Regulating membrane fusion with DNA origamis

1. State of the art

a. Vesicular trafficking in the cell

Membranes are ubiquitous among living organisms, defining physically the inside from the outside of cells and organelles and maintaining the inner physico-chemical conditions ensuring life[1]. Such fundamental character stems from the lipids, their building block, that self-assemble in a double leaflet because of the hydrophobic interactions of the lipid tails. However, cells need to extract chemical components from the exterior and to exchange biomolecules between their inner compartments. Vesicular trafficking is the most observed process to exchange in and outside components and it can be viewed in three steps[2]. First, a ~50 nm lipid vesicle that contains the molecular cargo to transport is formed at the donor compartment. Second, the travel of the vesicle to reach the acceptor compartment located at another location. Third, the fusion of the vesicle with the membrane of the target compartment leads to the opening and expansion of a fusion pore between the vesicle and the target membrane. Fusion between a vesicle and a lipid bilayer has a high energy barrier of 25-30 k_BT due to the important molecular rearrangement necessary to break the continuity of the two membranes[3].

b. Fusion is mediated by SNARE proteins

The fusion process is mediated *in vivo* by SNARE proteins, with the vSNAREs located on the vesicular membrane and the tSNAREs on the target membrane. The SNAREs mediating neurotransmission are the most studied and used to understand the fundamental mechanism of membrane fusion. vSNAREs, emanating from the vesicle membrane, and tSNAREs, from the target membrane, can hybridise by forming a four helixes complex called SNAREpin. Three helices come from the tSNARE (a hetero dimer with two proteins: Syntaxin1A and SNAP25) and one helix comes from the vSNARE (one protein: VAMP2). Upon complete hybridization, the SNAREpins force the two membranes to get in close proximity (2-3 nm) and provide enough energy to destabilise the two double leaflets, triggering the fusion.

c. Regulated fusion: Munc13 and Synaptotagmin, essential effector proteins

In vivo, fusion is a highly regulated process. SNARE proteins are surrounded by effector proteins that position the vesicles and the SNAREs in order to facilitate fusion. Two well-characterised effector proteins for the regulated fusion are Munc13 and synaptotagmin . Munc13 acts at the very beginning of the fusion process. By binding the vesicle and subsequently folding, this massive 182 kDa protein bridges and brings the vesicle and the target membrane close to each other (from 15 nm to 10 nm, see Figure panels a-c). This induced proximity is suspected to start the hybridization of vSNAREs and tSNAREs. Once Munc13 has triggered SNARE assembly, the SNAREpin will fold spontaneously and quickly. *In vivo*, complete SNAREpin assembly is clamped by synaptotagmin that blocks the vesicle in a “ready-to-fuse” state (Fig., panel c). Whenever neuron depolarization occurs, calcium ions enter the cell and bind to synaptotagmin (panel d). Calcium binding changes synaptotagmin conformation, thereby releasing the SNAREpins and allowing the completion of SNAREpin assembly and subsequent fast fusion (Fig. panel e).

2. Question emerging from our current knowledge - aims of the PhD

Despite intensive research on SNAREs since their discovery as the minimum machinery for fusion in the 90’s the molecular orchestration of this fusion is still poorly understood at a quantitative level. Many fundamental questions remain about SNAREs mediated fusion. For instance: Is there an optimal distance between the vesicle and the membrane for vSNARE and tSNARE hybridization? How fast does the fusion occur depending on the SNAREpin number? Is there an optimal inter SNAREpin distance for fast fusion? Is there an optimal exploration volume for Munc13 resulting from the balance between the probability to encounter a vesicle and the energy cost to bring the vesicle close to the target membrane ? How far must Synaptotagmin keep the membrane to prevent full zipping of the SNAREpin and stabilize the ready-to-fuse state? What is the optimal inter-membrane equilibrium distance in the ready-to-fuse state for fast fusion,

resulting from the balance between the diffusion of the synaptotagmin and the travel distance of the vesicle toward the target membrane?

Neurotransmission is a highly regulated and complex process *in vivo*. Although some information can be gathered partially by molecular alteration (e.g., deletion, mutation, etc.), it is impossible to omit undesired effects by various related molecules. Most attempts to determine the kinetics and energy landscape of SNARE proteins in presence of effectors have failed, creating a gap between *in vivo* observations and *in vitro* quantitative results obtained for fusion with SNAREs alone.

