Supporting Information

Poly(ethylene glycol)-Mediated Mineralization of Metal-Organic Frameworks

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MATERIALS AND METHODS

Materials. Ovalbumin (OVA), fluorescein isothiocyanate (FITC), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and fluorescein isothiocyanate (FITC) were obtained from Sigma-Aldrich (China). Zinc nitrate hexahydrate and 2-methylimidazole were supplied by Sinopharm Chemical Reagent Co. Ltd. (China). Doxorubicin hydrochloride (DOX, purity 98%) was obtained from 3A Chemicals (China). 8-arm-PEG terminated with hydroxyl groups (8-arm-PEG-OH) (10, 20, 40 kDa) and 8-arm-PEG terminated with amine groups (8-arm-PEG-NH_2) (40 kDa) were purchased from SINOPEG (China) and JenKem Technology (China), respectively. Dulbecco's phosphate-buffered saline (DPBS), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Alexa Fluor 647 carboxylic acid, succinimidyl ester (AF647), Alexa Fluor 488 carboxylic acid, succinimidyl ester (AF488), wheat germ agglutinin, Alexa Fluor 488 conjugate (WGA-AF488), and Hoechst 33342 was from Thermo Fisher Scientific (China). Poly-L-lysine (PLL, 16 kDa) was purchased from ALAMANDA Polymers, Inc. (USA). The water with a resistivity of 18.2 MΩ cm was prepared in a Milli-Q water apparatus (Integral 5).

Synthesis of ZIF-8 and PEG@ZIF-8 NPs. In a typical synthesis of PEG@ZIF-8 NPs, 8-arm-PEG-OH (10 mg) was dissolved in 2 mL of 2-methylimidazole (2-MIM, 160 mM), followed by the addition of 2 mL of zinc nitrate (40 mM) and incubation for 10 min under stirring at room temperature (25 ºC). The PEG@ZIF-8 NPs were obtained by centrifugation and washing with water three times. To investigate the distribution of 8-arm-PEG in ZIF-8 NPs, 8-arm-PEG-NH_2 was first labeled with AF647 or AF488

(molar ratio of PEG and dye, 15:1) in DMSO for 12 h, followed by dialysis against water and freeze-drying before using as the mineralizer described as above. For the preparation of ZIF-8 particles without using mineralizers, the synthetic procedure followed previously reported method,[1] where methanol was used as solvent.

Synthesis of Cargo-Encapsulated PEG@ZIF-8 NPs. The synthesis of DOX- and OVA-encapsulated ZIF-8 (denoted as DOX&PEG@ZIF-8 and OVA&PEG@ZIF-8, respectively) NPs was similar to the synthesis of PEG@ZIF-8 NPs. Briefly, DOX (4 mg) or OVA (2 mg) and 8-arm-PEG-OH (10 mg) was dissolved in 2 mL of 2-methylimidazole (2-MIM, 160 mM), followed by the addition of 2 mL of zinc nitrate (40 mM) and incubation for 10 min under stirring at room temperature (25 ºC). The DOX&PEG@ZIF-8 and OVA&PEG@ZIF-8 NPs were obtained by centrifugation and washing with water three times. The loading capacity of DOX was determined by UV-Vis spectroscopy, where DOX&PEG@ZIF-8 NPs were completely degraded in acetate buffer (pH 5). Drug release was monitored by measuring the fluorescence (λ_{em} = 560 nm) of the released DOX from DOX&PEG@ZIF-8 NPs in a Side-A-Lyzer mini dialysis device (10K MWCO, Thermo Fisher Scientific). In previous studies, organic solvent was typically involved to synthesize ZIF-8 NPs for the encapsulation of small drug molecules. Herein, the whole synthesis process was completed in aqueous solution, which provided a green strategy for the assembly of therapeutic NPs. The encapsulation of OVA was calculated based on a bicinchoninic acid (BCA) protein assay kit by measuring the supernatant of the non-encapsulated OVA.

To investigate the encapsulation of positively charged biomacromolecules, PLL and lysozyme were labeled with AF488 and FITC, respectively.[2] FITC-labeled lysozyme (or AF488-labeled PLL) (0.5 mg) and 8-arm-PEG-OH (10 mg) were dissolved in 2 mL of 2-MIM (160 mM), followed by the addition of 2 mL of zinc nitrate (40 mM) and incubation for 30 min under stirring. The resultant ZIF-8 (denoted as Lyz&PEG@ZIF-8 or PLL&PEG@ZIF-8, respectively) NPs were obtained by centrifugation and washing with water three times. Lyz&PEG@ZIF-8 or PLL&PEG@ZIF-8 NPs were degraded to release the biomacromolecules after incubation with phosphate buffer (pH 5) for the measurements of fluorescence spectra.

