

Electronic Supplementary Information for

ZIF-8 with cationic defects toward efficient $^{125}\text{I}_2$ uptake for in vitro radiotherapy of colon cancer

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Experimental

Materials and characterization

2-Methylimidazole (Im) ($\geq 98\%$, Aladdin), $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ ($\geq 99\%$, Sinopharm Chemical), CH_3I ($\geq 99.5\%$, Energy Chemical), and other solvents and reagents were obtained from commercial sources and used as received. Sodium iodide (Na^{125}I , 13 mCi) was bought from Shanghai Syncor Medicine Co. Ltd. Phosphate buffer solution (PBS) and Roswell Park Memorial Institute 1640 (RPMI 1640) were bought from Wisent Biotechnology (Nanjing) Co. Ltd. Cell Counting Kit-8 (CCK-8) was purchased from meilunbio (Dalian). Mouse colon cancer cell CT26 and human colon cancer cells HCT116 were purchased from the National Collection of Authenticated Cell Cultures.

Fourier transform infrared (FT-IR) spectra were recorded on a Bruker VERTEX 70+HYPERION 2000 spectrometer. Powder X-ray diffraction (PXRD) patterns were obtained with a Bruker AXS D8 Advanced SWAX diffractometer using $\text{Cu-K}\alpha$ (1.5406 Å) radiation. ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz NMR spectrometer. The scanning electron microscopy (SEM) images were obtained on a HITACHI S-4700 field-emission scanning electron microscope. Low-pressure gas adsorption measurements were obtained with a QUADRASORB Si automatic specific surface area and porosity analyzer. Thermogravimetric analysis (TGA) was performed on a TA Instruments Q500 thermogravimetric analyzer at a heating rate of $10^\circ\text{C min}^{-1}$ under a nitrogen gas flow in an Al_2O_3 pan. The determination of Zn content was carried out on a Varian 710-ES inductively coupled plasma mass spectrometer (ICP-MS). Radioactivity was measured with an activity meter (FJ-391A4). The count per minute (CPM) was measured with a γ counter (GC1200). The hydraulic diameter was determined by dynamic light scattering (DLS, Zetasizer Nano-ZS).

Synthesis of 2,3-dimethyl-1H-imidazol-3-ium iodide (Dmim)

To a THF (20 mL) solution containing Im (411 mg, 5 mmol) was added CH_3I (710 mg, 5 mmol) and the mixture refluxed overnight. The resulting solid was separated by filtration and washed with THF three times to remove excess CH_3I , before vacuum-drying in an oven at 70°C overnight. ^1H NMR (400 MHz, D_2O) δ 7.36 – 7.27 (m, 2H), 3.79 (d, $J = 0.9$ Hz, 3H), 2.61 (d, $J = 9.5$ Hz, 3H). IR (KBr disc; cm^{-1}): 3422 (m), 3083 (m), 2945 (w), 1603 (m), 1532 (w), 1432 (m), 1372 (s), 1260 (s), 1208 (w), 1138 (m), 1103 (s), 1035 (m), 930 (m), 753(vs), 637(s).

Synthesis of ZIF-8

Solutions of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (110 mg, 0.5 mmol) and Im (1230 mg, 15 mmol) in deionized water (5 mL) were separately prepared and then mixed. This mixture was allowed to stand at r.t. for 5 h to yield a white precipitate, which was filtered by centrifugation, washed three times with deionized water, and then lyophilized to obtain the final product ZIF-8. ^1H NMR (400 MHz, $\text{DMSO-}d_6$ and 10% D_2SO_4) δ 7.38 (s, 2H), 2.48 (s, 3H). IR (KBr disc; cm^{-1}): 3135 (w), 2452 (w), 1668 (w), 1584 (w), 1383 (m), 1310 (m), 1180 (m), 1146 (vs), 995 (s), 954 (w), 759 (s), 694 (m), 685 (m).

