ECOLE NORMALE SUPERIEURE DE LYON
Laboratoire de Physique
UMR CNRS 5672

Post-Doctoral offer in Biophysics @ ENS Lyon:

Physical properties of AAV viruses: an AFM study of capsid stability and disassembly at the single particle level

Context and Objective: Recent progresses in the characterization of physical properties of viruses allows for the first time to investigate distinct virus cycle events at the single particle level [1]. Using an original combination of experimental approaches based on Atomic Force Microscopy (AFM) and statistical physics modelization, we propose to study the physical properties of Adeno-Associated Virus (AAV) particles in particular to define the parameters leading to capsid disassembly and ejection of the viral genome. AAV is a nonpathogenic parvovirus which is currently used as a gene transfer vector in gene therapy applications [2]. The vectors are composed by a single-stranded DNA genome packaged into an icosahedral 20 nm-capsid which can be derived from at least 10 natural AAV serotypes (Figure 1). Interestingly, several studies indicate that the biological properties can greatly differ according to the AAV serotype used. Therefore, we would like to explore the physical properties of various AAV capsids serotypes to understand their differences in terms of stability, disassembly and release of their viral genome.

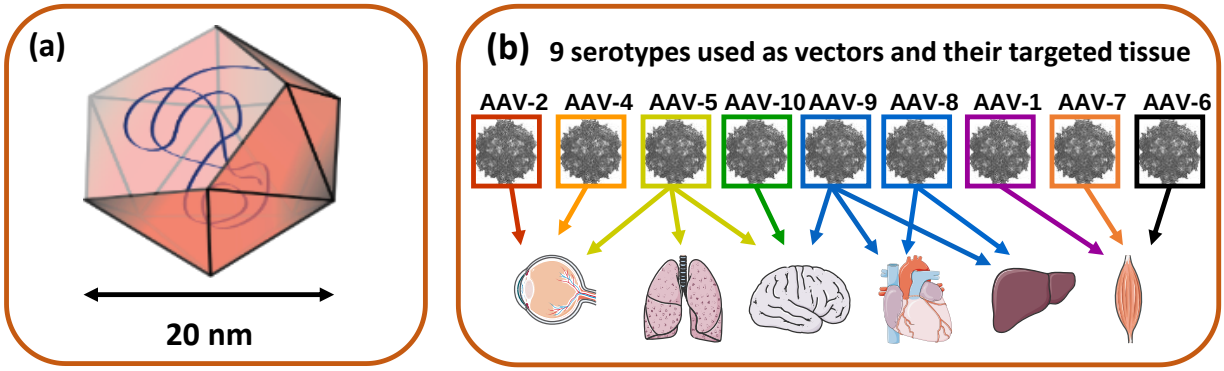

Figure 1: AAV viral vectors. *(a) Wild type (wt) AAV particles are composed by a non-enveloped icosahedral capsid containing a 4.7 kb single stranded DNA genome. (b) Currently, several AAV serotypes which differ for their capacity to target some organs in vivo are used as vectors for gene therapy.*

Scientific program: AFM experiments will be used to probe at a very high spatial resolution the morphology of viral capsids but also to exert mechanical constraints on viral particles thereby measuring their elastic response [3]. Using both AFM imaging and nano-mechanics, we will compare several AAV serotypes at thermodynamic equilibrium and relate their differences in term of physical properties with their biological behavior *in cellulo* (virus entry, stability of the capsid, disassembly inside the cell nucleus) as measured by our collaborators.

Then, we will vary the micro-environment of AAV capsids (temperature, pH, osmotic pressure,…) in order to study their influence on virus stability. Our preliminary results (Fig. 2) suggest that above a fixed temperature, single stranded DNA starts to be ejected from the capsid and is visible on AFM images. The amount of ejected single stranded DNA can be quantified as a function of temperature as well as the change in morphology and mechanical properties of capsids. These data will be useful to develop statistical modeling in order to relate the amount of ejected DNA with the conformation of DNA inside the viral capsid.

