Electronic Supplementary Information (ESI)
For

Tetraphenylethylene-based fluorescent Coordination Polymers for Drug Delivery

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Content

1 Material and Methods

Materials. All chemicals used during this investigation were reagent grade and used as received. N,N-dimethylformamide (DMF) was stored over activated molecular sieves and was distilledunder reduced pressure. Ultrapure water was prepared using a Millipore Simplicity System (Millipore, Bedford, USA).

Methods. The absorption spectra were obtained using a Shimadzu UV-2450 PC UV-Vis spectrophotometer at room temperature. Thermogravimetric analysis (TGA) was performed using a NetzchSta 449c thermal analyzer at the heating rate of $10^{o}Cmin^{-1}$ in air atmosphere. FTIR spectra were measured by Nicolet Impact 410 Fourier transform infrared spectrometer. Nitrogen (N_2) adsorption isotherm was measured on an Autosorb iQ_2 adsorptometer, Quantachrome Instruments. ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$ and DMSO-d_6. PXRD was recorded by Riguku D/MAX2550 diffractometer using Cu-*K*α radiation (40 kV, 200 Ma) with scanning rate of 0.4^{o} min^{-1}. TEM micrographs was recorded using a FEI Tecnai G2F20s-twin D573 with an acceleration voltage of 300 kV. SEM micrographs were performing on JEOL JXA-840 under an accelerating voltage of 15 kV.

2 Synthesis

2.1 Synthesis of H4TCPE

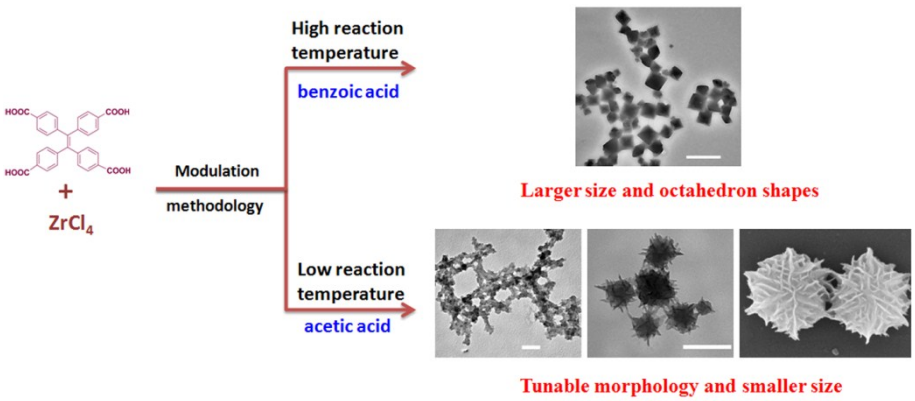
Tetrakis(4-carboxyphenyl)ethylene acids (H_4TCPE) was synthesized according to previous work.[S1]

2.2 Synthesis of NCP-1

A mixture of $ZrCl_4$ (26 mg), H_4TCPE (28 mg), benzoic acid (62.5 mg) and concentrated HCl (18 μL) in 2 mL of DMF was stirred for 2 h. The resulting solution was transferred to a 15 mL teflon-lined autoclave and kept under autogenously pressure at 120 ^oC for 2 days. After cooling to room temperature, the precipitated products were washed with DMF and MeOH for several times. The product was collected by centrifugation and drying under vacuum to give a white solid, yield 80%.

2.3 Synthesis of NCP-1-X (X = 20 μL, 50 μL, 100 μL, 150 μL and 300 μL of HAc)

$ZrCl_4$ (2.6 mg), H_4TCPE (3 mg) and DMF (1 mL) were added into a 10 mL vial. Then different amount of HAc were added into each of vial, dispersed through ultrasonic after sealed. The mixed solution was heated at 90 ^oC for 17 h. After cooling to room temperature, the products were collected by centrifugation with DMF and MeOH for several times and drying under vacuum to give a white solid.



Scheme S1 Schematic presentation of Zr-TCPE NCPs synthesis.

3 Characterization

3.1 The determination of fluorescence quantum yields

Fluorescence quantum yields (Φ_{NCPs}) of as-synthesized Zr-TCPE NCPs in MeOH were calculated in Eq. 1.

$$\Phi_{NCPs} = 2\Phi_{std}(A_{std}/A)(I_{NCPs}/I_{std})(\eta/\eta_{std})^2 \quad (1)$$

Φ_{NCPs} and Φ_{std}: fluorescence quantum yields of samples and rhodamine 6G (MeOH, $\Phi = 0.86$) as reference dyes. A and A_{std}: absorbances of samples and rhodamine 6G.[S2] I_{NCPs} and I_{std}: fluorescence intensity of samples and rhodamine 6G. η and η_{std} stand for the refractive index of MeOH (for the Zr-TCPE NCPs) and reference solvent.

