Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Rapid Synthesis of Lipid Nanoparticles Containing Hydrophobic Inorganic Nanoparticles

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Content:

1. Synthesis of IONP

0.72 g (2.66 mmol) $FeCl_3$. $6H_2O$, 0.266 g (3.32 mmol) $FeCl_2$. $4H_2O$ and 1.621 g (5.32 mmol) Na-oleate were added into a 50 mL round-bottomed flask in a mixture solvents containing ethanol : deionized water: toluene :: 8 mL : 6 mL : 14 mL followed by the addition of 7 mg (6.64 mmol) NaOH. The mixture was then magnetically stirred and refluxed at 80 °C for 4 h under N_2 atmosphere. Meanwhile, red–brown toluene layer was observed to change black. The flask was removed from oil bath and cooled to room temperature after refluxing. Excess of ethanol was added to precipitate IONP and the particles were separated after magnetic separation. The IONPs were further purified by re-dispersing into n-hexane and reprecipitating by magnetic separation in ethanol. The magnetically separated IONPs were stored in n-haxanes as 30 mg/mL stock solution at -20 °C.

2. Synthesis of GNP

$HAuCl_4$. 3H2O (0.3284 g, 0.833 mmol Au) was dissolved in 20 mL of DI-H2O. An organic phase was prepared separately by dissolving tetraoctylammonium bromide (0.3284 g, 12.0 mmol) in 80 mL of toluene. The aqueous and organic phases were both poured into a 125 mL Erlenmeyer flask and stirred vigorously with magnetic stirring for 1 hour. During this time, the aqueous phase turned colorless, indicating the complete phase transfer of gold ions. Subsequently, the purple toluene phase was collected by extraction, and the colorless aqueous phase was discarded. The toluene phase was placed in a clean 125 mL Erlenmeyer flask, stirring at 1500 rpm. A 0.600 mL 1-dodecanethiol (2.5 mmol) was injected into the stirring toluene phase and over the next 15 minutes, the stirring toluene phase turned from purple to colorless. Sodium borohydride (0.38 g, 10.0 mmol) in 10 mL was added rapidly into the above stirring toluene phase at 4 °C to form a dark brown microemulsion. The microemulsion was allowed to stir at

room temp for 6 h. The brown toluene phase was extracted into a clean glass centrifuge tube, and the colorless aqueous phase was discarded. The toluene phase was distributed into separate 30 mL high-strength glass centrifuge tubes along with methanol antisolvent, allowing each tube to have 5 mL toluene and 20 mL of methanol. The tubes were centrifuged at 8000 rpm for 5 minutes to completely precipitate the Au nanocrystals. The methanol/toluene supernatants were discarded, and the precipitates were briefly dried with a stream of Ar gas. About 1-2 mL of chloroform was used to disperse the entire mass of Au precipitate, and the above purification step was repeated once more. The purified gold nanoparticles were stored in chloroform (~30 mg/mL stock) at -20 °C.

