Simulation of cyclic and linear DNA chains moderately and strongly confined in nanochannels

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Motivation

Recent developments in **nanofluidic devices** fabricated by chip lithography provide a new impetus to study the confinement of principal biopolymers such as DNA [1]. Confining and stretching in nanoscale channels is used for sorting fragments of dsDNA of different length [2].

extended chains in arrays of channels,
free ends dangling out, or folded chain ends in channel

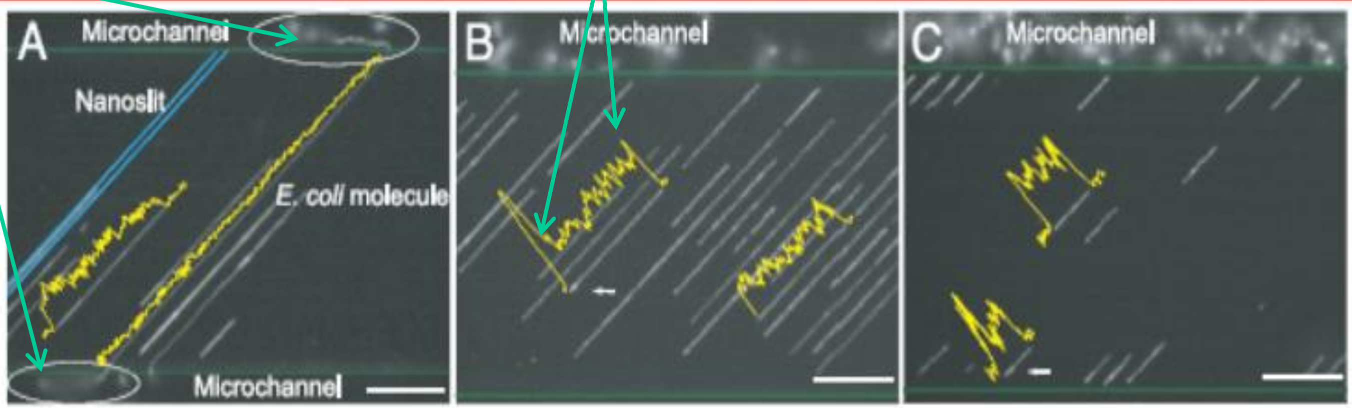
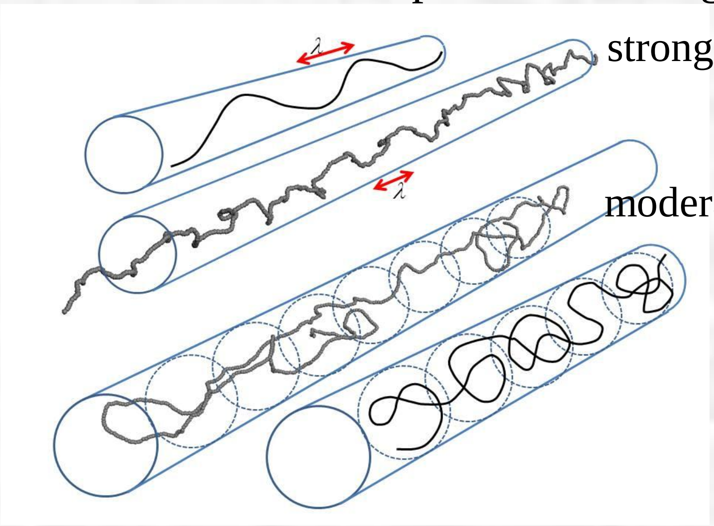


Fig. 2. Gallery of fluorescence micrographs shows stretched and relaxed DNA molecules within the nanoslit device after electrokinetic loading; images were taken a few minutes after the electric field was shut off. (*A*) A large *E. coli* DNA molecule spans across the 105-μm-long nanoslit (0.01× TE buffer) showing relaxed ends (circled) within abutting microchannels. (*B*) T4 DNA (166 kb) molecules; 0.05× TE. (*C*) λ DNA (48.5 kb) molecules; 0.01× TE. Green lines demarcate nanoslit–microchannel interfaces; blue indicates a nanoslit, and yellow lines show integrated fluorescence intensity profiles revealing folded ends (*B* and *C*, arrows). Relaxed molecules within the microchannel regions appear as diffuse, partly out-of-focus, fluorescent balls, whereas stretched molecules present as long, linear objects. (Scale bars, 20 μm.) Jo, Forrest et al, PNAS, 2007, 104, 2673

[1] Reisner, W. W. et al. *Phys. Rev. Lett.* **2005**, *94*, 196101. [2] Douville, N.; Huh, D.; Takayama, S. *Anal. Bioanal. Chem.* **2008**, *391*, 2395.

Properties of dsDNA in nanochannels deviate from their values in bulk, depend on confinement. To rationalize the DNA single molecule measurements experiment ought to be compared with the polymer physics theories and molecular simulations [3-5]. Mostly **linear macromolecules** were investigated in linearisation experiments though the **ring DNA** is also populated in biological systems.

