Biswas et al, http://www.jgp.org/cgi/doi/10.1085/jgp.200709932

Assay Resolutions

One question that arises is whether there is sufficient sensitivity to detect newly appearing fluorescence of our content probe during the observed times. If the increase in fluorescence is due to flux of dye through the pore and diffusion to the HAS lumen, one can calculate the time dye should take to redistribute in the ~15.5-μm radius HAS cell (estimated from capacitance) and whether the calculated distribution time is comparable to our time resolution of detected changes in fluorescence. From Einstein's relation, the time required for redistribution of aqueous dye in an HAS cell is $\tau = (r)^2/6D$ (Hille, 1992), where r is the radius of an HAS cell and D is the diffusion coefficient of CF. Inserting r = 15.5 μm and $D = 4 \times 10^{-6}$ cm^2/s for CF in water (Brink and Raman, 1985), τ is ~100 ms for CF. In our experimental setup, the fluorescence change above background noise can be detected with a time resolution (Fig. 6 A) for recording fusion events in the range of seconds. Thus we have sufficient sensitivity to detect a fluorescence change within our experimental times.

Image Acquisition and Data Analysis

Fluorescence images (video) of low pH–triggered dye transfer form labeled RBC to HAS cells were recorded with Cascade 512b camera (Roper Scientific, exposure time 10 ms, binning 4; 30,000 frames). Image frames were captured using IPLab 3.6 software (Scanalytic Inc.). Time zero in the video is the pH pulse. Beginning of the videos were clipped to show the difference qualitatively near the end of fusion reaction.

Cholesterol Affects Fusion Phenotype and/or Extent of Fusion

There was a difference in the extent of fusion between untreated and cholesterol pairs. To show the difference qualitatively near the end of the fusion reaction, a sequence of the video is presented on the website. In untreated pairs the RBC did not completely fuse with the HAS cell membrane with full content (CF) mixing. Thus the two distinct compartments (HAS and RBC) remained separate throughout the experiment, some amount of dye retained in the RBC could be seen at the end of the acquisition (Video 1, control).

With increased cholesterol, not only did bound RBC completely release their lumenal contents to HAS cells, but the membrane of the target RBC flattened and completely collapsed into the curve of the cholesterol-enriched HAS cell membrane, suggesting syncytium formation after complete opening of the omega-figured fusion pore (Video 2, cholesterol). Thus the presence of excess cholesterol allowed the fusion pathway to go to completion. Thus the effect of cholesterol on the fusion pore is on the late phases of pore expansion.