Synthesis of ZIF-8+(50) and ZIF-8+(38)

Dmim (112 mg, 0.5 mmol) and Im (1230 mg, 15 mmol, 30 equivalent) dissolved in 5 mL of deionized water was mixed with Zn(OAc)₂·2H₂O (110 mg, 0.5 mmol) in deionized water (5 mL) and allowed to stand at r.t. for 5 h to give a white precipitate, which was filtered by centrifugation, washed with deionized water three times, and lyophilized to obtain **ZIF-8+(50)**. Using a similar protocol, **ZIF-8+(38)** was prepared using Im and Dmim in a 20 : 1 ratio. Characterization data for **ZIF-8+(50)**: ¹H NMR (400 MHz, DMSO-*d*₆ and 10% D₂SO₄): δ 7.38 (s, 2H), 3.65 (s, 0.06H), 2.48 (s, 3H). IR (KBr disc; cm⁻¹): 3625 (w), 3135 (w), 2452 (w), 1668 (w), 1584 (w), 1383 (m), 1310 (m), 1180 (m), 1146 (vs), 995 (s), 954 (w), 759 (s), 694 (m), 685 (m). Characterization data for **ZIF-8+(38)**: ¹H NMR (400 MHz, DMSO-*d*₆ and 10% D₂SO₄): δ 7.37 (s, 2H), 3.64 (s, 0.08H), 2.48 (s, 3H). IR (KBr disc; cm⁻¹): 3625 (w), 3135 (w), 2452 (w), 1668 (w), 1584 (w), 1383 (m), 1310 (m), 1180 (m), 1146 (vs), 995 (s), 954 (w), 759 (s), 694 (m), 685 (m).

Iodine uptake by ZIF-8, ZIF-8+(50), and ZIF-8+(38) in the solution state

An I₂ stock solution (3000 mg L⁻¹) in *n*-hexane was prepared, and 30 mL was distributed into three sample bottles. Powders (10 mg each) of ZIF-8, **ZIF-8+(50)**, and **ZIF-8+(38)** were introduced and the mixtures were gently shaken before being left to stand. A portion of supernatant (0.5 mL) was taken out at intervals of 0 h, 2 h, 6 h, 9 h, 12 h, 24 h, and 48 h respectively, and diluted to 5 mL with *n*-hexane. The I₂ concentrations were determined from UV-Vis absorption measurements at 521 nm. The iodine adsorption capacity of the materials could then be derived.

The stability of I₂@ZIF-8+(38)

I₂@ZIF-8+(38) (2 mg) was dispersed evenly in water, PBS (1x) and RPMI-1640 with 10% FBS (2 mL) for 12 hours. The hydraulic particle size of each group of materials was determined by DLS as compared with the freshly prepared sample in water.

¹²⁵I₂ labeling efficiency of ZIF-8+(38)

Hydrogen peroxide (30%, 2 mL) was added to the bottom of a test tube, and *n*-hexane (2 mL) was then introduced along the wall of the tube to form a bilayer. Na¹²⁵I (1 mCi) and NaI (5 mg) were added and the mixture was gently shaken. The *n*-hexane layer was separated when the oxidation reaction was complete. **ZIF-8+(38)** (5 mg) was introduced and the test tube was shaken until the *n*-hexane became colorless. The precipitate obtained by centrifugation was denoted as ¹²⁵I₂@ZIF-8+(38). The *n*-hexane phase was also separated, and the radioactivities in the material and *n*-hexane were measured with an activity meter.

$$\text{Labeling Efficiency} = \frac{A_{\text{material}}}{A_{\text{material}} + A_{n\text{-hexane}}} \times 100\%$$

Where A_{material} is the radioactivity of the material, and $A_{n\text{-hexane}}$ is the radioactivity of the *n*-hexane.

Cell culture

The cellular uptake of **ZIF-8+(50)** and **ZIF-8+(38)** was evaluated with CT26 and HCT116 colon cancer cells. CT26 cells were cultured in RPMI-1640 and HCT116 cells were cultured in McCoy' 5A, both supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO₂. Cells grew as a monolayer and were detached upon confluence using trypsin (0.5% w/v in PBS). Cells were harvested from the culture medium by incubating in trypsin solution for 1 min. They were then centrifuged, and 1 mL serum-supplemented medium was added to neutralize residual trypsin after discarding the supernatant. The cells were re-suspended in serum-supplemented RPMI 1640 and seeded in a sterile petri dish. Cells were cultured at 37°C and 5% CO₂ for CCK-8 assay.

