Kinetic transitions in individual recombinant exocytotic fusion pores

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Understanding the mechanism(s) by which proteins catalyze the merger of lipid bilayers hinges on elucidating the structure and dynamics of the first crucial intermediate in the membrane fusion pathway, the fusion pore (Jackson and Chapman, 2006; Lindau and Almers, 1995). We recently developed new tools that allow us to interrogate, electrophysiologically, recombinant fusion pores held in their nascent open state, thus yielding the first µsec time-resolved measurements of these channel-like structures (Bao et al., 2018; Das et al., 2020). This was accomplished by reconstituting v-SNAREs into nanodiscs (ND) and t-SNAREs into planar lipid bilayers (called black lipid membranes [BLM]). As v- and t-SNAREs assemble into *trans*-SNARE complexes to form a fusion pore between the ND and BLM, the rigid membrane scaffold that forms a belt around the ND limits expansion, trapping the pore in an open state. We combined this approach with single molecule fluorescence experiments to address how regulatory proteins control the assembly and disassembly of *trans*-SNARE complexes to drive the formation and dissolution of fusion pores, respectively. These studies have begun to relate structural changes in the fusion machinery with kinetic transitions in fusion pores in a fully defined, experimentally accessible, model system.

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