Cell Viability Assay. Cell viability of DOX&PEG@ZIF-8 NPs was assessed using an MTT assay. HeLa cells were seeded into 96-well plates at a cell density of 10^4 cells per well (100 μL of DMEM media supplemented with 10% of FBS) and incubated overnight (37 °C, 5% CO_2) for cell attachment. Cells were then incubated with fresh media containing PEG@ZIF-8 NPs, free DOX, or DOX&PEG@ZIF-8 NPs loaded with equivalent DOX compared to the free DOX group. After 24 h incubation, the medium was replaced with 200 μL of MTT (0.5 g L^{-1}) and the cells were incubated for another 4 h. The resulting blue formazan was solubilized in 100 μL of DMSO and the absorbance at 570 nm was measured with a plate reader (TECAN Spark 10M). Cell viability of untreated cells was set as 100% and three independent experiments were performed.

Cell Imaging. To visualize the intracellular delivery of DOX, HeLa cells were seeded in 14 mm confocal dishes with a cell density of 3×10^4 cells per well in DMEM media

supplemented with 10% of FBS and incubated overnight. DOX&PEG@ZIF-8 NPs (equivalent DOX concentration, 4 μg mL^{-1}) were incubated with HeLa cells for 4 and 24 h, respectively. The cells were fixed with 4% paraformaldehyde in DPBS for 15 min, followed by staining with Hoechst 33342 for 10 min and WGA-AF488 for 5 min, respectively. Images of cells were taken using confocal laser scanning microscopy (CLSM).

Characterization Methods. The morphology of PEG@ZIF-8 NPs was assessed by transmission electron microscopy (TEM) (JEOL JEM-1400, Japan) and scanning transmission microscopy (SEM) (Carl Zeiss G300, Germany). For cryo-TEM characterization, 5 μL of the reaction suspension at different time points was dropped onto a TEM copper grid with carbon supporting film and then quickly plunged into a reservoir of liquid ethane (cooled by nitrogen) at −165 °C to freeze the samples. The copper grids were transferred into the cryo transfer holder (Gatan 626) before imaging with TEM. The diameters of PEG@ZIF-8 NPs were examined with a Malvern Zetasizer Nano ZS. UV−Vis absorption spectra were obtained on a Shimadzu UV−Vis spectrophotometer (UV-2600, Japan). Fluorescence spectra were measured on a Horiba fluorometer (FluoroMax-4, Japan). Power X-ray diffraction (PXRD) was performed using a Rigaku D/Max 2200-PC diffractometer (Rigaku, Japan). N_2 sorption isotherms were collected using an ASAP 2020 HD88 instrument (Micromeritics, USA). Thermo gravimetric analysis (TGA) was performed in N_2 atmosphere using a TGA5500 analyzer (TA, USA). Fluorescence spectra were measured using a Horiba FluoroMax-4 fluorescence spectrometer (excitation at 470 nm). The absorption for MTT assay were

measured with a plate reader (TECAN Spark 10M, Switzerland). Fluorescence microscopy images were taken using a Leica inverted fluorescence microscope (DMi8, Germany) equipped with a 63× oil immersion objective. Super resolution microscopy images of PEG@ZIF-8 NPs and cell were obtained using a Leica CLSM (Leica TCS SP8 STED 3X, Germany) (Cy5 channel for AF647 and LysoTracker Deep Red excited at 647 nm, PE channel for DOX excited at 488 nm, FITC channel for AF488 and FITC-labeled OVA excited at 495 nm, and DAPI channel for Hoechst 33342 excited at 358 nm).

