Supplementary Information

Enhancing the photoluminescence and cellular uptake of fluorescent carbon nanodots via cubosome lipid nanocarriers

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Additional method

Calculation of encapsuled C-dots inside a single cubosome

Assuming C-dots loading concentration is 3.2 wt%, C-dots with molecular weight of 2600 Da, the concentration of C-dots is 2.5×10^{-4} mol/L.

Assuming a single MO cubosome has a particle size diameter of r = 270 nm and a water content of 48 wt% based on the MO phase diagram by Qiu and Caffrey,[1] then

the volume of a particle $V = \frac{4}{3}\pi(\frac{270\ nm}{2})^3$

the mass of a MO particle = $(52\% \times M_{r=MO} + 48\% \times M_{r=water}) \times V \times 1 \times 10^6 = 9.98 \times 10^{-15}$ g

where $M_{r=MO}$ = 365 g/mol, $M_{r=water}$ = 18 g/mol

MO particle molecular weight = mass of a single MO particle × $6.02 \times 10^{23} = 6.01 \times 10^9$ g/mol.

Molar concentration of MO particles = $\frac{Concentration}{MO\ particle\ molecular\ weight} = \frac{20\ mg/ml}{6.01 \times 10^9 g/mol} = 3.3 \times 10^{-9}\ mol/L$.

With EE% of 95%, the number of C-dots loaded in a single MO cubosome = $\frac{2.5 \times 10^{-4} \times 0.95}{3.3 \times 10^{-9}} = 71969$

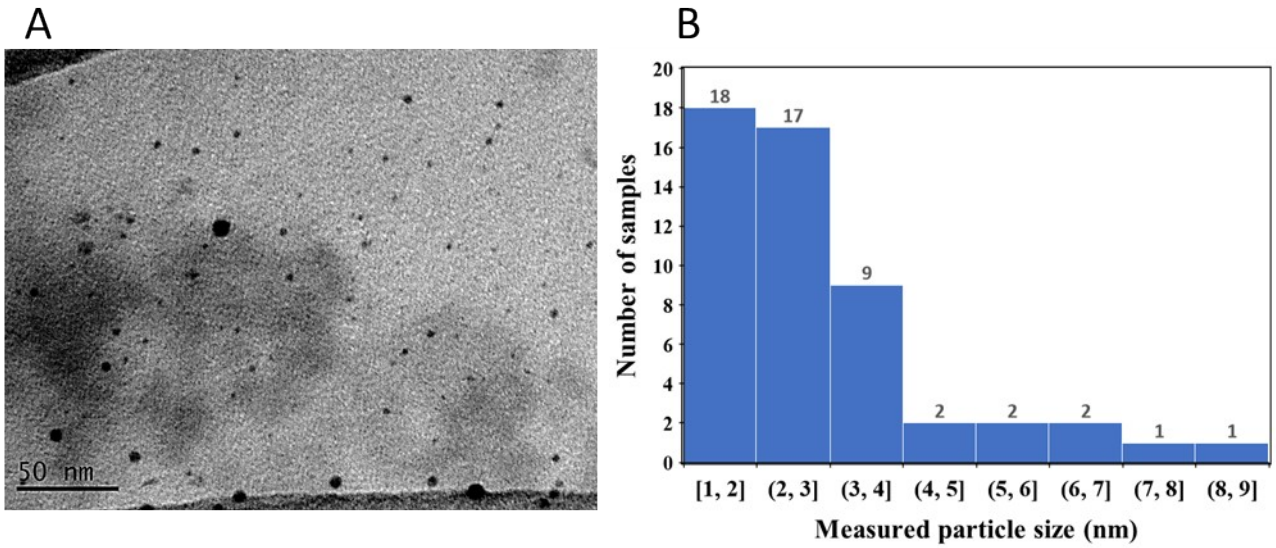

Figure S1. TEM image (A) and derived size distribution (B) of the C-dots. To derive the size distribution, ImageJ was used to analyse the TEM image.[2]

