Internship/PhD Project

Active transport at the nanoscale

Supervisors :

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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 [1]. 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 [2,3,4], but also the transport of viral particles [4].

To understand directional transport in biological nanopores we proposed here a mimetic approach that selects thermal fluctuations to exert an active translocation force on the species present upstream. During the PhD 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).

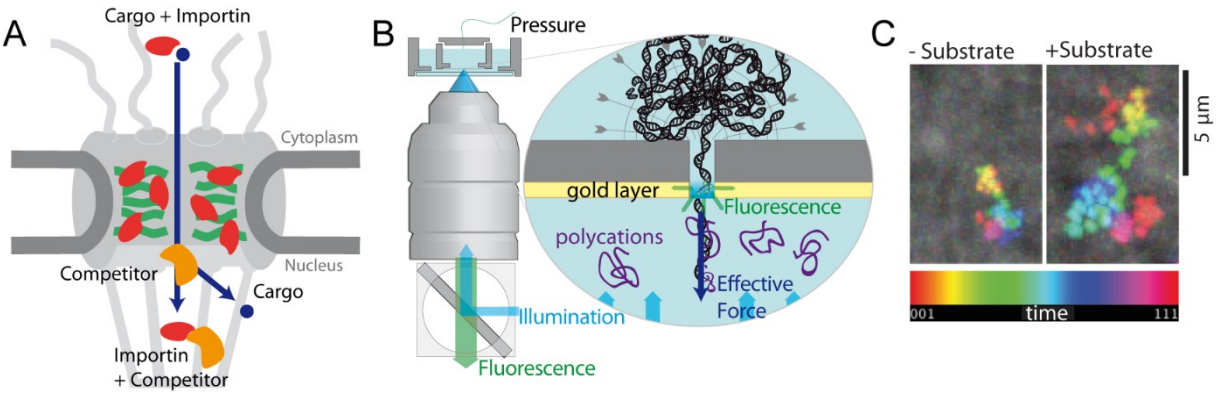

Figure 1 : (A) Mechanism of directional transport through the nuclear pore. The molecule to be transported (Cargo) is specifically recognised by a transporter (Importin). Once the complex is formed it can diffuse through the protein network (FG-nups) which acts as a barrier. The directionality of the 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]. C) 2D trajectories of a single enzyme (urease) with rainbow scale without and with the subtrate. Extracted from [7].

The ratchet agent 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).

In this internship/PhD project 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 [1,2], Figure 1b). Using a unique in France optical tweezers system coupled to a confocal microscope and a microfluidic system (Lumicks C-Trap) the forces involved in the transport will also be measured. From this measurement, we will extract the change in the translocation energy landscape in the presence of ratchet agents.

Complementarily to this ratchet strategy, other scenario involving active molecular responses may be envisioned to boost and direct translocation through nanopore. Indeed, it was recently suggested that unconfined enzyme molecules in presence of their energy-providing fuel molecule (substrate) exhibits a boosted diffusivity in solution as a result of the continuous binding/unbinding cycles with its substrate [6, 7]. In a second step, we will therefore investigate whether translocation of enzymes through nanopores can be sped up in presence of their substrate and whether directional transport can be achieved through gradients of this substrate between the two sides of the nanopore. Ultimately, this would allow us to address how this active enzymatic dynamics can couple to nearby macromolecules to assist their translocation across nanopores.

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 function of our cells.

References :

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