Supporting Information

One-Step Synthesis of Nanoscale Zeolitic Imidazolate Frameworks with High Curcumin Loading for Treatment of Cervical Cancer

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EXPERIMENTAL SECTION

Materials. All the chemicals and solvents were purchased from commercial sources and used without further treatment, unless indicated otherwise.

General measurements

The crystalline structure was recorded by using an X-ray diffractometer (XRD) (Bruker AXS D8 Focus), using Cu Ka radiation (λ = 1.54056 Å). TEM images were recorded with a FEI-TECNAI G^2 transmission electron microscope. SEM images were conducted by scanning electron microscopy (SEM, model JEOL JXA-840). Size distributions of the nanoparticles were determined by DLS with a vertically polarized He–Ne laser (DAWNEOS, Wyatt Technology, USA). Zeta potential measurement was performed using a Zetasizer Nano-ZS (Malvern Instruments Ltd.). UV-Vis absorption spectra were conducted on Shimadzu UV-2450 spectrophotometer.

Control experiments:

Method 1: Synthesis of CCM@NZIF-8 according to the previous method[1] with some modification. Entry 1: the solution of $Zn(NO_3)_2$ (150 mg/5mL MeOH) was added to the solution of MIM (330 mg) and CCM (5 mg) in MeOH (5mL). CCM@NZIF-8 NPs were formed after stirring for 10 min. The average size of the obtained NPs determined by SEM (Figure S2) is 200 nm, the DLC and DLE of CCM@NZIF-8 was calculated to be 0.33% and 2.05%, respectively.

Entry 3: The procedure is similar with method 1 except the weight of CCM is 48mg.

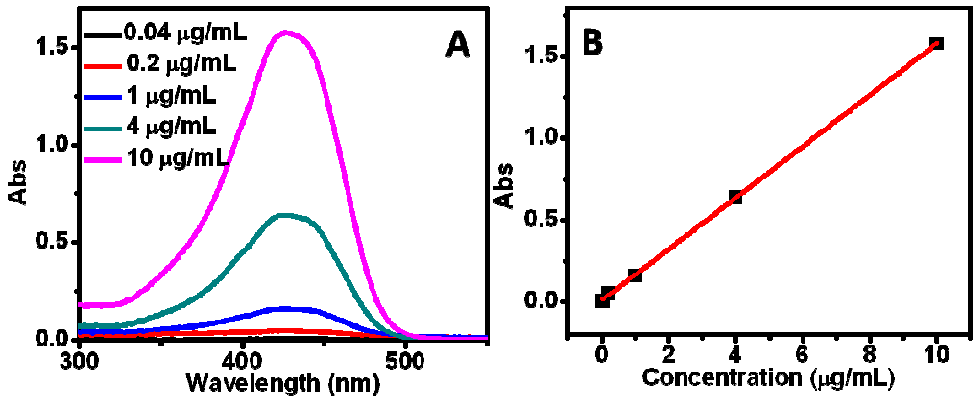

Figure S1. A) The UV-Vis absorption of CCM in ethanol at different concentrations. B) The linear relationship between the concentration of CCM and absorption intensity in ethanol, the linear range is 0.04 ~ 10 μg/mL (R^2 = 0.9999) and the regression equation is y=0.00841+0.15713x.

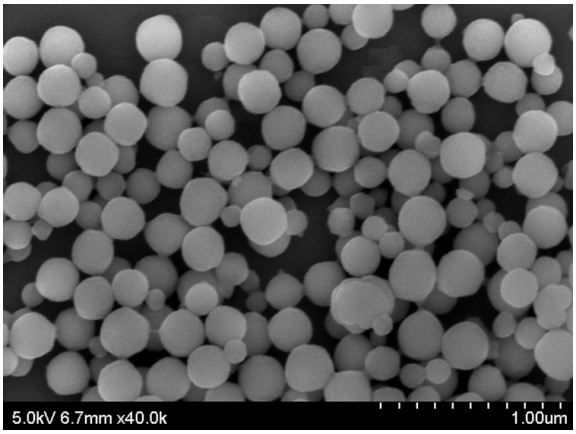

Figure S2. SEM image of CCM@NZIF-8 NPs prepared by entry 1.

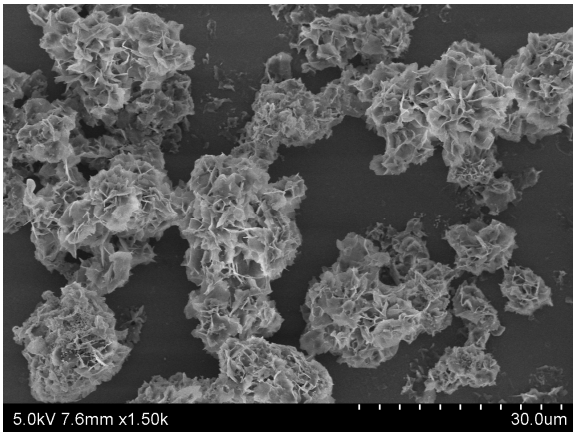

Figure S3. SEM image of CCM@NZIF-8 NPs prepared by entry 3.