Table S1. The max emission and luminescence quantum efficiency (Φ) of as-synthesized samples and TCPE ligands.

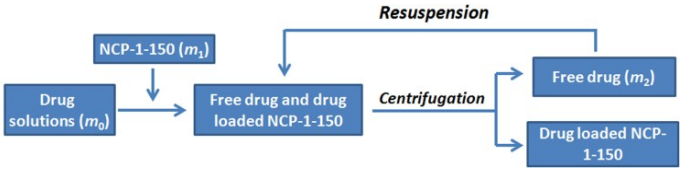
	H_4TCPE	NCP-1-20	NCP-1-50	NCP-1-100	NCP-1-150	NCP-1-300
$\lambda_{em\ (max)}$ (nm)[a]	488	492	482	474	468	466
Φ[b]	0.00114	0.061	0.091	0.115	0.124	0.297

a: λ_{ex} = 365 nm, dispersed in MeOH.

b: Rhodamine 6G (R6G) in MeOH (λ_{ex} = 488 nm, Φ_{std} = 0.86) was used as a fluorescence standard.

3.2 Preparation and determination of drug loading capacity and encapsulation efficiency

Typically, to a 5 ml of suspension of **NCP-1-150** (3.0 mg for loading Cur in MeOH) was added amount of drug (Table S2). The resulting mixture was stirred at room temperature for 12 h. After drug loading, the nanoparticles were centrifuged at 12000 rpm for 10 minutes to collect the supernatant for determining the drug content using UV spectra at 425 nm for Cur. All washings were combined to quantify the drug content. The detail determination of drug is presented in Scheme S2.



Scheme S2. Experimental determination of drug loading in **NCP-1-150** nanoparticles.

Table S2. The drug loading capacity and encapsulation efficiency of **NCP-1-150** nanoparticles.

	NCP-1-150 (mg)	Drug (μg)	Suprnatant	EE (%)	EC (%)	C (μg mg^{-1})
Cur@NCP-1-150-1	3	200	19.98	90.01	5.66	60
Cur@NCP-1-150-2	3	400	113.85	71.54	8.71	95.38
Cur@NCP-1-150-3	3	1000	350.2	64.98	17.8	216.6

EE and EC: loading efficiency and capacity determining the drug content using UV-vis spectra at 425 nm for Cur.

$$\text{Loading efficiency (\%)} = (m_0 - m_2)/m_0 \times 100\ \%\ (\mathbf{2})$$

$$\text{Loading capacity (\%)} = (m_0 - m_2)/m_1 \times 100\ \%\ (\mathbf{3})$$

m_0: the initialamountof Cur drug. m_1: the weight of the **NCP-1-150** carriers. m_2: the cumulative amount of non-absorbed drug.

3.3 *In vitro* drug release

Amount of freeze-dried drug@NCPs were dispersed into 2 mL of 0.01 M PBS at different pH 7.4 and 5.0 at 37 ^{o}C with gentle agitation. At suitable time intervals, samples were centrifuged and the supernatant were further analysed by UV at absorbance wavelength of 425 nm for Cur. The cumulative amount of drug released from NCPs samples was plotted against time.

3.4 Cell Culture

Human cervix carcinoma cells (HeLa), human lung cancer cells (A549) and human liver cancer cells (HepG2) were obtained from (Neurosurgery Department, University Hospital Carl Gustav Carus, Germany). Cells were cultured in Dulbecco minimum essential medium (DMEM) growth medium supplemented with 10 % (v/v) fetal calf serum, 2 mM glutamine, 1 mM sodium private, 100 μg mL^{-1} of penicillin and 100 μg mL^{-1} streptomycin. Cell were grown in a humidified incubator at 37 °C in 5 % CO_2 atmosphere. The medium was replaced every two days and cells were subcultured by trypsinization.

3.5 Cell viability-assay

The biocompatibility of as-synthesized NCPs was assessed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assays against HeLa, A549 and HepG2 cells as described previously.[S3] Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 1×10^5 cells

per well and incubated in DMEM for 24 h. The medium was then replaced by NCPs and drug@NCPs at various selected concentrations. The incubation was continued for 48 h. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg mL^{-1} was added. After 4 h incubation at 37 °C, the culture medium containing MTT were removed and 150 μL DMSO were added to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 5 min, and the absorbance of the solution was determined by a Bio-Rad 680 microplate reader at 490 nm. Cell viability was calculated based on the following equation: $(A_{sample}/A_{control}) \times 100$ %, where A_{sample} and $A_{control}$ denote as absorbencies of the sample well and control well, respectively. The inhibitory concentration of 50 % (IC_{50}) was obtained as the concentration of drug yielding 50 % of dye reduction compared to untreated control.

