Internship PROPOSAL

Laboratory name: Institut Cochin — CNRS identification code: UMR8104
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Innovative single-molecule methodology to improve the development of mRNA vaccines

All present and future mRNA vaccines will rely on efficient translation of mRNA. Therefore, both the stability and translatability of mRNA are essential determinants of vaccine efficiency. Moreover, it's well documented that these two properties are highly dependent on presence or absence of elements of three-dimensional structures adopted by mRNA (elements typically located at its 5' and 3' extremities, but also elsewhere in its sequence). We have undertaken to investigate these questions using a single-molecule force methodology that we have developed in recent years in our laboratory [1,2]. Funded by a Sanofi Innovation Awards grant, we will first use this methodology to investigate the structure of the 5'-untranslated region (5'-UTR) of existing and potential COVID-19 mRNA vaccines – in order to contribute to a better efficiency of these vaccines.

The single-molecule methodology that will be used during the proposed internship is schematized in Fig. 1 (and see also references 1-3). The optical-tweezers based assay relies on overstretching an RNA-DNA hybrid duplex (the mRNA of interest hybridised to its complementary DNA) that is tethered between two beads, such that three of the four strand ends are attached, while the fourth is left free. When forces above 60 pN are applied to the construct, the free (5') end of the RNA strand peels off, which generates a relaxed stretch of single-stranded RNA. Increasing the distance between the optical traps (black curve of Fig.1, right) generates a relaxed single-strand of increasing length, while reducing this distance (brown curve) results in progressive conversion of this strand back into the initial double-stranded configuration. The assay allows us to repetitively release the RNA molecule (starting from its 5' extremity) and let it fold in *in vivo*-like conditions. The corresponding force signals (more precisely, the difference between the force signal measured upon peeling and the one measured upon strand re-annealing) contain information about the structural elements present at the 5' extremity of the RNA molecule – corresponding to the 5'-UTR of the COVID-19 mRNA that we want to investigate.

The student will mostly be involved in single-molecule force measurements and their analysis: as such, a strong background in biophysics is important. He will also participate in the design and synthesis of the RNA/DNA molecules needed for the studies, and therefore he should have a good interest in the molecular biology techniques necessary for this part. Finally, the internship could potentially be followed by a PhD on the same topics.

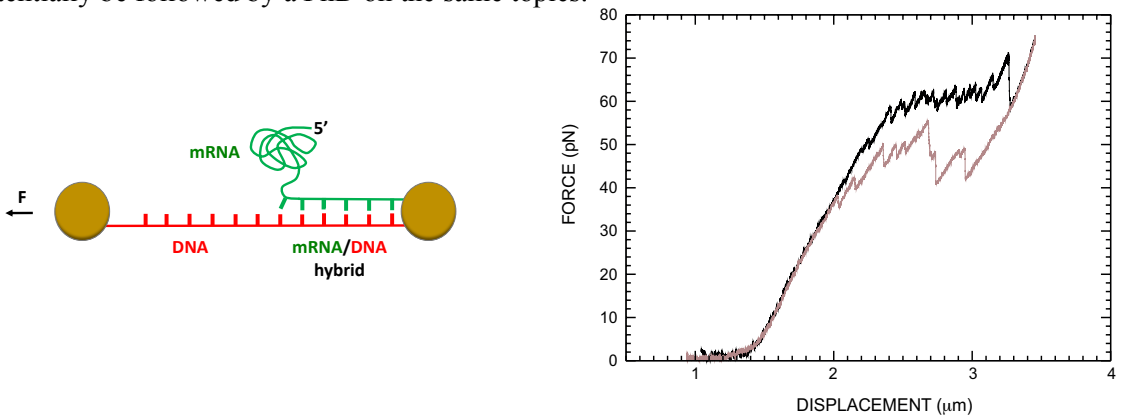

FIG. 1. Left: schematic depiction of the RNA-DNA construct undergoing peeling in the force measurement configuration. Right: force-extension relation measured upon peeling (black) and re-annealing (brown).

[1] L. Melkonyan et al, Biophys. J. 117, 509-519 (2019)
[2] U. Bockelmann, CNRS, International Patent Application WO 2020/141171 (2019)
[3] Gross et al, Nature Physics 7, 731-736 (2011)