Entry 4: The solution of $Zn(NO_3)_2$ (150 mg/5mL H_2O was added to the solution of MIM (330 mg) and CCM (5 mg) in THF (10 mL). CCM@NZIF-8 NPs were formed after stirring for 1 min. The average size of the obtained NPs determined by SEM (Figure S2) is 50 nm, the DLC and DLE of CCM@NZIF-8 was calculated to be 1.16% and 6.72%, respectively.

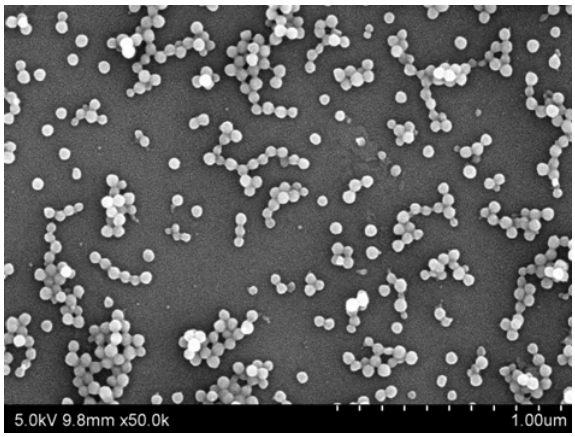

Figure S4. SEM image of CCM@NZIF-8 NPs prepared by entry 4.

Table S1. Synthesis of CCM@NZIF-8 NPs.

Entry	*$Zn(NO_3)_2$ solvent	*MIM+CCM solvent	CCM (mg)	Reaction time (min)	DLC (%)	DLE (%)
1	MeOH, 5mL	MeOH, 5mL	5	10	0.33	2.05
2	MeOH, 5mL	MeOH, 5mL	24	10	0.63	0.47
3	MeOH, 5mL	MeOH, 5mL	48	10	33.2	25.6
4	H_2O, 5mL	THF, 5mL	5	5	1.16	6.72
5	H_2O, 5mL	MeOH, 5mL	5	5	3.66	22.8
6	H_2O, 5mL	MeOH, 10mL	5	1	12.7	88.2

* $Zn(NO_3)_2$ (150 mg), MIM (330 mg)

MTT (3-(4,5-dmethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay.

Biocompatibility test

HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells/well and incubated in DMEM for 24 h. The medium was then replaced by NZIF-8, at a final equivalent concentration from 1 to 100 μg/mL. The

incubation was continued for 48 h. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plates were incubated for another 4 h at 37 ^{o}C, followed by removal of the culture medium containing MTT and addition of 150 μL of DMSO to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 10 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader.

Cytotoxicity test

HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells/well and incubated in DMEM for 24 h. The medium was then replaced by CCM or CCM@NZIF-8, at a final equivalent CCM concentration from 0.1 to 10 μg / mL for each drug. The incubation was continued for 48 h. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plates were incubated for another 4 h at 37 ^{o}C, followed by removal of the culture medium containing MTT and addition of 150 μL of DMSO to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 10 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader.

Confocal Laser Scanning Microscope Studies.

Cellular uptakes by HeLa cells were examined using a confocal laser scanning microscope (CLSM). HeLa cells were seeded in 6-well culture plates (a sterile cover slip was put in each well) at a density of 5 x 10^4 cells per well and allowed to adhere for 24 h. After that, the cells were treated with CCM or CCM@NZIF-8 for 0.5 h at 37 ^{o}C. After that, the supernatant was carefully removed and the cells were washed three times with PBS. Subsequently, the cells were fixed with 800 μL of 4% formaldehyde in each well for 20 min at room temperature and washed twice with PBS again. The slides were mounted and observed with a confocal laser scanning microscope imaging system (Zeiss LSM 780).

In vivo anticancer test.

Kunming (KM) male mice were obtained from Jilin University, China (56−84 d, 20−25 g) and maintained under required conditions. The xenograft tumor models of

U14 cervical cancer were established by injecting U14 cervical carcinoma cells into the right infra-axillary dermis of the mice. When the tumor grew to a size of ~200 mm^3, U14 bearing Kunming mice were randomly divided into three groups with 6 mice in each group. The free CCM or CCM@NZIF-8 groups were treated with CCM or CCM@NZIF-8 via tail-vein injection six times at 2 day intervals with the same dosage of (2.5 mg CCM)/(kg body weight), and the tumor sizes were measured every other day. The relative tumor volume V_t/V_0 as a function of time was used to investigate the inhibitory effect of CCM and CCM@NZIF-8.