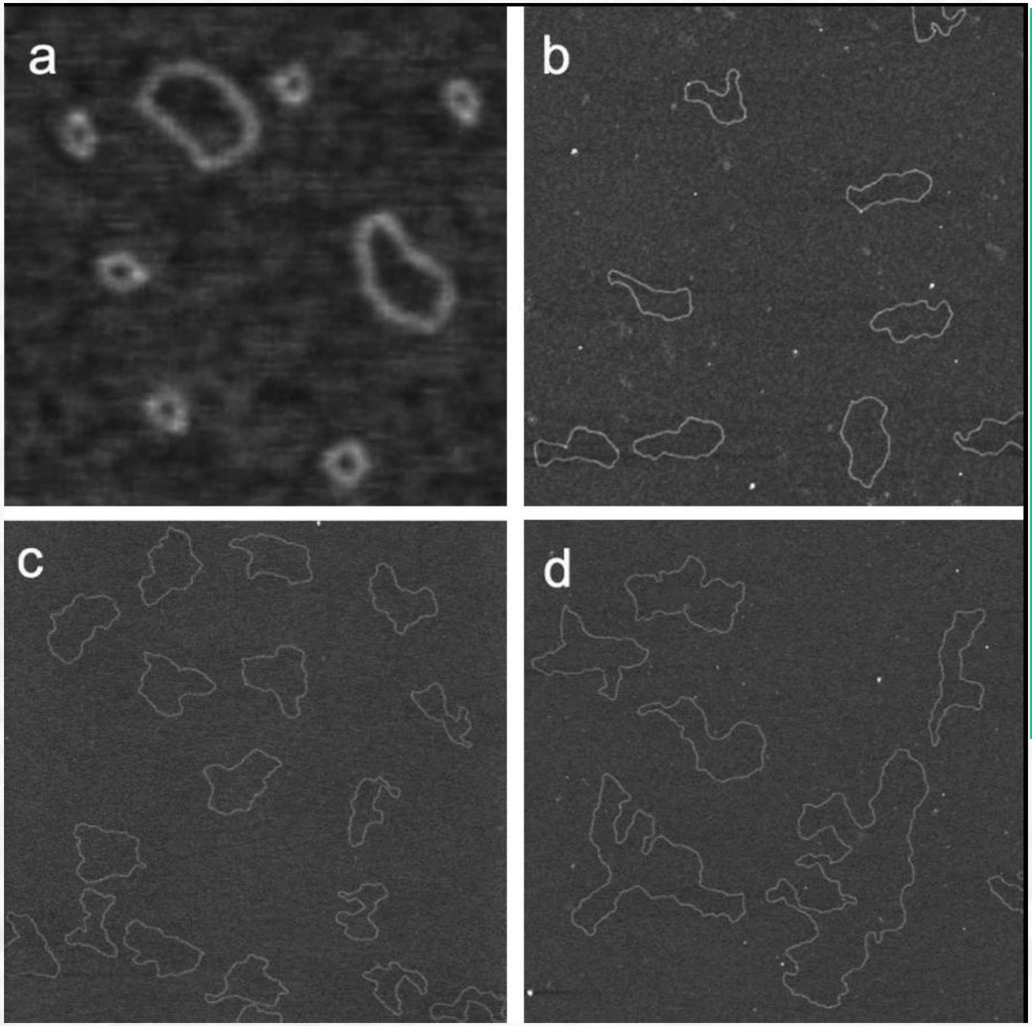


Constraints due to the chain closure in combination with geometrical constraints of a DNA molecule are inevitable for many biological processes. First experiments [6] and simulations [5] were reported for channel-confined rings and point to a modified behavior with respect to the linear chains.

Objective: Comparison of linearisation of ring and linear DNA macromolecules in nanochannels over the moderate and strong confinement regimes

[3] Cifra, P.; Benková, Z.; Bleha, T. *Faraday Disc.* **2008**, *139*, 377. [4] Cifra, P.; Benková, Z.; Bleha, T. *J. Phys. Chem. B*, **2009**, *113*, 1843. [5] Benková, Z.; Cifra, P. Macromolecules, **2012**, *45*, 2597. [6] Persson, F.; Utko, P.; Reisner, W.; Larsen, N. B.; Kristensen, A. *Nano Lett.* **2009**, *9*, 1382.

Semiflexible ring chains



G. Witz, K. Rechendorff, J. Adamcik, G. Dietler, *Phys. Rev. Lett*. **101**, 148103 (2008).

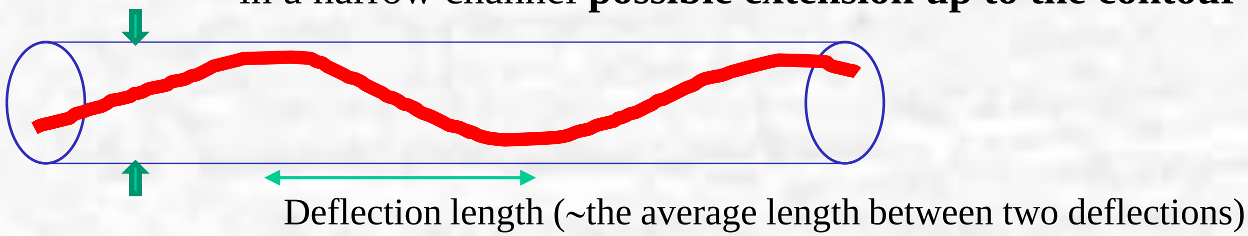
Relatively short loops of few persistence lengths: important role in many biological regulatory functions, such as loop regulated transcription of DNA, or in circular chains such as plasmids, used in a gene therapy.

