LPENS
LABORATOIRE DE PHYSIQUE
DE L'ÉCOLE NORMALE SUPÉRIEURE

UMR 8023
www.lpens.phys.ens.fr

Kinetics and energetics of molecular-size fusion pre-pores on asymmetric suspended membranes

1. Presentation and description of the project

A major pathway for trafficking in the cell is the fusion of small vesicles with target membranes. Typical examples are synaptic transmission and hormone release. Fusion is achieved by proteins that mechanically and cooperatively act to force the merging of the membranes. We have recently developed a microfluidic chip in which fusion can be recapitulated. Measuring pico-currents through the membrane, we observed series of metastable states triggered by the sequential action of these proteins. These states corresponds to pre-pores, with a size of a fraction of a nanometer. The purpose of the internship is to characterize these pre-pores. What is their dynamics? How are they molecularly arranged? What are the associated energy landscapes?

2. Techniques / methods used

Recapitulating membrane fusion quasi-physiologically was until recently technically impossible because the model membranes used did not satisfy all the required. Our laboratory has developed a membrane that meets all these criteria. A 100 µm diameter circular membrane is formed on a microfluidic chip built from 3D-printed molds. This membrane is suspended between two microfluidic channels. It can be observed by confocal microscopy and, simultaneously, its electrical properties can be measured, which makes it possible to follow the fusion pore with great spatial (~0.1nm) and temporal (10 µs) precisions. This membrane will be the template on which the research will be carried out.

3. Expected results

The molecular nature of the pre-pores remains unknown. For instance, it may be a hole that can be crossed by water and ions or a membrane defects, in which lipids are very dynamics. To determine this molecular organization, the membrane composition and ions in the buffers will be varied. In each situation, the durations and size variation of the pre-pores will be established, providing their complete energy landscape.

4. Skills

Experience in manipulating microfluidic chips, use of digital light processing/Stereolithography 3D-printer and confocal microscopy would be helpful but is not a requirement. All software for analysis are written in MatLab. English is required because our biologist collaborators that provide the proteins are Americans.

5. References and contact

More details and references will be provided to interested students, please contact Frédéric Pincet at the address indicated below.
Funding for a follow-up PhD is already secured.

Contact :
Frédéric Pincet
Laboratoire de Physique de l'ENS, Paris
E-mail: Frederic.pincet@ens.fr