One-pot, one-step synthesis of drug-loaded magnetic multimicelle aggregates.

Supporting information

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A)

	20% Power	30% Power	40% Power
Average size (nm)	81 and 234	118	277
PDI	0.239	0.185	0.182

B)

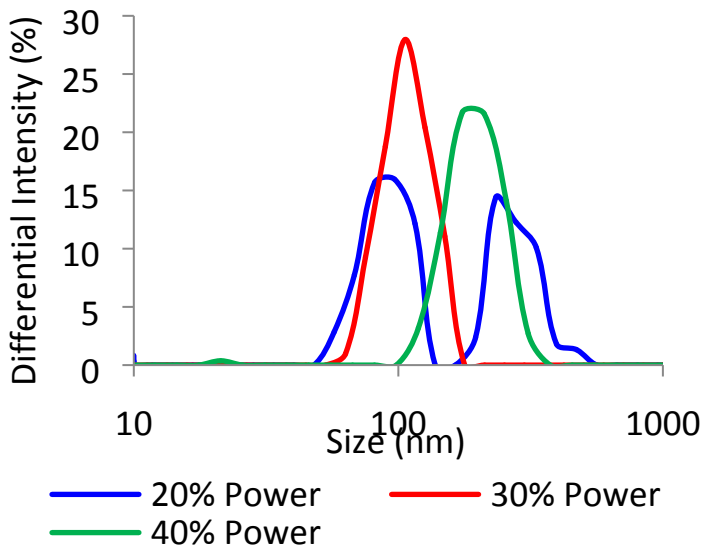

Figure S1. Characterization of multimicelle aggregates made using different power settings of the ultrasound probe. A) Average size and PDI as measured by DLS; B) size distributions (intensity) of the same batches.

A)

NP concentration (mg/mL)	Size (nm)	PDI	Zeta potential
0.1	118.70	0.19	-7.54
0.2	118.00	0.19	-6.68
0.4	118.33	0.14	-2.42
0.8	134.63	0.11	-11.68
1.6	146.40	0.10	-5.91

B)

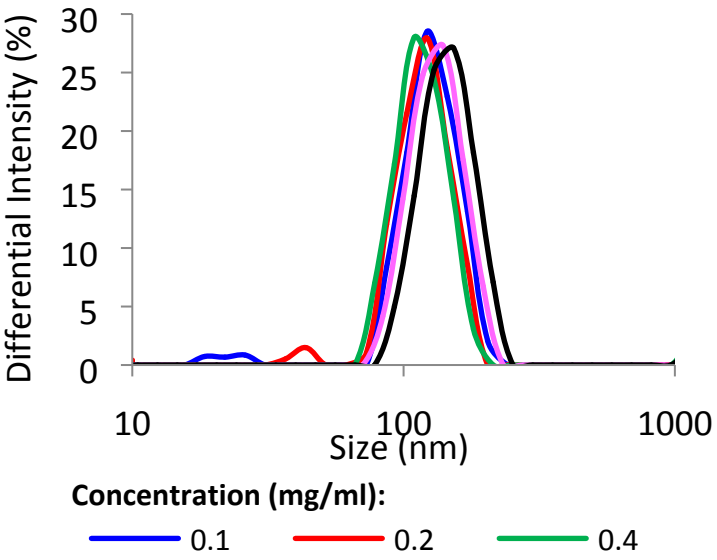

Figure S2. Changes in size of MaMAs at different iron oxide nanoparticle concentrations (mg/mL) in the infusion nanoparticle/lipid mixture. A) Average size and zeta potential of nanoparticles from triplicate samples. B) Corresponding representative size distributions (intensity).

