# Insights into DNA – protamines self-assembly


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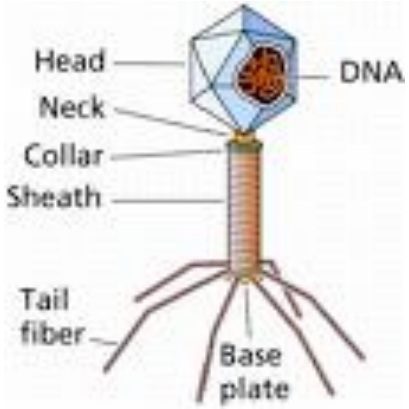
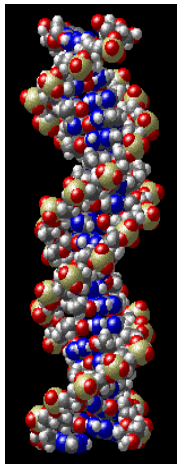
# DNA in vivo

## ds DNA Viruses




$C_{DNA}$ > 500 mg/ml (>50%)

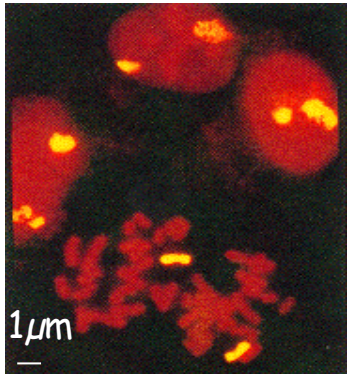
+polycations (polyamines)



+ proteins (histones) and polycations

## Eucaryotic chromosome

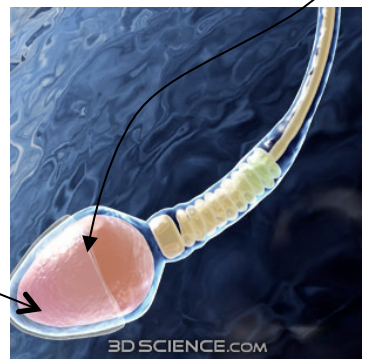
L = 1-10 cm




$C_{DNA}$ 50-250 mg/ml (5-25%)

+ proteins (protamines) and polycations

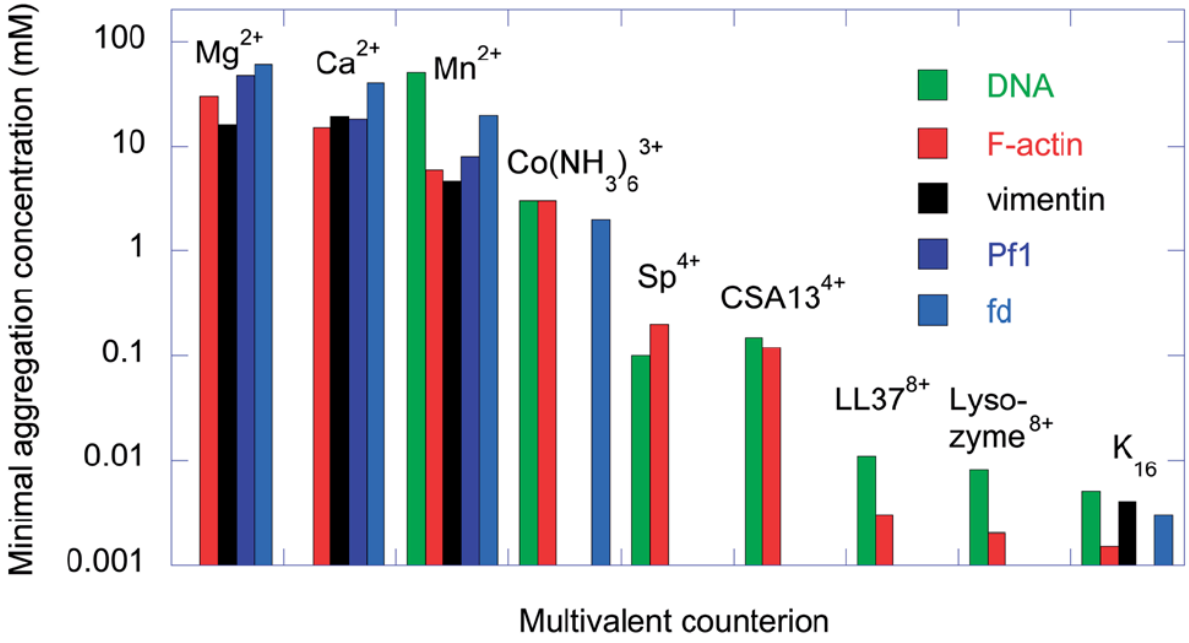
## Sperm cell



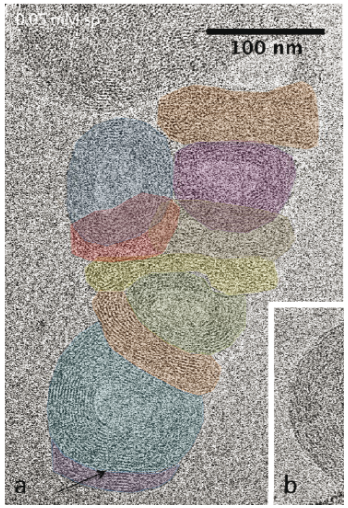

$C_{DNA}$ 250 - 400 mg/ml (25-40%)

- *Dense and complex soft matter*
- *Its organization controls the functionnal activities of the molecule*

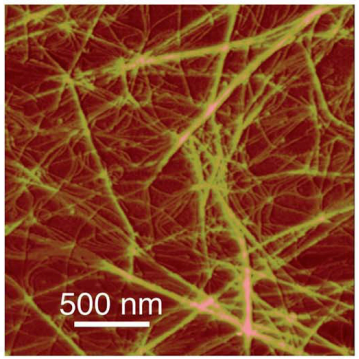
# Aggregation of different « rigid » biopolymers by different multications



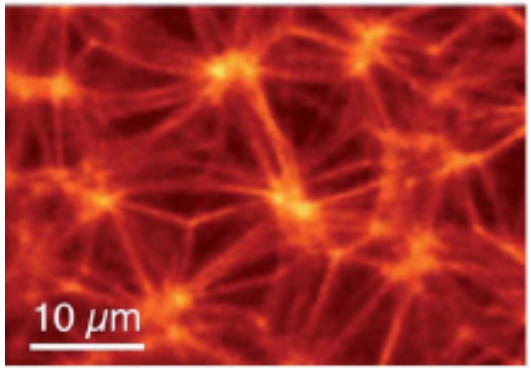

*Janmey et al. Soft Matter, 2014*




λ-DNA (Sung et al. )



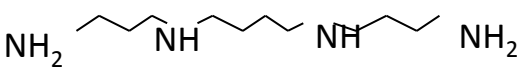

Pf1 network (Janmey et al, Soft Matter, 2014)



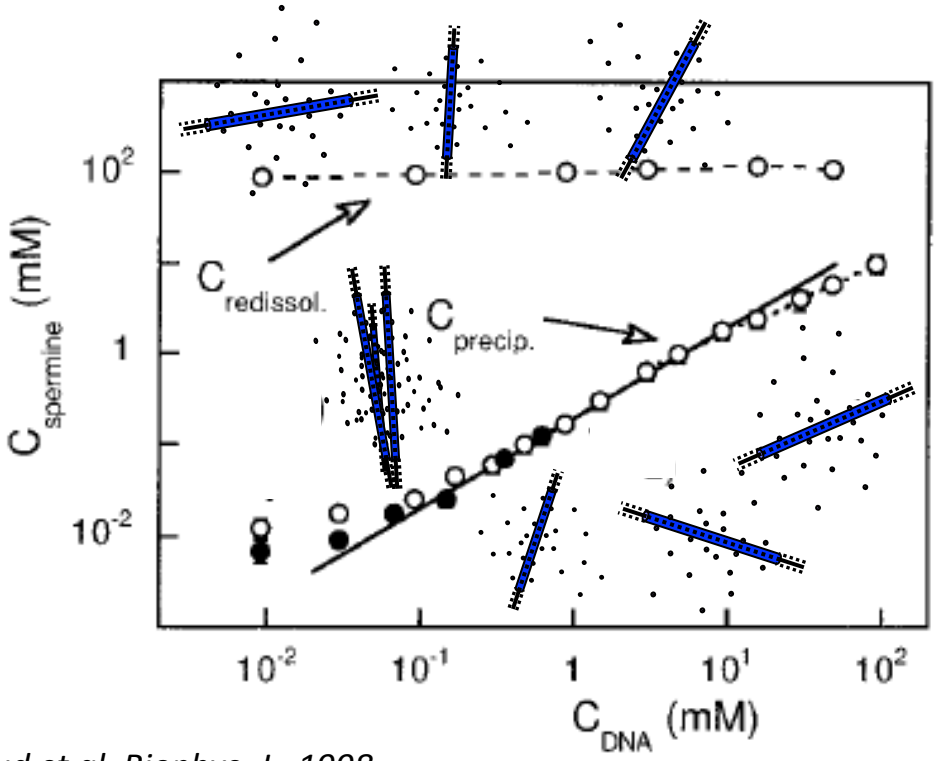

F-actin network (Hubert, Soft Matter, 2012)

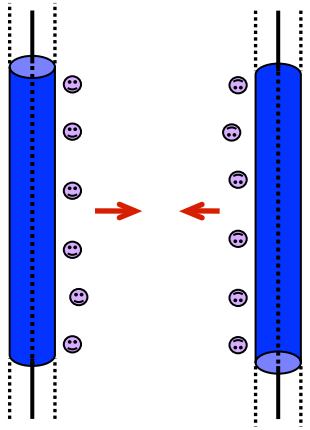
# *In vitro* aggregation of like-charged objects

short DNA fragments (150 bps), λ-DNA (48 kbp)
condensing agents: multivalent cations, small polycations ($3^+$, $4^+$)




spermine

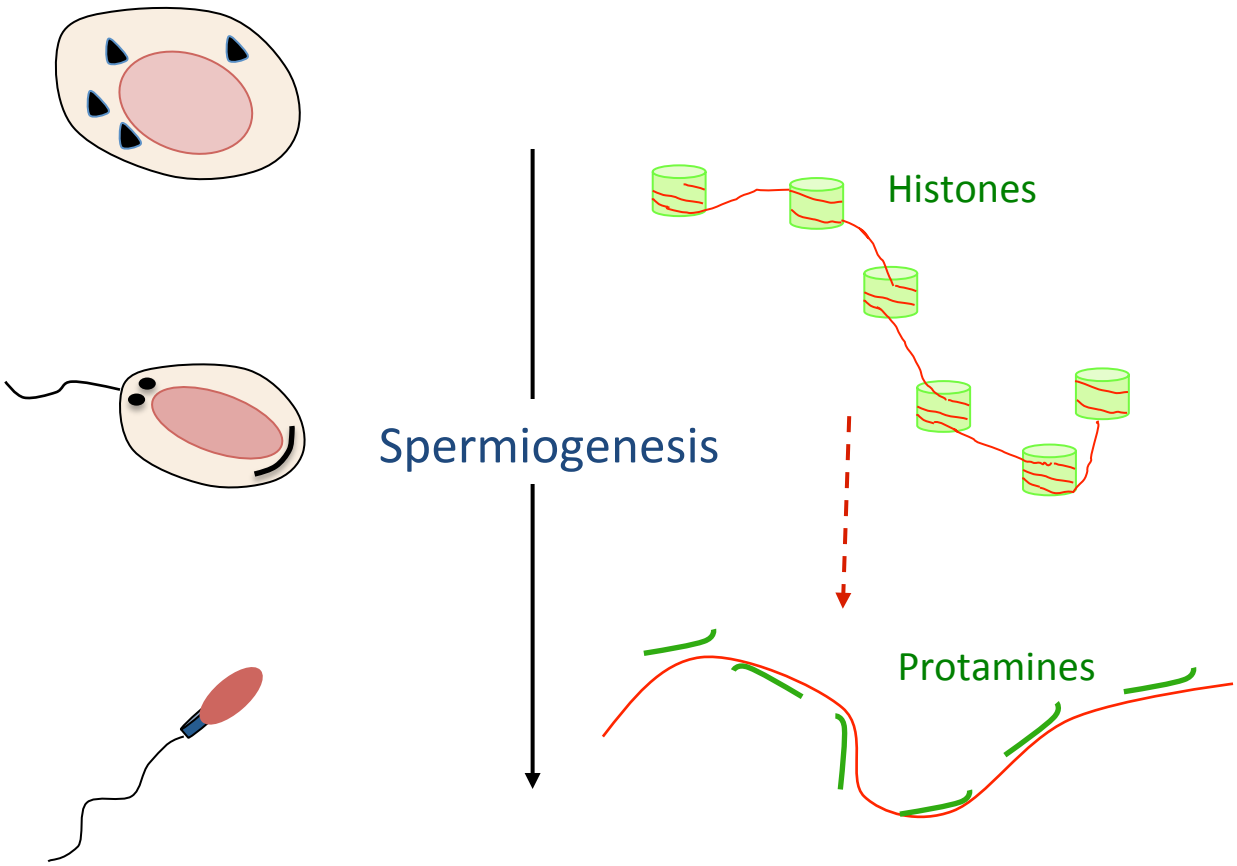


short-range attraction
(ion-correlation, ion-bridging …)

*Raspaud et al. Biophys. J., 1998*

# DNA in sperm cell



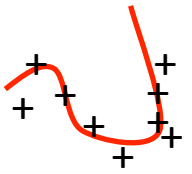

Many experimental studies
but
no complete phase diagram

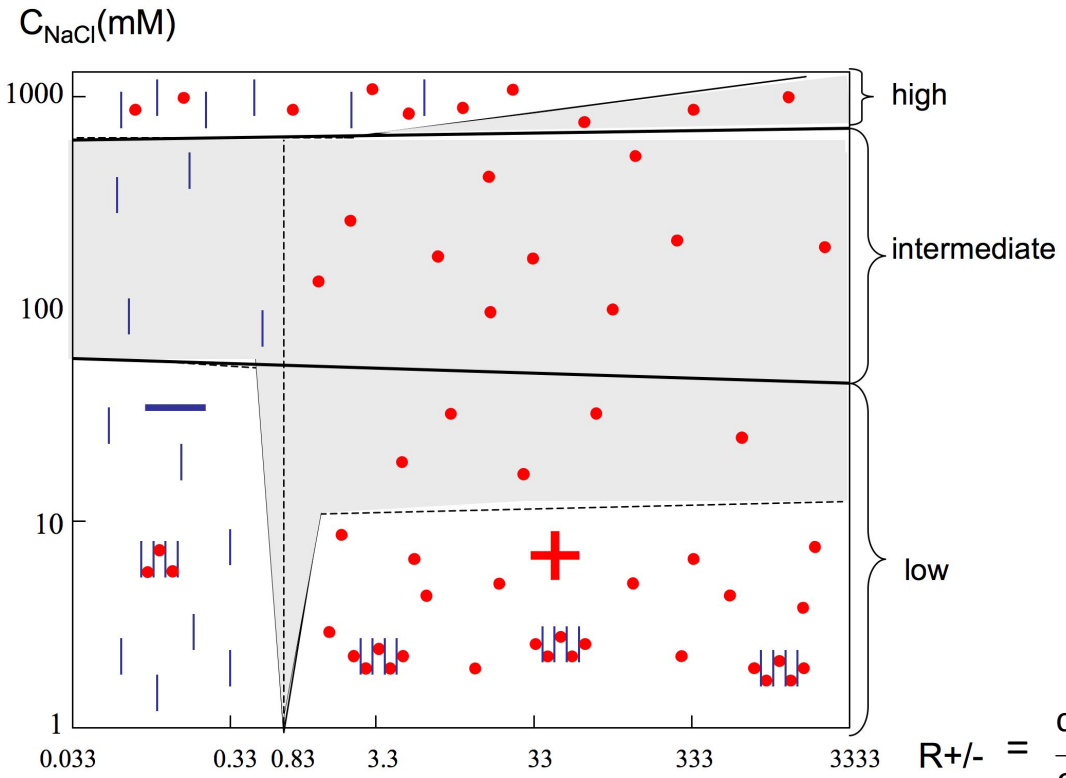
# Phase behavior for short dsDNA (50 nm) – small basic protein

salmon protamine
(30 amino acids, 21 positive)

