

## AFM studies of SNAREs interactions during membrane fusion and fission

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Liposomes reconstituted with SNARE proteins have been used to investigate membrane fusion. Membrane fusion is a physiologic process necessary for homeostasis. SNAREs are considered the minimal machinery for membrane fusion *in vitro*. We employed the atomic force microscope (AFM) to measure forces required to induce fusion between two apposing lipid bilayers during the approach step, and rupture forces associated with the dissociation of v- and t-SNARE complexes during the retract step. Egg phosphatidylcholine (egg PC) bilayers containing v-SNAREs (synaptobrevin 2) or binary t-SNAREs (syntaxin 1 and SNAP 25) were formed by lipid vesicle adsorption onto opposite glass dishes and glass microbeads attached to cantilever tips. Fusion and rupture (SNARE complexes) forces increased with loading rate (LR). The dynamic force spectrum (DFS) of the fusion process revealed a single linear loading regime in presence/absence of SNAREs. This suggests that, in the absence of applied force, membrane fusion is governed by one energy barrier under the current conditions. In the presence of SNAREs, the width of the energy barrier increased by ~3 fold compared to that in egg PC alone, whereas the activation potential was unchanged. This suggests that the interaction of cognate v- and t-SNAREs facilitates membrane fusion by reducing the slope of the energy barrier. On the other hand, the DFS of the SNARE complex dissociation revealed two loading regimes, a fast regime at high LRs and a slow one at low LRs, suggesting the presence of two energy barriers of the dissociation process of v-SNARE/t-SNARE complexes. The inner and outer barrier widths were 0.39Å and 2.9Å, and dissociation rate constants were 15.5 s<sup>-1</sup> and 0.3 s<sup>-1</sup>, respectively. These energy barrier parameters suggest that under applied external force, the dissociation of the v-SNARE/t-SNARE complex is effectively dominated by the inner barrier.