

Supplementary Materials for

Investigating Subcellular Compartment Targeting Effect of Porous Coordination Cages for Enhancing Cancer Nanotherapy

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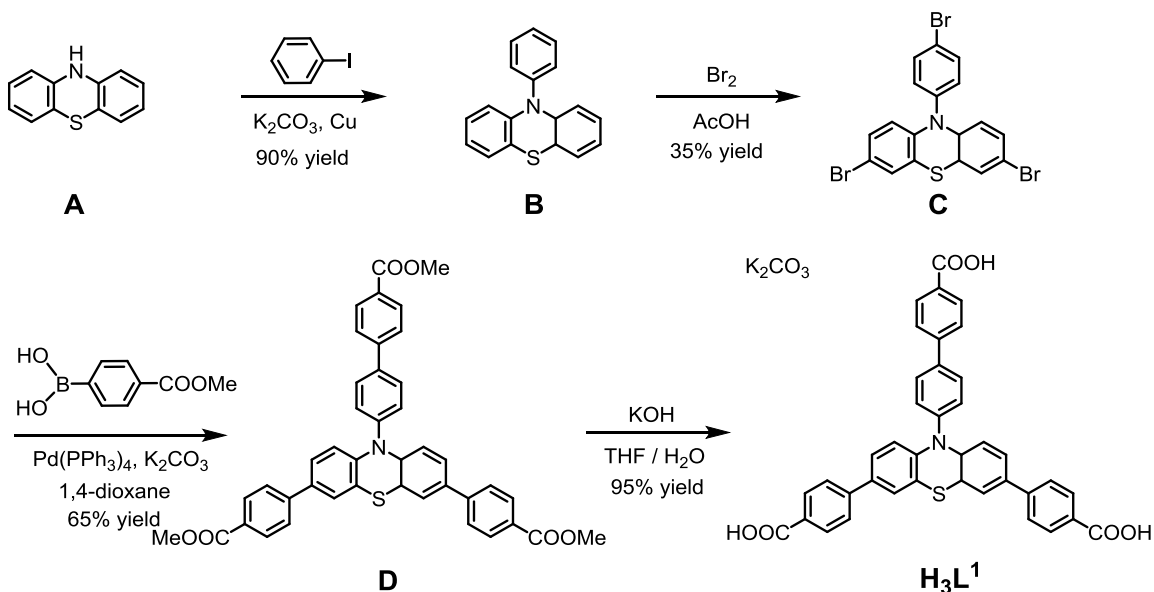
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Section 1. Materials and instrumentation

Phenothiazine (PTZ), *p*-tert-butylsulfonylcalix[4]arene (H₄TBSC, H₄V¹), 2,2'-bipyridine (V³), 1,3,5-Tris(4-Carboxyphenyl)Benzene (H₃L²), 2,4,5-Tri(4-Pyridyl)-1,3,5-Triazine (L³), ZnCl₂, CoCl₂, PdCl₂, N, N-dimethylformamide (DMF), Acetone, CHCl₃ were purchased from TCI USA and Sigma Aldrich. S-(+)-Camptothecin (CPT) were purchased from Sigma Aldrich. Na₄H₄V² was synthesized from H₄TBSC (H₄V¹) according to reported literature procedures.¹ All commercial chemicals were used without further purification unless otherwise mentioned. Single crystal X-ray diffraction was carried out Bruker Quest diffractometer equipped with a MoK α sealed-tube X-ray source (graphite radiation monochromator, $\lambda = 0.71073$) and a low temperature device (110 K). TEM was performed on a FEI Tecnai G2 F20 ST microscope at 200 kV equipped with a field emission gun. Thermogravimetric analyses (TGA) were carried out on a METTLER TOLEDO TGA/DSC 1 thermogravimetric analyzer from room temperature to 500 °C at a ramp rate of 2 °C/min in a flowing nitrogen atmosphere. Gas sorption measurements were conducted at different temperatures using a Micromeritics ASAP 2020 system. Nuclear magnetic resonance (NMR) data were collected on a Mercury 300 spectrometer. ESI Mass and MALDI-TOF-MS was conduct by Applied Biosystems PE SCIEX QSTAR system. The electronic absorption spectra were measured on a Hitachi U-4100 UV-Vis-NIR spectrophotometer. Steady-state luminescence spectra were acquired with a PTI Quanta Master Model QM-4 scanning spectrofluorometer equipped with a 75-watt xenon lamp, emission and excitation monochromators, excitation correction unit, and a PMT detector. The excitation and emission spectra have been corrected for the wavelength-dependent lamp intensity and detector response, respectively.

Section 2. The synthesis of panel ligand H_3L^1 .

Scheme S1 Synthetic procedures for ligand H_3L^1 .



A mixture of phenothiazine (**A**, 20.5 g, 0.10 mol), iodobenzene (42 g, 0.21 mmol), 45 g of K_2CO_3 and 5 g copper shavings were stirred at 190–200°C for 6 h in 100 ml of chlorobenzene and unreacted iodobenzene was then distilled off. Cold ethanol was then added, the mixture heated and filtered after dissolution of the product. The filtrate was cooled giving brownish red crystals of **B** (25.0 g, 0.09 mol, 90% yield).

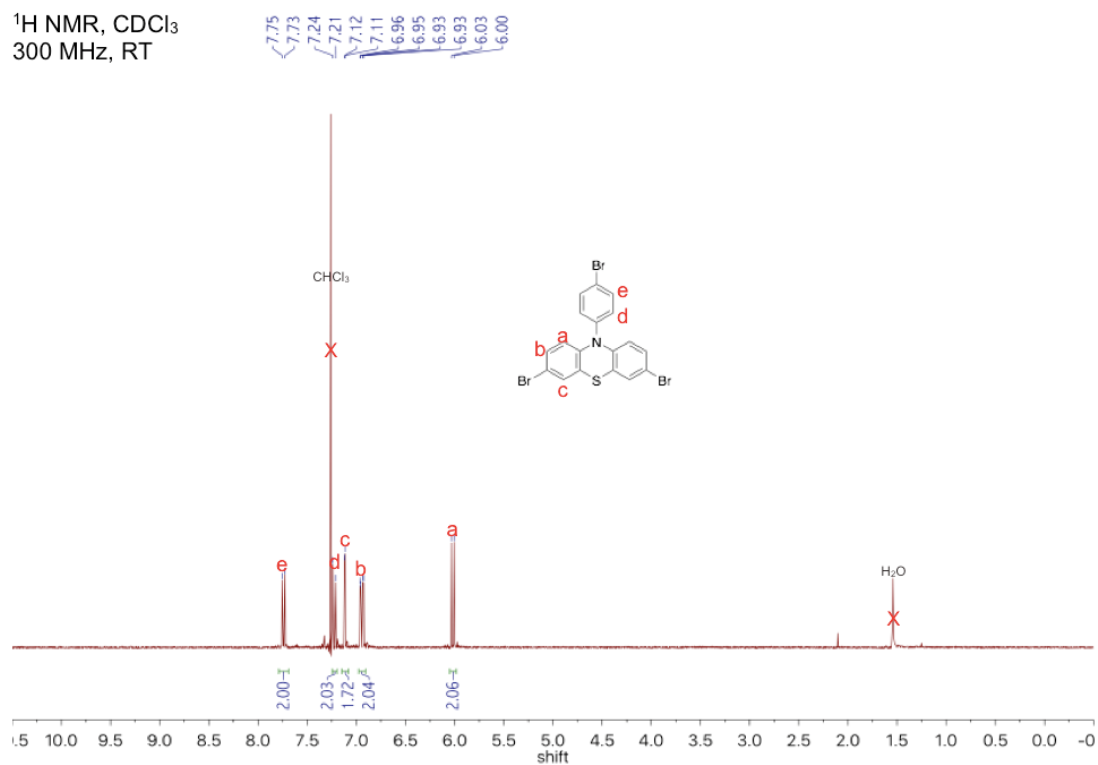
A stirred solution of **B** (2.0 g, 7.21 mmol) in acetic acid (78 mL) was flushed with nitrogen for 5 min, then bromine (1.8 mL, 92.8 mmol) in acetic acid (8 mL) was added dropwisely, and stirring was continued for 24 h at ambient temperature. The solution turned pink immediately, then changed gradually to deep red, next to green, and finally to black with the evolution of hydrogen bromide. After reaction complete, Na_2SO_3 (2.3 g) in 1.8 ml H_2O was added to the crude product and stirred for additional 2h. The mixture was poured to 200 mL H_2O of KOH (2.0 g). The solvent was filtrated and evaporated in vacuum to yield a

black oil. This oil was chromatographed on silica gel using benzene as the eluent to give our desired product **C** in acceptable yield (1.30 g, 2.52 mmol, 35% yield).

A mixture of **C** (2.86 g, 5.6 mmol), 4-(methoxycarbonyl)phenylboronic acid (4.0 g, 22.2 mmol), Pd(PPh₃)₄ (200 mg, 0.17 mmol), K₂CO₃ (6.0 g, 43.4 mmol) and dioxane (150 mL) was placed into a 250 mL sealed container with a magnetic stirring bar and evacuated and purged with nitrogen. Then the mixture was heated to reflux under nitrogen protection for 2 days. After the mixture was cooled to room temperature, the solvent was removed with a rotary evaporator. The crude product was purified by column chromatography to obtain the pure product **D** (2.47 g, 3.64 mmol, 65% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.15 (d, J = 8.1 Hz, 2H), 8.03 (d, J = 7.5 Hz, 4H), 7.88 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 8.1 Hz, 2H), 7.54-7.48 (m, 6H), 7.29 (d, J = 2.4 Hz, 2H), 7.11 (dd, J = 8.6, 7.2 Hz, 2H), 6.30 (d, J = 8.4 Hz, 2H), 3.93 (m, 9H). ¹³C NMR (75 MHz, CDCl₃) δ 166.88, 166.81, 143.89, 140.42, 140.28, 134.43, 131.20, 130.88, 130.30, 130.14, 129.84, 129.49, 128.79, 128.60, 127.09, 126.04, 125.79, 125.24, 120.37, 116.31, 52.23, IR, 2961, 2924, 2854, 1720, 1606, 1436, 1279, 1180, 1112, 1018, 737, 694, 563, 499, 433. ESI *m/z*: calculate for C₄₂H₃₃NO₆S: 679.20; found: 679.08; Elemental analysis: calculated (%) for C₄₂H₃₃NO₆S, C 74.21, H 4.89, N 2.06, S 4.72; found (%): C 73.46, H 4.27, N 2.20, S 5.03.

A mixture of **D** (0.54 g, 0.079 mmol), KOH (1.0 g, 17.8 mmol), 20 ml THF and 20 ml H₂O was placed into a 100 mL sealed container with a magnetic stirring bar. Then the mixture was heated to reflux until the solution turned transparent. After the mixture was cooled to room temperature, the solvent was removed with a rotary evaporator. Then add 6M HCl to

the mixture while vigorous strring to precipitate the crude product. The crude product **H₃L¹** (0.49 mg, 0.075 mmol, 95% yield) was collected by centrifugation and washed several time by water. ¹H NMR (300 MHz, *d*₇-DMF) δ 8.19 (d, J = 8.4 Hz, 4H), 8.07-8.01 (m, 6H), 7.83 (d, J = 8.7 Hz, 4H), 7.74 (d, J = 8.4 Hz, 2H), 7.59 (d, J = 2.4 Hz, 2H), 7.44 (dd, J = 8.7 Hz, 2H), 6.41 (d, J = 8.4 Hz, 2H). ¹³C NMR (75 MHz, *d*₇-DMF) δ 167.52, 167.48, 143.56, 143.35, 143.08, 140.37, 139.74, 133.91, 131.47, 130.50, 130.34, 129.68, 128.42, 128.11, 127.51, 126.38, 125.56, 125.27, 120.12, 116.82, IR 2961, 2926, 2854, 1720, 1606, 1436, 1279, 1180, 1112, 1016, 737, 694, 566, 486, 432. ESI *m/z*: calculate for C₃₉H₂₇NO₆S: 637.71; found: 638.03; Elemental analysis: calculated (%) for C₃₉H₂₇NO₆S·H₂O, C 71.44, H 4.46, N 2.14, S 4.89; found (%): C 71.27, H 3.91, N 2.05, S 4.63.



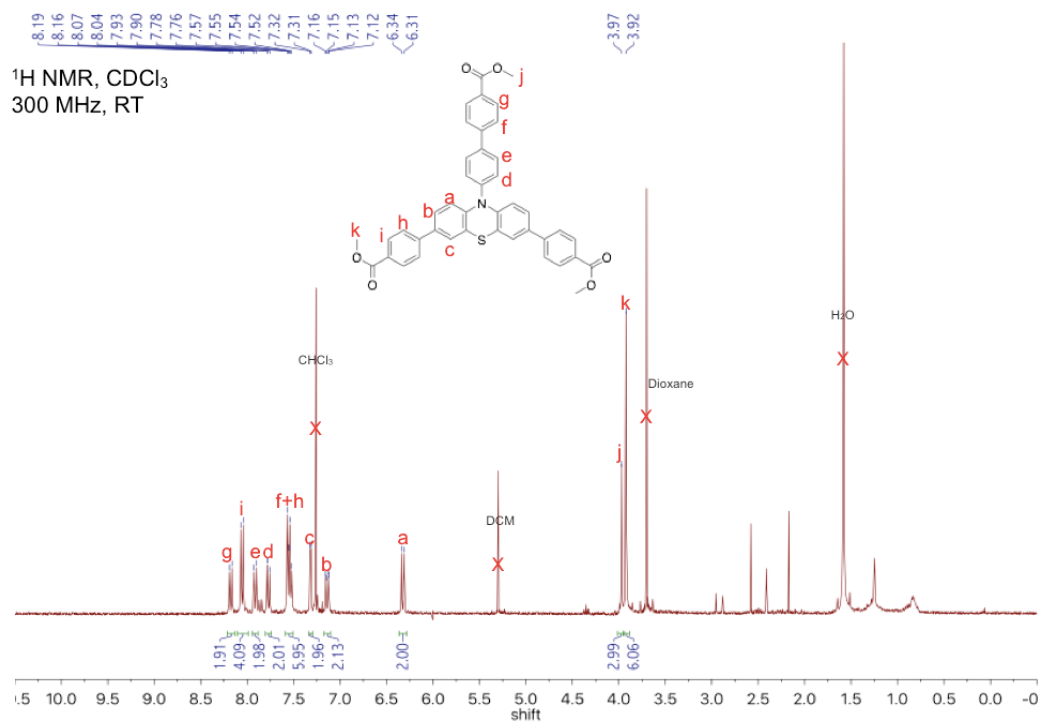


Fig. S2 ¹H NMR spectrum of compound **D**.