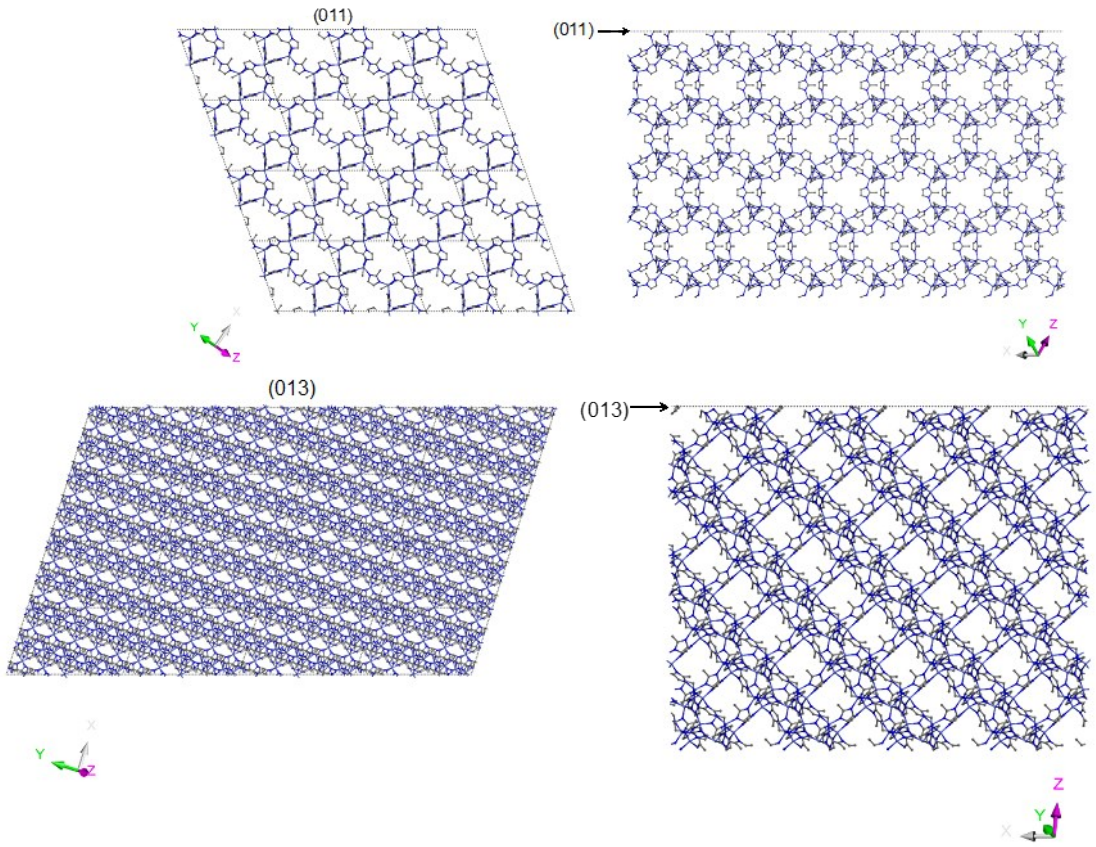

Fig. S1 Schematic illustration of the crystal structure of ZIF-8 via different projections. This result suggests that preferential packing of ZIF-8 may alternate the size of the windows/channels.

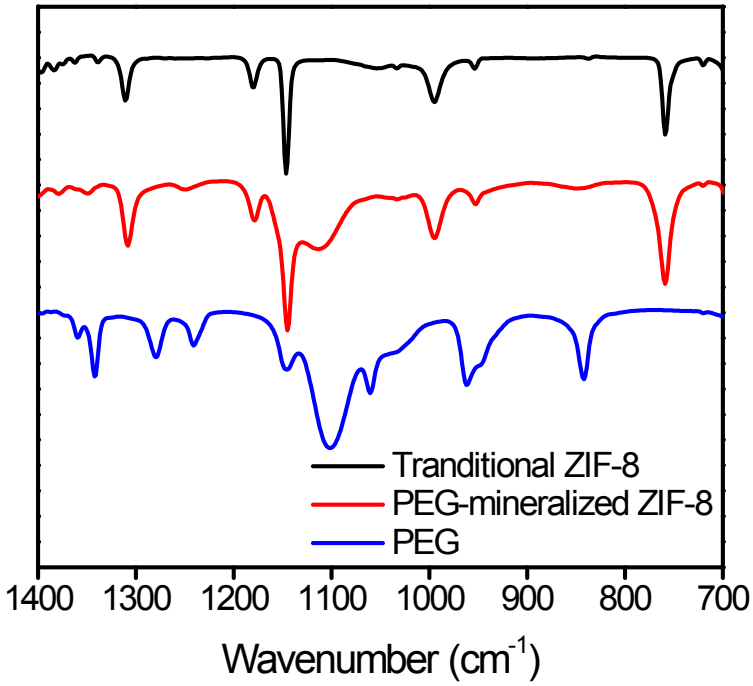

Fig. S2 FTIR spectra of PEG, PEG@ZIF-8 NPs, and traditional ZIF-8 NPs without using mineralizers.

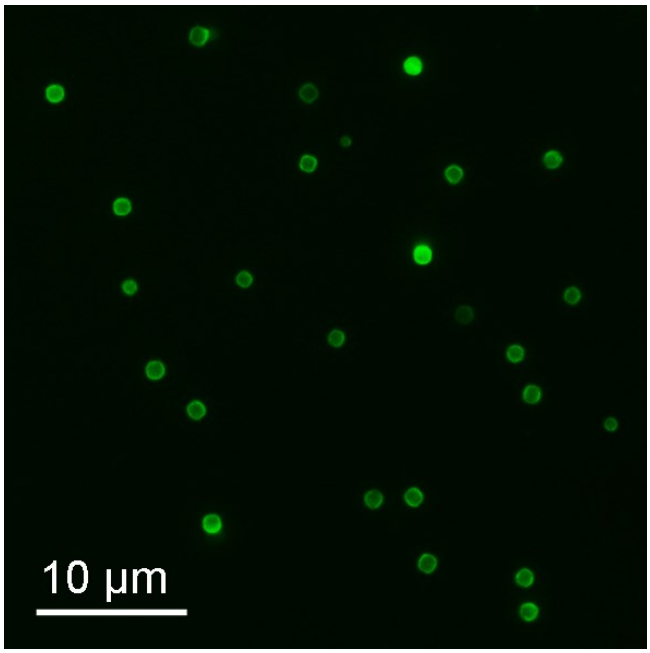

Fig. S3 Fluorescence image of ZIF-8 particles without using mineralizers after incubation with AF488-labeled 8-arm-PEG-NH_2.

Fig. S4 Photographs of the particle suspension at different time points after mixing 2-MIM and Zn^{2+} in the presence of 8-arm-PEG-OH (40 kDa, 2.5 mg mL^{-1}).