Determination of the cellular uptake of ZIF-8, ZIF-8+(50), and ZIF-8+(38) by CT26 and HCT116 with ICP-MS

CT26 and HCT116 cells were treated with ZIF-8, **ZIF-8+(50)**, or **ZIF-8+(38)** for 24 h and then washed twice with PBS and harvested via trypsinization. 1.0×10^7 cells in every treatment group were selected and dissolved in nitric acid, and then thermally concentrated. This residue was then made up to 15 mL using deionized water. The concentration of Zn²⁺ was measured by inductively coupled plasma mass spectrometry (ICP-MS).

In vitro cytotoxicity assayed by CCK-8

Cytotoxicity was determined using the CCK-8 assay. CT26 and HCT116 cells were seeded in 96-well plates (5000 cells per well) and then treated with different concentrations (20, 25, 30, 35, and 40 µg mL⁻¹) of ZIF-8, **ZIF-8+(50)**, or **ZIF-8+(38)** solutions for 24 h. CCK-8 (10 µL) was added to each well and incubation at 37°C with 5% CO₂ for 1 h. OD values were measured at 450 nm by using a Microplate reader (Synergy NEO of Bio Tek).

$$V\% = \frac{[A]_{\text{experimental}} - [A]_{\text{blank}}}{[A]_{\text{control}} - [A]_{\text{blank}}} \times 100\%$$

where V% is the percentage of cell viability, [A]_{experimental} is the absorbance of the wells culturing the treated cells, [A]_{blank} is the absorbance of the blank, and [A]_{control} is the absorbance of the wells culturing untreated cells.

Cancer cell uptake of ¹²⁵I₂@ZIF-8+(38)

CT26 cells were inoculated into 24 well plates at a density of 10⁵ per well. The culture medium was removed after cells had adhered to the walls, ¹²⁵I₂@**ZIF-8+(38)** was added and incubated at 37°C for 4 h. The medium was then removed and the cells were washed three times with PBS before lysing the cells with 0.5 mL of 1 M NaOH. The cell lysate was transferred to centrifuge tubes and the radioactivity was measured.

In vitro radiotoxicity assay

CT26 cells were seeded in 96-well plates (5000 cells per well) and then treated with free Na¹²⁵I and ¹²⁵I₂@ZIF-8+(38) with 0.3 mCi for each well. After 24 hours of incubation, 10 μL of CCK-8 solution was added to each well and incubated at 37°C with 5% CO₂ for 1 h. Finally, the absorbance at 450 nm was measured using a microplate reader.

Statistical analysis

Data are expressed as mean standard deviation (SD). Analysis of variance (ANOVA), followed by Student's *t*-tests, were used to determine the significant differences among the groups (**P* < 0.05, ***P* < 0.01, and ****P* < 0.001).

Table S1 Comparison of I₂ removal by various functionalized adsorbents.

Materials	Solvent	Time (h)	Capacity (mg g ⁻¹)	Reference
MIL-53-NH ₂	cyclohexane	48	170	1
MIL-101-NH ₂	cyclohexane	48	375	1
CAU-1	cyclohexane	48	300	1
ZIF-67@MCF	cyclohexane	10	1630	2
UiO-66-PYDC	cyclohexane	24	1250	3
Th-SINAP-8	cyclohexane	24	258	4
TMU-16-NH ₂	n-hexane	0.5	450	5
ZIF-8	n-heptane	24	900	6
ZIF-A61	n-hexane	24	1140	7
ZIF-8+(38)	n-hexane	48	2200	This Work

Table S2 IC₅₀ values of different samples against CT26 and HCT116 cell lines after 24 h incubation.

Formulation	IC ₅₀ ($\mu\text{g mL}^{-1}$)	
	CT26	HCT116
ZIF-8	35.56	59.27
ZIF-8+(50)	32.09	32.90
ZIF-8+(38)	29.06	32.24

Table S3 Cancer cell-binding after incubation of different concentrations of ¹²⁵I₂@ZIF-8+(38) with CT26 for 4 h.

Concentration ($\mu\text{g mL}^{-1}$)	Iodine-125 in cells (CPM)	Iodine-125 in culture medium (CPM)	Radioactivity bound (%)
30	132.7	18843.9	0.70
50	165.8	18698.0	0.88
60	35.0	18654.9	0.19

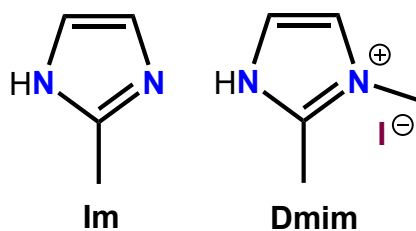


Chart S1 The structures of Im and Dmim.