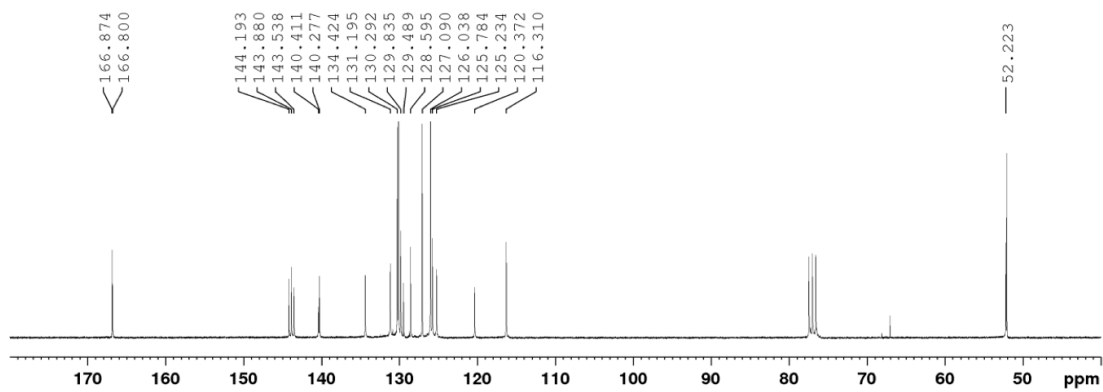


Fig. S3 ¹³C NMR spectrum of compound **D**.

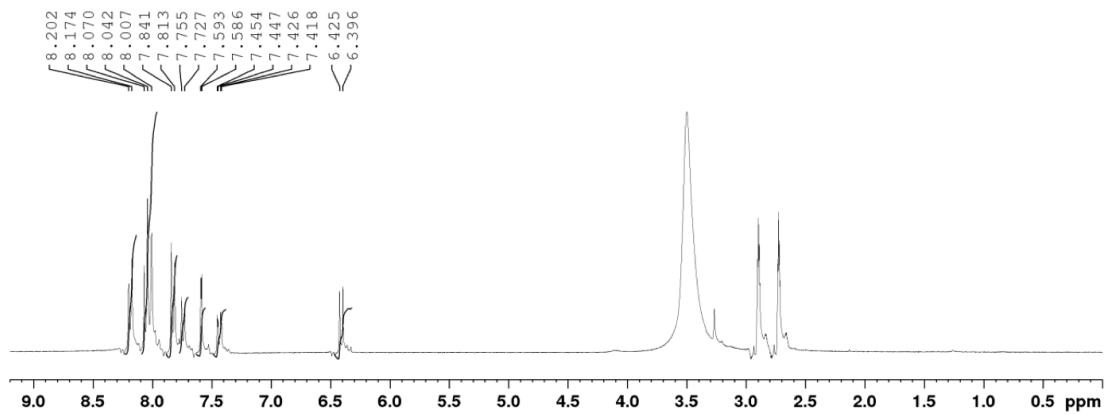


Fig. S4 ^1H NMR spectrum of compound H_3L^1 .

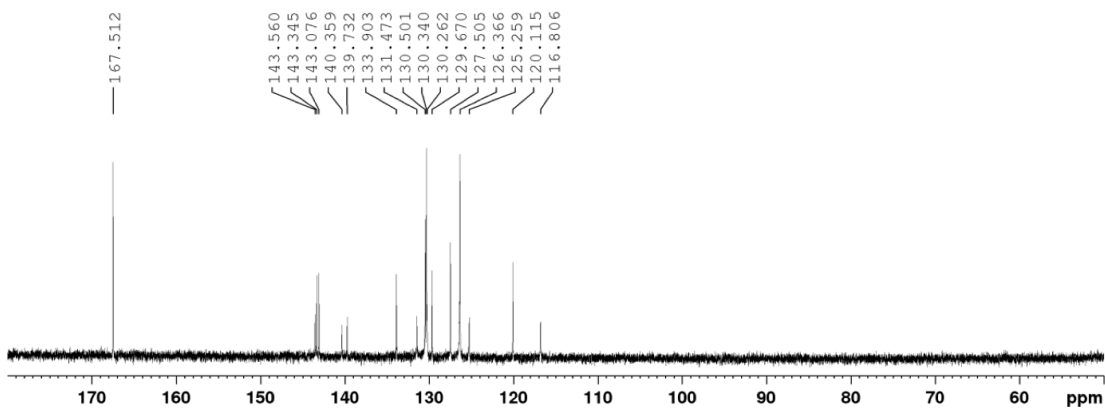


Fig. S5 ^{13}C NMR spectrum of compound H_3L^1 .

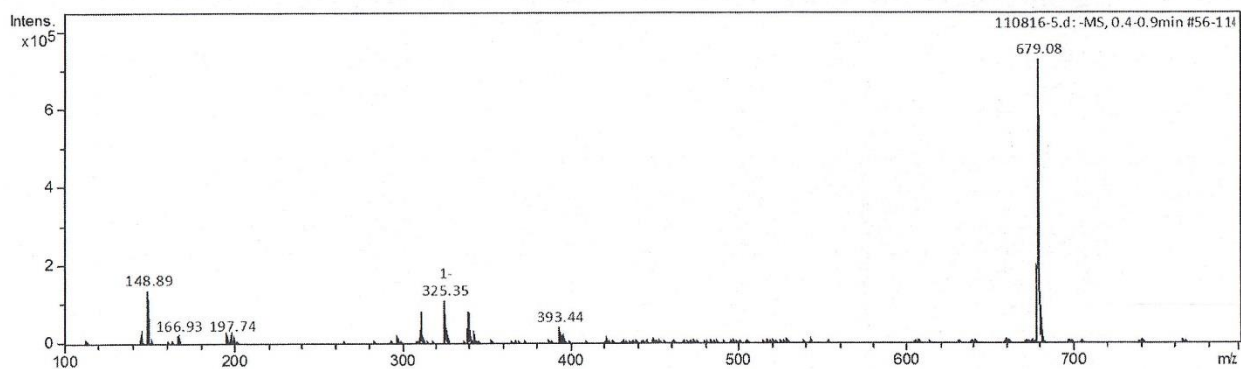


Fig. S6 ESI-MS Spectrum of compound **D**.

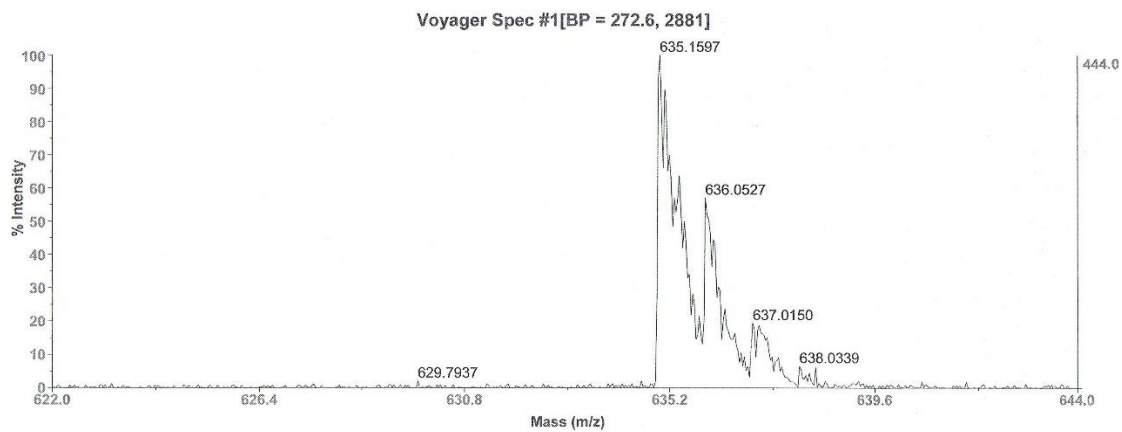
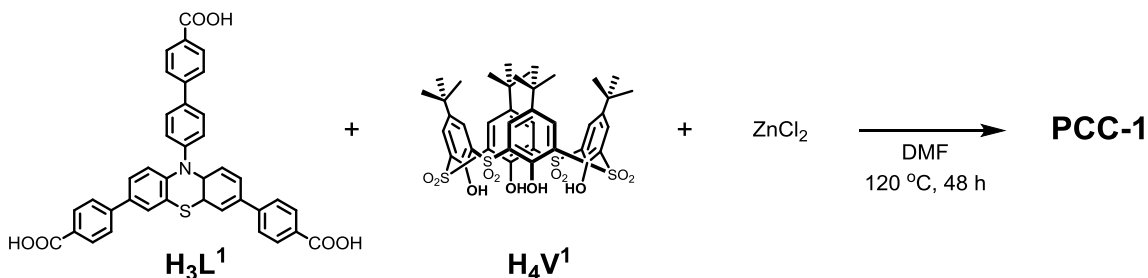


Fig. S7 ESI-MS Spectrum of ligand **H₃L¹**.

Section 3: The self-assembly of PCC cages.

Scheme S2 Synthetic procedures for PCC-1.



H₃L¹ (21 mg, 0.033 mmol) and **H₄V¹** (12 mg, 0.014 mmol) and **ZnCl₂** (14 mg, 0.103 mmol) were dissolved in 1 mL DMF. The mixture was heated up at 120 °C in an oven for 48 h. After cool down to ambient temperature, the bright yellow crystal were collected and washed by methanol. Yield of **PCC-1** (based on ligand): ~80% (according to ligand **H₃L¹**). ¹H NMR (300 MHz, *d*₇-DMF) δ 8.21 (br, 32H, **L¹**), 8.04 (br, 48H, **L¹**), 7.99 (br, 48H, **V¹**), 7.80 (br, 32H, **L¹**), 7.67 (br, 16H, **L¹**), 7.58 (br, 16H, **L¹**), 7.45 (br, 16H, **L¹**), 6.43 (br, 16H, **L¹**), 1.20 (s, 216H, **V¹**). ¹³C NMR (75 MHz, *d*₇-DMF) δ 167.52, 167.48, 143.56, 143.35, 143.08, 140.37, 139.74, 133.91, 131.47, 130.50, 130.34, 129.68, 128.42, 128.11, 127.51, 126.38, 125.56, 125.27, 120.12, 116.82, IR, 2963, 2926, 2854, 1720, 1606, 1436, 1278, 1180, 1112, 1016, 780, 736, 604, 563, 497, 480. MS, Elemental analysis: calculated (%) for [C₅₅₂H₄₅₂N₈O₁₂₆S₃₂Zn₂₄] · 8 DMF · 30 H₂O, C 53.49, H 4.43, N 1.73, S 7.93; found (%): C 53.88, H 4.13, N 1.25, S 8.17. MALDI-TOF-MS: 11764.30; found: 11764.41.

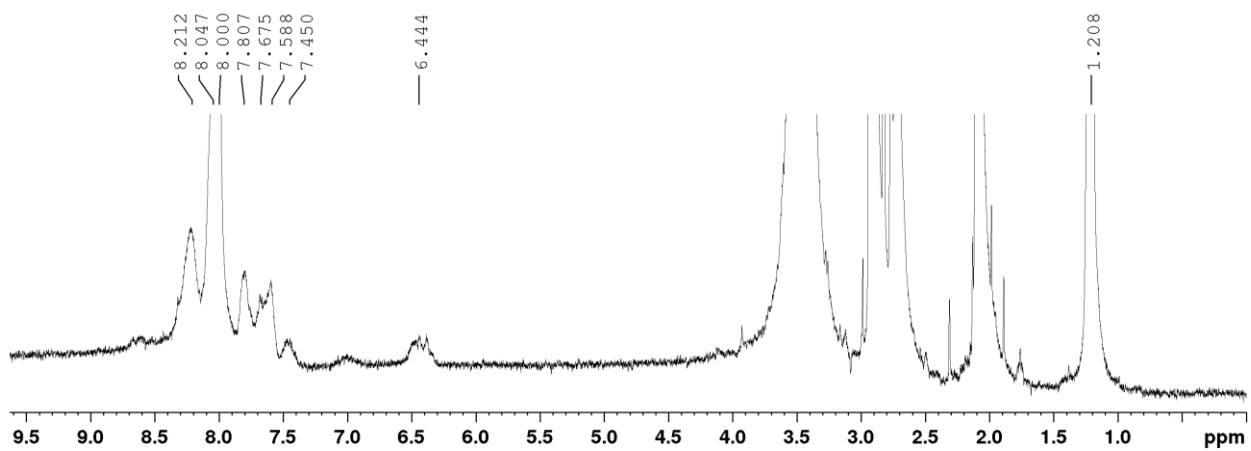


Fig. S8 ^1H NMR spectrum of PCC-1.