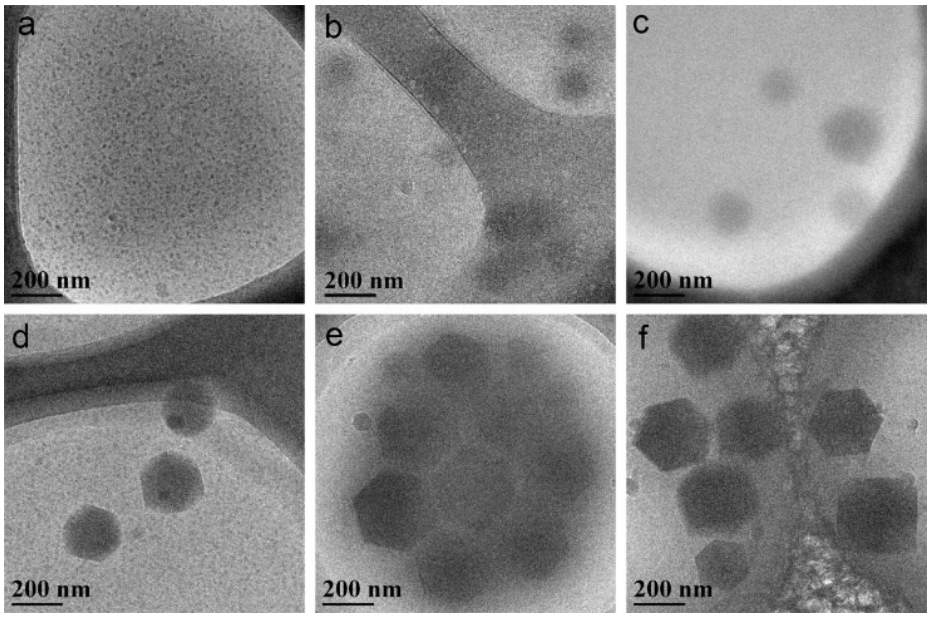

Fig. S5 Cryo-TEM images of PEG@ZIF-8 NPs using 8-arm-PEG-OH (40 kDa, 2.5 mg mL^{-1}) as mineralization agent at different time intervals of (a) 20 s, (b) 100 s, (c) 130 s, (d) 180 s, (e) 10 min, (f) 30 min after mixing 2-MIN and Zn^{2+}.

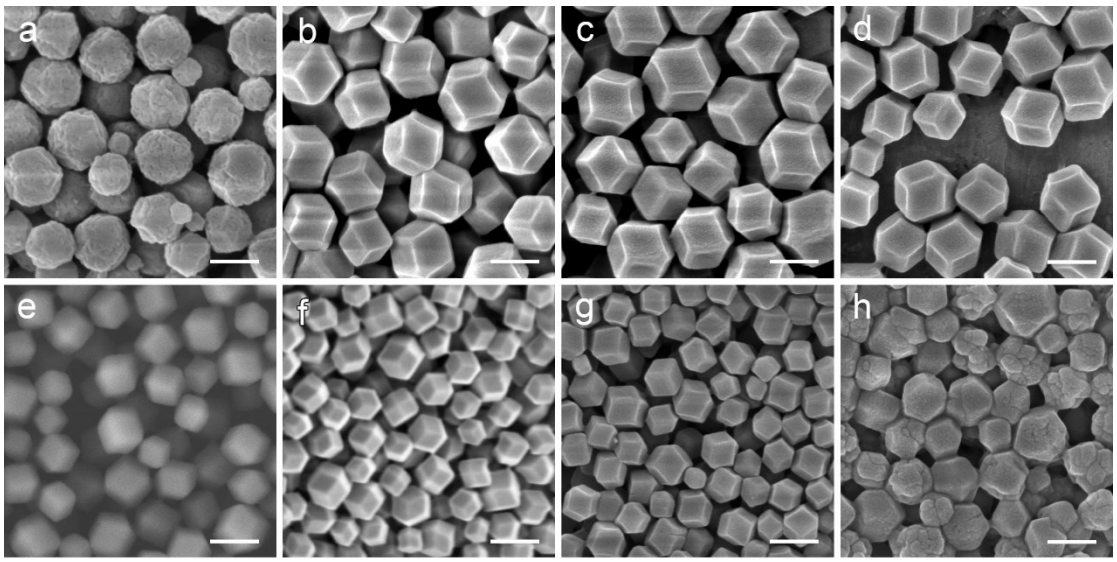

Fig. S6 SEM images of PEG@ZIF-8 NPs using 8-arm-PEG-OH (40 kDa) as the mineralizer to show the influence of PEG concentration on morphologies and diameters of ZIF-8 NPs. The 8-arm-PEG-OH concentrations were (a) 1.25 mg mL^{-1}, (b) 2.5 mg mL^{-1}, (c) 5 mg mL^{-1}, (d) 10 mg mL^{-1}, (e) 25 mg mL^{-1}, (f) 50 mg mL^{-1}, (g) 100 mg mL^{-1}, (h) 250 mg mL^{-1} in 4 mL solution. Scale bars are 200 nm.