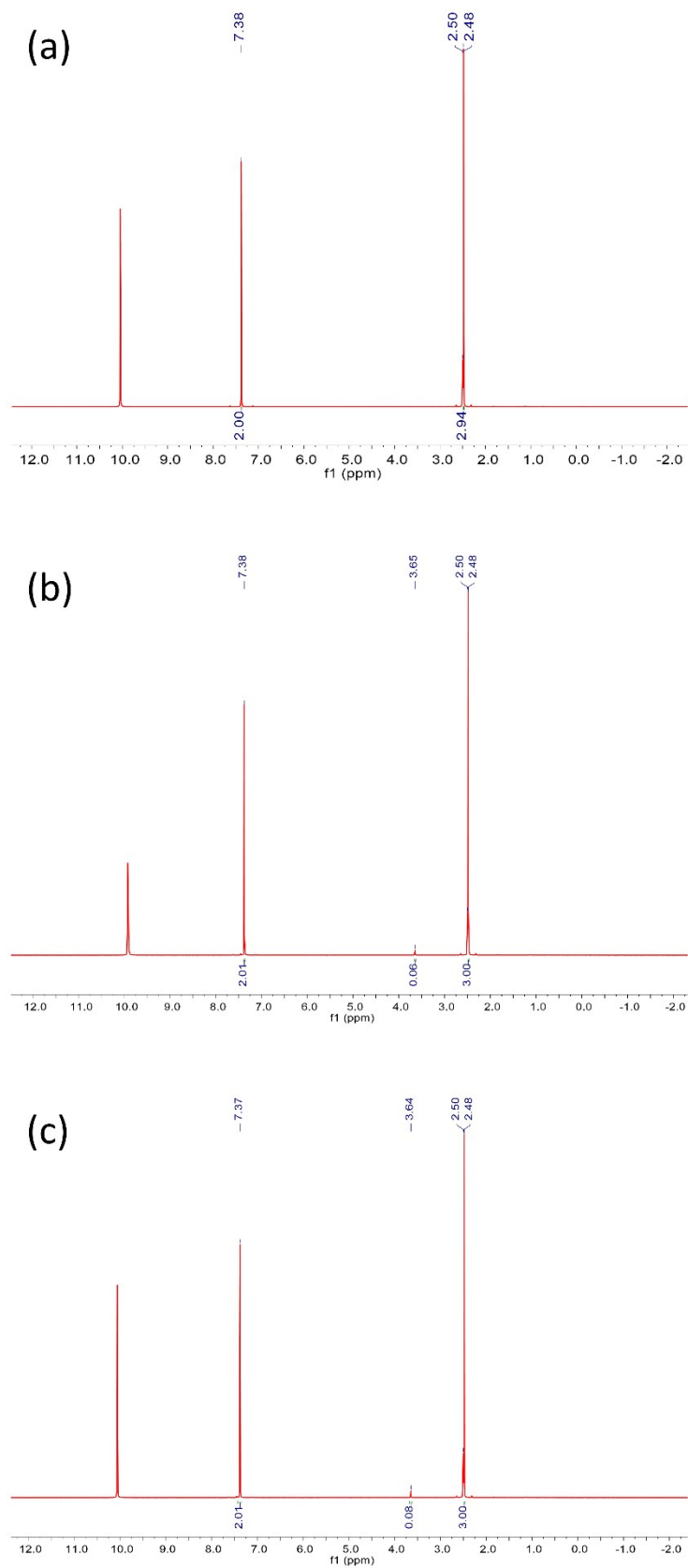


Fig. S1 The ^1H NMR spectra of (a) ZIF-8, (b) **ZIF-8+(50)**, and (c) **ZIF-8+(38)** in $\text{D}_2\text{SO}_4/\text{DMSO-}d_6$ (1 : 9, v/v).

