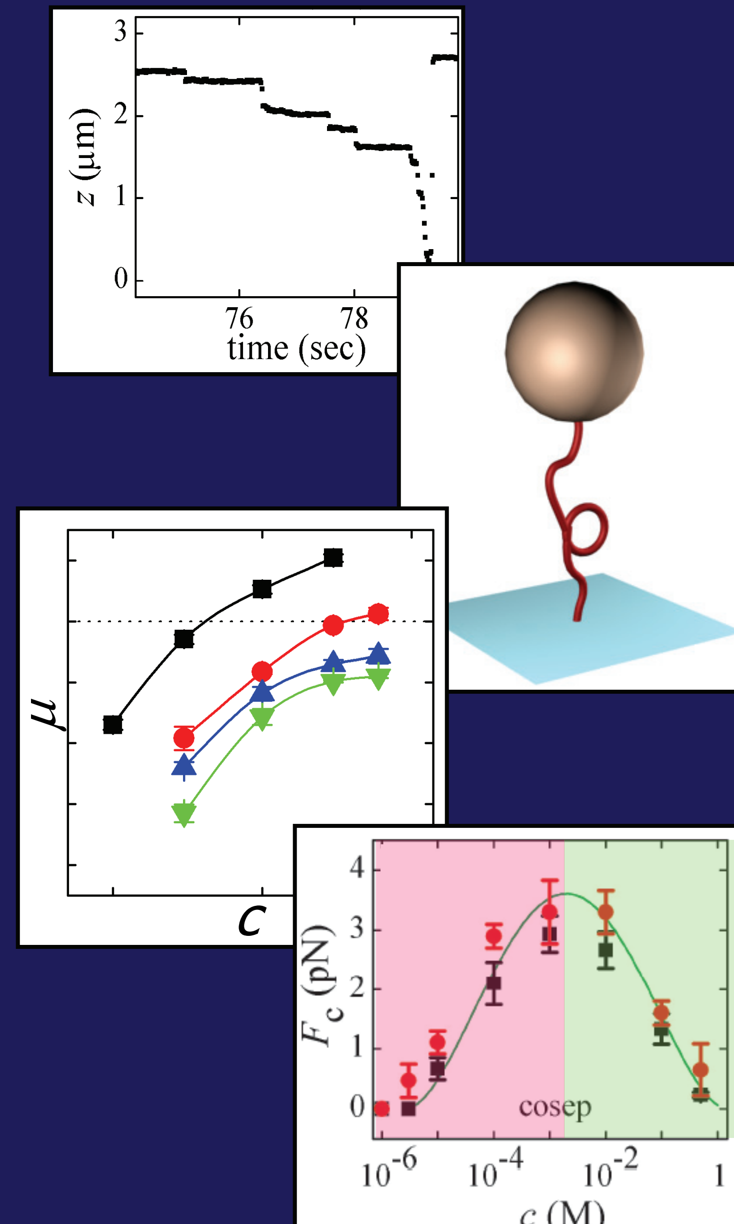
Conclusions

Condensation of DNA under tension occurs via a discrete, activated process

Transition state involves the formation of a loop

Multivalent ions can charge-invert DNA at low salt

Observations are qualitatively consistent with SCL mechanism



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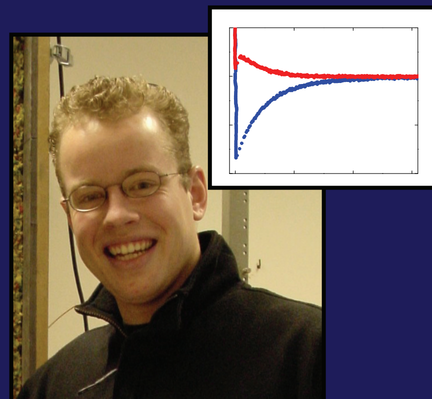
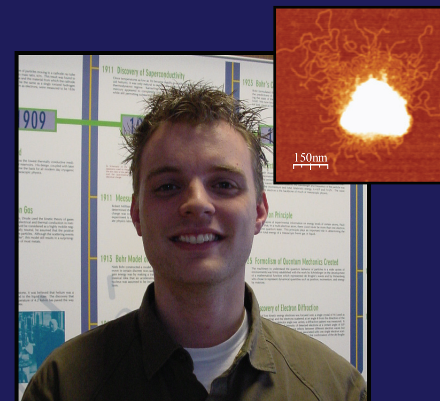
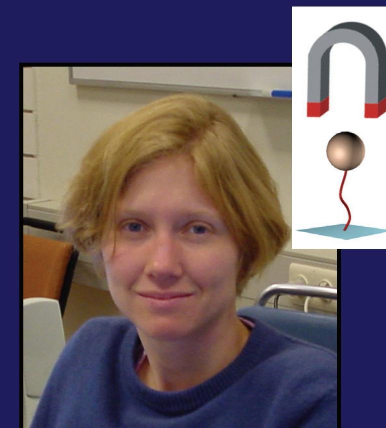
Koen Besteman



Marcel Zevenbergen



Koert van Eijk



Nynke Dekker

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Igor Vilfan, U. Ziese (TEM)

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Charge inversion accompanies DNA condensation by multivalent ions

I will discuss the problem of how stiff, highly charged DNA can be condensed into dense structures by multivalent ions. We studied in real time the condensation of single DNA molecules using magnetic tweezers and found that condensation occurs via discrete nucleated events. By measuring the influence of an imposed twist, we showed that condensation is initiated by the formation of a plectonemic supercoil. This demonstrates a strong interplay between the condensation transition and externally imposed mechanical constraints. We also performed electrophoresis measurements showing that DNA can be charge-inverted by tri- or quadrivalent ions at sufficiently low monovalent salt concentration. These results suggest a direct connection between charge inversion and DNA condensation, and are qualitatively consistent with the theoretical proposal that both effects are interdependent, purely electrostatic phenomena.

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