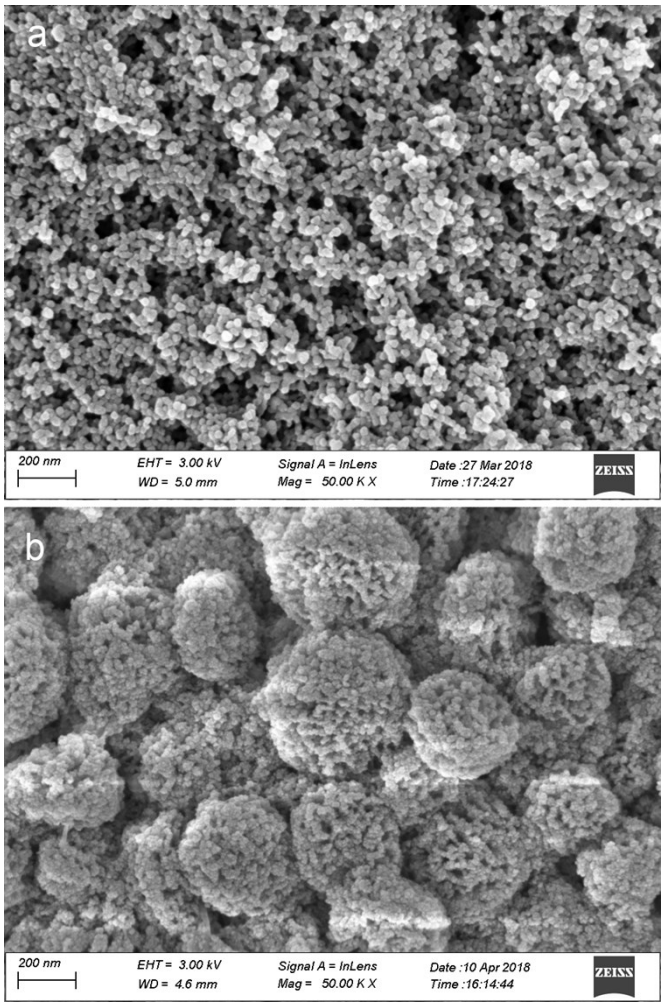

Fig. S7 SEM images of PEG@ZIF-8 NPs. The concentrations of 8-arm-PEG-OH (40 kDa) were (a) 0.25 mg mL^{-1} and (b) 375 mg mL^{-1}. When the PEG concentration was lower than 1.25 mg mL^{-1}, it could not result in the mineralization but form amorphous aggregates (Fig. S8) as like in the absence of PEG. In the opposite, when the PEG concentration was too high (e.g., 375 mg mL^{-1}), it could form a mass of pre-nucleation clusters, which fails to result in the formation of dodecahedron NPs and tend to form microparticles.

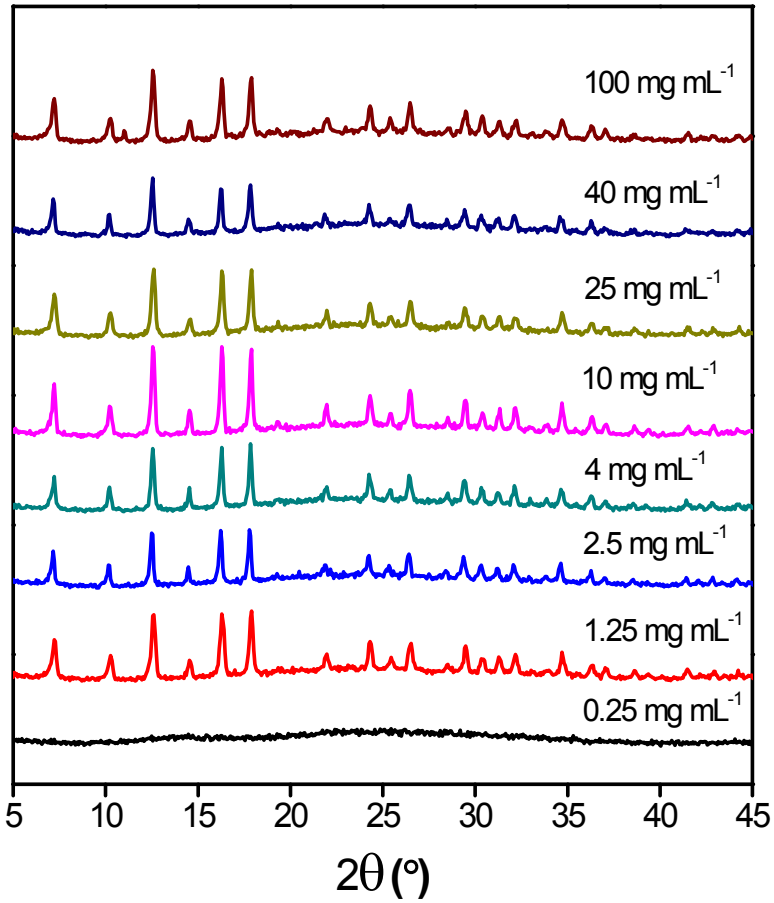

Fig. S8 XRD patterns of the PEG@ZIF-8 NPs obtained using 8-arm-PEG-OH (40 kDa) as the mineralizer with different concentrations.

Table S1 Influence of PEG concentration on size distribution of PEG@ZIF-8 NPs.

