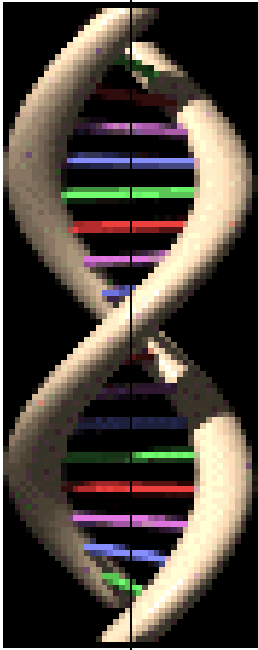# Electrostatic Interactions in Mixtures of Cationic and Anionic Biomolecules: Bulk Structures and Induced Surface Pattern Formation

Monica Olvera de la Cruz

F. J. Solis, P. Gonzalez-Mozuleos (theory)
E. Raspaud, J.L. Sikorav,
M.J. Bedzyk, Y. H. Cheng,
J. Libera (Experiments)
Y. Velichko (Simulations)

Double Stranded DNA
2 chains each with -1 charge every base pair 3.4 Å
Diameter 20 Å
Persistence length (rigidity) of 500 Å (150   basepairs)
Hydrophilic (hides hydrophobic units)

Projection of the two phosphates on the double-helix axis gives
linear charge density :
Double Stranded DNA: 1 e / 1.7 Å ~ 6 e / nm
(short=rod; long=semi-flexible)
Single Stranded DNA: 1 e / 3.4 Å
(flexible)

DNA is one of the most highly charged systems.
Strong polyelectrolyte

Synthetic example: Poly-Styrene-Sulphonate (100% S): 1 e / 2.5 Å ~ 4 e / nm.
Persisitence length 10 Å

Strong Polyelectrolyte Solutions Physical Properties

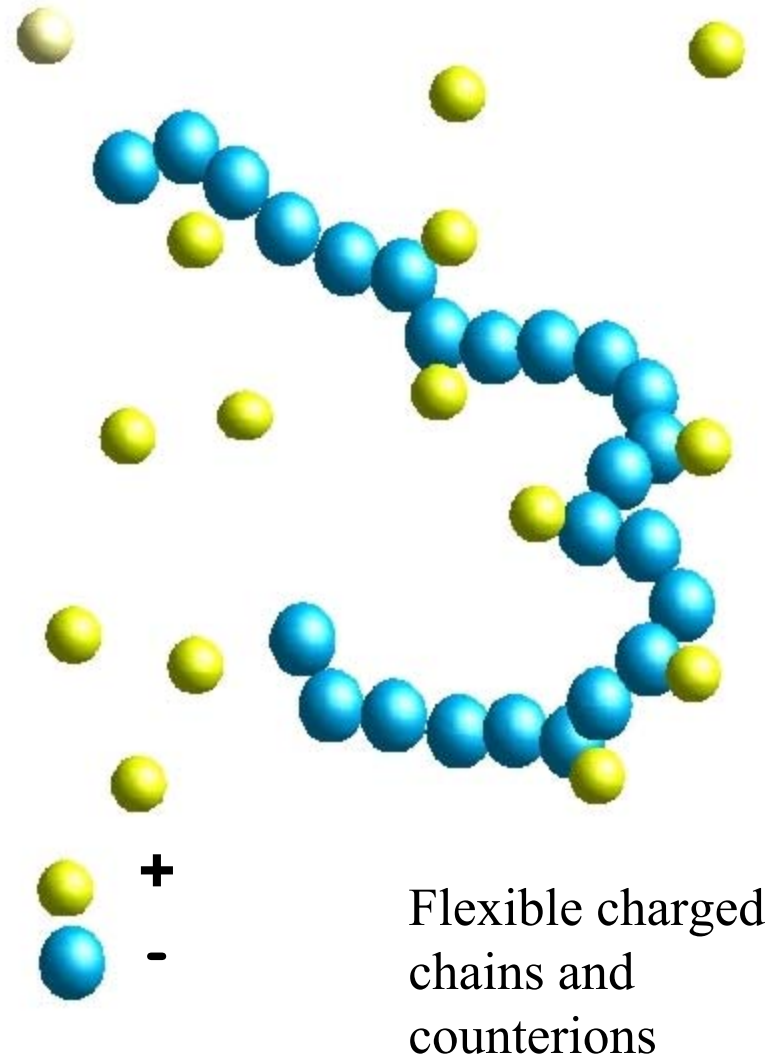**Water soluble in monovalent salts at low ionic strength solutions.**

Simple model:

charged chain (flexible, semiflexible or rod-like)

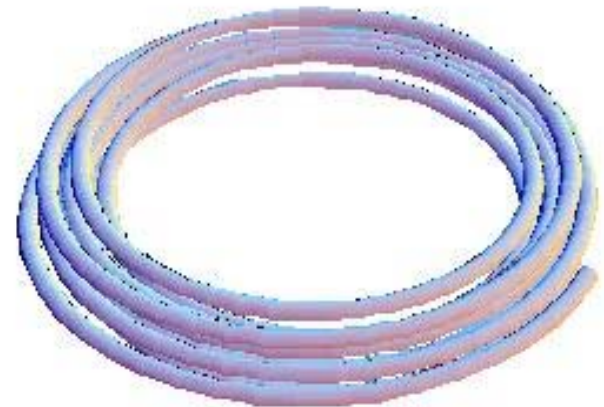**Solvated charge groups and ions**
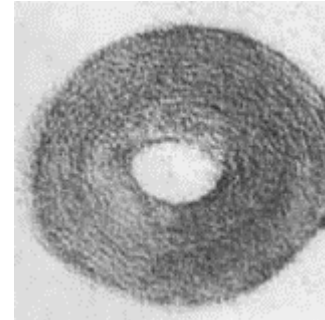
Water is structureless

Chains are stretched to decrease electrostatic repulsions

.

+

-

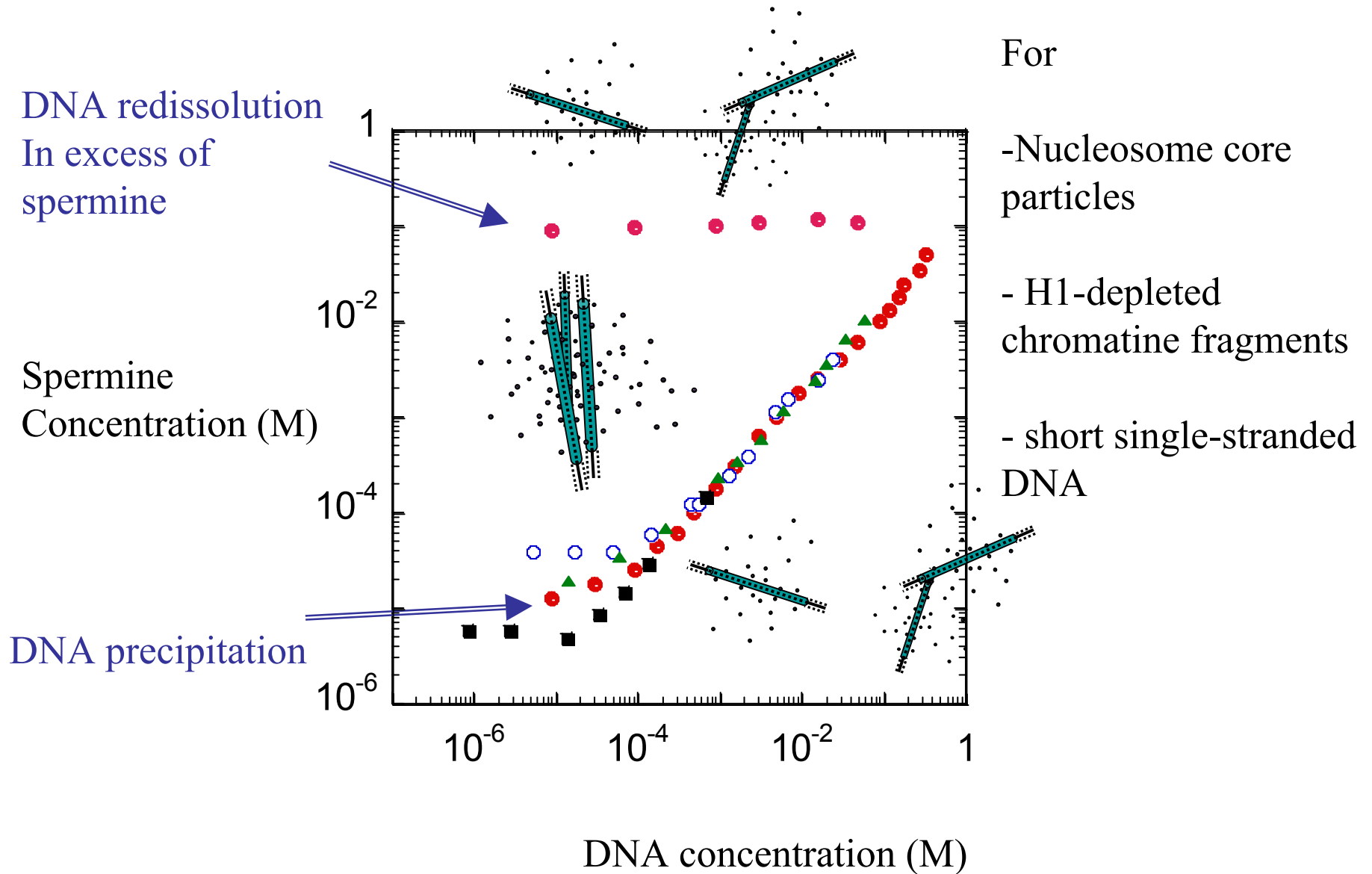Flexible charged chains and counterions

DNA Condensation: precipitation occurs upon the addition of multivalent salt

- Metallic or organic 3+, 4+, higher multivalent salts (Cobalt hexamine, Spermine, Spermidine) precipitate DNA into toroidal bundles.
- Single stranded DNA and other flexible polyelectrolytes form compact structures.

- J. Widom and R.L. Baldwin, *J. Mol. Biol.*, 144 (1980).
-

Phase diagram: nearly universal

DNA redissolution
In excess of
spermine

Spermine
Concentration (M)

DNA precipitation

For

-Nucleosome core
particles

- H1-depleted
chromatin fragments

- short single-stranded
DNA

DNA concentration (M)

(Raspaud et al., 1998-99)

Precipitation/condensation of polyelectrolytes

- Why do equal charges attract?

- The origin of the counterion driven attraction.

- Necessary elements to construct a theory.

Electrostatically driven self-assembly

- **Cationic-Anionic Biological Complexes**

  Viral assembly

  Chromosomes structure

  DNA packing for gene therapy

  Biotechnology (DNA condensation is used to increase DNA denaturation and cyclization rates by orders of magnitude as in vivo)

Virial Assembly: Helical and Sphere-like RNA Virus

(Tsuruta et al.)

