Phase Behavior of Mixed Lipid Monolayers on Perfluorocarbon Nanoemulsions and its Effect on Acoustic Contrast

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monolayer compositions (*purple*: 100:0:0; *black*: 60:30:10; *red*: 47.5:47.5:5). 1 Error bar: 1 SEM. Because concentrations must be adjusted prior to NTA, these concentrations in the figure are not the same as the as-made yields, which are calculated based on the dilution of each sample prior to NTA.



Figure S2. Size Distributions of 400 g fractionated PFH droplets for different monolayer compositions. Because concentrations must be adjusted prior to NTA, these concentrations in the figure are not the same as the as-made yields, which are calculated based on the dilution of each sample prior to NTA.

Shell	Mean	Std. Error	Std.
Composition	Diameter	(nm)	Deviation
	(nm)		(nm)

100:0:0	335.7	5.2	142.6
60:0:40	371.4	10.0	148.0
50:10:40	336.1	16.2	116.1
70:20:10	380.0	16.4	150.1
60:30:10	318.1	2.4	117.5
50:30:20	312.6	7.2	127.3
40:30:30	351.6	5.4	128.4
40:40:20	302.9	9.0	126.0
47.5:47.5:5	289.8	6.2	110.3
30:60:10	343.6	4.4	152.4

Table S1. Mean diameters, standard error, and standard deviation for droplets with different shell compositions.



Figure S3. Comparison between HIFU responses of liposomes (*red*) and droplets (*blue*) for representative lipid-cholesterol compositions.



Figure S4. (a) 100:0:0 PFH emulsions formulated with and without DSPE-PEG2000; (b) Microscopy image of 100:0:0 PFH droplets without DSPE-PEG2000. Scale bar: $5 \mu m$.



Figure S5. Fluorescence microscopy images of 50 g sample 47.5:47.5:5 PFH droplets with 20% (by mole) DSPE-PEG2000. Scale bar: 5 μ m.



Figure S6. Fluorescence microscopy images of 50 *g* 100:0:0 PFH droplets after 30 min of heating at 37^{0} C (a) and 15 min of cooling in an ice-bath (b) immediately after droplet preparation. Scale bar: 5 μ m.



Figure S7. TEM images of PFH droplets of representative monolayer compositions (mole % DPPC:DOPC:Cholesterol): 100:0:0 (a), 60:30:10 (b), and 47.5:47.5:5 (c-d). Scale bars for a, b, and d: 500 nm. Scale bar for c: 100 nm.



Figure S8. (a) Normalized size distributions of 400 *g* fractionated 100:0:0 droplets with 3.33% (*black*), 10% (*red*), and 20% (*blue*) DSPE-PEG2000 concentration; (**b**) HIFU response of 100:0:0 droplets of mean size 300-400 nm as a function of DSPE-PEG2000 concentration.



Figure S9. Variation of HIFU responses of 300-400 nm (mean diameter) PFH droplets, formulated out of 100:0:0 (*purple diamonds*) and 47.5:47.5:5 (*red circles*) compositions with number of HIFU cycles applied.



Figure S10. Size distributions of fractionated PFH droplets formulated out of 100:0:0 (a) and 47.5:47.5:5 (b) shell compositions; *solid line*: Day 1 of droplet preparation, *dashed line*: Day 7.