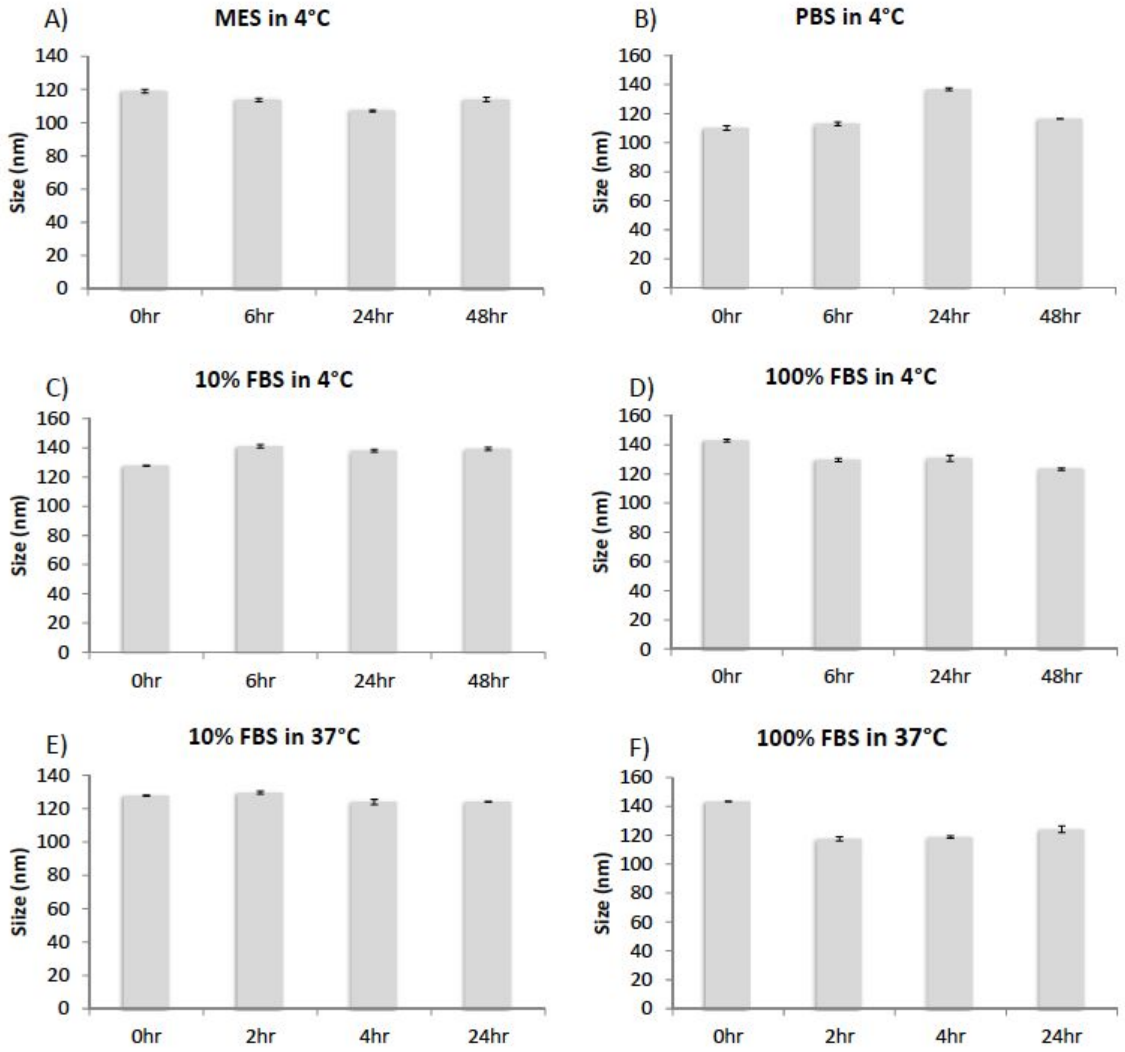

Figure S3. Stability testing of MaMAs in PBS (pH 7.4), MES (pH 6.5), 10% FBS, and 100% FBS over 6hr, 12hr, 24hr, and 48hr. Bars represent mean ± SD.