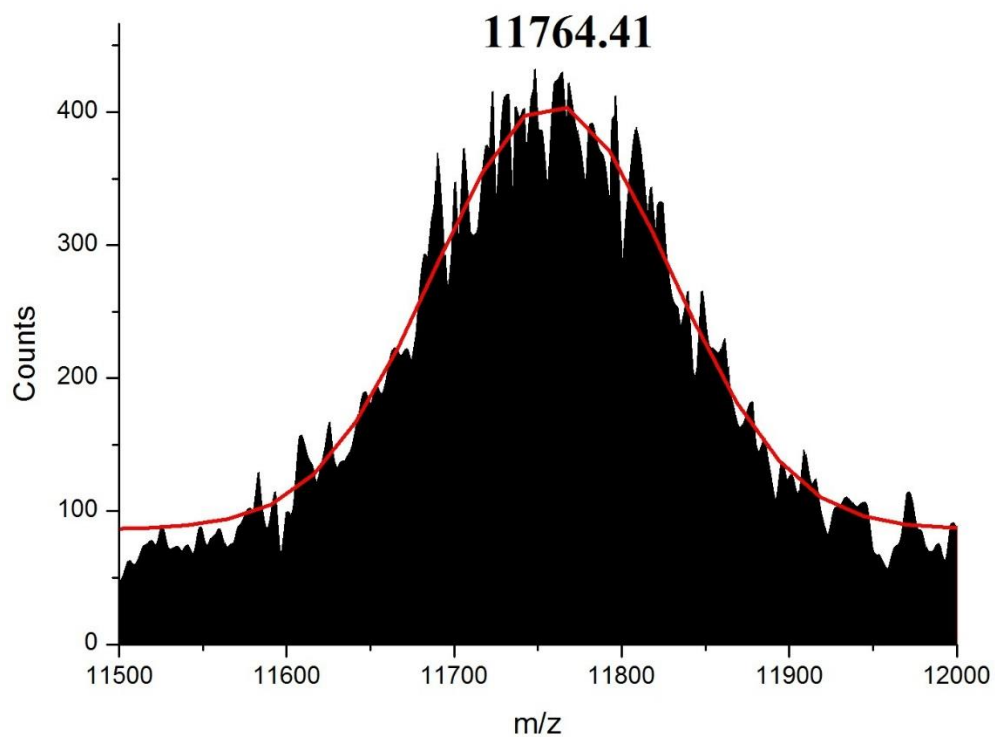
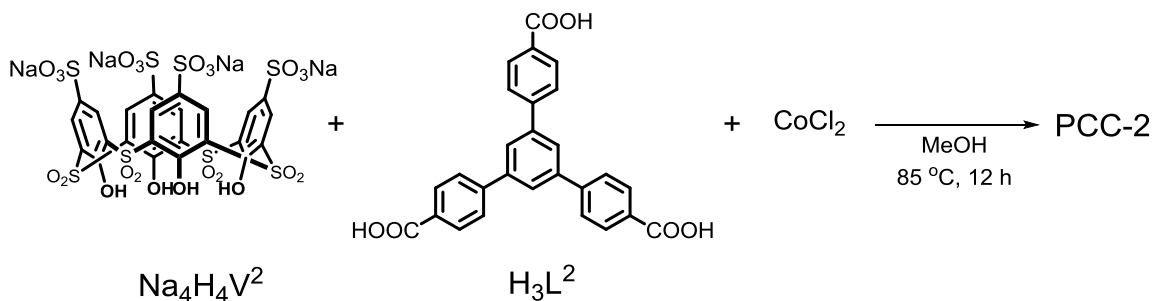


Fig. S9 MALDI-TOF MS spectrum of PCC-1.

Scheme S3 Synthetic procedure for **PCC-2**.



PCC-2 was synthesized according to literature [2] with minor modification as follows:

$\text{Na}_4\text{H}_4\text{V}^2$ (14.5 mg, 0.014 mmol), H_3L^2 (14.5 mg, 0.033 mmol), and CoCl_2 (13.3 mg, 0.103 mmol) were suspended in 2 mL MeOH with 2 drops of Et_3N . The mixture was heated at 85 °C in an oven for 12 h. After cooling to ambient temperature, large purple crystals were collected and washed with methanol to yield **PCC-2** in about 95% (according to $\text{Na}_4\text{H}_4\text{V}^1$). Elemental analysis: calculated (%) for $\text{Na}_{24}[\text{Et}_3\text{NH}]_6[\text{C}_{360}\text{H}_{222}\text{S}_{48}\text{O}_{198}\text{Co}_{24}] \cdot 5 \text{ MeOH} \cdot 10 \text{ H}_2\text{O}$, C 39.56, H 2.96, N 0.69, S 12.64; found (%): C 39.28, H 2.92, N 0.70, S 12.86. Other characterizations, please refer to literature [2].

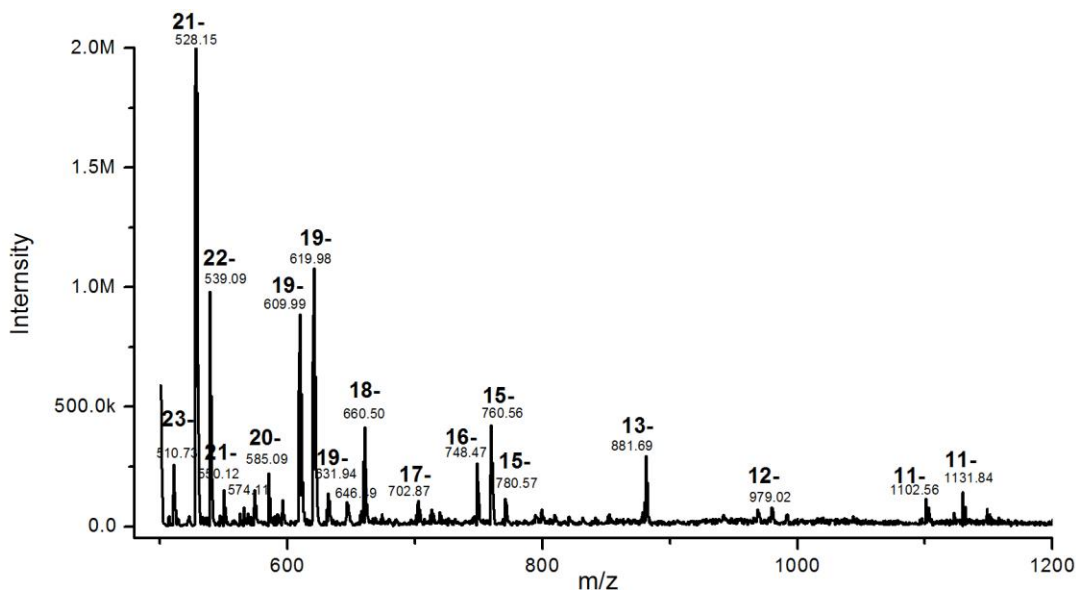
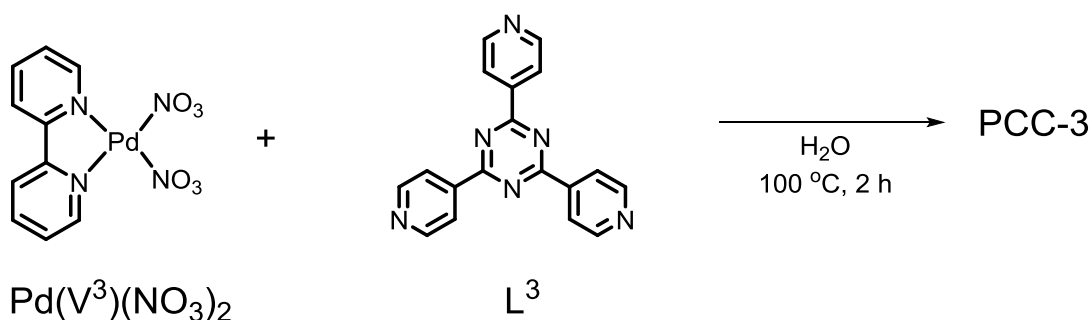


Fig. S10 ESI-MS Spectrum of **PCC-2a**.

Table. S1 Mass fragment table for **PCC-2a**.

$$M = C_{360}H_{174}CO_{24}O_{198}S_{48}Na_{24}(C_6H_{15}NH)_6 = 11833.78$$

Formula	Charge z	Calculated m/z	Experimental m/z
(M-23Na ⁺)+ 14 MeOH	23-	511.01	510.73
(M-22Na ⁺)+ 9 MeOH	22-	528.00	528.15
(M-22Na ⁺)+ 17 MeOH	22-	539.65	539.09
(M-21Na ⁺)+ 6 MeOH	21-	549.66	550.12
(M-21Na ⁺)+ 22 MeOH	21-	574.07	574.11
(M-20Na ⁺)+ 10 MeOH	20-	584.70	585.09
(M-19Na ⁺)+ 6 MeOH	19-	609.95	609.99
(M-19Na ⁺)+ 12 MeOH	19-	620.06	619.98
(M-19Na ⁺)+ 19 MeOH	19-	631.86	631.94
(M-18Na ⁺)+ 7 MeOH	18-	646.89	646.49
(M-17Na ⁺)+ 15 MeOH	18-	661.12	660.50
(M-17Na ⁺)+ 16 MeOH	17-	703.25	702.87
(M-16Na ⁺)+ 16 MeOH	16-	748.64	748.47
(M-16Na ⁺)+ 22 MeOH	15-	760.65	760.56
(M-15Na ⁺)+ 7 MeOH	15-	780.87	780.57
(M-14Na ⁺)+ 26 MeOH	14-	881.75	881.69
(M-12Na ⁺)+ 6 MeOH	12-	979.16	979.02
(M-11Na ⁺)+ 17 MeOH	11-	1102.30	1102.56
(M-11Na ⁺)+ 27 MeOH	11-	1131.42	1131.84

Scheme S4 Synthetic procedures for **PCC-3**.

PCC-3 was synthesized according to literature [3, 4] with minor modification as follows: $[Pd(V^3)(NO_3)_2]$ (20 mg, 31 mmol) and 2,4,6-tri(4-pyridyl)-1,3,5-triazine (L^3 , TPT; 6.3 mg, 20 mmol) were added in H_2O (4.0 mL). The resulting suspension was stirred at 100 °C for 2 h. A trace amount of insoluble material was filtrated, and the clear colorless solution was evaporated to dryness to give **PCC-3** as a white powder (yield: 99%). The product was

dissolved in D₂O and confirmed by NMR spectroscopy. The single crystal X-ray structure was obtained by slowly vaporized the aqueous solution of **PCC-3**. ¹H NMR (500 MHz, D₂O) δ 9.49 (d, 24H, **L**³), 8.90 (d, 24H, **L**³), 8.50-8.35 (m, 24H, **V**³), 7.70-7.60 (m, 24H, **V**³), 4.79 (s, H₂O). Other characterizations, please refer to literature [3, 4].

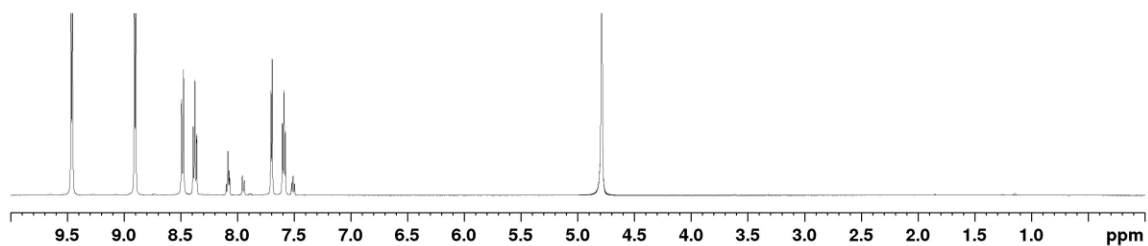


Fig. S11 ¹H NMR spectrum of **PCC-3**.

Section 4: Structure refinement of PCCs by single crystal XRD.

All as-synthesized crystals were taken from the mother liquid without further treatment, transferred to oil and mounted into a loop for single crystal X-ray data collection. Diffraction was measured on a Bruker Quest diffractometer equipped with a MoK α sealed-tube X-ray source (graphite radiation monochromator, $\lambda = 0.71073$) and a low temperature device (110 K). Data were collected at 110 K and were integrated and scaled using APEX3 software package.⁵ XPREP⁶ was used to determine the space group based on the systematic absence and E-statistics. The structures were solved using direct methods with SHELXT³ and refined with SHELXL⁷ under an Olex 2⁸ graphical interface.

All non-hydrogen atoms were found in the electron density difference map and refined anisotropically. All hydrogen atoms were calculated and refined as a riding model. Unrestrained atomic displacement parameters sometimes produces high U_{eq} values during the refinement thus ISOR, DELU and SIMU commands were introduced as appropriate with careful adjustment of their standard deviations.

PCC-1 crystallizes in the central-symmetric tetragonal space group $I4/m$ (Figure S12 & S13). The cage molecule consisted of six tetranuclear metal clusters as vertices (Zn_4V^1) and eight panel ligands as faces (L^1) assembled into an octahedron cage. Each Zn atom in the cluster was located at the four corner of the cluster square plane and held in a pseudo-octahedral configuration by coordination with six oxygen atoms. The six oxygen atoms includes one sulfonic oxygen and two phenolate oxygen atoms from V^1 , two carboxylate oxygens from two adjacent L^1 and a μ_4 -OH₂.