3.6 Cellular uptake

A549 cells were seeded in 6-well culture plates (a clean cover slip was put in each well) at a density of 5×10^4 cells per well and allowed to adhere for 24 h. The medium was then replaced by NCPs and drug@NCPs at a final concentration of 10 μg mL^{-1} for each. After incubation 1 h at 37 °C, the supernatant was carefully removed and the cells were washed three times with PBS. Subsequently, the cells were fixed with 1 mL of 4 % formaldehyde each well for 10 min at room temperature and washed twice with PBS. The cellular localization was visualized under CLSM by LSM 780 (Carl Zeiss, Jena, Germany) with 10 × eyepiece and 40 × objective. Lyso Tracker®Red CM-H2XRos was also used to stainlysosomes.

4 Results

4.1 Characterization of as-synthesized NCP-1 (Fig. S1-S8)

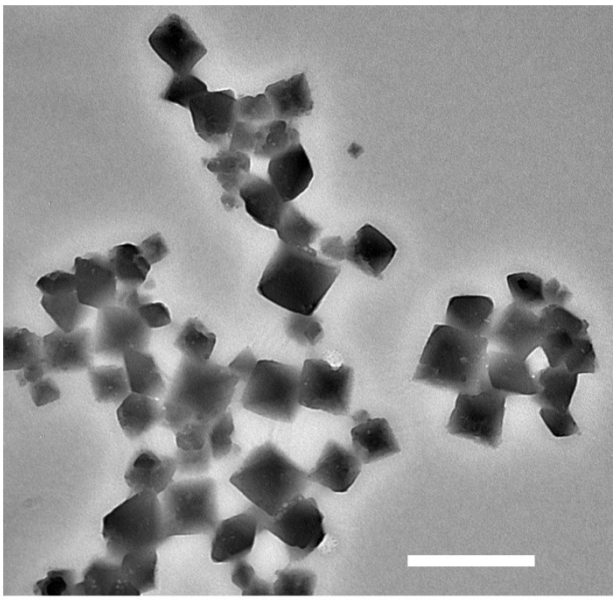


Fig. S1 TEM image of as-synthesized **NCP-1**. scale bar: 1 μm.

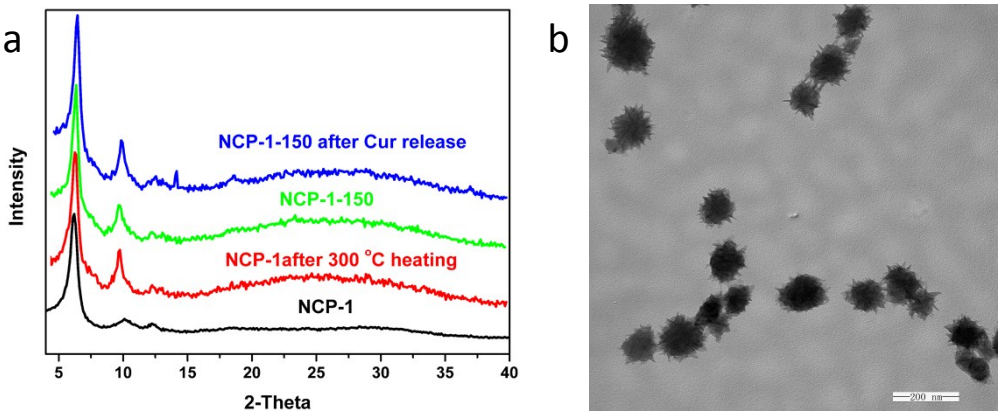


Fig. S2 (a) PXRD patterns of **NCP-1** (black), **NCP-1** after heating (red), **NCP-1-150** (green) and **NCP-1-150** after Cur drug release (blue). (b) TEM image of as-synthesized nanocarriers dispersed in water for 48 h.