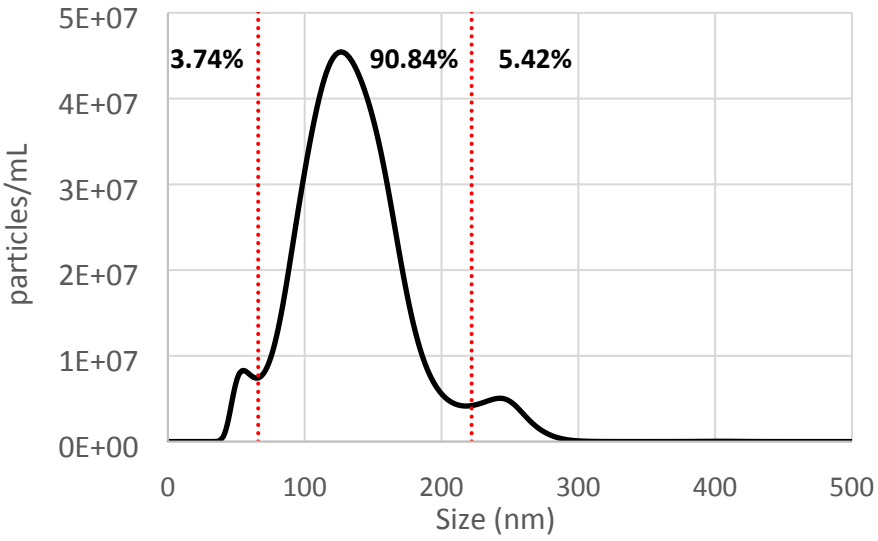

Figure S4. Distribution of MaMAs' size measured using NanoSight NS300 NTA device. Percentage of total nanoparticles were calculated for three subpopulations separated using full width of half maximums for first and third peaks (dashed lines). Over 90% of the MaMAs were in the main subpopulation with the mean peak of 133 nm.

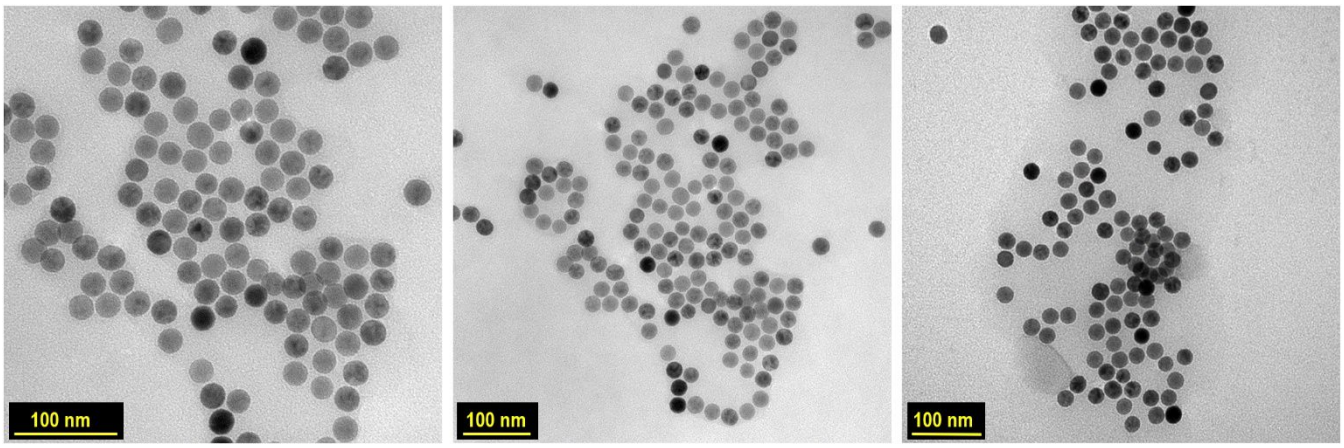

Figure S5. TEM images of MaMAs without negative staining.

