Fusion-Dependent Formation of Lipid Nanoparticles Containing Macromolecular Payloads

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SUPPORTING INFORMATION

STATISTICAL COMPARISONS

Table 1. 2-way ANOVA comparison of LNP-nucleic acid size

Tukey's multiple comparisons test	Mean Diff.	99% CI of diff.	Significant?	Summary	Adjusted P Value
mRNA:Liposomes pH 4 vs. mRNA:Electron-dense pH 4	-20.37	-26.96 to -13.79	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. mRNA:PBS pH 7.4	-36.24	-41.94 to -30.53	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. mcDNA (1.4 kbp):Liposomes pH 4	0.4641	-5.241 to 6.169	No	ns	> 0.9999
mRNA:Liposomes pH 4 vs. mcDNA (1.4 kbp):Electron-dense pH 4	-28.25	-34.70 to -21.79	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. mcDNA (1.4 kbp):PBS pH 7.4	-48.81	-54.52 to -43.11	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. mcDNA (3.4 kbp):Liposomes pH 4	-1.851	-7.556 to 3.854	No	ns	0.9878
mRNA:Liposomes pH 4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	-36.92	-49.02 to -24.81	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-48.46	-54.16 to -42.75	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. pDNA (5.6 kbp):Liposomes pH 4	3.910	-1.795 to 9.614	No	ns	0.2987
mRNA:Liposomes pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	-32.57	-38.76 to -26.39	Yes	****	< 0.0001
mRNA:Liposomes pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-44.74	-50.45 to -39.04	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. mRNA:PBS pH 7.4	-15.87	-22.45 to -9.278	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. mcDNA (1.4 kbp):Liposomes pH 4	20.84	14.25 to 27.43	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. mcDNA (1.4 kbp):Electron-dense pH 4	-7.873	-15.12 to -0.6246	Yes	**	0.0028
mRNA:Electron-dense pH 4 vs. mcDNA (1.4 kbp):PBS pH 7.4	-28.44	-35.03 to -21.85	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. mcDNA (3.4 kbp):Liposomes pH 4	18.52	11.94 to 25.11	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	-16.54	-29.08 to -4.001	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-28.09	-34.67 to -21.50	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. pDNA (5.6 kbp):Liposomes pH 4	24.28	17.70 to 30.87	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	-12.20	-19.21 to -5.191	Yes	****	< 0.0001
mRNA:Electron-dense pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-24.37	-30.96 to -17.78	Yes	****	< 0.0001
mRNA:PBS pH 7.4 vs. mcDNA (1.4 kbp):Liposomes pH 4	36.70	31.00 to 42.41	Yes	****	< 0.0001
mRNA:PBS pH 7.4 vs. mcDNA (1.4 kbp):Electron-dense pH 4	7.992	1.535 to 14.45	Yes	***	0.0002
mRNA:PBS pH 7.4 vs. mcDNA (1.4 kbp):PBS pH 7.4	-12.58	-18.28 to -6.870	Yes	****	< 0.0001
mRNA:PBS pH 7.4 vs. mcDNA (3.4 kbp):Liposomes pH 4	34.39	28.68 to 40.09	Yes	****	< 0.0001
mRNA:PBS pH 7.4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	-0.6768	-12.78 to 11.42	No	ns	> 0.9999
mRNA:PBS pH 7.4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-12.22	-17.92 to -6.515	Yes	****	< 0.0001

