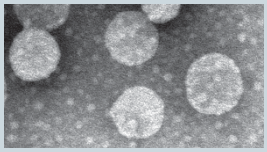
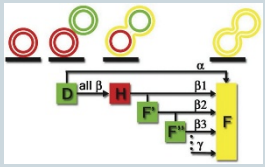
Earnshaw, W.C. *J. Cell Sci.* **117**, 5461–5477 (2004).
5. Wigge, P.A. & Kilmartin, J.V. *J. Cell Biol.* **152**, 349–360 (2001).
6. McCleland, M.L. *et al. Curr. Biol.* **14**, 131–137 (2004).
7. Wei, R.R., Sorger, P.K. & Harrison, S.C. *Proc. Natl. Acad. Sci. USA* **102**, 5363–5367 (2005).
8. Deluca, J.G. *et al.* **16**, 519–531 *Mol. Biol. Cell* (2004).
9. McCleland, M.L. *et al. Genes Dev.* **17**, 101–114 (2003).
10. Stradal, T., Kranewitter, W., Winder, S.J. & Gimona, M. *FEBS Lett.* **431**, 134–137 (1998).
11. Hayashi, I. & Ikura, M. *J. Biol. Chem.* **278**, 36430–36434 (2003).
12. Kline-Smith, S.L., Sandall, S. & Desai, A. *Curr. Opin. Cell Biol.* **17**, 35–46 (2005).
13. Hill, T.L. *Proc. Natl. Acad. Sci. USA* **82**, 4404–4408 (1985).
14. Tanaka, T.U. *et al. Cell* **108**, 317–329 (2002).
15. Shang, C. *et al. Mol. Biol. Cell* **14**, 3342–3355 (2003).
16. Cimini, D., Wan, X., Hirel, C.B. & Salmon, E.D. *Curr. Biol.* **16**, 1711–1718 (2006).
17. Joglekar, A.P., Bouck, D.C., Molk, J.N., Bloom, K.S. & Salmon, E.D. *Nat. Cell Biol.* **8**, 581–585 (2006).
18. Emanuele, M.J., McCleland, M.L., Satinover, D.L. & Stukenberg, P.T. *Mol. Biol. Cell* **16**, 4882–4892 (2005).

All mixed up

In eukaryotic cells, membrane-encased organelles exchange information by pinching off vesicles, which fuse with a target organelle's membrane and release their contents into it. The process of membrane fusion involves a class of proteins known as SNAREs. Vesicles that are budded from the endoplasmic reticulum have v-SNAREs on their surface, whereas target membranescontain t-SNAREs. The interaction of v- and t-SNAREs leads to the formation of a joint coiled coil from unstructured regions of each SNARE, and the energy released drives fusion.





Studies have indicated that fusion may involve an intermediate (hemifusion) state in which the outer membranes, but not the inner membranes, are fused. Other work has suggested that hemifusion intermediates represent off-pathway, reversible events. Distinguishing between these models requires a method to look at individual fusion events, as the intermediates are difficult to characterize by bulk solution methods.

Recent work from Ha and colleagues describes the use of a single-molecule approach to follow the process of membrane fusion in real time (*Proc. Natl. Acad. Sci. USA*, published online 13 December 2006, doi:10.1073/pnas.0606032103). Liposomes are differentially labeled with fluorophores and contain either a yeast t-SNARE or a v-SNARE. The v-SNARE liposomes are attached to a surface and the t-SNARE liposomes are flowed through the cell. When the two SNARE liposomes dock, an increase in FRET signal is observed, the amplitude of which corresponds to the degree of lipid mixing of the two membranes. Control experiments show a low background FRET signal when SNARE liposomes can dock but not fuse owing to the absence of Sec9c, a yeast protein required for a complete SNARE complex. The authors observed that the magnitude of the FRET signal is dependent on the level of Sec9c; at lower Sec9c concentration, where hemifusion has been reported to occur, the FRET efficiency is lower.

Fusion was next followed in real time. The initial docking reaction requires about 4 min, suggesting that this step is rate limiting, as fusion occurs more rapidly. Three types of traces were seen. In the first type (α), docking led to full fusion without passing through any discernable intermediate, although it is possible that the dwell time in the intermediate states is too short to be detected. The second type (β) showed evidence of one or more distinct intermediate states before full fusion. The final type (γ) contained intermediate forms, but as a continuum rather than as distinct states. The β type tracings had a common intermediate, which was identified as the hemifusion state. Its presence suggests the hemifusion is on the pathway to full fusion. The other β intermediates are proposed to be new states that have not been detected in bulk experiments, which could be associated with a phenomenon known as fusion-pore 'flickering' — closing of the pore before lipid mixing is complete. More rarely, tracings were obtained that were interpreted as partially restricted hemifusion or 'kiss-and-run' events.

These results show that single-molecule approaches can be used to examine the quantitative and dynamic aspects of the pathways followed during membrane fusion. For example, the dwell times of the hemifusion and subsequent intermediate states were progressively longer than the docking state, suggesting that there is a greater energetic barrier between hemifusion and pore-formation states than between docking and hemifusion states. Such information is obtainable only through single-molecule measurements. ***Angela K Eggleston***