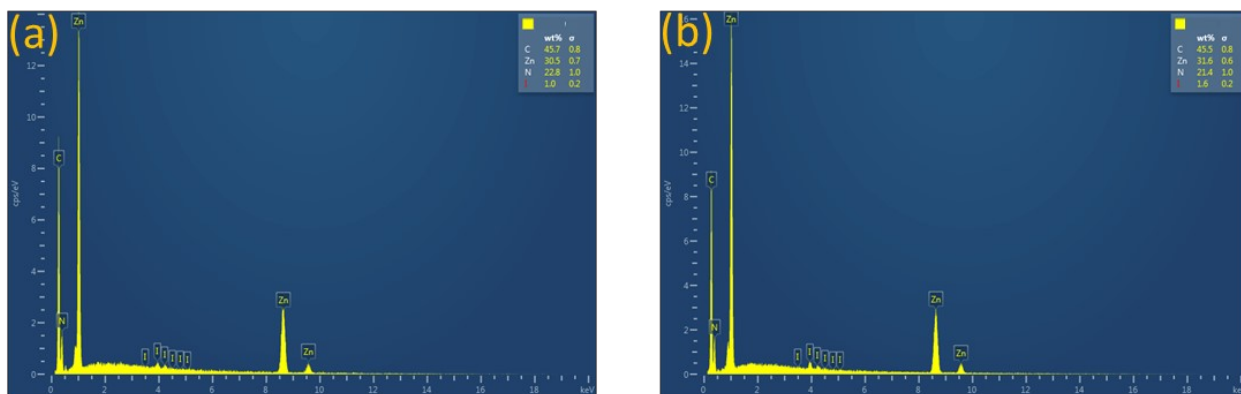


Fig. S2 The EDS diagrams of **ZIF-8+(50)** (a) and **ZIF-8+(38)** (b). The Zn : I weight ratios are 30.5 : 1 for **ZIF-8+(50)** (a) and 31.6 : 1.6 in **ZIF-8+(38)** (b), corresponding to the atomic ratios of 59 : 1 for **ZIF-8+(50)** (a) and 38 : 1 in **ZIF-8+(38)** (b).

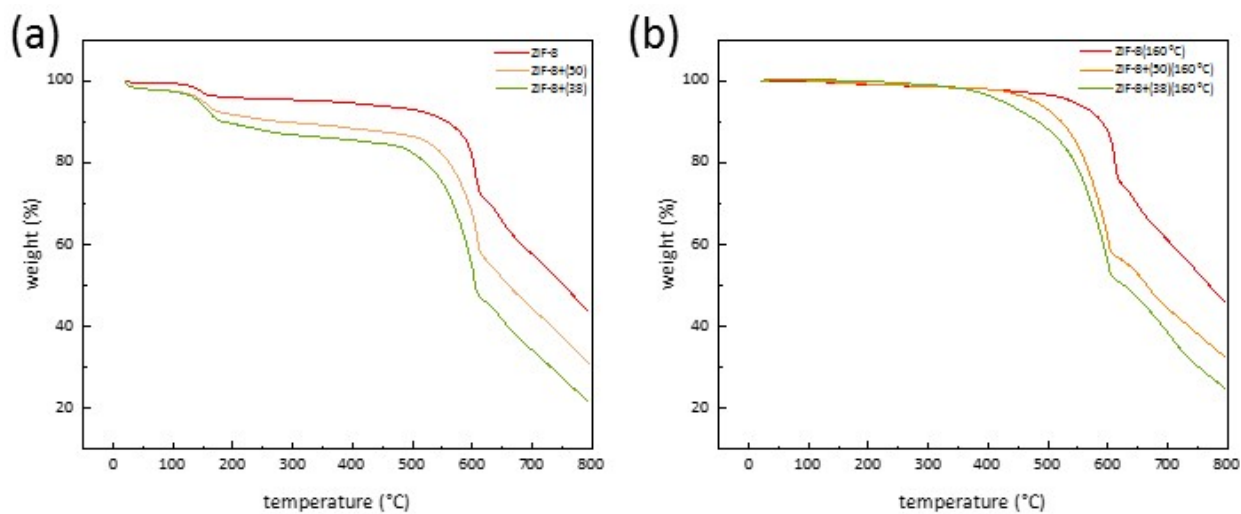


Fig. S3 TGA curves of ZIF-8, **ZIF-8+(50)**, and **ZIF-8+(38)** (a), and of ZIF-8, **ZIF-8+(50)** and **ZIF-8+(38)** dried at 160°C for 1 h (b).