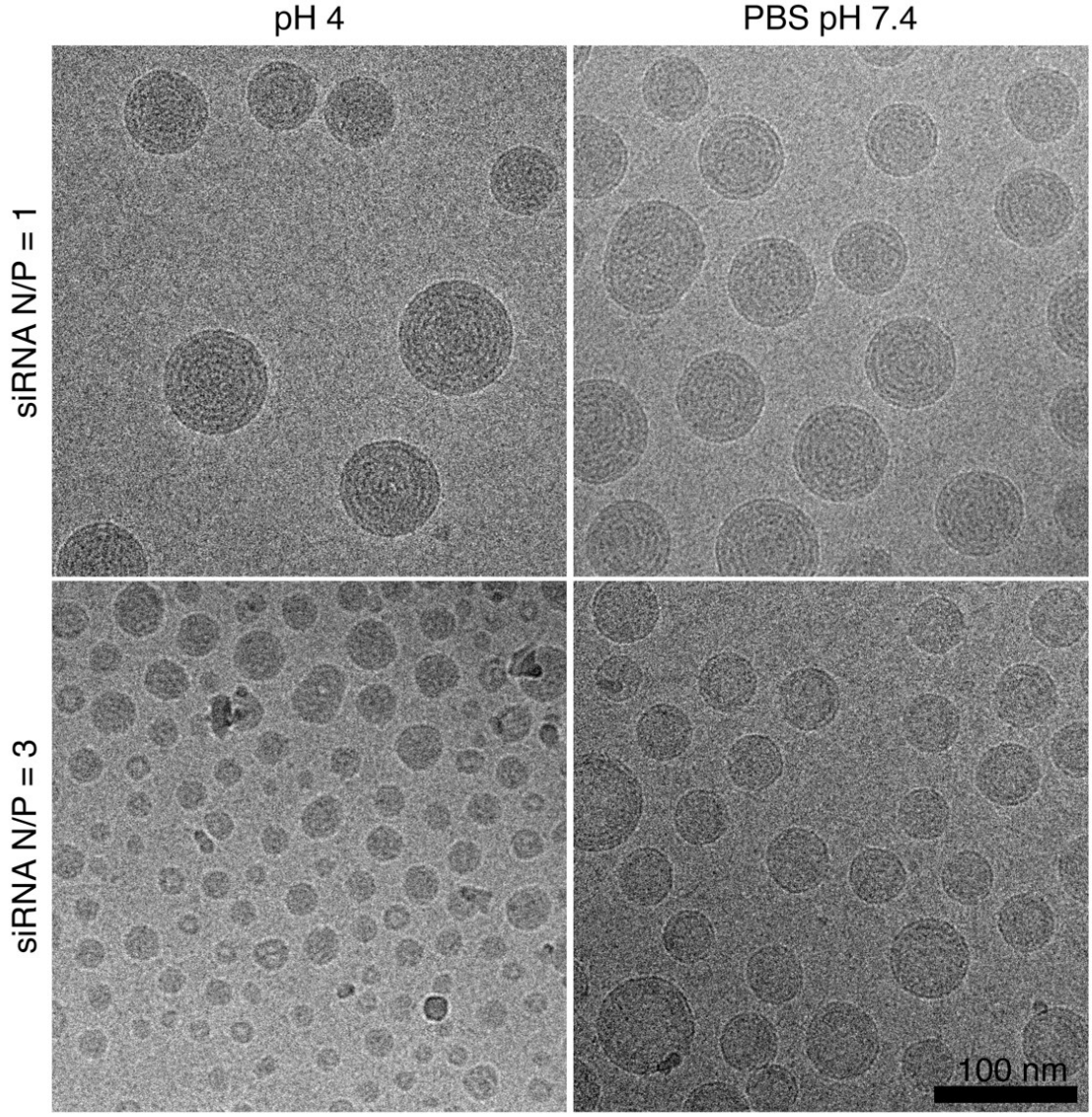
mRNA:PBS pH 7.4 vs. pDNA (5.6 kbp):Liposomes pH 4	40.15	34.44 to 45.85	Yes	****	< 0.0001
mRNA:PBS pH 7.4 vs. pDNA (5.6 kbp):Electron-dense pH 4	3.667	-2.519 to 9.852	No	ns	0.5342
mRNA:PBS pH 7.4 vs. pDNA (5.6 kbp):PBS pH 7.4	-8.505	-14.21 to -2.800	Yes	****	< 0.0001
mcDNA (1.4 kbp):Liposomes pH 4 vs. mcDNA (1.4 kbp):Electron-dense pH 4	-28.71	-35.17 to -22.25	Yes	****	< 0.0001
mcDNA (1.4 kbp):Liposomes pH 4 vs. mcDNA (1.4 kbp):PBS pH 7.4	-49.28	-54.98 to -43.57	Yes	****	< 0.0001
mcDNA (1.4 kbp):Liposomes pH 4 vs. mcDNA (3.4 kbp):Liposomes pH 4	-2.315	-8.020 to 3.390	No	ns	0.9351
mcDNA (1.4 kbp):Liposomes pH 4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	-37.38	-49.48 to -25.28	Yes	****	< 0.0001
mcDNA (1.4 kbp):Liposomes pH 4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-48.92	-54.63 to -43.22	Yes	****	< 0.0001
mcDNA (1.4 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):Liposomes pH 4	3.445	-2.259 to 9.150	No	ns	0.5036
mcDNA (1.4 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	-33.04	-39.22 to -26.85	Yes	****	< 0.0001
mcDNA (1.4 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-45.21	-50.91 to -39.50	Yes	****	< 0.0001
mcDNA (1.4 kbp):Electron-dense pH 4 vs. mcDNA (1.4 kbp):PBS pH 7.4	-20.57	-27.02 to -14.11	Yes	****	< 0.0001
mcDNA (1.4 kbp):Electron-dense pH 4 vs. mcDNA (3.4 kbp):Liposomes pH 4	26.40	19.94 to 32.85	Yes	****	< 0.0001
mcDNA (1.4 kbp):Electron-dense pH 4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	-8.669	-21.14 to 3.805	No	ns	0.2779
mcDNA (1.4 kbp):Electron-dense pH 4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-20.21	-26.67 to -13.75	Yes	****	< 0.0001
mcDNA (1.4 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):Liposomes pH 4	32.16	25.70 to 38.61	Yes	****	< 0.0001
mcDNA (1.4 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	-4.325	-11.21 to 2.560	No	ns	0.4385
