Electronic Supplementary Information (ESI)

for

A functional preservation strategy produced highly photoluminescent emerald carbon dots for lysosome targeting and lysosomal pH imaging

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The change of Gibbs free energy for preparing CDs.

The Gibbs free energy of the reactant (P-benzoquinone and ethanediamine) and product were calculated by B3LYP method in solid-state density functional theory (DFT) calculations at standard temperature and pressure. The sum of electronic and thermal Free Energies of P-benzoquinone is 0.067857 Hartree, ethanediamine is 0.069798 Hartree and the product is 0.310299 Hartree. So the ΔG=0.310299 - (0.067857 + 0.069798*4) = -0.03674 Hartree = -94.28 kJ/mol < 0.

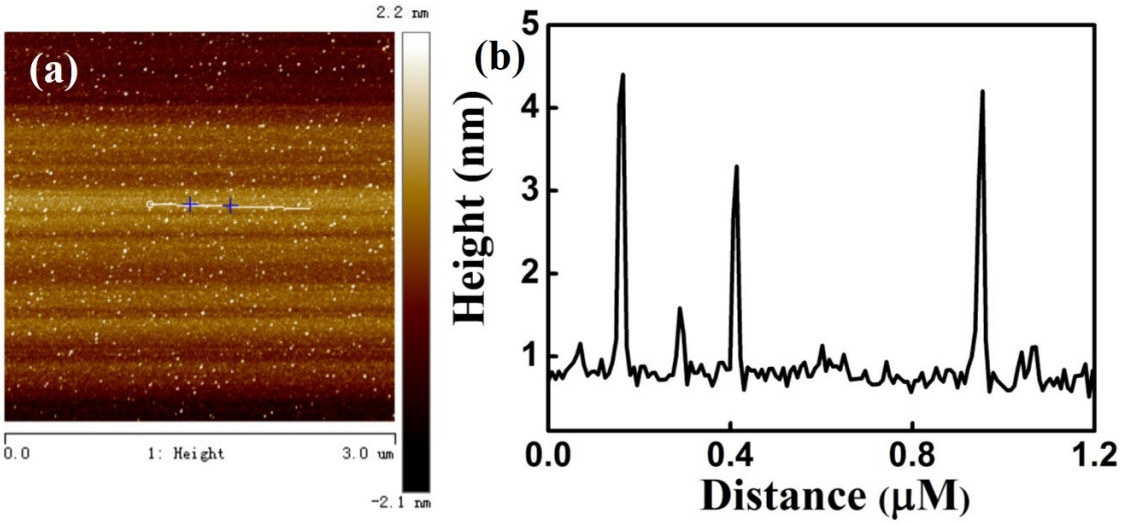


Fig. S1 The AFM of CDs. (a) The AFM image and (b) the height distributions.

Note: Drops of a dilute aqueous solution of the CDs were deposited on mica substrates for AFM. The AFM image showed that the height of the CDs was approximately 1.6-4.4 nm, about 4-11 graphene layers.

