Synaptotagmin interactions with membranes: measuring the force of calcium triggering of neurotransmission

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A critical step of neurotransmission is the rapid and synchronized fusion of synaptic vesicles with the pre-synaptic plasma membrane of the neuron upon calcium entry, allowing the release of neurotransmitters immediately upon arrival of an action potential. A calcium binding protein anchored in the synaptic vesicle, the Synaptotagmin-1 (Syt1), has been identified as the calcium sensor of this process, being able to accelerate fusion by more than four orders of magnitude in presence of calcium. Despite this crucial role, the molecular mechanism involved remains unclear.

The cytosolic domain of Syt1 consists of tandem Ca2+- binding C2 domains (C2A and C2B) attached to the membrane via a juxtamembrane linker domain. Different sites of the protein are responsible for its specific roles. First, the interaction of the polybasic region of the C2B domain with the anionic lipid PIP2 is needed for the initial docking of the synaptic vesicle at the plasma membrane. Second, upon calcium binding, the aliphatic loops on each C2 domains partially insert into the membrane, enabling the SNARE proteins to complete membrane fusion.

While the mechanistic details described above are mostly well-accepted, importantly, the binding energies of Syt1 with membranes have never been reported. Therefore, the aim of our work is to measure the energetics of the major membrane binding sites of Syt1, i.e. the polybasic motif and the calcium-loop insertions of C2B and C2A. For this we use a Surface Force Apparatus (SFA), a device that provides a direct measurement of the interaction force between two surfaces as a function of their separation distance, with nanoscale resolution. This system has successfully been used to measure the energy of the SNARE proteins assembly, but the previous experiments did not include Syt1. In our set up, both surfaces are coated with a lipid bilayer. One of them mimics the synaptic vesicle membrane, on which we bind Syt1. The opposing bilayer mimics the inner leaflet of the plasma membrane and contains PIP2 and PS lipids. We will present results obtained with various lipid compositions and relevant mutations of the protein and how these interactions are impacted by the presence of calcium. Ultimately, we plan to provide a complete mapping of the energetics of the critical membrane interaction sites of Syt1.