Our aim is to bridge this gap by combining conventional *in vitro* SNARE-mediated fusion technique with DNA origami to mimic the mechanical and structural role of Munc13 and synaptotagmin. The simple rule of nucleic acid base pairing gives to DNA origami a wide structural and functional modularity that allows using them as machines that act on their surroundings and/or as dynamic building blocks in complex structures[4, 5]. Using DNA origami, it is also possible to preorganize protein at nanometric scale just like effector proteins are doing on SNARE. In this PhD proposal, we propose to use this approach by mimicking Munc13 and synaptotagmin with dynamic DNA origami on a novel model membrane suspended between two microfluidic channels[6, 7] to access the kinetics and energy landscape of regulated fusion. This will be the first example in which the sequential action of two proteins is fully reproduced at the molecular level by synthetic material. It will open the door to the ability to replace a whole protein pathway by a series of artificially triggered events. Proteins that are difficult to purify could also be replaced by this type of material in model systems.

3. Methodology

We envision mimicking Munc13 and synaptotagmin by placing a DNA ring on the target membrane functionalized with vertical DNA pillars facing the aqueous phase. The ring will have the role of controlling the tSNARE number as well as the inter-tSNARE distances (Fig. panel f). The pillars will reproduce the mechanical functions of both the Munc13 and synaptotagmin. In their initial state the pillars are composed of a rigid part, bound to the DNA ring and a flexible part composed of non-hybridized single strand DNA (t-ssDNA) (Fig. panel f). The t-ssDNA will be able to diffuse around till it encounters a vesicle bearing v-SNARE and single-stranded DNA oligonucleotides (v-ssDNA) complementary to the t-ssDNA. The hybridization of the t-ssDNA and the v-ssDNA will trigger the folding of the t-ssDNA trapping the vesicle at 10 nm from the target membrane, mimicking the function of Munc13 (Fig. panel g and h). At this distance, SNAREpins can initiate their formation but cannot zip completely because of the rigidity of the pillars. The structure is clamped in a stable ready-to-fuse state, like synaptotagmin does *in vivo*. To move towards fusion, the clamp needs to be released. This release will be achieved by adding oligos able to unfold the pillars (Fig. panel i) thereby freeing the SNAREpins and mediating membrane fusion (Fig. panel j). This final step is identical to the binding of calcium on synaptotagmin. Such design provides the tools to address the questions stated above by changing the diameter of the ring, the number of bound tSNARE, their orientation, the length of the pillars and the length of v and t-ssDNA.

4. Complementarity of the two laboratories where the PhD will take place

The project envisioned here will require techniques and knowledge coming from various fields of science. The PhD will take place in two laboratories in Paris, the Institut de Psychiatrie et Neurosciences de Paris (IPNP, Inserm) and the Laboratoire de Physique de l'Ecole Normale Supérieure (LPENS, associated to SU). The PhD student will also have the opportunity to visit the lab of C. Lin (2nd co-PI) at Yale School of Medicine to design, construct and characterize by electron microscopy the DNA nanoscaffold that will mimic Munc13 and synaptotagmin. The Molecular Biology aspect of the project will be supervized by Paul Heo (co-PI) at IPNP. There SNAREs will be purified and characterized biochemically and functionally. The Biophysics part of the project will be done with Frédéric Pincet (PI) at LPENS. There, the experiments on the suspended

membrane in the microfluidic chip will be performed by using the SNAREs and the DNA scaffold to perform measurements on the regulated fusion mimic system.