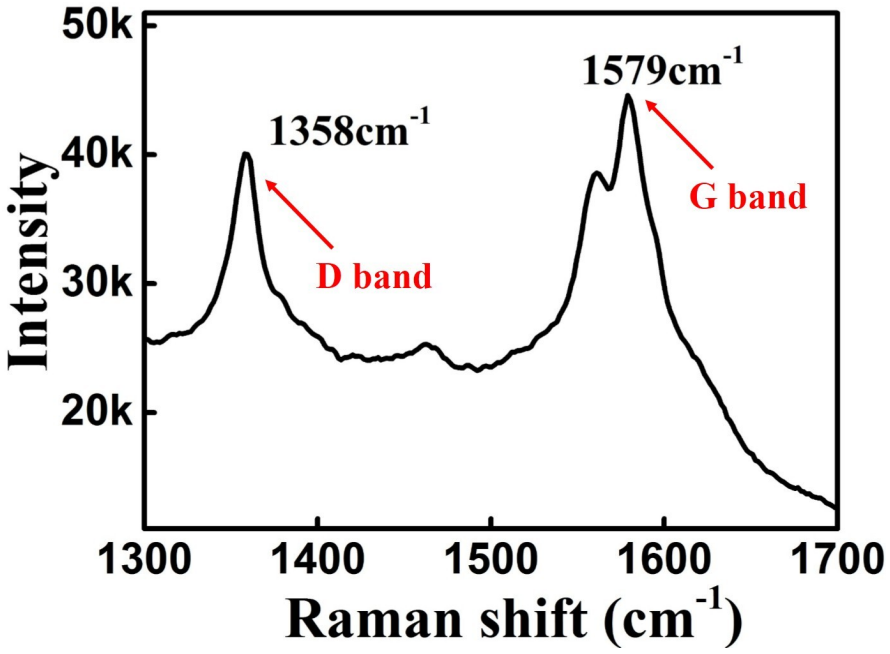


Fig. S2 The Raman spectra of CDs.

Note: AgNPs were used as the Raman enhancement matrix. The Raman spectra showed that the D-band at 1358 cm^{-1} was due to sp^3 carbons, while the G-band at 1579 cm^{-1} was due to sp^2 carbons.

Table S1 Percentage of C, N, and O atoms in CDs, as determined by XPS measurements.

	CDs
C/%	68.40
C=C	79.86
C–O	17.36
C=N/C=O	2.78
N/%	7.49
Aromatic N	87.80
N–H	12.20
O/%	24.47

Note: The as-prepared CDs were composed of C, N and O atoms, with the C atom of 68.40 %, O atom of 24.47 % and N atom of 7.49 %. And the percentage of C=C was the highest in the C1s, which showed the formation of abundant graphene layer structure of CDs. The 12.20 % of N–H, 17.36 % of C–O and 2.78 % of C=N/C=O clearly proven the luxuriant functional groups of CDs, like $–NH_2$ and –COOH.

