Fermeture/ouverture de bulles de dénaturation dans l'ADN en solution

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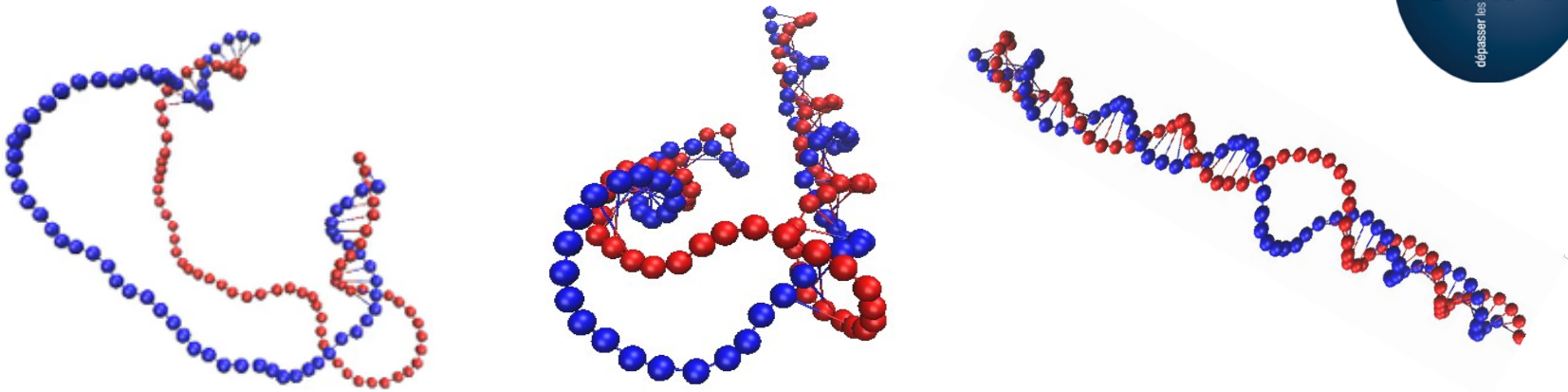

Many thanks to…

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References:
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* A.K. Dasanna, ND, J. Palmeri, M. Manghi, **Phys. Rev. E** 87, 052703 (2013)
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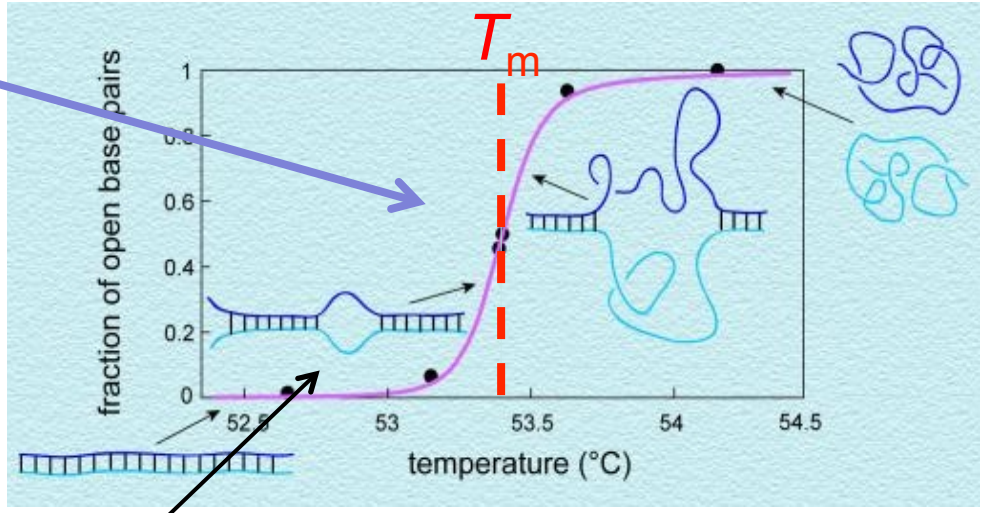
Biophysical context –

Denaturation bubbles *at equilibrium*

DNA thermal denaturation – mesoscopic viewpoint

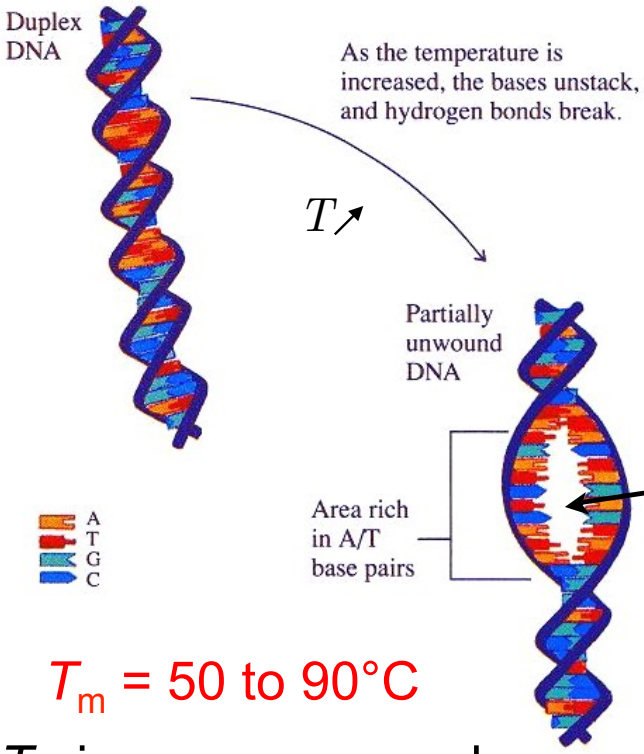
For long homo-polynucleotides:

$$T_{\rm m} = \frac{2}{k_{\rm B}} \frac{\mu + K}{\ln\left(\frac{a_{\rm ds}\epsilon_{\rm ds}\kappa_{\rm ds}\sqrt{C_{\rm ds}}}{a_{\rm ss}\epsilon_{\rm ss}\kappa_{\rm ss}\sqrt{C_{\rm ss}}}\right)}$$

