Supporting Information

Amidoxime-Functionalized Covalent Organic Nanosheets for Sequestration of Uranium in Vivo

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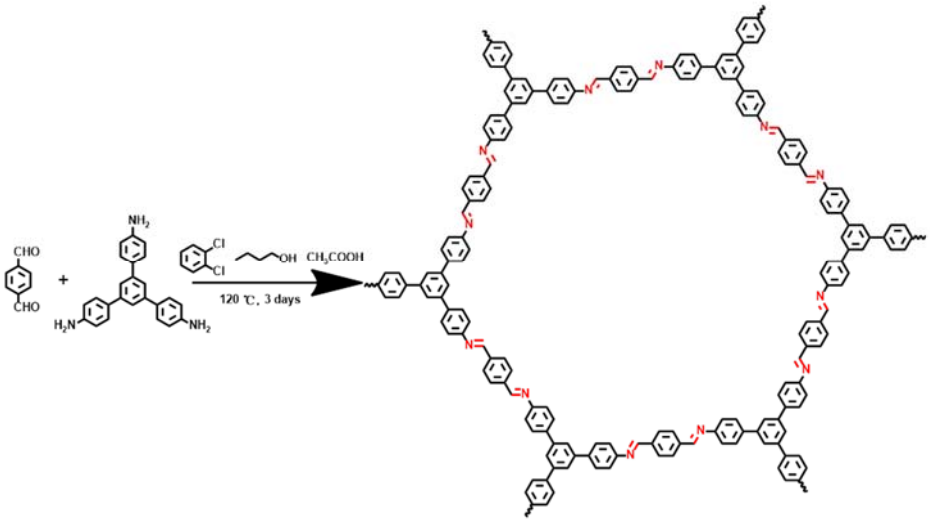
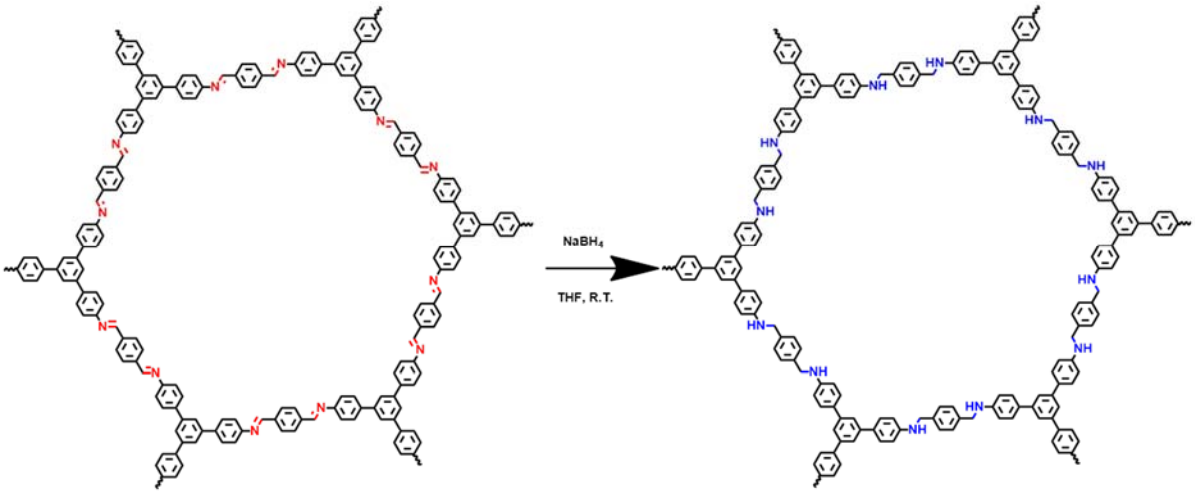
The synthesis of CON-AO.

COF-Imine:



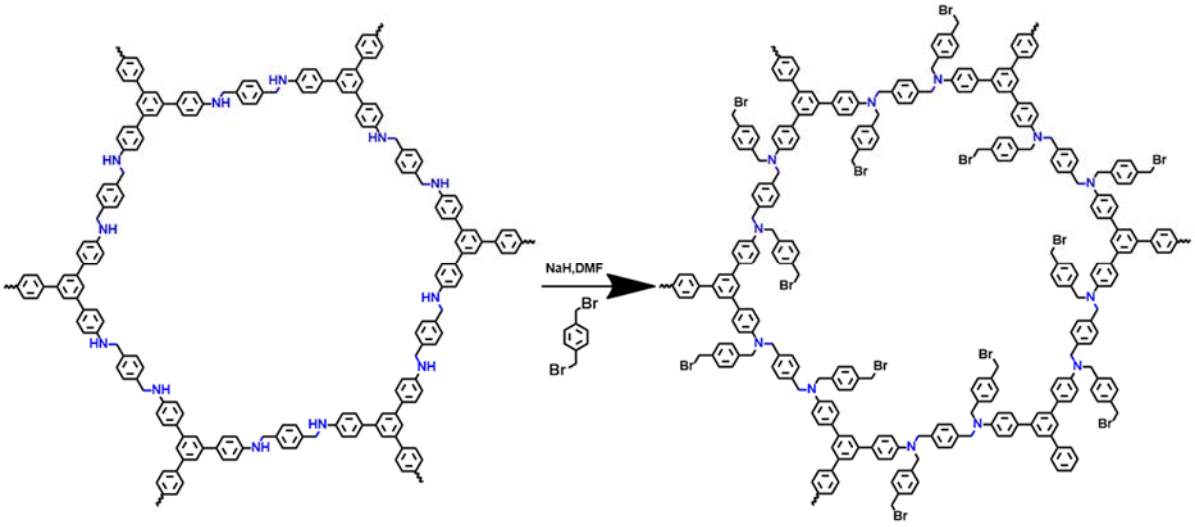
A pyrex tube (10 mL) was charged with terephthalaldehyde (32 mg, 0.24 mmol), tris(4-aminophenyl)benzene (56 mg, 0.16 mmol), o-dichlorobenzene (0.5 mL), 1-butanol (0.5 mL) and aqueous acetic acid (0.1 mL, 6 M). The tube was degassed for three freeze-pump-thaw cycles and then sealed. The reaction was heated at 120 ˚C for 72 h and yielded a yellow solid. The solid was isolated by filtration and washed with tetrahydrofuran (THF, 5 mL × 3) and ethanol (EtOH, 5 mL × 1). Then the material was activated with THF in a Soxhlet extractor for 24 h following drying under vacuum at 50 ℃ for 6 h.[1]