Tobacco Mosaic Virus. One RNA (-) molecule of 6,300 base + 2,000 identical capsid (+) proteins

RNA   protein subunit

Capsid

Hole   2 RNA molecules (3kB)

Density ≈ RNA crystal
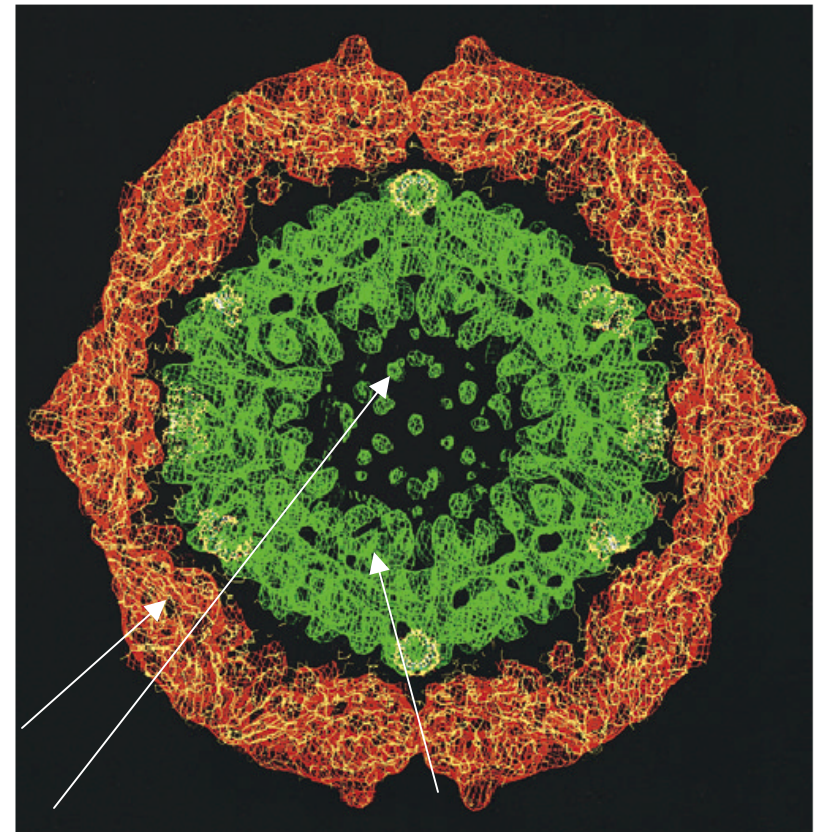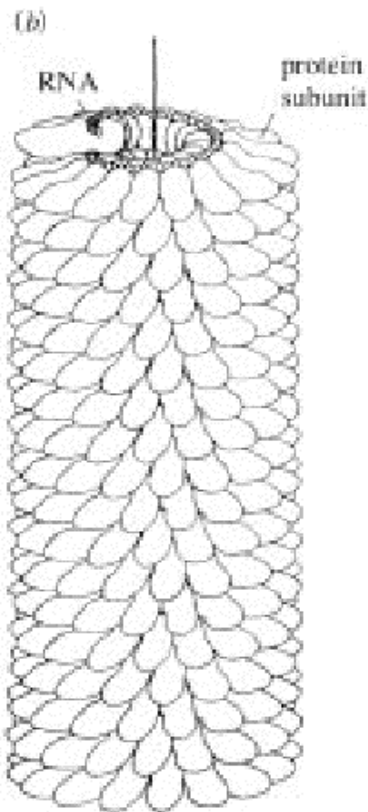
Self-assembles from capsid protein + viral RNA solution

Chromosome

Condensation Transition

DNA/Histone electrostatic attraction versus Bending Stiffness.
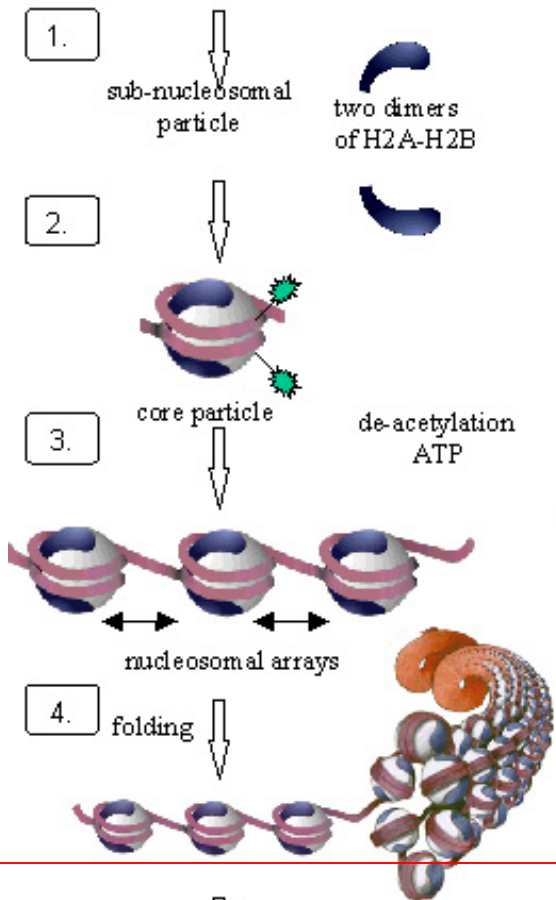
Known structures

Nucleus: 23 chromosomes

tetramer H3-H4*

1.
sub-nucleosomal particle

two dimers of H2A-H2B

2.
core particle

de-acetylation ATP

3.
nucleosomal arrays

4. folding

5.
domains in the nucleus
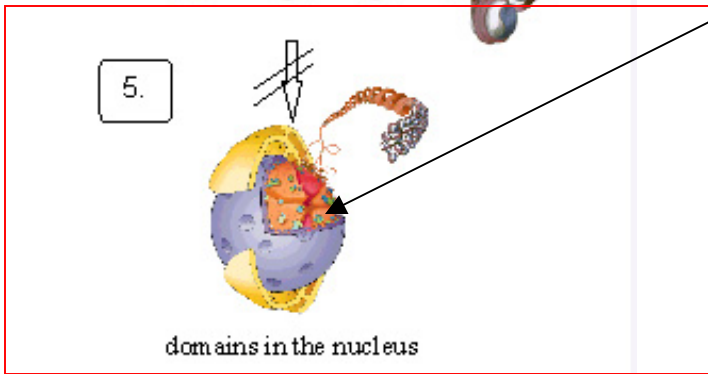
Characteristic length
for ionic dissociation/association in water:

The Bjerrum length l_B

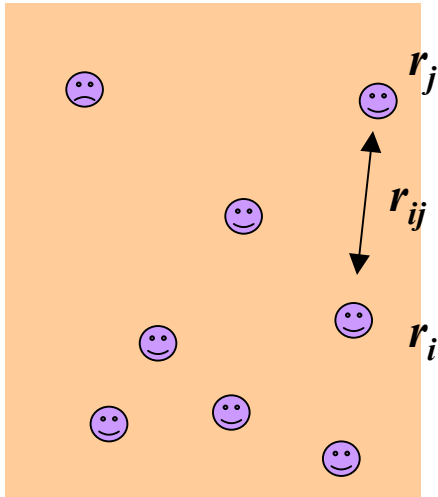$$kT = \frac{e^2}{4\pi\varepsilon\varepsilon_0 l_B}$$
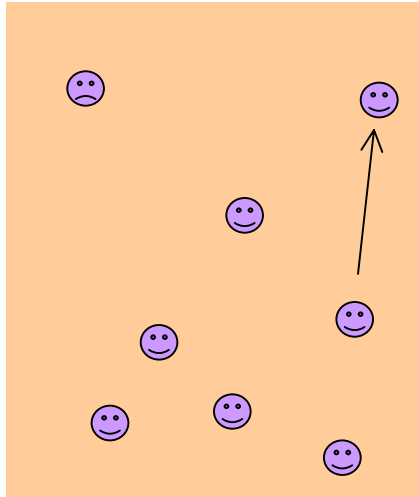
**In water,
l_B = 7.14   Å**

if $d < l_B$ then
ion pairing occurs

For a multivalent salt
$z_i : z_j$

*Association energy
increases by $|z_i z_j|$ and
$l_B \uparrow$*

Potential at the arbitrary point r :

$$\psi(r) = \int \frac{\rho(r')dr'}{4\pi\varepsilon_{\varepsilon_0}|r-r'|}$$

with $\rho(r)$ the total charge density at r

How does the charges distribution $\rho(r)$ change with respect to the distance r from a central ion ?

$\rho(r)$ is related to $\psi(r)$ by the Poisson's eq.,

$$\Delta\psi(r) = -\rho(r)/\varepsilon\varepsilon_0$$
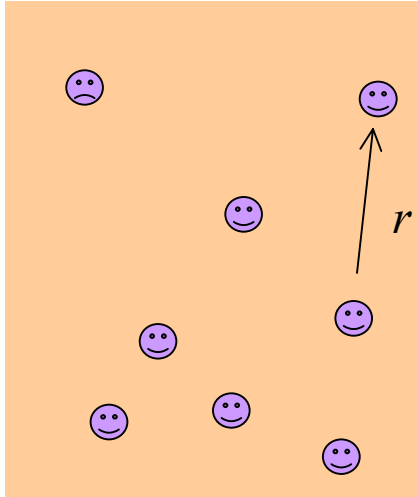
If short range correlations are ignored (as in point ions), One can use the Boltzmann's distribution,

$$\rho(r) = z_+e\, C_+^0\, e^{-z_+e\psi(r)/kT} + z_-e\, C_-^0\, e^{-z_-e\psi(r)/kT} \quad,$$

The Poisson-Boltzmann (PB) equation.
Expanding exponential gives lineaqrized PB or Debye-Hückel (DH) theory:

$$\Delta\psi = \begin{cases} 0 & \text{if} \quad r<a \\ \kappa^2\psi & \text{if} \quad r>a \end{cases}$$

$$\kappa^2 = \frac{4\pi\, l_B\rho^*}{a^3} = \frac{4\pi\,\rho^*}{a^2 T^*}$$

T*=a/l$_B$

Salt-free polyelectrolyte solutions, monovalent counterions

In polyelectrolytes the linearized theories are not valid. The chains are strongly perturbed by electrostatics

$$\xi \sim 1/\kappa^2 \approx (\rho^*)^{-1}$$

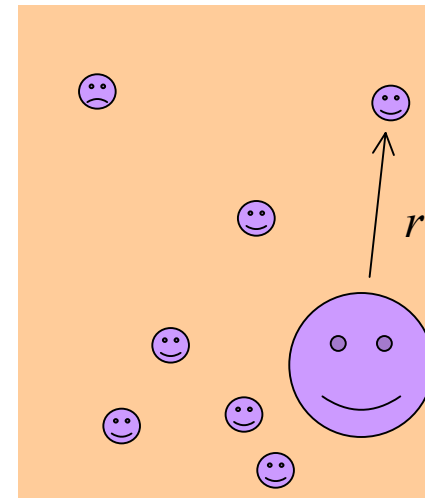**They are stretched due to the repulsions**