Now rings also analysed in nanochannels

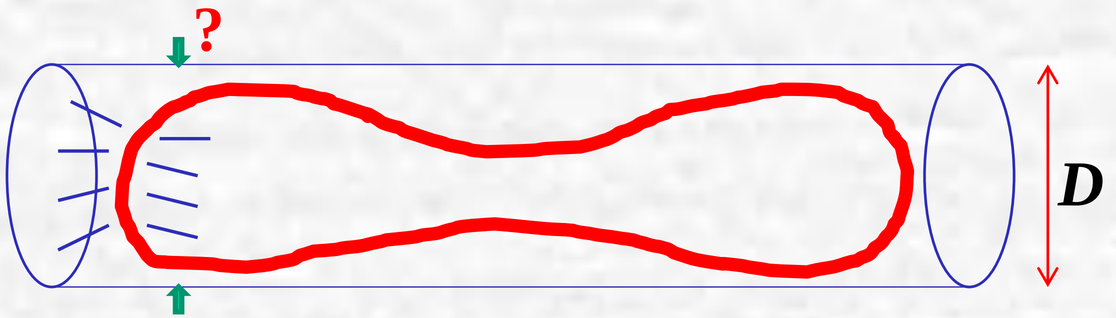
AFM snapshots of cyclic DNA, plasmids on Mg^{2+} mica surface

Strong confinement of a linear stiff macromolecule in channel

in a narrow channel **possible extension up to the contour length**



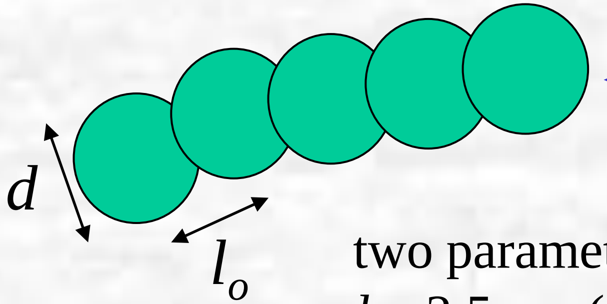
Strong confinement (***D*** < ***P***) for semiflexible rings?

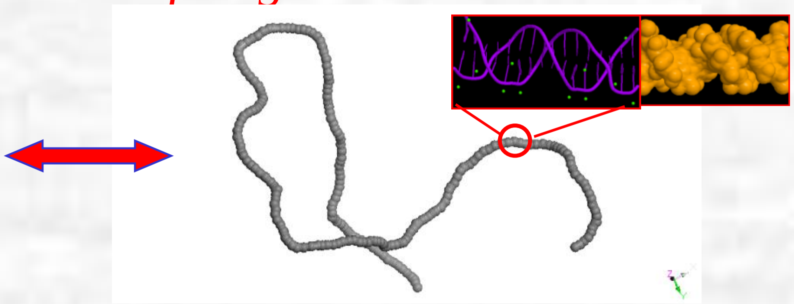


- Does the strong confinement regime exist for semiflexible rings?
- Is there a transition between the moderate and strong confinement? Where it would be situated compared to linear chains?
- Experimental results [6] for rings exist only for the moderate confinement

Coarse-grained Model

Bead–spring wormlike chain model of dsDNA





two parameters of the model:
d = 2.5 nm (7.4 base pairs)
P = 50 nm (the persistence length of dsDNA at high salt concentration)

Monte Carlo simulation

Metropolis method with the energy $U = U_F + U_M + U_b$ at $T > T_\theta$

U_F - bond length variations (FENE potential)

U_M - nonbonded repulsive interactions of beads

$U_b = b(1-\cos\phi)$ -bending energy, -the stiffness parameter $b = P/l_0$,-the complementary valence angle ϕ

Ring chain lengths N=300 beads (the contour length L) 0.75μm
linear chains up to 5 μm

long-runs, 9 x 10^7 configurations, MC updates

Channels: hard-wall interactions of a chain with the walls of the cylindrical channels, variation of the channel diameter down to D/d=6.8, i.e. to the width D=17 nm.

Results

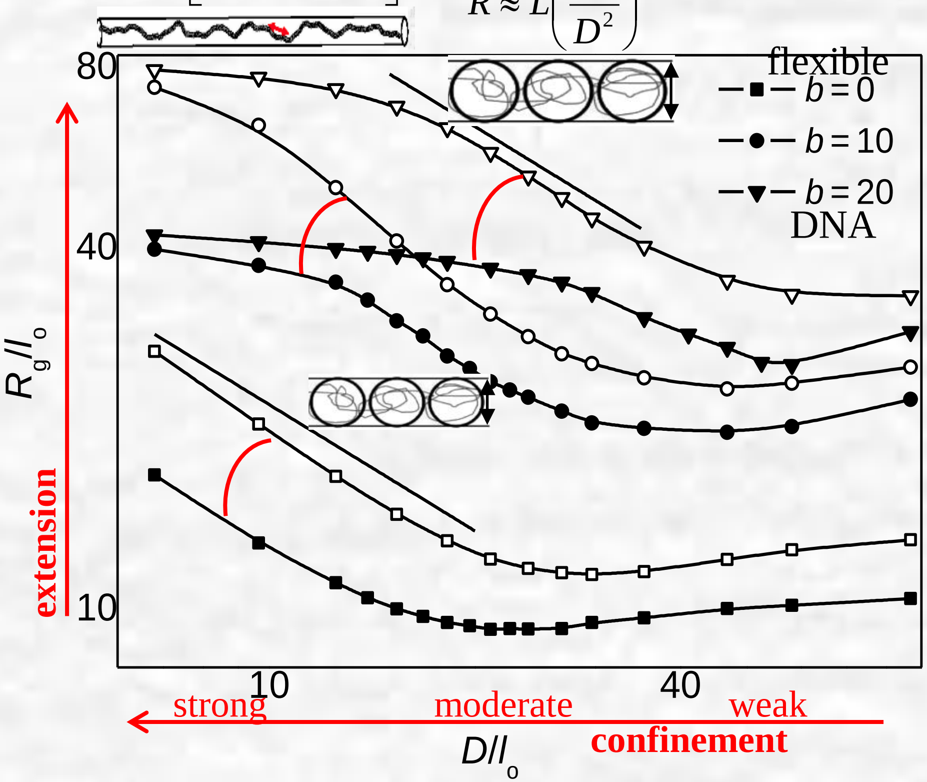
Mean span of linear and cyclic chains along channel