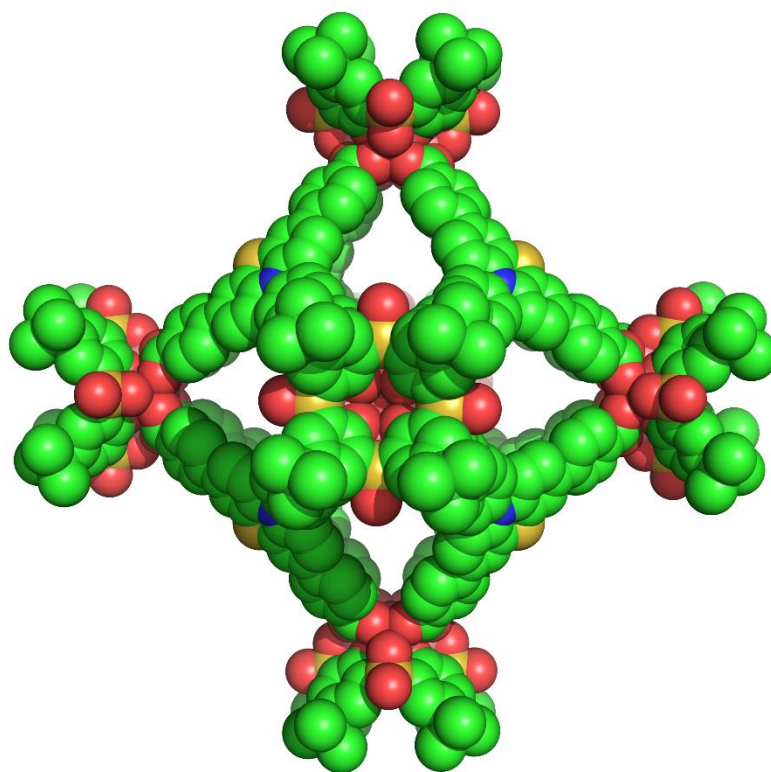


Fig. S12. Space filling model of PCC-1 crystal structure. Graphic was generated using Pymol.

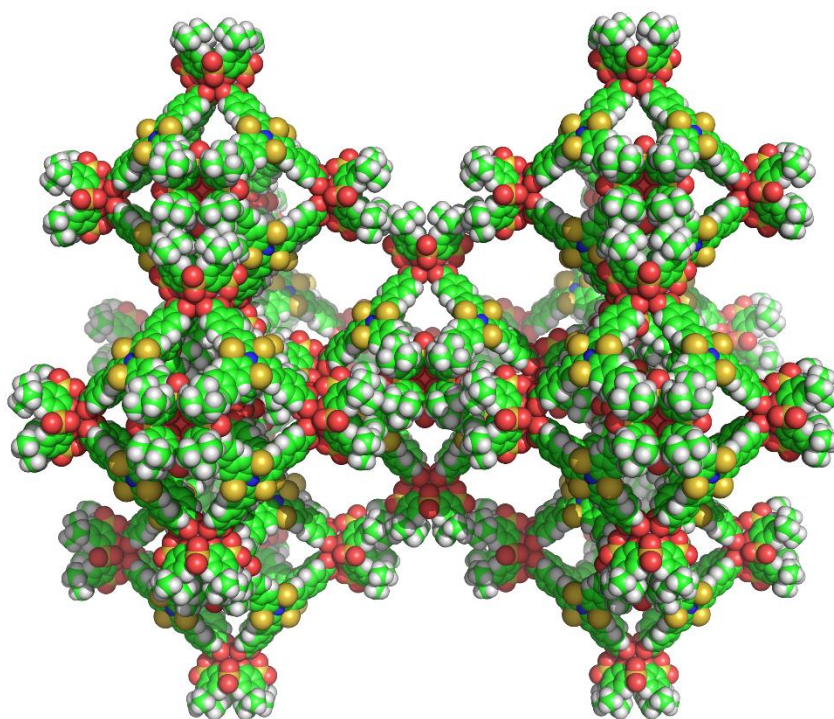


Fig. S13. Crystal Packing of PCC-1. Graphic was generated using Pymol.

For PCC-1, the sulfur atom of the panel ligand was found to be disordered over three non-crystallographic-symmetry-related sites with equal electron density (Figure S14). The three sites are related to the molecular C_3 rotational axis of the cage molecule (and the panel ligand), however, due to the packing of these cages in solid state, this molecular symmetry element is absent in the crystal structure. Based on the equal electron density of the three sites and the molecular point group symmetry of the cage molecule, the sulfur atom is determined to have equal occupancy. The location of the three sulfur sites were slightly away from the panel ligand plane, defined by its centroid nitrogen atom and the three peripheral carboxyl carbon atoms. Thus, each of the two phenyl rings that are directly attached to the sulfur atom must be twisted to be coplanar with it. The conformation of the third central phenyl ring is modeled to be perpendicular to the panel ligand plane to give the minimal steric hindrance. Overall, taking consideration of the disorder, each central phenyl ring effectively has three orientations with equal probability. These phenyl rings are refined as ideal hexagons and their atomic displacement parameters of the three equivalent sites are constrained to be the same.

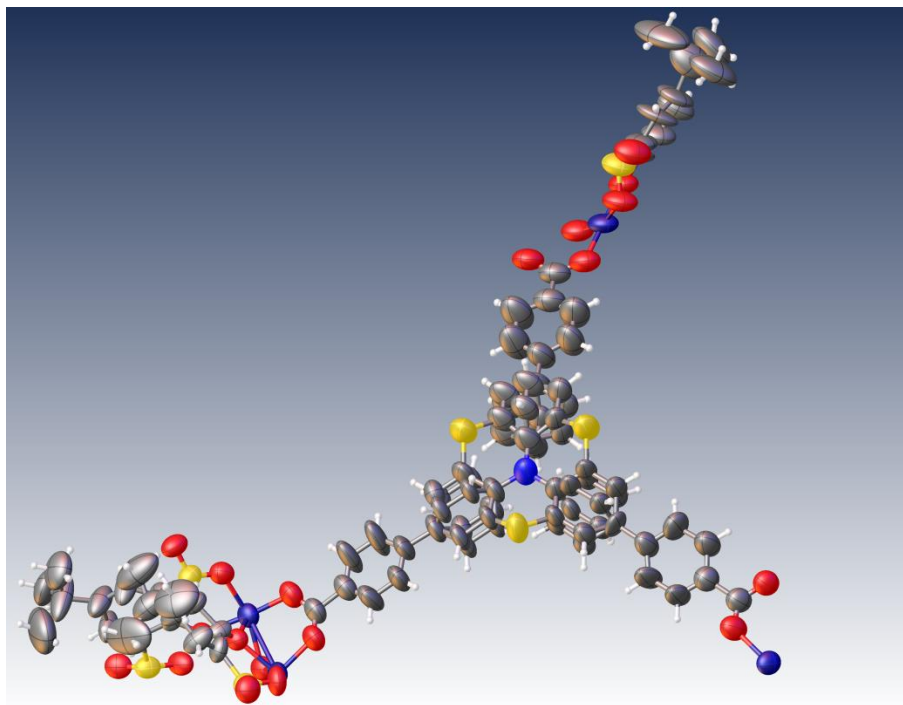


Fig. S14. ORTEP diagram of the crystallographic asymmetric unit of **PCC-1** as determined by SC-XRD, showing 50% probability of the atomic displacement ellipsoids. Graphic was generated using Olex 2.

The structure was finally treated with SQUEEZE using PLATON to remove 14220 electrons, which accounts for 177.75 DMF molecules per unit cell (Table S2).

Table S2. Crystal data and structure refinement

Name	PCC-1
Empirical formula	$C_{552}H_{440}N_8O_{126}S_{32}Zn_{24}$
Formula weight	11795.89
Temperature/K	110.0
Crystal system	Tetragonal
Space group	$I4/m$
$a/\text{\AA}$	37.945(3)

$b/\text{\AA}$	37.945(3)
$c/\text{\AA}$	60.835(8)
$\alpha/^\circ$	90
$\beta/^\circ$	90
$\gamma/^\circ$	90
Volume/ \AA^3	87593(17)
Z	2
ρ_{calc} (g/cm^3)	0.447
μ/mm^{-1}	0.385
$F(000)$	12096.0
Crystal size/ mm^3	$0.12 \times 0.08 \times 0.08$
Radiation	MoK α ($\lambda = 0.71073$)
2θ range for data collection/ $^\circ$	5.118 to 36.998
Index ranges	$-33 \leq h \leq 33, -33 \leq k \leq 33, -54 \leq l \leq 54$
Reflections collected	420955
Independent reflections	16497 [$R_{\text{int}} = 0.2413, R_{\text{sigma}} = 0.0979$]
Data/restraints/parameters	16497/474/913
Goodness-of-fit on F^2	1.057
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0905 (0.2776)^1, wR_2 = 0.2120 (0.5770)^1$
Final R indexes [all data]	$R_1 = 0.1495 (0.4511)^1, wR_2 = 0.2422 (0.6633)^1$
Largest diff. peak/hole / $e \text{\AA}^{-3}$	0.84/-0.33

Note: values in the parentheses are the refinement factors before SQUEEZE.

PCC-2 crystallizes in the rhombohedral $R\bar{3}$ as large, purple blocks (Figure S15 & S16).

Each PCC-2 cage molecule comprises six tetranuclear cobalt clusters $[\text{Co}_4(\mu_4\text{-OH})]$ and six vertex ligands as vertices, and eight panel ligands as faces of an octahedral cage, giving a anionic host as $\{[\text{Co}_4(\mu_4\text{-OH})\text{TCAS}]_6(\text{BTB})_8\}^{30-}$. Counter cations are Na^+ and Et_3NH^+ . Each cobalt atom coordinates one sulfonic oxygens and two phenolate oxygen atoms from V, two carboxylate oxygens from L, and a $\mu_4\text{-OH}$, giving rise to an octahedral coordination environment. Based on the crystal structure, the longest inner-cavity dimension of PCC-2 is 25.1 Å (the distance between two $\mu_4\text{-OH}$ of two opposite Co_4 clusters), and the diameter of the sphere in which the octahedral cage is inscribed is 42.2 Å.

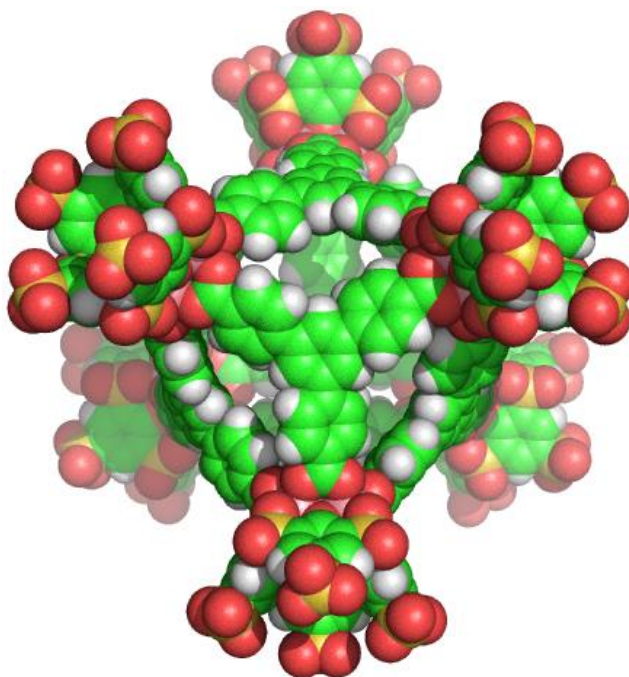


Fig. S15. Space filling model of **PCC-2** as determined by SC-XRD. Graphic was generated using Pymol.

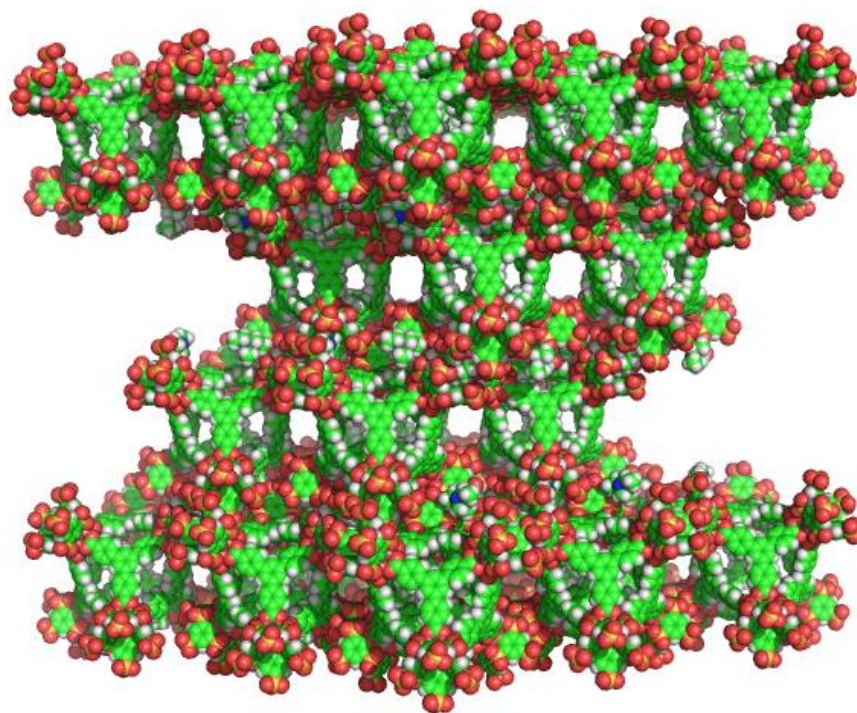


Fig. S16. Crystal packing view of **PCC-2** as determined by SC-XRD. Graphic was generated using Pymol.

For PCC-2, a triethylamine molecule was found in each the calix[4]arene cavity. We assumed there were more disordered triethylamine molecules in the outer calix cavity. All counter-cations (Na^+) could not be assigned due to the disorder. The central hollow cavity was filled with solvent molecules (MeOH). The structure was finally treated with SQUEEZE using PLATON to remove solvent electrons (Table S3).