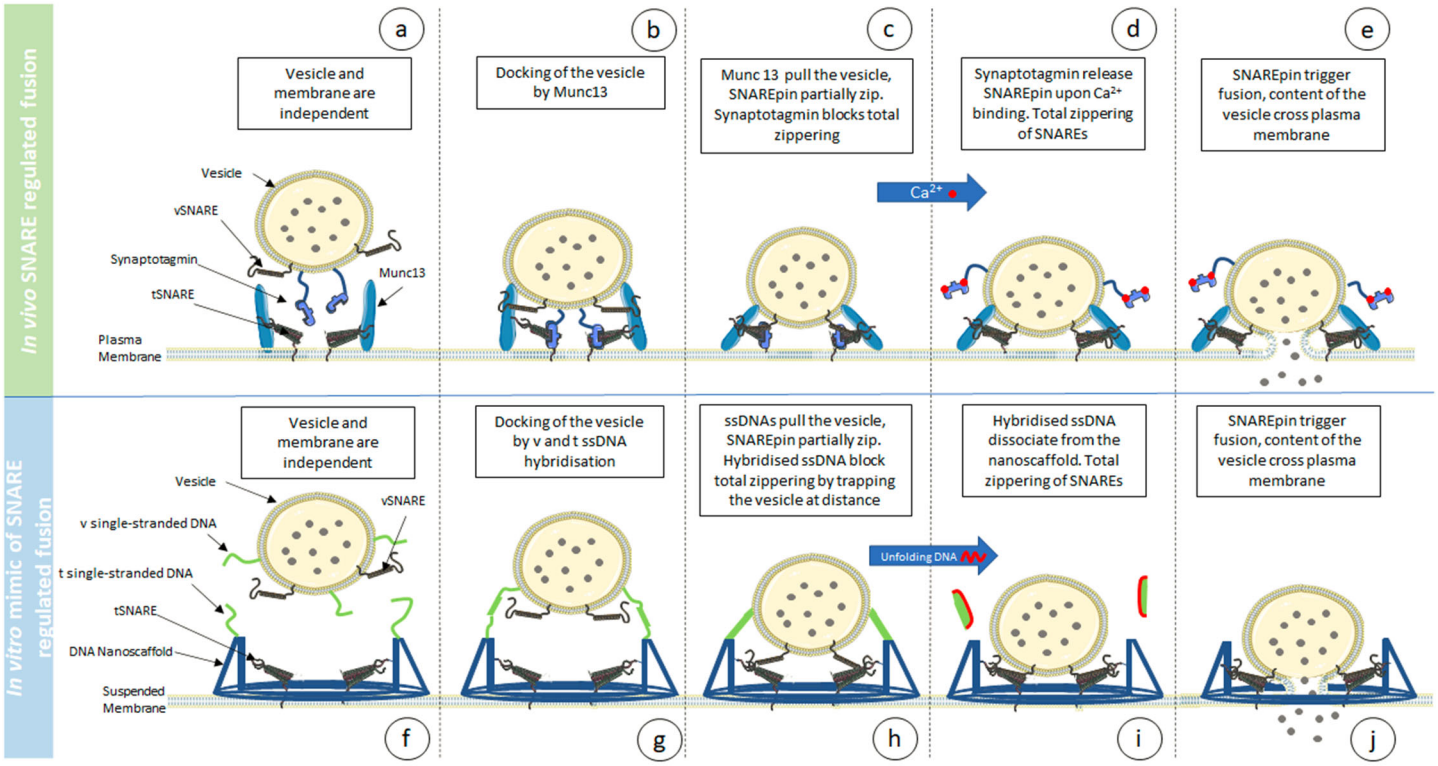

Figure: Top panels: SNARE regulated fusion pathway *in vivo*. (a) a vesicle functionalized with vSNARE and Synaptotagmin and the plasma membrane functionalised with Munc13 and tSNARE are getting closer. (b) Once the vesicle is ~20 nm far from the plasma membrane, Munc13 is docks the vesicle to the plasma membrane. (c) Munc13 folds in direction of the plasma membrane pulling the vesicle toward the plasma membrane at ~10 nm. vSNARE and tSNARE start hybridizing into a SNAREpin. Synaptotagmin blocks the full hybridization of SNAREpins trapping the vesicle in a ready-to-fuse state. (d) Incoming calcium ions bind to Synaptotagmin that changes conformation, freeing the SNAREpins that fully zippers at this point, leading to the opening of a fusion pore and the release of the cargo molecules (e).
Bottom panels: *in vitro* mimic system of the SNARE regulated fusion: (f) a vesicle functionalised by vSNARE and v-single stranded DNA (v-ssDNA) and a suspended asymmetric membrane functionalised by tSNARE and a DNA nanoscaffold are getting closer. The DNA origami is composed of a DNA ring disposed on the membrane that constraint the movements of tSNARE and present vertical pillars with at their very end t-single stranded DNA (t-ssDNA). The t-ssDNA are complementary strand of the v-ssDNA. (g) Once the vesicle is close enough, the t-ssDNA and v-ssDNA get in contact and start hybridizing, the vesicle is now docked to the DNA nanoscaffold. After the docking step, v-ssDNA and t-ssDNA continue to hybridize pulling the vesicle toward the plasma membrane, initiating SNAREpin formation. (h) Upon total hybridisation v-ssDNA and t-ssDNA form a rigid structure that imposes a distance large enough to avoid full SNAREpin assembly. (i) Addition of unfolding DNA strand detaches v- and t-ssDNA from the vesicle and the nanoscaffold, allowing total SNAREpin hybridization, leading to the opening of a fusion pore and the release of the cargo molecules (panel j).

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