Odijk regime

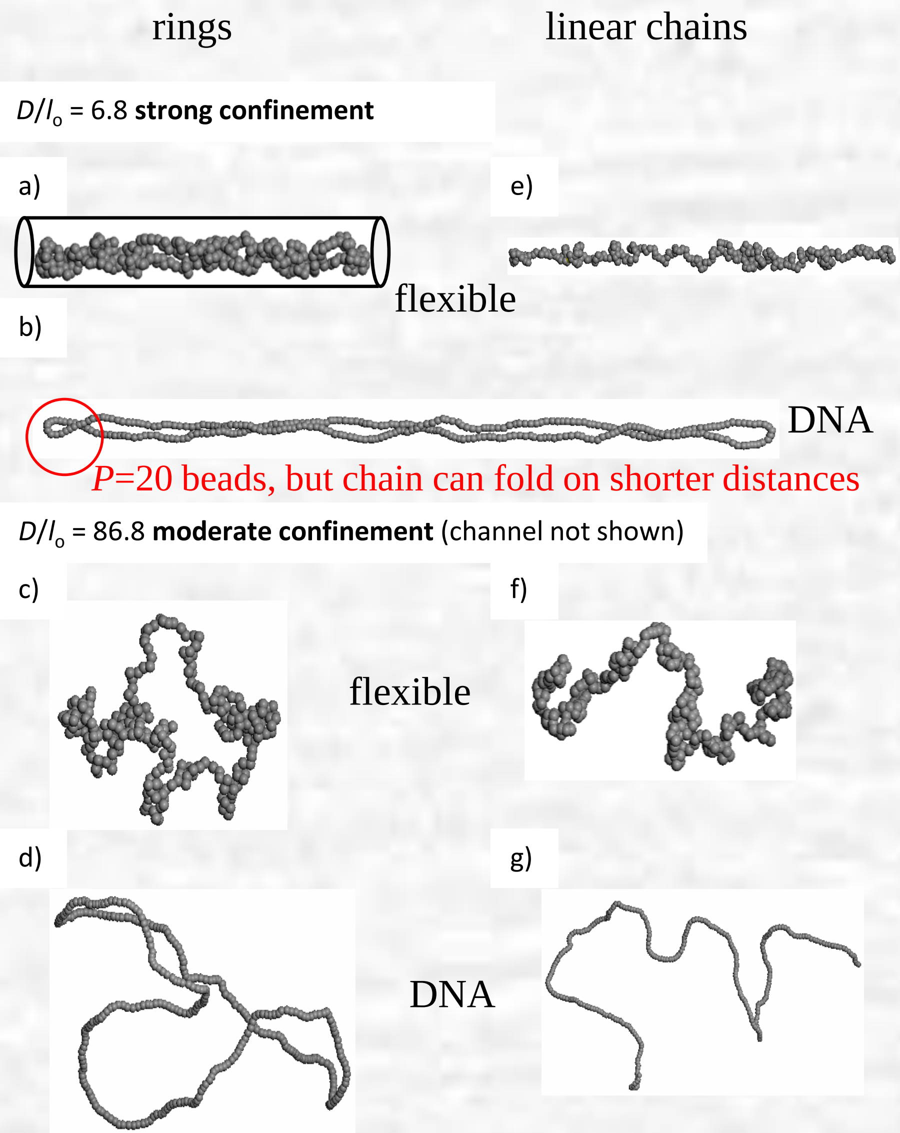
$$R = L\left[1 - A\left(\frac{D}{P}\right)^{2/3}\right]$$

blob (de Gennes) regime

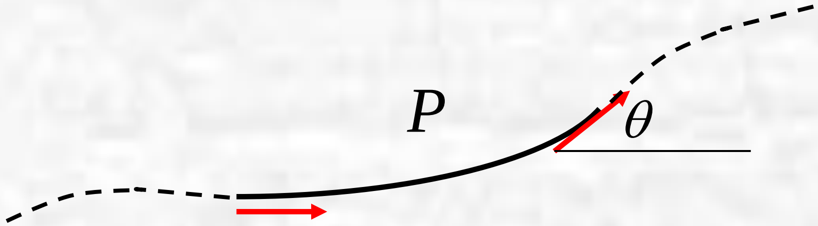
$$R \approx L\left(\frac{Pd}{D^2}\right)^{1/3}$$



rings (solid symbols)
linear (open symbols)



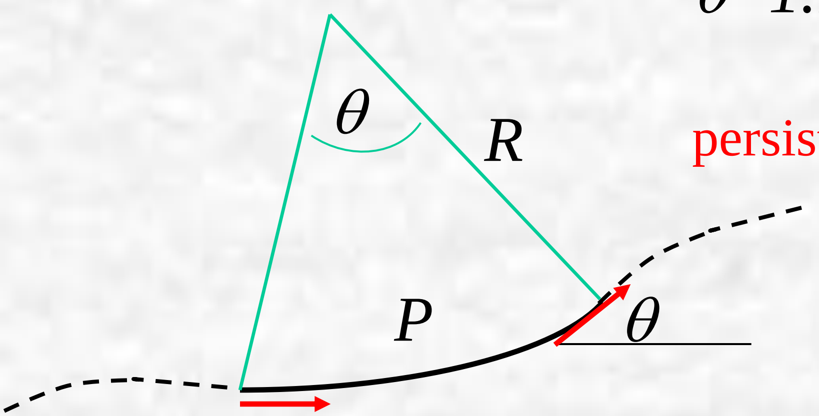
Within one persistence length P the chain is not completely rigid. This allows for semiflexible rings to reach strong confinement regime



