Probing DNA conformational changes at the single molecule level

Impact of local defects include in the DNA molecule and of solvent physicochemical conditions

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ANR project :TPM-on-a-chip

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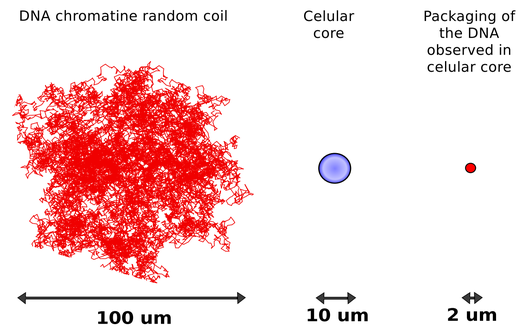
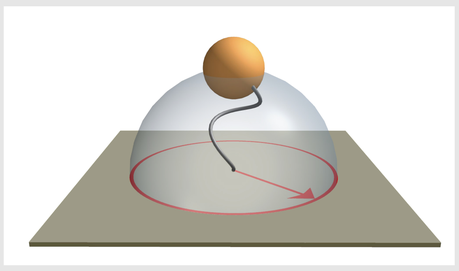
Global context

The description of the living with theoretical models provides quantitative information, that are indispensable to get a better understanding of the biological phenomena. However biological processes are very complex. In particular, DNA related phenomena require further studies combining experimental and theoretical approaches. Here we propose to explore the mechanical properties of DNA in terms of statistical physics. We will probe the intrinsic DNA bending or the impact of changes in physicochimical condition set by the solvent at the single molecule level using parallelized TPM method.



Single molecule approaches

Tethered Partucle Motion (TPM) Technique

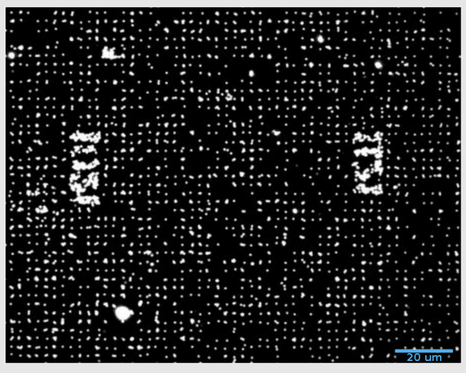


TPM : Single molecule technique.

Principle : Tracking the motion of a bead tethered by a DNA molecule to a surface.

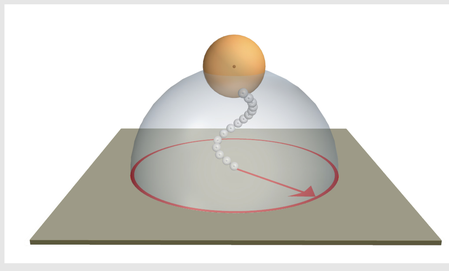
Observable : Apparent end-to-end distance of a DNA molecule

High Throughput TPM-chip [1]



Creation of parallelized-TPM. ~ 1000 DNA/particle complex followed and analyzed simultaneously in real time.

Mesoscopic statistical model



DNA-particle complex model as a chain of **N** (N=50) connected small spheres of radius **a** and a larger final particle of radius **R** (from 20 to 150 nm). Displacement of the labeled particle is obtained by performing a **Kinetic Monte Carlo Simulation [2].**

- **Bent DNA molecules** : fixed angle between 3 successive monomers at the center of the DNA.

  => Values of angles ranging from 0 to 180 °

- **Changes in ionic strength conditions** : changes in the global DNA persistence lenght.

  => Values of Lp ranging from 107 bp to 207 bp

Analysis of the impact of ionic strenght on the persistence lengtht

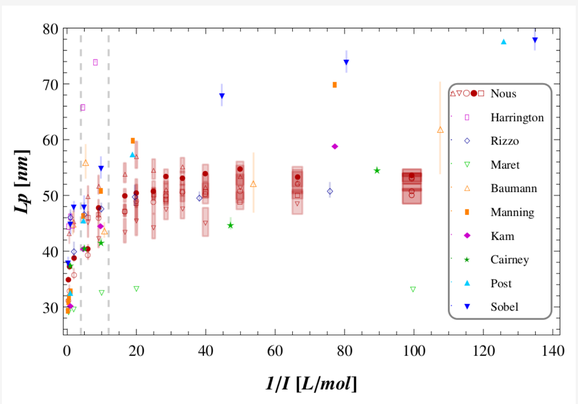
Global effect, changes in ionic strenght

