EFFECTS OF BINDING LIGANDS AND ARCHITECTURAL PROTEINS ON DYNAMICS, FLOW, AND GELATION OF DNA

In organisms, the genetic material is often, if not always in a crowded and congested state. Folding of DNA is facilitated by a myriad of biophysical processes, which is only partially understood. Here, we focus on an exemplary selection of key players responsible for folding of the genome in viruses and bacteria, that is a series of polyamines and nucleoid associated proteins (NAPs). These ligands bind on DNA, modify the secondary structure and mechanical properties of the double helix, and mediate bridging interactions between different segments of the same or different DNA molecules. In particular, DNA folding and compaction are thought to be related to protein and/or ligand mediated bridging interactions. Cross-linking by bridging interactions is expected to affect DNA dynamics and the properties of its flow. Gelation might also occur if (semi)-permanent bridges are formed.

The project encompasses a systematic investigation of the effect of cross-linking bridging interactions on the dynamics and concomitant rheological properties of DNA.
The specific aims are:

- Do condensing ligands and proteins affect genome dynamics?
- Is this related to bridging interactions between different DNA molecules or segments thereof?
- Does this result in gelation of the genome with implications for the machinery of life?

In order to achieve these objectives, a selection of biologically relevant compaction agents need to be investigated. These agents are a series of polyamines and two bacterial nucleoid associated proteins (NAP), that is H-NS and Hfq. They differ in their modes of operation, but the key factors are charge and specific ligand interaction. The effects of all of these agents on the dynamics of the genome will be evaluated using a combination of passive and active micro-rheology assays.

For *active* microrheology measurements, magnetic wires with diameter 500 nm and length 5 µm to 50 µm will be synthesized (Figure 1).

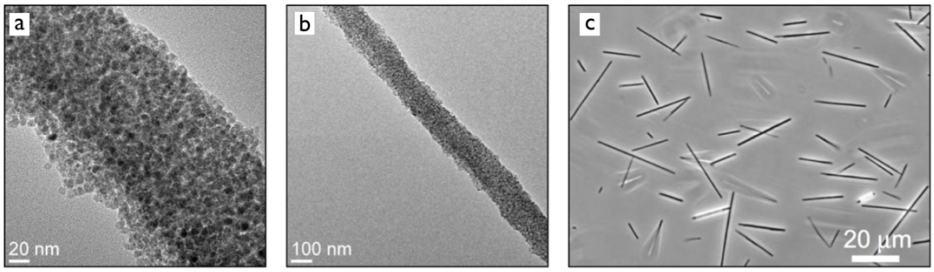

Figure 1. a and b) Transmission electron microscopy of magnetic wires made from 10 nm iron oxide nanoparticles. c) Phase-contrast image of wires observed by optical microscopy.

In active mode, the wires are submitted to an external rotating magnetic field. The technique is known as Magnetic Rotational Spectroscopy (MRS). MRS consists in monitoring the wire motion as a function of the actuating frequency

The shear viscosity η can be determined from the wire motion recorded by video-microscopy as a function of the time. The advantage of MRS is its broad frequency range, from 10^{-4} and 10^{2} rad s^{-1}. Crowded media, such as crosslinked DNA dispersions can be characterized by long relaxation times (>minutes) that can only be accessed with low frequency testing. In a recent paper on cell biomechanics, we have shown that the interior of living cells can be described as a viscoelastic liquid, and not as a soft solid (characterized by a yield stress). This conclusion was made possible by gaining access to a frequency range not explored before.

The Berret group has also provided the constitutive equations of viscoelasticity for magnetic wires in nonlinear materials. In this project, the wire-based magnetic rotation spectroscopy, a powerful technique to explore soft matter dynamics and flow, will be complementary to the passive microrheology method as discussed above.

Related references

1. Fresnais, J., J.-F. Berret, B. Frka-Petesic, O. Sandre, R. Perzynski, Adv. Mater. **2008**, 20, 3877.
2. Frka-Petesic, B., K. Erglis, J.-F. Berret, A. Cebers, V. Dupuis, J. Fresnais, O. Sandre, R. Perzynski, J. Magn. Magn. Mater. **2011**, 323, 1309.
3. Chevry, L., N. K. Sampathkumar, A. Cebers, J. F. Berret, Phys. Rev. E **2013**, 88.
4. Berret, J.F., Nature Communications **7**, 10134 (2016), DOI: 10.1038/ncomms10134
5. Malabirade, A., K. Jiang, K. Kubiak, A. Diaz-Mendoza, F. Liu, J. A. van Kan, J.-F. Berret, V. Arluison, and J. R. C. van der Maarel, Nucleic Acids Research **2017**