COF-Amine:



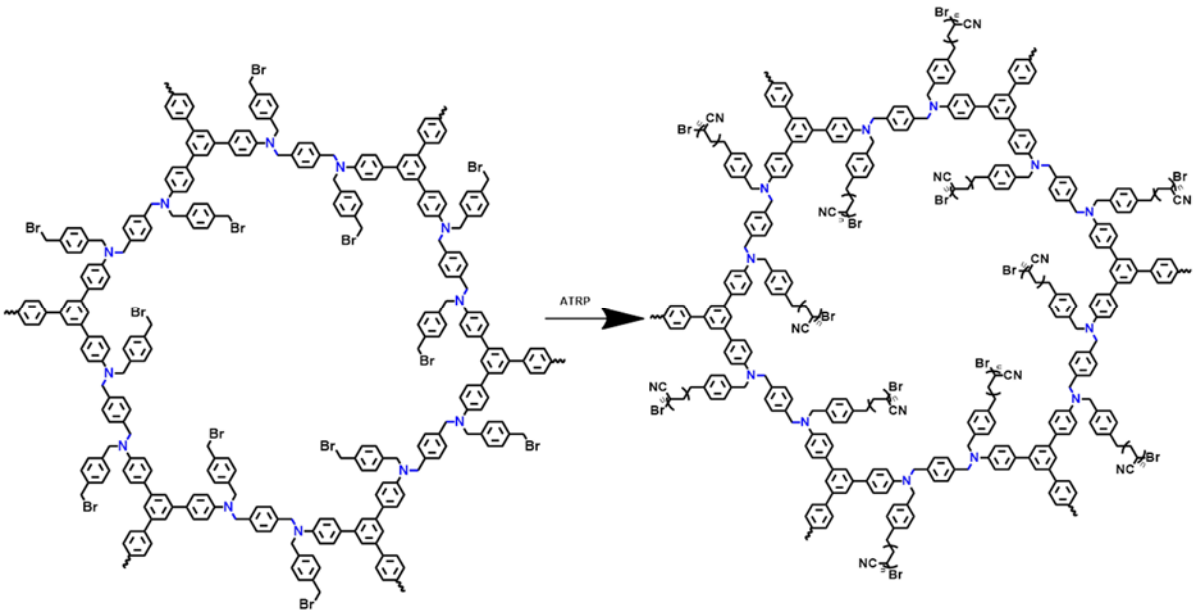
COF-Imine (250 mg, 0.75mmol) and $NaBH_4$ (1130 mg, 29.87 mmol) were mixed in 25 mL of anhydrous THF in a 50 mL flask under N_2 atmosphere. After sonication for 30 min, the reaction mixture was stirred for 12 h at room temperature. After the reaction finished, the mixed solution of H_2O and EtOH (4:1, v/v) was added into the reaction and stirred for 30 min to remove excess $NaBH_4$. The obtained yellow solid was isolated by centrifugation, and then washed with H_2O (20 mL × 4), THF (10 mL × 3) and EtOH (10 mL × 3), respectively. The solid was dried in a vacuum oven at 50 ℃ for 6 h.[2]

CON-Br:



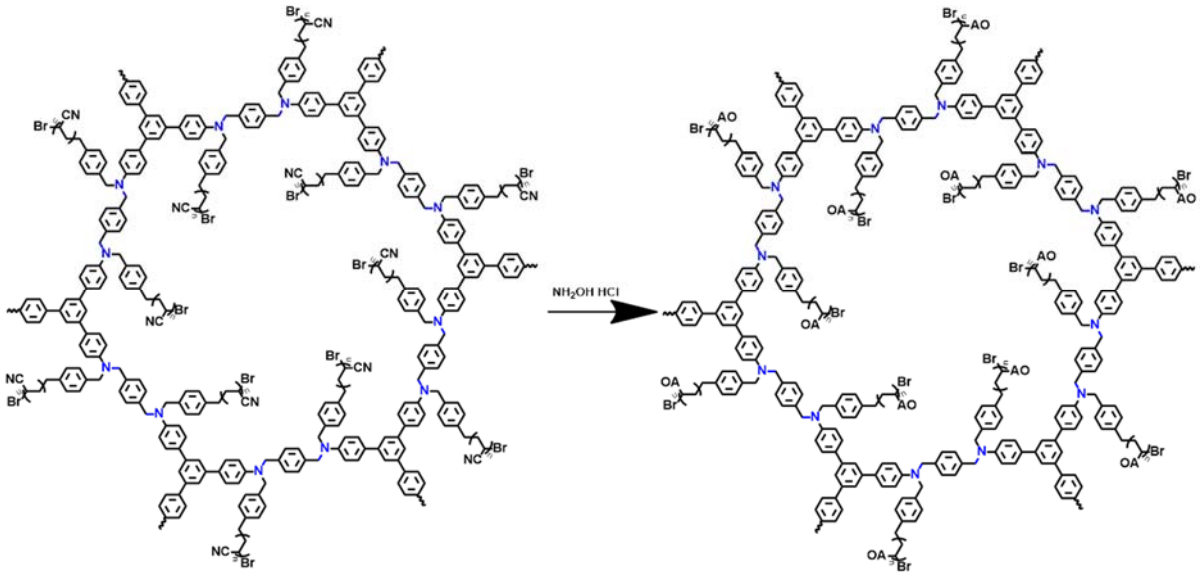
COF-Amine (250 mg, 0.75 mmol) and NaH (600 mg, 25 mmol) suspended in 25 mL of anhydrous N, N-dimethylformamide (DMF) and stirred for 30 min at room temperature. Subsequently, 1,4-bis(bromomethyl)-benzen (3.96 g, 15 mmol) was added to the mixed system and stirred for 12 h in nitrogen atmosphere. After the reaction finished, EtOH (20 mL) was added gradually to the orange suspension and stirred for 10 min in order to completely remove residual NaH. Then the orange solid was isolated by centrifugation, washed with EtOH (10 mL × 1), THF (10 mL × 3) and EtOH (10 mL × 1), respectively. The solid was dried in a vacuum oven at 50 ℃ for 6 h.[3]

CON-CN:



CON-Br (25 mg, 0.052 mmol), acrylonitrile (AN, 2.89 mL, 41.51 mmol), Me_6TREN (38 mg, 0.16 mmol), $CuBr_2$ (1 mg, 0.005 mmol) and DMSO (1.615 mL, 33.80 mmol) were mixed in a pyrex tube, and the mixture was bathed in ice-water for 15 min. Subsequently, the mixture was bubbled with nitrogen for 15 min to remove the dissolved oxygen from the solution. CuBr was washed with glacial acetic acid and diethyl ether to remove any impurities and then dried in vacuum before use. Then CuBr (14.3 mg, 0.10 mmol) was added to the tube and the mixture was degassed by freeze-pump-thaw for three times. The tube was put into an oil bath at 338 K and reacted for 24 h. After cooling the reaction mixture to ambient temperature, the yellow solid was acquired by centrifugal separation, and washed with DMF (5 mL × 3) and EtOH (5 mL × 3). Finally, the solid was dried in a vacuum oven at 50 ℃ for 6 h.[4]

CON-AO:



CON-CN (20 mg, 0.188 mmol), hydroxylamine hydrochloride (130 mg, 0.94 mmol) and triethylamine (190 mg, 0.94 mmol) were charged in a flask containing 25 mL of EtOH. Then the reaction was stirred at 80 ºC for 24 h at N_2 atmosphere, the yellow solid was isolated by centrifugation and washed with EtOH (5 mL × 3). The product was dried in a vacuum oven at 50 ºC for 6 h.

Uranium sorption kinetics study. 30 mg of CON-AO powder was put into 30 mL of HEPES buffer solution (20 mM, pH 7.40) containing U (VI) (4.56 ppm). The mixture was shaking during the experiment and an aliquot UO_2^{2+} solution was extracted at various sorption times (1, 5, 30, 60, 180, 360, 600, 1440 min). Each sample was filtered immediately by a nylon membrane filter (0.22 μm) and the concentration of uranium was determined by ICP-AES. The value of distribution coefficient (K_d, mL g^{-1}) under specific conditions was determined as follows:

$$K_d = \frac{Co-Ce}{Ce} \times \frac{V}{m},$$

where Co is the original concentration of U(VI), Ce is defined as the concentration when reaching equilibrium, V is the total volume of uranyl solution (mL), and m is the mass of CON-AO (g).

Sorption isotherm study. The UO_2^{2+} sorption isotherm experiments were carried out at pH 5. For each experiment, 3 mg of CON-AO was immersed into 3 mL of uranium solution of a certain concentration in a vial, followed by stirring for 24 h at room temperature. Finally, the sample was filtered by a nylon membrane filter and diluted with 3% HNO_3 for ICP-AES analysis.

pH-dependent uranium adsorption. 5.0 mg of CON-AO was added into 10 mL of U(VI) solution (8 ppm, pH 3~9) and then the mixture solution was shaken for 1 h, 5 h, and 24 h respectively. The mixture was filtered, and the concentration of uranium was determined by ICP-AES.

The adsorption selectivity. In a simulated physiological environment of HEPES (20 mM), uranyl ions and other divalent ions were mixed to evaluate the selectivity of CON-AO toward uranyl. The first set of bicomponent competitive adsorption experiment was carried out by mixing CON-AO (5 mg), uranyl (10 ppm), and each divalent ion (50 ppm, Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Zn(II)) in 10 mL of HEPES solution. The second experiment was carried out by mixing CON-AO (5 mg), uranyl (4 ppm), and a group of divalent ions (10 ppm, Mg(II), Ca(II), Mn(II), Co(II), Ni(II), Zn(II)) in 10 mL of HEPES. All samples were shaken for 24 h. The concentrations of each metal ion were analyzed by ICP-AES.

The Stability of CON-AO in HEPES (pH 7.4). CON-AO powder (60 mg) was put into the buffer solution (20 mM) and soaked for 24 h. The yellow solid was collected by centrifuging for 2 min (6000 rpm) and dried in a vacuum oven at 50 ℃ for 6 h. Finally, the stability of CON-AO was characterized by N_2 adsorption isotherms and FT-IR spectra.

DLS (Dynamic Light Scattering). CON-AO powder (1 mg) was ultrasonically dispersed in EtOH (1 mL) for 6 h and then ultrapure water (2 mL) was added. The particle size of the nanosheet was determined by DLS.

TEM and AFM measurements. 1 mg of CON or COF materials was ultrasonically dispersed in 3 mL THF or EtOH for 3 h. For TEM test, the suspension was dropped on a copper grid and dried on a glass slide. For AFM measurement, the suspension was dropped on a mica plate and dried at room temperature.

Cell line and cytotoxicity assays: The cell viability assay of uranyl and CON-AO was performed to evaluate its comprehensive cytotoxicity using the renal proximal tubular epithelial cells (NRK-52E). The cell line was received from Chinese Academy of Sciences and reared in the mixture of F-12 nutrient, fetal bovine serum (10%, volume ratio), and Penicillin-Streptomycin (1%) in a humid atmosphere containing 5% CO_2 at 37 ℃, which was propagated every two days.

The comprehensive cytotoxicity of CON-AO and $UO_2(NO_3)_2{\cdot}6H_2O$ was carried out to assess the bio-toxicity of CON-AO. The uranyl cytotoxicity assay has been reported in our previous study,[5] and 12.4 μM of $UO_2(NO_3)_2{\cdot}6H_2O$ was treated as the suitable concentration. The cells were firstly reared in a 96-well plate for 24 h, and then added the medium mixture containing $UO_2(NO_3)_2{\cdot}6H_2O$ (12.4 μM) and various concentrations of CON-AO or $ZnNa_3$-DTPA from 3 to 50 μg/mL, while the control group was cultured only with medium. The cells were continuously reared for 48 h. Whereafter, CCK-8 (10.0 μL) was added into each well. NRK-52E cells were allowed to rear for 1 ~ 2 h. The cell survival rate was determined on the basis of the reported formula, and $*p < 0.05$ was regarded as statistical significance.[6]