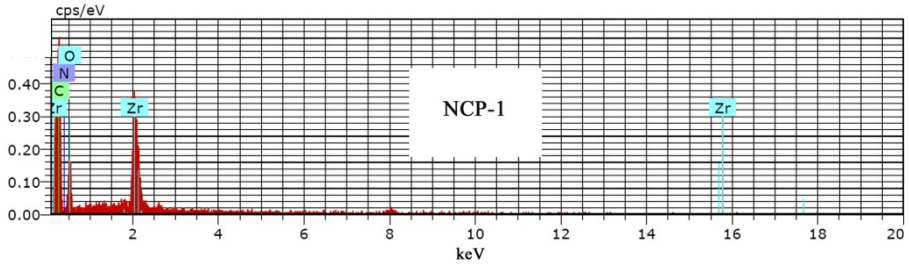


Fig. S3 EDS spectra of **NCP-1**.

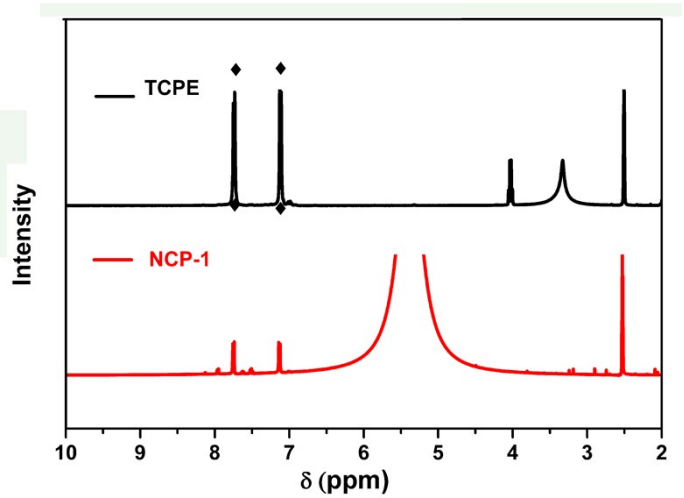


Fig. S4 ^{1}H NMR spectra of TCPE ligands (black) measured in DMSO-d_6, **NCP-1** (red) measured in HF/DMSO-d_6.

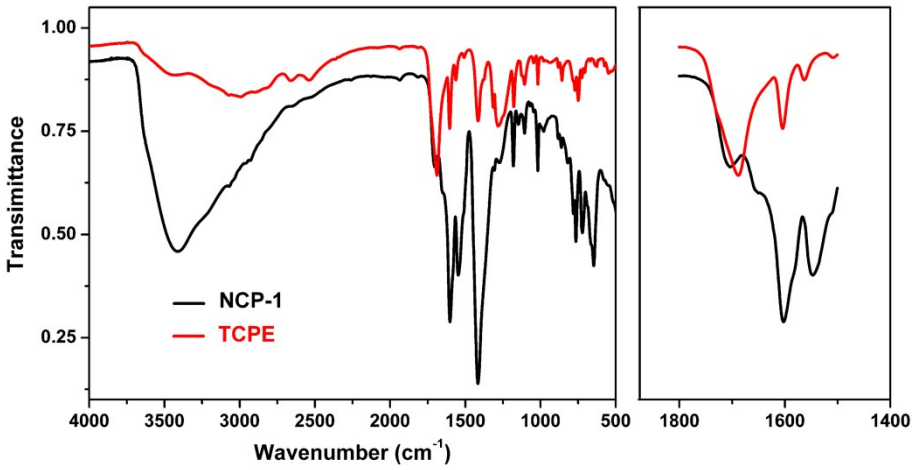


Fig. S5 FTIR spectra of **NCP-1** (black) and TCPE ligands (red).

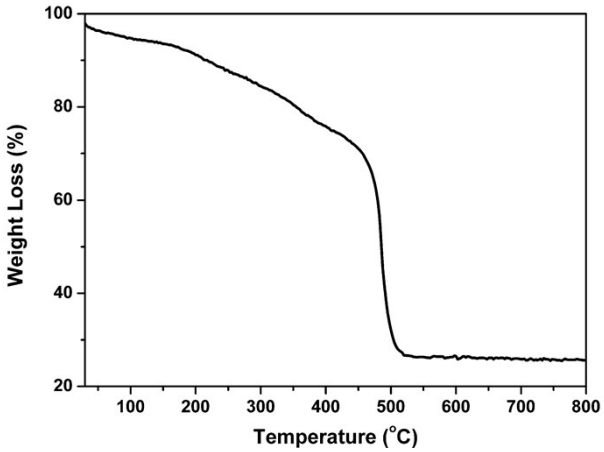


Fig. S6 TGA curves of as-synthesized **NCP-1** under Air atmosphere.