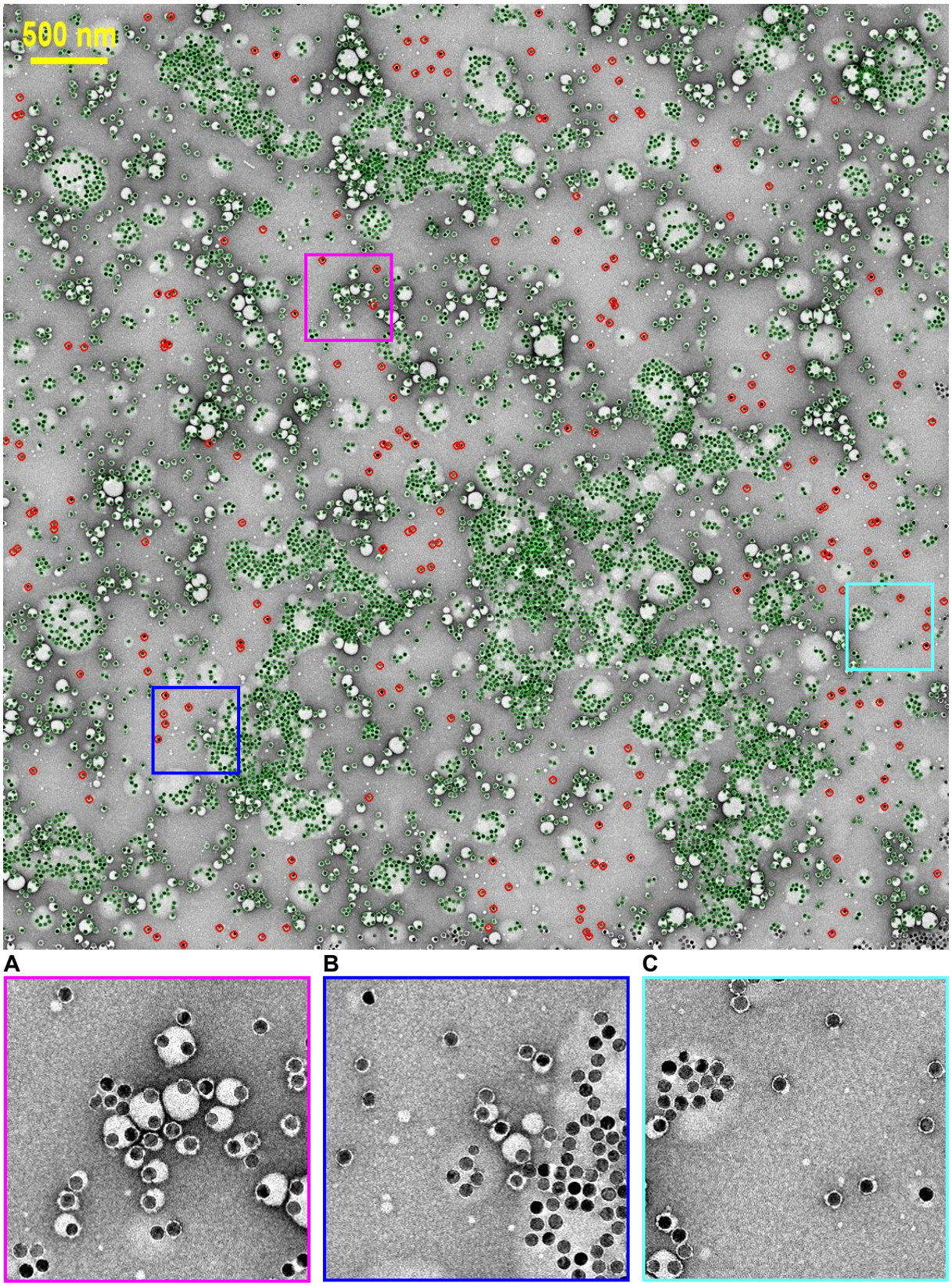

Figure S6. Composite TEM image of MaMAs with IONPs classified as (i) either coated with a clearly visible lipid layer or residing within larger negatively stained structures (green, 8218 out of 8414, 97.7%) or (ii) as not having a clearly identifiable visible negatively stained layer on their surface (red, 196 out of 8414, 2.3%). Three zoomed insets without the color coding (A, B, C) are showed in the bottom.

Supplementary methods description for composite TEM imaging and analysis in Figure S6.