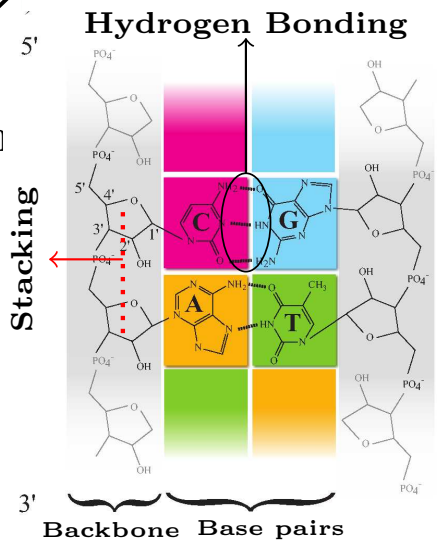


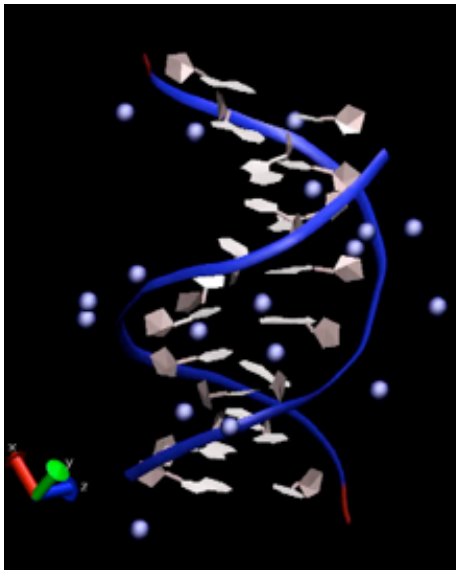
Exp.: polydA-dT, N=1815 Wartell et Montroll (1972)

Fit: $\beta\mu = 1.79$; $K = 0$; $\beta J = 3.67$; $\rightarrow T_{\rm m} = 326.4$ K



denaturation **bubble**



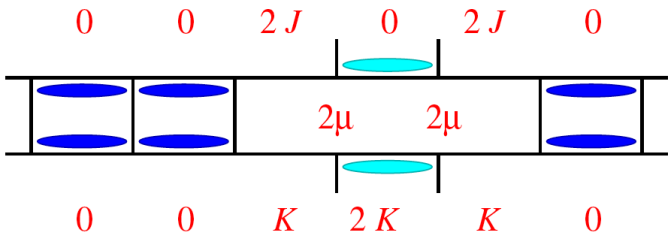


$T_{\rm m}$ = 50 to 90°C

$T_{\rm m}$ is sequence- and salt-dependent

Coupled mesoscopic model(s)

1D Ising model with applied field + discrete elastic rod (Worm-like Chain)



$\sigma =$ +1 +1 +1 −1 −1 +1 +1

$$\mathcal{H}_{\rm Ising} = -\sum_{i=1}^{N-1}\left[J\sigma_i\sigma_{i+1} + \frac{K}{2}(\sigma_i+\sigma_{i+1})\right] - \mu\sum_{i=1}^{N}\sigma_i$$

stacking energies — base pairing

$$\mathcal{H}_{\rm DWLC} = \sum_{i=1}^{N-1}\kappa(\sigma_i,\sigma_{i+1})(1-\hat{\mathbf{t}}_i\cdot\hat{\mathbf{t}}_{i+1})$$

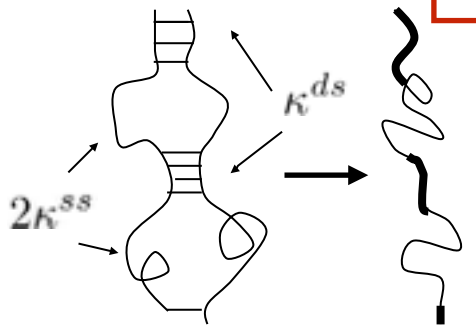
$$\mathcal{H}_{\rm DWLC} = \frac{1}{2}\sum_{i=1}^{N-1}C(\sigma_i,\sigma_{i+1})(\phi_{i,i+1}+\psi_{i,i+1})^2$$

$$\mathcal{H}_{\rm stretch} = \frac{1}{2}\sum_{i=1}^{N}\frac{\epsilon(\sigma_i)}{2}[|\mathbf{t}_i|^2 - a^2(\sigma_i)]^2$$

DNA mechanical parameters depend on base-pair state

$$\frac{\kappa_{\rm ds}}{\kappa_{\rm ss}} \approx \frac{C_{\rm ds}}{C_{\rm ss}} \approx 50$$

$$\frac{a_{\rm ds}}{a_{\rm ss}} \approx 0.5$$

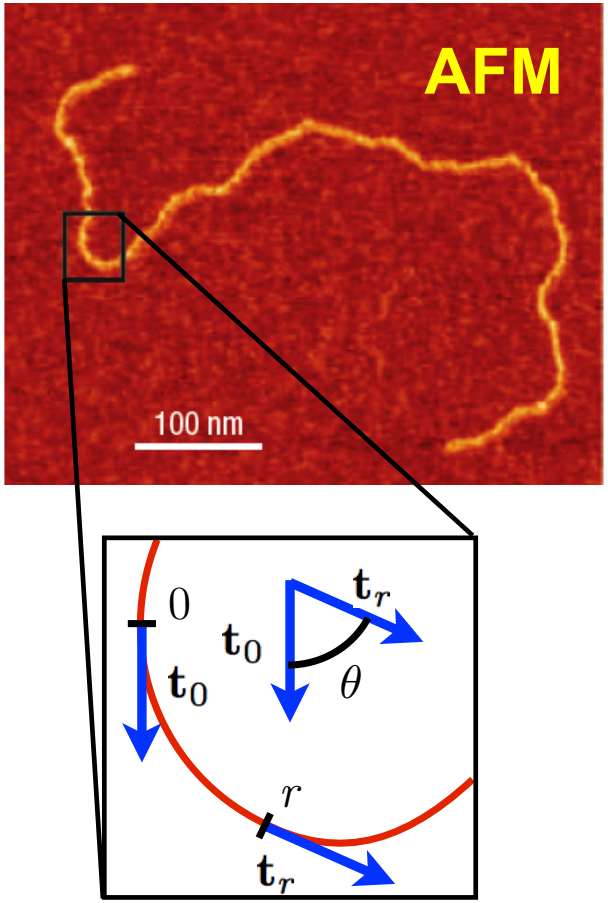


Exactly solvable Transfer Matrix techniques (quantum rigid rotator)

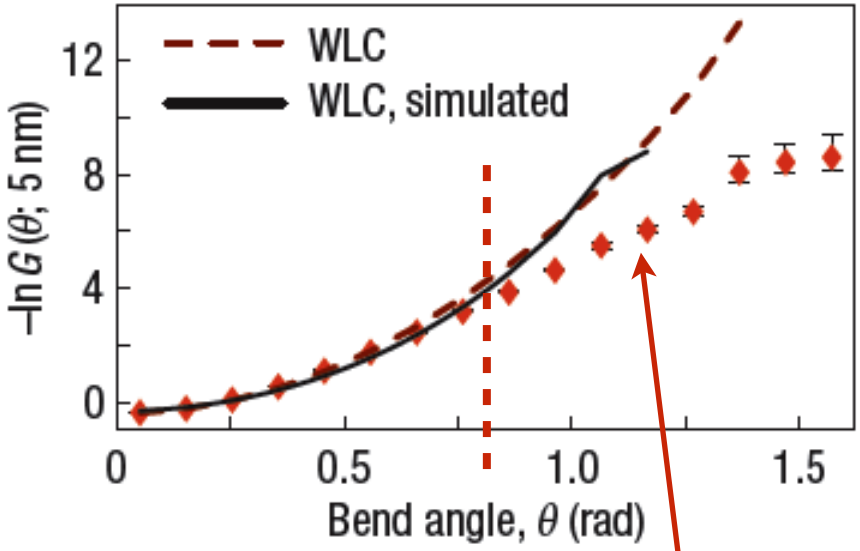
→ denaturation transition vs. mechanical parameters