mcDNA (1.4 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-16.50	-22.95 to -10.04	Yes	****	< 0.0001
mcDNA (1.4 kbp):PBS pH 7.4 vs. mcDNA (3.4 kbp):Liposomes pH 4	46.96	41.26 to 52.67	Yes	****	< 0.0001
mcDNA (1.4 kbp):PBS pH 7.4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	11.90	-0.2032 to 24.00	No	*	0.0126
mcDNA (1.4 kbp):PBS pH 7.4 vs. mcDNA (3.4 kbp):PBS pH 7.4	0.3552	-5.349 to 6.060	No	ns	> 0.9999
mcDNA (1.4 kbp):PBS pH 7.4 vs. pDNA (5.6 kbp):Liposomes pH 4	52.72	47.02 to 58.43	Yes	****	< 0.0001
mcDNA (1.4 kbp):PBS pH 7.4 vs. pDNA (5.6 kbp):Electron-dense pH 4	16.24	10.06 to 22.43	Yes	****	< 0.0001
mcDNA (1.4 kbp):PBS pH 7.4 vs. pDNA (5.6 kbp):PBS pH 7.4	4.070	-1.634 to 9.775	No	ns	0.2402
mcDNA (3.4 kbp):Liposomes pH 4 vs. mcDNA (3.4 kbp):Electron-dense pH 4	-35.07	-47.17 to -22.96	Yes	****	< 0.0001
mcDNA (3.4 kbp):Liposomes pH 4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-46.61	-52.31 to -40.90	Yes	****	< 0.0001
mcDNA (3.4 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):Liposomes pH 4	5.761	0.05590 to 11.47	Yes	**	0.0087
mcDNA (3.4 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	-30.72	-36.91 to -24.54	Yes	****	< 0.0001
mcDNA (3.4 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-42.89	-48.60 to -37.19	Yes	****	< 0.0001
mcDNA (3.4 kbp):Electron-dense pH 4 vs. mcDNA (3.4 kbp):PBS pH 7.4	-11.54	-23.64 to 0.5584	No	*	0.0186
mcDNA (3.4 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):Liposomes pH 4	40.83	28.72 to 52.93	Yes	****	< 0.0001
mcDNA (3.4 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	4.344	-7.992 to 16.68	No	ns	0.9767
mcDNA (3.4 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-7.828	-19.93 to 4.273	No	ns	0.3902
mcDNA (3.4 kbp):PBS pH 7.4 vs. pDNA (5.6 kbp):Liposomes pH 4	52.37	46.66 to 58.07	Yes	****	< 0.0001
mcDNA (3.4 kbp):PBS pH 7.4 vs. pDNA (5.6 kbp):Electron-dense pH 4	15.89	9.701 to 22.07	Yes	****	< 0.0001
mcDNA (3.4 kbp):PBS pH 7.4 vs. pDNA (5.6 kbp):PBS pH 7.4	3.715	-1.990 to 9.420	No	ns	0.3792