ξ
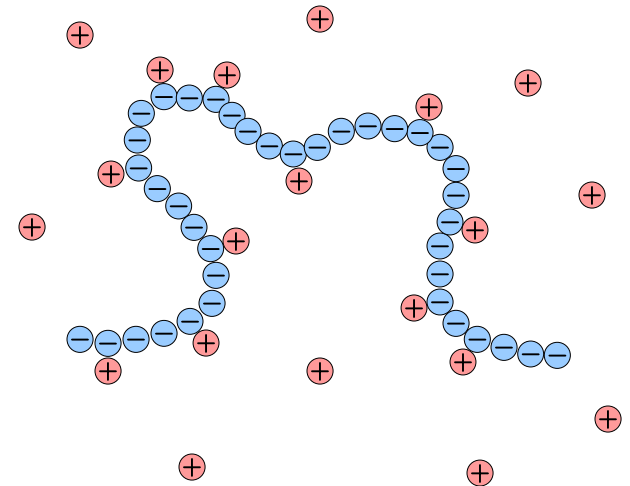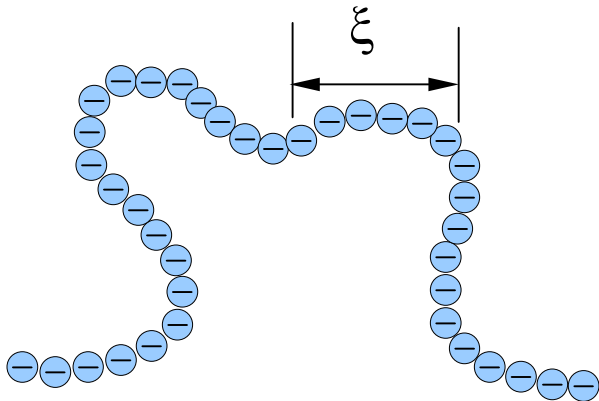
BUT ion association along the chains: Shape depends on how many ions are around the chains

Non-linear effects in polyelectrolytes

Simple salt
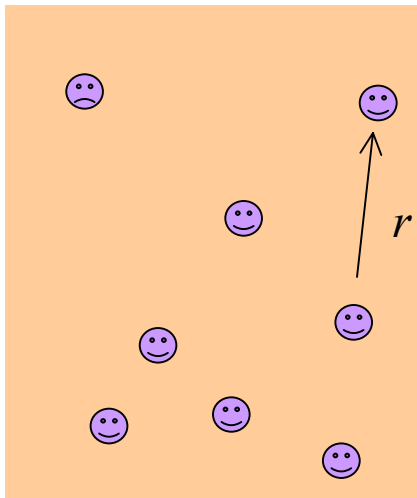
Polyelectrolyte
in ionic solution

r

Apply
similar
calculation ?

r

-Start from the Poisson-Boltzmann equation

- BUT linearization is not possible because the approximation $z_i e\, \psi(r)$ $<< kT$ is not valid $\rightarrow$ ion pairing or ion condensation even at high T*

In colloids the"condensed"ions renormalized the charge like a double layer in charged surfaces.
Away from the macroion the interaction with other charges reflects only the effective charge of the macroion, and follows mean field values (Debye-Huckel, DVLO).
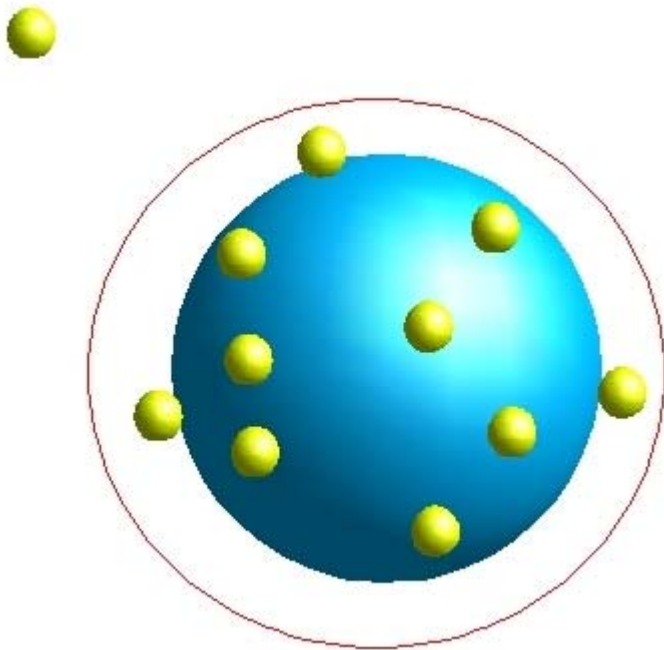
Warning. Poisson Boltzmann ignores short range correlations, which can be important in the dense ionic region or double layer.

Infinite rods the charge density in Bjerrum length l_B units is

Monomers
$z_m = -1$

$$\xi_M = l_B / b \text{ the Manning parameter}$$

For B – DNA, $b = 1.7$ Å : $\xi_M = 4.2$
if $> 1/z_c$ ion condensation

Counterions
$z_c = +1$

« free » ions treated in the Debye-Hückel approximation.

« condensed » ions reducing the monomer charge to an effective charge $q_{eff.}$
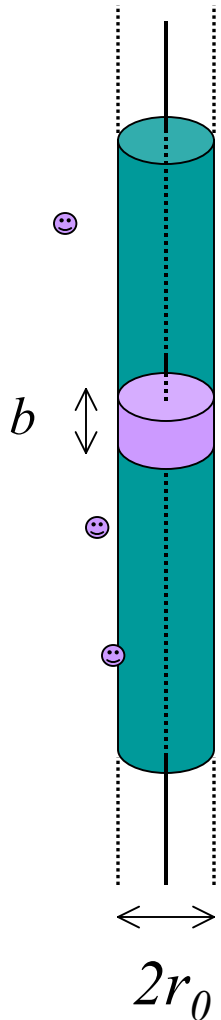
b

$2r_0$

Counterion charge renormalization in 3D, only at finite concentration

(Alexander et al.,1984)

Size and concentration effects

L

$1/\kappa$
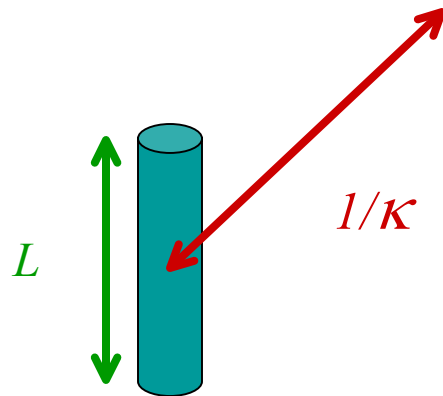
For <u>charged spheres of size R</u>, ion chemical potential far from the spheres, mainly entropic
$$kT \ln C,$$
on its surface, mainly enthalpic (electrostatic)
$$-q_{eff}e^2/4\pi\varepsilon\varepsilon_0 R$$

$$q_{eff} \sim - R \ln C$$

The effective charge increases or the fraction of condensed ion decreases with dilution
For rods R~N and the chemical potential ln N term

PB similar to equate chemical potential of free and condensed ions to determine the effective charge of the colloid.
This can be done for any input charge distributions. For a fractal chains we input the distribution from the center of mass and allow the ions to "penetrate" the fractal.

+

-
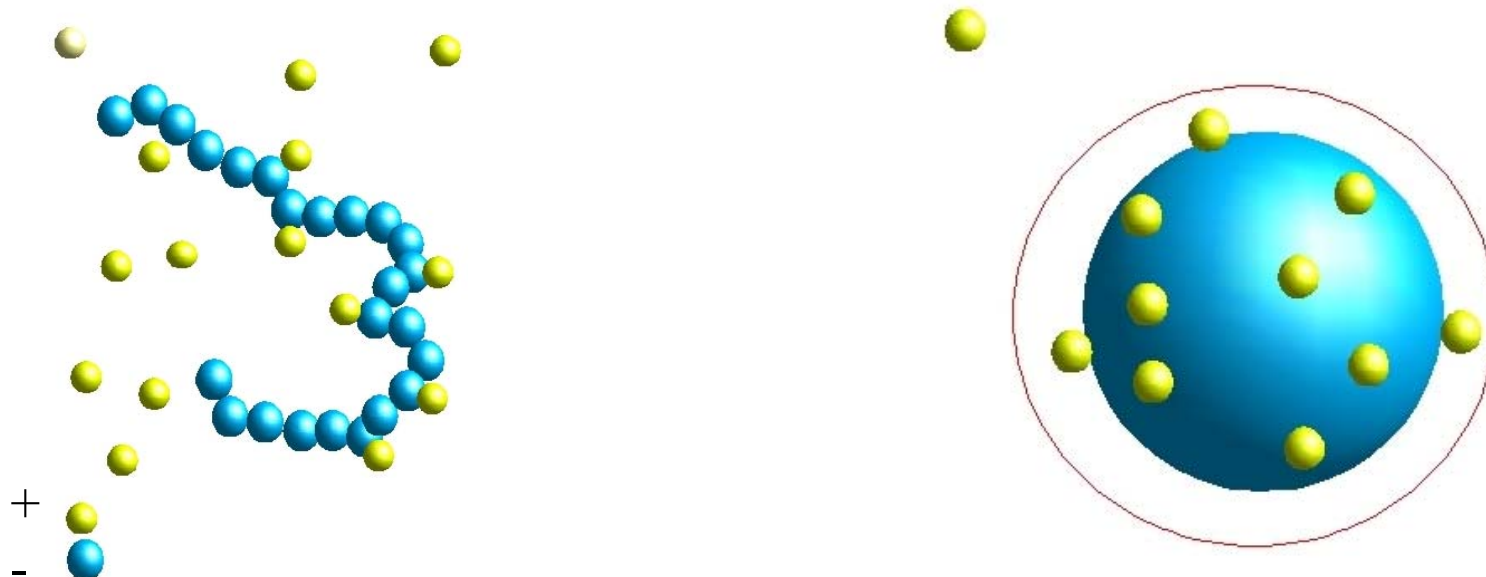
$R \sim N^{\nu}$ $\nu = 1$ rod

$\nu = 1/2$ random walk

$\nu = 1/3$ dense ionic

structure

Conformation effects: fraction of condensed counterions (DH for ions only, no chain RPA contribution)

$q_{eff} \sim - R \ln C$

$1/\kappa$

$1/\kappa$

$1/\kappa$

1.0

0.8

0.6

f^c

0.4

0.2

0.0
-10.0 -15.0 -20.0 -25.0 -30.0

(a)

