Internship/PhD Project

NanoRatchet : Active transport at the nanoscale

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Biological nanopores are uncanny molecular machines that perform a wide variety of cellular functions, from sorting biomolecules to building cellular osmotic pressure and folding newly synthesised proteins. Their performance, as measured by their energy efficiency, directionality or selectivity, is unmatched by any other artificial system. In recent years, we have focused on one such nanopore, the nuclear pore, which consumes chemical energy (ATP hydrolysis) to transport macromolecules (proteins, DNA and RNA). In particular, we have studied the contribution of confinement, which dominates the transport properties for this type of object [1,2,3] but also for the transport of viral particles [4].

To go further and better understand the functioning of the nuclear pore we propose here a mimetic approach that selects thermal fluctuations to exert an active translocation force on the species present upstream. In this project, following the thesis work of Bastien Molcrette [3], we use a molecule placed downstream of the membrane, the ratchet agent, which allows to induce a directional transport similar to the transporters of the natural system (Figure 1a).

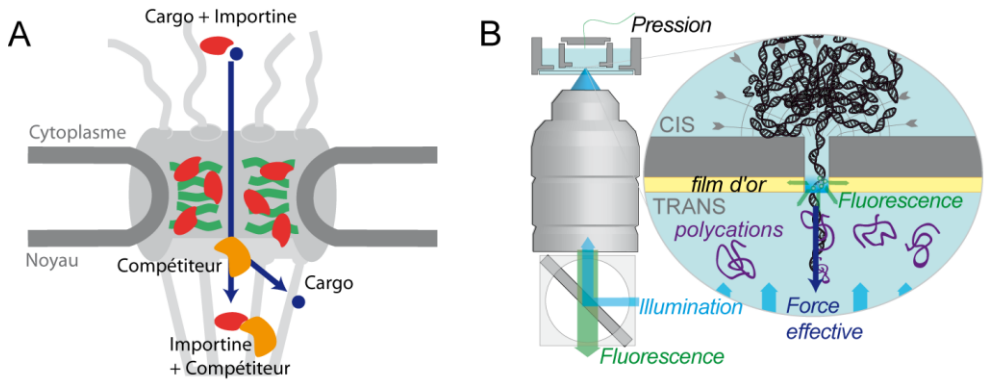

Figure 1 : (A) Mechanism of directional transport through the nuclear pore. The molecule to be transported (Cargo) is specifically recognised by a transporter (Importin). Once the complex is formed it can diffuse through the protein network (FG-nups) which acts as a barrier. The directionality of the movement is induced by the presence of a competitor that separates the complex and prevents the cargo from moving backwards. (B) The Zero Mode Waveguide technique allows the transport of individual molecules in single nanopores to be monitored in real time. The use of ratchet agents in the form of polycations allows to reproduce the directionality of transport observed in biological pores. Extracted from 3].

These molecules are able to bind strongly to DNA once they leave the upstream compartment but cannot diffuse into the upstream compartment (size exlusion). Their association with DNA therefore induces a bias in polymer diffusion and thus active transport of the polymer to the downstream compartment (Figure 1b). We will characterise these active nano-pumps for biomacromolecules such as DNA at the single molecule scale.

The transport of single macromolecules will be measured by a near-field optical technique developed in the laboratory (Zero-Mode Waveguide for nanopores [1], Figure 1b) and the forces involved will be measured using a unique in France optical tweezers system coupled to a confocal microscope and a

microfluidic system (Lumicks C-Trap). From this measurement, we will extract the change in the translocation energy landscape in the presence of ratchet agents.
In a second step, we will use the same experimental setup with nuclear Xenope envelopes and associated transporters. This will allow us to measure the energy efficiency of the transport in an ex-vivo system and to compare it directly to the phase landscape obtained with the mimetic system.
This work will provide access to the boundary parameters of nano-pumps and guide the understanding of natural nano-pumps such as the translocon and the nuclear pore. It will open the possibility of building minimal systems that reproduce the behaviour of these natural systems that are essential for the proper functioning of our cells.

Bibliography :

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3] Molcrette B, Chazot-Franguiadakis L, Liénard F, Balassy Z, Freton C, Grangeasse C, and Montel F. Experimental study of a nanoscale translocation ratchet. **Proceedings of the National Academy of Sciences** 2022, 119 (30) e2202527119.

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