In vivo uranyl sequestration: The uranyl stock solution was prepared by dissolving $UO_2(NO_3)_2{\cdot}6H_2O$ (3.2 mg) in brine solution (20.0 mL, 0.14 M). $ZnNa_3$-DTPA (25.8 mg) dissolved in brine solution (2.8 mL, 0.14 M) to prepare the $ZnNa_3$-DTPA (60 mg/kg) stock

solution with pH at 7 ~ 8. The CON-AO (60 mg/kg) stock solution was prepared by dispersing CON-AO (36.8 mg) into 4.0 mL of brine solution (0.14 M) with pH at 7 ~ 8. In this study, the animals used for the in vivo uranyl decorporation assays are female Kunming mice, which are 84 ~ 86-days old and the body weight is (30.7 ± 1.5) g. All the assays have been confirmed by the Animal Care and Use Committee in Soochow University. In addition, all the animal experiments are performed according to the National Institutes of Health guidelines. The uranium decorporation assays were performed by intravenously (i.v.) injection of CON-AO in prophylactic administration and prompt injection modes. For the prophylactic administration groups, the experiments group were i.v. injected 0.2 mL CON-AO (or $ZnNa_3$-DTPA) stock solution, followed by i.v. injection of U(VI) stock solution (0.2 mL) 1 h later, while the control groups were given the corresponding volume of NaCl solution (0.14 M); the CON-AO group with prompt injection was performed by i.v. injection of CON-AO stock solution (0.2 mL) immediately after i.v. injection of uranyl stock solution (0.2 mL) (**Table S5**). In order to further understand the uranium removal efficacy of CON-AO in prompt injection, another assay was performed by i.v. injecting $ZnNa_3$-DTPA stock solution (0.2 mL) immediately after i.v. injection of U(VI) stock solution (0.2 mL), while the control group was injected NaCl solution (0.2 mL, 0.14 M) similarly (**Table S6**). The statistical analyses were carried out by following the published methods.[6]

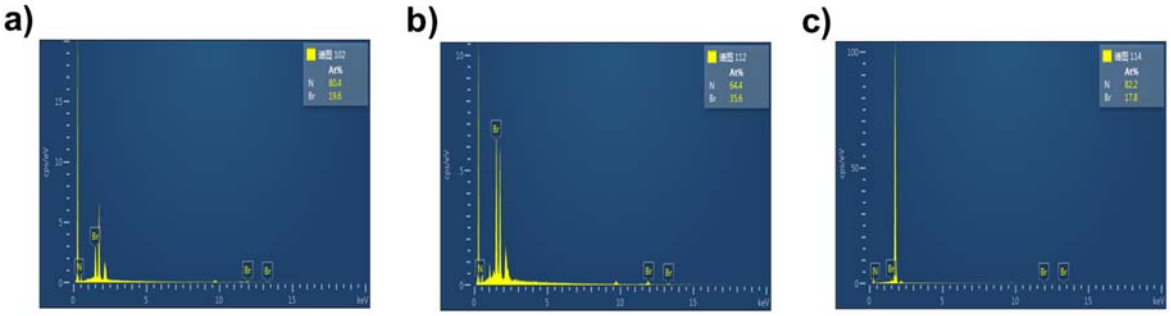


Figure S1. EDS analysis of the bromine contents of CON-Br samples obtained from reactions in different solvents: a) THF, b) DMF, c) THF : DMF (v : v = 1 : 1).

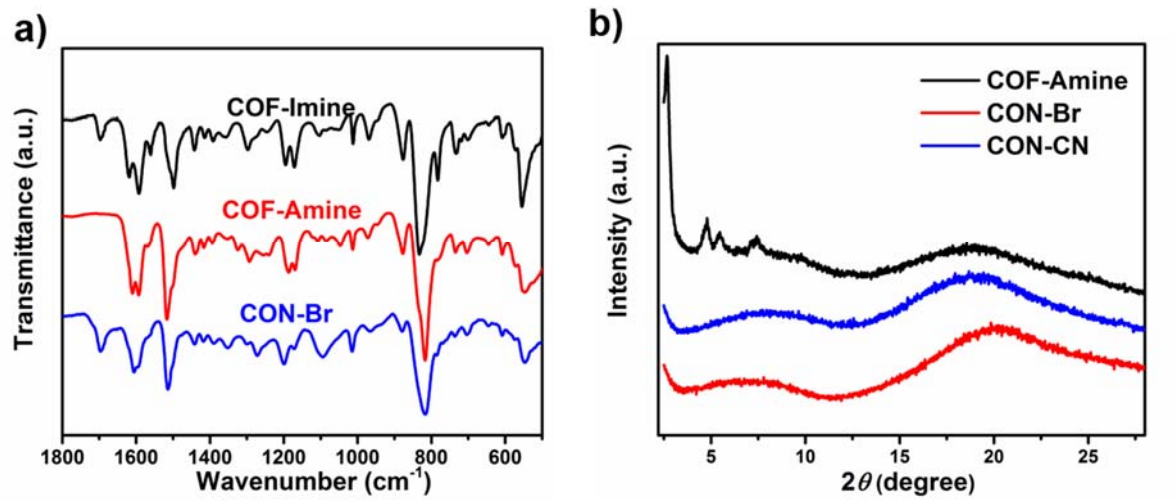


Figure S2. (a) The FT-IR spectra of COF-Imine, COF-Amine, and CON-Br. (b) The PXRD patterns of COF-Amine, CON-Br, and CON-CN.

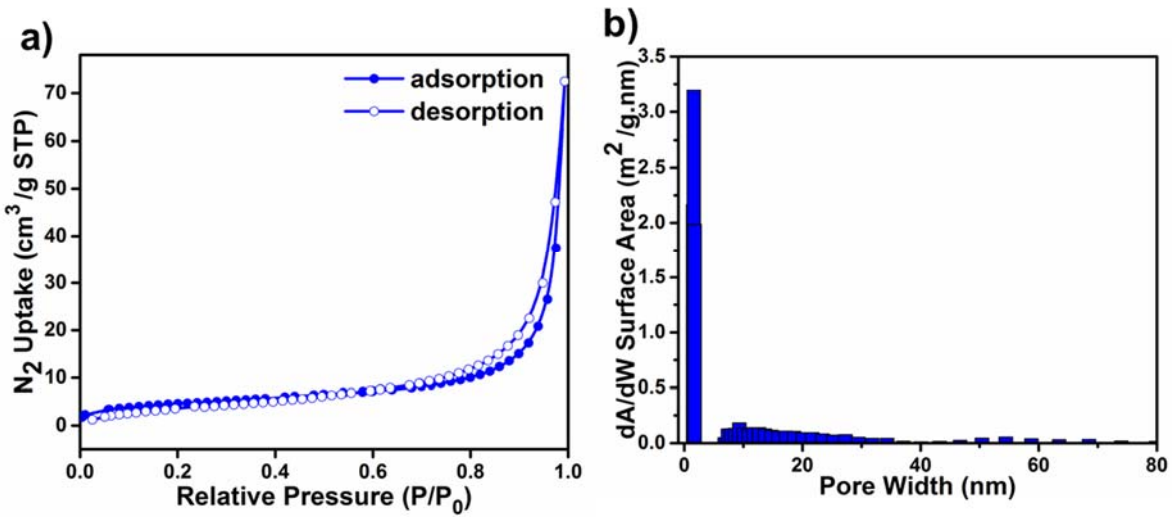


Figure S3. (a) N_2 adsorption-desorption isotherms of CON-AO. (b) The pore-size distribution of CON-AO.