→ conformations vs. T

Mechanically induced denaturation bubbles



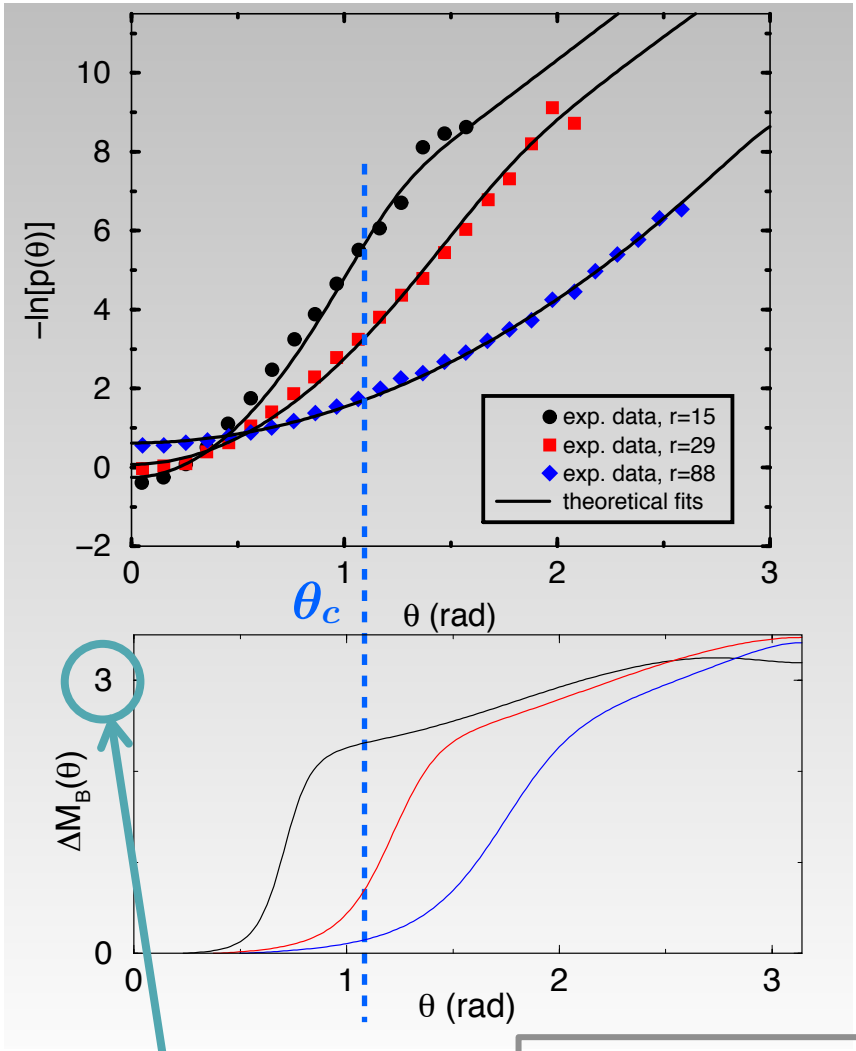
[Wiggins et al., Nature Nanotech. 2006]



over-abundance of large θ (r = 5 nm)

- Anomalous elasticity? Why??
- Local denaturation? But denaturation probability is $\approx 10^{-7}$ at room temperature!?

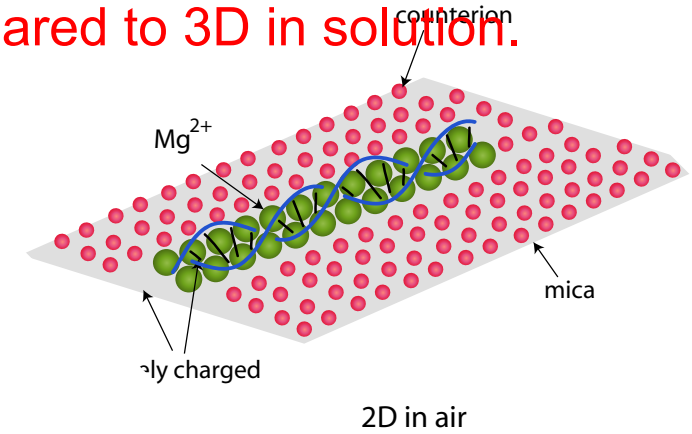
Our modeling and the “straw”-like mechanism



defect = 3-4 bp denat. bubble

But: requires significantly modified elastic parameters as compared to 3D in solution. Why?

Beware of AFM!