Wormlike chain: $\langle \mathbf{u}(r)\mathbf{u}(0)\rangle = \cos(\theta) = \exp(-r/P) = e^{-1}$

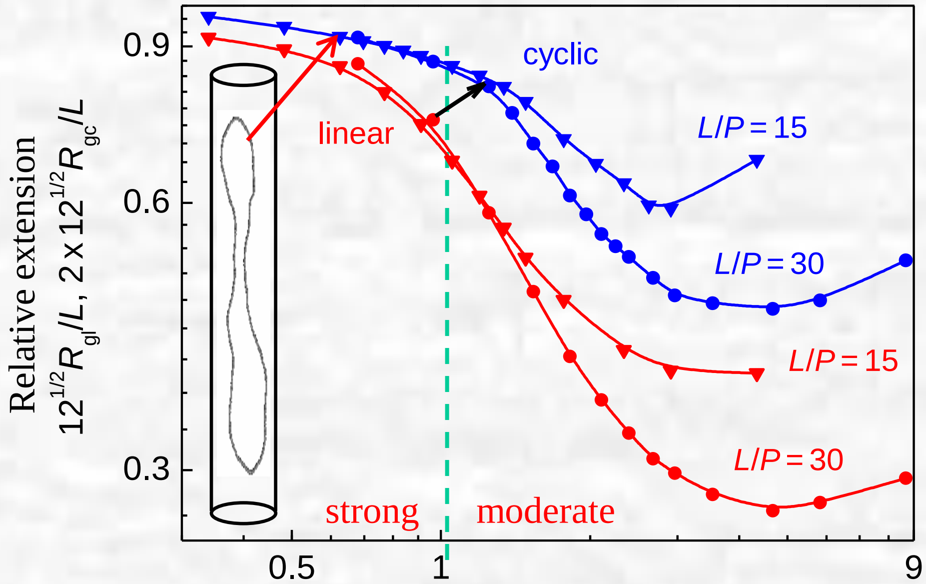
$$\cos(\theta) = 0.368, \theta = 68^{o} = 1.194 rad$$

$$\theta = 1.19\ rad = P/R$$

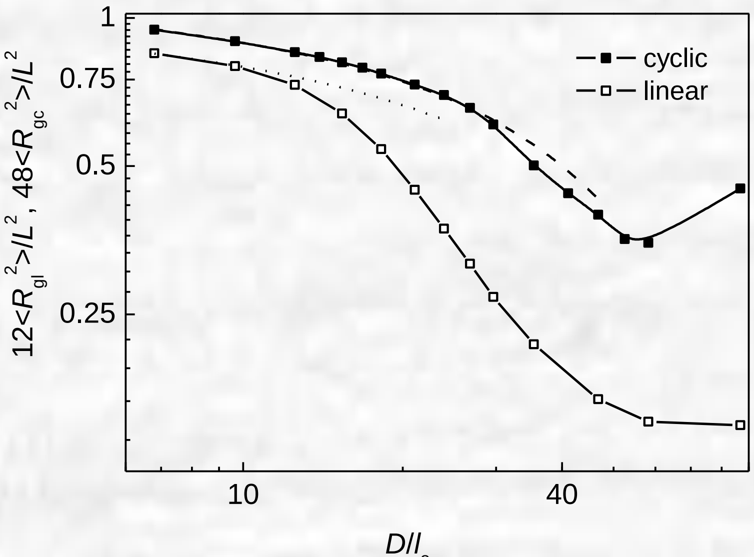


persistence length $P \cong$ radius of curvature R

Differences due to chain topology



Relative confinement D/P
(channel width/chain persistence length)



- **transition** between the strong and moderate confinement known for linear chains (at $D/P = 1$) **exists also for rings**
- strong confinement regime is wider for cycles
- relative extension is larger for cycles than for linear chains
- decay rate in moderate confinement is weaker for cycle, as reported also from experiment [6]

Both larger extension and earlier onset of strong confinement regime on increasing confinement results from **stronger self-avoidance in confined cycle (doubled local density in confined cycle)** (noticed already by Ha et al. Soft Matter, 2012, 8, 2095 and Sheng, Luo, Soft Matter, 2012 $R_{II,r} / R_{II,l} = 0.561$)

Instead of original Odijk relation for end-to-end chain extension of linear chains along channel

$$R = L\left[1 - A(D/P)^{2/3}\right]$$

analogous relation applies for rings for radius of gyration R_g

$$R_{g,cycle} = \frac{L}{2\sqrt{12}}\left[1 - A(D/P)^{2/3}\right]$$

Structure

Dependence of **orientation correlations** $<\boldsymbol{u}_i\boldsymbol{u}_j>$ on the segment separation n along the chain backbone for **ring** (black lines) and **linear** (gray lines) chains of indicated stiffness parameters b confined in a **narrow cylindrical channel**

