LPENS
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DE L'ÉCOLE NORMALE SUPÉRIEURE

UMR 8023
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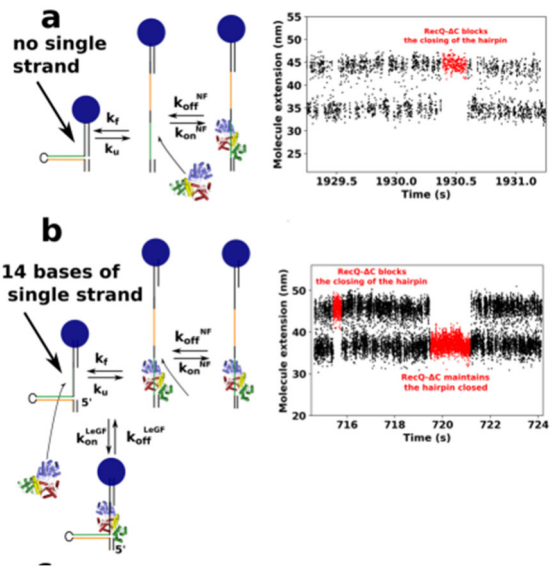
Micromanipulation study of RNA energy landscape modulation by epigenetics

The project proposed is interdisciplinary. It requires an interest in statistical physics, signal analysis, molecular biology, chemistry and has some potential applications in drug development.

Magnetic tweezers are a tool, initiated in our group, enabling real-time monitoring of nucleic acid extension on a single-molecule scale, at several hundred Hz. They are perfectly suited for tracking the temporal evolution between different molecular conformations of nucleic acids and measuring their energy differences as we already demonstrated. What's more, by imposing a tensile force on the molecules studied, it is possible to modify the energy profile and bring out minority conformations, i.e., those of higher energy. In the past, the application of force has already enabled us to observe the binding to DNA of proteins bound in a stable manner or with a lower affinity, as illustrated in the figure on the right in the case of a helicase, a molecular motor, in the absence of ATP, the fuel of the motor. In this example, the protein can bind to the single strand and block folding fluctuations of a stem-loop presenting two conformations (it is a two-level system), or hook onto the end of the stem-loop and limit its spontaneous opening (red dots in the curves).



Each of our cells contains our entire genetic heritage. Yet our organism is made up of very different cells. The differentiated expression of this genome according to the cell type relies on so-called "epigenetic" modifications. These modifications are essential to cell function, and their deregulation is associated with the development and progression of numerous pathologies. Understanding the mechanisms of epigenetic modifications is currently at the heart of new therapeutic approaches aimed at restoring deregulated mechanisms associated with human pathologies.

Of the more than 150 epitranscriptomic RNA modifications listed, methylation at position 6 of the RNA base adenosine (m6A) is the most widespread, whether in viral, bacterial, yeast, plant or mammalian RNAs. Whereas the methylation step adds only one carbon to the RNA, this modification will induce a change in the conformational landscape that the RNA can cover without greatly altering its thermodynamic stability. As with the methylation step, this increased structural flexibility seems to play an important role in the binding of proteins targeting this sequence in its methylated target. In order to analyze this mechanism in detail, it is essential to characterize the stable, but transient and often minority states in solution, recognized by the proteins targeting the RNA.

The proposed project will first study of the influence of methylation on RNA conformational flexibility at the single molecule level with magnetic tweezers. After the quantification of the conformational energy profile of the RNA, the influence of RNA methylation on protein binding on the sequence used will be investigated.

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On a longer term, during a PhD, potential drugs will be injected in the system and their influence on the binding properties of the proteins will be investigated. Such an approach will ultimately enable us to propose new therapeutic approaches based on molecules formally capable of restoring an unmethylated epigenetic phenotype, without modifying the RNA methylation state.

The M2 internship will be supervised by Pr. Jean-François Allemand, Ass. Pr. Jessica Valle-Orero and Dr. Vincent Croquette in LPENS. The project is a collaboration between the LPENS team and Dr. Laurent Micouin and Dr. Erica Benedetti from Paris Cité University at LCBPT lab.

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