Supplementary information

	Tracks per capture number						Valid
Aqueous: organic phase ratio	1	2	3	Δ	5	Total	tracks (total particle count)
1 atio	1	Z	5	4	5	tracks	count)
1.3:1	2773	1676	1493	1515	1731	9188	2774
1.5:1	1906	1779	1809	1533	1495	8522	3623
2:1	6770	3570	4048	6766	2493	23647	4976
3:1	3207	3984	6882	3131	2926	20130	5056

Table S1: NTA data particle trajectories (tracks).



Figure S1: GFP fluorescence quantification from mGreenLantern-loaded LNPs. Comparing the different aqueous:lipid phase ratios in HEK-293 (A-B), THP-1 (C-D) and BMM (E-F) cells for LNPs manufactured with ALC-0315 (A, C, E) and SM-102 (B, D, F) as the ionisable lipid. Values quantified using ImageJ, as an average of 5 images from each sample. Significance values shown calculated from one-way ANOVA.



Figure S2: In vivo size versus mRNA expression of LNPs. Comparing LNPs manufactured at sizes smaller than 120d.nm (left) and larger than 120d.nm (right). Error bars shown as \pm S.E.M. Statistically analysed using a two-sample unpaired T-test.