Supporting Information

Quantitative Fluorescence Microscopy to Determine Molecular Occupancy of Phospholipid Vesicles

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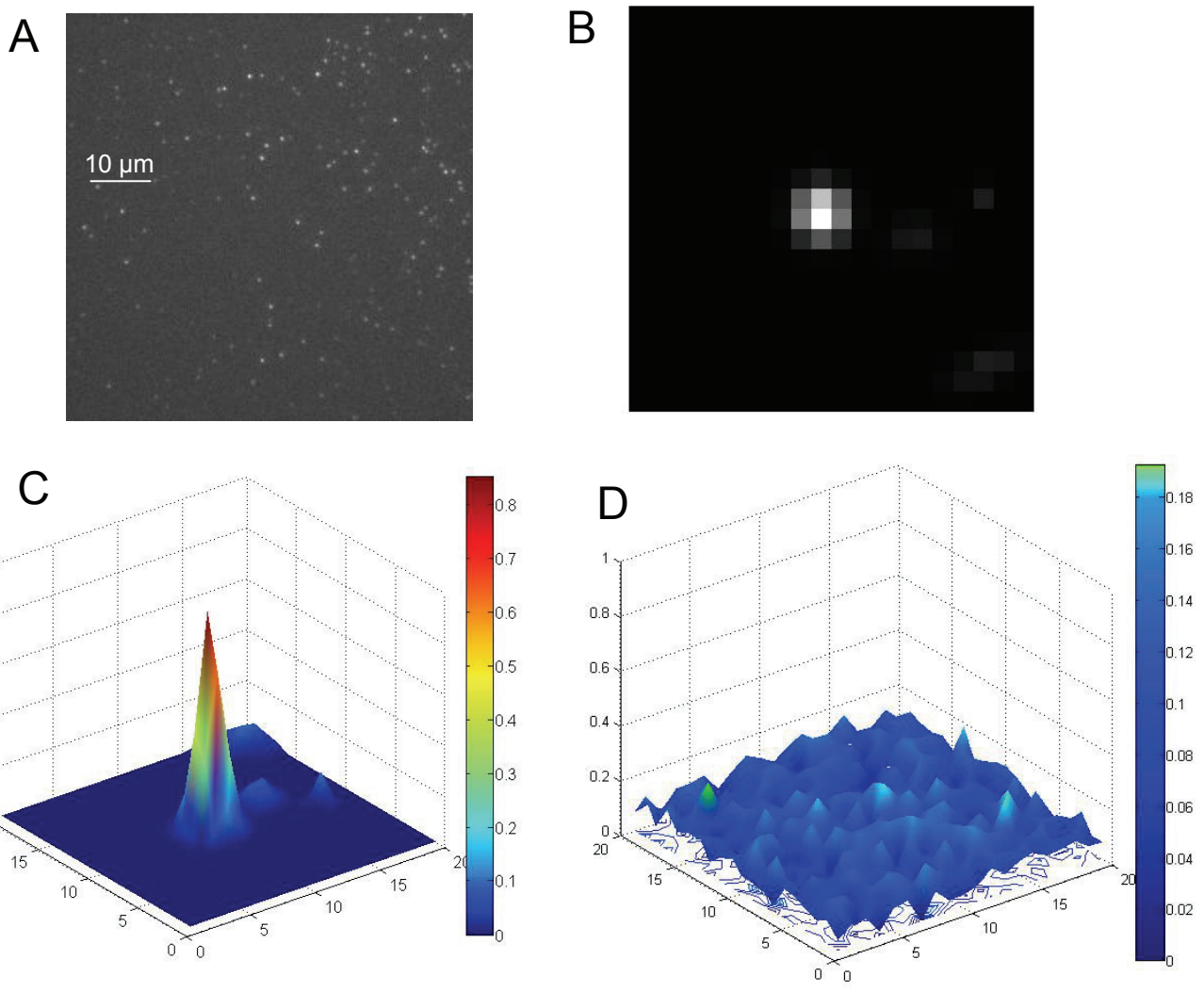


Figure S1

Optical resolution (point spread function) determination

A. Fluorescence image of single sulforhodamine B molecules dispersed on the surface of a glass coverslide.
B. Image from a single sulforhoramine B molecule (20x20 pixels)
C. Point spread function from 2-dimensional Gaussian fit.
D. Residuals after subtracting the 2-dimensional Gaussian fit from the data.