$\log (\rho_m b^3/N_m)$
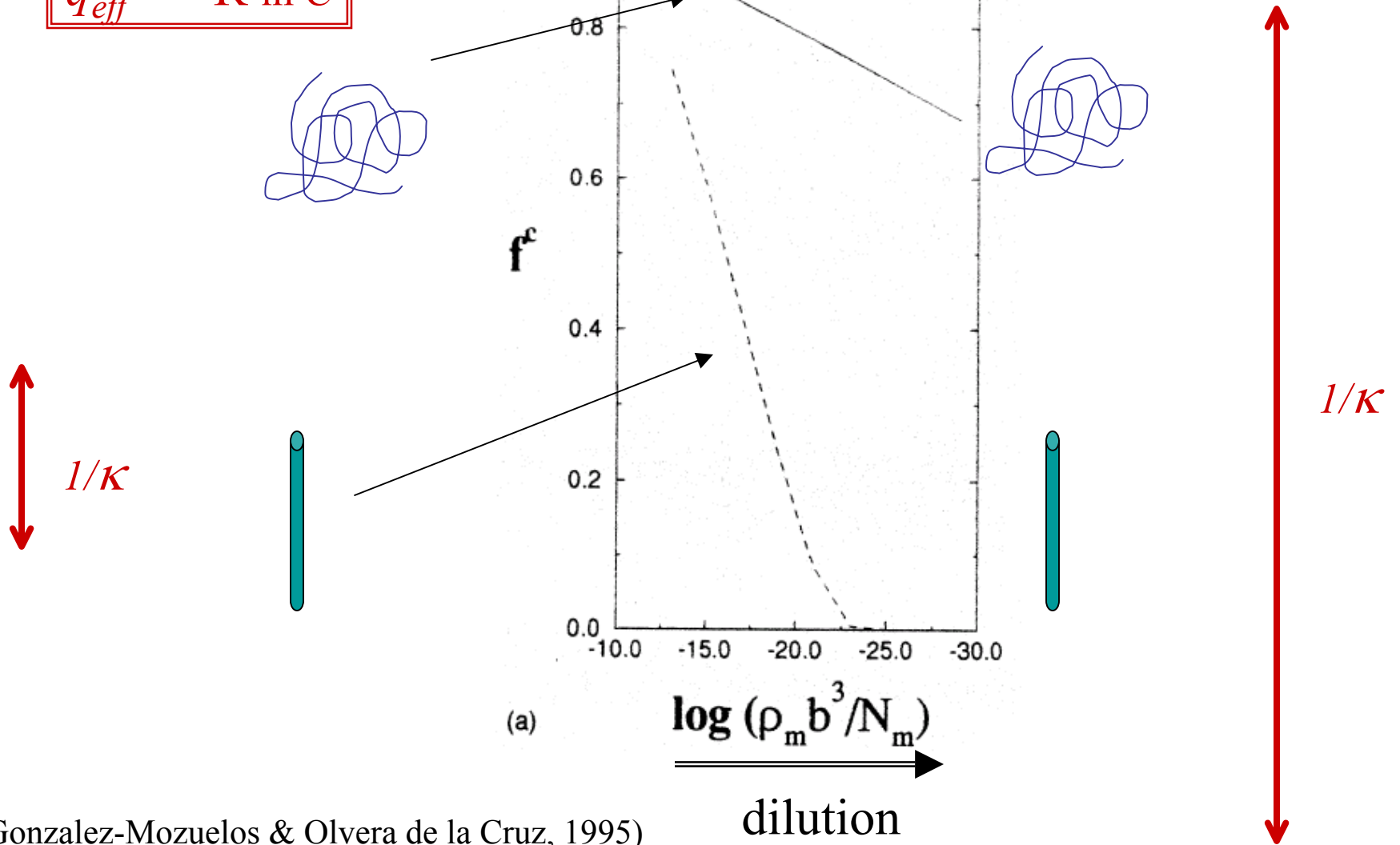
dilution

(Gonzalez-Mozuelos & Olvera de la Cruz, 1995)

Poison-Boltzmann gives rod lowest energy though more ions are condensed in collapsed than in rod.

IF SHORT RANGE CORRELATIONS
as charge density increases or valence of
counterions increases then collapse chains

Collapse to denser system of charges due
to counterion induced attractions

Z=4.
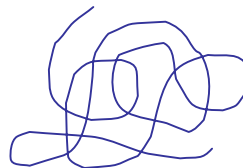
Z=1.

Two-dimensional lattice of the multivalent counterions around the surface (Rouzina and Bloomfield 1996) and around rods (Arenson et al 1999, Solis and Olvera de la Cruz, 1999)
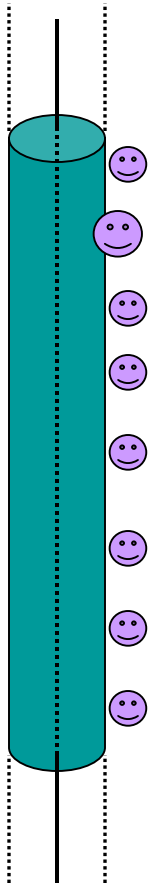
Correlation energy ε_c per ion < 0 (attractive)
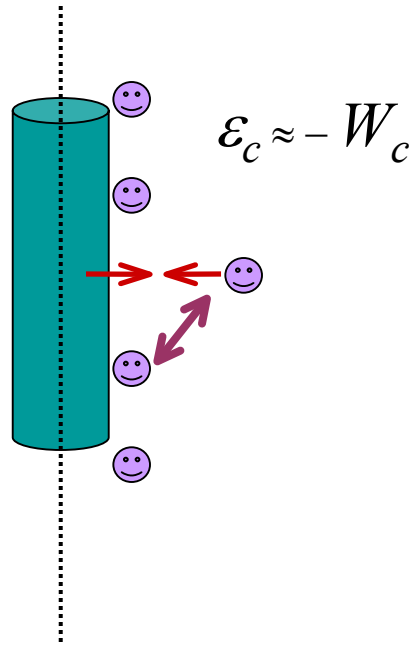
If n : local concentration of the correlated liquid

$$\varepsilon_c \approx - W_c$$
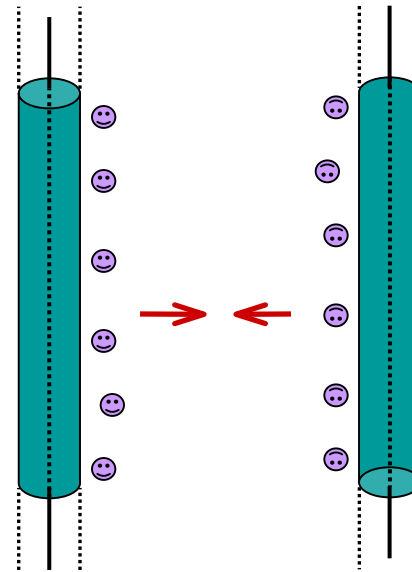
$$\varepsilon_c(2n) < \varepsilon_c(n)$$

Attraction between chains

Phase separation of polyelectrolytes with the addition of multivalent ions

In dense systems

Highly correlated ions leads to attractions (Modified D-H gives no transition in salt solutions)
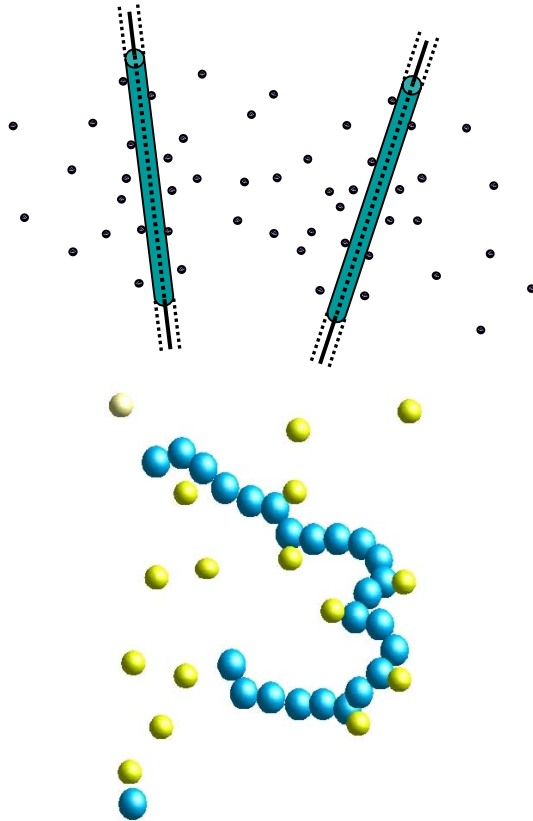
$e^{-\kappa r}$ Repulsions only in dilute with point ions

(Brenner & Parsegian, 1972)
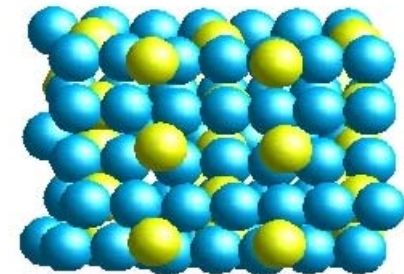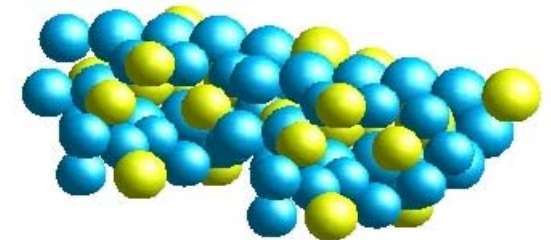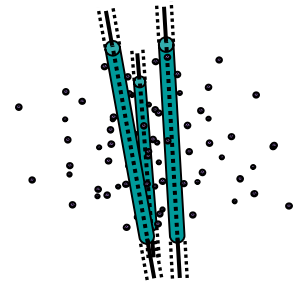
Ionic glass

(Solis + M.O. de la Cruz, 2000, 2001)

Phase diagram: nearly universal

DNA redissolution
in excess of
spermine

Spermine
Concentration (M)

DNA precipitation

In the
precipitated
region the
chains form
an ionic
crystal

DNA concentration (M)
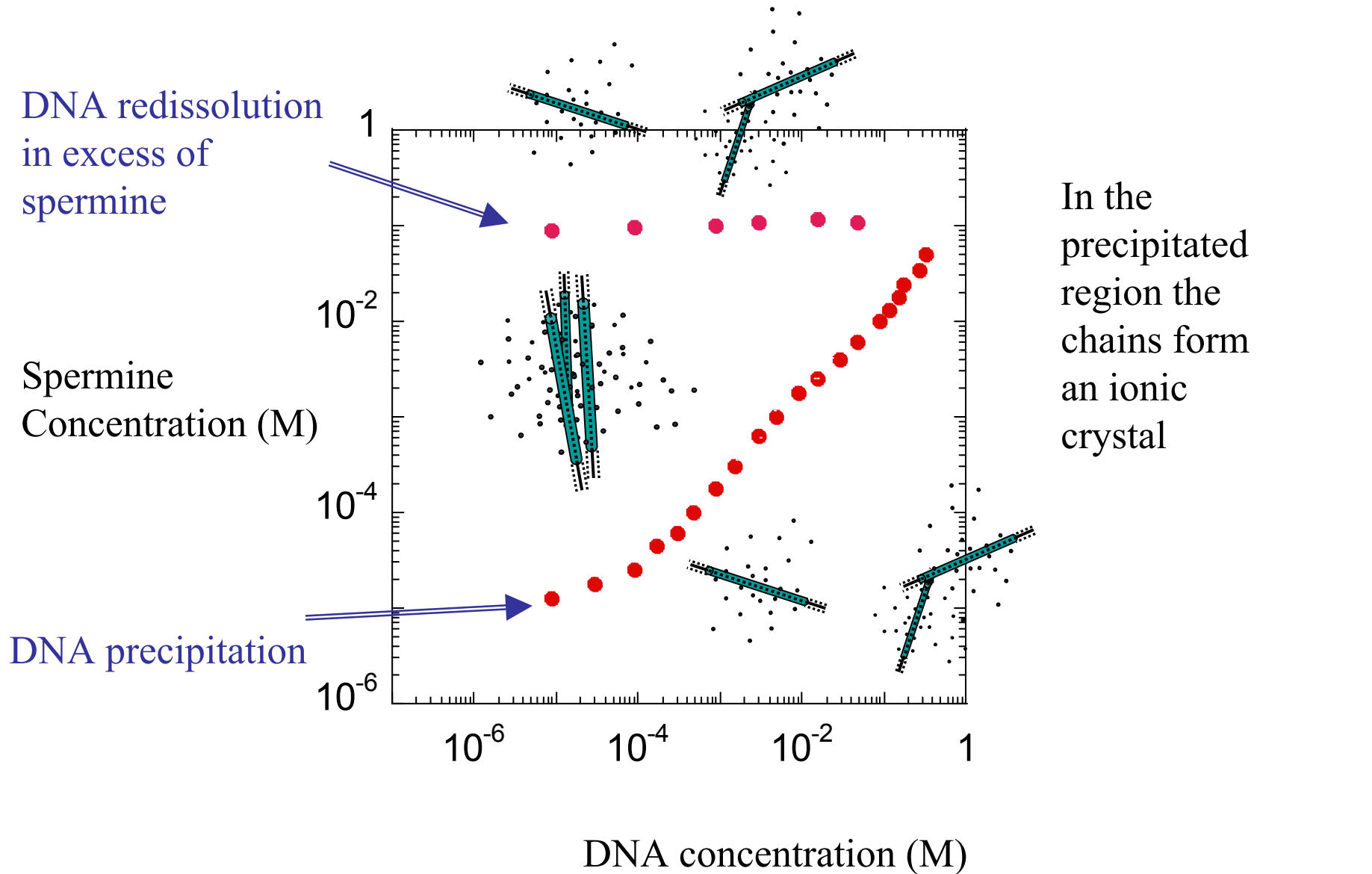
(Raspaud et al., 1998-99)