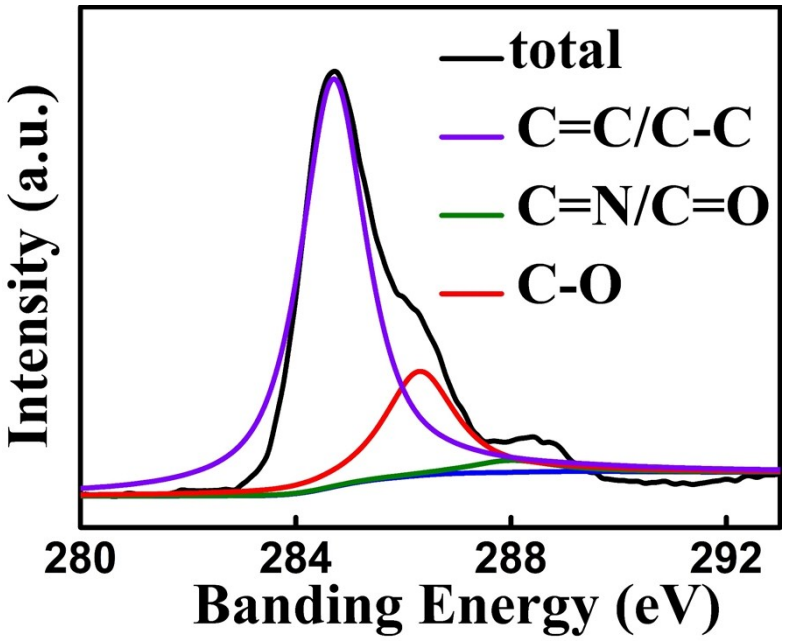
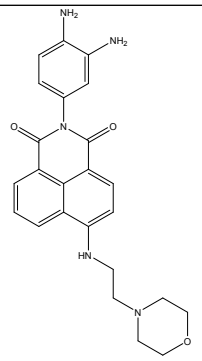
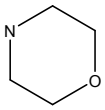
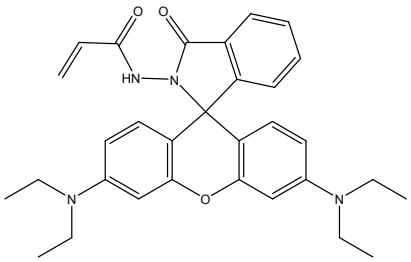
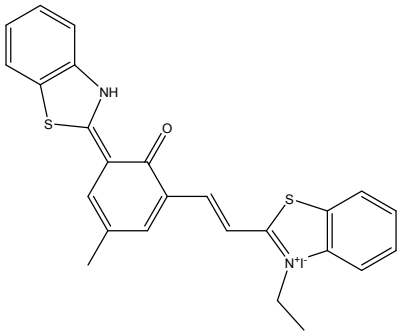
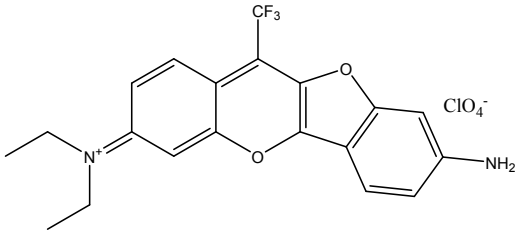
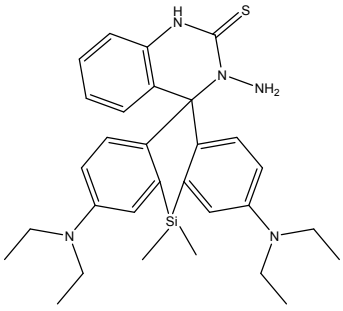
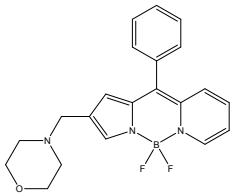
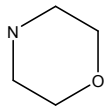


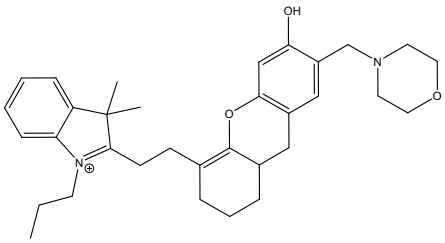
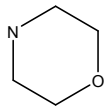
Fig. S3 The C1s XPS spectra of CDs.

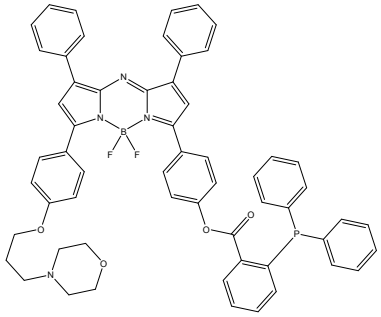
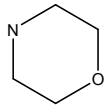
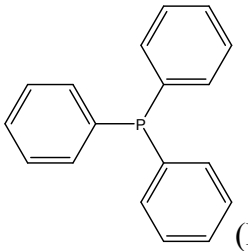
Note: There were three peaks in the high resolution scan of the C1s region, including sp^2 C (C–C or C=C) in graphene at 284.7 eV[1], C–O at 286.3 eV[2] and C=N/C=O at 288.0 eV[3].

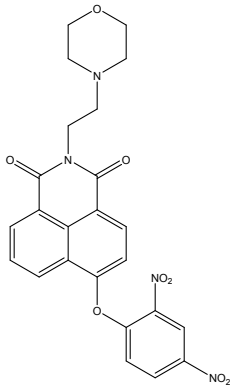
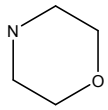
Table S2 Summary of Lysosome-targeting dyes reported previously.

