Electronic Supplementary Material (ESI) for Environmental Science Nano

Supporting Information for

Decorporation of uranyl in kidneys using an engineered nanocomposite

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S1. Synthesis of Bn-Cm-3,2-HOPO-COOH and 3,2-HOPO-COOH.

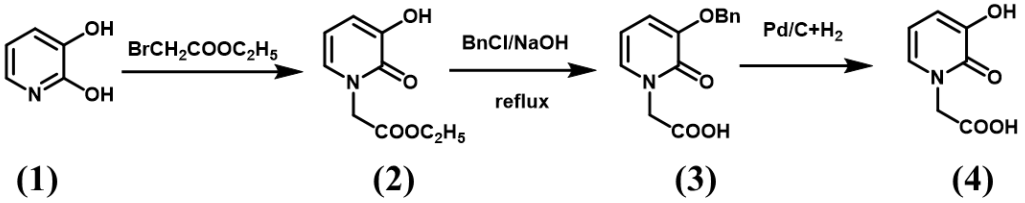

Fig. S1. The synthesis procedure of Bn-Cm-3,2-HOPO-COOH and 3,2-HOPO-COOH.

Synthesis of compound 2: 1,2-dihydro-2,3-pyridinediol (compound 1, 22.6 g, 0.2 mol) and ethyl bromoacetate (133.6 g, 0.8 mol) were stirred together and refluxed under nitrogen for 48 h. Then the solution was poured into a watch glass with a diameter of 20 cm to volatilize in fuming cupboard until yellow solid obtained. After that, the crude was then recrystallized with 95% ethanol to yield colorless solid, ethyl 2-(3-hydroxy-2-oxopyridin-1(2H)-yl) acetate (compound 2) (27.4 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.14 (s, 1H), 7.13 (d, J = 6.8 Hz, 1H), 6.72 (d, J = 7.2 Hz, 1H), 6.11 (t, J = 7.0 Hz, 1H), 4.71 (s, 2H), 4.14 (q, J = 5.3 Hz 2H), 1.20 (t, J = 7.0 Hz, 3H).

Synthesis of compound 3 (Bn-Cm-3,2-HOPO-COOH): Compound 2 (10.0 g, 0.05 mol) was first dissolved in 300 mL of 90% methanol (10% water), then benzyl chloride (25.0 g, 0.2 mol) was added. The reaction was refluxed at 100℃ for 6 h, with the pH of the solution maintaining above 12 by constantly addition of 10 mol/L NaOH. Until the TLC plates indicated the reaction was completed, methanol was removed by rotary evaporation. The remaining solution was diluted with 50 mL deionized water, followed by extracted with dichloromethane for at least three times. After extraction, the water layer was adjusted to pH ~ 1 with concentrated hydrochloric acid until white precipitate was formed. Compound 3 (10.8 g, 83%) was obtained by filtering the white precipitate and dried at 50℃ under vacuum for 24 h. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.44–7.32 (m, 5H), 7.26 (d, J = 6.4 Hz, 1H), 6.93 (d, J = 7.2 Hz, 1H), 6.15 (t, J = 7.2 Hz, 1H), 5.01 (s, 2H), 4.61 (s, 2H).

Synthesis of compound 4 (3,2-HOPO-COOH): Briefly, 5% Pd/C (100 mg) was added slowly to a solution of compound 3 (1.0 g, 3.86 mmol) in 50 mL methanol, and the reaction mixture was magnetically stirred in a H_2 atmosphere at room temperature for 6 h until the TLC plate indicated that the reaction was complete. Then, the product 3,2-HOPO-COOH was obtained by filtrating Pd/C, concentrated and washed with dichloromethane. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.97 (s, 1H), 9.07 (s, 1H), 7.12 (dd, J = 6.9, 1.7 Hz, 1H), 6.71 (dd, J = 7.2, 1.7 Hz, 1H), 6.09 (q, J = 7.0 Hz, 1H), 4.63 (s, 2H).

S2. The ^{1}H NMR and LC-MS of Bn-2LI-Cm-3,2-HOPO and 2LI-Cm-3,2-HOPO.