3. Synthesis of Cholesterol palmitate

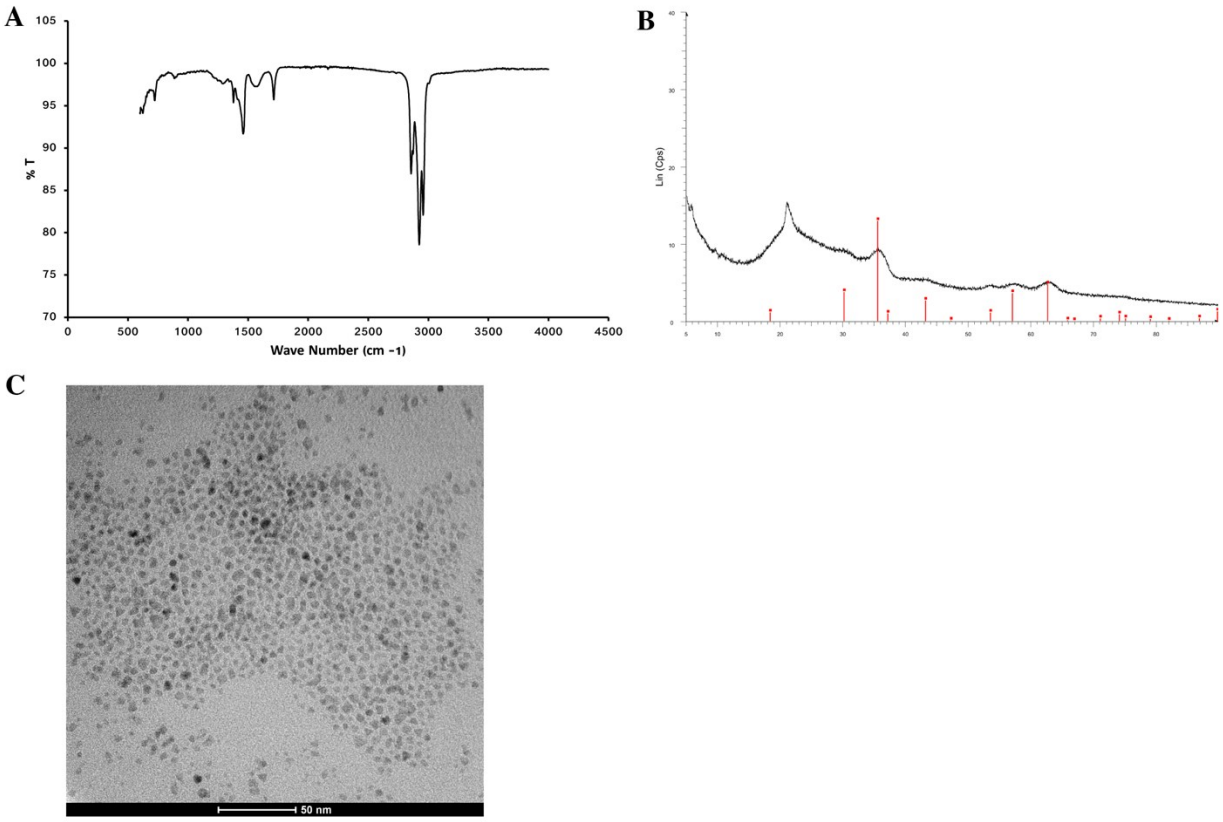
Palmitic acid (846 mg, 3.30 mmol, 1.1 equiv.) was added to a room temperature CH_2Cl_2 (15 mL) solution of dicyclohexylcarbodiimide (681 mg, 3.30 mmol) in a round bottom flask under argon and stirred for 10 min. The mixture was then cooled in an ice bath, cholesterol (1.16 g, 3.00 mmol) and 4-dimethylaminopyridine (403 mg, 3.30 mmol) were added and the resulting mixture was allowed to warm up over 14 h. The reaction mixture was diluted with Et_2O (25 mL), filtered through Celite to remove the white precipitate, and the filtrate concentrated on a rotary evaporator to provide the crude as brown, waxy solid. This material was redissolved in 95:5 hexanes/EtOAc and suction filtered through a plug of silica gel (30 mL) to afford the title compound as an off-white, waxy solid (0.98 g, 52% yield). R_f 0.53 (silica gel, 95:5 hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz): δ 5.46-5.31 (m, 1H), 5.69-4.56 (m, 1H), 2.40-2.19 (m, 4H), 2.11-1.76 (m, 5H), 1.74-0.68 (m, 63H), 0.69 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.3, 139.7,

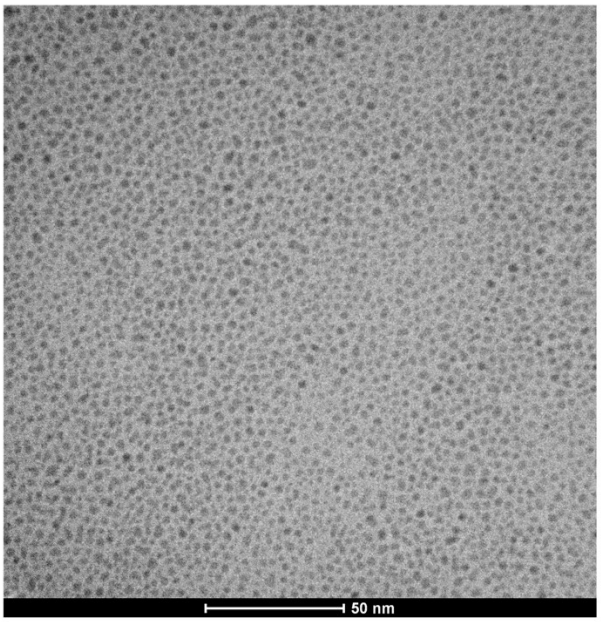
122.5, 73.6, 56.7, 56.1, 50.0, 42.3, 39.7, 39.5, 38.1, 37.0, 36.2, 35.8, 34.7, 31.92, 31.88, 31.83, 29.68, 29.65, 29.57, 29.44, 29.36, 29.24, 29.1, 28.2, 28.0, 27.8, 25.0, 24.3, 23.8, 22.8, 22.7, 22.5, 21.0, 19.3, 18.7, 14.1, 11.8 ppm.