Finally, we will examine the effect of viral genome length and nature (single or double stranded) on the AAV capsid disassembly process [4].

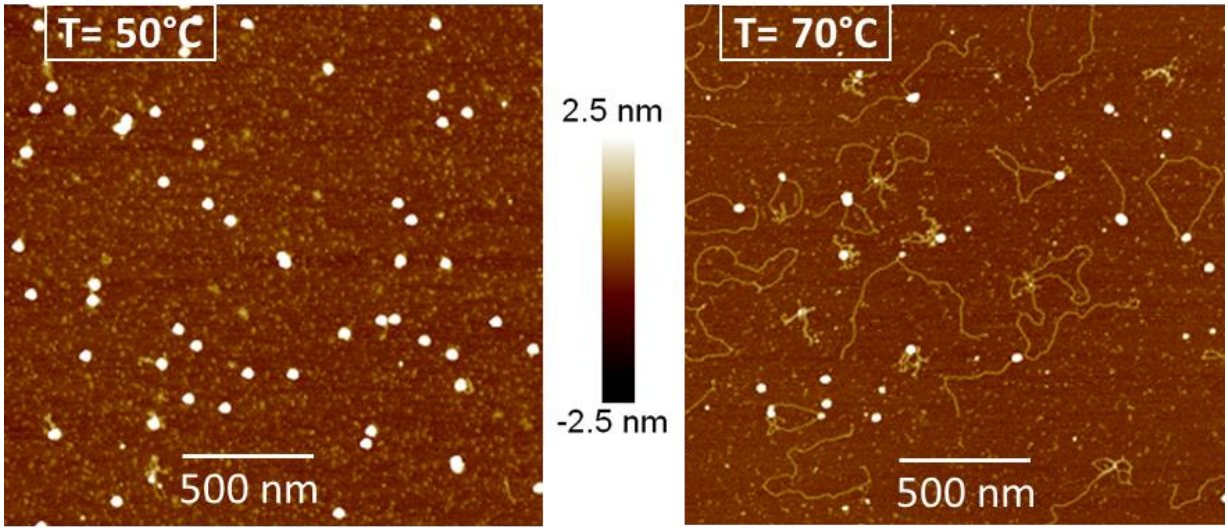

Figure 2: Example of AFM image of AAV8 capsids incubated for 15 minutes at 50°C or 70°C*. Above 60°C, some single stranded DNA ejected from the virus can be observed. Using image analysis tools, we are able to quantify the length of DNA ejected as a function of temperature and/or time of incubation.*

Interdisciplinary project: This post-doc project will benefit from the interdisciplinary collaboration we have initiated recently with the team of Anna Salvetti (CIRI, ENS Lyon), a specialist of AAV virus biology and AAV vector development. The research work will take place in the *Laboratoire de Physique*, in close collaboration with Martin Castelnovo for the physical modelization, and Anna Salvetti for the biology/virology part of the project.

Profile: The candidate should have a PhD in Soft Matter Physics or Biophysics. An experience with AFM and/or single molecule techniques will be an asset. The position is available in October 2015 and funding is for 12 months. To apply, please send a CV with a list of publications, a brief summary of previous research activities and the name of 2 or 3 references to **Cendrine Moskalenko** (Cendrine.Moskalenko@ens-lyon.fr).

[1] Roos W.H., Bruinsma R., Wuite G.J.L; Physical virology, *Nature Physics* **2010**, *6*, 633.
[2] Mingozzi F. and High K.A. *Nat. Rev. Genet.* **2011**, 12, 341.
[3] Castellanos M, Pérez R, Carrasco C, Hernando-Pérez M, Gómez-Herrero J, de Pablo PJ, Mateu MG.; *Proc Natl Acad Sci U S A*. **2012**, *109*, 12028.
[4] Nurmemmedov E. ; Castelnovo M. ; Catalano C.E. ; Evilevitch A.; *Quaterly Reviews of Biophysics* **2007**, *40*, 327.