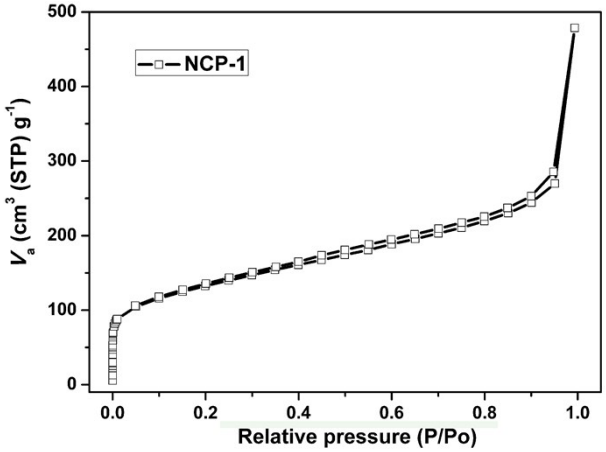


Fig. S7 N_2 adsorption and desorption curves at 77 K for **NCP-1**.

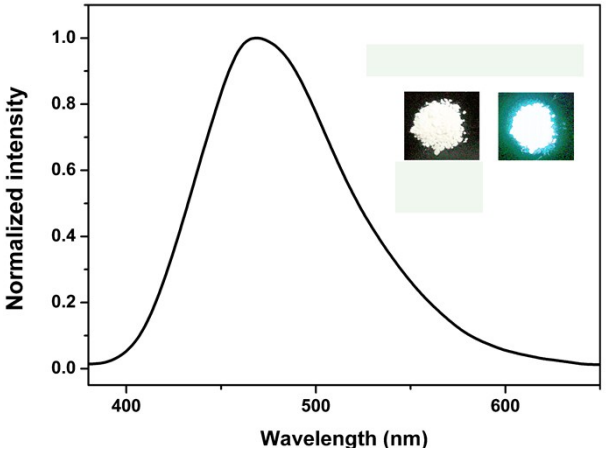


Fig. S8 The solid luminescence spectrum of **NCP-1** excited at a wavelength of 367 nm, insert: the digital photographs of **NCP-1** samples under the illumination of natural light and UV lamp (λ_{ex} = 365 nm).

4.2 Characterization of as-synthesized NCP-1-X (Fig. S9-S17)

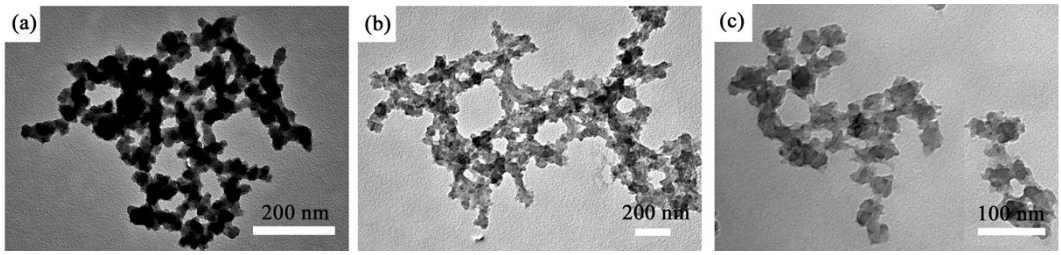


Fig. S9 TEM images of as-synthesized **NCP-1-20** (a), **NCP-1-50** (b) and **NCP-1-100** (c).

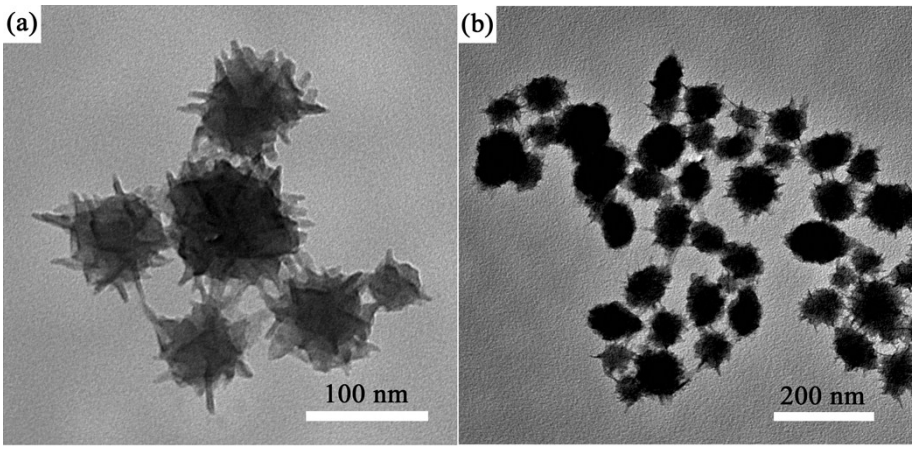


Fig. S10 TEM images of as-synthesized **NCP-1-150**.