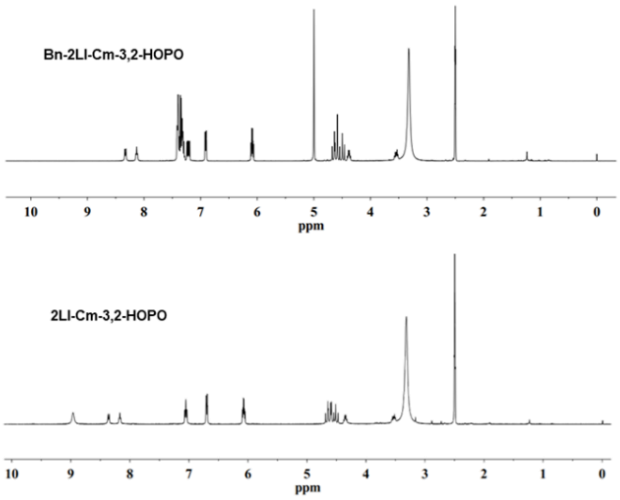

Fig. S2. NMR characterizations of Bn-2LI-Cm-3,2-HOPO and 2LI-Cm-3,2-HOPO.

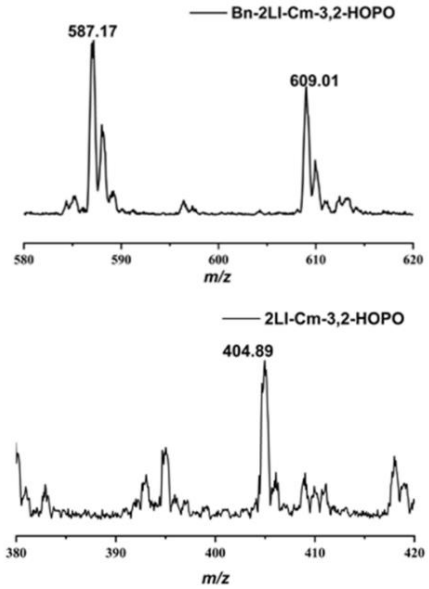

Fig. S3. LC-MS characterizations of Bn-2LI-Cm-3,2-HOPO and 2LI-Cm-3,2-HOPO.

S3. TEM, DLS and FT-IR characterizations.

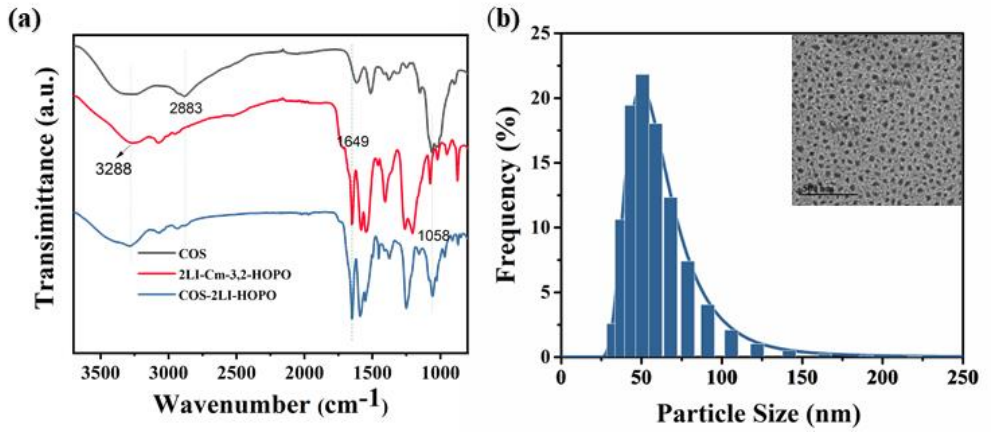

Fig. S4. (a): FT-IR spectra of COS, 2LI-Cm-3,2-HOPO and COS-2LI-HOPO; (b): TEM image (inset) and DLS data of COS-2LI-HOPO.

S4. Elemental analysis.

Table S1. Comparison of the elemental analysis results of COS,[1] 2LI-Cm-3,2-HOPO^{calcd} and COS-2LI-HOPO.

	C (%)	N (%)	H (%)
COS	34.39	6.76	7.073
2LI-Cm-3,2-HOPO	50.25	13.79	4.47
COS-2LI-HOPO	46.79	10.89	5.478

S5. Hydroxyl radicals scavenging ability of 3,2-HOPO-COOH and 2LI-Cm-3,2-HOPO.

To compare the hydroxyl radical scavenging ability of COS-HOPO and COS-2LI-HOPO, electron paramagnetic resonance (EPR) assay was conducted.