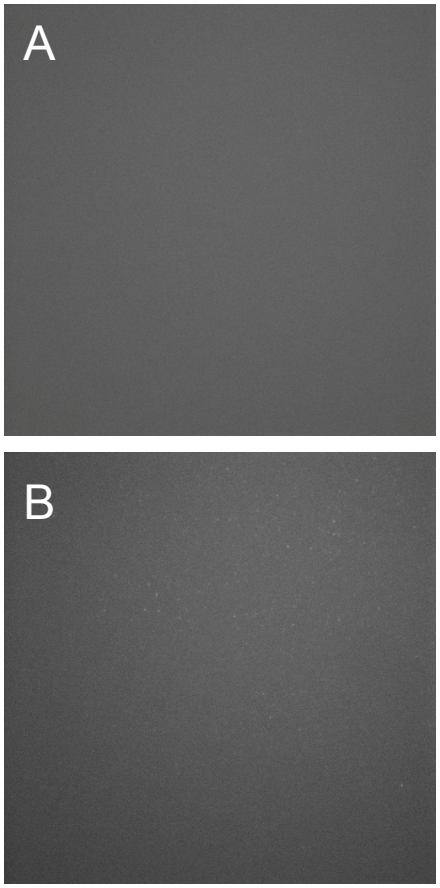


Figure S2

Blank controls.
A. Image of a coverslide formerly exposed to aqueous SFRB, and subsequently rinsed with buffer (as the vesicle samples were treated to remove the free dye).
C. Image of a coverslide with 20 nM Nile red in the solution above it.

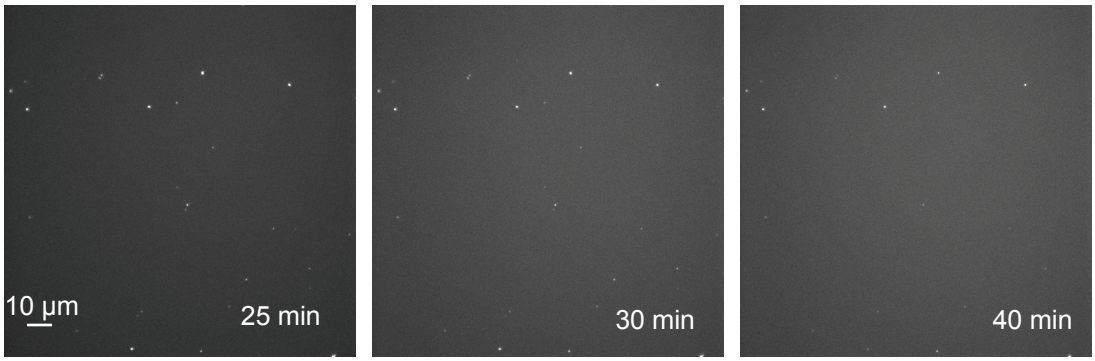
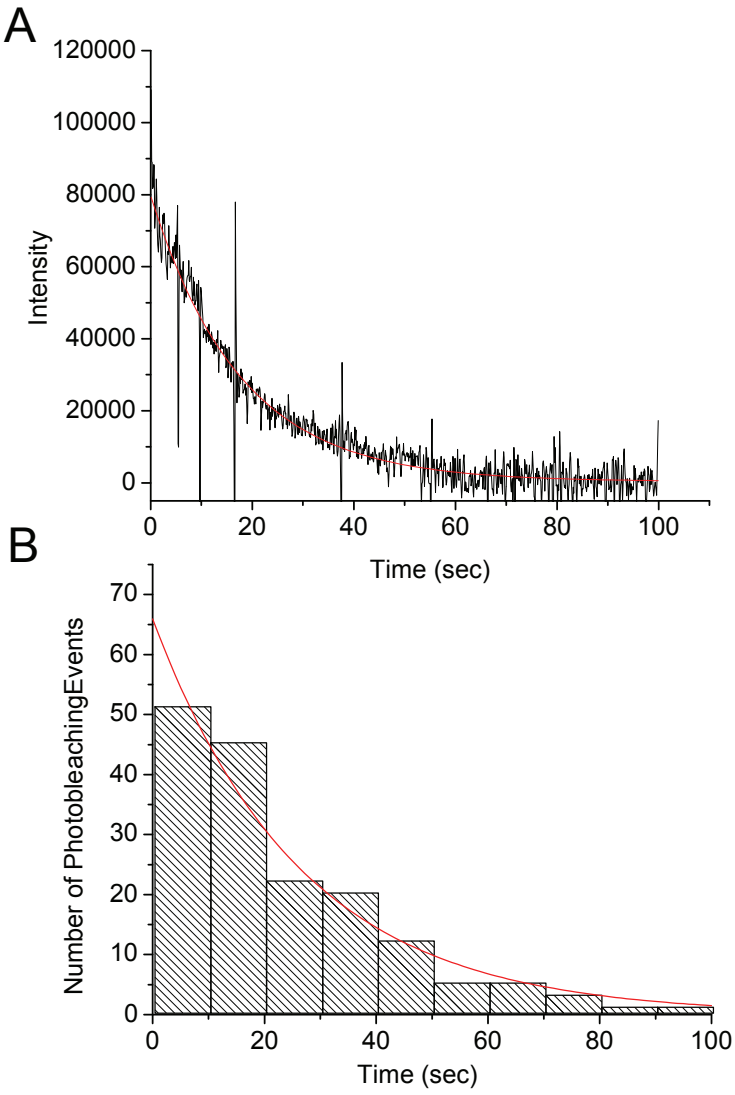