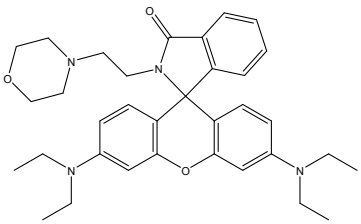
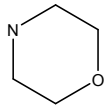
Dyes	Molecular structure	Lysosome-targeting unit	Ref.
Lyso- NINO		$-NH_2$ and 	Ref. 4
RhB-1		-NH	Ref. 5
Compound 1		-NH	Ref. 6
AFR		$-NH_2$	Ref. 7
SiRB-Cu		$-NH_2$	Ref. 8

Lyso-V   Ref. 9

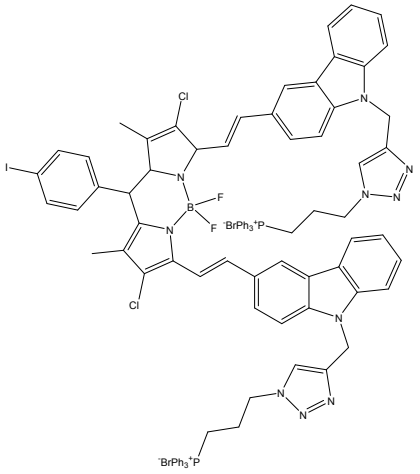
Lyso-pH   Ref. 10

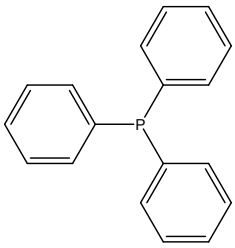
Lyso-JN   and  (PPh_3) Ref. 11

Lyso-NHS   Ref. 12

RM   Ref. 13

Lyso-NIR

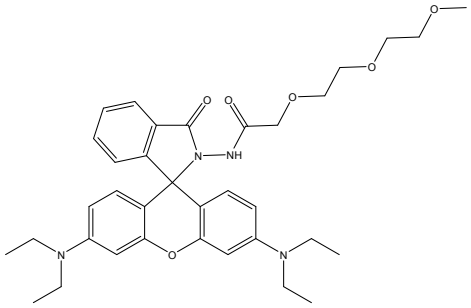


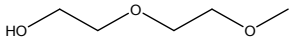


(PPh_3)

Ref. 14

Rlyso





Ref. 15

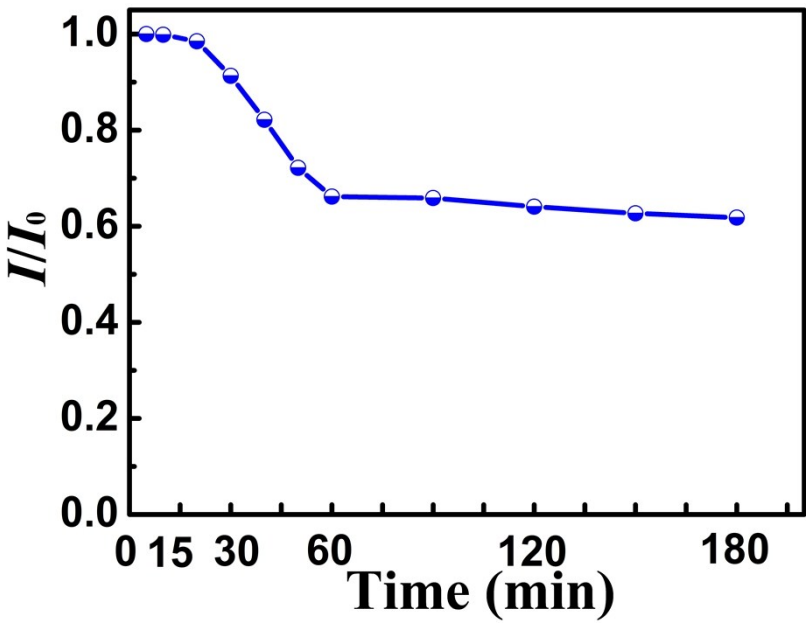


Fig. S4 The stability of the CDs under the light of a 150 W Xe lamp for 180 min; c_{CDs}, 2 μg/mL; λ_{ex}, 400 nm; λ_{em}, 530 nm.