PRRRRSSSRP VRRRRRPRVS RRRRRRGGRR RR

R = arginine



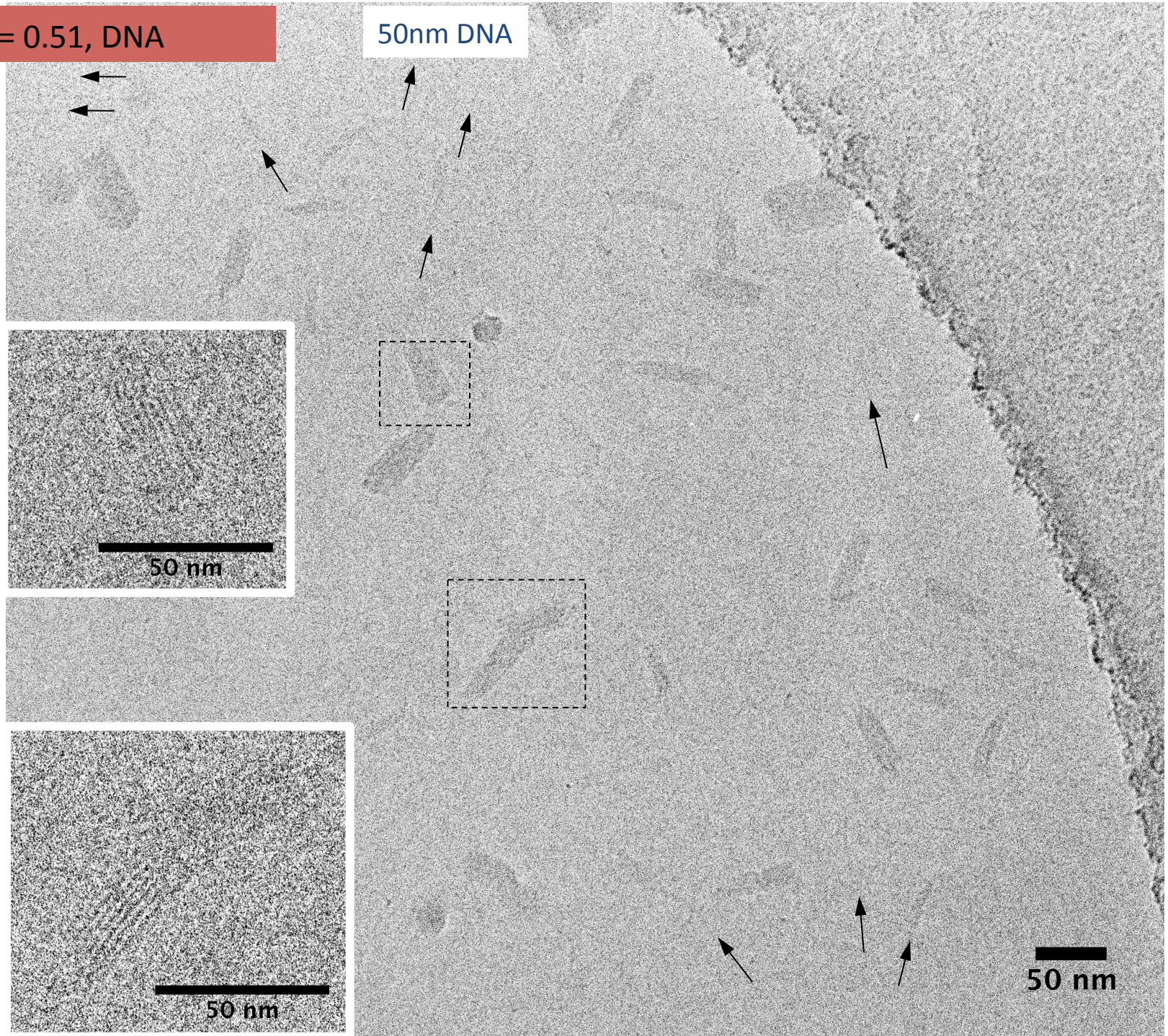




$C_{DNA}$ ~ 30 μM

$$R+/- = \frac{\text{charges of protamines}}{\text{charges of phosphates}}$$

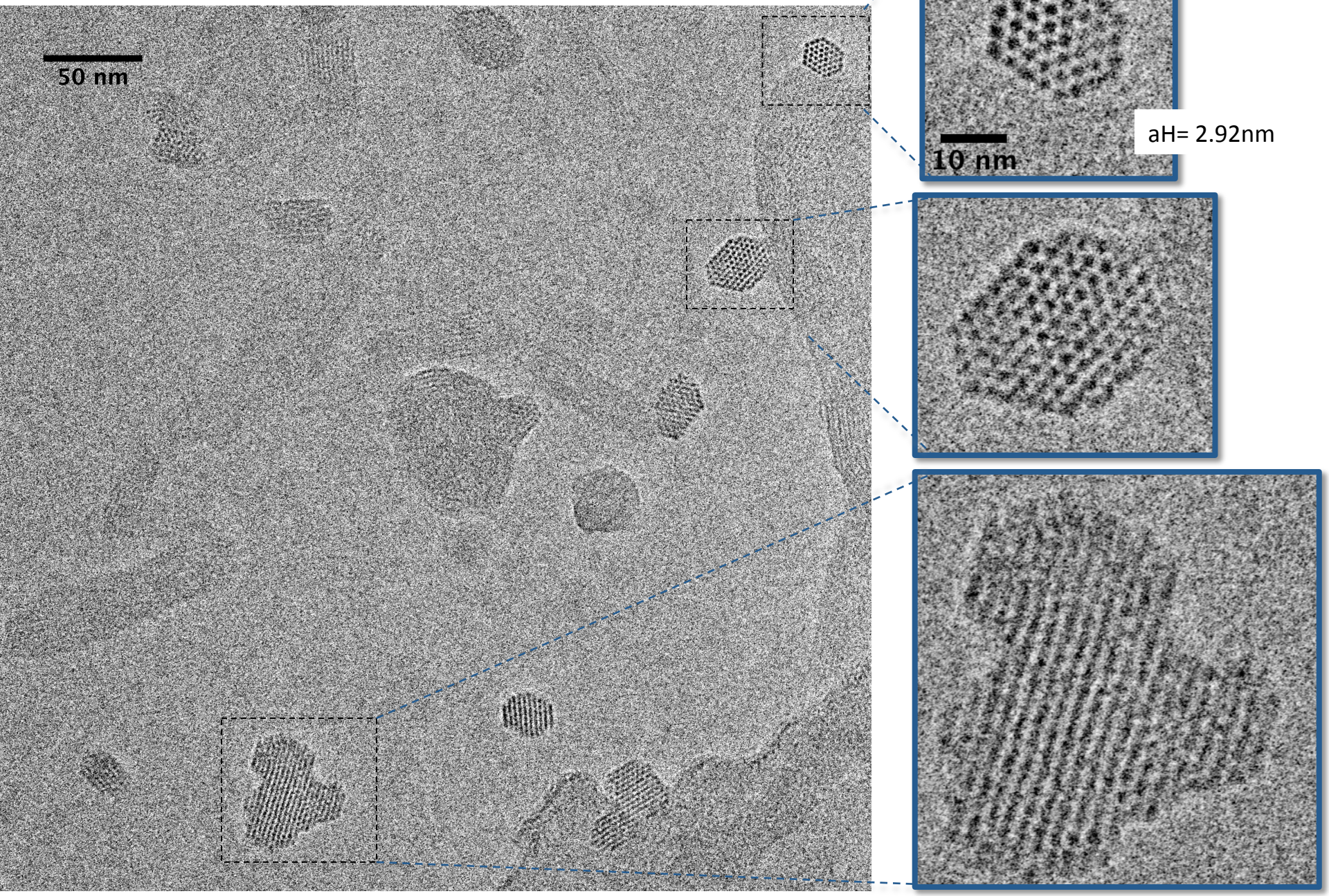
Toma et al., Biomacromolecules (2009); Soft Matter (2011)

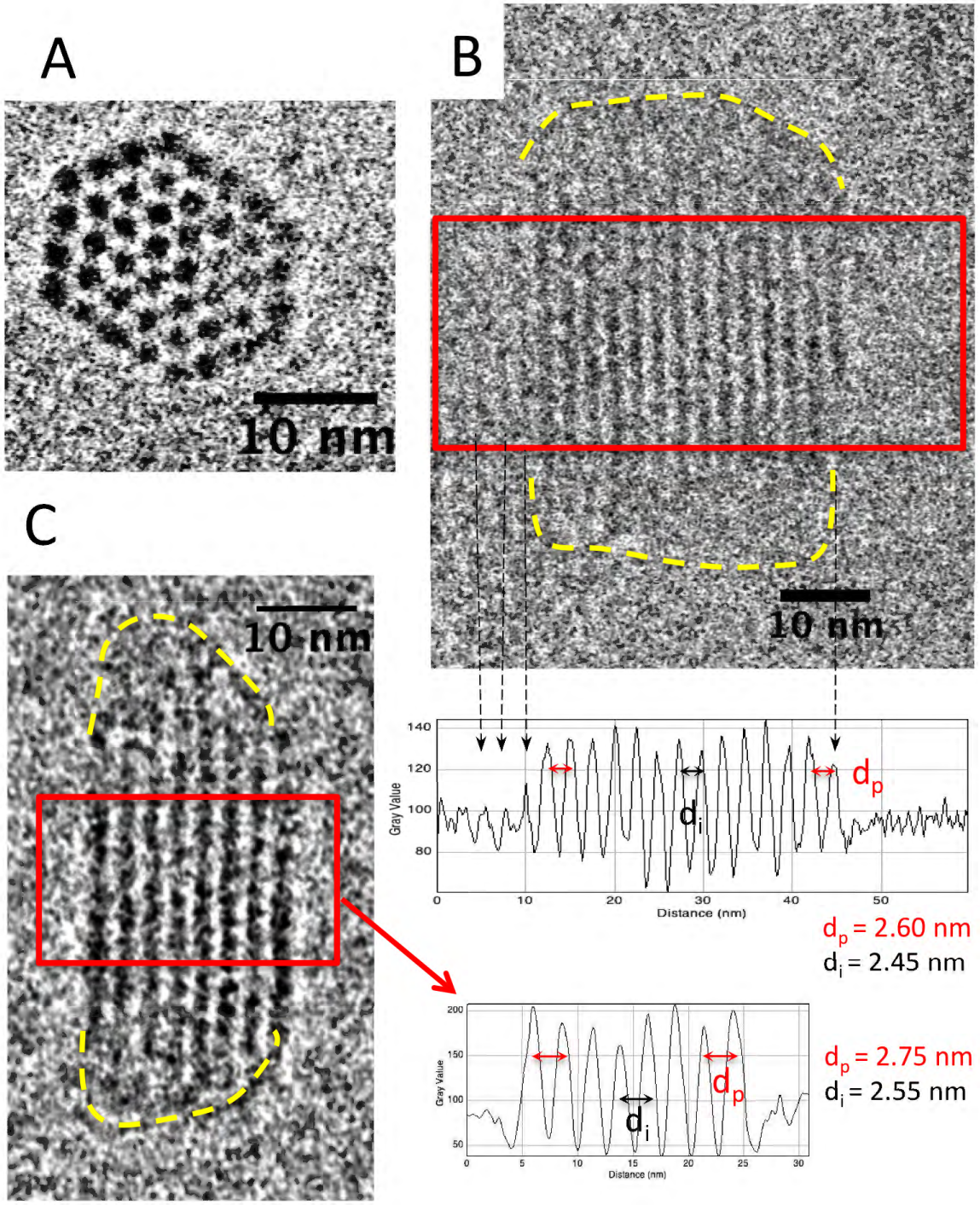
R(+/-) = 0.51, DNA excess



R(+/-) = 1.36, protamine excess

50nm DNA




A
10 nm
B
10 nm
C
10 nm
Gray Value
Distance (nm)
d_p
d_i
d_p = 2.60 nm
d_i = 2.45 nm
d_p = 2.75 nm
d_i = 2.55 nm

Can an idealized model help in interpreting the experiments?

- model
- DNA interactions mediated by protamines
- molecular dynamics (MD) simulations using different initial conditions
- some characteristics of the bundles
- MD simulations more relevant to experiments
- summary

# Idealized model

DNA: $L_{DNA}$ ~ 147 x 3.4 Å

$q_{DNA}$ = - 147 x 2e

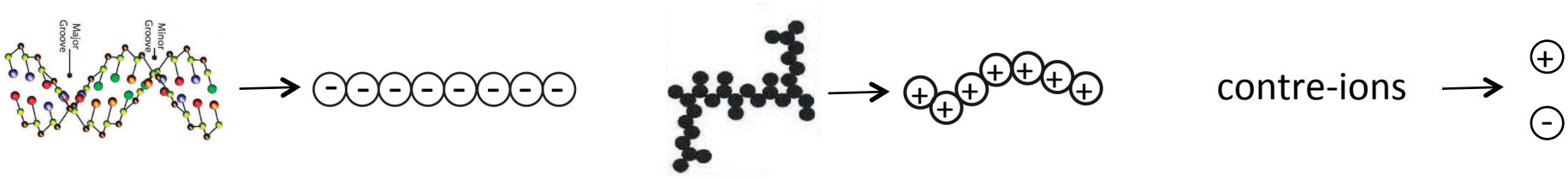
$\lambda_{DNA}$ = - e / 1.7 $A^{-1}$

protamines: $L_{PRO}$ ~ 31 x 3.8 Å

$q_{PRO}$ = 21 e

$\lambda_{PRO}$ = e / 5.6 $A^{-1}$

→ bead model



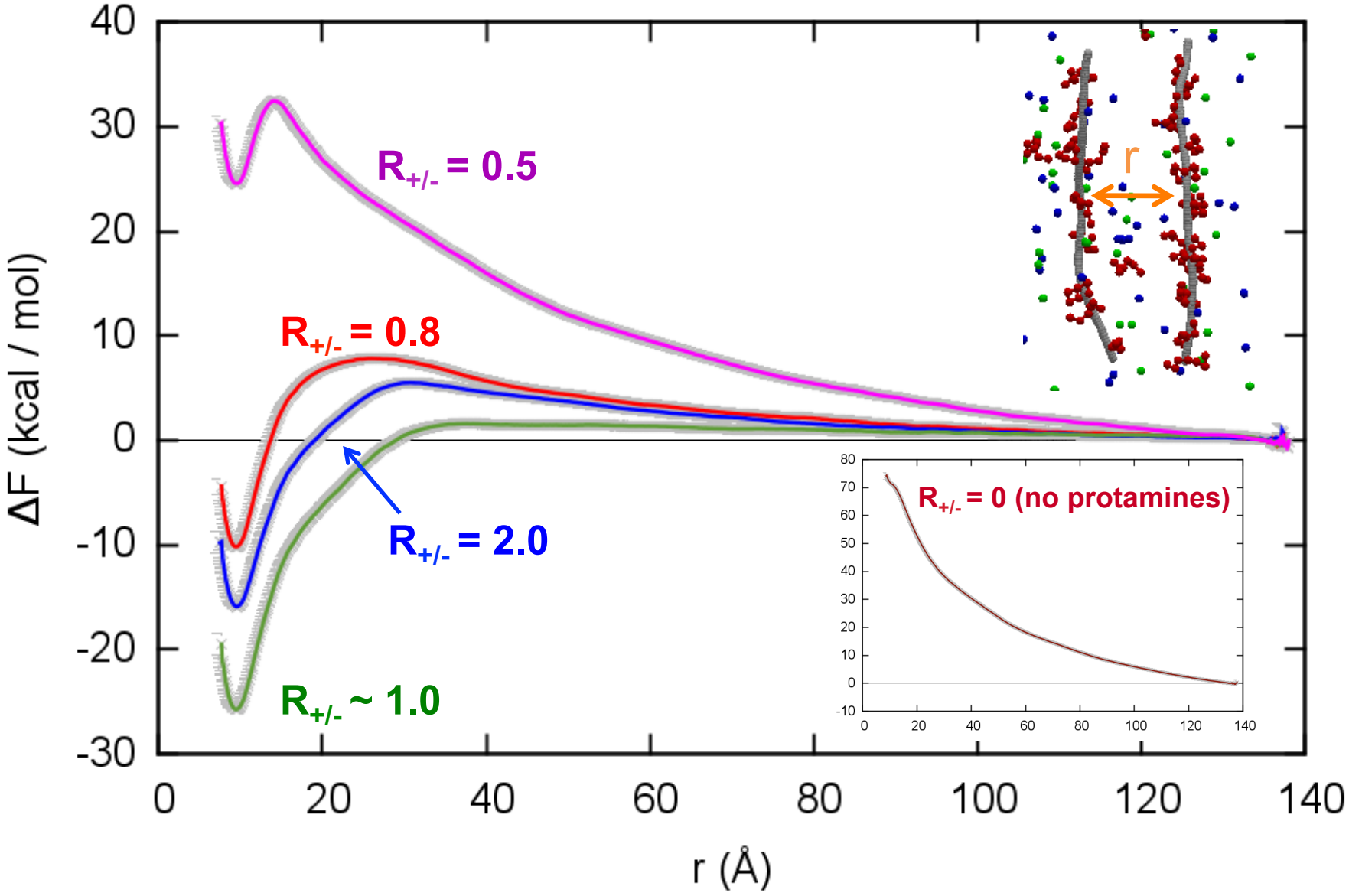

Non-bonded interactions: long-range electrostatics and short-range repulsion (excluded volume)

(implicit solvent $\varepsilon_r$ ~ 80)

