Supporting Information

Vesicles tethered to microbubbles by hybridized DNA oligonucleotides: Flow cytometry analysis of this new drug delivery vehicle design

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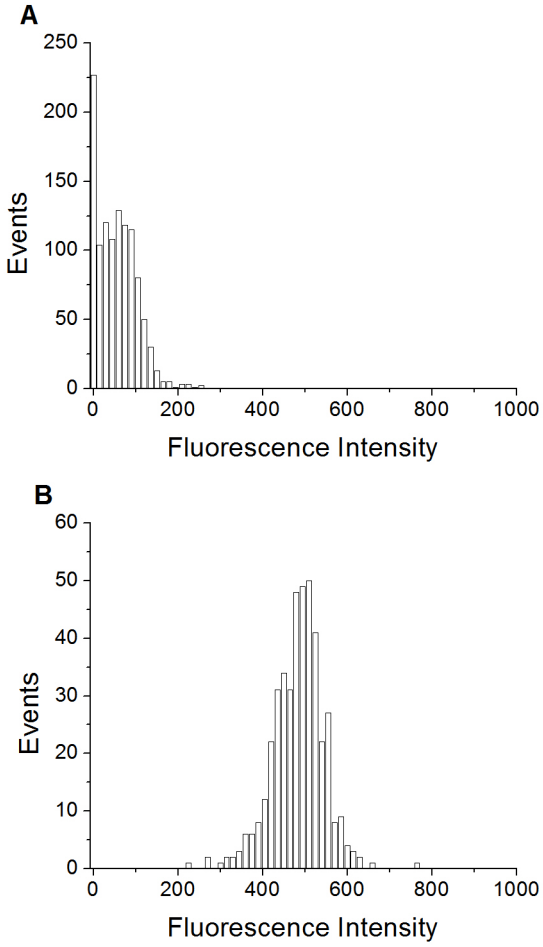

Figure S1. Events vs. fluorescence intensity histograms for large-sized (FS=200-300) **A)** DSPC/DPPS microbubbles; and, **B)** DSPC/DPPS microbubbles incubated with fluorescently labeled DSPC/DiC18-B' SUVs for 20 min (0nM DiC18-B – 20 min). Fluorescent probe was DiOC18.