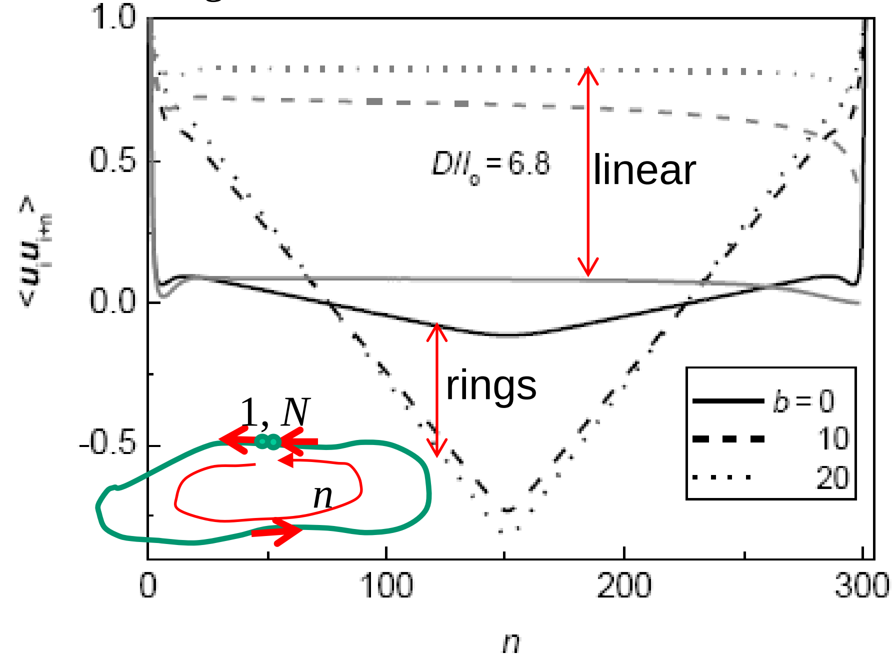
- Initial (local) decay related to P *as*

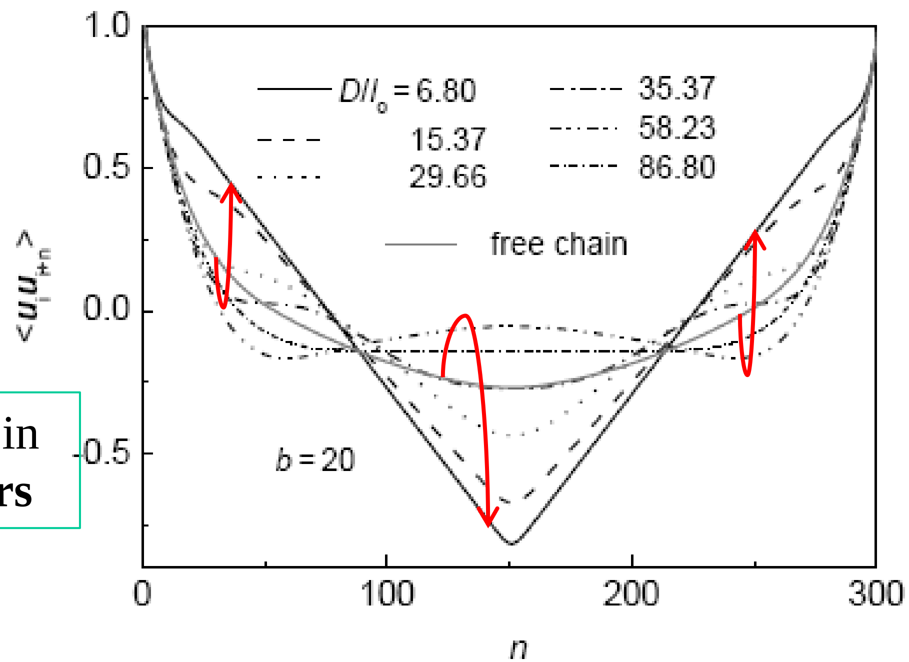
$$\langle \boldsymbol{u}_i \boldsymbol{u}_j \rangle = \langle \cos\theta_{ij} \rangle = \exp\left[-\frac{|i-j|\langle l \rangle}{P} \right]$$

Globally differences between ring and linear chain

- **Minimum** due to the U-turn in ring, sharper minimum for stronger confinement
- **Recovery of correlations** (only) for ring due to the circular topology