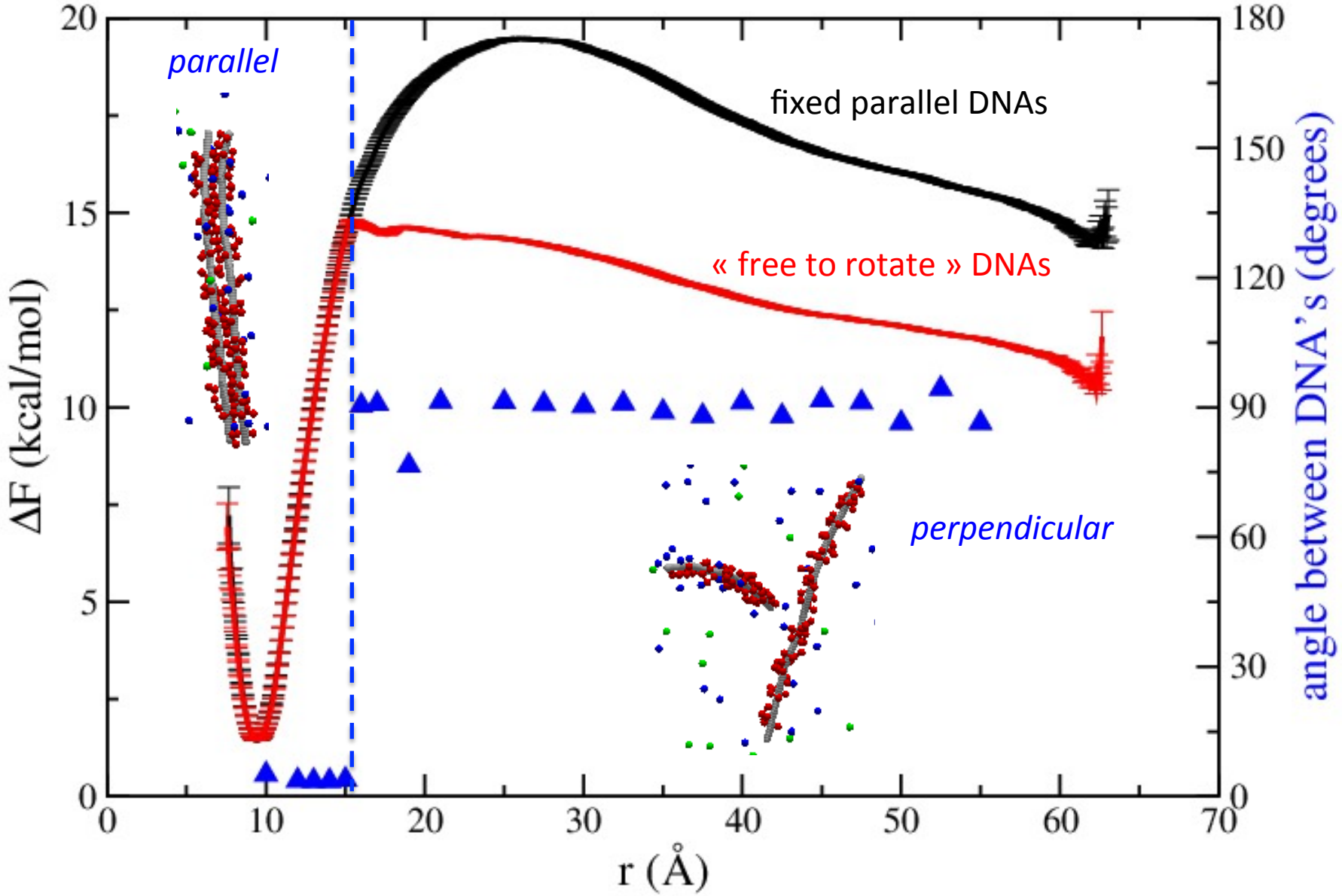
→ our model: the lengths of DNA and protamines are ~3 times shorter than in experiments

- DNA: 100 beads, $\sigma$ = 4Å, q = - e with $r_{eq}$ = 1.7 Å (+ bending potentiel, $\theta_{eq}$ = 180°) ($L_{DNA}$ ~ 169Å)
- protamines: 7 beads, $\sigma$ = 4Å, q = +e with $r_{eq}$ = 5.6Å (fully flexible) ($L_{PRO}$ ~33Å)
- counterions: 1 bead, $\sigma$ = 4Å, q = ± e)
- all the bead have the same mass (in MD)

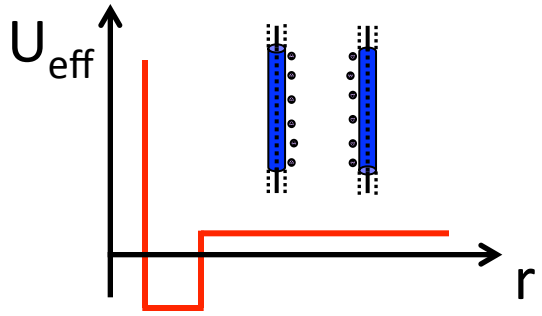
Free energy difference for 2 DNA molecules « parallel » to each other
(umbrella sampling calculation)

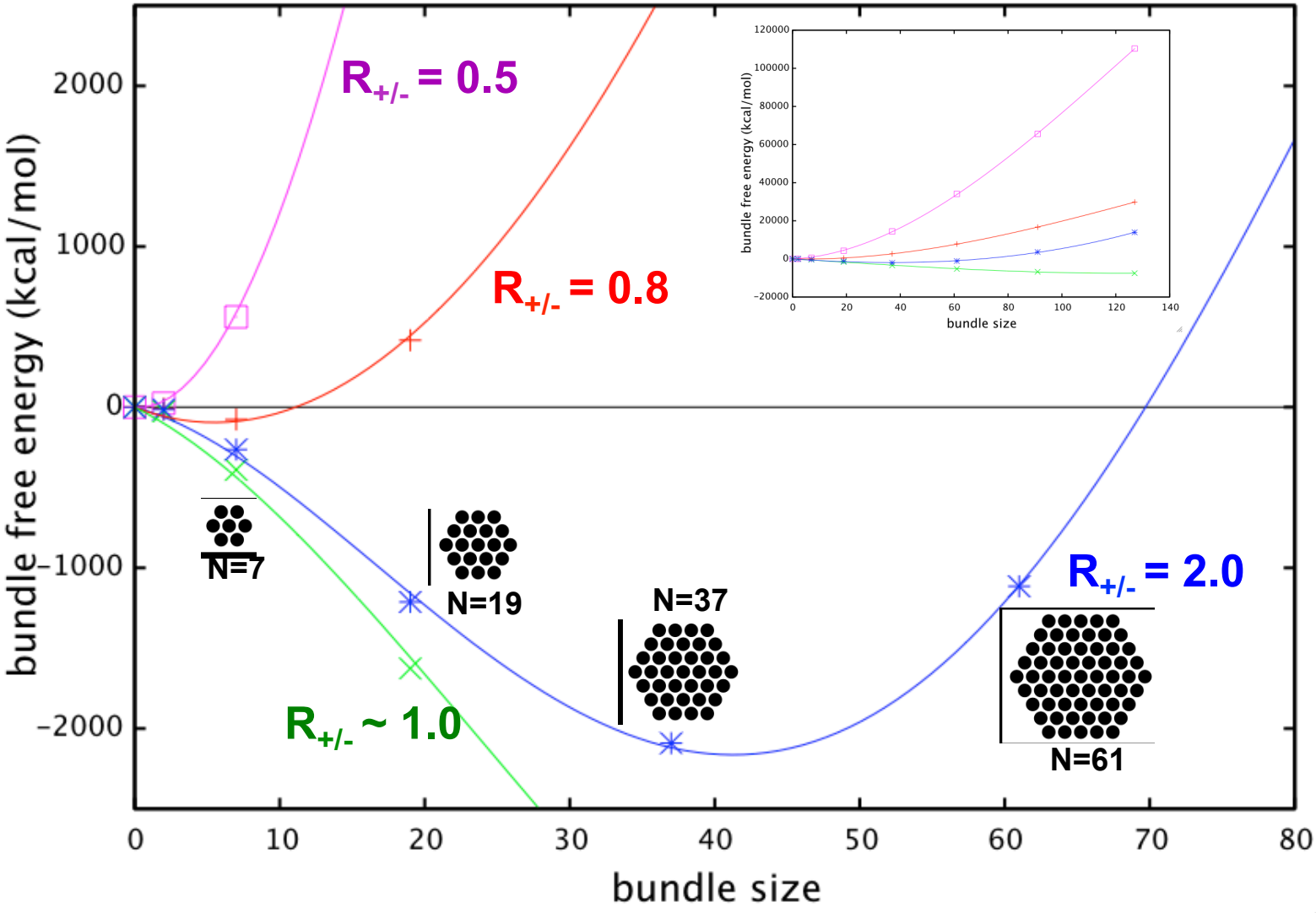


Free energy difference for 2 DNA molecules free to rotate ($R_{+/-}$ = 0.8)



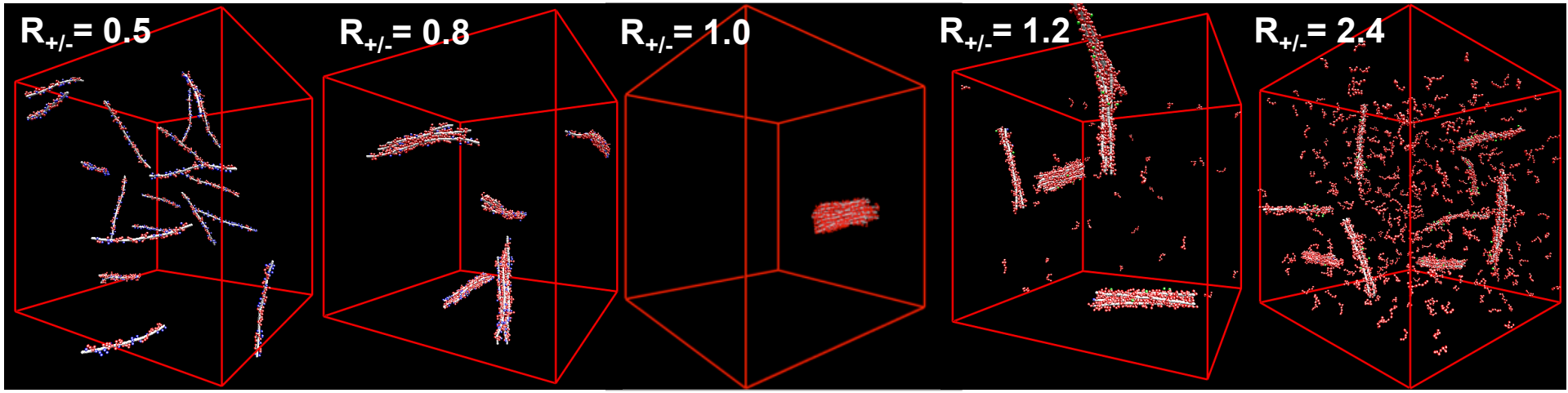
# Free energy of a bundle (pairwise additivity hypothesis)



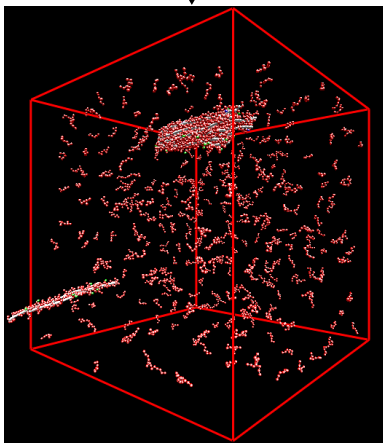
# DNA-protamines NVT MD (or REMD) simulations
# I- isotropic initial state ($N_{DNA}$ = 20, x1000 exp. concentration)

Later stage of the evolution (t* = $10^7$ timesteps) from an initial isotropic condition




excess of DNA — Isoelectric point — excess of protamines

later stages



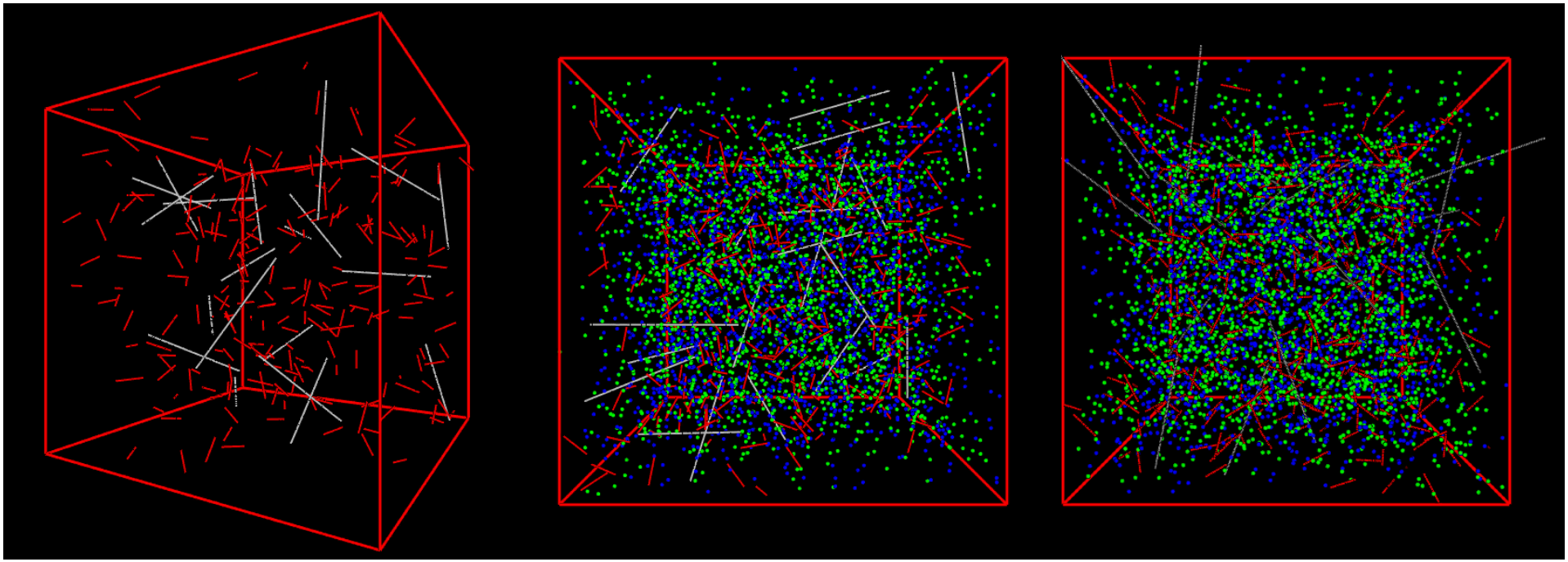
($R_{+/-}$=2.4; 9 $10^7$ ts)

- protamines induced attraction between DNA (correlations, bridging)
- bundles are charged in excess of DNA or in excess of protamines
- bundles form only if there is enough protamines
- bundles in excess of protamines tend to aggregate

# DNA-protamines MD (or REMD) simulations
# I – Isotropic initial state
# Dynamical formation of the bundles

NVT simulation with $\mathbf{N_{DNA} = 20}$ in a cubic box of side 48 nm

Time evolution (first 3 $10^6$ timesteps of ~9-14 $10^7$, $\Delta t$ = 1 fs) from an initial isotropic condition

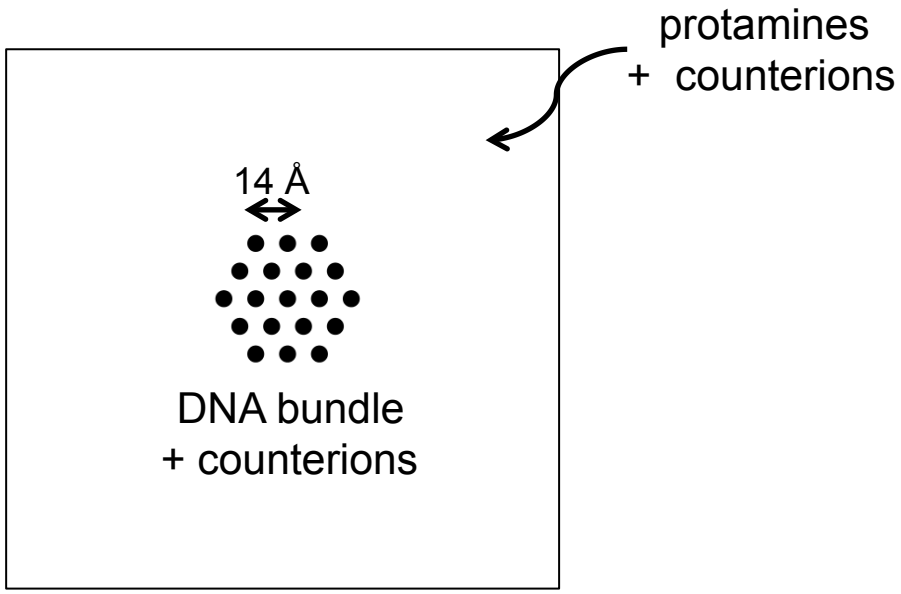


$R_{+/-}$ = 0.8 (DNA excess)

$R_{+/-}$ = 1 (precipitation)

$R_{+/-}$ = 1.2 (protamine excess)

