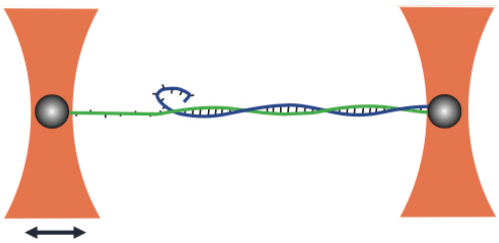
Single-molecule force measurements: a new methodology to improve the development of mRNA vaccines

We propose to improve the efficiency of mRNA vaccine development and consequently the quality of the resulting vaccines using a novel, biophysics-based-methodology (single-molecule force measurements), which allows to investigate mRNA structure with high precision (references 1 and 2).

mRNA vaccines development is undoubtedly one of the most promising medical fields - as underlined by the 2023 medicine Nobel prize. As exemplified by the COVID-19 mRNA vaccines, this technology mostly relies on the efficient translation of the mRNA into the desired antigen to drive the targeted protective immune response (reference 3). Therefore, both the stability and translatability of mRNAs 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 mRNAs (elements typically located at its 5' and 3' extremities, but also elsewhere in its sequence – reference 4).

To investigate the likely *in vivo* three-dimensional structure of an mRNA molecule, we use an optical-tweezers based assay (see figure below). The mRNA molecule of interest is annealed to a complementary DNA. This RNA/DNA hybrid duplex is chemically modified at its extremities and tethered between two microspheres. Three of the four strand ends are attached to the microspheres, while the fourth (at the RNA 5' end) is left free. When the microspheres are moved away from each other by two optical traps, the RNA strand progressively peels off, generating a single-stranded mRNA of experimentally-controlled length and *in vivo*-like three-dimensional structure. Afterwards, the beads are moved back closer to each other so that the double-stranded hybrid nucleic acid molecule reanneals. The force exerted on the nucleic acid is continuously measured during peeling and reannealing, the force signals are composed of sawtooth-shaped peaks containing detailed information about the structure of the mRNA. We perform several peeling/reannealing cycles on a single molecule during a timeframe of a few hours and measure tens of single molecules, both of which contribute to a very reliable experimental signal.

This biophysics-based-methodology is already well established in our laboratory: we have recently applied it successfully to perform preliminary investigations of vaccine-like SARS-CoV-2 mRNA structures (research supported by a Sanofi iAward 2021 grant). More generally, we have widely-recognized experience in the field of high precision force measurements and dispose of all necessary bioinformatics, biochemical, molecular biology and biophysics skills to perform them.



Schematic representation of the measurement configuration. An RNA/DNA hybrid molecular construct (mRNA in blue, its complementary DNA in green) is attached by three of its four single-strand extremities to two microscopic beads (beads and molecule are not at the same scale). The two beads of each dumbbell are captured in separate optical traps (orange). Force versus displacement curves are obtained by measuring the position of one bead within the trap to nanometre precision, whereas the other trap is displaced.

For the proposed internship, the student will be in charge of the following tasks: 1- measurements of single mRNA/DNA molecule force versus displacement curves with the optical traps. 2- analysis of step1 data: examination of the measured curves will reveal whether or not signatures of mRNAs structural elements are present and whether they're predicted to impact on the stability and translatability of the mRNA.

mRNA molecules investigated in this project will be vaccine-like SARS-CoV-2 mRNAs with (or without) modified nucleotides - as these modifications played such a crucial role in the success of COVID-19 mRNA vaccines (reference 5). Investigation of the impact of these chemical modifications on the structure of the mRNAs will thus be the main objective of the project, with the perspective to predict the impact of these modifications on mRNA stability and translatability.

These investigations can be carried on and extended during a subsequent PhD thesis. During the thesis, the student will also investigate RNA secondary structure modeling directed by our force measurement data: to establish the quantitative link between the measurements and the mRNA structure, we will follow a global empirical approach that uses an experimentally-derived pseudo-free energy change term, similar to the well-established SHAPE-directed RNA structure prediction procedures (reference 6).

References

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Contact
Ulrich Bockelmann
Equipe Biologie comparative des apicomplexes
Institut Cochin
22 rue Méchain, 75014 Paris
ulrich.bockelmann@inserm.fr
01.40.51.64.20

Institut Cochin
recherche biomédicale