Table S3. Crystal data and structure refinement

Name	PCC-2
Empirical formula	$\text{C}_{360}\text{H}_{168}\text{O}_{198}\text{S}_{48}\text{CO}_{24} \cdot 6(\text{C}_6\text{H}_{15}\text{N})$
Formula weight	11221.27
Temperature/K	100.0
Crystal system	Trigonal

Space group	$R\bar{3}$
$a/\text{\AA}$	33.542 (11)
$c/\text{\AA}$	80.869(5)
Volume/ \AA^3	78794(7)
Z	3
ρ_{calc} (g/cm ³)	0.709
μ/mm^{-1}	0.507
F(000)	17028.0
Crystal size/mm ³	0.06 × 0.05 × 0.05
Radiation	MoK α ($\lambda = 0.71073$ nm)
2 θ range for data collection/ $^\circ$	5.118 to 46.512
Index ranges	$-37 \leq h \leq 37, -37 \leq k \leq 37, -89 \leq l \leq 89$
Reflections collected	357724
Independent reflections	25048 [$R_{\text{int}} = 0.0897, R_{\text{sigma}} = 0.0463$]
Data/restraints/parameters	25048/796/1022
Goodness-of-fit on F^2	1.383
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.1087$ (0.2307), $wR_2 = 0.3222$ (0.5455)
Final R indexes [all data]	$R_1 = 0.1384$ (0.3058), $wR_2 = 0.3542$ (0.6153)
Largest diff. peak/hole / e \AA^{-3}	2.91/-0.76

Note: values in the parentheses are the refinement factors before SQUEEZE.

PCC-3 crystallizes in the rhombohedral T_d as large, pale yellow blocks (Figure S17 & S18). Each PCC-3 cage molecule comprises six Pd metal and six bipyridyl ligands as vertex,

and four TPT panel ligands as faces of an octahedral cage, giving a canionic host as $\{[\text{Pd}(\text{Bpy})]_6(\text{TPT})_4\}^{12+}$. Counter cations are NO_3^- . Total formula is $\{[\text{Pd}(\text{Bpy})]_6(\text{TPT})_4\}^{12+}(\text{NO}_3^-)_{12}$ (M.W.= 3569.05). Each Pd atom coordinates two nitrogen atoms from bipyridyl ligands and two nitrogen atoms from TPT panel ligand. Based on the crystal structure, the longest inner-cavity dimension of PCC-3 is 12.7 Å (the distance between one Pd atom and one opposite N atom in TPT ligand), and the diameter of the sphere in which the octahedral cage is inscribed is 28.0 Å.

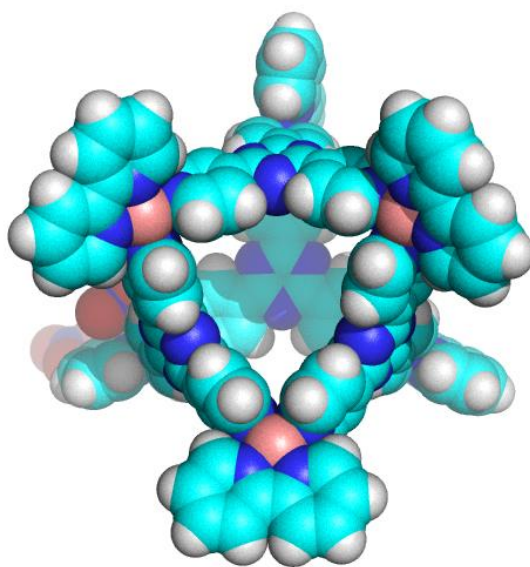


Fig. S17. Space filling model of **PCC-3** as determined by SC-XRD. Graphic was generated using Pymol.

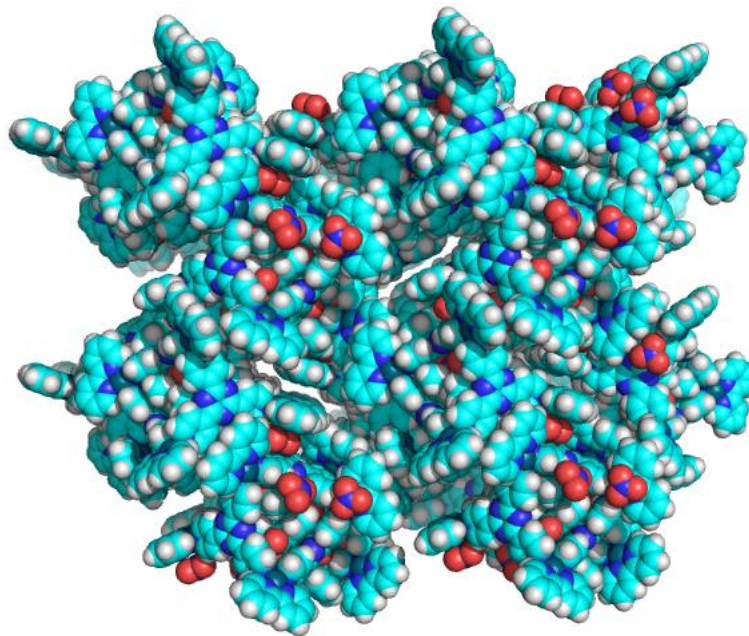


Fig. S18. Crystal packing view of **PCC-3** as determined by SC-XRD. Graphic was generated using Pymol.

Section 5: Sample activation and low pressure adsorption of PCC-1

Activation of PCC-1

As-synthesized samples of **PCC-1** were immersed in the methanol solution for 2 days, during which the solution/solvent was decanted and freshly replenished 3 times per day. Obtained samples were immersed in acetone for 12 h, during which the activation solvent was replenished more than 3 times. The sample was extracted by supercritical CO₂. And then the chamber containing the sample and liquid CO₂ was heated up around 40 °C and kept under the supercritical condition (typically 11 MPa) for 2 h. CO₂ was slowly vented (ca. 6 h) from the chamber at around 40 °C, yielding activated **PCC-1**.

Low-pressure gas sorption measurements.

Low-pressure gas sorption experiments were carried out on a Micromeritics ASAP-2020 automatic volumetric instrument. Ultrahigh-purity-grade N₂ gases were used in all adsorption measurements. The N₂ isotherms were measured using a liquid nitrogen bath (77 K). The adsorption and desorption results is shown in **Fig. S19**.

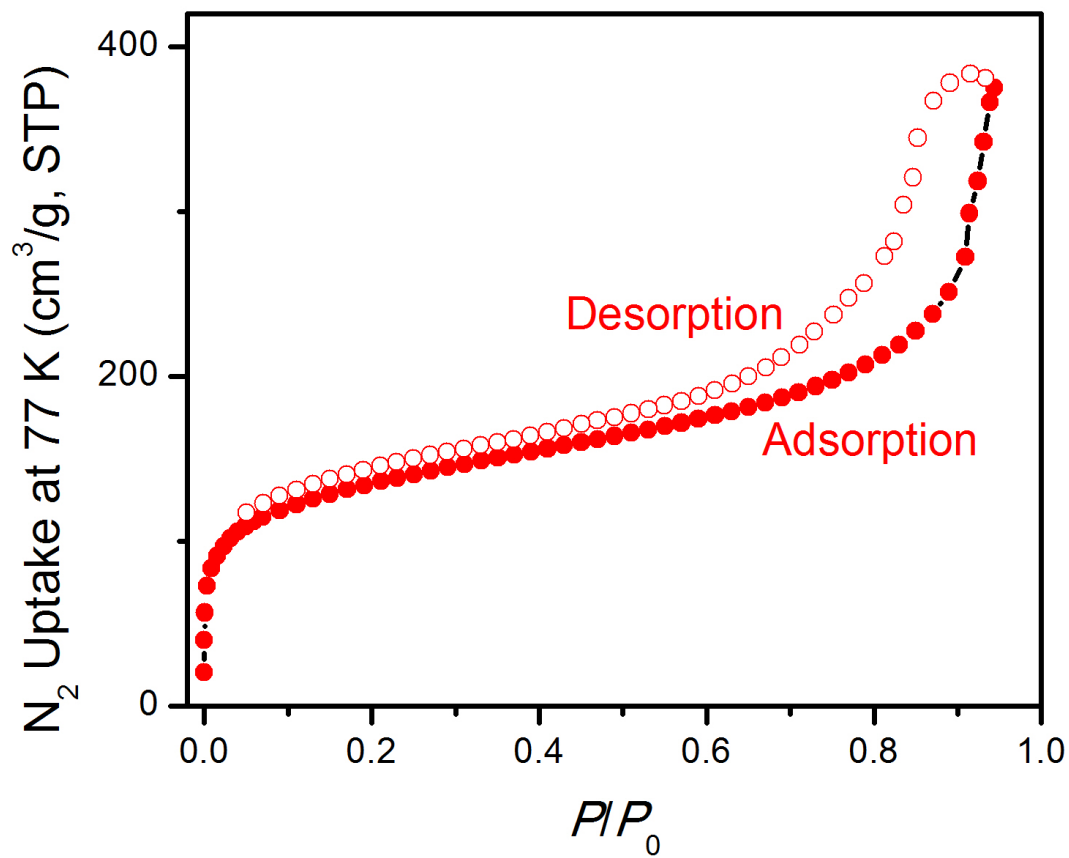


Fig. S19. N_2 adsorption (filled circles) and desorption (open circles) isotherms at 77 K of activated **PCC-1**.

Section 6. Thermogravimetric Analysis of PCCs

TGA-DSC curves for as-synthesized **PCC-1** were shown in Figure S20 & S21. The sample of as-synthesized **PCCs** was taken out from the mother liquor and washed with methanol and dried in air. The apparent weight loss in temperature range from 25 °C to 440 °C can be attributed to the loss of solvent molecules for PCC-1. The weight loss percentage is 7.75% for as-synthesized **PCC-1**, which are solvent molecules.

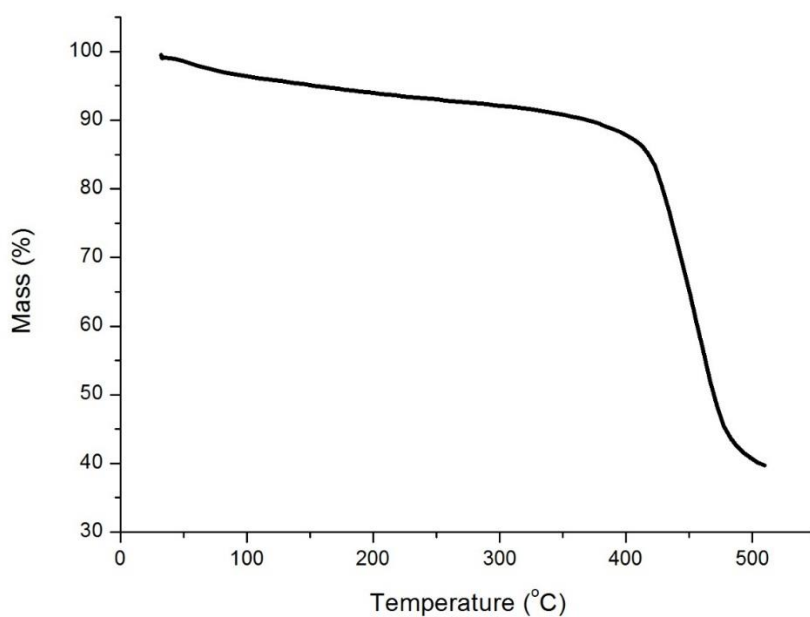


Fig. S20 TGA curve for **PCC-1** as synthesized (Temperature VS Mass percentage).

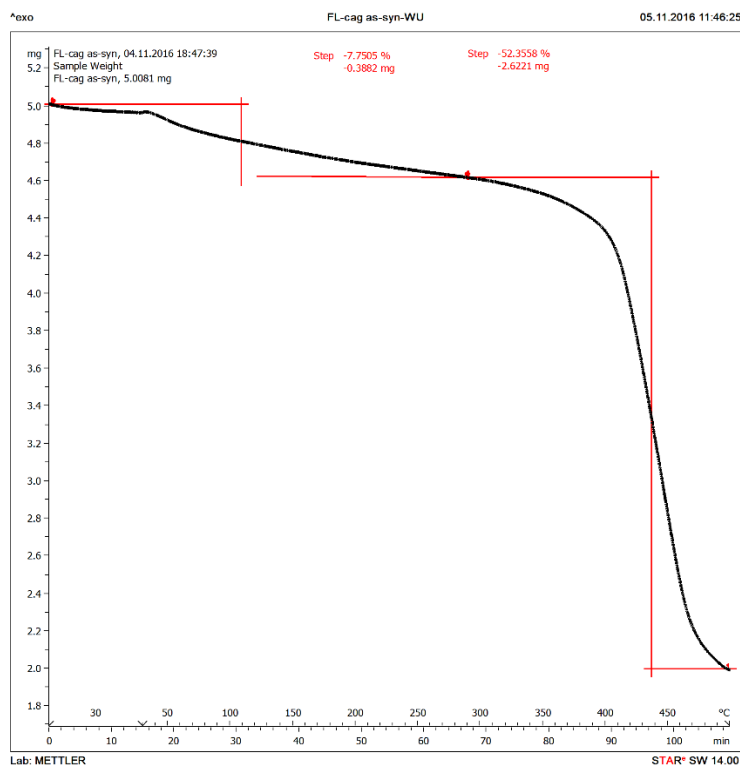


Fig. S21 TGA curve for **PCC-1** as synthesized (Temperature VS Absolute Mass).