Polyelectrolyte (PE) Chains in multivalent ions (Gonzalez-Mozuelos et al 95;Solis et al 00, 01; Lee + Thirumalia 00; Liu + Muthukumar 02))

E. Luijten 04

$\sigma_m \approx 2.5\text{Å}$; water at 298K: $l_B \approx 7.1\text{Å}$ $\Rightarrow$ $l_B / \sigma_m \approx 3$; $C_m = 0.008\sigma_m{-3} \sim 1M$

PE(*N*=32) + 8(4:1)salt

Scaling description: $R_g \sim N^{\nu}$
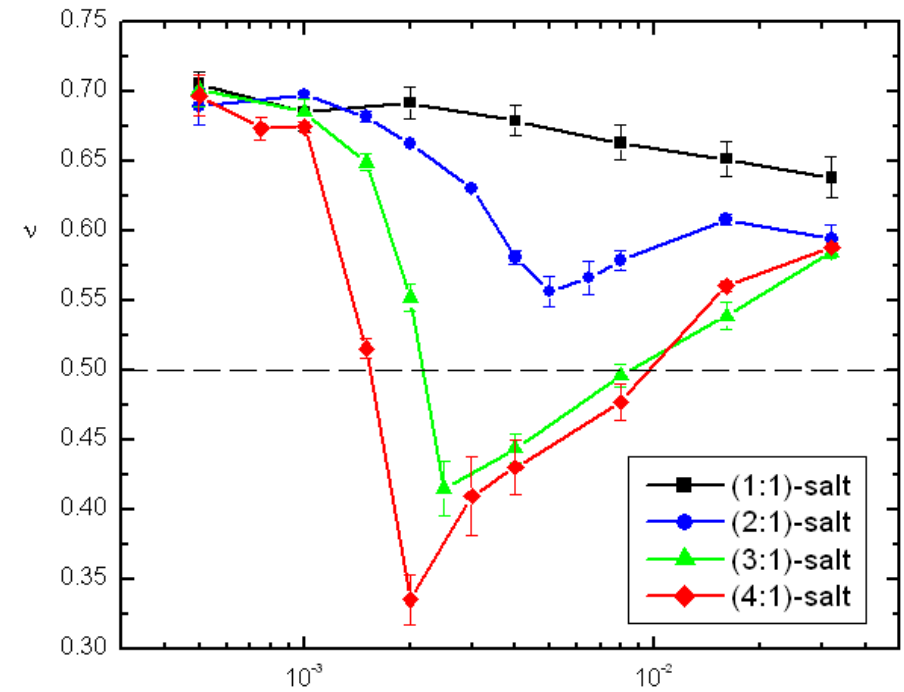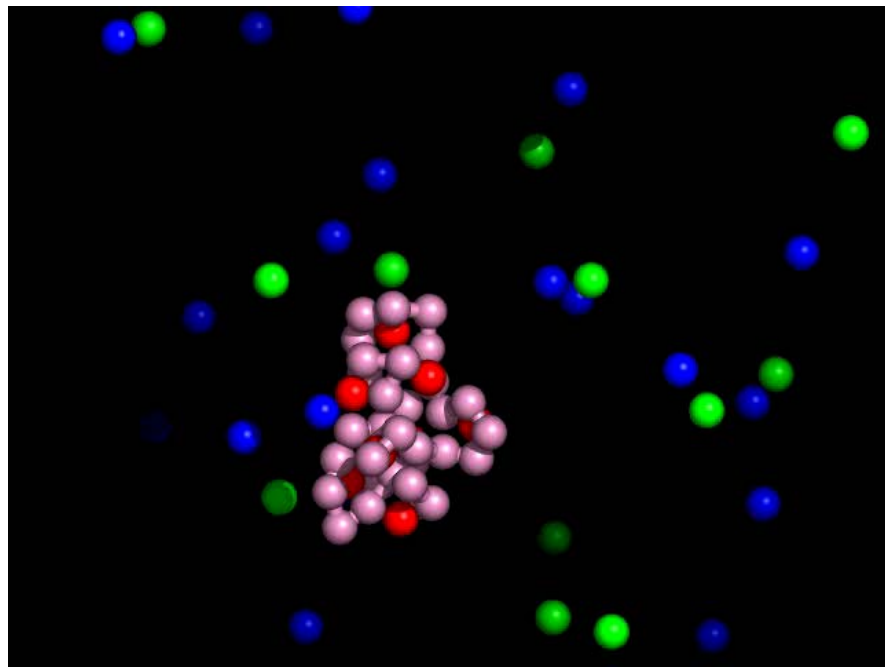
−1	mer	Charged rods (DD DNA) the precipitation is into bundles
+1	(+1)	
+4	(+4)	
−1	coion	

$\nu = 1/3$ sphere

$\nu = 0.5$ ideal chain

$\nu = 0.588$ coil

$\nu = 1$ rigid rod

Effective charge

+z   -1   +1

a   b   c

d   e   f
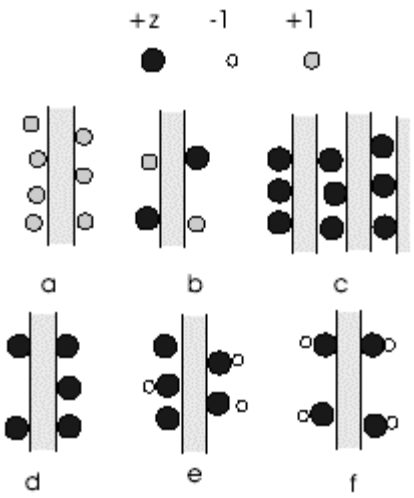
d=positive; e=Neutral, f=negative

Solis + MO de la Cruz 00, 01;
Mesina, Holm and Kremer 00; Solis
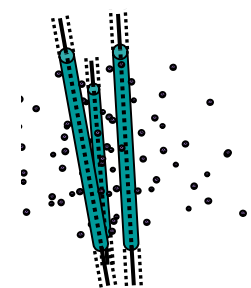02, Grosberg et al 02, Grosberg +
Tanaka 01

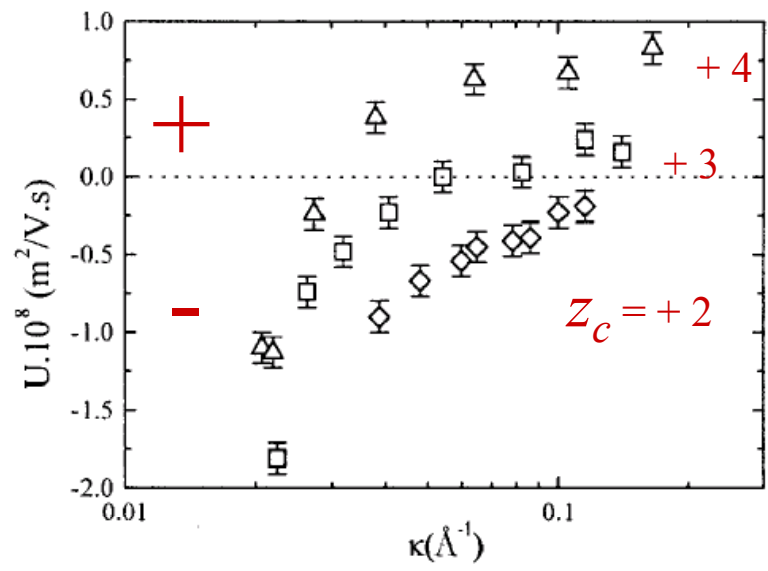**Stability of complexes to segregation or to dissolution**

Polyelectrolytes collapse; beyond neutralization, re-expansion occurs because the multivalent ions can interact more with co-ions Counterion size has crucial role.
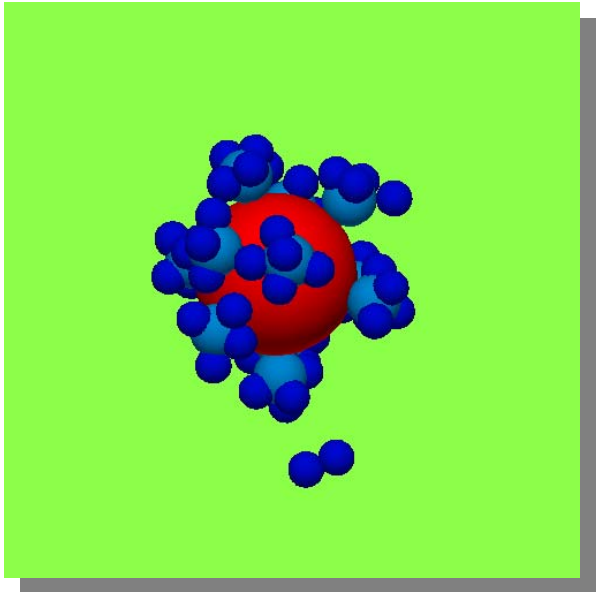
Experiments on charge inversion,

T4 DNA
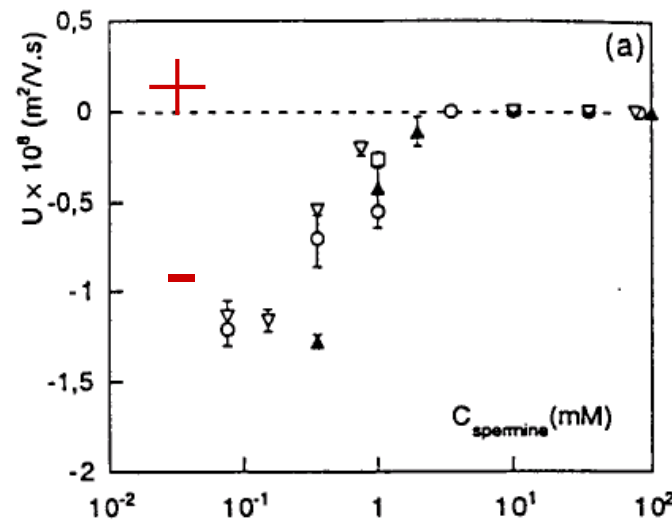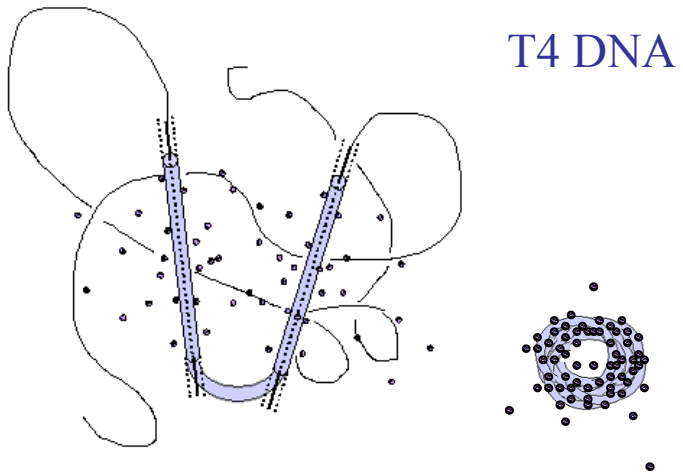
Short DNA fragments