Note: The as-prepared CDs showed good photostable properties within 15 min even if illuminated using a 150 W Xe lamp.

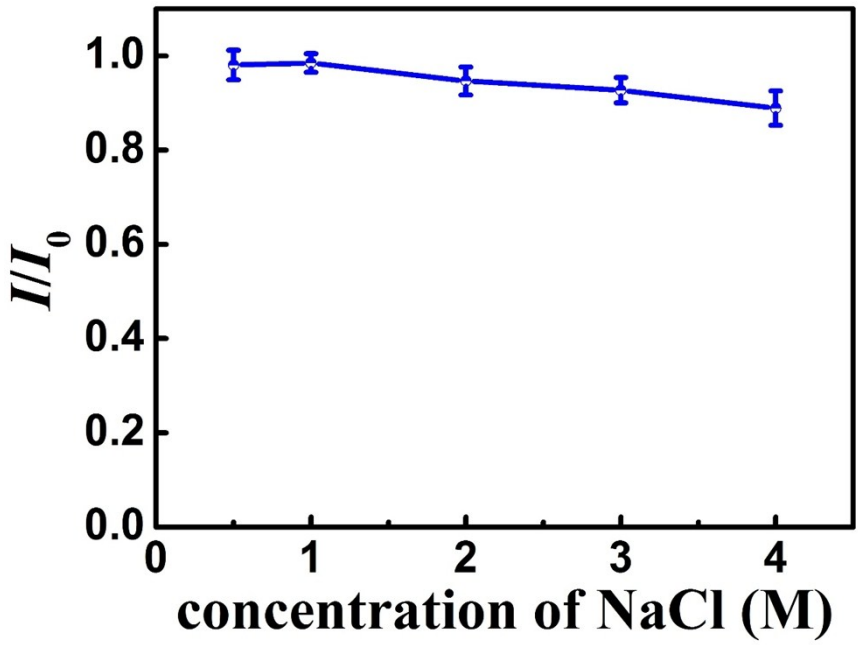


Fig. S5 The stability of the CDs in a salty medium; c_{CDs}, 2 μg/mL; λ_{ex}, 400 nm; λ_{em}, 530 nm.

Note: The as-prepared CDs remained stable in salt solution even with the concentration of NaCl as high as 4 M, which indicated the excellent stability of the CDs prepared.

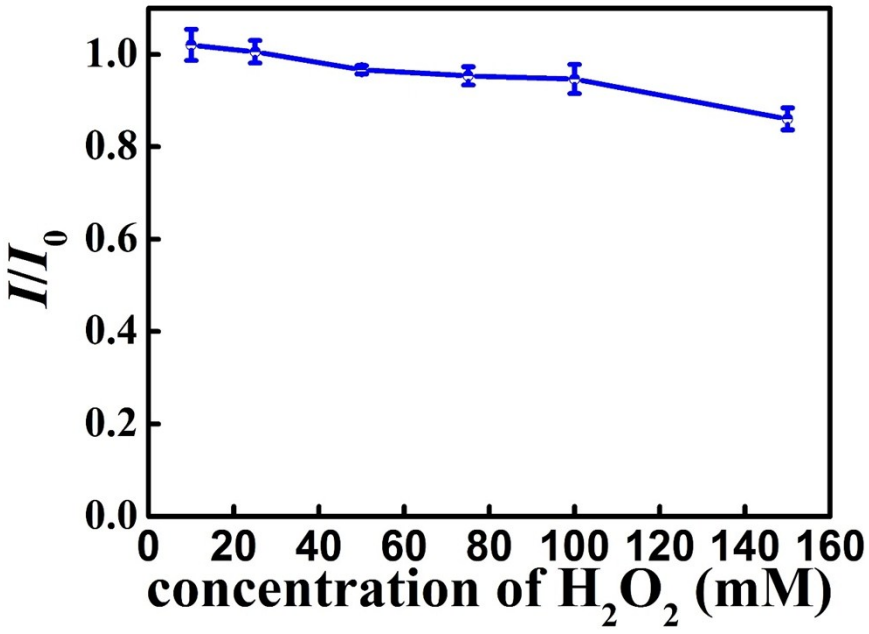


Fig. S6 The antioxidant capacity of CDs; c_{CDs}, 2 μg/mL; λ_{ex}, 400 nm; λ_{em}, 530 nm.