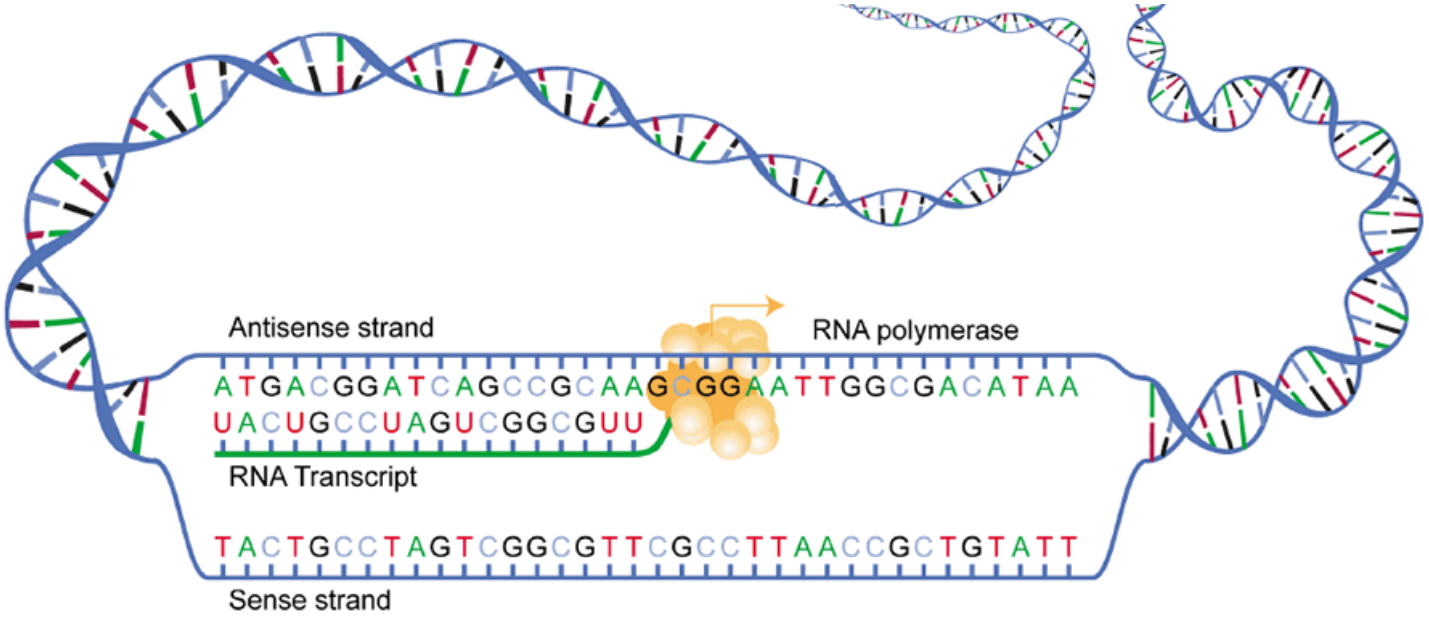


Conclusion: coupling between internal (base-pairing) and external (chain) degrees of freedom

Out-of equilibrium denaturation bubbles

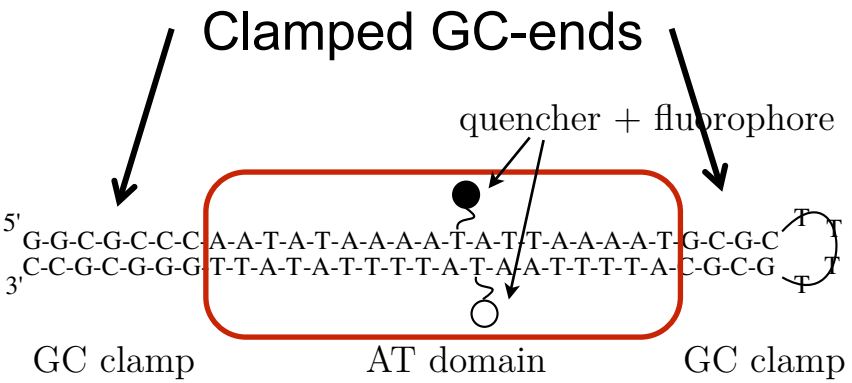
bubble closure and nucleation below T_m

Example of biological motivation: DNA transcription



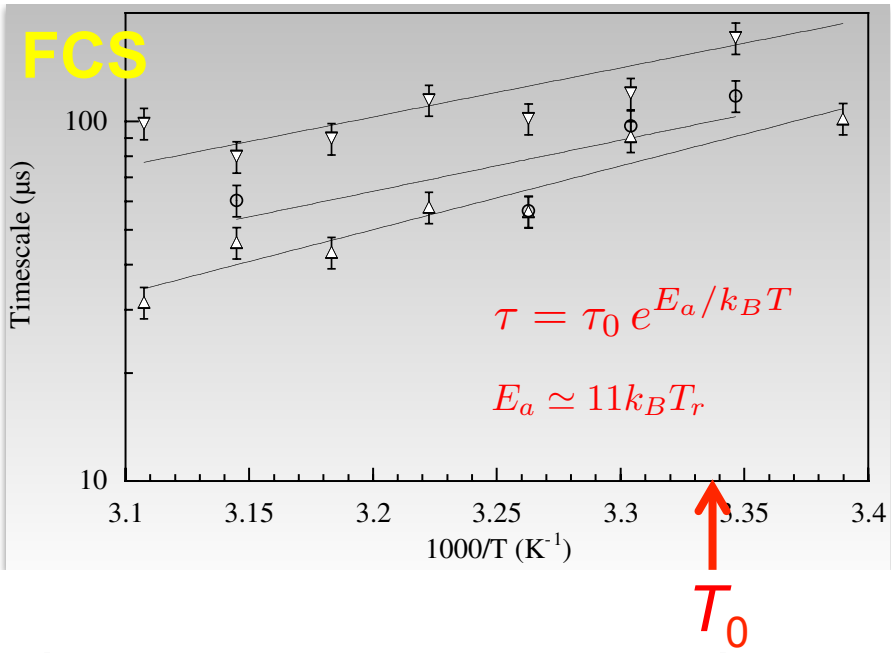
How does DNA close *in vivo* at the end of the day?

In vitro – Fluorescence Correlation Spectroscopy (FCS)



long time scales! $\tau \simeq 20 - 100\ \mu s$

[Altan-Bonnet et al., PRL 2006]



Conclusion: very long closure time scales as compared to diffusion times for a short 29-bp construct!

Question: Does it mean that for k-bp or larger constructs, bubble closure times will become so large that it will become an issue from a biological perspective?

NMR experiments (imino-proton exchange)

Recent NMR experiments on short hairpins :

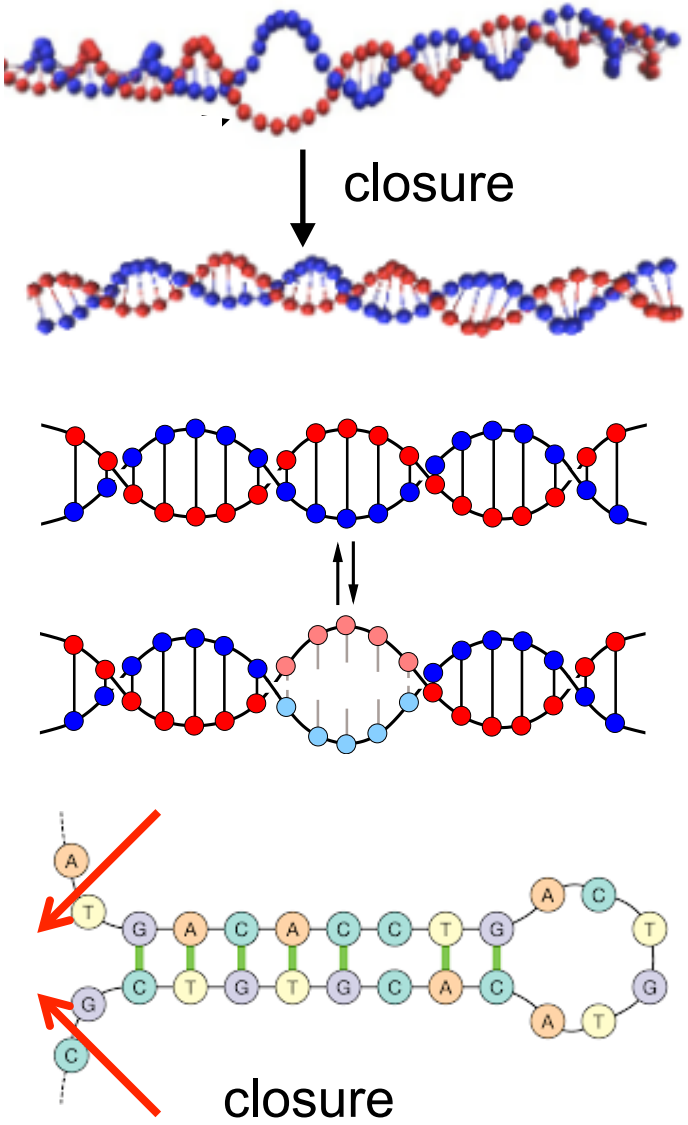
- short-lifetime openings (< 1 ns): “breathers”
- long-lifetime openings (~1 µs): same mechanism?