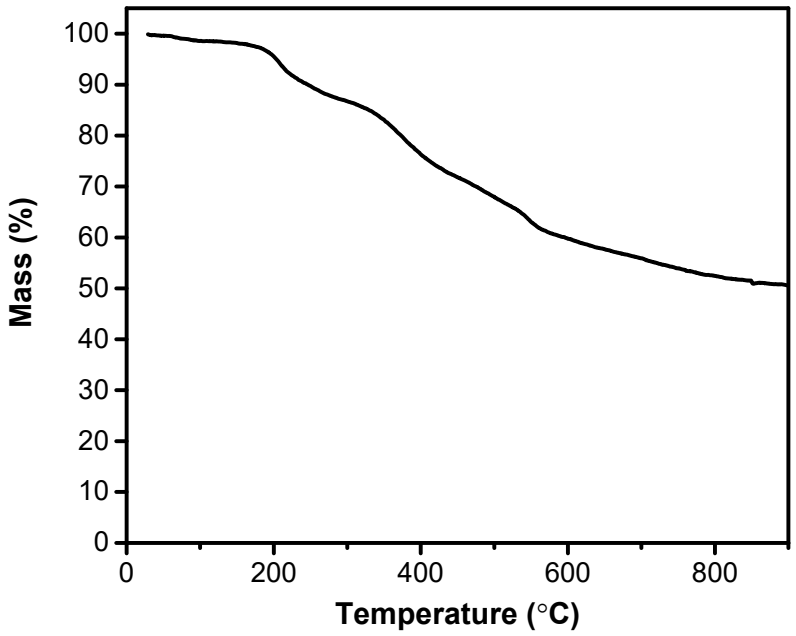


Figure S4. The TGA analysis of CON-AO in N_2.

Table S1. The elemental analysis results of the COF and CON materials.

Materials	N(%)		C(%)		H(%)	
	Found	Calculated	Found	Calculated	Found	Calculated
COF-Imine	8.07	8.43	83.62	86.72	5.12	4.85
COF-Amine	8.06	8.33	83.34	85.68	5.43	5.99
CON-Br	6.12	3.99	75.95	68.39	5.31	4.88
CON-CN	9.90	10.98	74.55	68.24	5.68	5.13
CON-AO	11.56	9.93	66.20	61.70	6.10	5.71

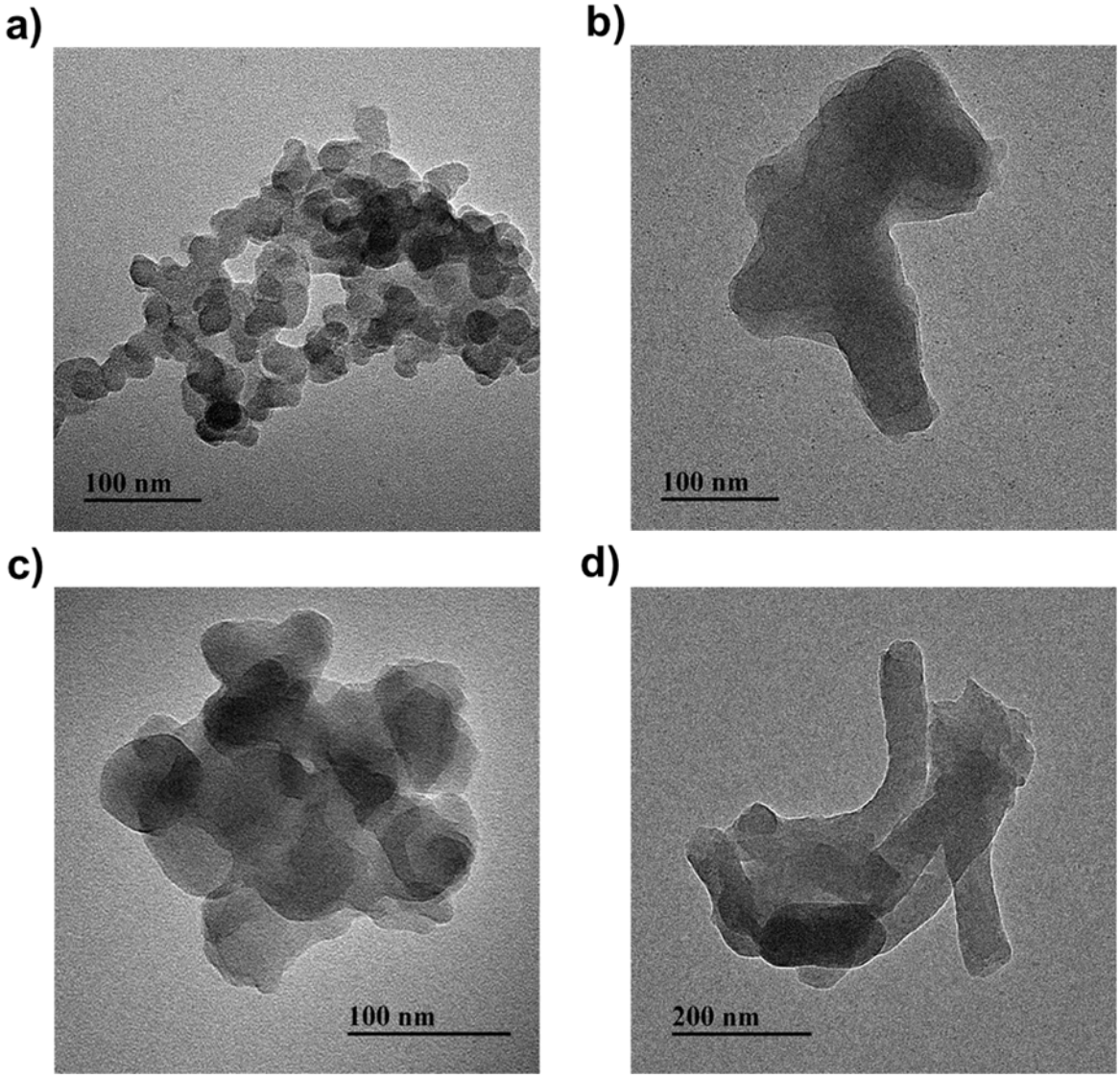


Figure S5. The TEM images of (a) COF-Imine, (b) COF-Amine, (c) CON-Br, and (d) CON-CN.