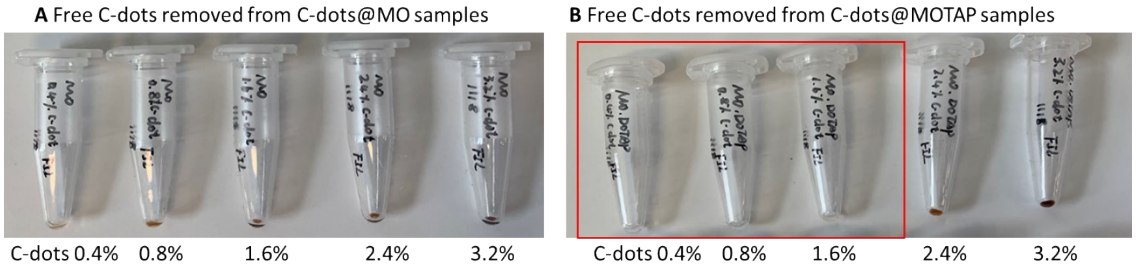

Figure S2. Pictures of dried filtrates after centrifugation of (A) MO and (B) MO/DOTAP loaded with various concentrations of C-dots (0 – 3.2 wt% C-dots to MO). The red box indicates that there was no visible C-dots in the filtrates of the MO/DOTAP NPs containing 0.4 – 1.6 wt% C-dots.

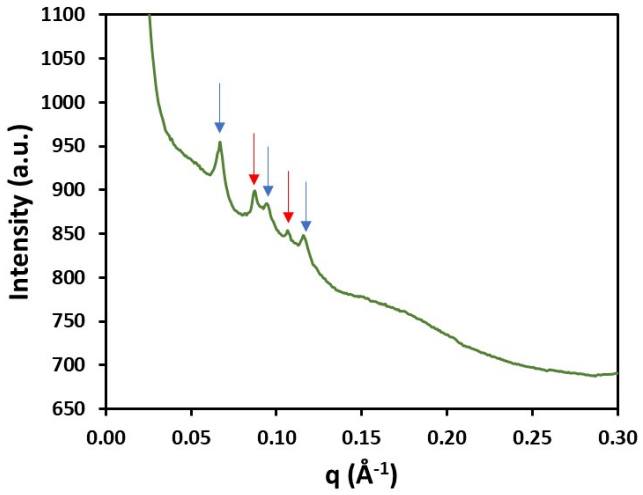

Figure S3. SAXS pattern of C-dots@MO/DOTAP (C-dot concentration =1.6 wt%). The primitive cubic phase with the crystallographic symmetry group *Im3m* was identified by the the peaks indicated by blue arrows with spacing ratios of √2, √4, and √6. The double diamond cubic phase with the crystallographic symmetry group *Pn3m* was identified by the the peaks indicated by red arrows with spacing ratios of √2 and √3. The *Im3m* phase and the *Pn3m* phase had lattice parameters of 13.2 nm and 10.2 nm respectively.