Concentration (mg mL^{-1})	PEG Molecular weight (kDa)	NP size (nm)
1.25	40	235 ± 3
2.5	40	242 ± 2
5	40	245 ± 2
10	40	235 ± 3
25	40	185 ± 4
50	40	172 ± 5
100	40	165 ± 5
250	40	195 ± 5

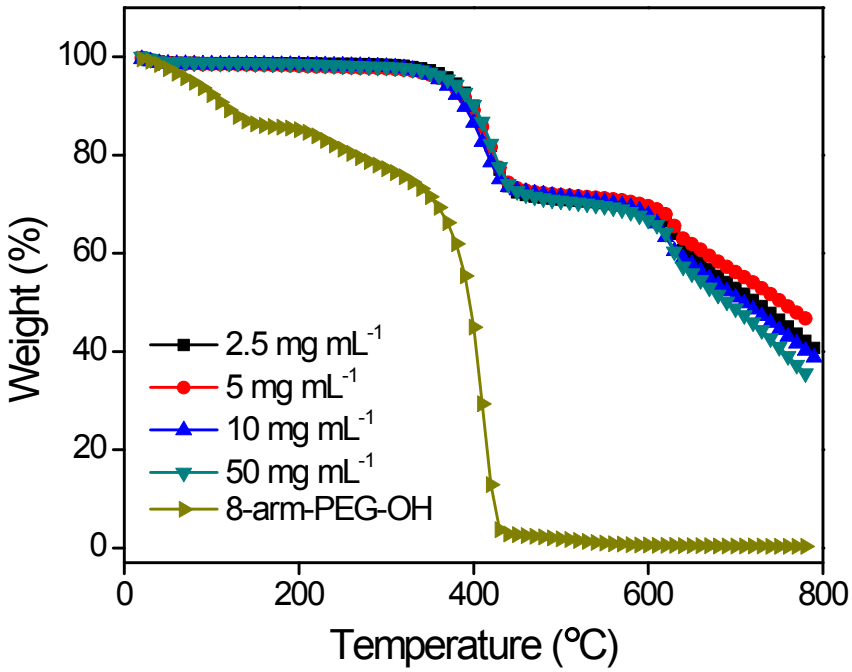

Fig. S9 Thermogravimetric analysis of 8-arm-PEG and the PEG@ZIF-8 NPs using 8-arm-PEG-OH (40 kDa) as the mineralizer with different concentrations.

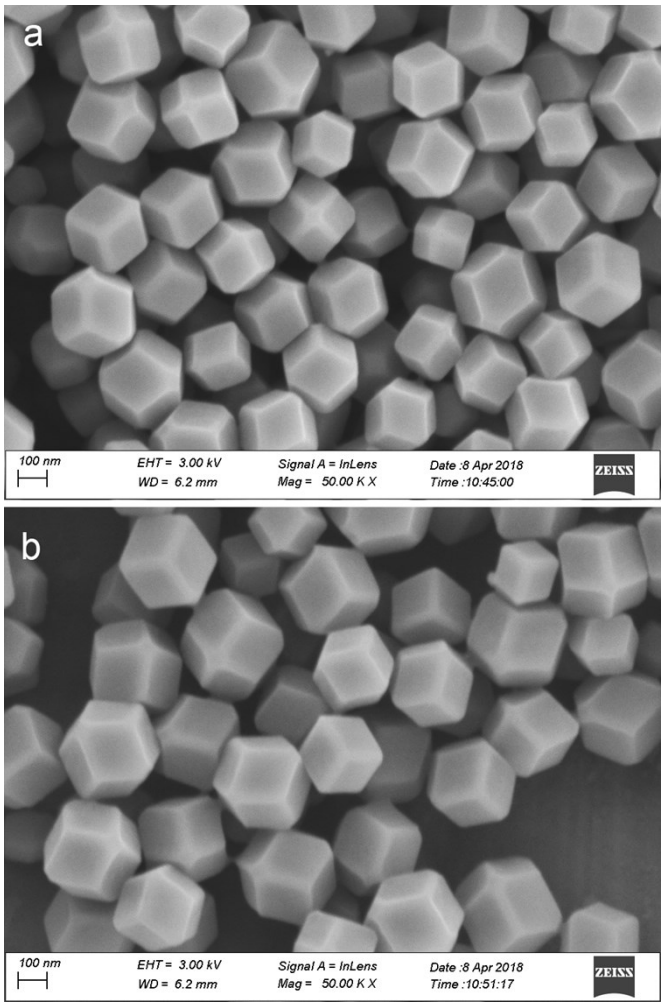

Fig. S10 SEM images of PEG@ZIF-8 NPs using (a) 8-arm-PEG-OH (10 kDa, 2.5 mg mL^{-1}) and (b) 8-arm-PEG-OH (20 kDa, 2.5 mg mL^{-1}) as the mineralizer.

Table S2. Influence of temperature on size distribution of PEG@ZIF-8 NPs.