Note: The as-prepared CDs kept stable in H_2O_2 solution even with the concentration of H_2O_2 up to 0.15 M, which indicated the excellent stability of the CDs prepared.

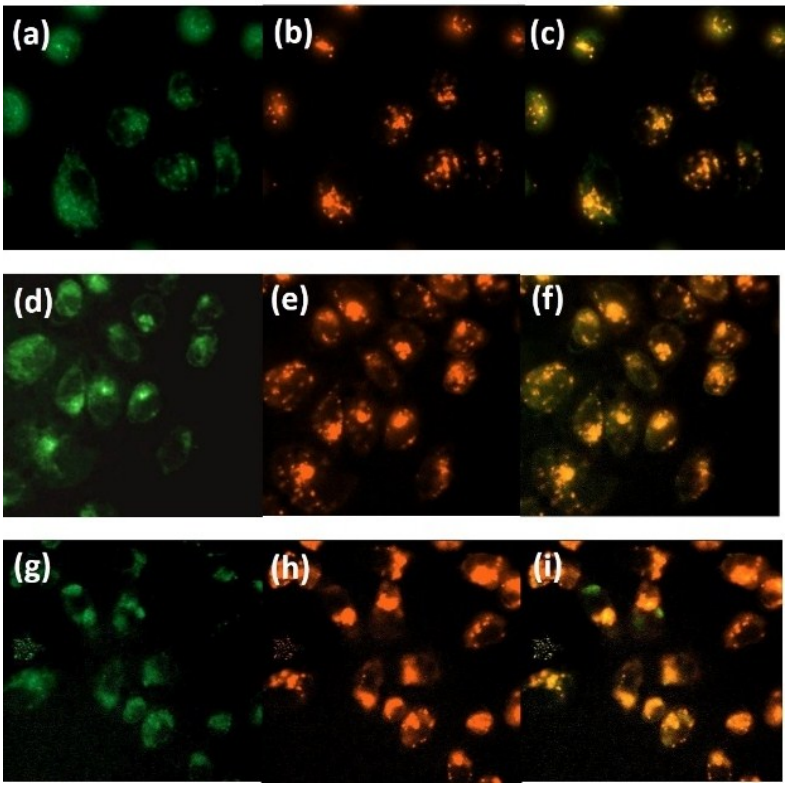


Fig. S7 CDs localizing to lysosomes in live A549. (a, b, c) Images of A549 cell incubated for 12h. Cells were stained with: (a) 10 μg/mL CDs, (b) Lyso-Tracker Red. (c) Areas of co-localization appear in yellow. (d, e, f) Images of A549 cell incubated for 24h. Cells were stained with: (d) 10 μg/mL CDs, (e) Lyso-Tracker Red. (f) Areas of co-localization appear in yellow. (g, h, i) Images of A549 cell incubated for 48h. Cells were stained with: (g) 10 μg/mL CDs, (h) Lyso-Tracker Red. (i) Areas of co-localization appear in yellow. CDs and Lyso-Tracker Red were excited at 470-490 nm and 510-560 nm, respectively. The fluorescence images were recorded at 510-550 nm (CDs) 570-650 nm (Lyso-Tracker Red), respectively.

Note: Incubated with CDs for even 48 h, the lysosome was tagged very well, which confirmed the well lysosome-targeting properties and capability of CDs for long-time lysosome imaging (Fig. S7)..

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