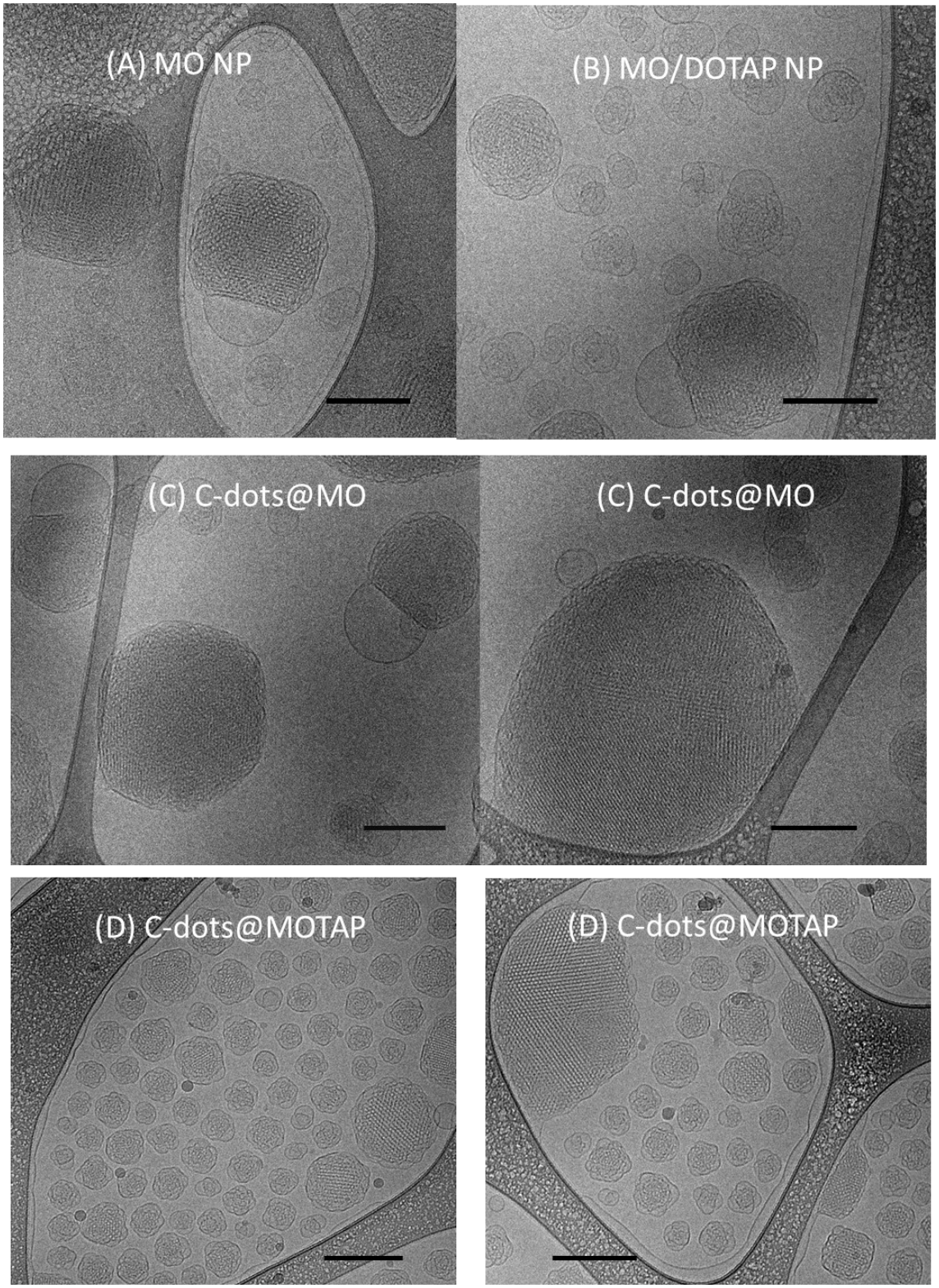

Figure S4. Representative cryo-TEM images of (A) MO NPs, (B) MO/DOTAP NPs, (C) C-dots@MO and (D) C-dots@MOTAP.

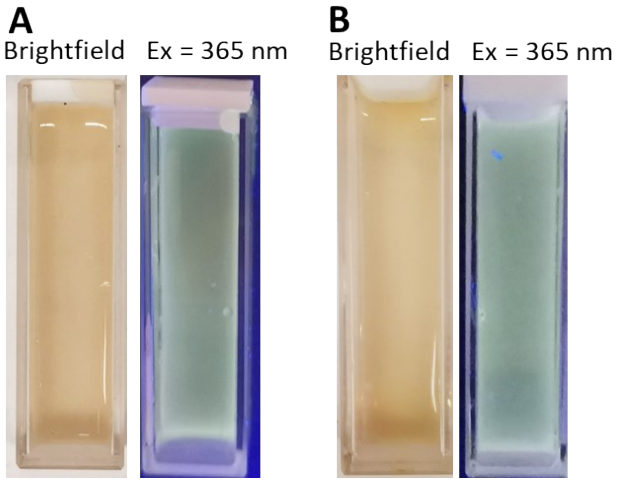

Figure S5. Images of (A) C-dots@MO and (B) C-dots@MO/DOTAP samples under brightfield or under a portable UV lamp with excitation at 365 nm. The concentration of C-dots in both samples was 3.2 wt%. Excitation wavelength was 360 nm.