Figure S3

Test of vesicle stability. Intermittent images of vesicles adsorbed to a the glass coverslip. The vesicles retained their contents for greater than 40 min, demonstrating stability without vesicle rupture or fusion for time periods much longer than is required for the photobleaching experiment to be completed.

Figure S4

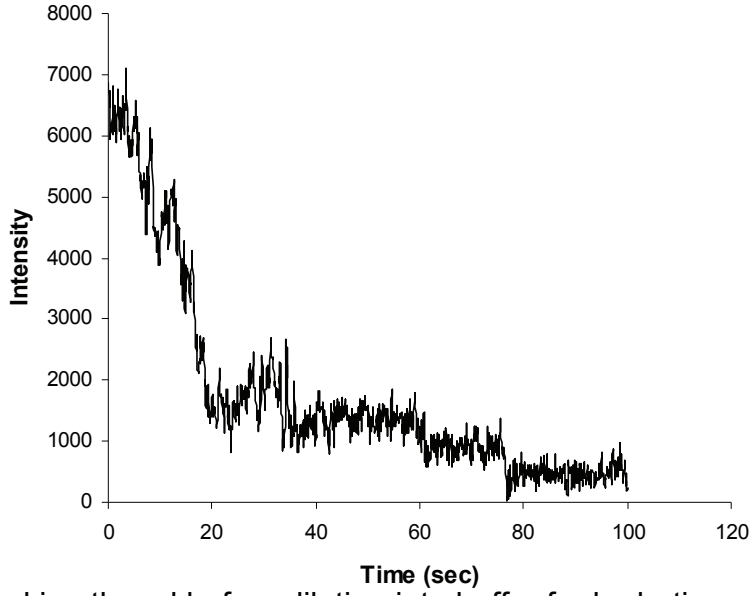


A. A sum of the intensities of vesicles encapsulating 0.5 μM SFRB shows exponential photobleaching with a decay time τ =17.9 ±0.3 seconds. Complete photobleaching was accomplished in relatively short time.

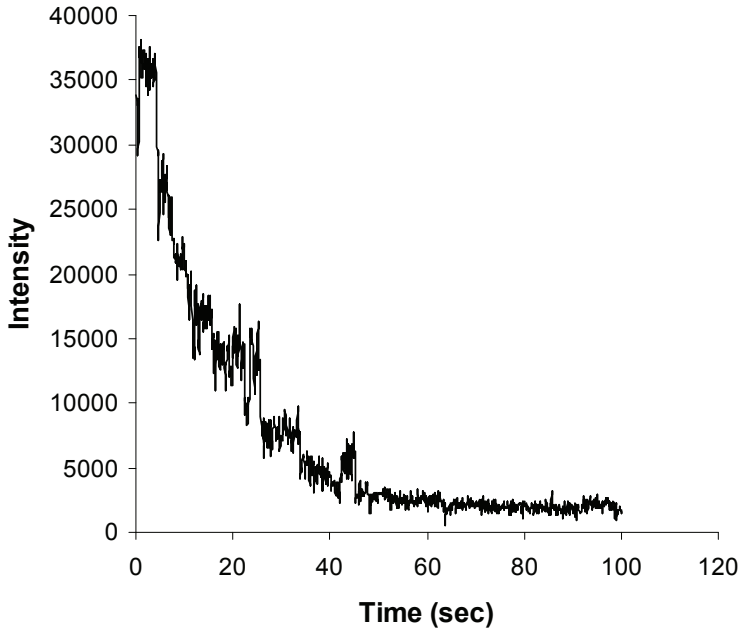
B. A histogram of decay times for single photobleaching events allowed determination of the probability that SFRB would photobleach in the first integration period (100 ms) and indicated that the probability of two photobleaching events occuring simultaneously was negligibly small.

Figure S5

Photobleaching tests for dye aggregates in vesicles for different preparation methods.



SFRB dissolved in ethanol before dilution into buffer for hydrating and extrusion showed indications of aggregation in photobleaching traces, like the example plotted above.



SFRB prepared by dissolving in DMF prior to dilution into aqueous buffer for vesicle preparation also exhibited aggregation.