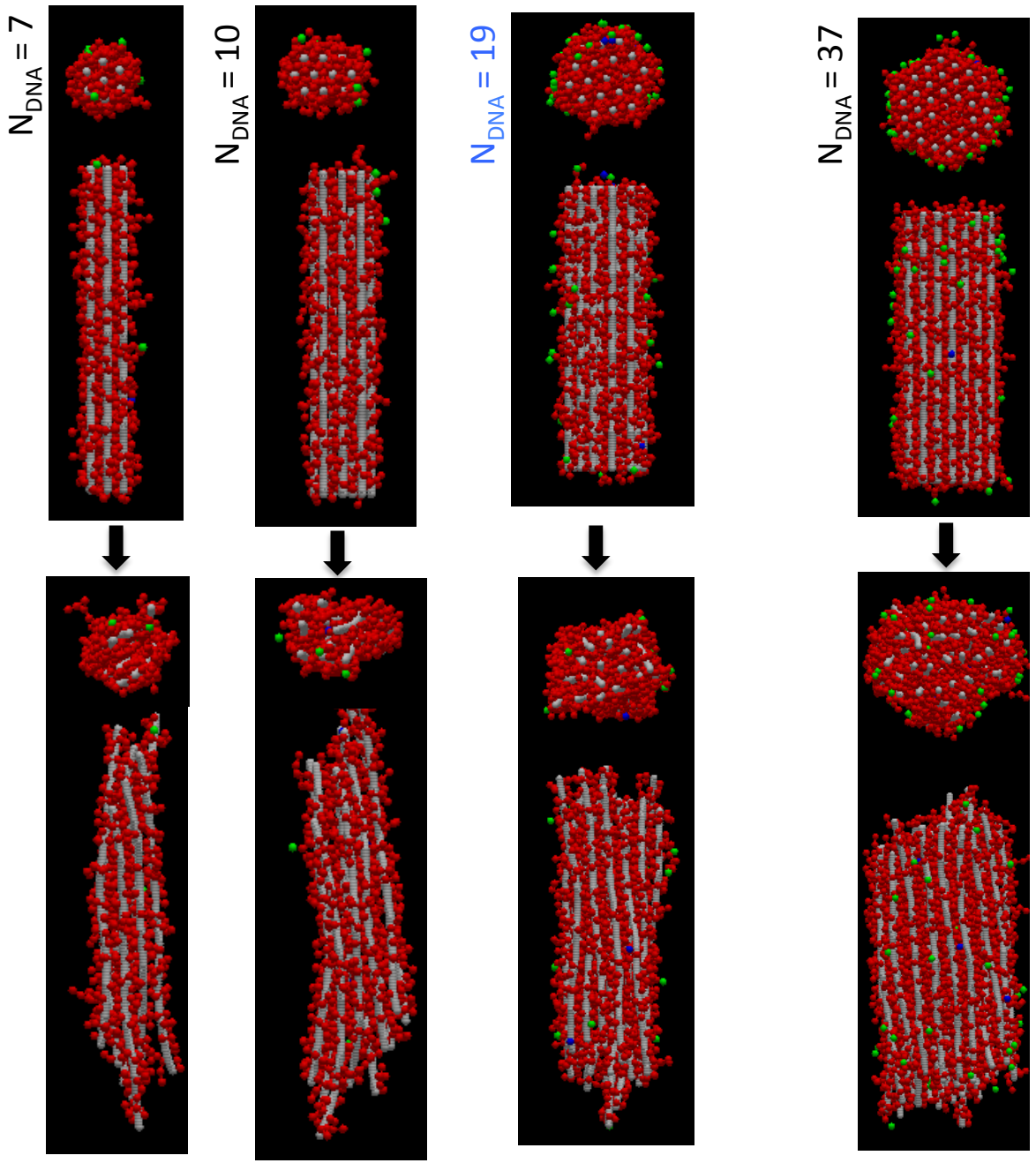
# DNA-protamines MD simulations
# II- preformed bundle as initial state




Simulation

1 – equilibration: fixed DNA and free protamines/counterions
2 – production: free DNA, protamines and counterions

Pre-assembled cluster for $R_{+/-}$ = 2.0 (protamines/counterions in solution not shown)

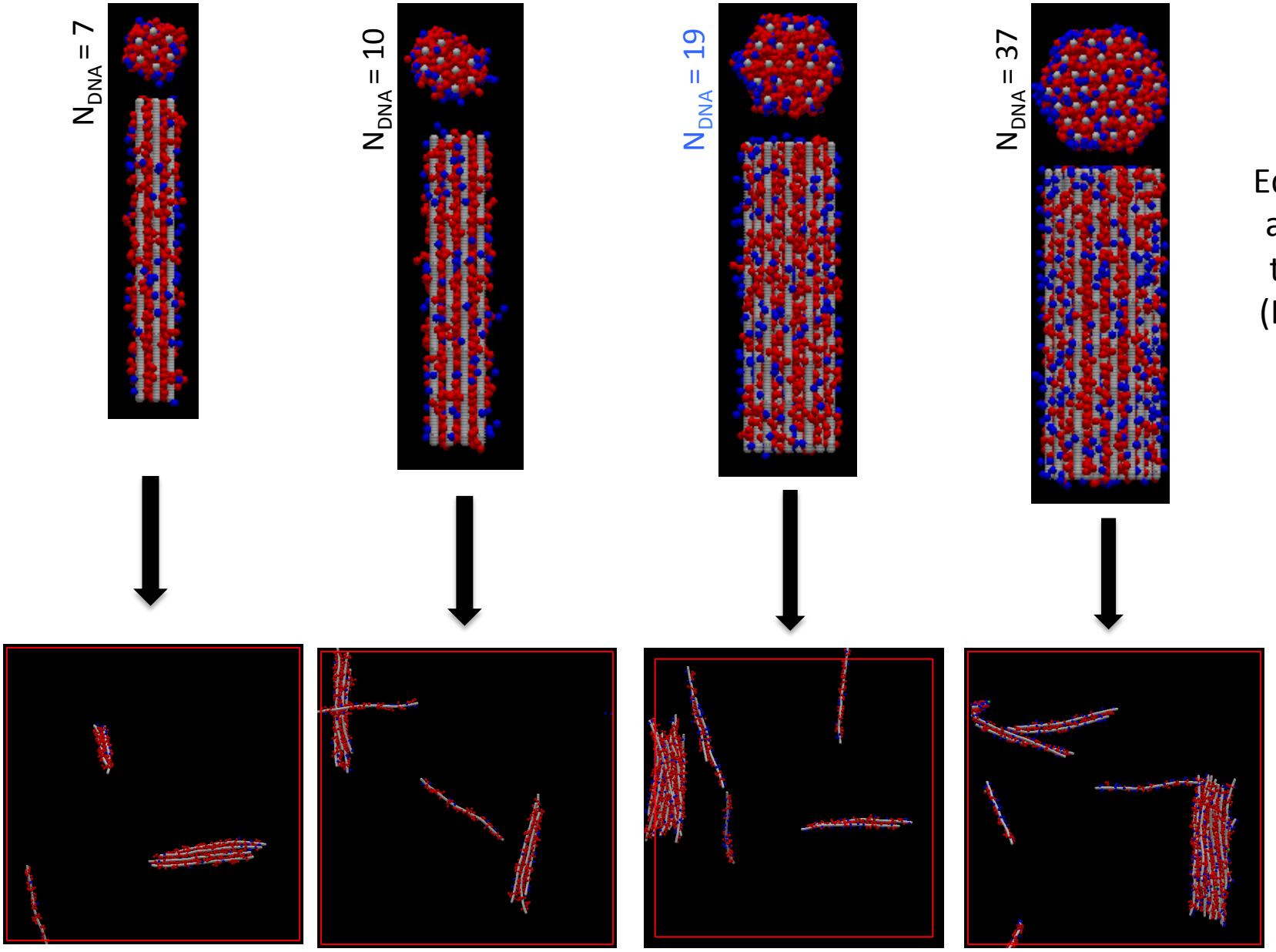



Equilibrated after 5 $10^6$ timesteps (DNA fixed)

State after 2 $10^7$ timesteps

**Bundles mechanically stable**

Pre-assembled cluster for $R_{+/-}$ = 0.8 (counterions in solution not shown)

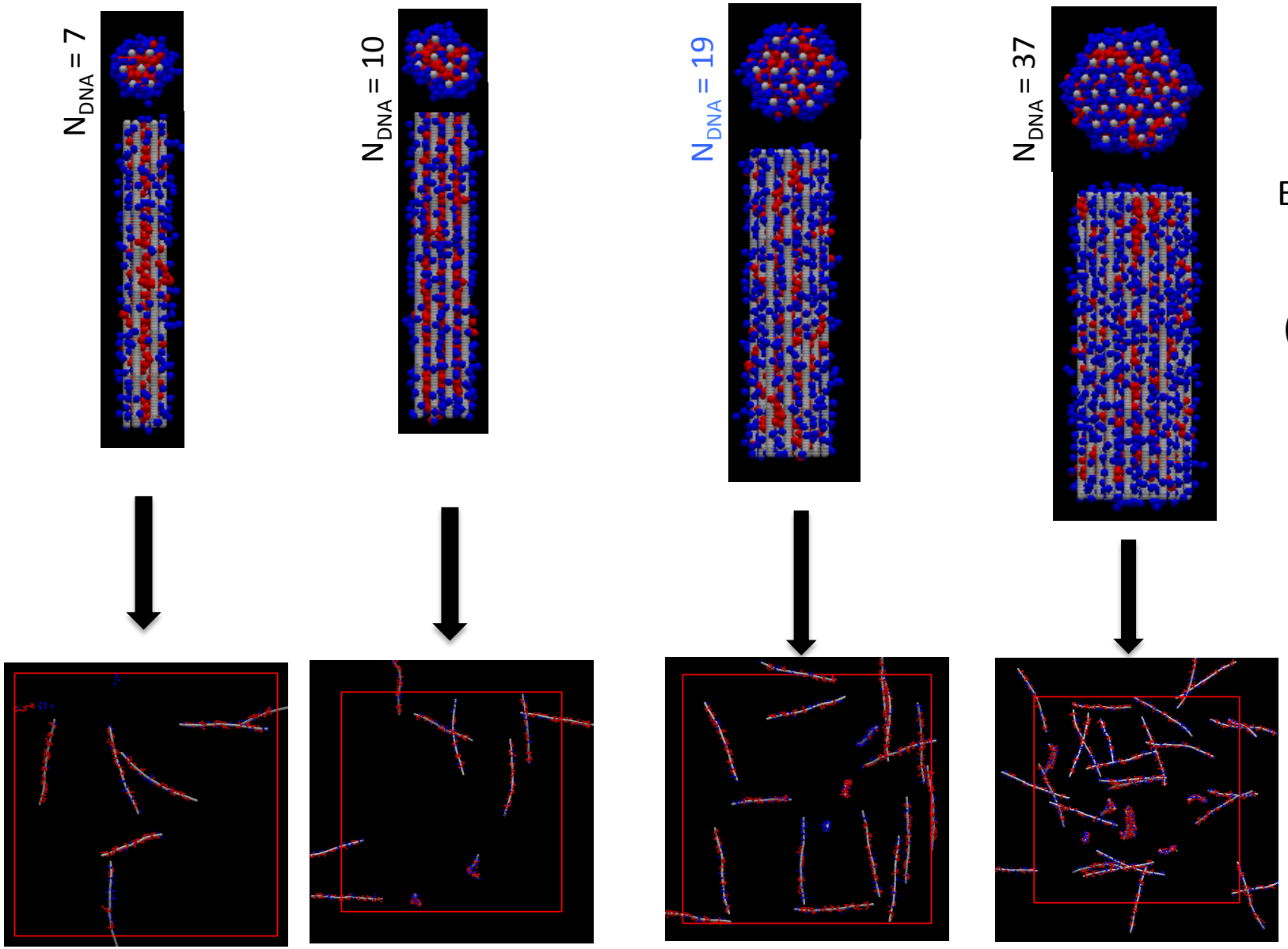



Equilibrated after 5 $10^6$ timesteps (DNA fixed)

State after 2 $10^7$ timesteps

**Bundles fission**

Pre-assembled cluster for $R_{+/-}$ = 0.5 (counterions in solution not shown)



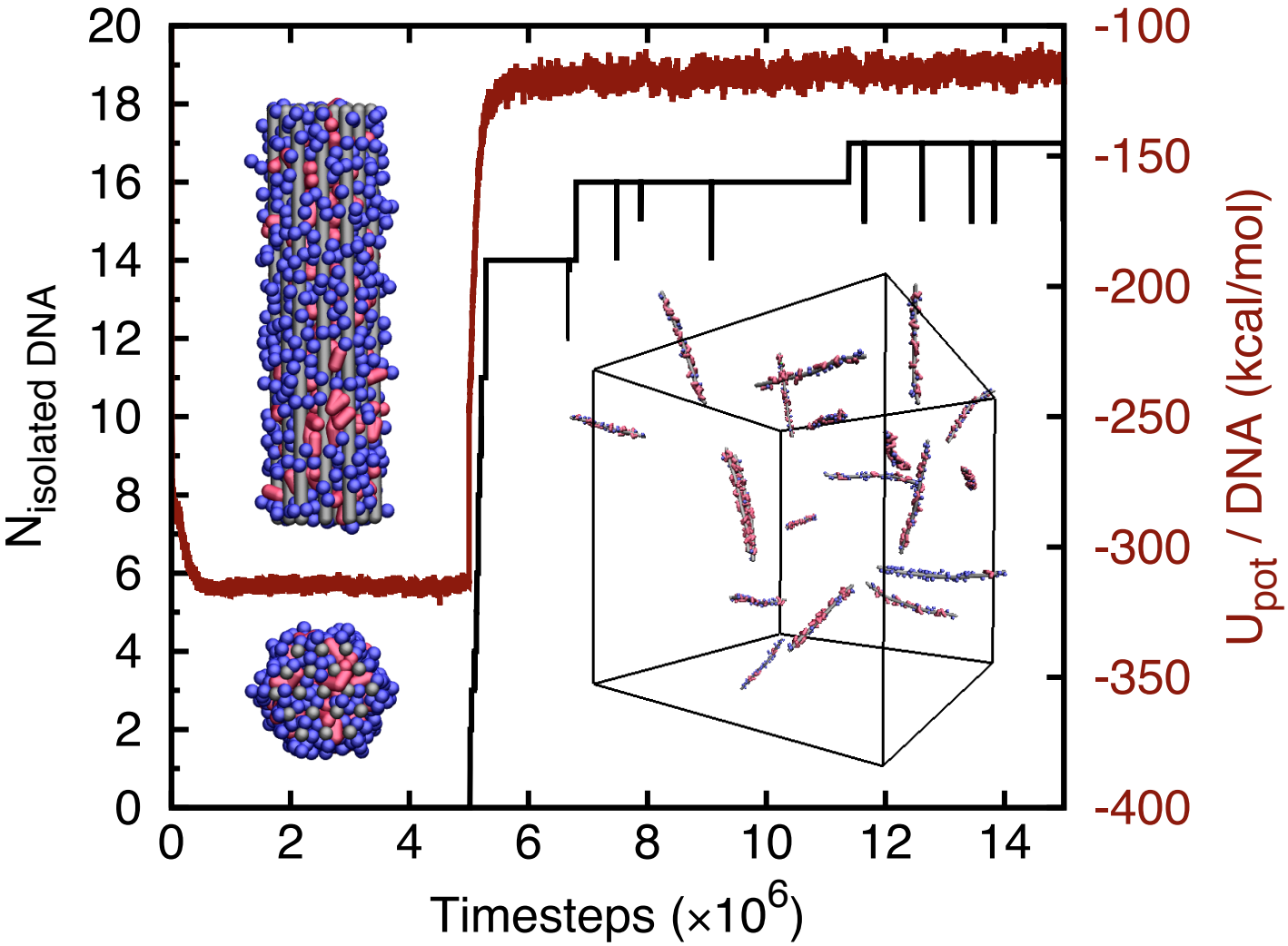

Equilibrated after 5 $10^6$ timesteps (DNA fixed)

State after 2 $10^7$ timesteps

**Bundles mechanically unstable**

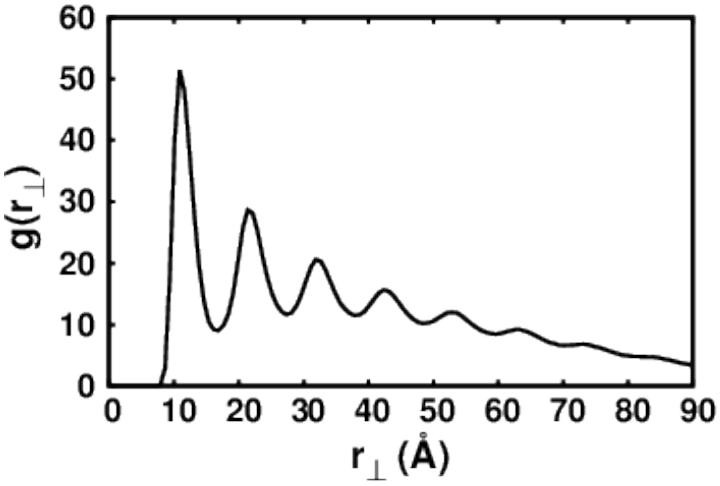
# Entropy stabilizes the DNA bundles

Time evolution of the potential energy
Preformed (mechanically unstable) bundle, $N_{DNA}$ = 19, $R_{+/-}$ = 0.5