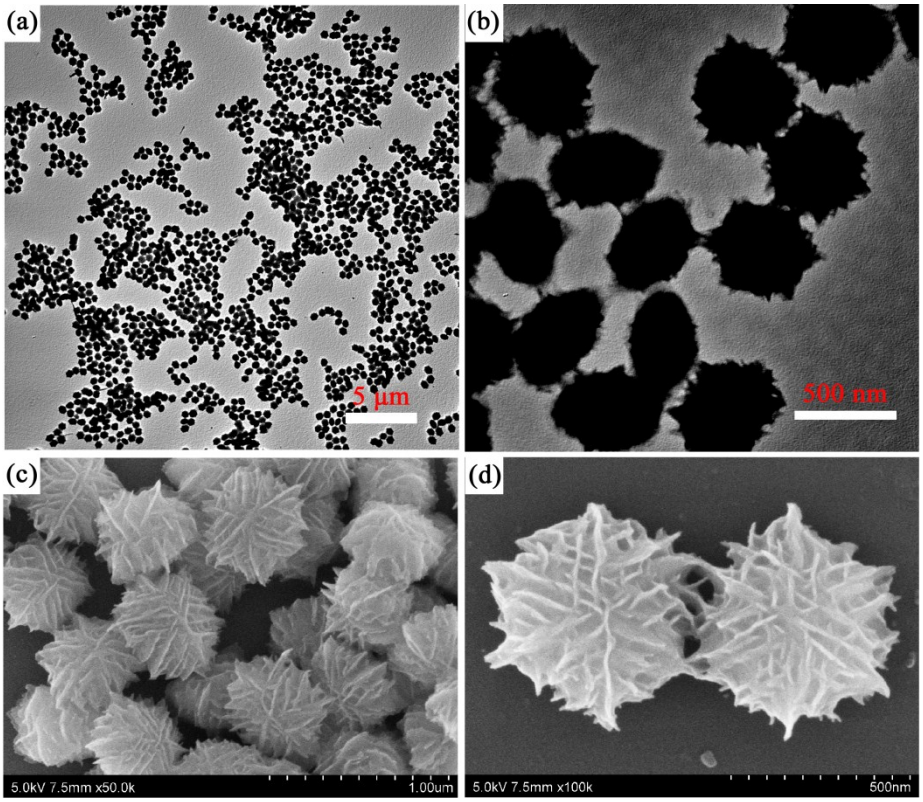


Fig. S11 TEM (a, b) and SEM (c,d) images of as-synthesized **NCP-1-300**.

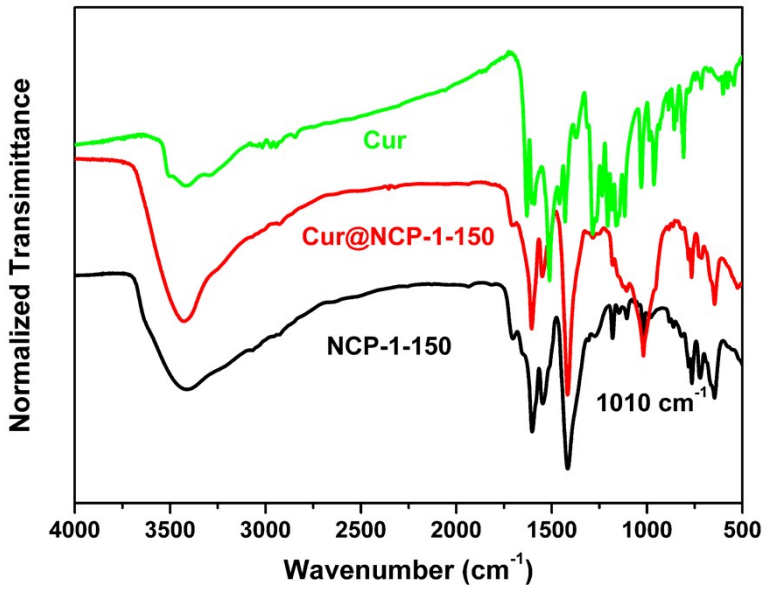


Fig. S12 FTIR spectra of **NCP-1-150** (black), **Cur@NCP-1-150** (red) and Cur (green).