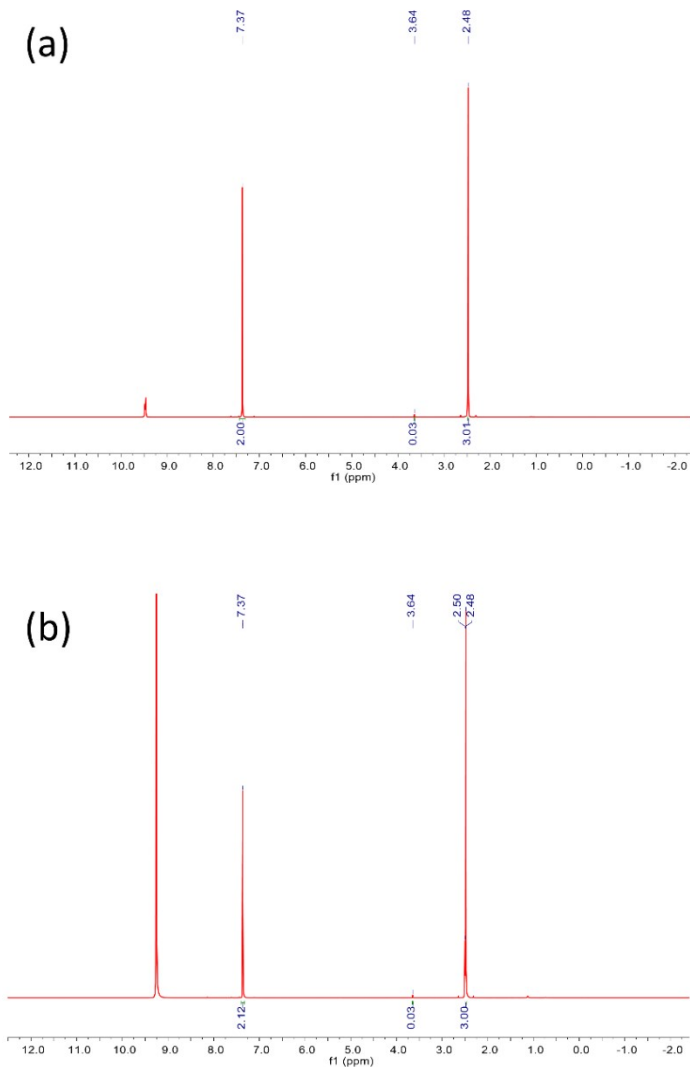


Fig. S4 ^1H NMR spectra of (a) ZIF-8+(50) and (b) ZIF-8+(38) upon drying at 160°C for 1 h.

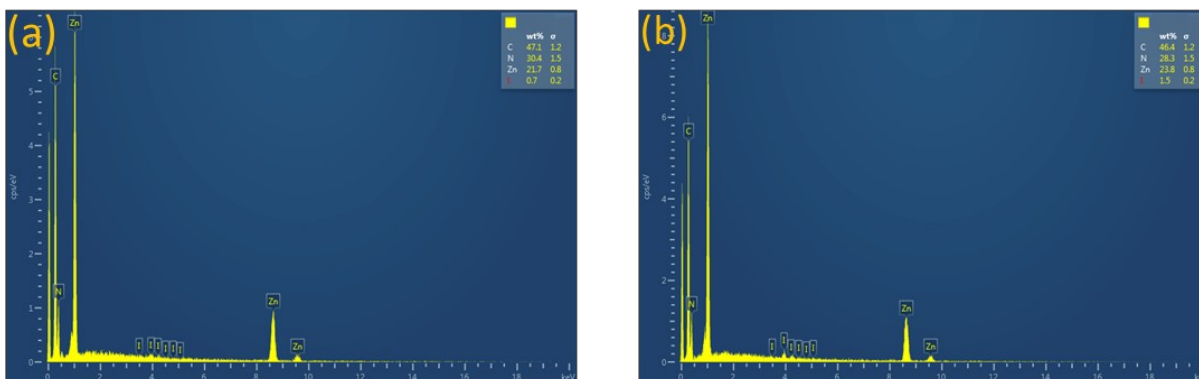


Fig. S5 EDS diagrams of ZIF-8+(50) and ZIF-8+(38) upon drying at 160°C for 1 h. The Zn : I weight ratios are 21.7 : 0.7 for ZIF-8+(50) (a) and 23.8 : 1.5 in ZIF-8+(38) (b), corresponding to the atomic ratios of 60 : 1 for ZIF-8+(50) (a) and 31 : 1 in ZIF-8+(38) (b).