[Wärmländer et al., Biochemistry 2000]

Warning:

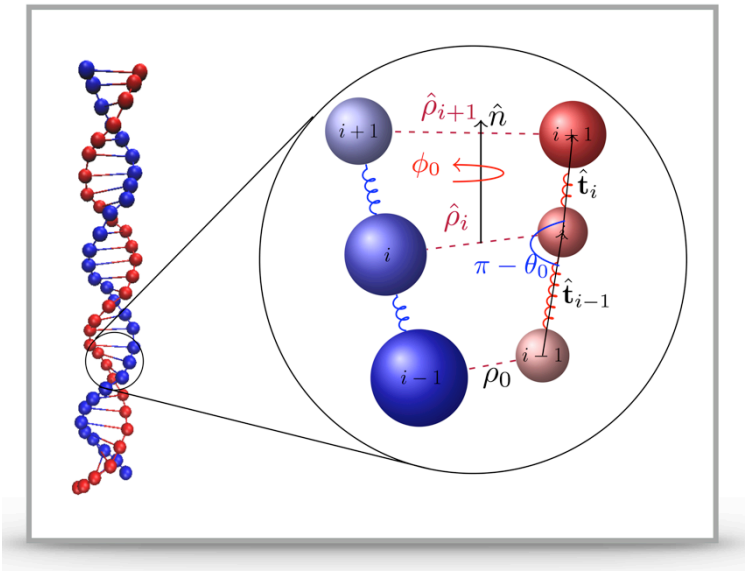
To be distinguished from:

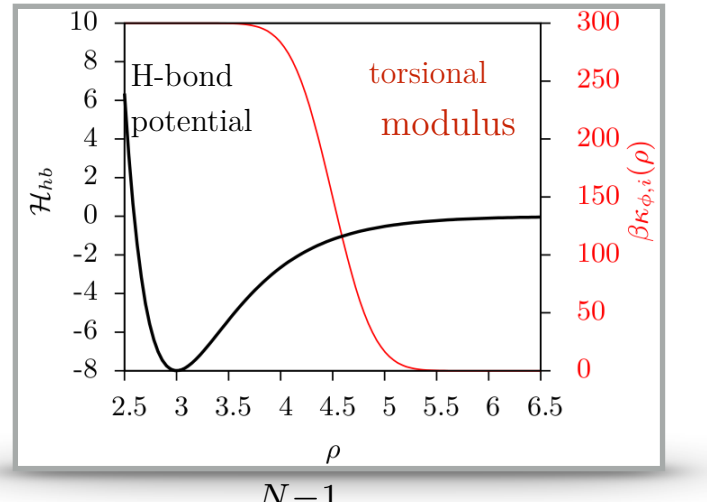
- breathers (< 1 ns opening) polymer chain frozen
- processive short hairpin closure (1 to 10 µs for <20 bp hairpins)



In silico – Brownian dynamics simulations

Coarse-grained heical model: 2 interacting and inter-wined freely rotating chains of *N* beads with effective stacking and relevant persistence lengths





$$\mathcal{H}_{\text{torsion}} = \frac{1}{2}\sum_{i=1}^{N-1} \kappa_{\phi,i}(\phi_i - \phi_0)^2$$

Overdamped langevin equation:

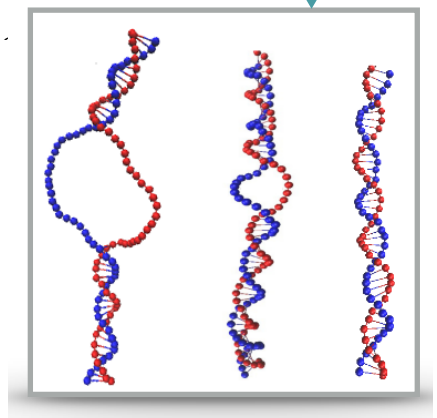
$$\zeta \dot{\mathbf{r}}_i(t) = -\nabla_{\mathbf{r}_i}\mathcal{H}(\{\mathbf{r}_j\}) + \xi_i(t)$$

stretching+bending +inter-strand potential

thermal noise

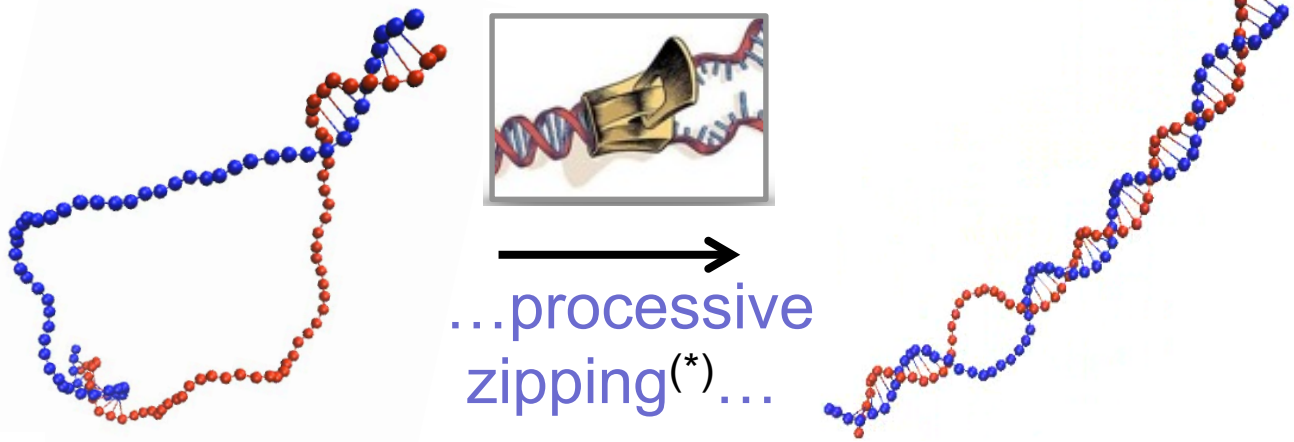
Advantages:

- $N \approx 100$ bp
- $t_{\text{sim}} \approx 1$ ms
- minimal set of parameters



Bubble Closure dynamics

Starting from a large equilibrated bubble…

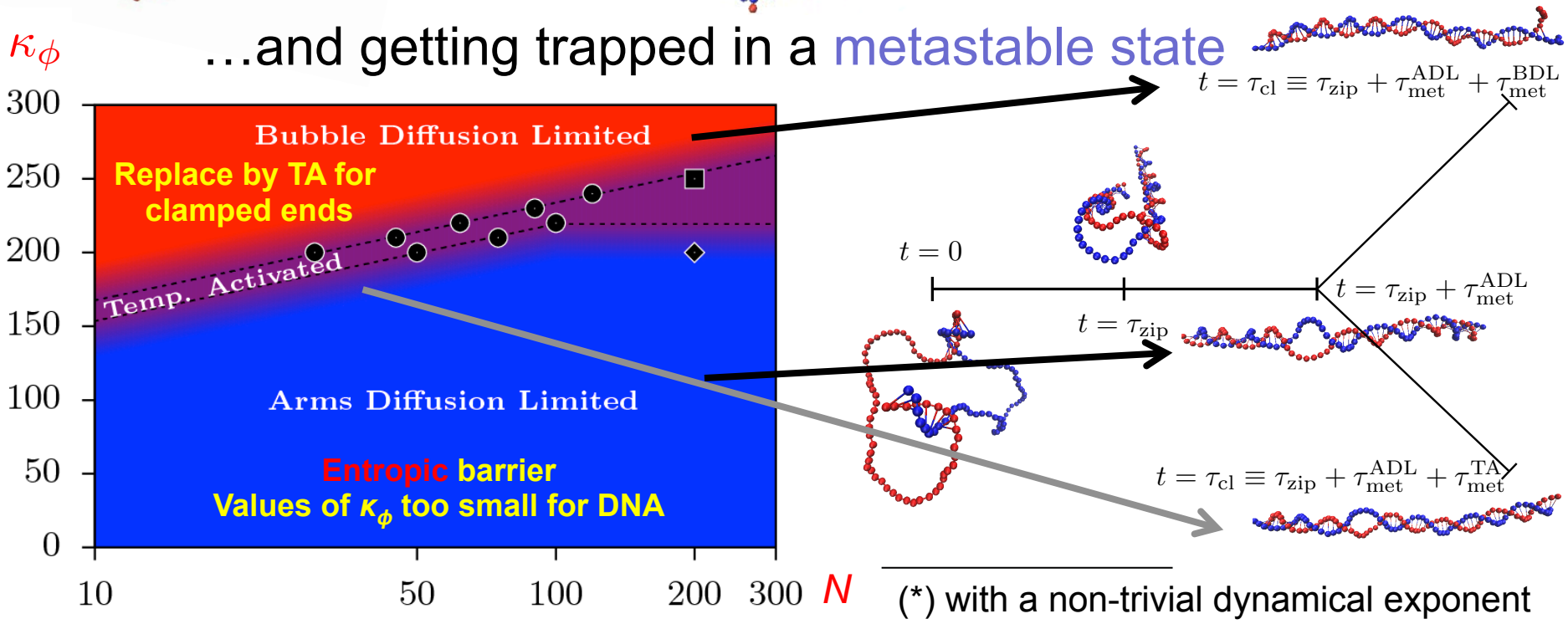


…processive zipping(*)…

Q.: Where does the (free) energy barrier come from?

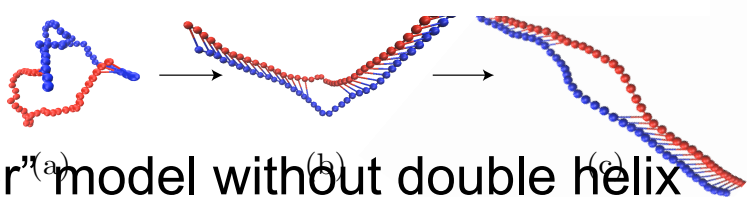
A.: It depends on the parameters

…and getting trapped in a metastable state



(*) with a non-trivial dynamical exponent

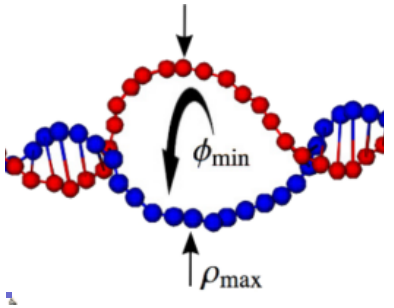
- ADL: entropic barrier (alignment requirement) $\Longrightarrow$ dependency on *N*
- TA: energetic barrier $\Longrightarrow$ indep. of *N* and Arrhenius: $\tau_{\mathrm{TA}} \sim \exp(E_{\mathrm{a}}/k_{\mathrm{B}}T)$



What is the origin of the barrier?

- No *energy* (only entropic) barrier in a "ladder" model without double helix
- Barrier for *strong enough torsional modulus* κ_ϕ

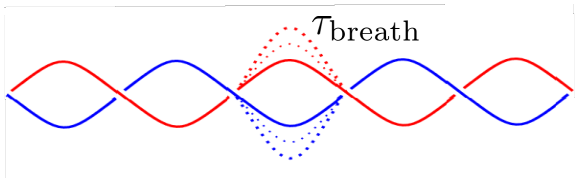
$\Longrightarrow$ should be related to torsional elasticity

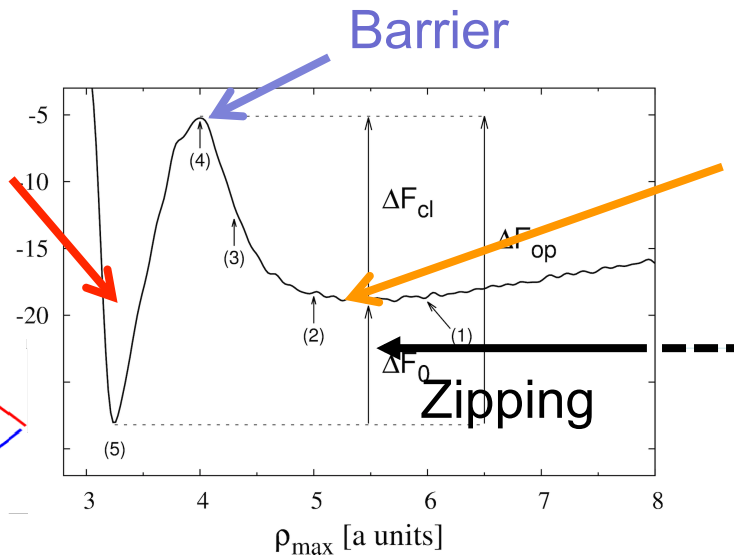


But the height of the barrier prevents a "thermal" exploration of the free-energy landscape by classical Brownian dynamics.