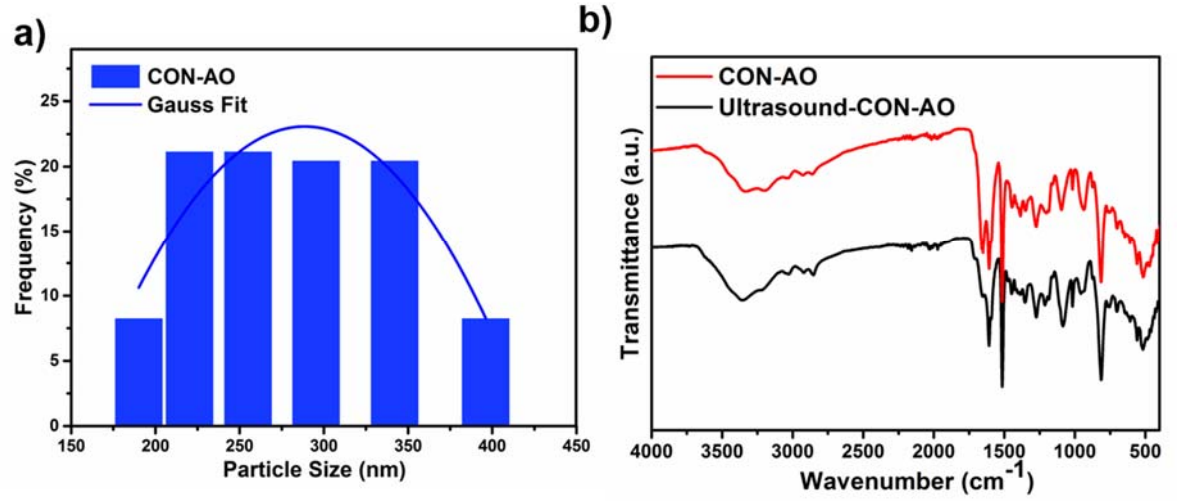


Figure S6. (a) The DLS data of CON-AO. (b) The FT-IR spectra of the CON-AO (red line) before and after (black line) ultrasonication for 6 h.

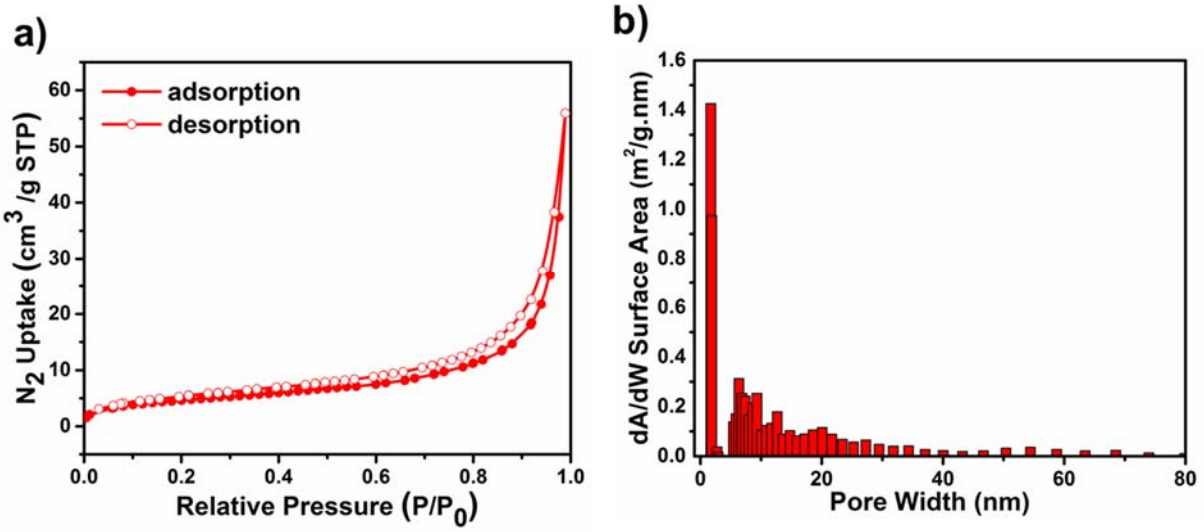


Figure S7. (a) N_2 adsorption-desorption isotherms and (b) pore-size distribution of CON-AO after soaking in HEPES for 24 h.

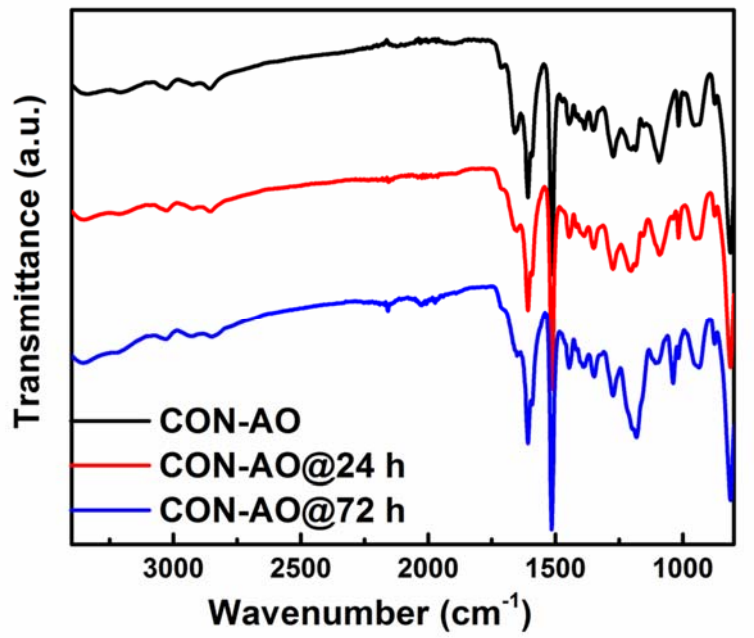


Figure S8. The FT-IR spectra of CON-AO after soaking in HEPES solution for 24 h and 72 h.