pDNA (5.6 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):Electron-dense pH 4	-36.48	-42.67 to -30.30	Yes	****	< 0.0001
pDNA (5.6 kbp):Liposomes pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-48.65	-54.36 to -42.95	Yes	****	< 0.0001
pDNA (5.6 kbp):Electron-dense pH 4 vs. pDNA (5.6 kbp):PBS pH 7.4	-12.17	-18.36 to -5.986	Yes	****	< 0.0001

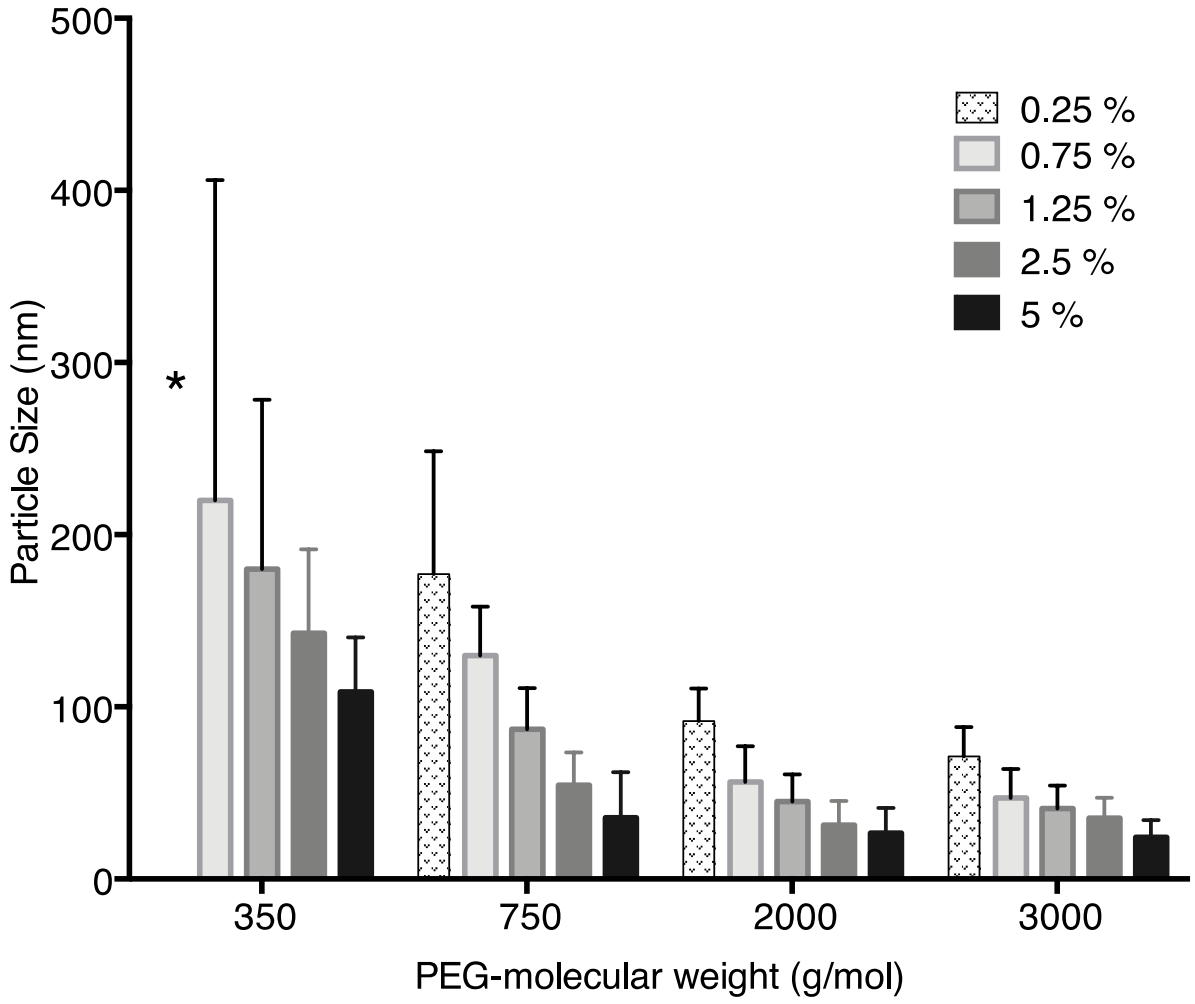
Table 3. 2-way ANOVA comparison of LNP-GNP size