4. ***In vitro*** **r2* measurement**

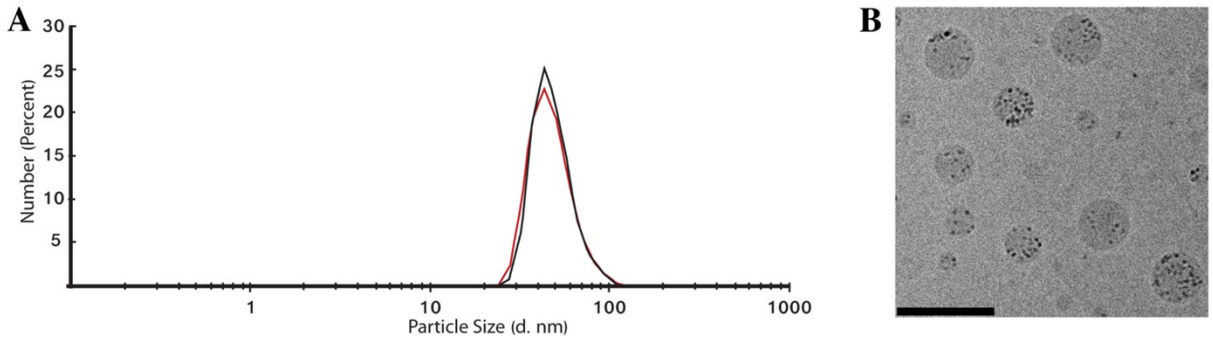
Phantoms of different doses in 12 mm diameter polypropylene vials were immersed in a container of water. Fieldmap-based shimming was performed (both first and second order shims). T2*-weighted images were acquired with a multi-gradient echo sequence (MGE, TR = 55ms, TE1 = 2.71ms, 12 echoes, echo spacing = 4.15ms, matrix size = 512 x 256 x 96, FOV = 58.9 x 58.9 x 22.1 cm, flip angle 20 degrees, 1 average). For each pixel, a single exponential decay was fit to extract the T2* relaxation time. T2-weighted images were acquired with a single-slice multi-spin echo sequence (TE = 6.553, 32 echoes, TR = 1500ms, 2 averages, matrix size = 128 x 128, FOV = 6 x 6 cm, slice thickness = 2 mm, alternating descending crusher gradients with composite refocusing pulse). Pixel fits of the exponential decay was fit in the same way to extract the T2 relaxation time. Relaxivity constants were calculated as the slope of the inverse relaxation times versus dose.



Supplemental Figure S1. Characterization of IONP. (A) Fourier Transform Infrared Spectroscopy of oleic-acid coated IONP show characteristics peaks at 2964, 2932, 2874, 1629 and 1469 cm^{-1}. (B) X-ray diffraction. (C) A representative TEM image of IONP particles. Scale bar = 50 nm.

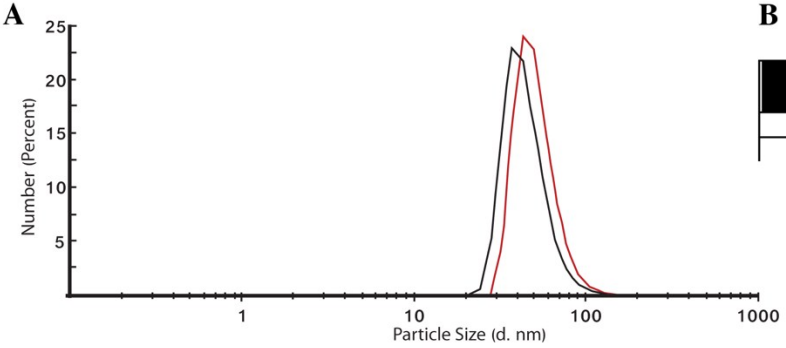


Supplemental Figure S2. Representative TEM image of gold nanoparticles. 10μL of GNP solution was pipetted onto a copper grid and imaged using a TEM at 200 kV acceleration voltage. Images were acquired using a high-speed AMT 2K side mount CCD camera.



Supplemental Figure S3. LNP-IONP are stable in storage for at least 3 months. (A) Representative particle size distributions of LNP-IONP (generated at M/L ratio of 0.107 mg IONP/ mg lipid) immediately prior to storage (black), and after 3 months storage at 4˚C (red). (B) Cryo-TEM image of LNP-IONP after 3 months storage. Scale bar = 100nm.