***A Brunet**, A., Tardin, C., Salomé, L., Rousseau, P., Destainville, N., Manghi,M., **Dependence of DNA persistence length on ionic strength of solutions with monovalent and divalent salts : a joint theory-experiment study**, **Macromolecules**, **2015**, 48 (11), pp 3641-3652, DOI : 10.1021/acs.macromol.5b00735*

Ions play an important role in many biological processes affecting the DNA molecule, both for binding activities of DNA-protein interaction, and the DNA packaging in viral capsids or cell nucleus.

We probe the effect of the ionic strength induced by the presence of monovalent Na^+ and divalent Mg^{2+} ions.

Lp near physiological salt



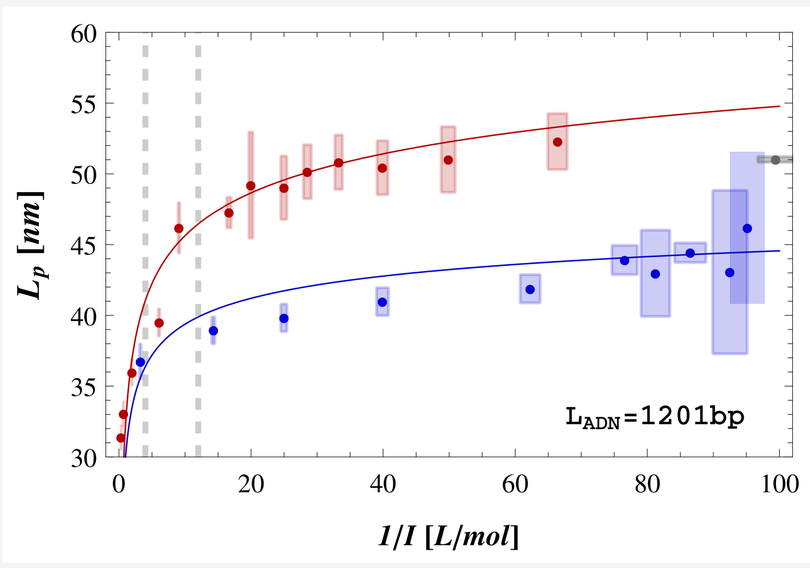
=> Our Lp=48+/-6nm measurement are in agreement with mean published values of Lp=47+/-4nm

Quantification of Lp(I) dependancy

Lp(I) dependancy can not be explained by OSF, Manning condensation, Netz Orland or the crossover of Muthukumar theories.

=> Validation of the new Manning theory propose in 2006 [3] on the all large range of Na+ ionic strenght

This theory failed on $Mg2^+$



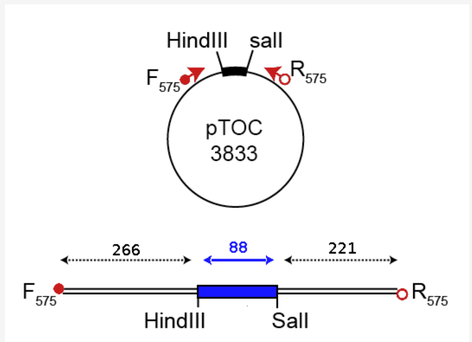
Quantificative charaterization of DNA bending

Local effect, an intrinsic curvature

***A Brunet**, A., Chevalier, S., Destainville, N., Manghi, M., Rousseau, P., Salhi, M., Salomé, L., Tardin, C, **Probing a label-free local bend in DNA by single molecule tethered particle motion-experiment study**, **Nucleic acids researchs**, **2015**, 42 (11), pe 72 (7)DOI : 10.1093/nar/gkv201*

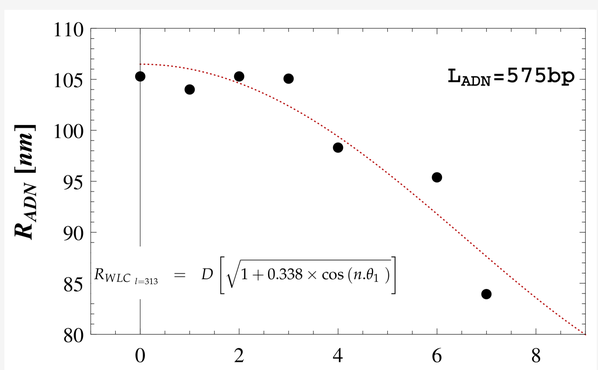
Curvature and flexibility of DNA molecules are sequence dependent.