Spermine concentration ($z_c = +4$)

Linear DNA
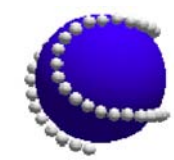
Spermidine concentration ($z_c = +3$)

No charge inversion

$+4$

$+3$

$z_c = +2$

Nucleosome Core Particles

(Raspaud et al., 1999; De Frutos et al., 2001)

Adsorption of Strongly Charged Polyelectrolytes onto Oppositely Charged Surface

Driving force: counterion release
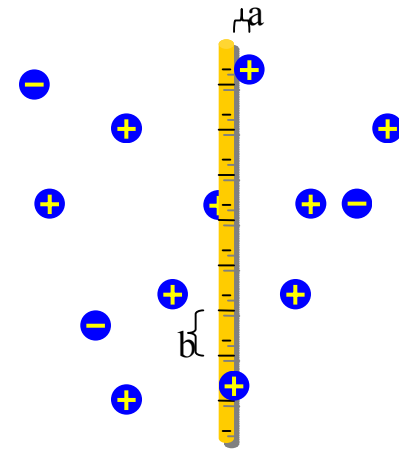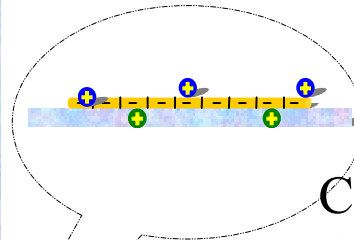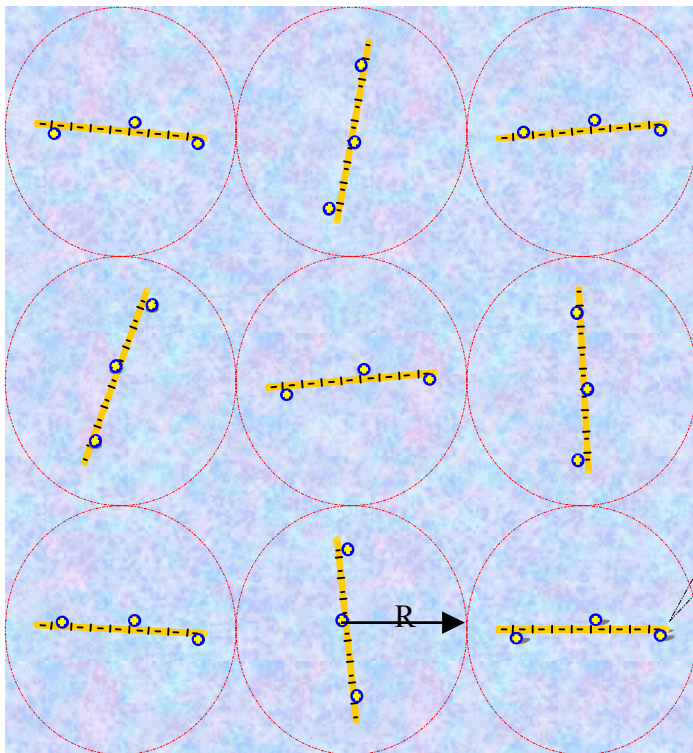
Correlations along surface if distance between rods larger than adsorbed layer thickness (break down of PB) leads to charge inversion (Netz+ Joanny 99; Dobrynin+ Rubinstein 00).
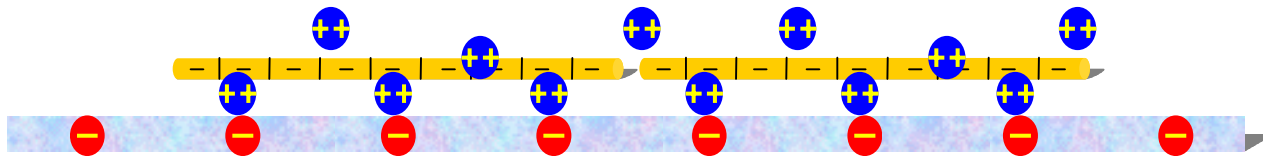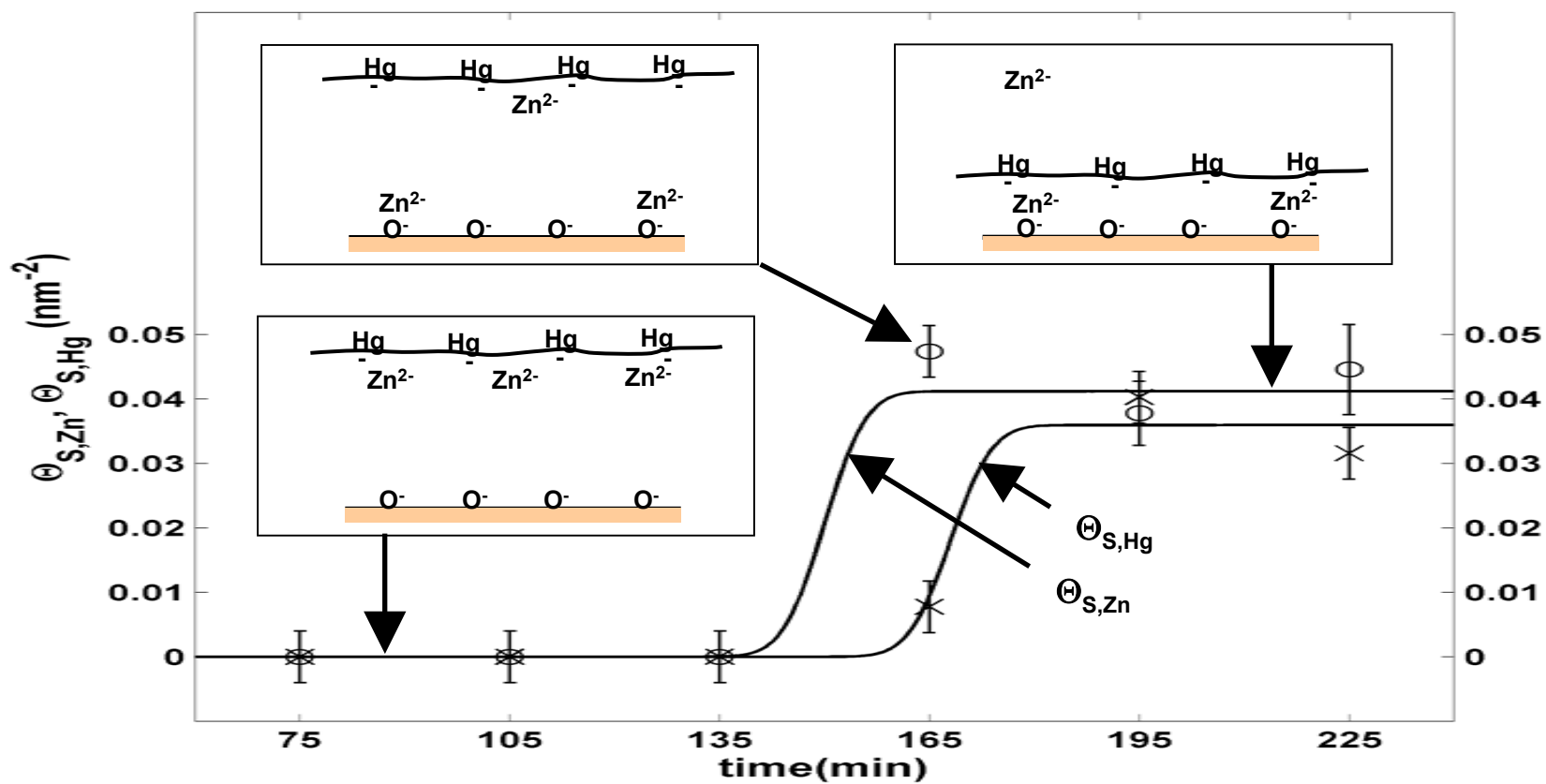
Strongly charged rods in divalent solution

$b/(1-f)$

b
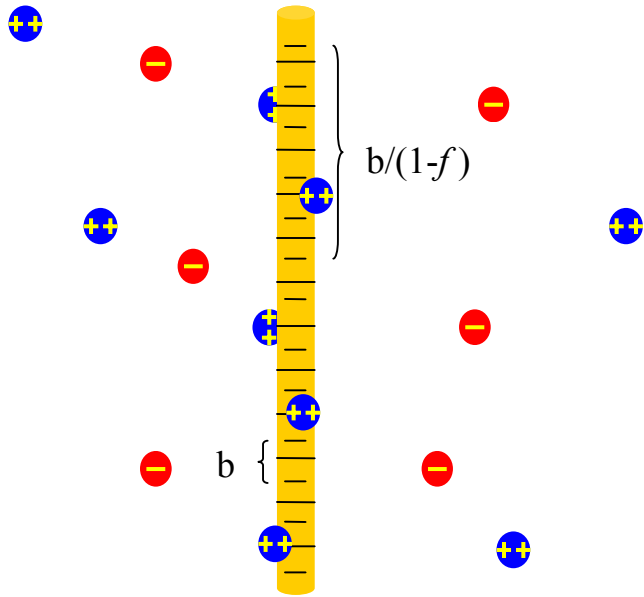
Polyelectrolytes adsorption onto same charged surfaces studied experimentally via X-Ray Standing waves (Libera, Cheng, Bedzyk).
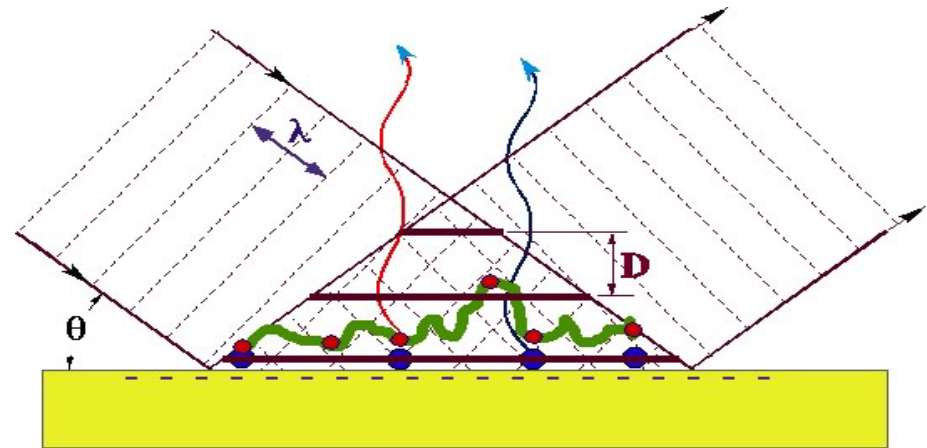
Adsorbed rods via short range attractions

Time dependence of the Zn and Hg surface condensed layer coverages.The line is drawn to guide the eye.