The preparations were diluted to 2 × 10^{12} MaMAs/mL concentrations with HEPES buffer. 3 µl of sample was applied to a carbon Cu 200 mesh grid that had been glow discharged for 15 seconds using a PELCO easiGlow system (Ted Pella, Inc.) at 15 mA and 0.3 mBar. The sample was applied to grid, allowed to adsorb for 20 seconds and then blotted. Blotting was immediately followed by the application of 0.7% Uranyl Formate (aq) stain and then blotted again after a 5 second wait time. Staining and blotting in this manner was repeated four more times in succession. A final application of stain was then applied with a 20 second wait time and then blotted. The grid was then allowed to air dry for 1-2 minutes before imaging in the electron microscope. A Talos L120C TEM (Thermo Fischer Scientific) equipped with a 4k x 4k Ceta CMOS camera was used at an accelerating voltage of 120 kV and a nominal magnification of 36,000x to record micrographs at a raster of 2.94 Å/pixel and a defocus value of ~ -5 µm. SerialEM software was used for data collection. Total 100 images were collected and large area montage image was stitched and the nanoparticles were then examined manually in IMOD software to determine whether they were surrounded by a lipid coating. The nanoparticles were added to one of two Model Objects in IMOD software to classify and enumerate how many were within a lipid boundary and how many were not.

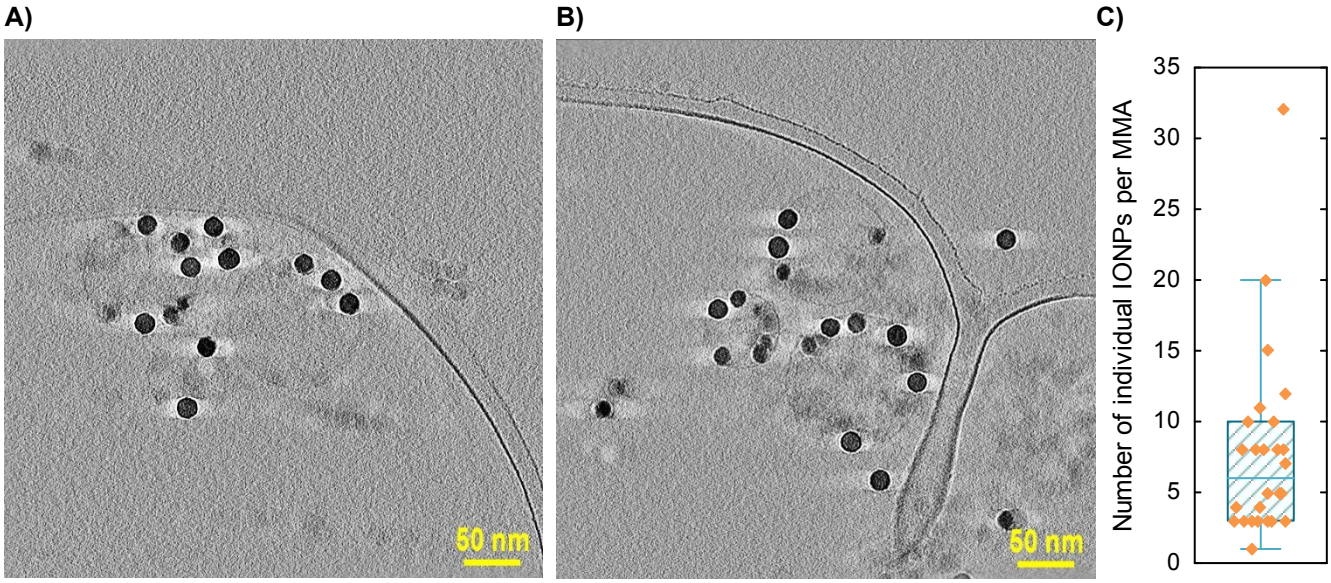

Figure S7. (A, B) Additional cross-sectional cryo-EM images of MaMAs preparations; (C) Four stacks of cross-sectional cryoEM images were used to determine the average number of IONPs per MaMA (total 26 MaMAs analyzed, median 8 IONPs per MaMA, interquartile range (25%-75%) 3-10). Each orange dot represents an individual MaMA.

Supporting movies 1-4. Stacks of cross-sectional images from cryo-EM imaging of MaMAs. These stacks were used for calculation of the median number of IONPs per MaMA in Figure S7, C. Size of the scale bar in the bottom right corner is 46 nm.