

New and Notable

Imaging Fast SNARE Mediated-Membrane Fusion in Planar-Supported Bilayers

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Seven years ago, Rothman and co-workers reported that the pairing of SNARE molecules constitutes a minimal machinery for the fusion of two membranes (1), providing support for the earlier formulated SNARE hypothesis. Their conclusion was drawn from experiments *in vitro* in which the plasma membrane protein complex syntaxin1A/SNAP25 as t-SNARE and the synaptic vesicle membrane protein vAMP2 as v-SNARE were reconstituted into lipid vesicles. Although this assay clearly demonstrated that t- and v-SNAREs were sufficient to fuse liposomes, the kinetics of the reaction were disturbingly slow. The half-time of fusion was on the order of 10 min, i.e., $>10^5$ times slower than Ca^{2+} -triggered synaptic vesicle fusion in neurons (2).

On pages 2458–2472 of this issue, Liu et al. present a vesicle-planar bilayer fusion assay with a time constant for vesicle fusion of 25 ms (3). To achieve this relatively fast fusion rate, the authors reconstituted syntaxin1A/SNAP25 into glass-supported lipid model membranes composed of the synthetic lipids POPC and DOPS. Planar-supported membranes provide an interesting and very useful new approach to study the complex molecular machinery of exocytosis and membrane fusion. The model system allows the investigator to adjust specific lipid and protein compositions and the planar

geometry supports the use of total internal reflection fluorescence microscopy to monitor vesicle docking and fusion. In 1994, Hinterdorfer et al. first reported a supported membrane assay to observe influenza hemagglutinin mediated viral fusion by total internal reflection fluorescence microscopy (4). The first successful reconstitution of SNARE proteins into planar membranes was achieved by Wagner et al. in 2001 (5). New fast and sensitive charge-coupled device cameras made it possible to image individual vesicles while they dock and fuse with a target membrane and therefore overcome the limitation of the previous ensemble measurements. First experiments of this kind were reported last year by Fix et al. (6) and Bowen et al. (7). Although faster fusion kinetics were observed in these studies than in the vesicle-vesicle fusion assays, they were still slow compared to *in vivo* fusion measurements.

By acquiring fast images with a time resolution of 5 ms and tracking individual vesicles as they appear at the bilayer surface, Liu et al. now show that 77% of synaptobrevin-containing vesicles fuse within 100 ms after they have docked to the membrane. The remaining vesicles fused within 4 s after docking. The vesicles were labeled with the lipid probe TMR-DHPE, and fusion events were recognized as sudden bursts of fluorescence intensity mainly due to dequenching and polarization effects, followed by subsequent diffusion of the probes into the plane of the target membrane. The decrease of the fluorescence signal at a certain fusion site allowed for determination of the diffusion constant of the transferred lipid probes and demonstrated the high quality of the supported membrane—a prerequisite for this kind of assay. The vesicle-to-planar membrane fusion the authors observed was independent of divalent cations and independent of SNAP25. The surprising independence on the presence of SNAP25 is consistent with the results of Bowen et al. (7), but unusual for reconstituted vesicle-vesicle

fusion or synaptic fusion *in vivo*. In addition to determining rates of fusion, Liu et al. were also able to determine the rate of docking as well as the density of effective docking sites. A comparison of conditions in the three systems might help explain qualitative and quantitative differences of the fusion behavior observed by the three groups.

Most conspicuous are differences in composition of the target membranes. Fix et al. (6) used synthetic POPC lipids and a higher density (lipid/protein: ~ 3000) of coexpressed syntaxin1A/SNAP25 complex. Fusion was triggered by adding Ca^{2+} ions in their assay. Bowen et al. (7) used extracted lipid mixtures of eggPC and brainPS and a low density (lipid/protein: $\sim 14,000$) of syntaxin in their membranes and triggered fusion thermally. Liu et al. were able to achieve high fusion rates without an external trigger when they used a very low density (lipid/protein: $\sim 30,000$) of either syntaxin1A or syntaxin1A/SNAP25 complexes in a membrane consisting of the synthetic lipids POPC and DOPS. Although the authors showed that a high density of t-SNAREs prevents fusion in their system, the influence of the lipid environment was not studied systematically. By comparing the experiments of the different groups, it seems that the lipid composition indeed plays a crucial role in vesicle membrane fusion.

The observation of fast fusion events coupled with rates of lipid diffusion similar to those observed in cell membranes are evidence for the high quality of Liu et al.'s planar-supported membranes and prove that this constitutes an excellent experimental system to address questions that remain to more fully understand exocytotic membrane fusion at the molecular mechanistic level. The variation of lipid and protein compositions as well as the use of membrane and content labels should further unveil details about the molecular mechanisms and kinetics of pore

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formation during membrane fusion. It will be interesting to see if further perfection of this assay will resolve remaining questions about the role of SNAP25 and other accessory proteins in synaptic membrane fusion and eventually raise the fusion rate further to those observed in cells.

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