Linear *vs* ring chain in narrow channel

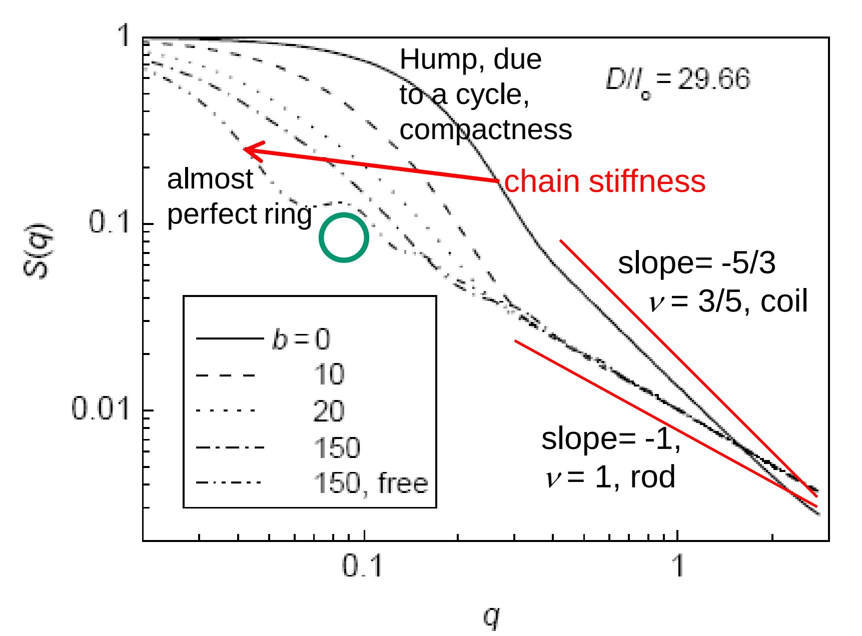




Ring DNA-like chain (b=20) in channels of **various diameters**

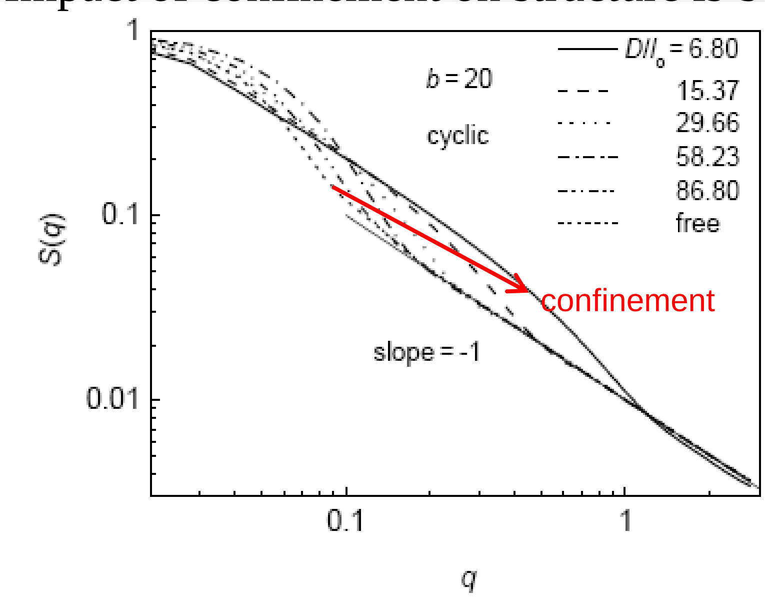
Form factor

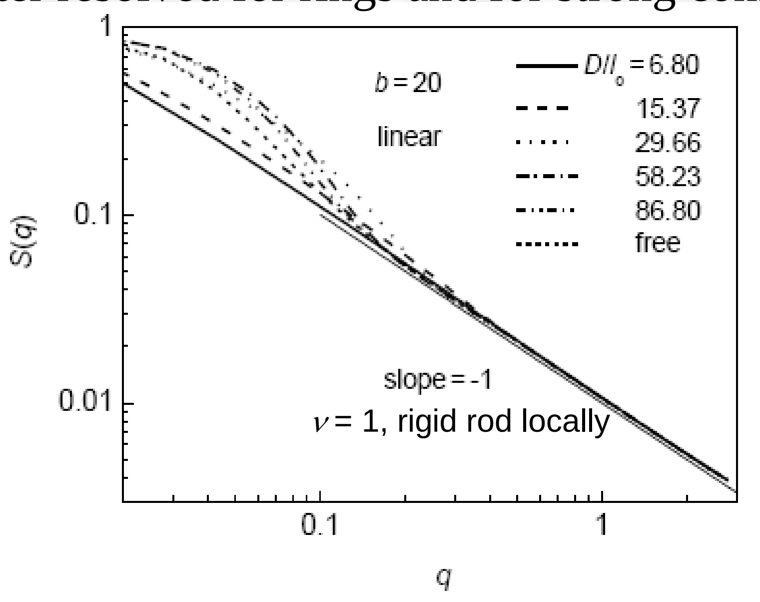
$S(q) = \frac{1}{N^2}\left\langle \sum_{i=1}^{N}\sum_{j=1}^{N} \sin(qR_{ij})/qR_{ij} \right\rangle$ (measured) , $S(q) \sim q^{-1/\nu}$ (predicted by theory for polymer coil)



Cycle under moderate confinement, the effect of chain stiffness

Impact of confinement on structure is better resolved for rings and for strong confinement





Conclusions

- The channel induced elongation profiles $R_g(D)$ of linear and ring polymers exhibit both Odijk and blob regimes (for the strong and moderate confinement). **Transition between the moderate and strong confinement regime of ring semiflexible chains is described for the first time** [5].

- For rings the relative chain extension is stronger, the Odijk regime extends to larger channel diameters D and under moderate confinement the chain extension declines less steeply. All three findings are explained in terms of **stronger self-avoidance (excluded volume) in confined rings relative to linear analogs** [5-6], the last finding is consistent with the reported experimental measurements [6].

- In the Odijk regime the **chain extension of cycles is governed by the same analytical function as for linear chains** provided half of the contour length for a cyclic chain is considered at full extension. The radius of gyration satisfactorily represents the stretching of both chain topologies.

- Orientation correlations and form factor of chains characterize structural differences arising from the interplay of confinement, chain topology and backbone stiffness

- Investigation of implications for segregation of rings in bacterial chromosomes are under way (monomers of cyclic chains are more susceptible to the self-avoidance under confinement than those of linear chains what will affect also the segregation). Focus on effect of chain stiffness.

References: [1] Reisner, W. W. et al. *Phys. Rev. Lett.* **2005**, *94*, 196101. [2] Douville, N.; Huh, D.; Takayama, S. *Anal. Bioanal. Chem.* **2008**, *391*, 2395. [3] Cifra, P.; Benková, Z.; Bleha, T. *Faraday Disc.* **2008**, *139*, 377. [4] Cifra, P.; Benková, Z.; Bleha, T. *J. Phys. Chem. B*, **2009**, *113*, 1843. [5] **Benková, Z.; Cifra, P. Macromolecules, 2012, *45*, 2597**. [6] Persson, F.; Utko, P.; Reisner, W.; Larsen, N. B.; Kristensen, A. *Nano Lett.* **2009**, *9*, 1382.