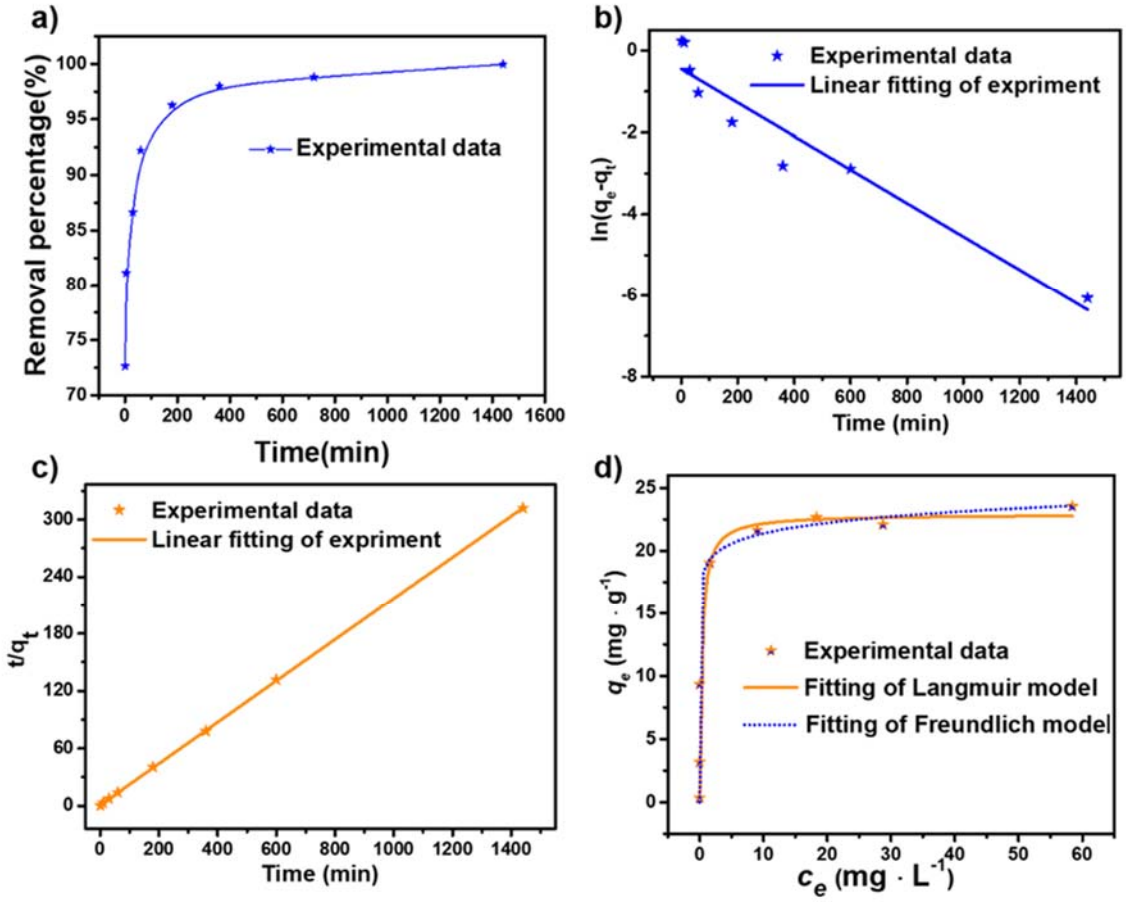


Figure S9. (a) The kinetics of uranyl adsorption with an initial concentration of 4.56 ppm U(VI) in buffered solution, m/V = 1 g/L. (b) Pseudo-first-order model fitting for the uranyl sorption kinetics by CON-AO. (c) Pseudo-second-order model fitting for the uranyl sorption kinetics by CON-AO. (d) Uranium sorption isotherms for CON-AO, m/V = 1 g/L.

Table S2. Kinetic parameters of pseudo-first-order and pseudo-second-order models for uranyl sorption by CON-AO.

	CON-AO	
Pseudo-first-order	k_1	0.0041
	q_e	0.6375
	R^2	0.9147
Pseudo-second-order	K_2	0.0531
	q_e	4.6258
	R^2	0.9999

The adsorption kinetics of uranium are fitted by the following two equations:

pseudo-first-order model: $\ln(q_e - q_t) = \ln q_e - k_1 \mathrm{t}$,

pseudo-second-order model: $\frac{\mathrm{t}}{\mathrm{q_t}} = \frac{1}{\mathrm{k_2 q_e}^2} + \frac{\mathrm{t}}{\mathrm{q_e}}$,

where q_t [mg·g^{-1}] is the amounts of adsorbed uranium (mg) for per gram of CON-AO at contact time and q_e [mg g^{-1}] is the adsorbed amounts at equilibrium time, t [min] is contact time, k_1 [min^{-1}] and k_2 [g mg^{-1} min^{-1}] are the rate constants for pseudo-first-order and pseudo-second-order, respectively.

Table S3. Parameters for Langmuir and Freundlich model fitting for uranyl sorption isotherms of CON-AO.

	CON-AO	
Langmuir	q_{max} (mg g^{-1})	22.90
	R^2	0.82
Freundlich	K_f (mg g^{-1})	18.81
	R^2	0.82

The adsorption isotherm was fitted by the following two models:

Langmuir sorption isotherm model: $\frac{1}{q_e} = \frac{1}{q_{max}} + \frac{1}{K_L q_{max} C_e}$

Freundlich sorption isotherm model: $lnq_e = lnK_f + \frac{1}{n} lnC_e$

where q_e (mg g^{-1}) is the adsorption capacity at equilibrium concentration, Ce (mg L^{-1}) is the equilibrium concentration, q_{max} (mg $g^{-1)}$ is the maximum sorption capacity, K_L and K_f are the constants of Langmuir and Freundlich model, respectively.