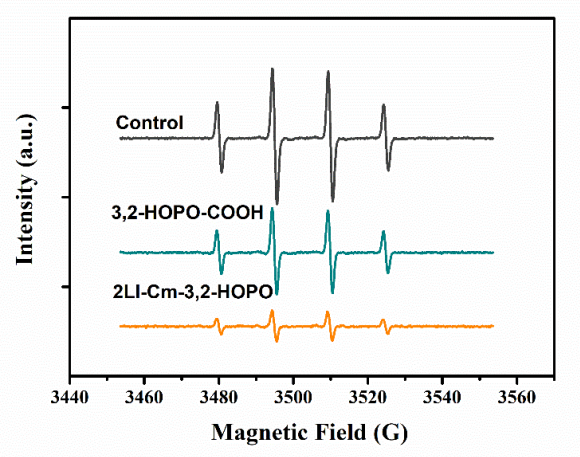

Fig. S5. EPR spectra of hydroxyl radical captured by 50.0 mM of DMPO after treatment with 12.5 mM of H_2O_2 and 3,2-HOPO-COOH (0.3 mg/mL) or 2LI-Cm-3,2-HOPO (0.7 mg/mL).

S6. Preparation and characterizations of Cy5.5-COS-2LI-HOPO.

Cy5.5 labeled COS-2LI-HOPO was prepared by amidation of the residual amine groups of COS and the NHS ester of Cy5.5. Briefly, 2.5 mg of Cy5.5 NHS ester (APE×BIO) was dissolved in 0.5 mL dried DMSO, then 18.8 mg of COS-2LI-Cm-3,2-HOPO was added, following by the addition of 10 μL triethylamine. The blue solution was stirred overnight in the dark. The resulting mixture was dialyzed (Mw = 500 – 1000 Da) against DI water for 48 h. Then the resulting blue solution was lyophilized for further study.

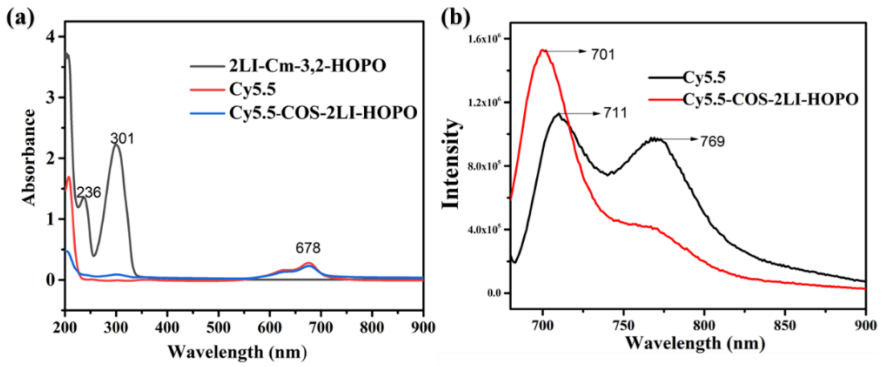

Fig. S6. (a): UV-Vis spectra of Cy5.5, COS-2LI-HOPO, and Cy5.5-COS-2LI-HOPO; (b): Fluorescence emission spectra of Cy5.5 and Cy5.5-COS-2LI-HOPO.

S7. *In vivo* biodistribution of Cy5.5-COS-2LI-HOPO.

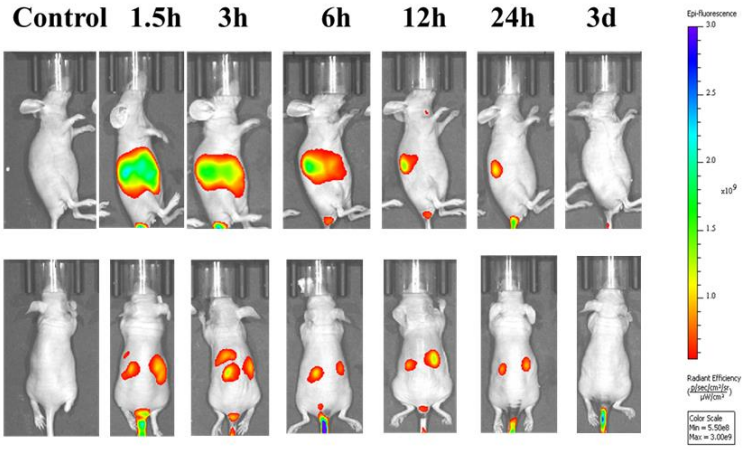

Fig. S7. *In vivo* fluorescence images of female nude mice after intravenously injected of Cy5.5-COS-2LI-HOPO (4.0 mg/kg) at 1.5 h, 3 h, 6 h, 12 h, 24 h and 3 d after administration.

S8. Cell viability assays.

Table S2. Dosage-dependent growth rate of NRK-52E cells treated with U[(VI), 12.4 μM] + 2LI-Cm-3,2-HOPO, U[(VI), 12.4 μM] + DTPA-$ZnNa_3$, and U[(VI), 12.4 μM] + COS-2LI-HOPO.