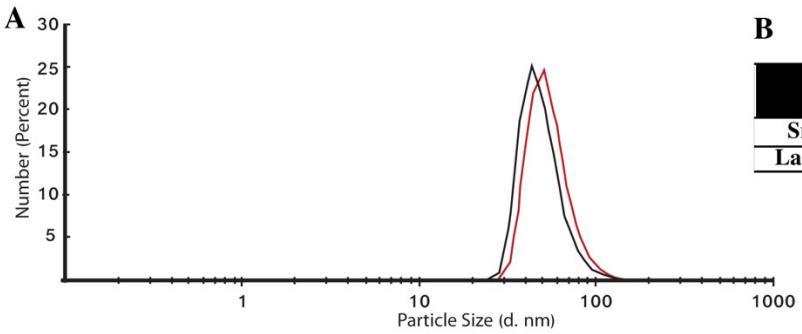
A



B

Formulation	Particle Size (± SD, nm)	PDI
LNP-GNP	45.45 ± 1.83	0.122 ± 0.010
LNP-QD	51.89 ± 1.43	0.124 ± 0.008

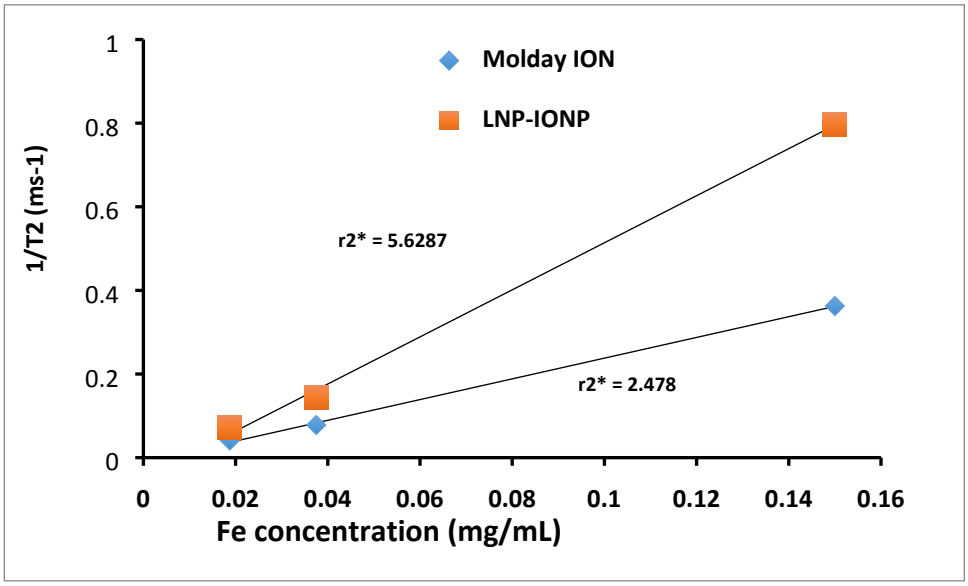
Supplementary Figure S4. LNP-GNP and LNP-QD display similar particle size as LNP-IONP systems. Number-weighted particle sizing analysis of LNP-GNP and LNP-QD generated through rapid mixing of lipids and HNPs in 40% THF in ethanol (v/v) and water at a total flow rate of 20mL/min. LNPs composed of triolein/POPC/PEG-DSPE at a ratio of 72/25/3 mol% were generated using M/L ratios corresponding to 0.107 mg HNP / mg lipid. (A) Representative number-weighted particle size distributions for LNP-GNP (black), and LNP-QD (red). (B) Corresponding particle size averages and polydispersity indices (PDI) for LNP-GNP and LNP-QD as measured by DLS. Results represent the mean ± s.d of three experiments.



B

Formulation	Particle Size (± SD, nm)	PDI
Small scale (8 mL)	48.84 ± 3.84	0.133 ± 0.008
Large scale (160 mL)	54.83 ± 1.46	0.105 ± 0.009

Supplementary Figure S5. Large-scale LNP-IONP formulations have similar characteristics to small-scale formulations. Number-weighted particle sizing analysis of LNP-IONP generated through rapid mixing of lipids and IONPs in 40% THF in ethanol (v/v) and water at a total flow rate of 20mL/min. LNPs composed of triolein/POPC/PEG-DSPE at a ratio of 72/25/3 mol% were generated using M/L ratios corresponding to 0.430 mg IONP / mg lipid. (A) Representative number-weighted particle size distributions for small-scale 8mL formulations (black), and a large-scale formulation (160 mL, red). (B) Corresponding particle size averages and polydispersity indices (PDI) for LNP-IONP as measured by DLS. Results represent the mean ± s.d of three experiments.



Supplementary Figure S6. Spin–spin 1/T2* relaxation rates of LNP-IONP (orange) is compared to compared to commercially available T2 contrast agent Molday ION (blue) at different iron (Fe) concentrations. Transverse relaxivity rates r2* of LNP-IONP and Molday ION were obtained by measuring calculating the slopes.