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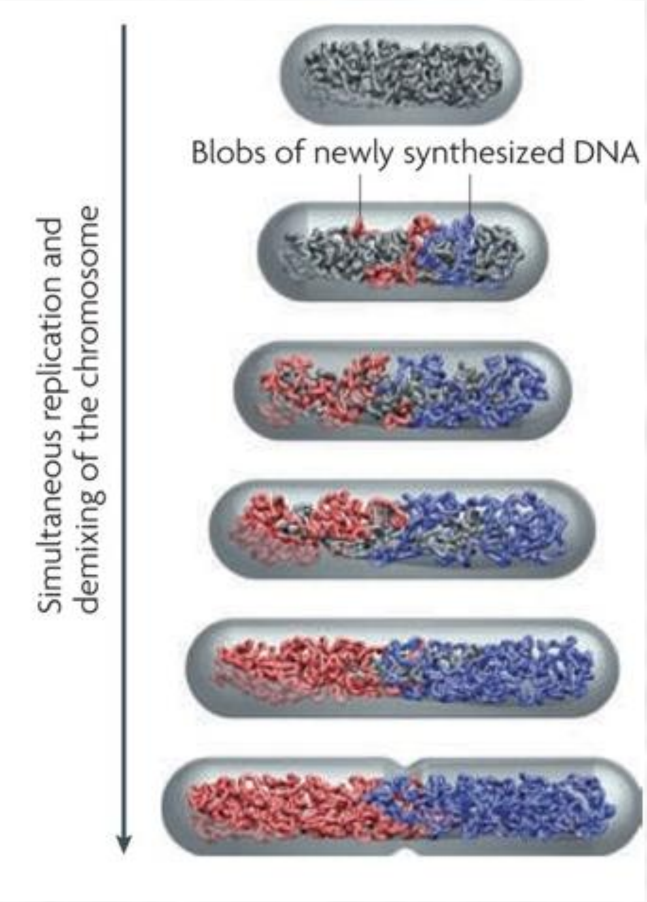
Future plans with stiffer confined macromolecules
(two examples)

I. Segregation of confined macromolecules in tubular capsids

these systems allow to specify geometric and thermodynamic conditions for segregation and duplication in bacterial chromosomes.

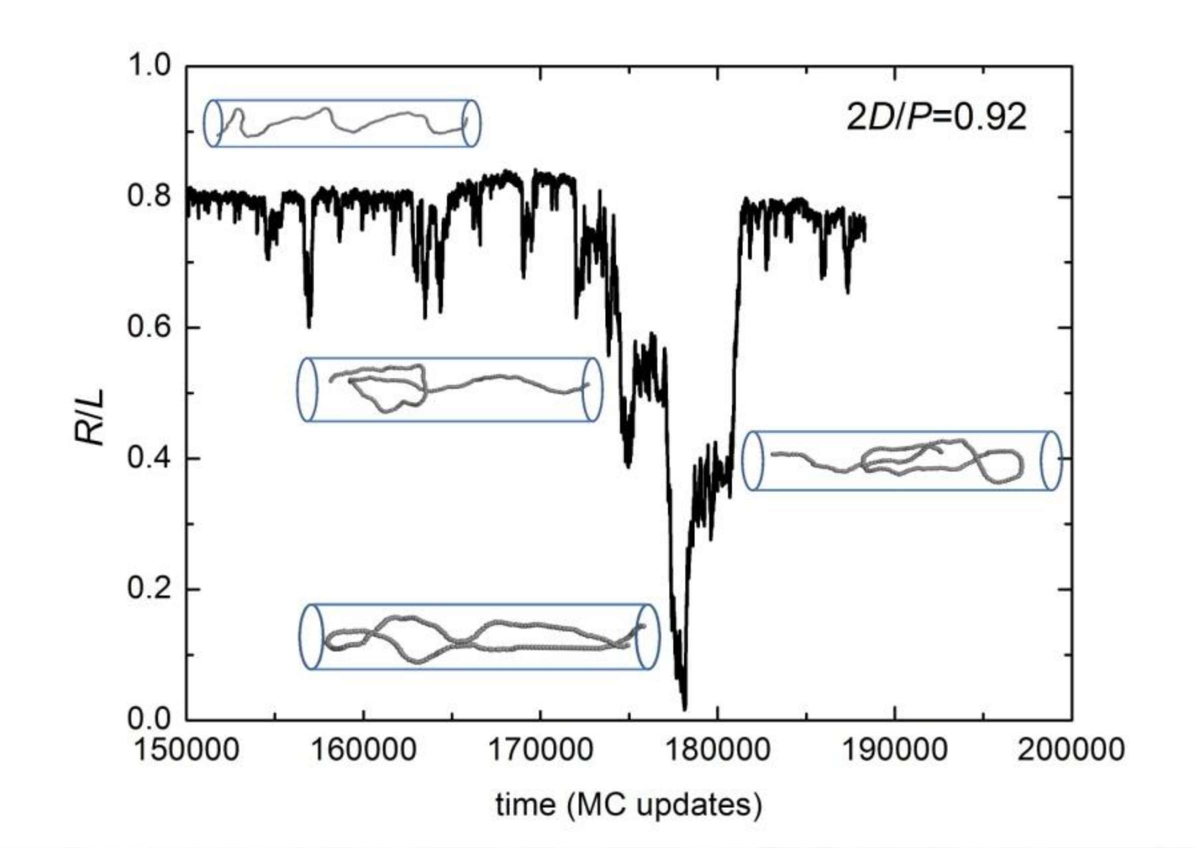
can bring also a broader insight for process of compartmentalization in cell biology.

Effect of **chain stiffness on segregation?**



Jun, Mulder, PNAS, 2006

II. For linear chains metastable **events** in time evolution of extension due to hairpinlike structures



For rings?

- no chain ends
- need for longer chains