Concentration (μg/mL)	**U(VI) + 2LI-Cm-3,2-HOPO Survival Rate (%)**	**U(VI) + DTPA-$ZnNa_3$ Survival Rate (%)**	**U(VI) + COS-2LI-HOPO Survival Rate (%)**
18.8	93.3 ± 7.2	92.4 ± 3.0	98.3 ± 6.4
37.5	89.3 ± 5.9	90.1 ± 3.1	98.6 ± 5.1
75.0	85.4 ± 6.8	84.2 ± 3.1	97.9 ± 6.0
150.0	74.4 ± 2.6	81.3 ± 2.4	88.8 ± 4.5
300.0	63.5 ± 7.5	80.2 ± 3.1	77.1 ± 6.2

S9. ROS scavenging assays.

Table S3. Example of reagent list and flow cytometer setup.

Instrument: Becton Dickinson-FACS Verse			
Probe	DCFH-DA	Vendor/Cat.No	Beyotime Biotechnology/# S0033S
Laser lines	488 nm	Emission filters	525 nm

Table S4. ROS level in cells after incubated with U(VI), U(VI) + DTPA-$ZnNa_3$, U(VI) + COS, U(VI) + 2LI-Cm-3,2-HOPO, U(VI) + COS-2LI-HOPO.

Group	**ROS**
Control	5303.0 ± 430.6
U(VI)	7039.7 ± 1189.2
U(VI)+DTPA-$ZnNa_3$	7110.0 ± 1504.2
U(VI)+COS	5311.3 ± 1114.8
U(VI)+ 2LI-Cm-3,2-HOPO	3807.0 ± 1086.8
U(VI)+COS-2LI-HOPO	4402.3 ± 484.4

S10. *In vitro* and *in vivo* uranyl decorporation assays.

Table S5. U(VI) content of NRK-52E cells treated with U[(VI) 12.4 μM], U[(VI), 12.4 μM] + 100.0 μg/mL DTPA-$ZnNa_3$, and U[(VI), 12.4 μM] + 100.0 μg/mL COS-2LI-HOPO by prompt administration. *p < 0.05, **p < 0.01, ***p < 0.001 are compared with the U(VI)-treated control group, n = 3.

Group	U(VI) Content (ng/10^6 cells)
U(VI)	263.0 ±79.5
U(VI)+DTPA-$ZnNa_3$	223.8 ± 22.7
U(VI)+COS-2LI-HOPO	71.0 ± 7.2*

Table S6. U(VI) content of NRK-52E cells treated with U[(VI) 12.4 μM], U[(VI), 12.4 μM] + 100.0 μg/mL DTPA-$ZnNa_3$, and U[(VI), 12.4 μM] + 100.0 μg/mL COS-2LI-HOPO by delayed administration. *p < 0.05, **p < 0.01, ***p < 0.001 are compared with the U(VI)-treated control group, n = 3.

Group	U(VI) Content (ng/10^6 cells)
U(VI)	33.7 ± 2.2
U(VI)+DTPA-$ZnNa_3$	17.3 ± 7.9
U(VI)+COS-2LI-HOPO	8.0 ± 5.5**

Table S7. U(VI) retention in kidneys and femurs for the prophylactic administration. *p < 0.05, **p < 0.01, ***p < 0.001 are compared with the U(VI)-treated control group, n = 5.

(μg/g)	U (VI) + NS	U (VI) + DTPA-$ZnNa_3$	U(VI) + 2LI-Cm-3,2-HOPO	U (VI) + COS-2LI-HOPO
Kidneys	10.98 ± 6.72	11.89 ± 2.72	10.52 ± 4.75	4.96 ± 1.64
Femurs	8.47 ± 2.92	8.71 ± 6.66	5.65 ± 3.03	7.36 ± 1.99

Table S8. U(VI) retention in kidneys and femurs for the delayed administration. *p < 0.05, **p < 0.01, ***p < 0.001 are compared with the U(VI)-treated control group, n = 5.

(μg/g)	U(VI) + NS	U (VI) + DTPA -$ZnNa_3$	U (VI) + COS-2LI-HOPO
Kidneys	14.11 ± 1.52	12.98 ± 4.66	9.15 ± 2.60**
Femurs	4.85 ± 1.84	6.09 ± 3.17	4.89 ± 1.48

REFERENCES

1. C. Shi, X. M. Wang, J. M. Wan, D. Zhang, X. Yi, Z. L. Bai, K. Yang, J. Diwu, Z. F. Chai and S. A. Wang, 3,2-Hydroxypyridinone-Grafted Chitosan Oligosaccharide Nanoparticles as Efficient Decorporation Agents for Simultaneous Removal of Uranium and Radiation-Induced Reactive Oxygen Species in Vivo. *Bioconjugate. Chem*., 2018, **29**(11), 3896-3905.