Conclusions on precipitation

**We have proposed a mechanism for attractions
of purely electrostatic nature that describes the phase
diagram.**

**Smaller multivalent ions are more efficient condensing
polyelectrolytes. In order to observe re-dissolution by adding
more multivalent ions one needs small association constants.**

Mixture of mono and z_c-valent cations condensed onto the DNA induce non-universal phase diagrams

The non-ideal behavior of the "free" multivalent salt (determine by effective ionic sizes) reduced and may suppressed the DNA overcharging

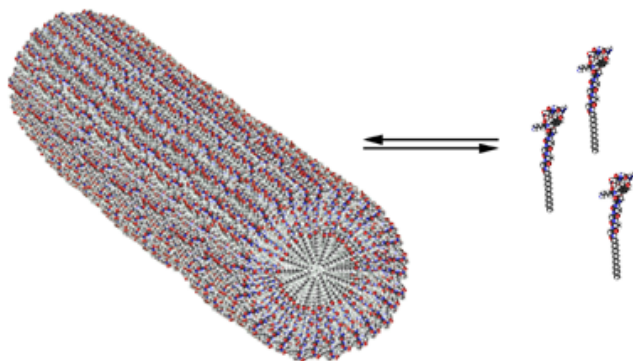Surface adsorption of polyions described by correlations.

Equal charge surface adsorption is possible via oppositely charged ions of valence 2+ (like – cell membranes to – DNA)
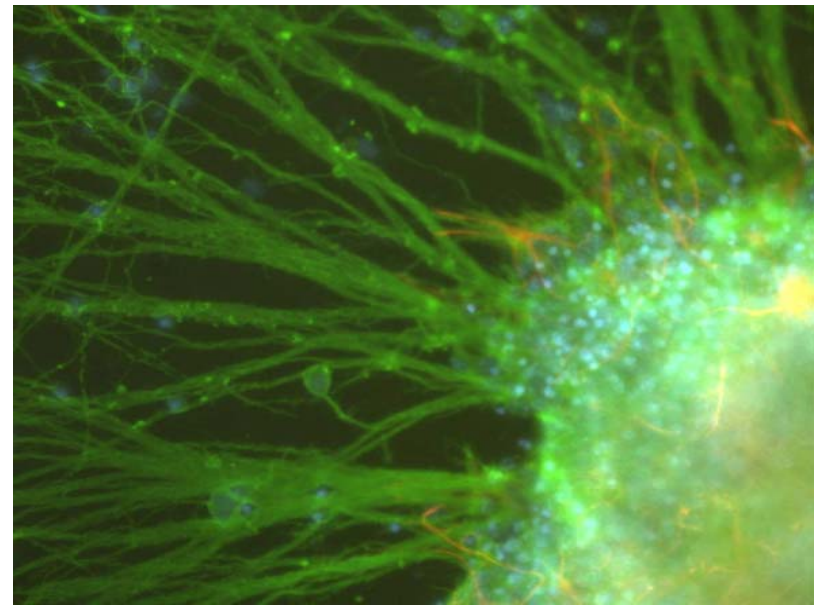
Mixed PAs
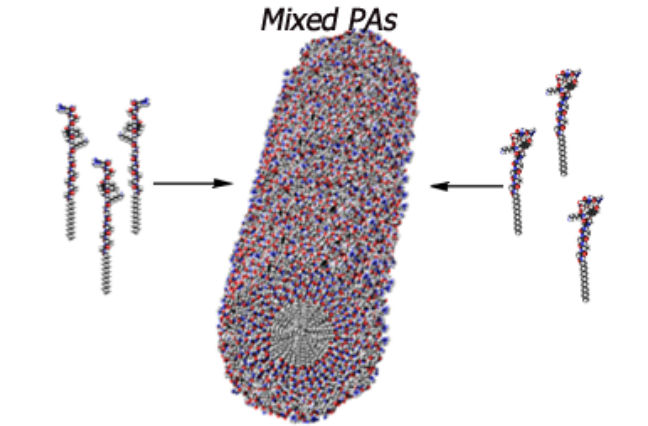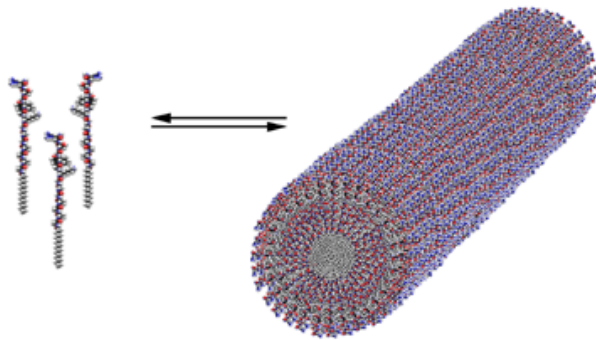
Basic PA

Acidic PA

pH<4　　　　　pH>9

Peptide Amphiphiles (PA) nanofibers formed from acidic PAs by dropping the pH below 4.5 (low). Basic PAs self-assembled above pH 9 (middle).
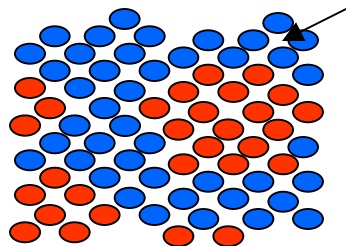
Combining acidic and basic PAs at neutral pH self-assembly (top).

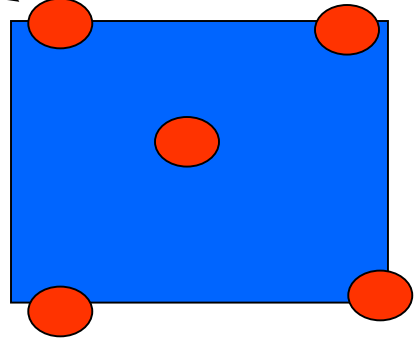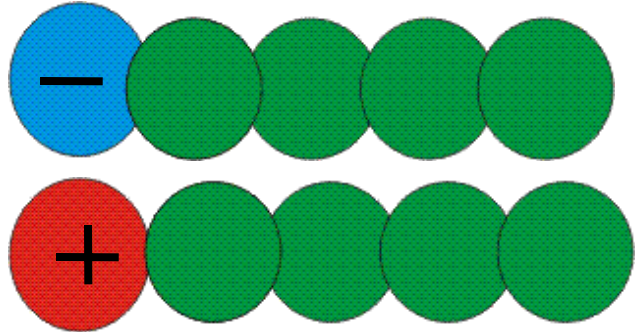**Only form cylinders. (S. Stupp lab for use as cell support such as laminin)**

Multi-component micelles? (Solis, de la Cruz, Stupp 04)

Use oppositely charge units to built stable nano-aggregates with surface structure if units are otherwise immiscible.
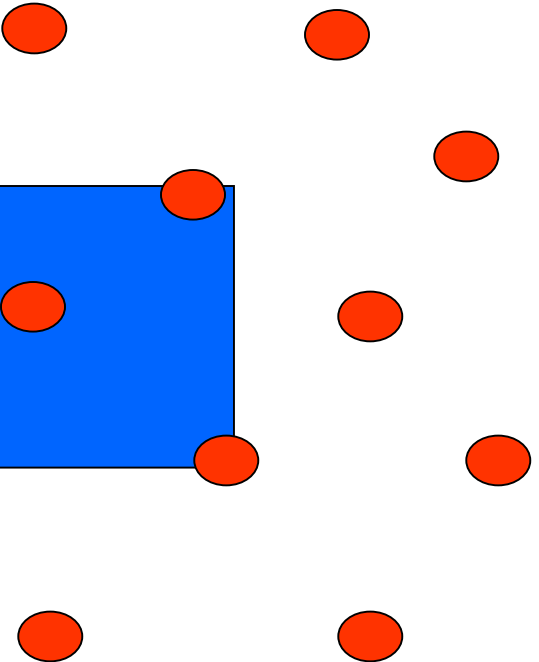
γ_o

Flat surface pattern due to the competition of surface tension γ_o and charge density $\bullet$:
domain of size $L \sim A^{1/2}$ is $L \sim (\gamma_o/\bullet^2)^{1/2}$
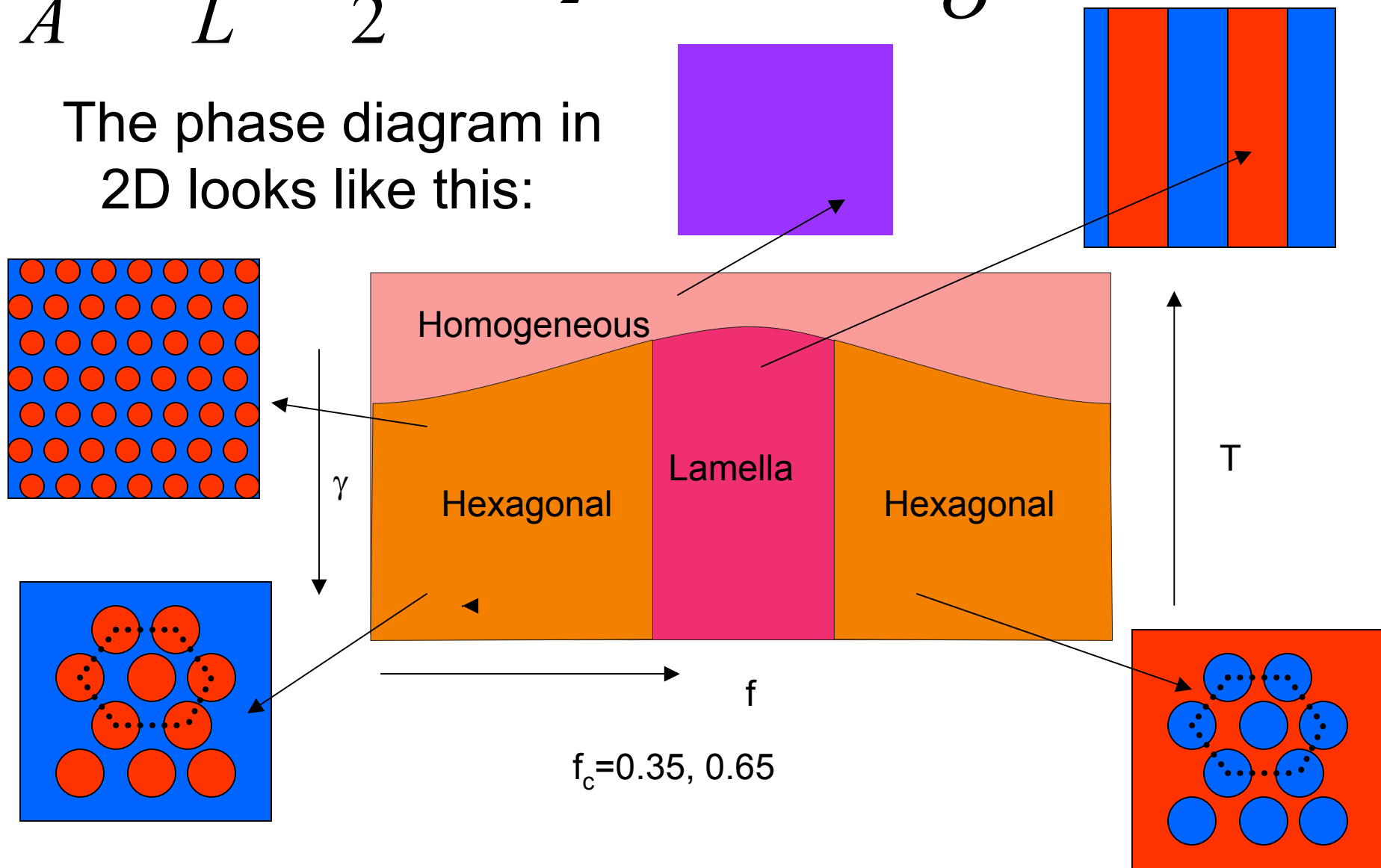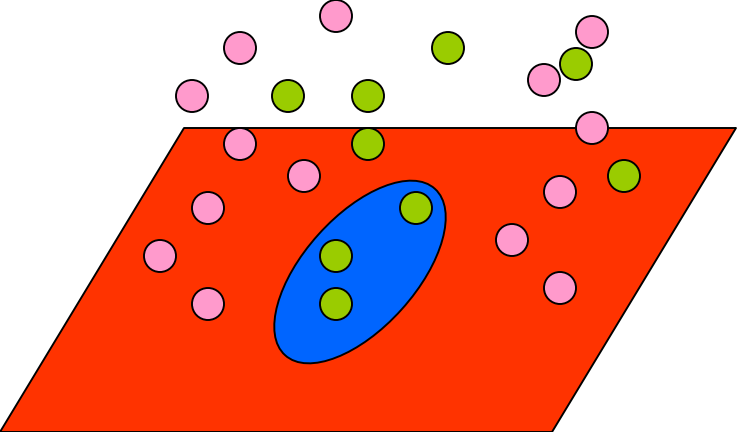The fraction of area f of one unit in the cell of are A determines the pattern structure.