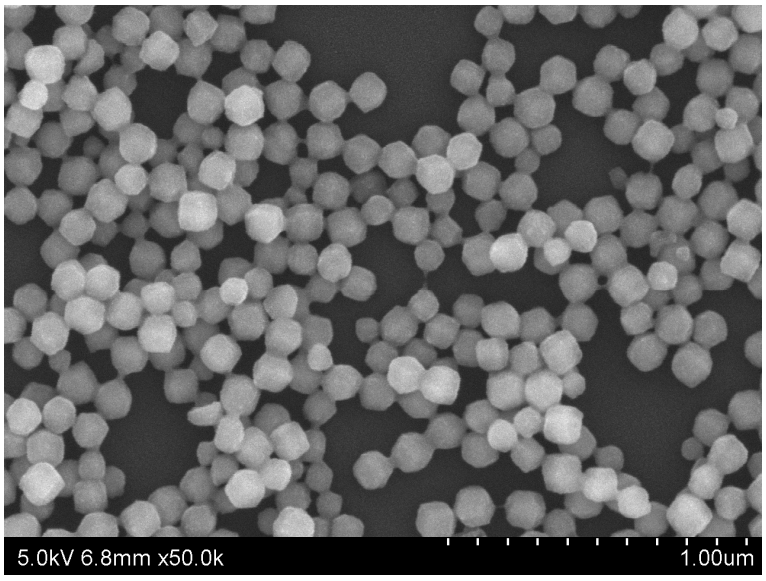

Figure S5. SEM image of NZIF-8 NPs.

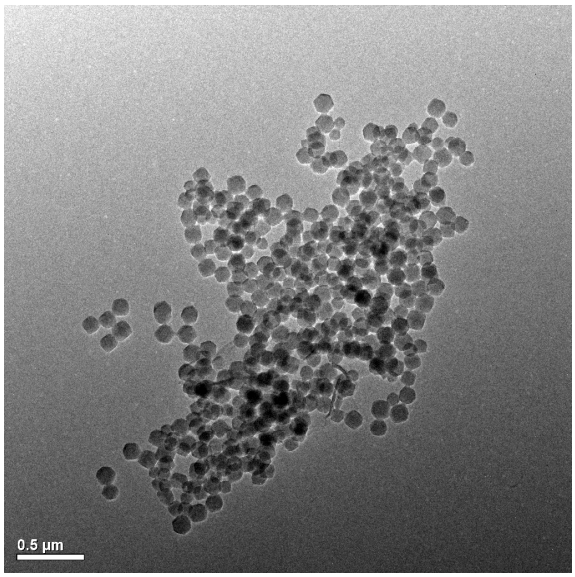

Figure S6. TEM image of NZIF-8 NPs.

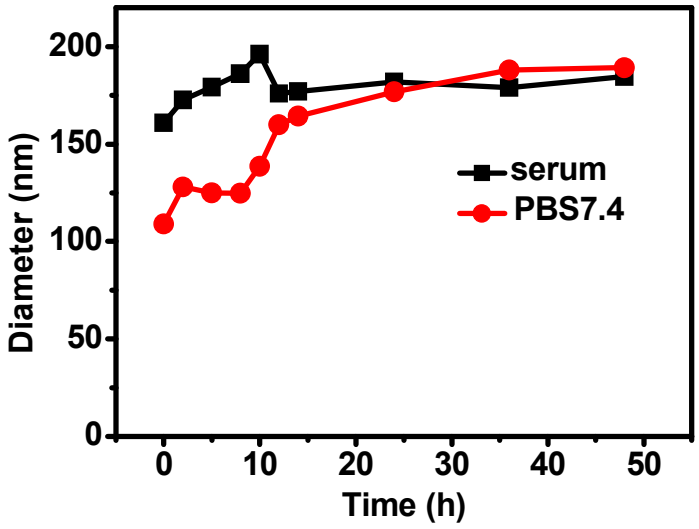

Figure S7. Hydrodynamic diameters of NZIF-8 NPs in PBS (pH 7.4) and fetal calf serum.