Temperature (ºC)	PEG molecular weight (kDa)	NP size (nm)
10	40	590 ± 2
20	40	350 ± 2
30	40	160 ± 5
40	40	115 ± 2
50	40	85 ± 3
70	40	60 ± 4

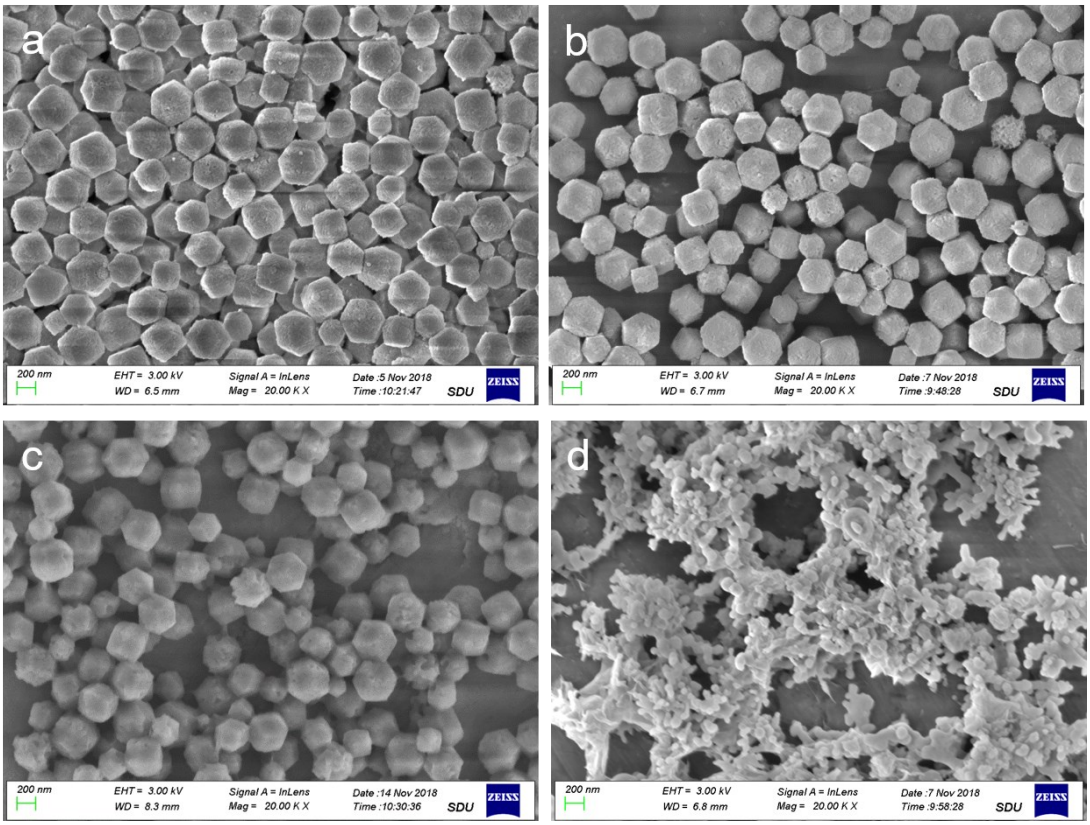

Fig. S11 Stability of PEG@ZIF-8 NPs using 8-arm-PEG-OH (40 kDa, 2.5 mg mL^{-1}) as the mineralizer after incubation in DMEM medium supplemented with 10% fetal bovine serum for (a) 1 day, (b) 4 days, and (c) 14 days. (d) SEM image of traditional ZIF-8 NPs after incubation for 1 day.

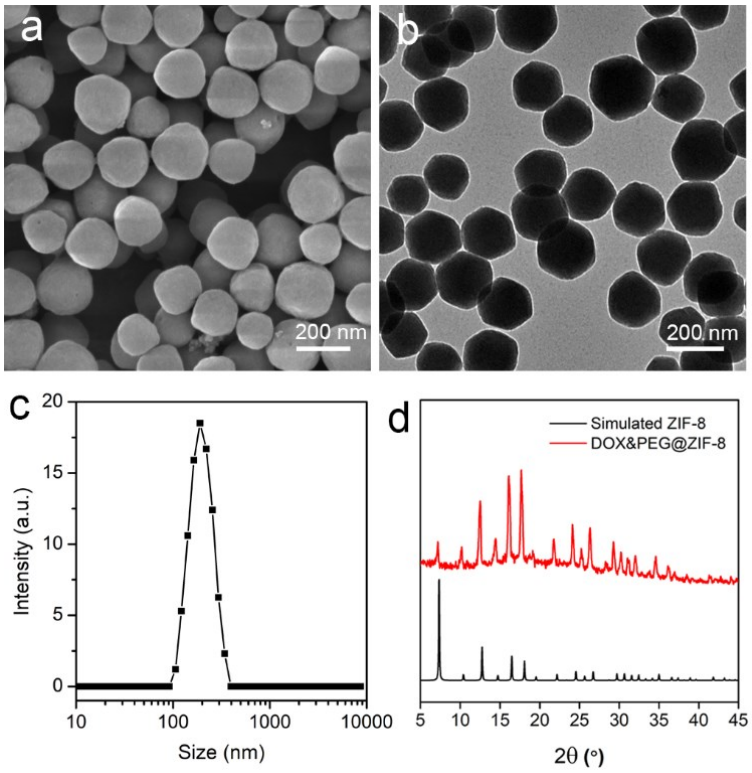

Fig. S12 (a) SEM image, (b) TEM image, (c) size distribution, (d) XRD pattern of DOX&PEG@ZIF-8 NPs using 8-arm-PEG-OH (40 kDa, 2.5 mg mL^{-1}) as the mineralizer.