DNA molecules containing A-tracts, runs of four or more contiguous adenine residues, are known to be curved if the A-tracts are repeated in phase with the helix screw[4].



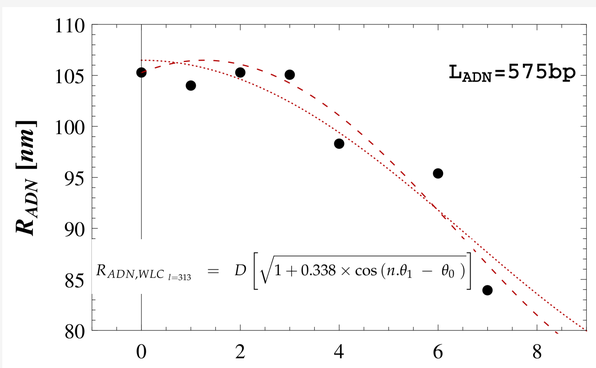
6An0P CCATCTCGAACCGACGTTGCTGGCCGTACATGTGGCTAGCACCATGGAGGATCCCTTAACGCTATGGAACTCGCCGCCCGACTGGGCT
6An1P CCATCTCGAACCGACGTTGCTGGCCGTACATGTGGCTAGCAAAAAACGGGATCCCTTAACGCTATGGAACTCGCCGCCCGACTGGGCT
6An2P CCATCTCGAACCGACGTTGCTGGCCGTAGGCTAGCAAAAAACGGCAAAAAACGGGATCCCGCTATGGAACTCGCCGCCCGACTGGGCT
6An3P CCATCTCGAACCGACGTTGCTGGCGCTAGCAAAAAACGGCAAAAAACGGGCAAAAAACGGGATCCGGAACTCGCCGCCCGACTGGGCT
6An4P CCATCTCGAACCGACGTTGGCTAGCAAAAAACGGCAAAAAACGGGCAAAAAACGGCAAAAAACGGGATCCTCGCCGCCCGACTGGGCT
6An4O CCATCTCGAACCGCTAGCAAAAAACGGACGTACAAAAAACGGACGTACAAAAAACGGACGTACAAAAAACGGGATCCCCGACTGGGCT
6An7P CCGACGTTGGCTAGCAAAAAACGGCAAAAAACGGGCAAAAAACGGCAAAAAACGGGCAAAAAACGGCAAAAAACGGGATCCTCGCCGC
6An6P TTGGCTAGCAAAAAACGGCAAAAAACGGGCAAAAAACGGCAAAAAACGGGCAAAAAACGGCAAAAAACGGGCAAAAAACGGGATCCTC

Considering a single DNA local bending



=> A-tract Bent angle = 16° +/-2°

plus a global curvature



=> A-tract Bent angle = 19° +/-4°
DNA Global angle = 25° +/-19°

Summary

=> We show here that **TPM measurements** combined with a mesoscopic statistical modelling permits the **detection** and **quantification** of:

- **DNA intrinsic bends** with **good accuracy +/- 1.8° &** on a **range lying between 30° and 150°**
- the decrease of **persistence length** from **55 to 30 nm** induced by the increase of **ionic strength** ranging from **10^{-2} to 3 mol/L** in presence of **monovalent** and **divalent** ions.

[1] Plénat, T., Tardin, C., Rousseau, P., Salomé, L., 2012. *High-throughput single-molecule analysis of DNA-protein interactions by tethered particle motion*. **Nucleic Acids Research**. 40 (12) : e89

[2] M. Manghi, C. Tardin, J. Baglio, P. Rousseau, L. Salomé, N. Destainville, 2010. *Probing DNA conformational changes with high temporal resolution by tethered particle motion*, **Phys. Biol.** 7, 046003

[3] G. S. Manning. *The persistence length of DNA is reached from the persistence length of its null isomer through an internal electrostatic stretching force*. **Biophysical journal**, 91(10) :3607-16, November 2006.

[4] Koo, H.S., Wu, H.M., and Crothers, D.M. 1986. DNA bending at adenine-thymine tracts. Nature 320, 501-506