TGA-DSC curves for as-synthesized **PCC-2** were shown in **Fig. S22**. The sample **PCC-2** was taken out from the mother liquor and washed with methanol and dried in air. The apparent weight loss in the temperature range 25 °C to 240 °C can be attributed to the loss of solvent molecules. The solvent weight loss percentage is 5.99%. It is noticeable that there are two weight loss curve from 240 °C to 440 °C, which should be ascribed to the partial and complete decomposition of the cage framework. These weight loss percentages are 15.47% and 33.43%, respectively. The experimental gas adsorption data is reasonably lower than simulated gas adsorption data.

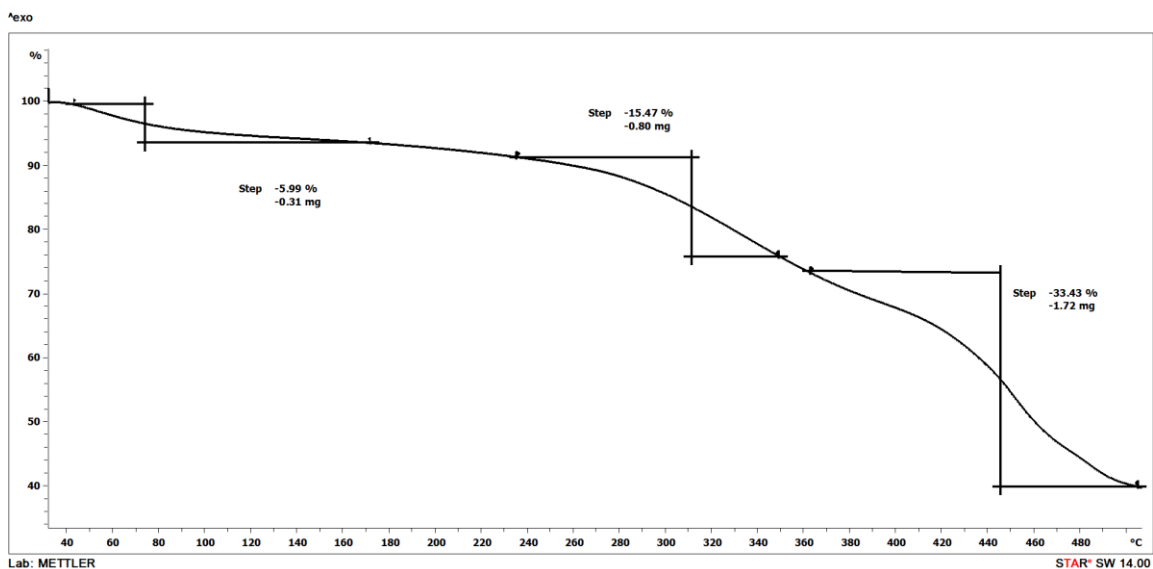


Fig. S22 TGA curve for **PCC-2** as synthesized (Temperature vs Mass percentage).

Section 7. UV-Vis spectra and fluorescent spectra of PCCs

The samples were prepared by dissolving the H_3L^1 and PCC-1 in DMF solution. Then put the solution in 1mL cell for UV-Vis and fluorescent experiments (Fig. S23 and S24).

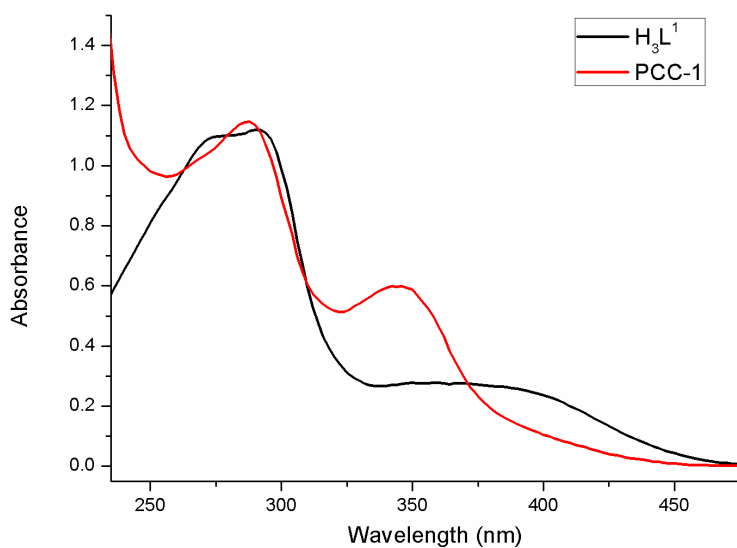


Fig. S23 UV-Vis spectrum of H_3L and PCC-1.

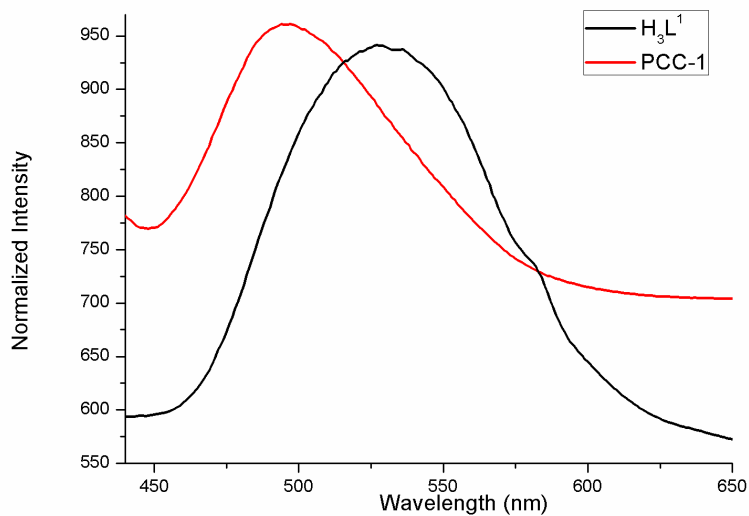


Fig. S24 Fluorescent spectrum of H_3L and PCC-1.

Section 8. Guest encapsulation of PCCs

Guest molecule selection

Three guest molecule were selected. The single crystal data of (+)-Camptocecin (CPT), Rhodamine B (RB) and Nile Red (NR) were downloaded from CCDC. The molecular dimension was based on their crystal structure. The molecular dimension were considered to be suitable for the cavity and pore opening of PCCs.

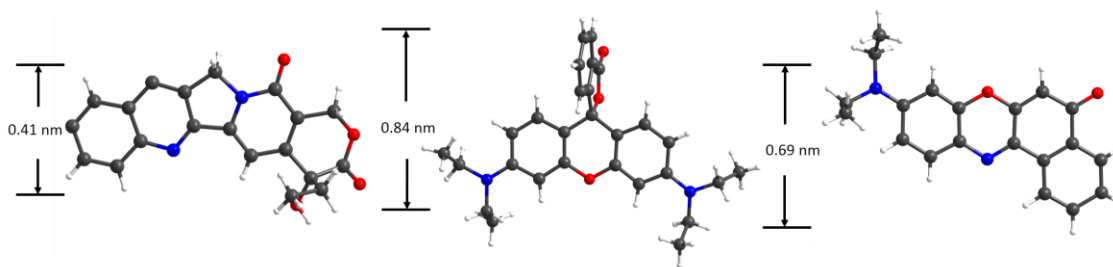


Fig. S25 Molecular dimension of crystal structure of guest molecules.

Formation of CPT@PCC-1

For investigating the size between the anticancer drug **CPT** and the cavity of **PCC-1**, we firstly run a molecular modeling. And we found that four molecules of **CPT** can be encapsulated within the cavity of **PCC-1**. Each drug molecule packed at one of the eight triangular ligand and the four drug molecules located orthogonal within the cavity of **PCC-1** (**Fig. S26**).

For **PCC-1**, MW = 11808.63, each gram of **PCC-1** is 8.47×10^{-5} mol. For **CPT**, MW = 348.35, so the maximum loading is $4 \times (8.47 \times 10^{-5}) \times 348.35 = 0.118$ g/g. The maximum loading is 400% mol/mol.

Typical drug encapsulation procedure is: 50 mg of activated single crystal of **PCC-1** was soaked in a solution of 6 mg of **CPT** in 10 mL Ethanol, and stirred at room temperature for 24 h. Then the supernatant was separated by centrifugation and analyzed by UV-Vis spectrum (**Fig. S27**). By comparing the initial and final concentration, the encapsulated

drug amount is 5.5 mg for 50 mg **PCC-1**. The loading amount is and ratio is 0.110 g/g (93% loaded). After encapsulation of **CPT**, the fluorescent spectrum of **CPT@PCC-1** doesn't change and show exactly the same as **Fig. S24**.

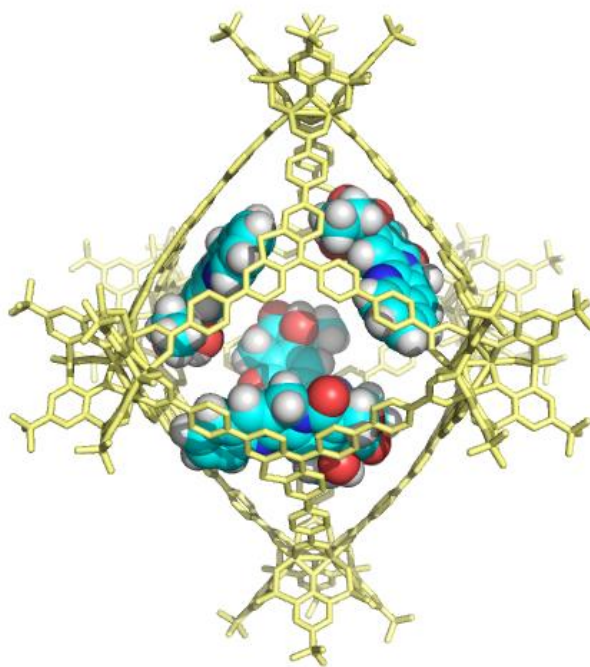


Fig. S26 Molecular modeling of **CPT@PCC-1**.

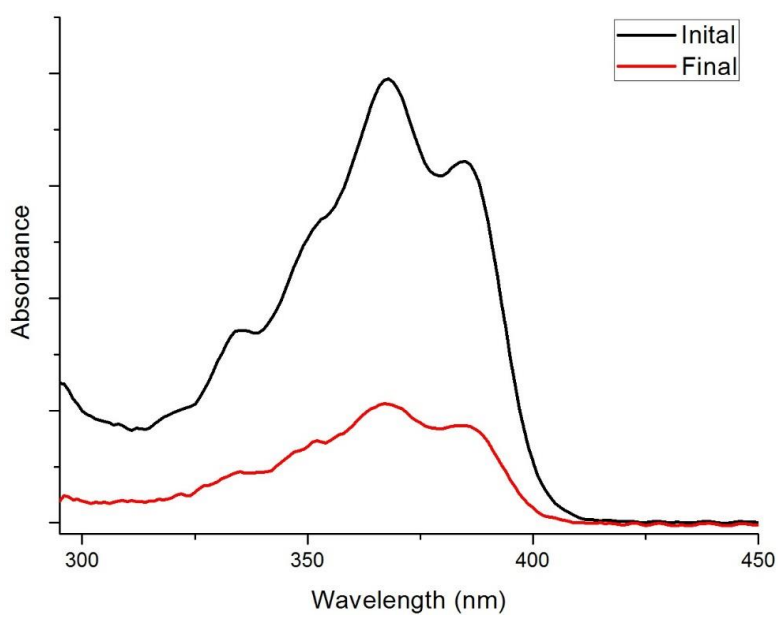


Fig. S27 UV-Vis spectrum of **CPT** solution before and after soaking with **PCC-1**.

After drug encapsulation, the drug included crystal **CPT@PCC-1** was washed by ethanol for three times and dried in air. The after washes crystal of **CPT@PCC-1** was also put into ethanol solution for another 24 h to check the guest leaching, but no signal was found. This crystal was then prepared for drug release experiment.

Formation of RB@PCC-2

For investigating the size between the fluorescent dye **RB** and the cavity of **PCC-2**, we firstly run a molecular modeling. And we found that two molecules of **RB** can be encapsulated within the cavity of **PCC-2**. Both drug molecule located orthogonal within the cavity of **PCC-2** (**Fig. S28**). For **PCC-2**, MW = 11833.78, each gram of **PCC-2** is 8.45×10^{-5} mol. For **RB**, MW = 479.02, so the maximum loading is $2 \times (8.45 \times 10^{-5}) \times 479.02 = 0.081$ g/g. The maximum loading is 200% mol/mol.

Typical dye encapsulation procedure for PCC-2 is: 5 mg of activated single crystal of PCC-2 was soaked in a solution of 2 mg of Rhodamine B (**RB**) in 10 mL Ethanol, and stirred at room temperature for 24 h. Then the supernatant was separated by centrifugation and analyzed by UV-Vis spectrum. By comparing the initial and final concentration, the encapsulated drug amount is 4.1 mg for 50 mg **PCC-1**. The loading amount is 3.8 mg and ratio is 0.076 g/g (94% loaded). (**Fig. S29**). After encapsulation of **RB** dye, the UV-Vis spectrum of **RB@PCC-2** was shown in **Fig. S30**. Comparing to free dye, the peak of **RB@PCC-2** blue shifted for 3.8 nm. After encapsulation of **RB** dye, the fluorescent

spectrum of **RB@PCC-2** was shown in **Fig. S31**, which has maximum emission peak at 594.1 nm.