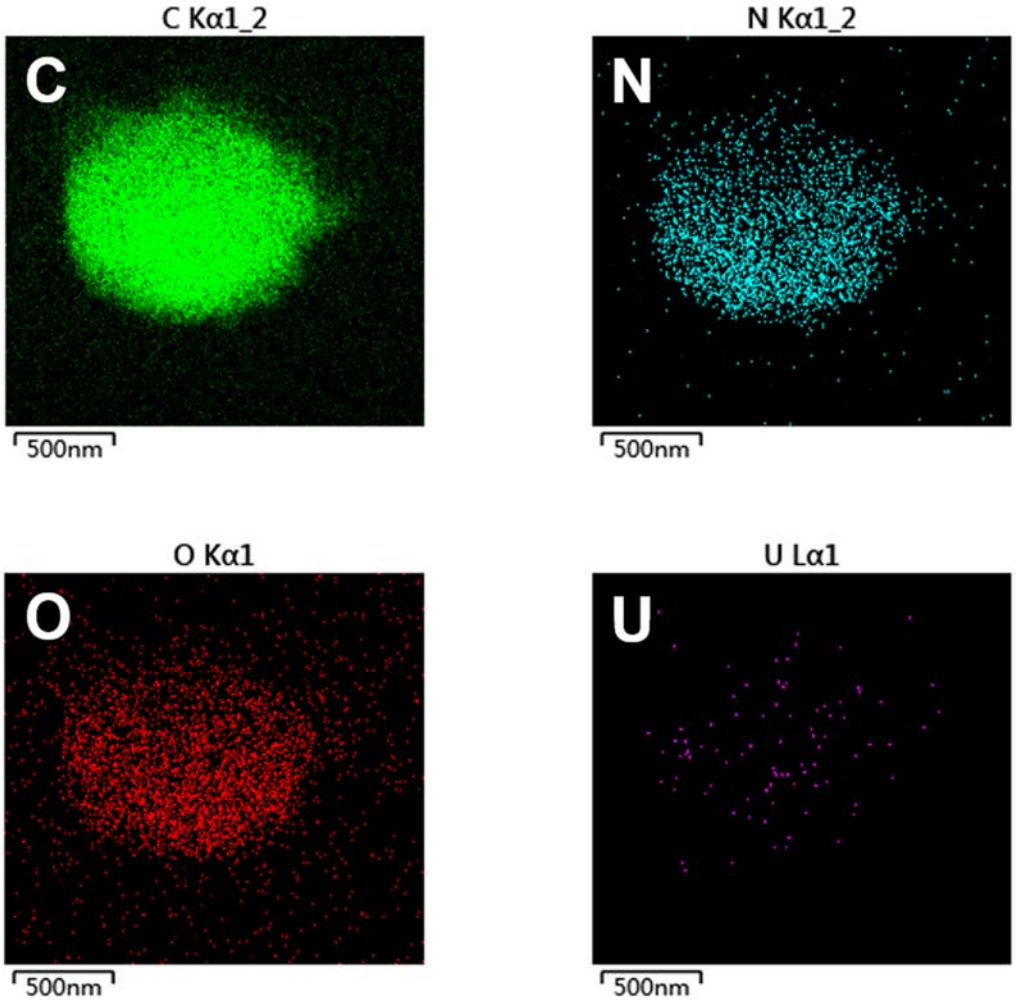


Figure S10. The TEM mapping of CON-AO before the uranyl adsorption.

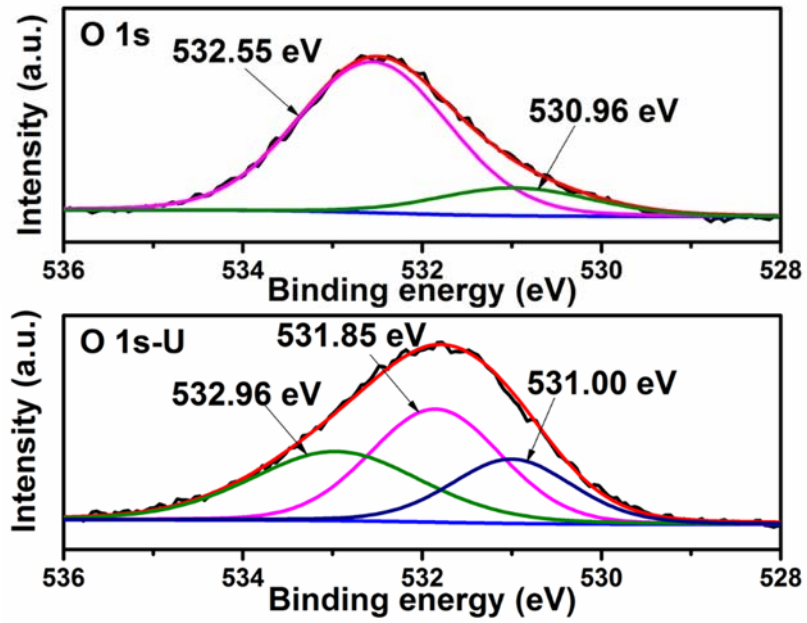


Figure S11. Expanded XPS spectra O1s of U(VI)@CON-AO.

Table S4. The comprehensive cytotoxicity study of CON-AO at the cellular level.

Concentration ($\mu g \cdot mL^{-1}$)	U(VI) + $ZnNa_3$-DTPA Survival Rate (%)	U(VI) + CON-AO Survival Rate (%)
3.13	89.96 ± 6.52	88.82 ± 8.60
6.25	93.61 ± 4.24	85.98 ± 6.31
12.50	82.97 ± 5.94	82.77 ± 7.95
25.00	81.27 ± 10.0	79.05 ± 9.00
37.50	77.19 ± 9.72	72.49 ± 4.37
50.00	84.51 ± 2.57	64.40 ± 8.07

Table S5. The amount of U(VI) in kidneys and femurs (μg per g).

	U (VI) + NS-prophylactic administration	U (VI) +$ZnNa_3$-DTPA-prophylactic administration	U (VI) + CON-AO-prophylactic administration	U (VI) + CON-AO-prompt injection
Kidneys	9.76 ± 1.03	8.28 ± 3.13	7.11 ± 0.57	4.89 ± 1.15
Femurs	3.28 ± 0.45	3.23 ± 0.80	2.37 ± 0.34	3.08 ± 0.40
Liver + spleen + muscle	1.28 ± 0.27	0.51 ± 0.05	1.05 ± 0.44	0.72 ± 0.23

Table S6. The amount of U(VI) in kidneys and femurs (μg per g).

	U (VI) + NS-prompt injection	U(VI) + $ZnNa_3$-DTPA -prompt injection
Kidneys	9.28 ± 1.64	8.22 ± 2.08
Femurs	2.86 ± 0.45	3.19 ± 0.60
Liver + spleen + muscle	1.11 ± 0.09	0.87 ± 0.44

Reference

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