New Strategy to Study a Single SNARE Mediated Membrane Fusion Event

<u>Jean-Baptiste Fleury</u>¹, Jose Nabor Vargas¹, Kewin Howan², Ralf Seemann^{1,3}, Andrea Gohlke^{2,4}, James E. Rothman⁴, and Frederic Pincet^{2,4}

¹Experimental Physics, Saarland University, Saarbrücken, Germany, ²Laboratoire de Physique Statistique, Ecole Normale Supérieure, 75005 Paris, France, ³Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany and ⁴Department of Cell Biology, School of Medicine, Yale University, CT 06520 New Haven, USA

We present an approach to explore the properties of a single SNARE mediated membrane fusion event in a microfluidic chip. In a first step, a single free standing lipid membrane is generated at a defined position with the Droplet Interface Bilayer technique (DiB). In a second step, we inject a solution of divalent cations (Calcium, Ca2+) and small unilamellar vesicles functionalized with T-SNARE proteins (T-SUVs) around the planar membrane using a volume controlled flow. The presence of calcium mediates the direct fusion of the vesicles with the planar membrane, which is incorporating the proteins into the membrane. In a third step, we remove the calcium and the T-SUVs with a buffer solution. After this washing step, a solution of small unilamellar vesicles functionalized with V-SNARE proteins (V-SUVs) is injected around the planar membrane. And finally, we study single fusion event with good optical and electrical access.

(in preparation)