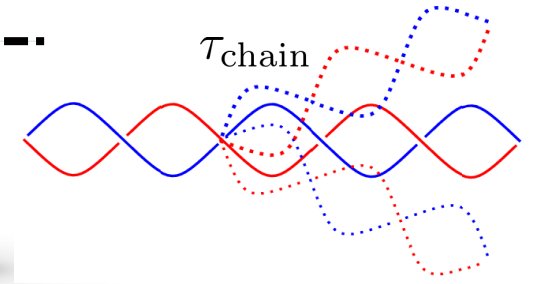
$\Longrightarrow$ biased dynamics wanted: Metadynamics [Laio, Parinello, PNAS 2002]

Breathing, transient bubbles as studied, e.g., by non-linear physics (ns lifetimes)



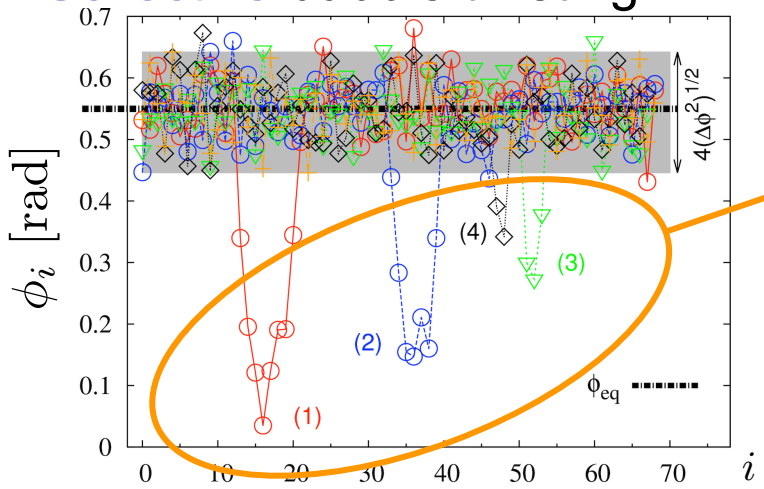


Long-lived, metastable bubbles of interest here and presumably biologically relevant



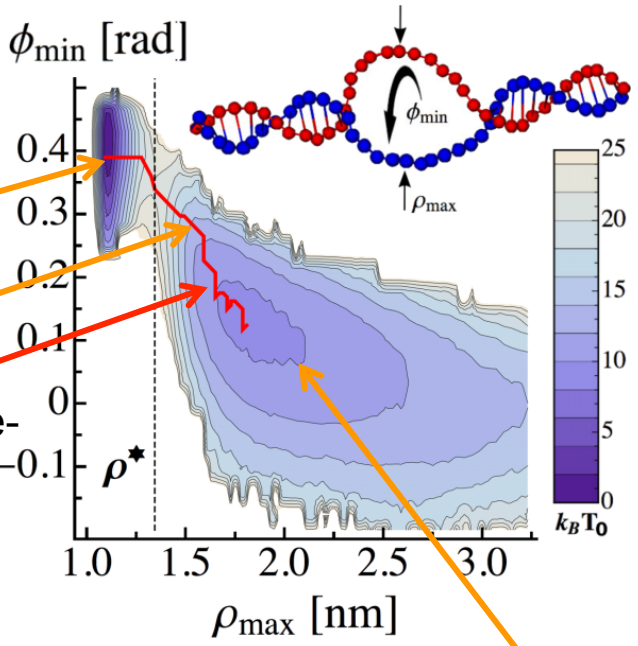
Origin of the barrier

1. Collective bubble twisting

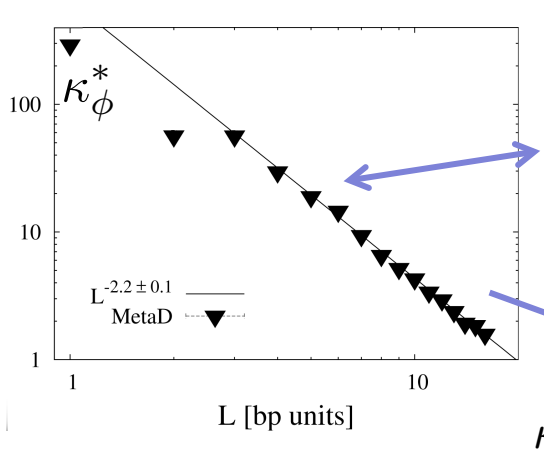


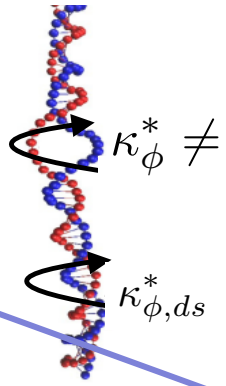
Breathing

Typical minimal free-energy path

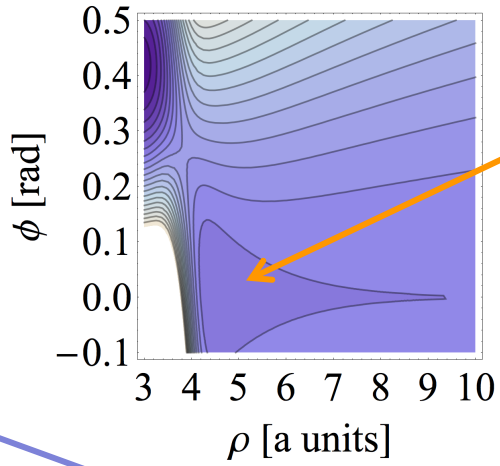


2. The effective bubble torsional modulus $\kappa^*_\phi(L)$ explains the energy barrier





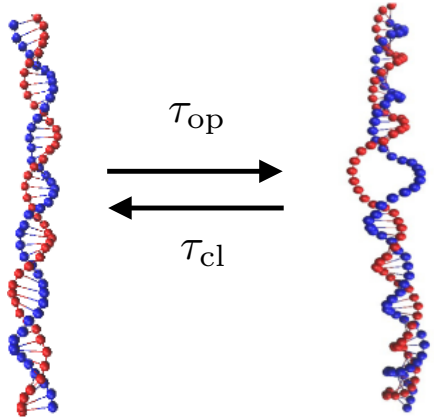
$\kappa^*_{\phi,ds} \simeq 400 k_B T$

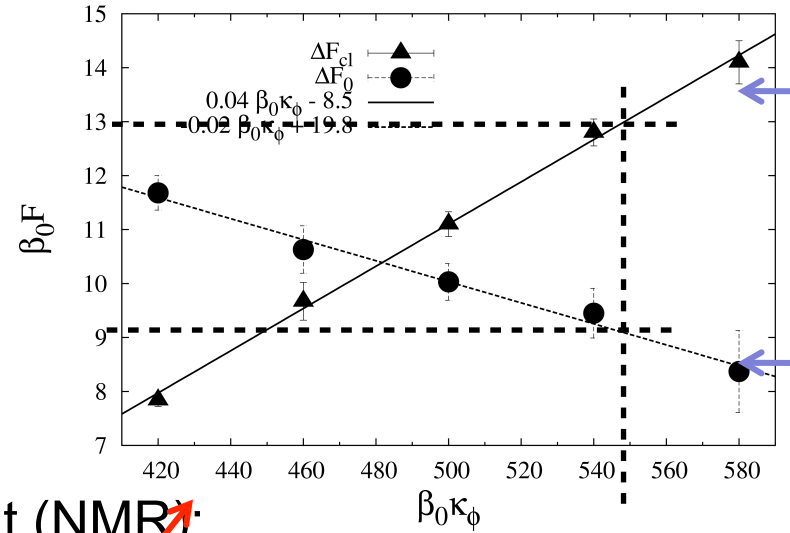


metastable basin

Mean-field model: $\mathcal{H}(\rho,\phi) = V_{\mathrm{Morse}}(\rho) + \frac{\kappa^*_{\phi,\mathrm{ds}}}{2}(\phi-\phi_{\mathrm{eq}})^2 + \frac{\kappa^*_\phi(\rho)}{2}\phi^2 \qquad (\rho \propto L)$