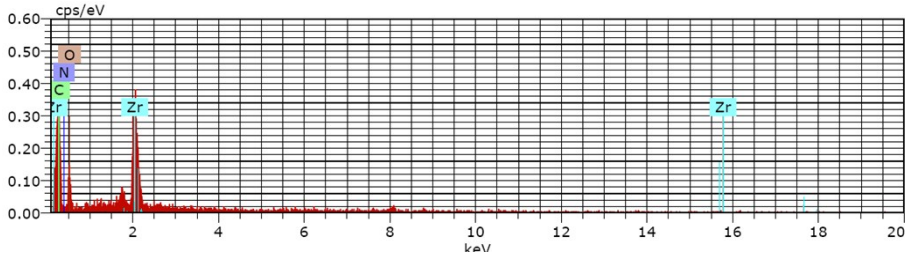


Fig. S13 EDX spectra of **NCP-1-150**.

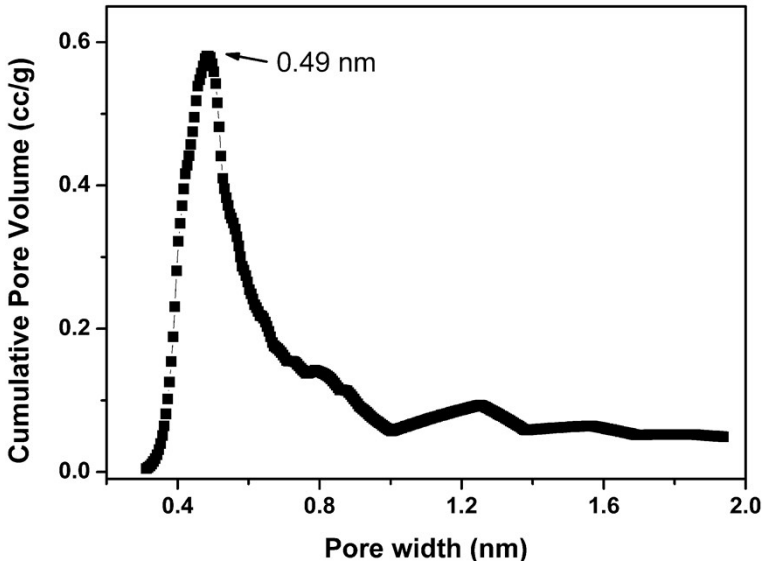


Fig. S14 The pore size distribution (HK method) of **NCP-1-150** determined from nitrogen uptake measurements at 77 K.

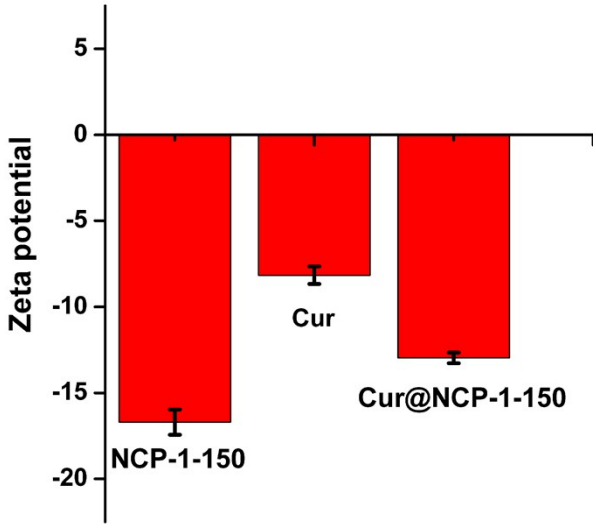


Fig. S15 zeta potentials of **NCP-1-150** measured in PBS.