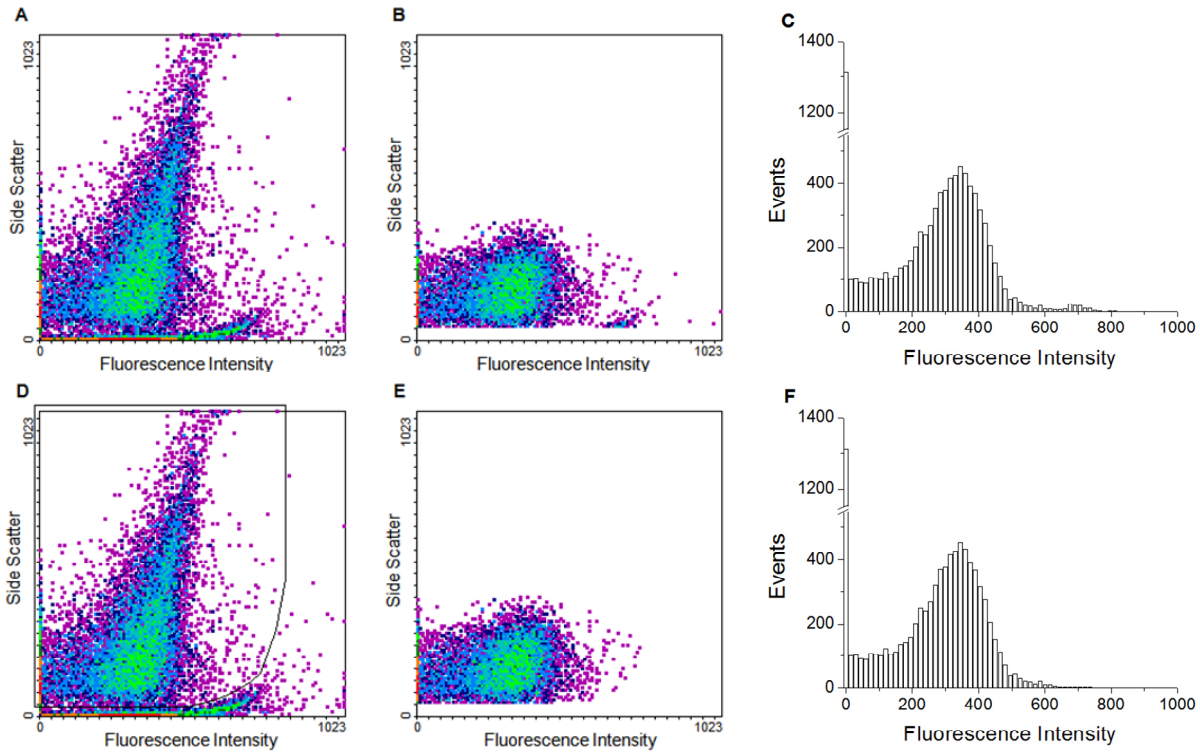

Figure S2. Side scatter vs. fluorescence intensity density dot plot for DSPC/DPPS microbubbles prepared with 0nM DiC18-B incubated with fluorescently labeled DSPC/DiC18-B' SUVs for 20min (0nM DiC18-B – 20 min) **A)** before the application of any gate, and **B)** after the application of FS = 0-100 gate and **C)** concomitant events vs fluorescence intensity histogram illustrating the presence of a small peak at FI ~ 700 corresponding to aggregated vesicles. Side scatter vs. fluorescence intensity density dot plot **D)** before the application of FS = 0-100 gate illustrating the location of the secondary gate, and **E)** after the application of FS = 0-100 and secondary gates and **F)** concomitant events vs. fluorescence intensity histogram illustrating the absence of the small peak at FI ~ 700 corresponding to aggregated vesicles. Highest density to lowest density order is red, green, blue, purple.

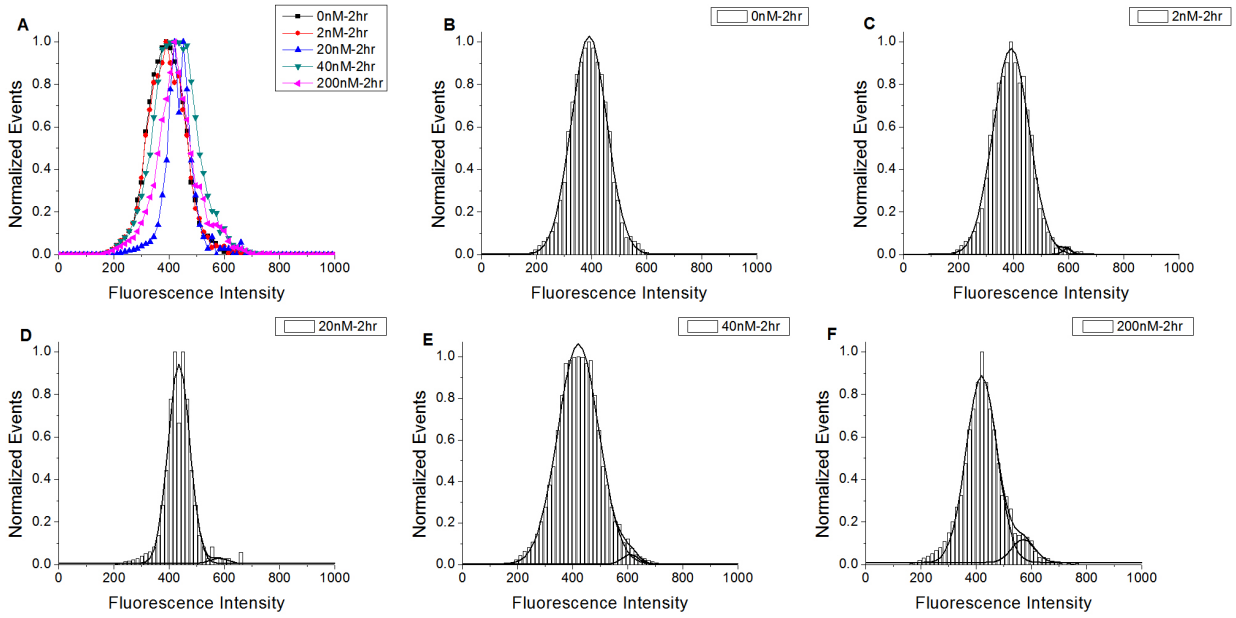

Figure S3. Normalized events vs. fluorescence intensity histograms for small sized (FS=0-100) DSPC/DPPS microbubbles prepared with increasing DiC18-B concentrations and incubated with fluorescently labeled DSPC/DiC18-B' SUVs for 2 h. **A)** All histogram shapes plotted without Gaussian fits; and, histogram with Gaussian fits for **B)** 0, **C)** 2, **D)** 20, **E)** 40, and **F)** 200 nM DiC18-B. Fluorescent probe was DiOC18.

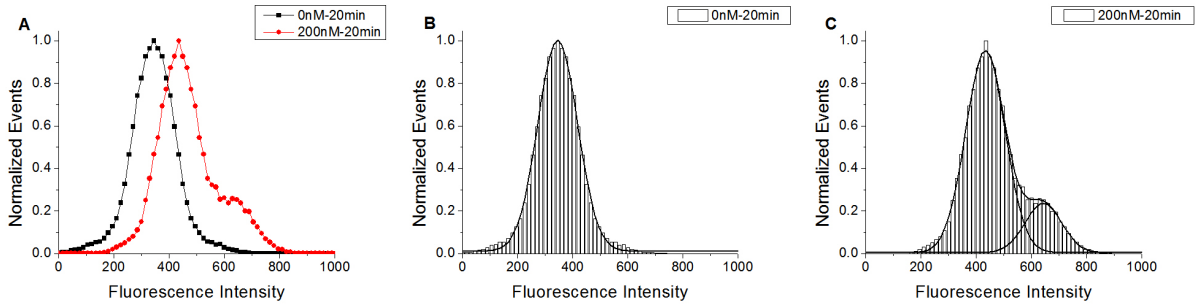

Figure S4. Normalized events vs. fluorescence intensity histograms for small sized (FS=0-100) DSPC/DPPS microbubbles prepared with 0 or 200 nM DiC18-B and incubated with fluorescently labeled DSPC/DiC18-B' SUVs for 20 min. **A)** Both histogram shapes plotted without Gaussian fit; and, histogram with Gaussian fits for **B)** 0 and **C)** 200 nM DiC18-B. Fluorescent probe was DiOC18.