$$\frac{F}{A} = \frac{\gamma\, s_1}{L} + \frac{1}{2}\sigma^2 L s_2 \qquad A_o = \frac{\gamma}{\sigma^2}$$
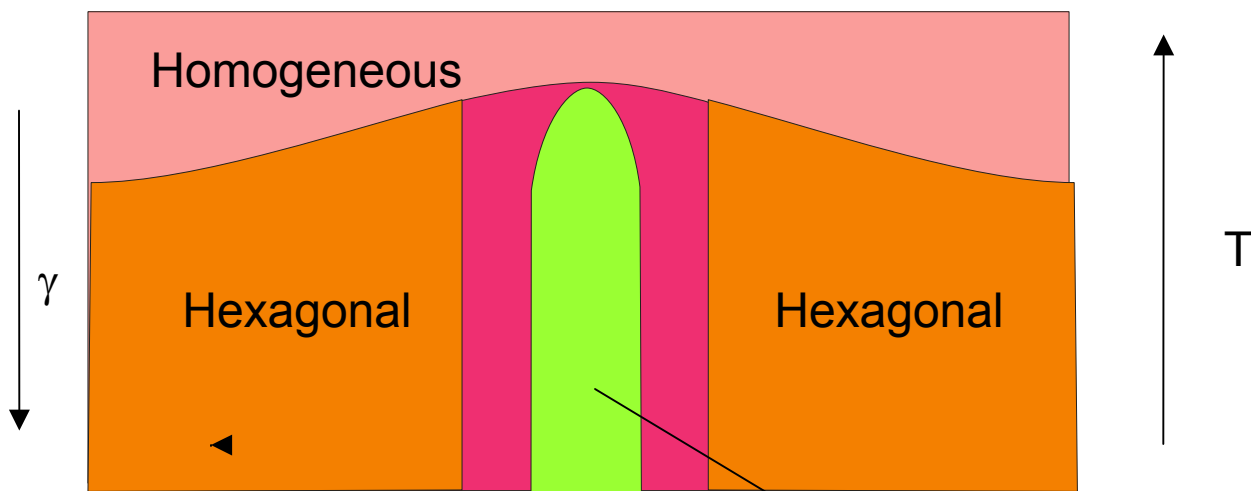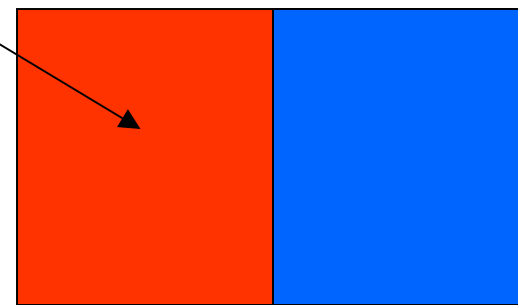
The phase diagram in 2D looks like this:

γ

T

Homogeneous

Hexagonal

Lamella

Hexagonal

f

f_c=0.35, 0.65

Pure Coulomb   F ~ L
Screened   F ~ L, L<1/𝜘 or F ~ 1/𝜘, L>1/𝜘

Macrophase segregation
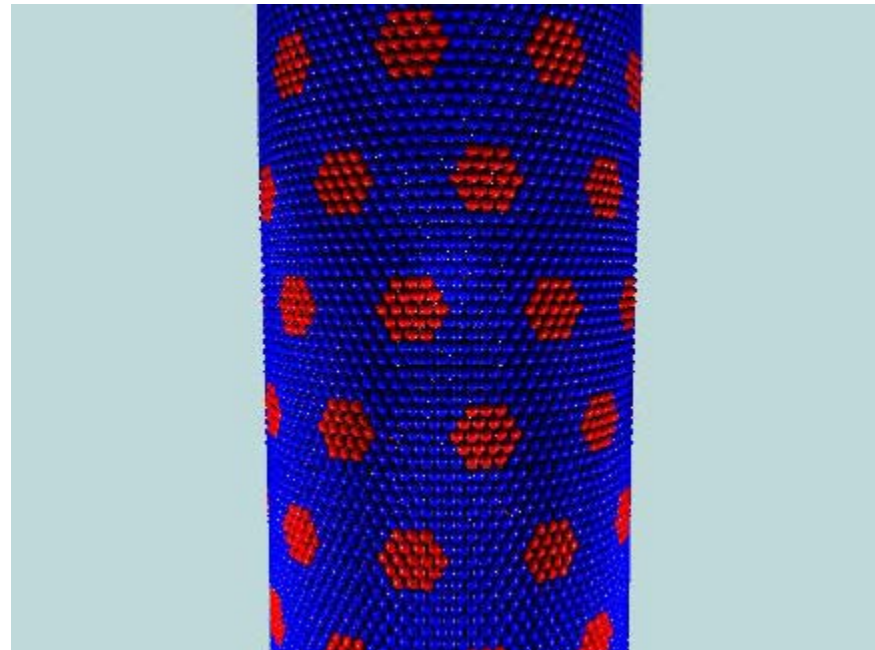
Homogeneous

γ

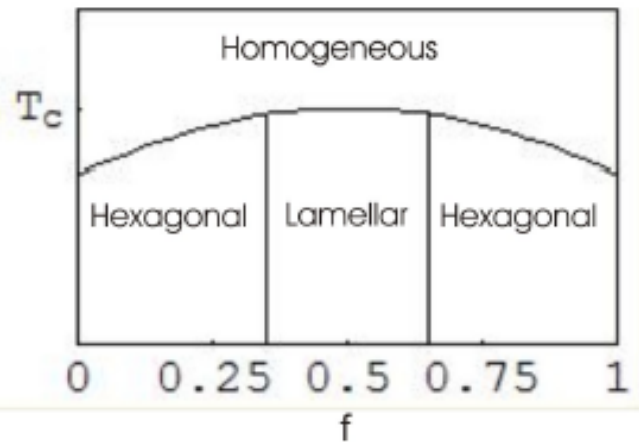Hexagonal
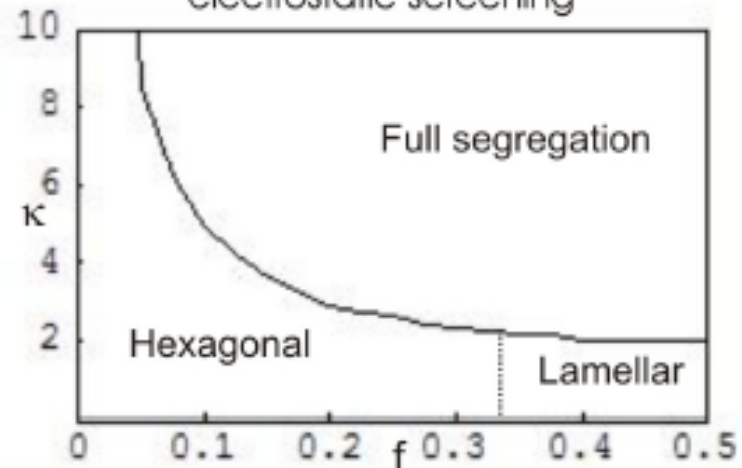
Hexagonal

T

f

Full segregation

Electrostatics generate multicomponent micelles and vesicles with surface patterns

Phase diagram for a flat geometry

With screening the domain size jumps from finite to macroscopic segregation

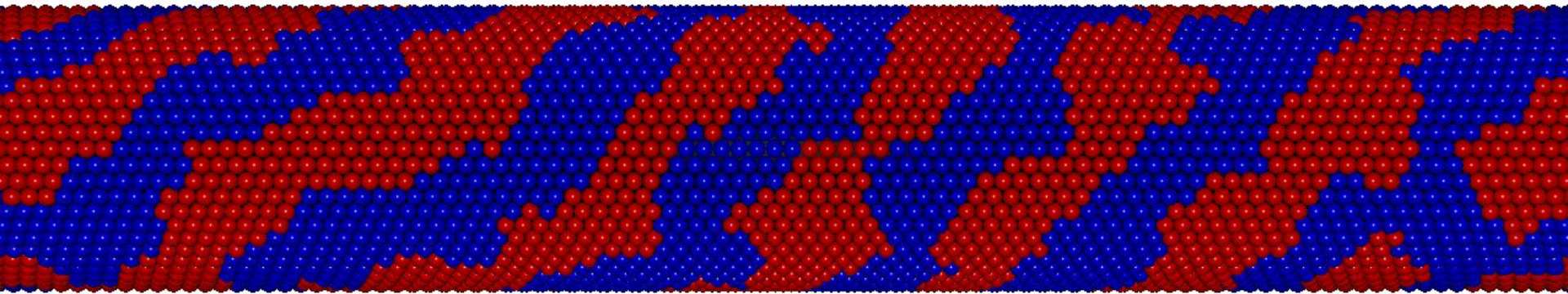Phase boundaries in the presence of electrostatic screening

MONTE CARLO EQUAL SIZE AND OPPOSITE CHARGE

+1 and -1

Sort range = 3KT ,

Electrostatic   1/ε ~ .1

Conclusion: in two dimensions one can create periodic patterns
using the competition among electrostatic and van der Waals interactions