Quantifying closure and nucleation times





Exptal equilibrium constant (NMR):

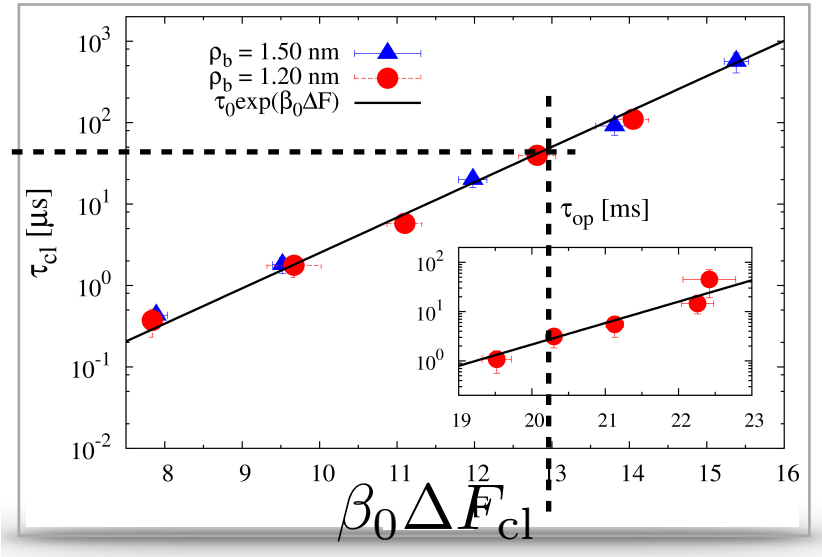
$$K = \frac{\tau_{\rm cl}}{\tau_{\rm op}} \sim 10^{-3}$$

- Opening/closure free energies
- Arrhenius activation as expected
- Quantitative agreement with experiments :

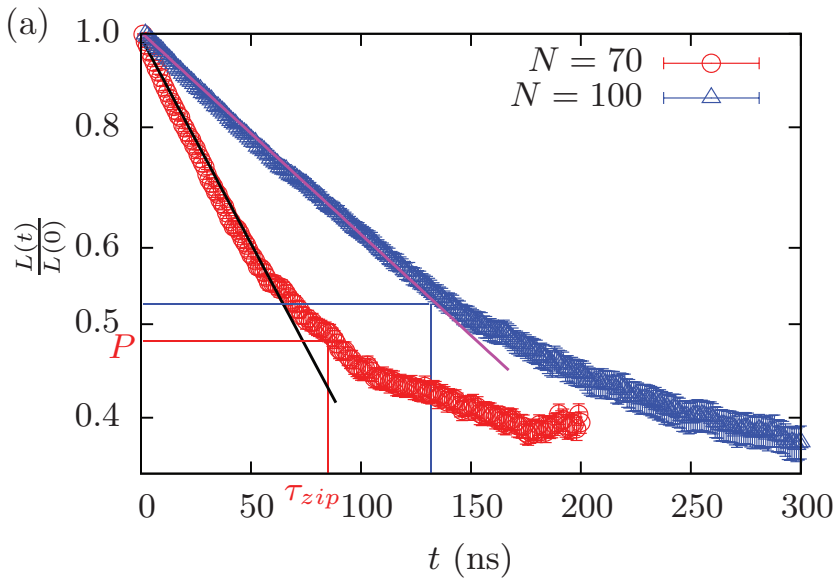
$$\tau_{\rm cl}^{\rm exp} \simeq 40\ \mu{\rm s} \Rightarrow \Delta F_{\rm op} \approx 22\ k_{\rm B}T_0$$

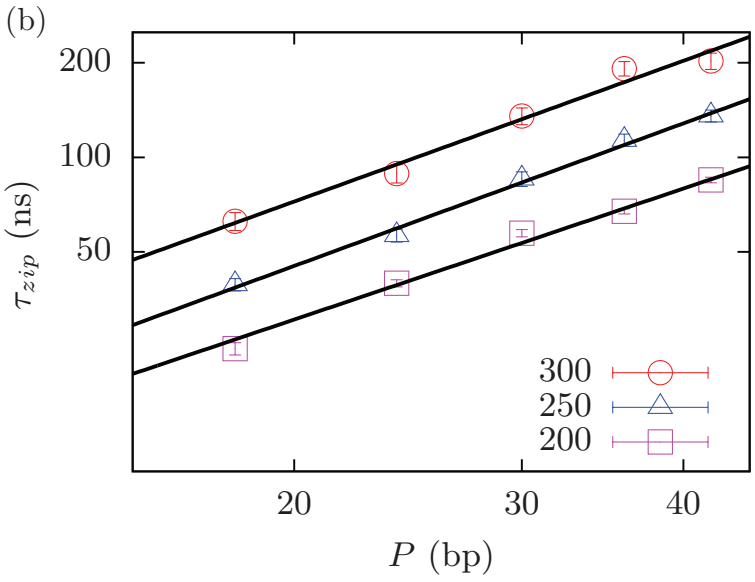
$$\Rightarrow T_{\rm op} \approx 15\ {\rm ms}$$ (consistency check)

Metadynamics can also give access to real dynamics [Tiwary, Parinello 2013]:



Bonus: zipping dynamical exponent





$$L(\tau_{\rm zip}) = P$$

where

$$P \equiv \frac{3}{5}[L(0) - \bar{L}]$$

$$\tau_{\rm zip} \propto P^{\gamma}$$

with $1.4 \leq \gamma \leq 1.5$

$$\gamma = 1 + \nu \ ?$$

Conclusion and outlook

★ Coupling between bending/torsion and base-pairing is essential when addressing several biophysical properties of DNA. Effective 1D models are then to be ruled out.

★ Coarse-grained models can be compulsory because all-atom approaches are limited with respect to biological relevant scales (size and/or time-scale)

★ Metadynamics enable the exploration of parameter regimes out-of-rich by classical, unbiased techniques

★ No issue from a biological perspective

★ A prediction: closure times independent of DNA/hairpin length. Could easily be tested experimentally.