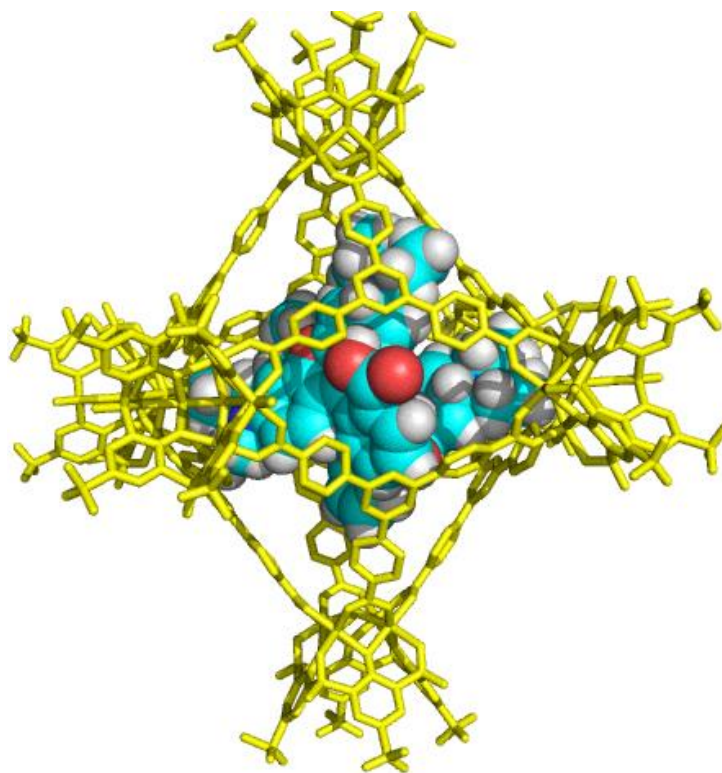


Fig. S28 Molecular modeling of **RB@PCC-2**.

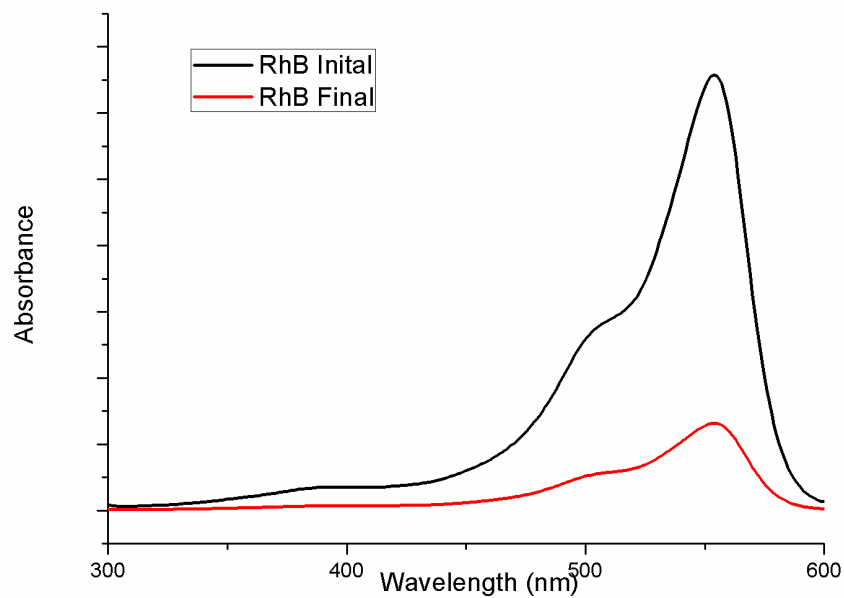


Fig. S29 UV-Vis spectrum of **RB** solution before and after soaking with **PCC-2**.

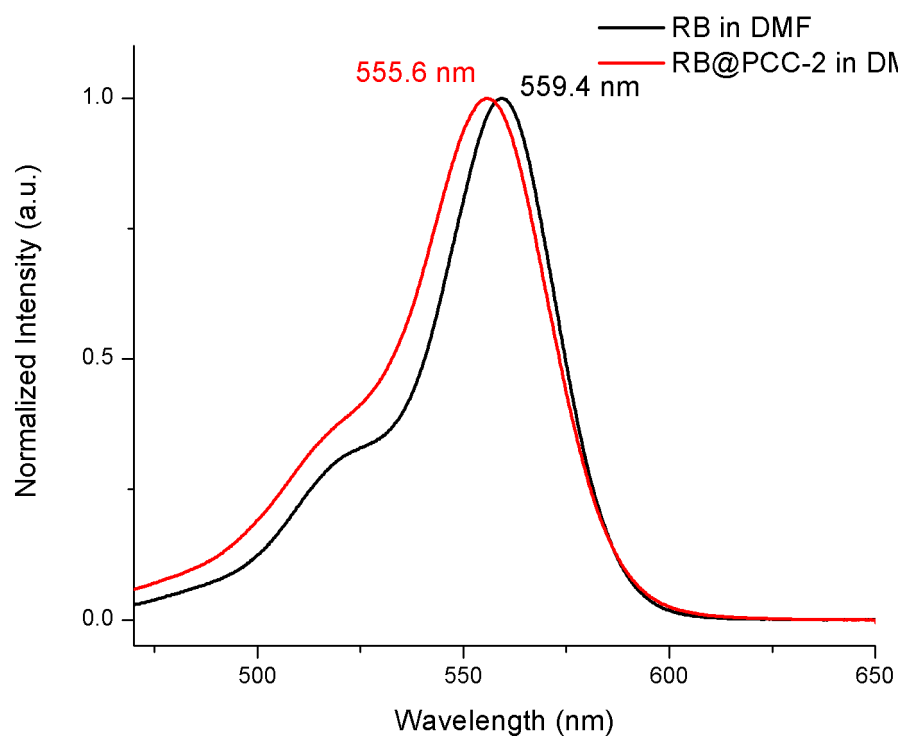


Fig. S30 UV-vis spectrum of **RB** and **RB@PCC-2**.

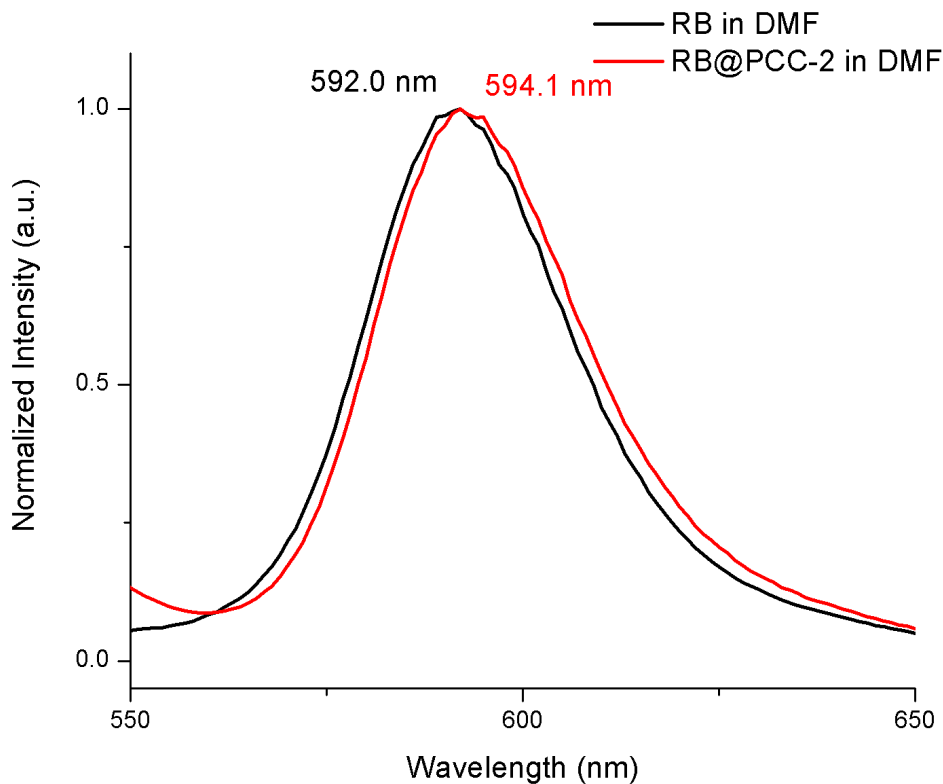


Fig. S31 Fluorescent spectrum of **RB** and **RB@PCC-2**.

Formation of **NR@PCC-3**

For **PCC-3**, MW = 3569.05, each gram of **PCC-2** is 2.80×10^{-4} mol. For **NR**, MW = 318.369, so the maximum loading is $2 \times (2.80 \times 10^{-4}) \times 318.369 = 0.178$ g/g. The maximum loading is 200% mol/mol.

Typical dye encapsulation procedure for **PCC-3** is: When a solid Nile Red (**NR**; 8.9 mg; insoluble in water) was suspended in a H₂O solution (50 mg, 10 mL) of **PCC-3** and the resulting solution was stirred at 80 °C for 1 h, the solution color turned from pale yellow to blue. After removal of residual **NR** by filtration, ¹H NMR confirmed the formation of dye-cage complex **NR@PCC-3** (**Fig. S32 & S33**). From the NMR integration, we found there are two **NR** molecules were encapsulated within the cavity of **PCC-3**. After

encapsulation of **NR** dye, the UV-Vis spectrum of **NR@PCC-3** was shown in **Fig. S34**. Comparing to free **NR** dye, the maximum absorption peak red shifted 46.0 nm. After encapsulation of **NR** dye, the fluorescent spectrum of **NR@PCC-3** was shown in **Fig. S35**, which gives a maximum emission peak at 656.0 nm.

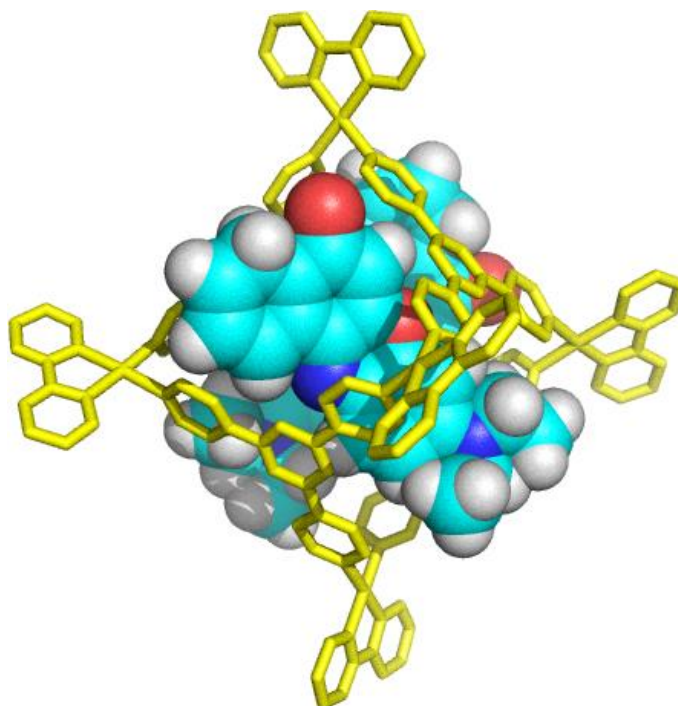


Fig. S32 Molecular modeling of **NR@PCC-3**.

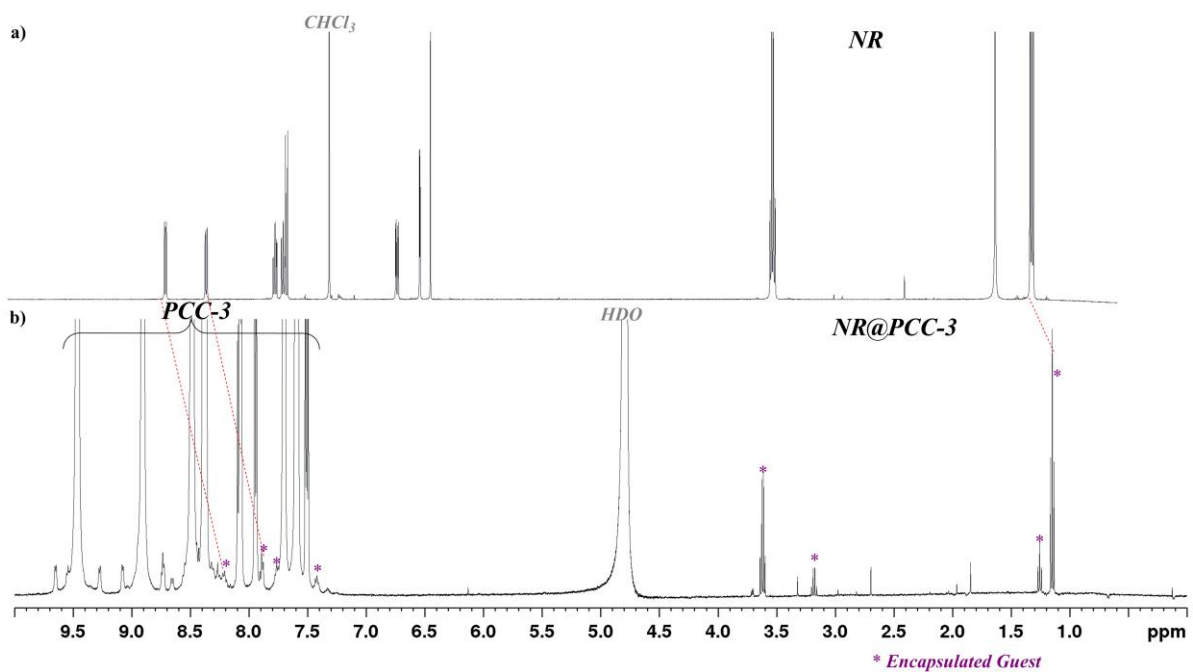


Fig. S33 ^1H NMR spectrum of a) NR in CDCl_3 and b) NR@PCC-3 in D_2O .

