Internship/PhD Project

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Active transport through mimetic nanopores

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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, for instance, by their energy efficiency, is unmatched by any other artificial system. Some biological nanopores as the nuclear pore complex (Fig 1A) induce the directionnal transport of macromolecules such as DNA, RNA and proteins.

In previous years, we have developed bottom-up approaches to monitor the transport of biomolecules in synthetic nanopores that mimic their biological counterparts [1]. Depending on the type of molecule being transported (DNA, synthetic polymers, proteins, viruses), we have been able to identify the physical phenomena that control transport in each case [2-6]. For example, confinement entropy for nucleic acids [2-4] and binding between transported objects for viruses [5,6].

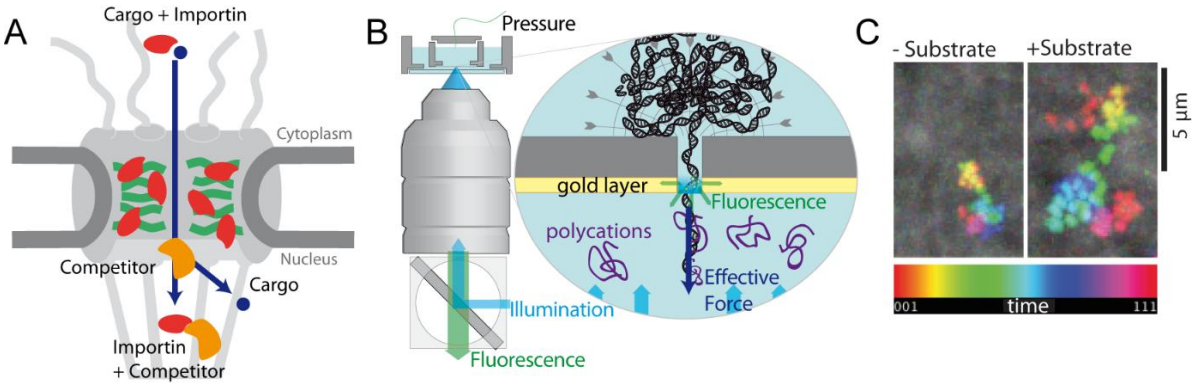

Figure 1 : (A) Mechanism of directional transport through the nuclear pore. The molecule to be transported (Cargo) is specifically recognised by a transporter (Importin). Once the complex is formed it can diffuse through the protein network (FG-nups) which acts as a barrier. The directionality of the 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].

In this internship/PhD project, our aim is to investigate directional transport using to two distincts scenario :

- **Translocation ratchet** : A molecular agent present downstream bind to the transported molecule and exert an effective translocation force on the species present upstream ([3], Fig 1B).
- **Translocation induced by the enhanced mobility of enzymes** in presence of their substrate ([7, 8], Fig 1C).

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], Fig1B). 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.

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.

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References :

1] Molcrette B, Chazot-Franguiadakis L, Auger T and Montel F. Quelques éléments de physique autour des nanopores biologiques. **Reflets de la Physique** 2023, Vol. 75, p18

2] Zero-mode waveguide detection of flow-driven DNA translocation through nanopores. Auger T, Mathé J, Viasnoff V, Charron G, Di Meglio JM, Auvray L, Montel F. **Physical Review Letters**. 2014 Jul 11;113(2):028302.

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.

4] Kolbeck PJ, Benaoudia D, Chazot-Franguiadakis L, Delecourt G, Mathé J, Li S, Bonnet R, Martin P, Lipfert J, Salvetti A, Boukhet M, Bennevault V, Lacroix JC, Guégan P and Montel F. Thermally switchable nanogate based on polymer phase transition. **NanoLetters** 2023, 23, 11, 4862–4869

5] Chazot-Franguiadakis L, Eid J, Socol M, Molcrette B, Guégan P, Mougel M, Salvetti A, and Montel F Optical Quantification by Nanopores of Viruses, Extracellular Vesicles and Nanoparticles. **NanoLetters** 2022, 22, 9, 3651–3658

6] Chazot-Franguiadakis L, Eid J, Delecourt G, Kolbeck PJ, Brugère S, Molcrette B, Socol M, Mougel M, Salvetti A, Démery V, Lacroix JC, Bennevault V, Guégan P, Castelnovo M, Montel F. Soft jamming of viral particles in nanopores. **Nat Commun.** 2024 Jul 23;15(1):6180. doi: 10.1038/s41467-024-50059-9. PMID: 39039059

7] Xu, M., Ross, J. L., Valdez, L. & Sen, A. Direct Single Molecule Imaging of Enhanced Enzyme Diffusion. **Physical Review Letters (2019),** 123, 128101.

8] Jee, A.-Y., Cho, Y.-K., Granick, S. & Tlusty, T. Catalytic enzymes are active matter. **Proceedings Of The National Academy Of Sciences Of The United States Of America (2018),** 115, E10812–E10821.