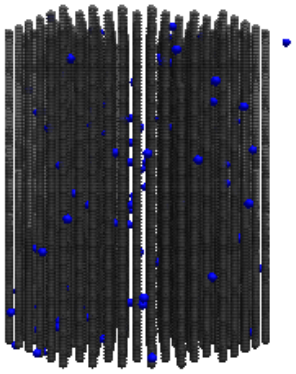


# Bundles are dynamical structures

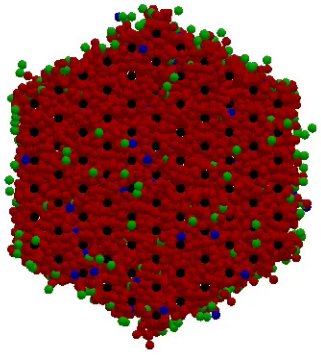
$R_{+/-} = 2$, $N_{DNA} = 91$ (preformed bundle)




bundles are fluidlike (no positional order) with orientational order: liquid crystal state

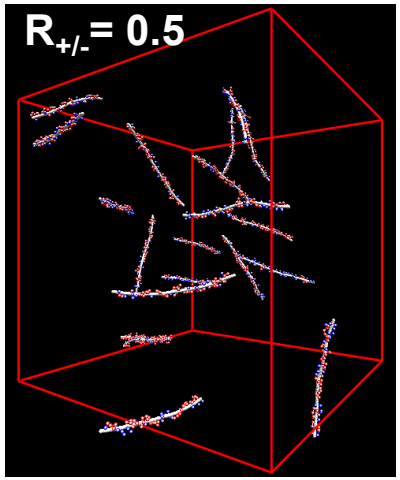


bundle lateral view
(only DNA and their counterions shown)

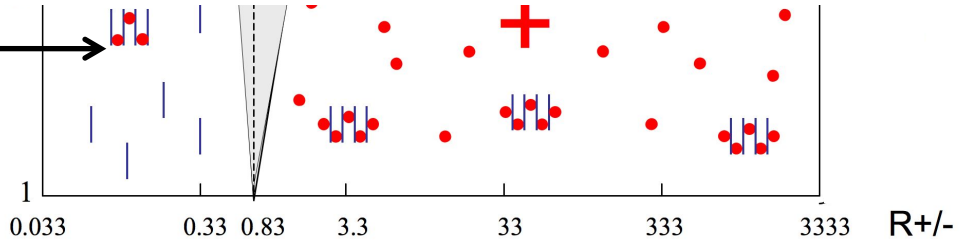


bundle central transverse plane view

# Simulations do not lead to bundles at « low » protamine concentration

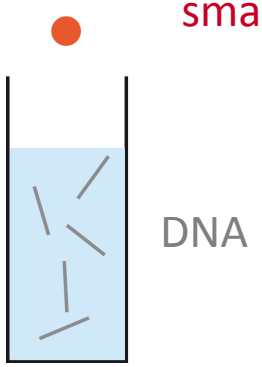



?




small protamine droplet

Inhomgeneous experimental conditions



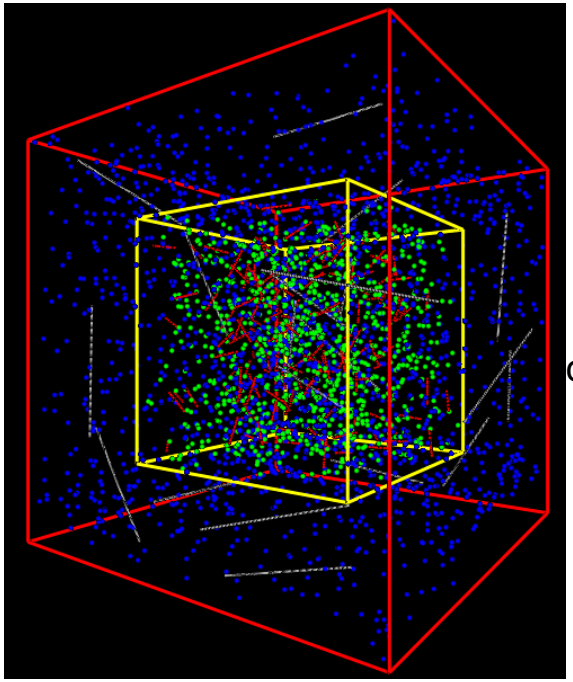

➡ always in *local* excess of protamines

- bundle formation ~independant of $R_{+/-}$ in excess of DNA ($R_{+/-} < 1$)
- bundles in coexistence with « naked » DNA for $R_{+/-} < 1$

# Mimicking the experiments with MD
# I – « chamber experiment »

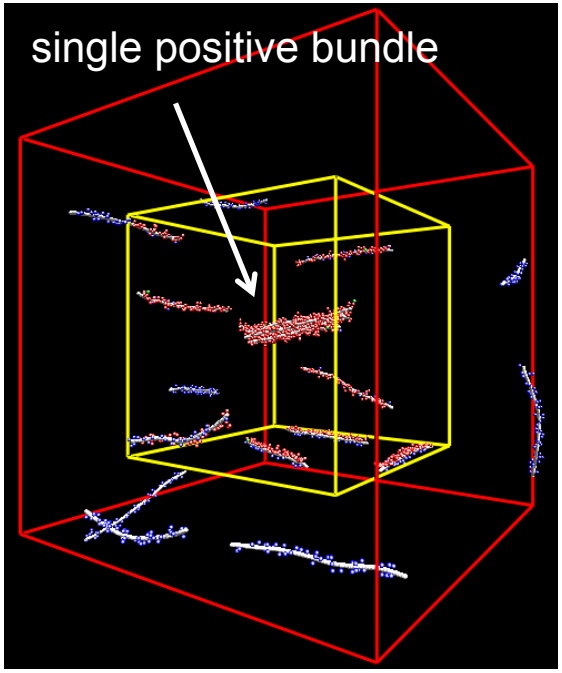
→ MD simulations in DNA excess: $R_{+/-} = 0.5$ $(R_{+/-}^{chb} > 1)$

$(N_{DNA} = 20,\ V_{ch} = V/4,\ R_{+/-}^{chb} = 2)$



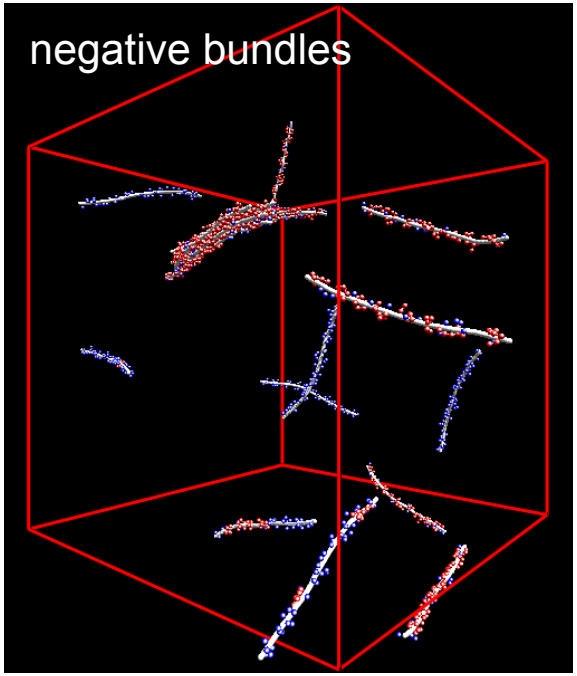
initial state

→ closed

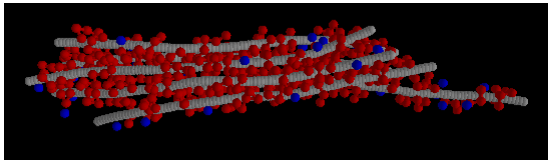



final chamber state ($t^* = 5\ 10^6$)

→ open



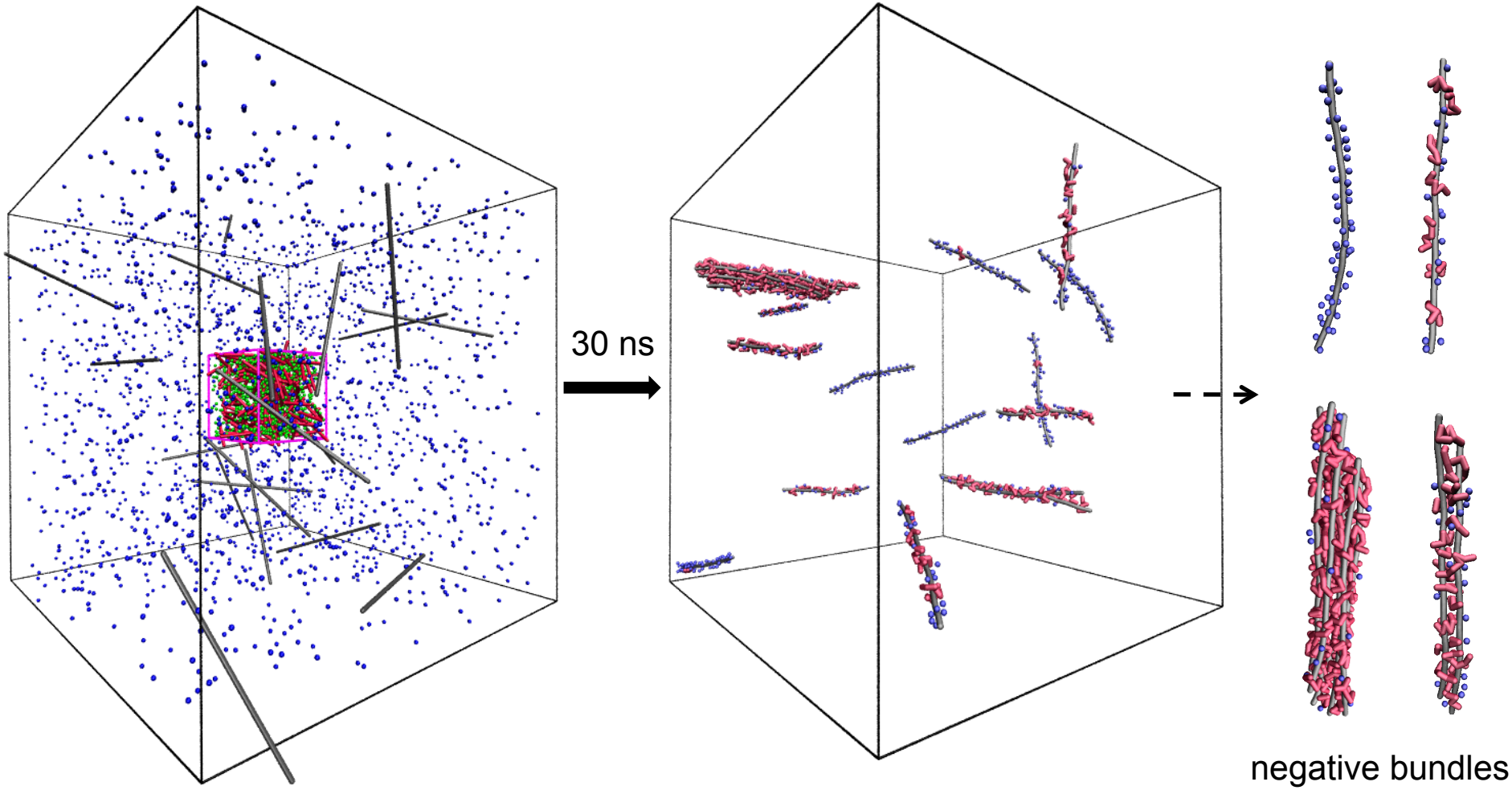

final state ( $t^* = 3\ 10^7$ )



# Mimicking the experiments
# II - Langevin dynamics

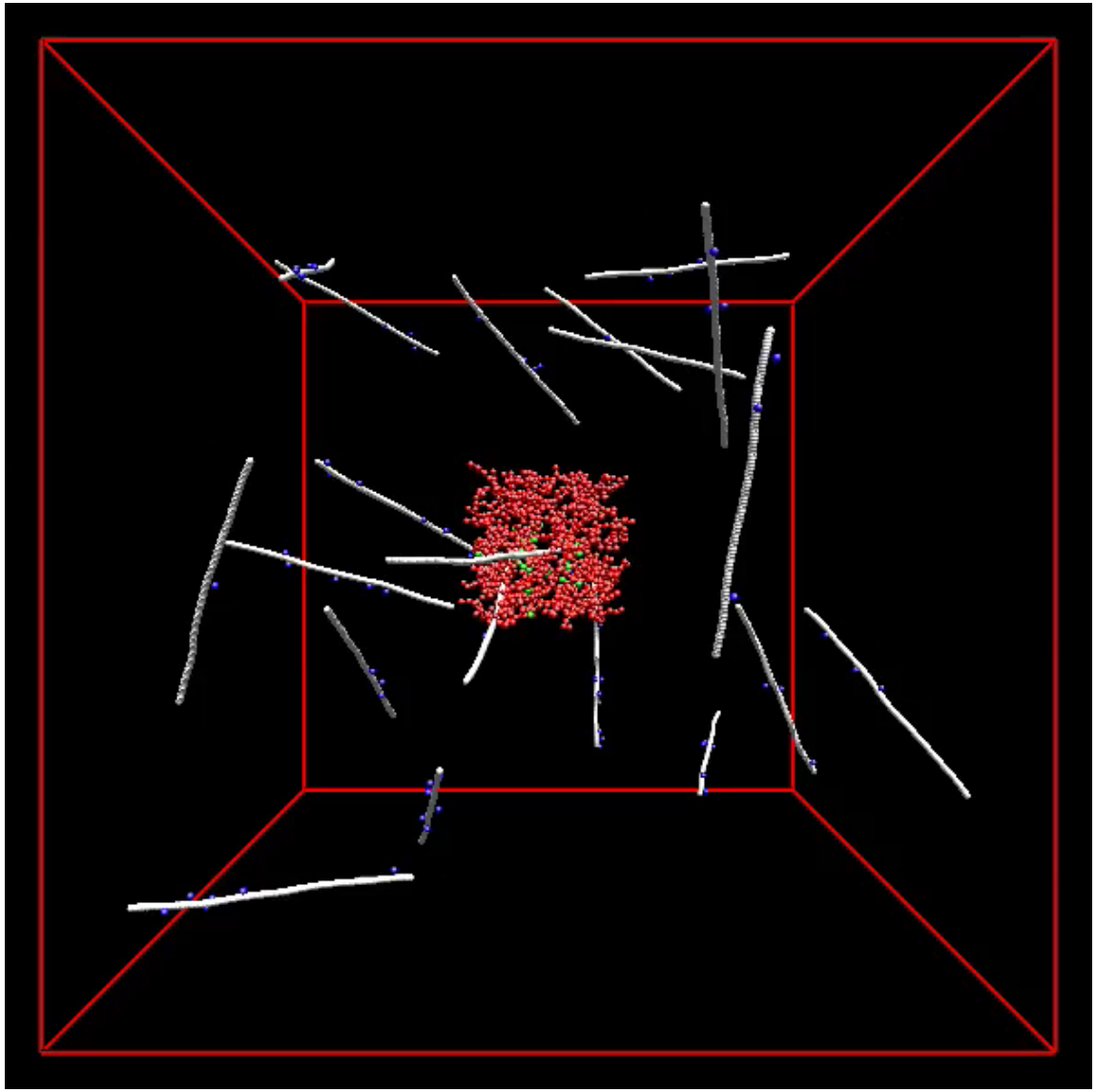
- translational diffusion: $D_{protamine} \sim 10\ D_{146bp}$

$N_{DNA} = 20$, $V_{drop} = V/100$, $R_{+/-} = 0.5$ ($R_{+/-}^{drop} = 50$)



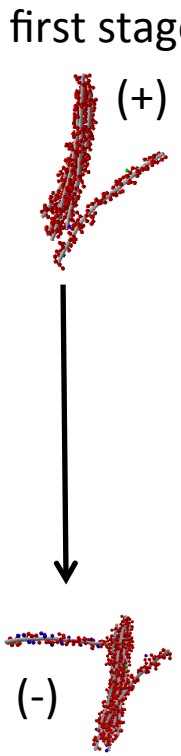
Langevin dynamics: $N_{DNA}$ = 20, $V_{drop}$ = V/100, $R_{+/-}$ = 0.5 ($N_{PRO}$ = 143)