Tukey's multiple comparisons test	Mean Diff.	99% CI of diff.	Significant?	Summary	Adjusted P Value
5 nm GNP:pH 4.0 vs. 5 nm GNP:PBS pH 7.4	-22.96	-26.51 to -19.41	Yes	****	< 0.0001
5 nm GNP:pH 4.0 vs. 12 nm GNP:pH 4.0	9.240	5.691 to 12.79	Yes	****	< 0.0001
5 nm GNP:pH 4.0 vs. 12 nm GNP:PBS pH 7.4	-22.64	-26.19 to -19.10	Yes	****	< 0.0001
5 nm GNP:PBS pH 7.4 vs. 12 nm GNP:pH 4.0	32.20	28.65 to 35.75	Yes	****	< 0.0001
5 nm GNP:PBS pH 7.4 vs. 12 nm GNP:PBS pH 7.4	0.3159	-3.233 to 3.865	No	ns	0.9925
12 nm GNP:pH 4.0 vs. 12 nm GNP:PBS pH 7.4	-31.88	-35.43 to -28.34	Yes	****	< 0.0001

SUPPORTING FIGURES



Supporting Figure 1. LNP-siRNA containing MC3 display similar morphology and formation steps as those containing KC2. LNP-siRNA formulations composed of MC3, DSPC, Cholesterol and PEG-lipid (50/1038.5/1.5 mol% respectively) were prepared using rapid mixing techniques and then dialyzed against pH 4 buffer (to remove solvent) or PBS pH 7.4 (to remove solvent and neutralize the pH). Previous work from our group[1] has shown that LNP-siRNA composed of the ionizable lipid KC2 also form in this manner.



Supporting Figure 2. PEG-molecular weight and molar fraction dictate particle size. LNP formulations composed of KC2, DSPC, Chol and PEG-lipid were generated at the molar ratios of 40/11.5/48.5-X/X, where X covered the range of 0.25 to 5 mol%. The resulting suspension was dialysed against PBS overnight. Particle sizing was performed using dynamic light scattering. Results indicate number mean ± standard deviation. * indicates a formulation that resulted in flocculation; no particle sizing data was obtained.

REFERENCES

1. Kulkarni, J. A.; Darjuan, M. M.; Mercer, J. E.; Chen, S.; van der Meel, R.; Thewalt, J. L.; Tam, Y. Y. C.; Cullis, P. R.,*ACS Nano* **2018,** *12*, 5, 4787-4795.