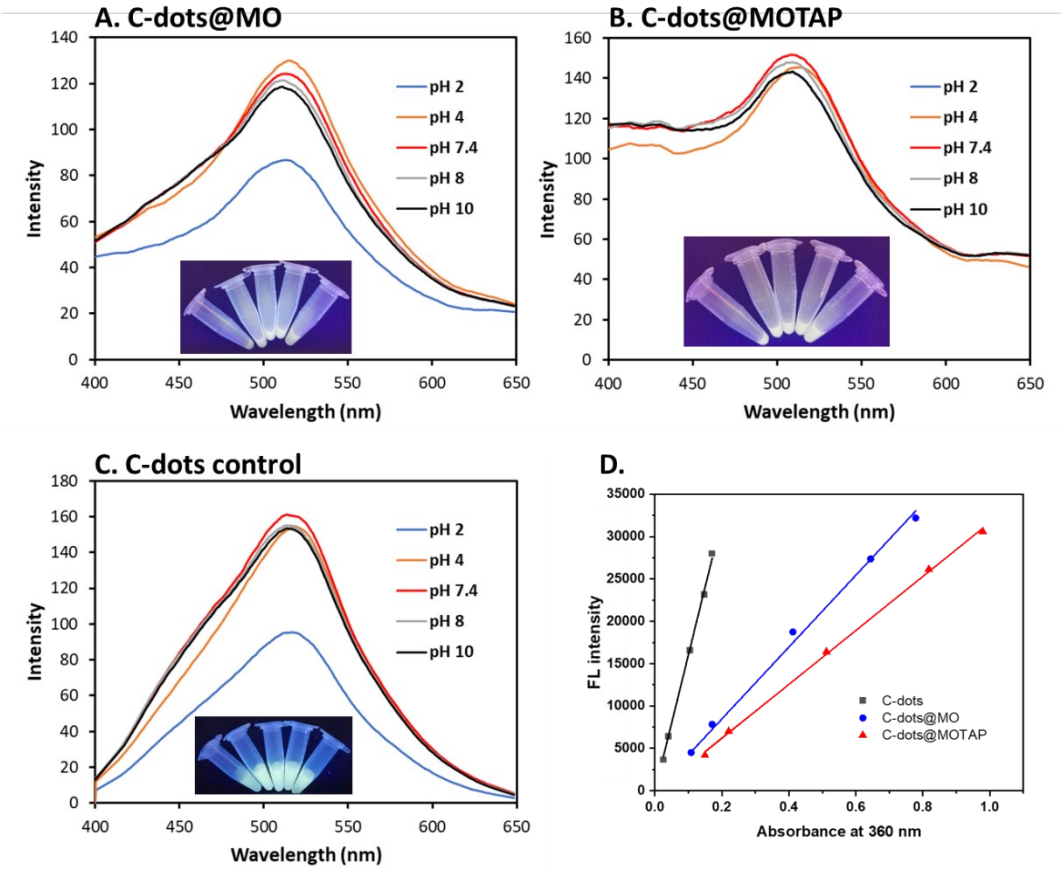

Figure S6. PL spectra of (A) C-dots@MO, (B) C-dots@MO/DOTAP, (C) C-dots control under excitation of 360 nm, and (D) The correlation of UV-vis absorbance at 360 nm with

FL intensity. Insets are theimages of respective samples under 365 nm UV lamp illumination with pH was increased from left to right. . The concentration of C-dots was 3.2 wt%. The spectra are an average of two independent samples.