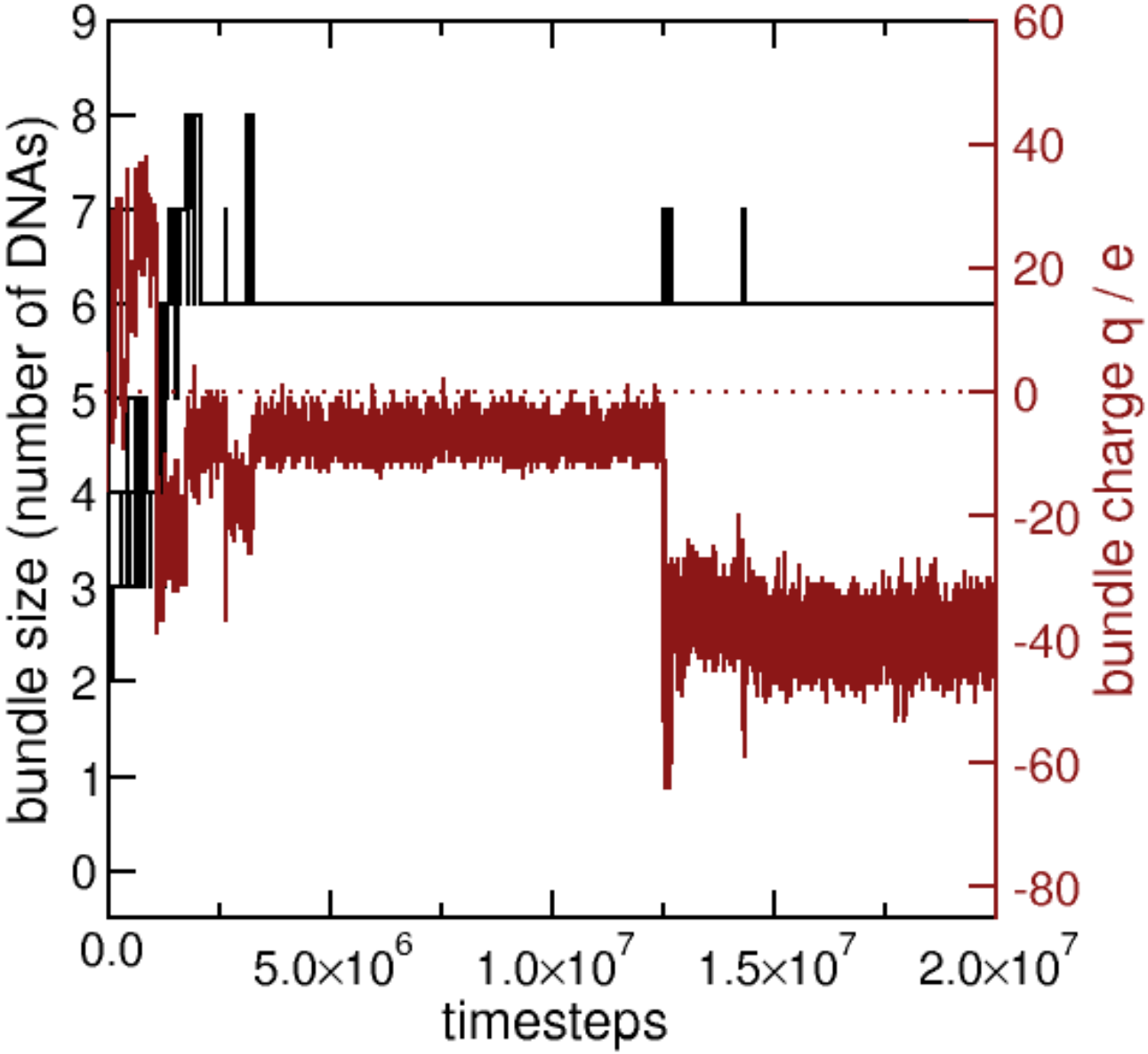
*only bundles and protamines are shown*



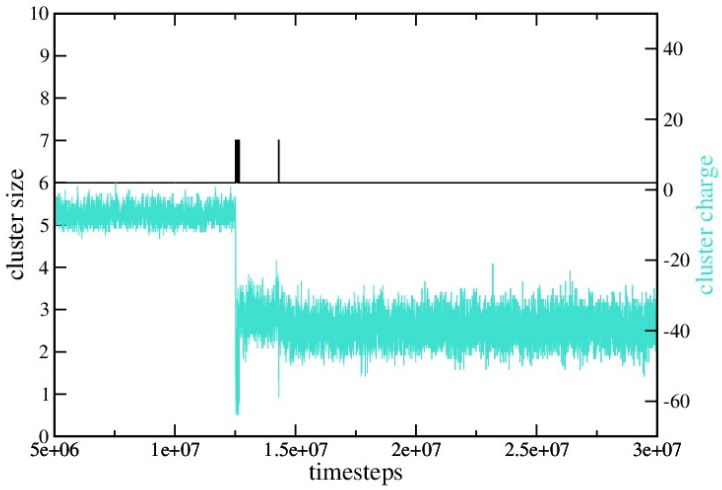
# Time-evolution of the « largest » bundle in Langevin simulation

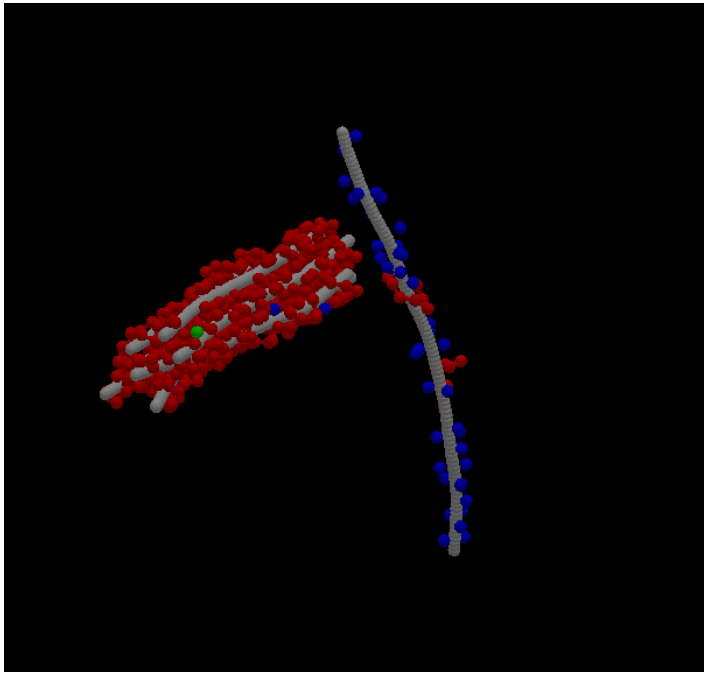
inhomogenous system, R+/- = 0.5 (excess of DNA)

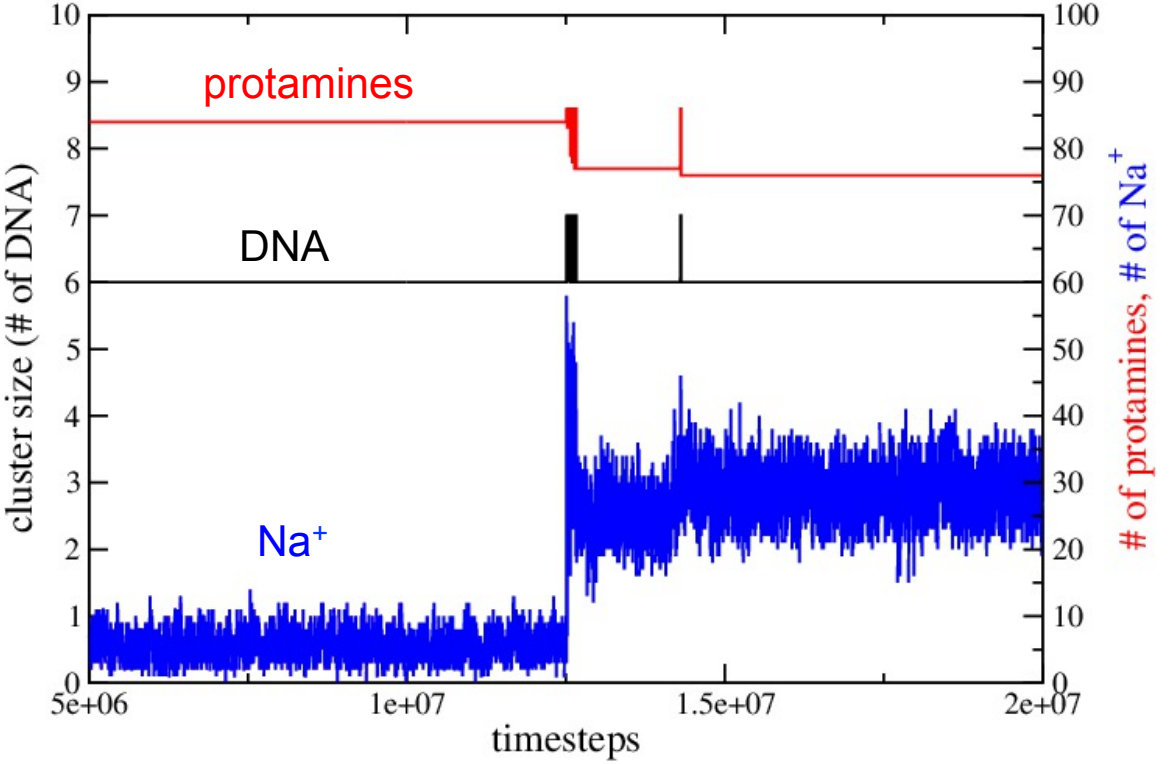


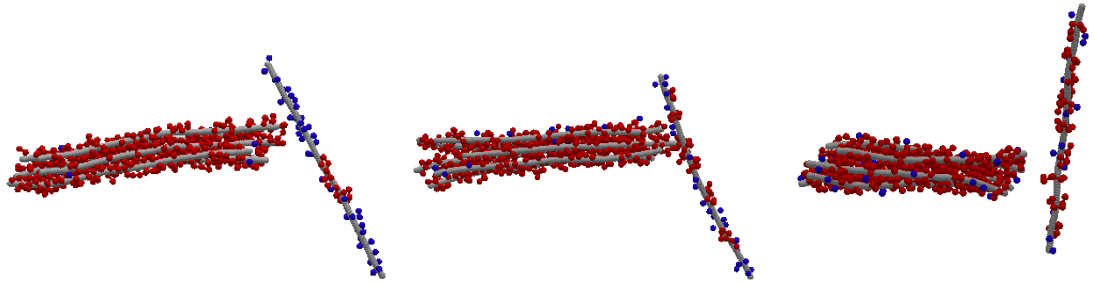



# Kinetics plays an important role in the aggregation





transient aggregation and protamine transfer

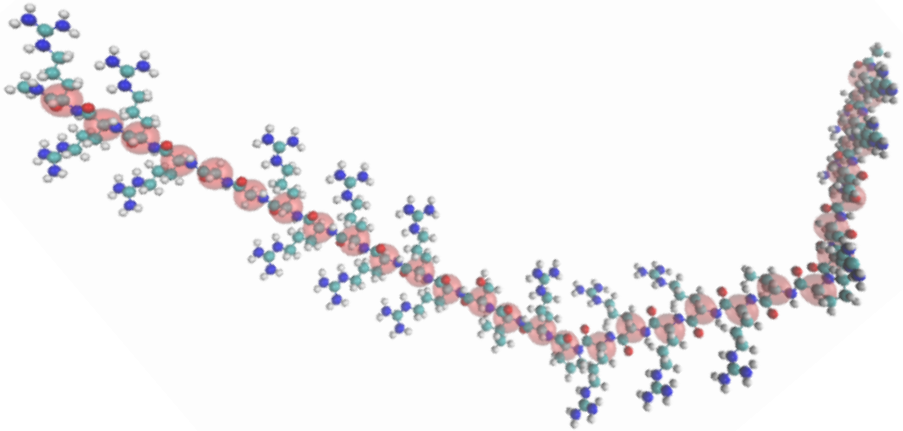
# Summary 1

- Inhomogeneous simulations are in good agreement with experiments
  - ✓ bundles coexist with « naked » DNA for R+/- < 1 (even at low $R_{+/-}$)
  - ✓ bundles coexist with protamines in solution for $R_{+/-} > 1$
  - ✓ bundles are positively charged for $R_{+/-} > 1$, negatively charged for $R_{+/-} < 1$
  - ✓ bundle size in part governed by kinetics (barrier due to electrostatic repulsion)

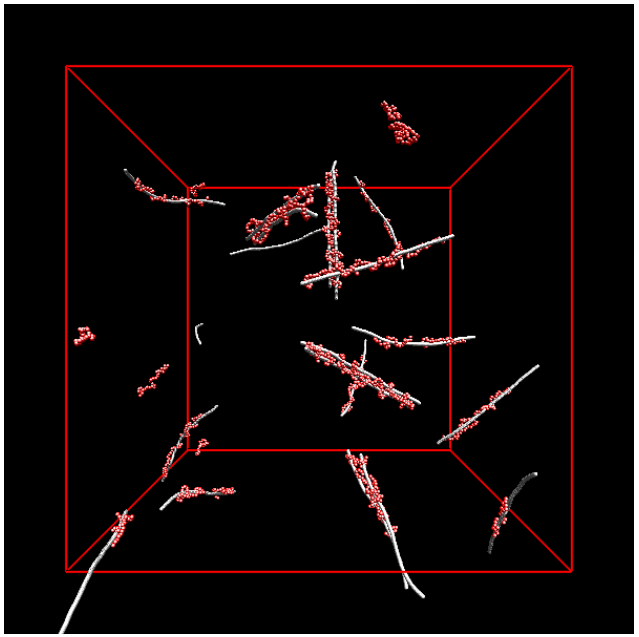
- Bundles are always formed in excess of protamines in the experiments
  - negative bundles are stable due to electrostatic repulsion
  - positive bundles tend to aggregate (protamine & counterions entropy?)

- Route to design soluble charged complex of controlled size?

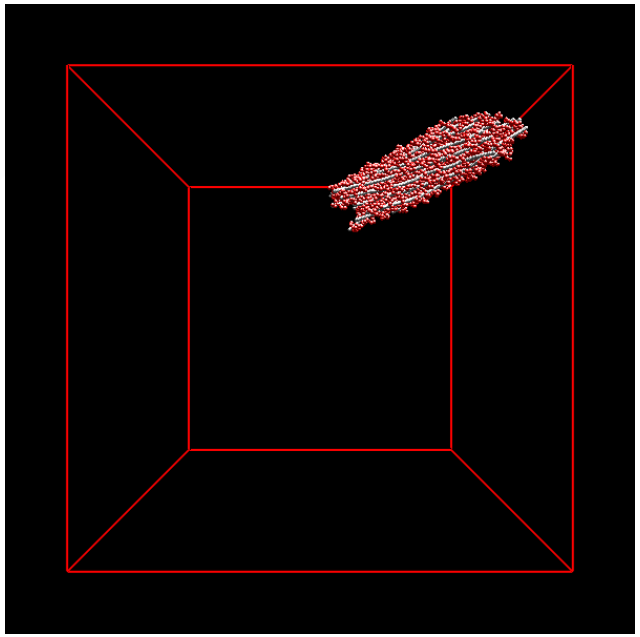
# Importance of the length of the condensing agent (protamine)



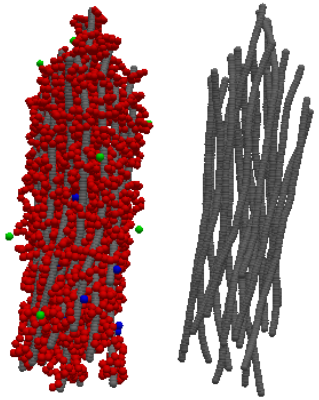
beads on $C_\alpha$ ("arginine beads" are carried a +e charge)



**$R_{+/-}$ = 0.5**



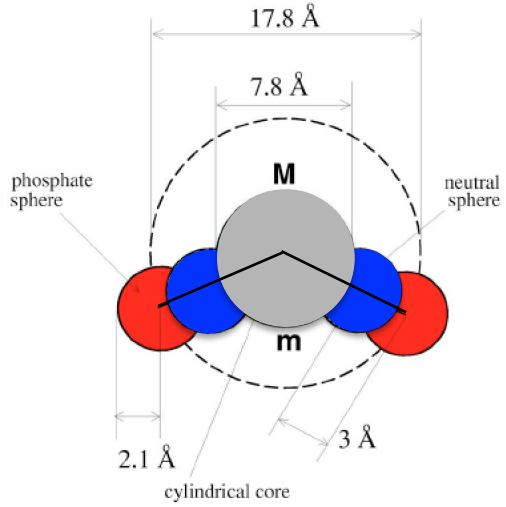
**$R_{+/-}$ = 1.0**

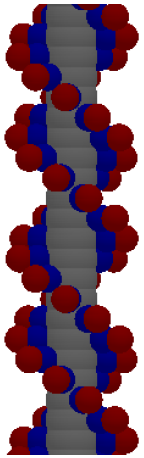


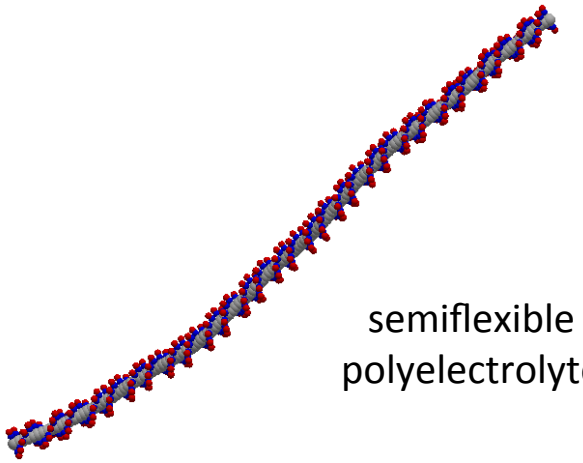
The change in length and charge distribution of the protamine do not seem to modify our previous qualitative findings

A more structurally detailed model but with only steric and electrostatic interactions