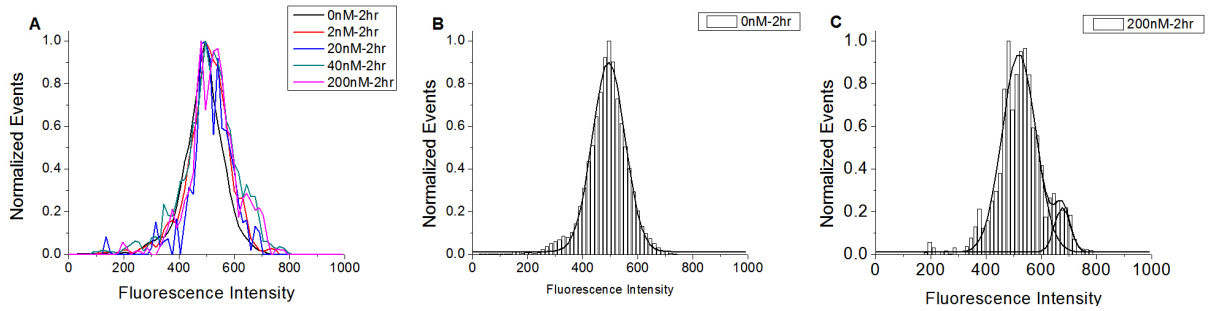

Figure S5. Normalized events vs. fluorescence intensity histograms for large sized (FS=200-300) DSPC/DPPS microbubbles prepared with increasing DiC18-B concentrations and incubated with fluorescently labeled DSPC/DiC18-B' SUVs for 2 h. **A)** All histogram shapes plotted without Gaussian fit; and, histogram with Gaussian fits for **B)** 0 and **C)** 200 nM DiC18-B. Fluorescent probe was DiOC18.

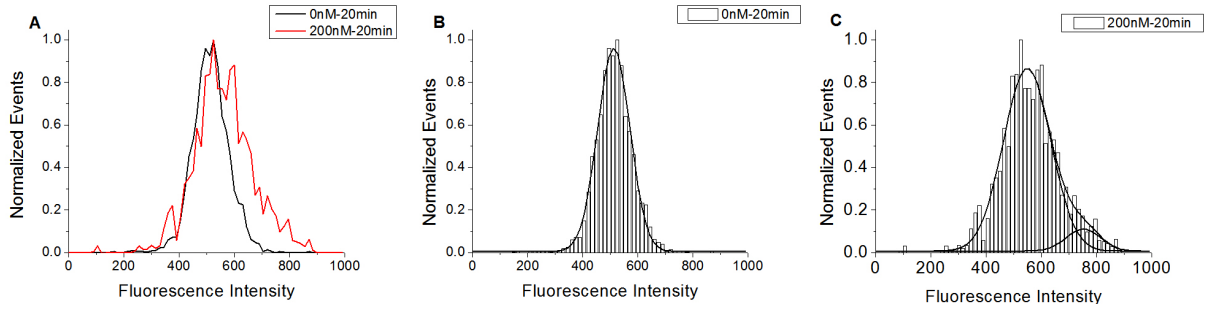

Figure S6. Normalized events vs. fluorescence intensity histograms for large sized (FS=200-300) DSPC/DPPS microbubbles prepared with 0 or 200 nM DiC18-B concentrations and incubated with fluorescently labeled DSPC/DiC18-B' SUVs for 20 min. **A)** Both histograms without Gaussian fit; and, histogram with Gaussian fits for **B)** 0 and **C)** 200 nM DiC18-B. Fluorescent probe was DiOC18.

Table S1. Summary of fitting parameters (p, μ, and σ) obtained from one- or two-peak Gaussian fits to histograms in Figures S5 and S6 corresponding to large sized (FS=200-300) microbubbles, where p, μ, and σ represent population fraction of each peak (i.e. $p_1 + p_2 + p_3 = 1$), mean fluorescence intensity (FI), and standard deviation respectively.

DiC18-B Concentration [nM]	μ_1 **FI**	p_1	$\mu_2 \pm \sigma_2$ **FI**	p_2	$\mu_3 \pm \sigma_3$ **FI**	p_3
0 (2 h)	/	/	493 ± 119	1	/	/
200 (2 h)	/	/	518 ± 122	0.905	676 ± 57	0.095
0 (20 min)	/	/	512 ± 118	1	/	/
200 (20 min)	/	/	547 ± 172	0.92	752 ± 125	0.080