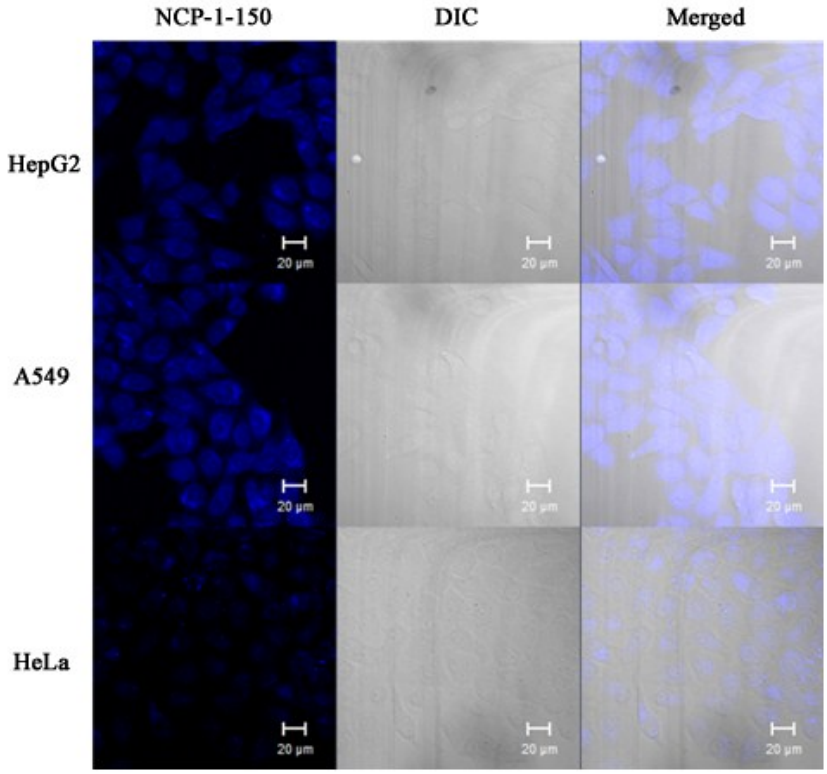


Fig. S16 Cellular uptakes of **NCP-1-150** after incubation with HepG2, A549 and HeLa cells for 1 h at a concentration of 10 μg mL^{-1} detected by CLSM. Scale bars represent 20 μm in all images.

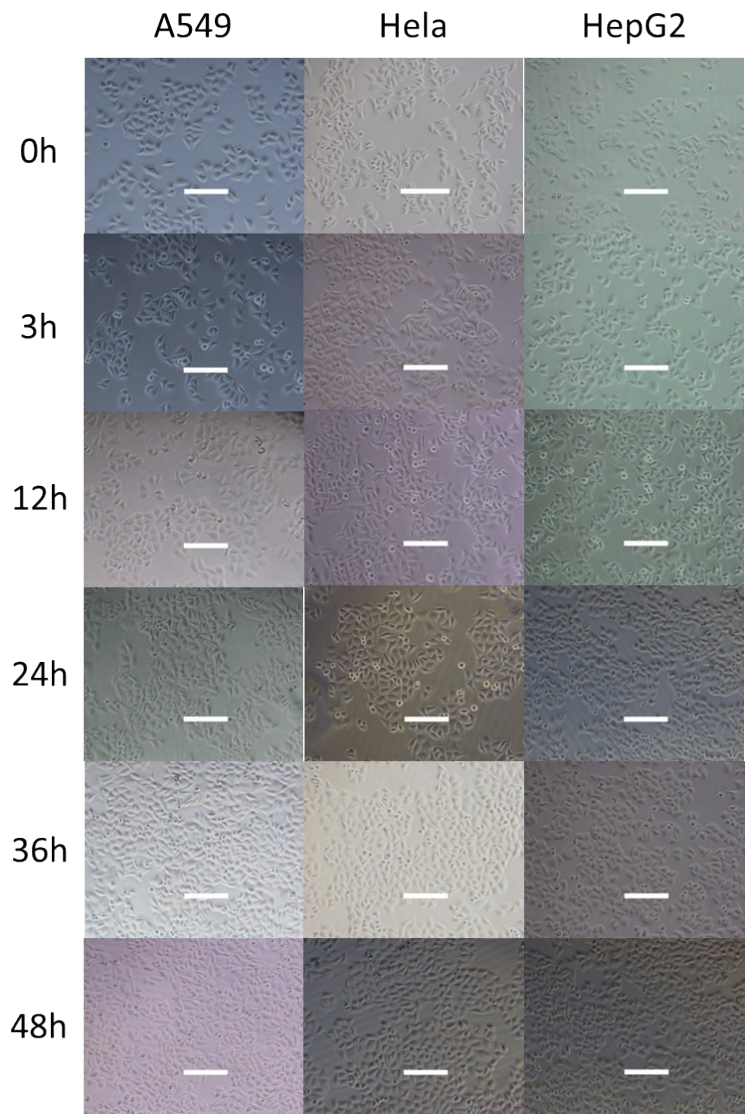


Fig. S17 Morphology changes with time monitored by optical microscopy before and after incubation **NCP-1-150** with HepG2, A549 and HeLa cells for 1 h at a concentration of 10 μg mL^{-1}. Scale bars represent 50 μm in all images.

5 Reference

S1 Y. Liu; C. Deng; L. Tang; A. Qin; R. Hu; J. Z. Sun; B. Z. Tang, *J. Am. Chem. Soc.* **2011**, *133*, 660-663

S2 I. John Olmsted, *J. Phys. Chem.* **1979**, *83*, 2581-2584.

S3 T. Mosmann, *J. Immunol. Methods.* **1983**, *65*, 55-63.