DNA : extension of the Montoro-Abascal model *(Gil Montoro & Abascal, J. Chem. Phys. 1995)*

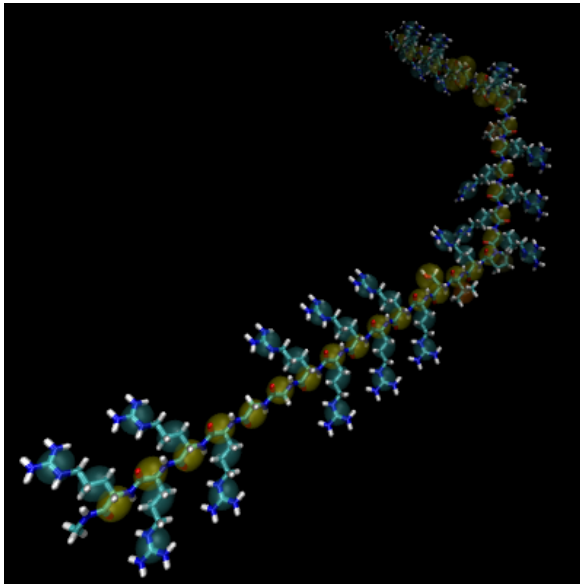


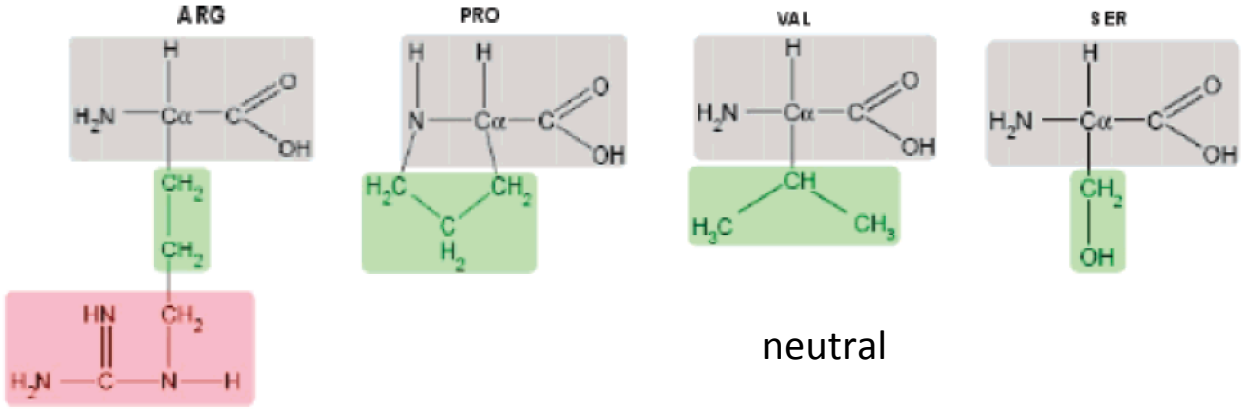





semiflexible polyelectrolyte

Protamine : Inspired by the SCORPION model *(Basdevant et al. J. Phys. Chem. B 2007)*






positive

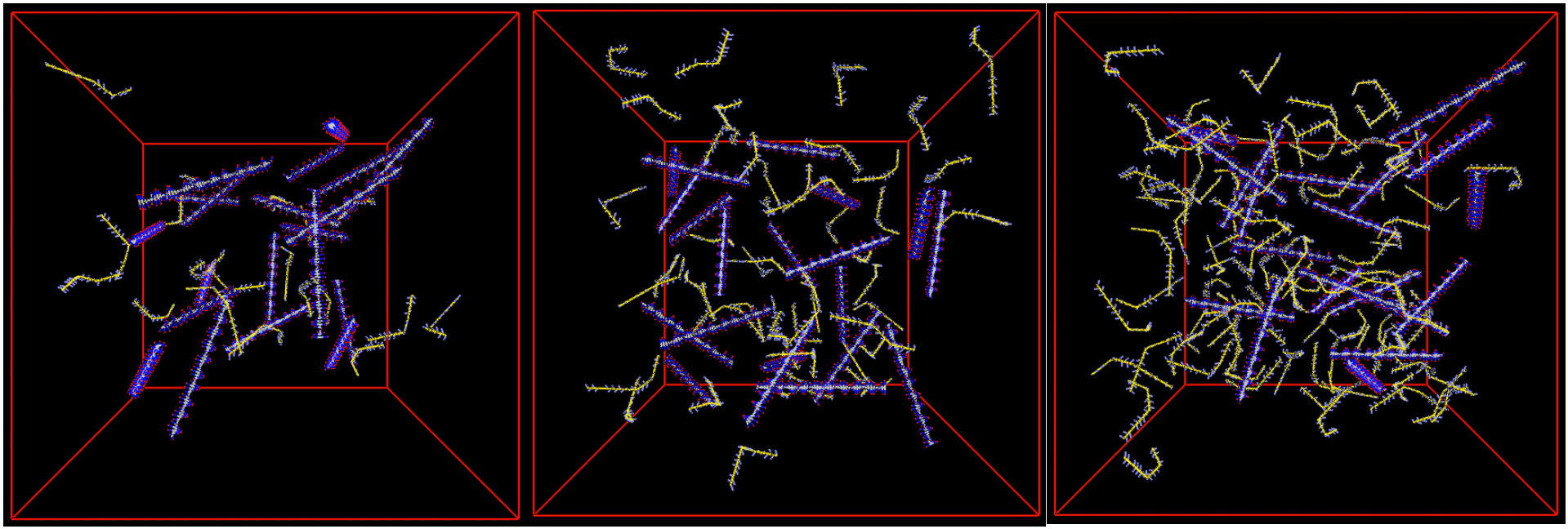
neutral

# 20 DNA (50 bps + protamines) in a 48 nm-side cubic box

## steric and electrostatic interactions and implicit solvent

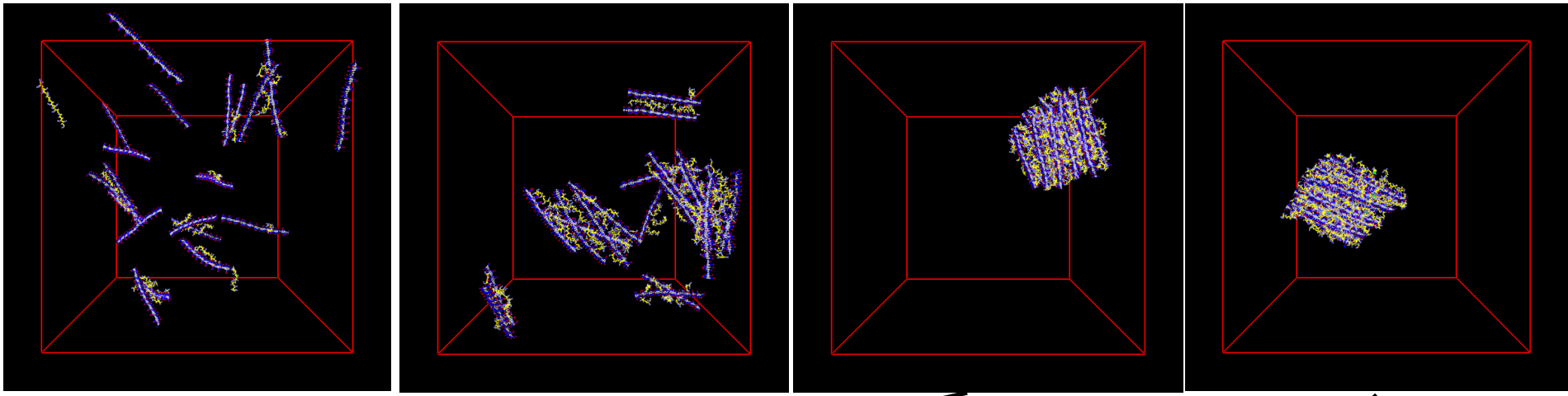
Isotropic initial conditions (counterions not shown, protamines in yellow)

**R = 0.2** **R = 0.5** **R = 1.0**

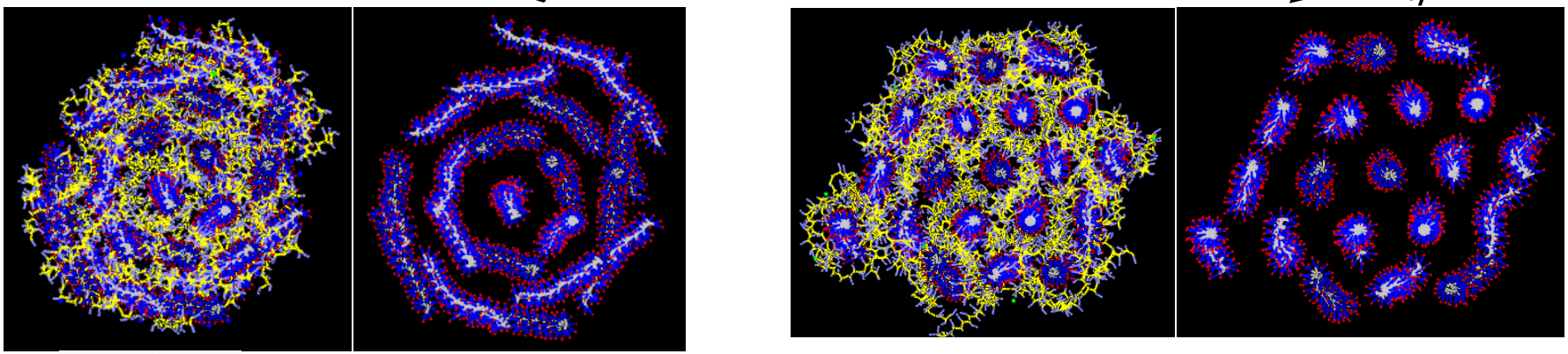


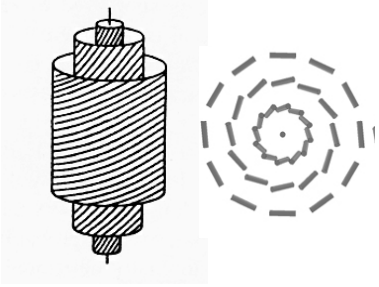
- Self-assembly into bundles of "significant size" occur at lower $R_{+/-}$
- Single bundle formation at $R_{+/-} = 0.8$ (precipitation around this value in experiment)
- No significant aggregation at $R_{+/-} \leq 0.2$

$R_{+/-} = 0.2$ $R_{+/-} = 0.5$ $R_{+/-} = 0.8$ $R_{+/-} = 1.0$

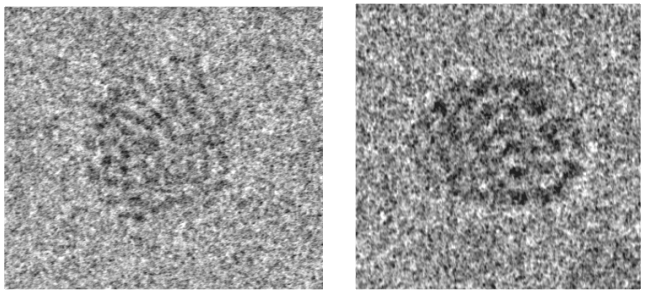


$R_{+/-} = 0.8$ $R_{+/-} = 1.0$





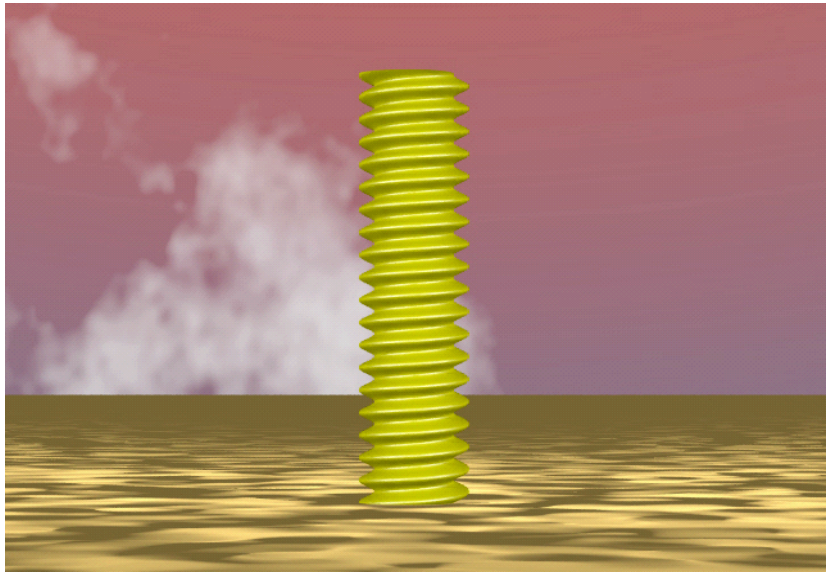
double-twist cylinder

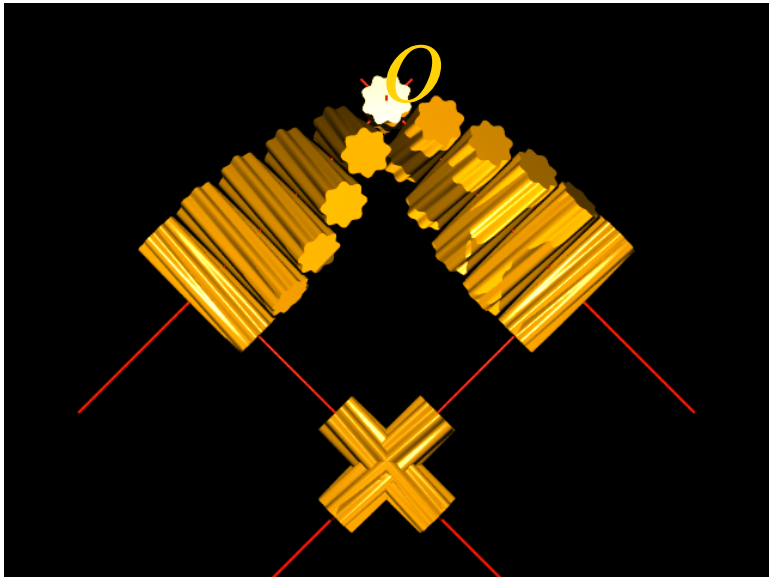


Excess of DNA
(J. Degrouard, F. Livolant)

Grason *et al.*, PRL, 99 (2007); PRE 79 (2009)