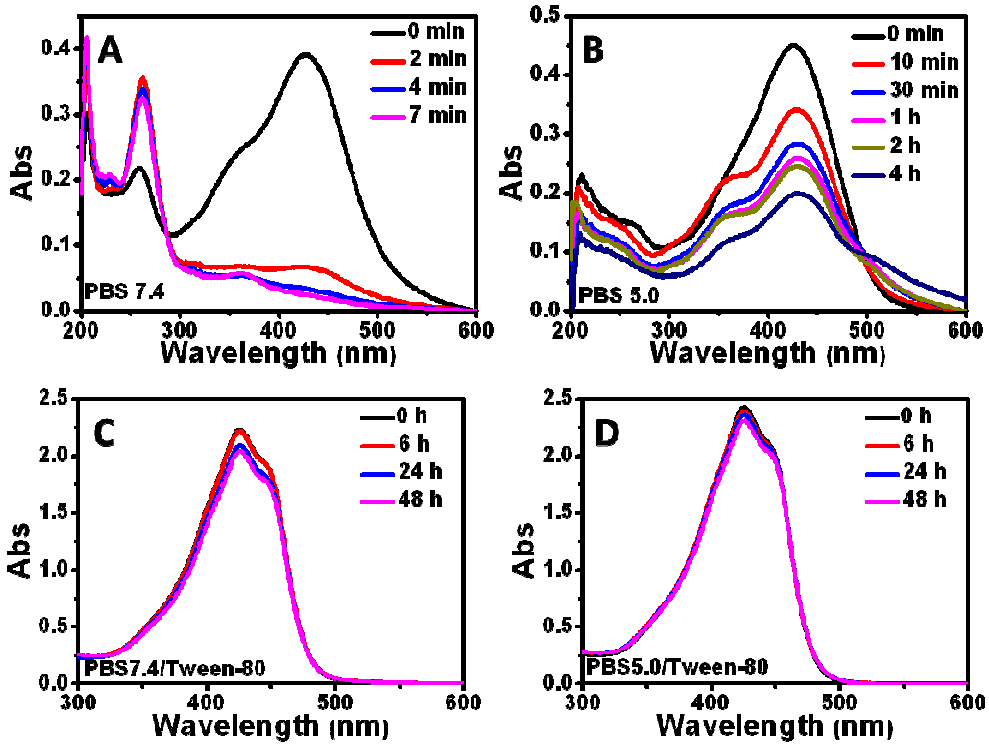

Figure S8. The UV-Vis absorption spectra of CCM in (A) 0.02 M PBS (pH 7.4), (B) 0.02 M PBS (pH 5.0), (C) 0.02 M PBS (pH 7.4)/Tween-80 (1 wt%) and (D)0.02 M PBS (pH 5.0)/Tween-80 (1 wt%).

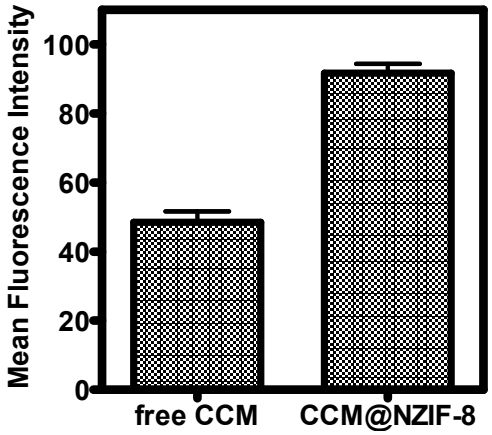

Figure S9. The mean fluorescence intensity of free CCM and CCM@NZIF-8 determined by Image-pro plus 6.0.

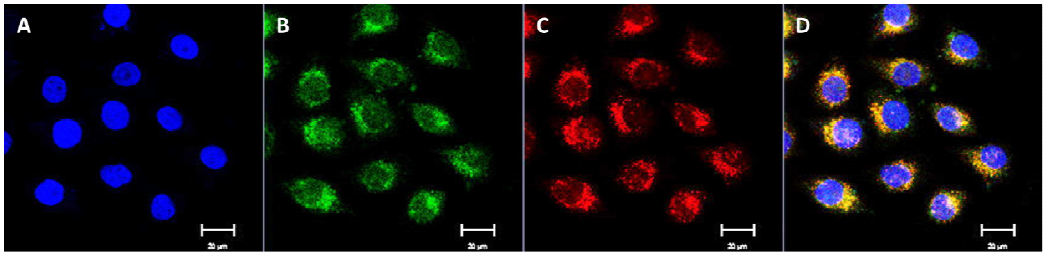

Figure S10. Co-localization of CCM and the Lysosome Tracker Red: HeLa cells were incubated for 0.5 h with CCM@NZIF-8 and Lysosome tracker red, the nuclei were stained by DAPI. CLSM images of HeLa cells with DAPI (A), CCM@NZIF-8 (B), Lysosome tracker red (C) and (D) the overlay image of (A)-(B). All scale bars are 20 μm.

Reference

(1) Zhuang, J.; Chou, L.; Liu, D.; Weerapana, E.; Tsung, C. Optimized Metal Organic-Framework Nanospheres for Drug Delivery: Evaluation of Small-Molecule Encapsulation. *ACS Nano* **2014**, *8*, 2812–2819.