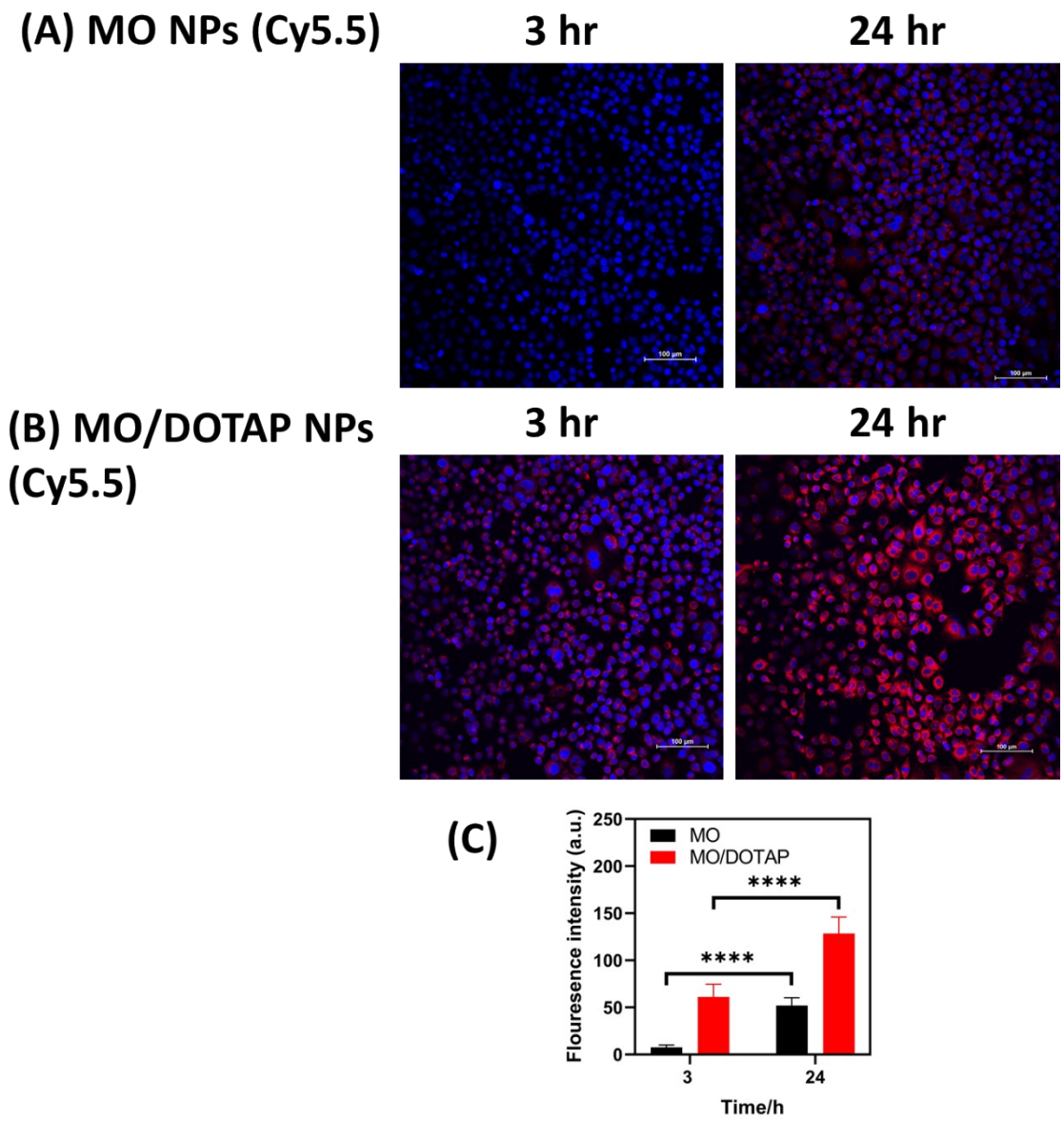

Figure S7: Enhanced uptake of lipid NPs labelled with the fluorescent 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(Cyanine 5.5) in AGS cells. (A) CLSM images of AGS cells treated with MO NPs. (B) CLSM images of AGS cells treated with MO/DOTAP NPs. (C) Quantitative analysis of the fluorescence intensity of the signal in the cells. NPs were added to the cells at approximately 20 μg/mL. Incubation time was 3 hrs or 24 hrs followed by fixing and staining the cell nuclei with NucBlue Fixed Cell Stain DAPI reagent. Images were merged from the DAPI channel and the Cy5 channel. Scale bar was 100 μm.

Table S1: IC50 values of lipid NPs and C-dot-loaded NPs in AGS and THP-1 cells

	AGS cells		**THP-1 cells**	
	IC50	**R^2**	**IC50**	**R^2**
MO NPs	165.6	0.8942	238.5	0.5523
MO/DOTAP NPs	163.9	0.7830	309.3	0.7317
C-dot@MO	247.5	0.9135	341.4	0.8459
C-dot@MOTAP	453.3	0.8363	207.4	0.8610

Note : IC50 values refer to the MO concentration in μg/mL. Nonlinear regression fit was performed to the cytotoxicity data (Figure 6) using GraphPad Prism 9.0.1 for Windows, GraphPad Software, San Diego, California USA.

References

(1) Qiu, H.; Caffrey, M. The phase diagram of the monoolein/water system: Metastability and equilibrium aspects. *Biomaterials* **2000,** *21*, 223-234.
(2) Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. Nih image to imagej: 25 years of image analysis. *Nature Methods* **2012,** *9*, 671-675.