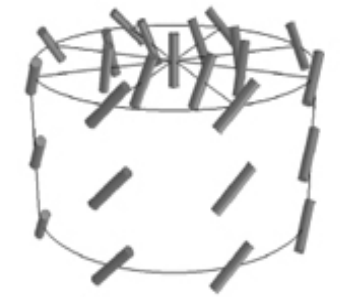
# Dense phase made of chiral molecules

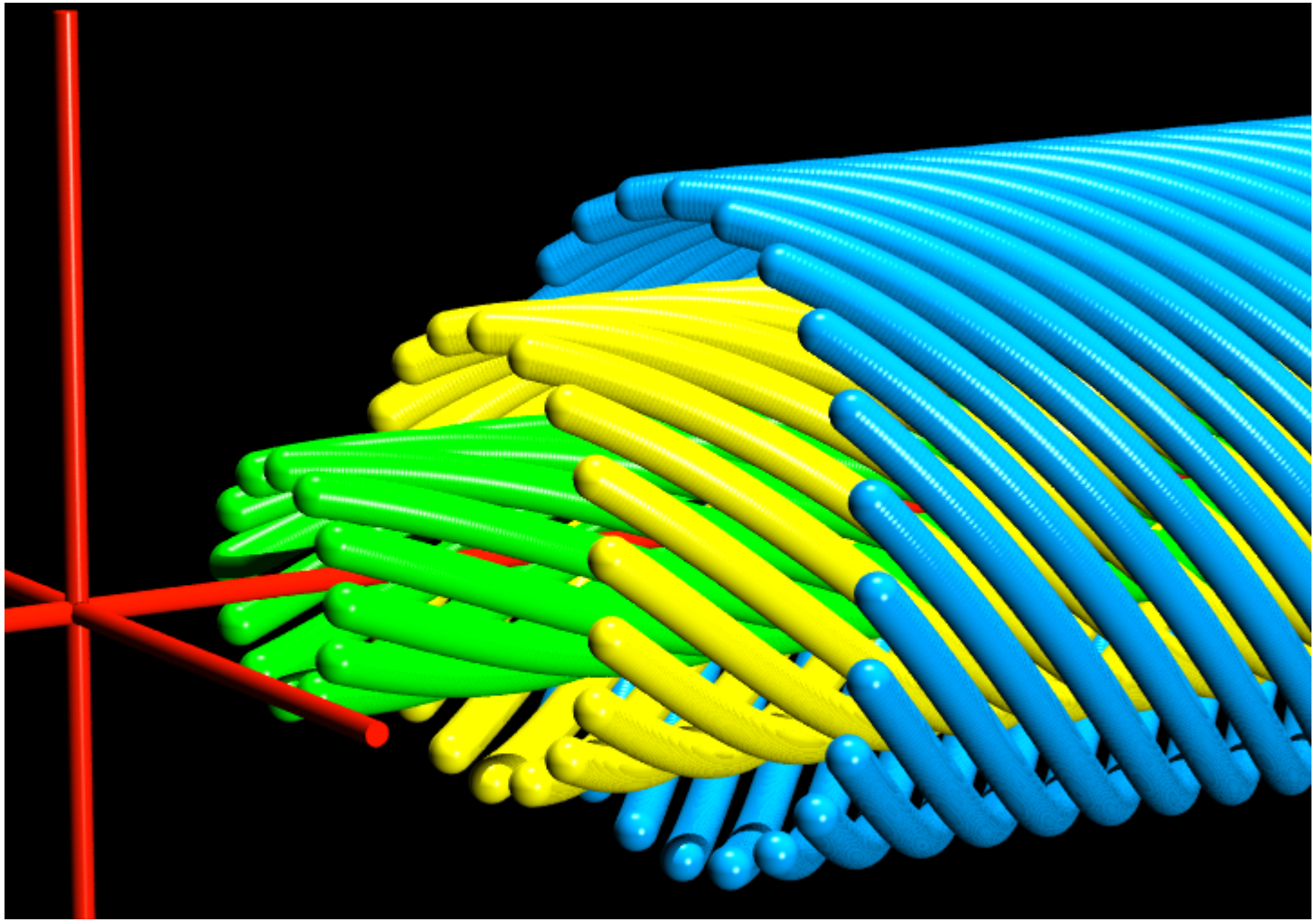




Molecules are trying to find a compromise

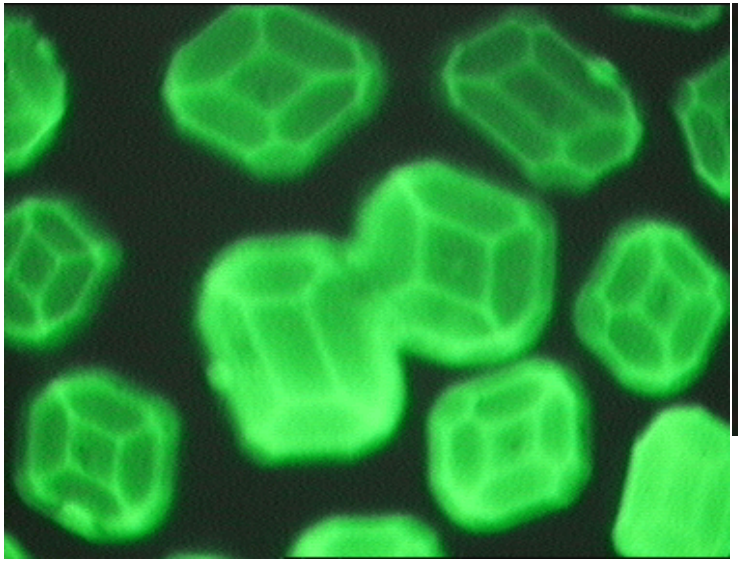
# A solution: the double-twist cylinder

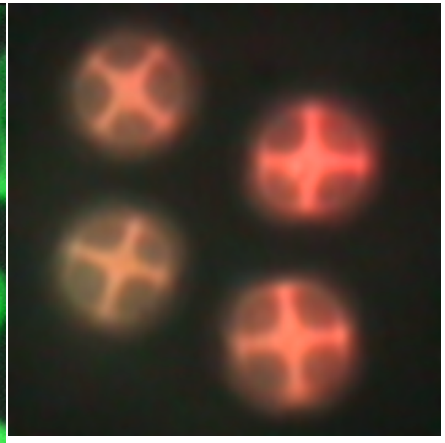




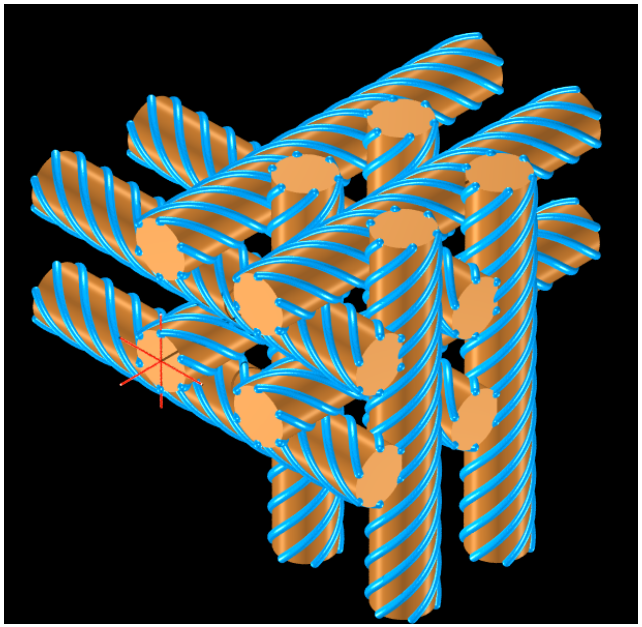
# Self-assembly of double-twist cylinder = blue phases

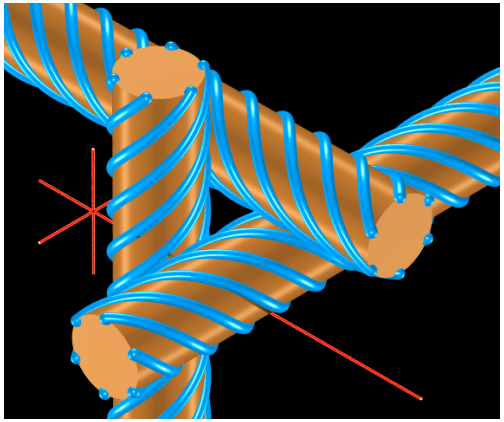
*Liquid crystal phases with cubic symmetries*

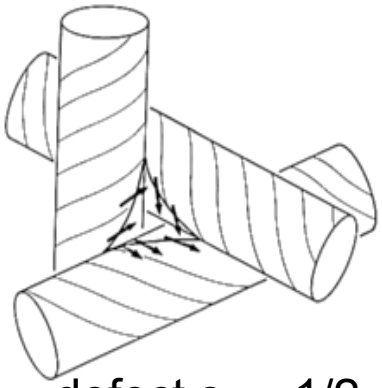




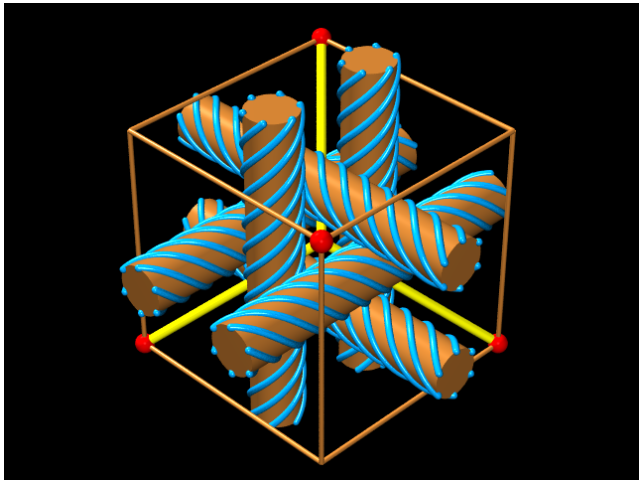
(B. Pansu;
R. Barbet-Massin
University Paris 11
Orsay, France)





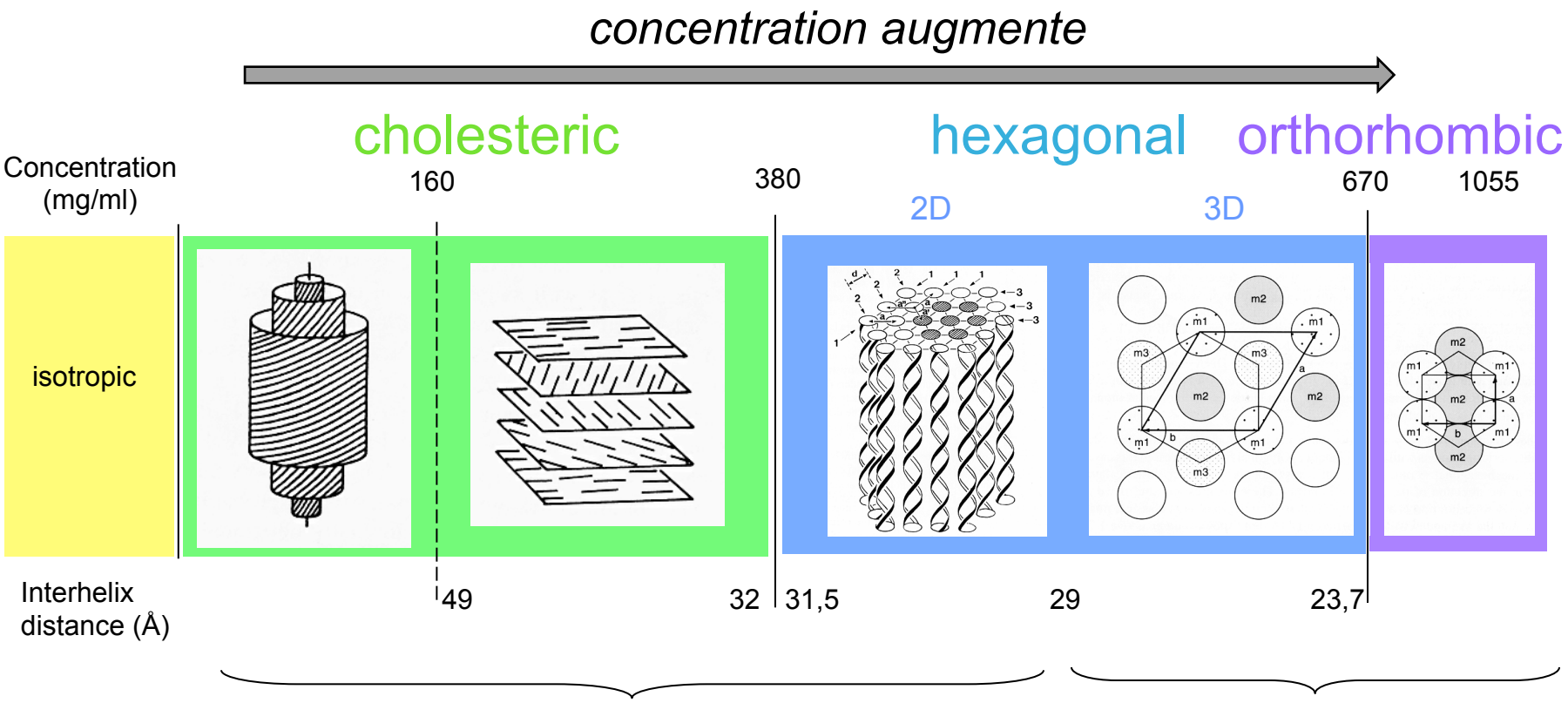


defect s = -1/2



# DNA in solution (50nm fragments)

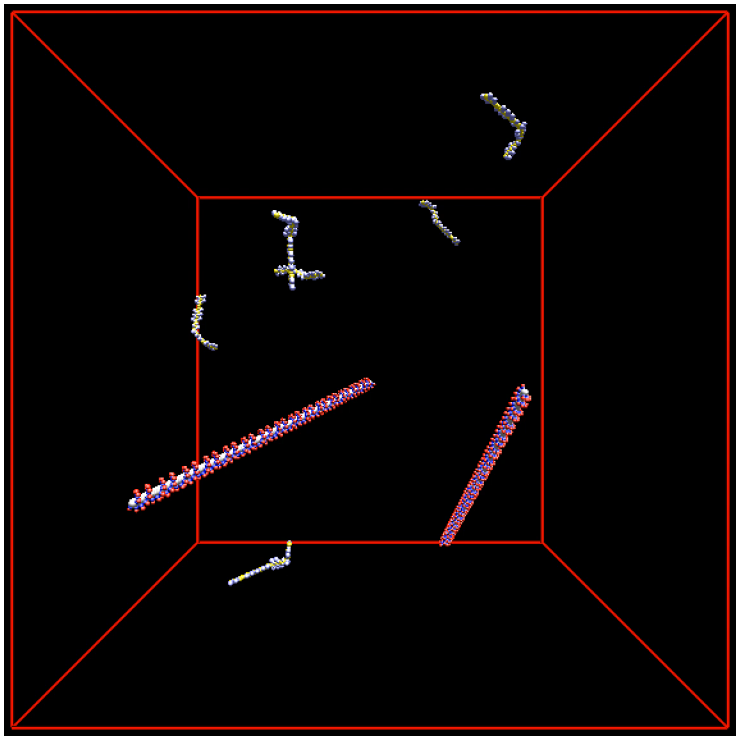
*Robinson C. (1961, 1966); Luzzati & Nicolaieff (1959, 1963)*

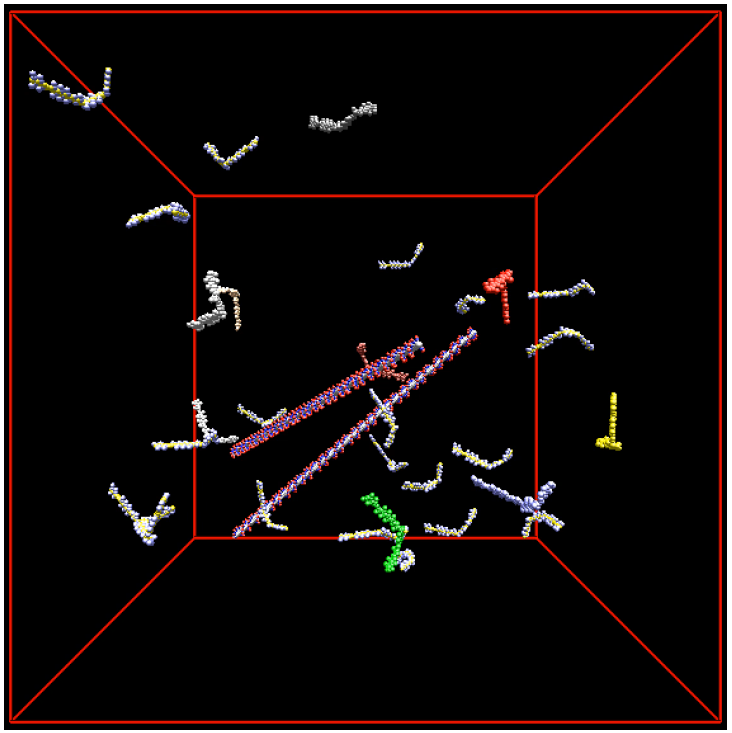


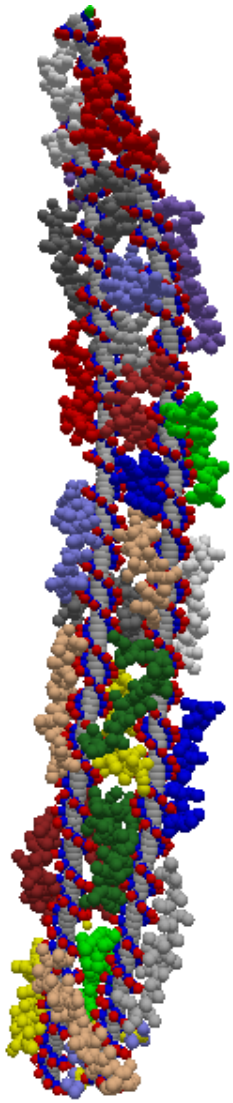

*(Durand, Doucet, Livolant, Orsay, France)*

# A pair of DNA (150 bps + protamines) in a 65 nm-side cubic box

steric and electrostatic interactions and implicit solvent

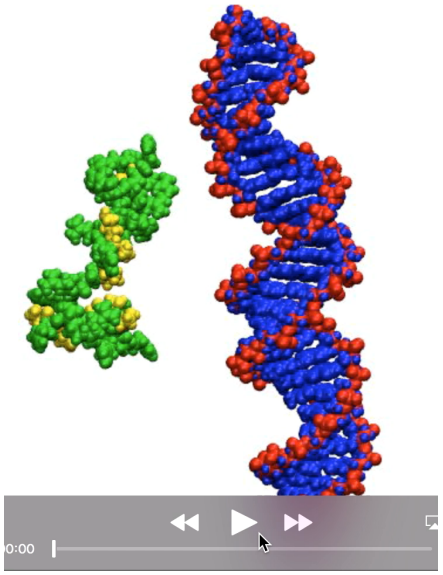






Results seem to agree with the same model for shorter DNA (but density is lower here)

# Summary



- Models at different coarse-grain levels
(polybeads / modified Montoro-Abascal / Martini model / all-atoms)

- Self-assembly in dilute or concentrated systems with long-range electrostatics (e,g. LC phases, crowding) in presence of different types of condensing agents

- Qualitative behavior of the DNA-protamine system for a given model

- Self-assembly in strongly charged systems (e.g. DNA – protamine) poorly understood especially in dilute regime

  - Nature of the polyelectrolyte (e.g. charge, persistence length) and of the condensing agents, effect of added salt, concentration, etc

  - Improve statistics on self-assembly (optimized electrostatic; MC, MD, Langevin/BD coupled to KMC)

  - Going beyond the mechanistic stability → free energy of bundle formation? (stable or kinetically-trapped bundles? size? etc)


Thank you very much!