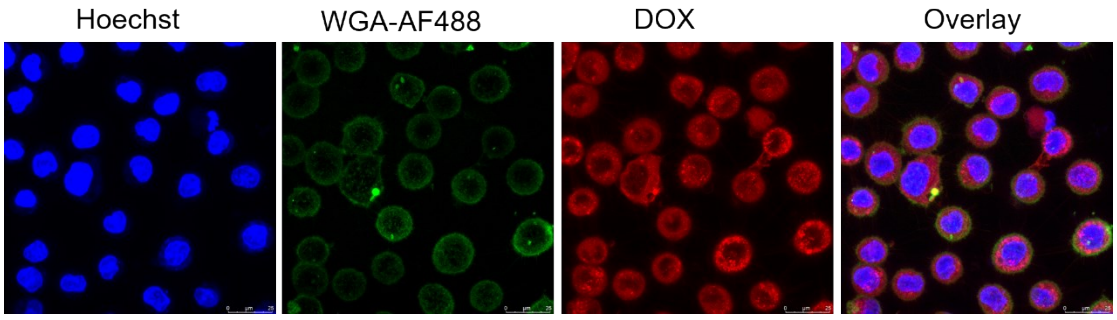

Fig. S13 CLSM images of HeLa cells after 24 h incubation with DOX&PEG@ZIF-8 NPs. Nuclei and cell membranes were stained with Hoechst 33342 (blue) and WGA-AF488 (green), respectively. Red fluorescence was from DOX. Scale bars are 25 μm.

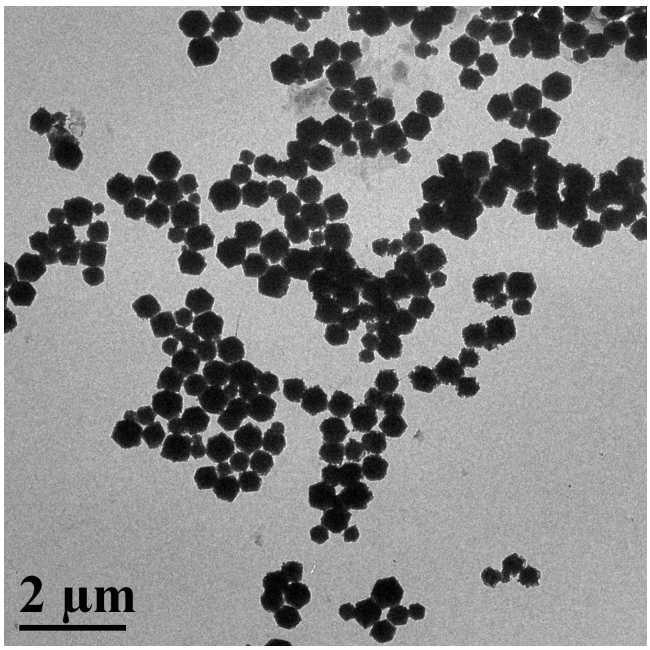

Fig. S14 TEM image of OVA&PEG@ZIF-8 NPs.

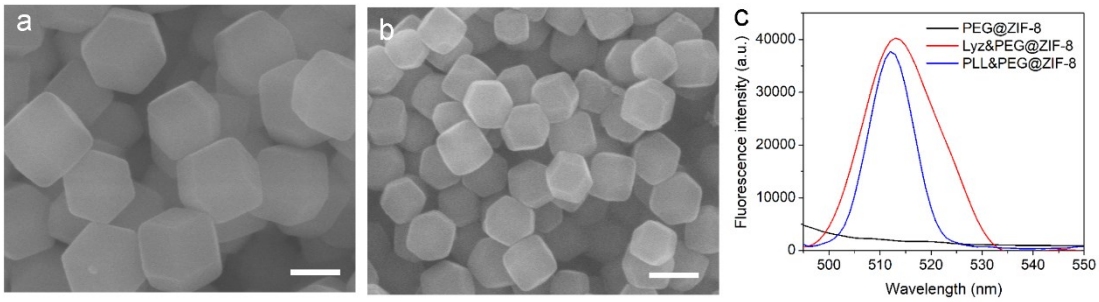

Fig. S15 (a,b) SEM images of PLL&PEG@ZIF-8 (a) and Lyz&PEG@ZIF-8 NPs (b). Scale bars are 200 nm. (d) Fluorescence spectra of the solutions after degradation of PEG@ZIF, PLL&PEG@ZIF-8, and Lyz&PEG@ZIF-8 NPs.

References:

1 N. L. Torad, M. Hu, Y. Kamachi, K. Takai, M. Imura, M. Naito and Y. Yamauchi, *Chem. Commun.*, 2013, **49**, 2521.

2 J. Pan, Y. Wang, C. Zhang, X. Wang, H. Wang, J. Wang, Y. Yuan, X. Wang, X. Zhang, C. Yu, S.-K. Sun and X.-P. Yan, *Adv. Mater.*, 2018, **30**, 1704408.