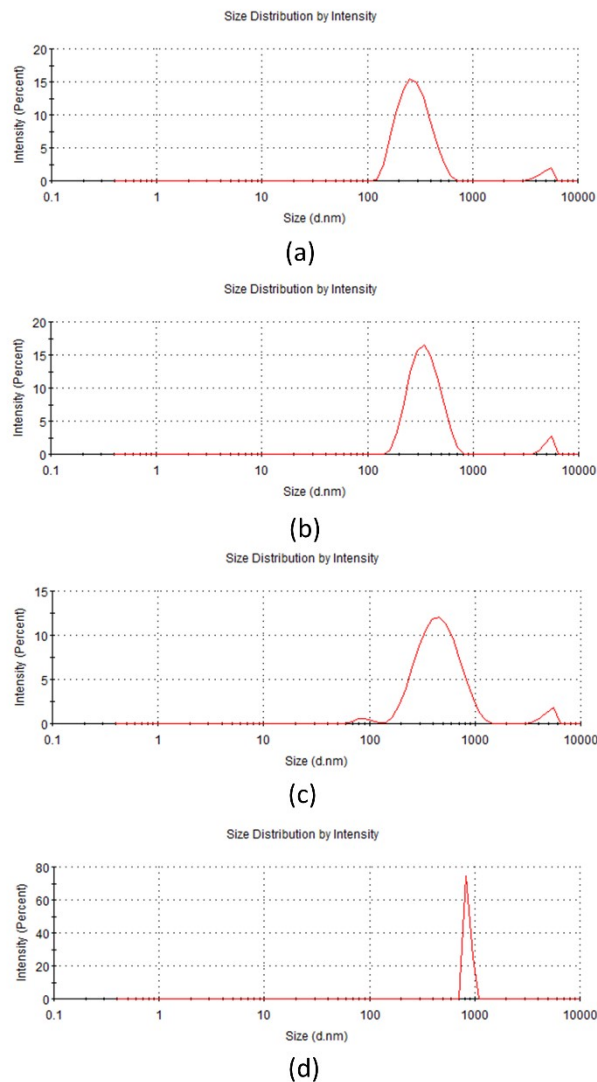


Fig. S6 Initial hydraulic diameter distribution of $I_2@ZIF-8+(38)$ (a). Hydraulic diameter distribution diagram of $I_2@ZIF-8+(38)$ after soaking in water, RPMI-1640 + 10% FBS and PBS for 12 h (b-d).

References

1. C. Falaise, C. Volkringer, J. Facqueur, T. Bousquet, L. Gasnot and T. Loiseau, *Chem. Commun.*, 2013, **49**, 10320–10322.
2. L. Chen, J.-Y. Qian, D.-D. Zhu, S. Yang, J. Lin, M.-Y. He, Z.-H. Zhang and Q. Chen, *ACS Appl. Nano Mater.*, 2020, **3**, 5390–5398.
3. Z. Wang, Y. Huang, J. Yang, Y. Li, Q. Zhuang and J. Gu, *Dalton Trans.*, 2017, **46**, 7412–7420.
4. Z.-J. Li, Z. Yue, Y. Ju, X. Wu, Y. Ren, S. Wang, Y. Li, Z.-H. Zhang, X. Guo, J. Lin and J.-Q. Wang, *Inorg. Chem.*, 2020, **59**, 4435–4442.
5. V. Safarifard and A. Morsali, *CrystEngComm*, 2014, **16**, 8660–8663.
6. A.-N. Au-Duong and C.-K. Lee, *Mater. Sci. Eng. C*, 2017, **76**, 477–482.

7. Y. R. Lee, X. H. Do, K. Y. Cho, K. Jeong and K.-Y. Baek, *ACS Appl. Nano Mater.*, 2020, **3**, 9852–9861.