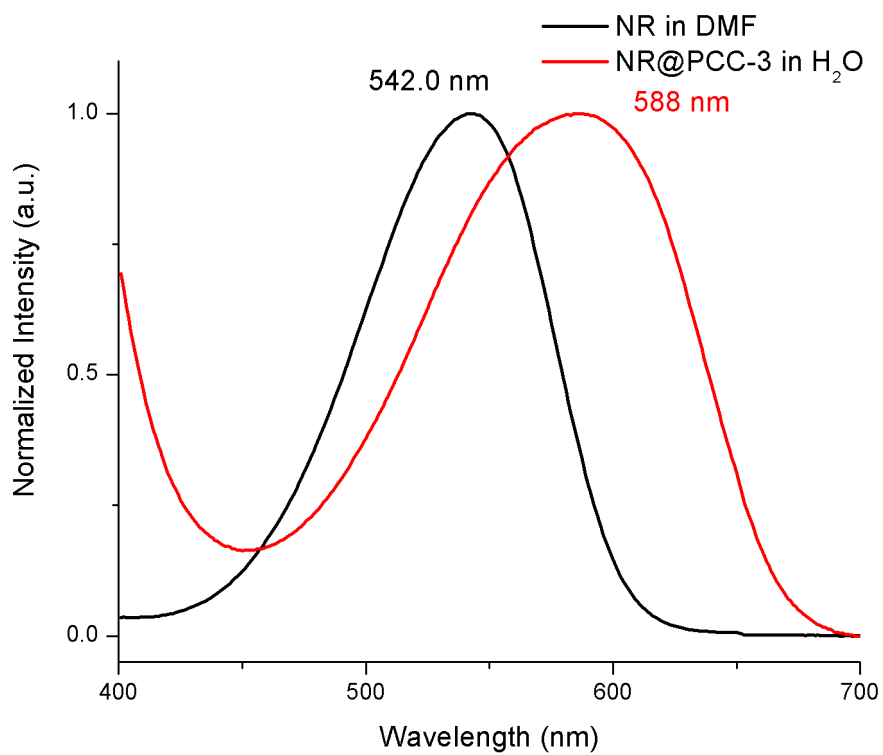


Fig. S34 UV-vis spectrum of NR and NR@PCC-3.

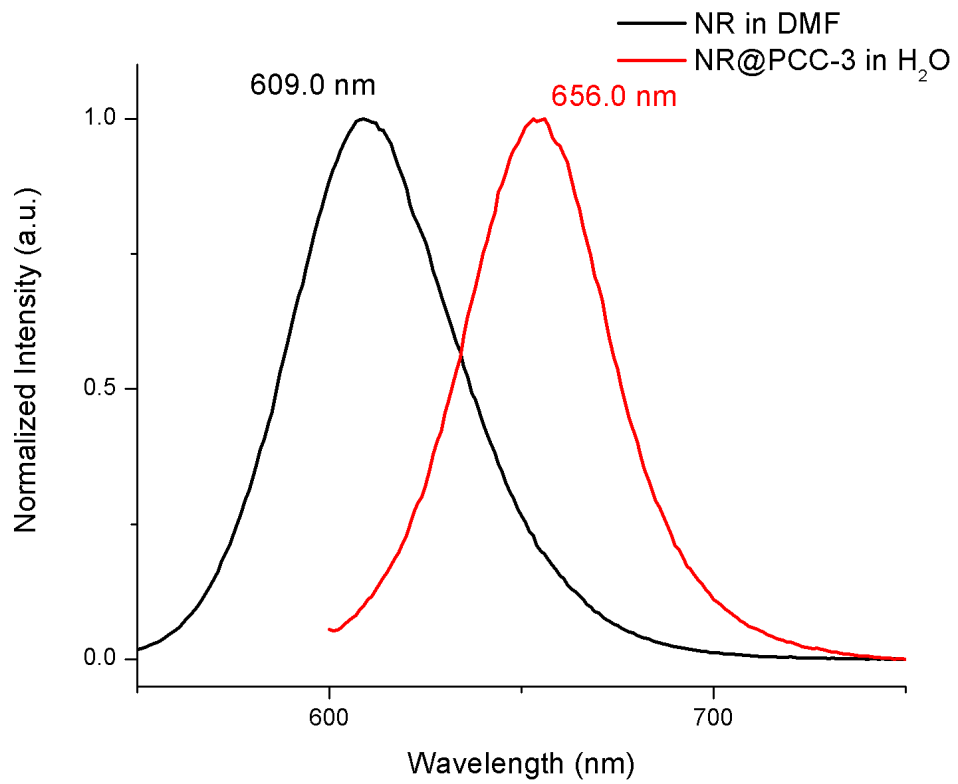


Fig. S35 Fluorescent spectrum of **RB** and **RB@PCC-2**.

Section 9. Cargo molecule release of cargo@PCC complex

A typical instant release system was prepared by suspending 10 mg of **CPT@PCC-1** material in 20.0 mL of buffer solution (pH 8.0, 7.4, 7.0, 6.5, 6.0, 5.5, and 5.0, respectively) at 37 °C. The release system was then maintained at 37 °C under shaking (shaking frequency = 150 rpm). One mL of release medium was sampled at each time point, and UV–Vis spectrophotometry was used to determine the percentage of cargo molecules that had been released, after which the sample was returned to the original release system. In a long time release experiment, 10 mg of **CPT@PCC-1** material was tested in a 20.0 mL of 10% (v/v) FBS buffer solution (pH 8.0, 7.4, 7.0, 6.5, 6.0, 5.5 and 5.0, respectively) at 37 °C for 48 h. The amount of cargo molecules loaded was determined from the UV–vis absorbance at 368 nm. The release percentages of cargo molecules were calculated according to the formula, release percentage (%) = (released guest) / (loaded guest). The UV-Vis spectra of accumulated release and delayed release were shown in **Fig. 36**.

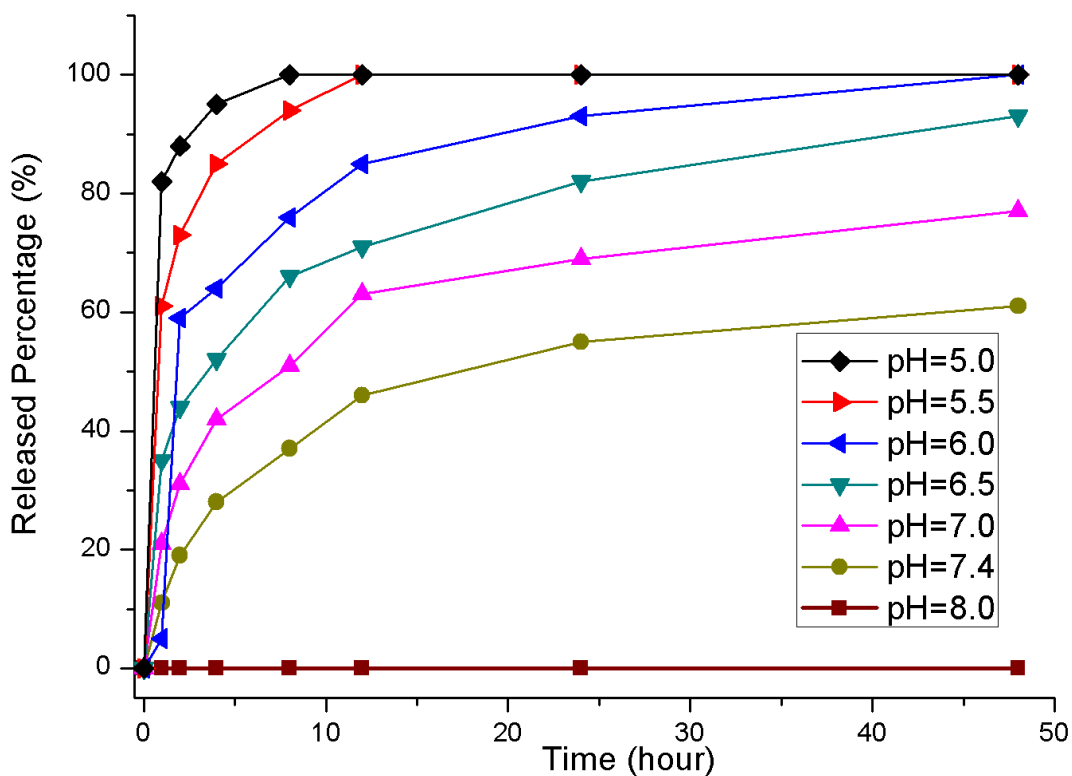


Fig. S36 Accumulated drug release percentage of **CPT@PCC-1** under different pH values.

The cargo release of **RB@PCC-2** and **NR@PCC-3** was conducted in similar ways. The release curve was shown in **Fig. 2f**.

Section 10. *In vitro* experiments

Human cell lines

Human breast cancer cell lines (Hela, Skov3, H1299 and HepG2) was purchased from the American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 µg/mL penicillin and 100 mg/mL streptomycin at 37 °C with 5% CO₂ in the atmosphere as conventional method. The medium was changed twice a week. The cells were harvested with trypsin.

Cell Uptake observed by confocal laser scanning microscopy (CLSM)

Cells were seeded and grown to 80-90% confluency in 1-2 days on a 48-well plate. The cells were incubated with free CPT, PCC-1, CPT@PCC-1, RB@PCC-2 and NR@PCC-3 (2.5 µg/mL Leibovitz's L-15 media that did not contain cysteine, nrL-15) for different period of time (typically 30 mins). Cells were washed with nrL-15 for twice. Images were collected using a Rolera-MGI Plus back-illuminated electronmultiplying charge-coupled device (EMCCD) camera (Qimaging). Images were acquired using bright-field imaging and a standard fluorescence filter sets: CFP (excitation (Ex) = 436 ± 10 nm/emission (Em) = 480 ± 20 nm. The fluorescence intensities of different cells were measured with the SlideBook 4.2 software (Olympus), and the average fluorescence intensity was determined for each condition.

Permeabilized cell cellular transportation observed by Epi-fluorescent microscopy (EFM)

For microscopy, flow cells were transferred to an 8-well glass plate. Cells were washed with Leibovitz's L-15 media that did not contain cysteine, nrL-15, permeabilized for 2 min with 40 µg/mL digitonin in the buffer, and washed again with the buffer twice. A cover slip was added immediately on the top of the pad, and the obtained slide was analyzed by microscopy using a Delta-Vision microscope. Typical time-lapse movies were shot for 5 min with frames captured every 2 s. For fluorescence microscopy, the images were captured with the FITC-filter or Rhodamine-filter. To avoid blue light toxicity, the time of illumination was limited to 0.5 s in the presence of a 50% neutral density filter. The fluorescent intensity of nuclear and cytoplasm was measured and recorded by PALM, followed by making figures by using Image J software. Movies were obtained by processing the series of images collected with the Quick Pro Time software (See Video 1-3).

Cytotoxicity Assays (Sytox Green assay)

The cells were seeded in 96 well plates at a density of about 2000 cells/mL and cultured for 24 hours. CPT, PCC-1 and CPT@PCC-1 (2.5, 5.0, 10.0 and 15.0 µg/mL DMEM solution) were incubated with cells for 24 hours. Then the media was removed and cells were washed with PBS for twice. Then, the cells were soaked in nrL-15 and treated with dyes.

Cell nuclei were stained with Hoechst 33342 for 15 min and dead cell nuclei were stained with SYTOX Green for 15 min. Images were collected using a Rolera-MGI Plus back-illuminated electronmultiplying charge-coupled device (EMCCD) camera (Qimaging). Images were acquired using bright-field imaging and two standard fluorescence filter sets: DAPI (excitation (Ex) = 360 ± 10 nm/emission (Em) = 460 ± 20

nm); FITC (Ex = 488 ± 10 nm/Em = 520 ± 20 nm). Blue and green images were collected with all samples. ImageJ was used to count the number of blue and green cells. Multiple times of tests were done to test repeatability.

Data and error bars of statistical analysis for uptake and cytotoxicity studies represent the mean \pm SEM of at least 5 independent experiments. Statistical analyses for comparison of free drug (CPT), PCC-1 and CPT@PCC-1 were carried out using Student's t-test. Results with $P < 0.05$ were considered significantly different and $P < 0.01$ were considered extreme significantly different. Curve fitting of the cytotoxicity data was performed using Graph Pad Prism 6.0. Best nonlinear regression curve fittings were chosen by evaluating R², sum of square, and the standard deviation of residuals.

After respective treatment of free drug CPT, PCC-1 and CPT@PCC-1, the cancer cells showed obvious differences in cell amounts, morphology and fluorescence behavior. For CPT@PCC-1 group, the amounts of HeLa cells are dramatically reduced compared with those treated with CPT, PCC-1. This suggests the high cytotoxicity of CPT@PCC-1 towards HeLa cells, and the moderate toxicity of CPT, PCC-1. Cancer cells remained high viability and proliferation ability after treatment of CPT, PCC-1. Benefiting from the intranuclear delivery ability of CPT@PCC-1, cancer cells were effectively killed during incubation, and most of the dead cells washed out during washing steps. As a result, only a few cells remained in the wells. Moreover, it is also noticeable that even the remained cells showed apoptotic morphology such as round-up, shrinking and detached (bright field images of **Fig. 4**).

After dye staining, the amount of the viable cells was counted statistically. As shown in the fluorescence images of **Fig. 4**, it is clear that cell viability was significantly different

for control groups and CPT@PCC-1 group. Significant more green fluorescent cells were observed for CPT@PCC-1 group than control groups, indicating these cancer cells were killed by CPT@PCC-1. These results indicated that CPT@PCC-1 showed much enhanced toxicity to the tested cells, comparing to free drug CPT and empty PCC-1 drug carrier.

Cytotoxicity Assays (MTT assay)

The cells were seeded in 96 well plates at a density of about 2000 cells/mL and cultured for 24 hours. CPT, PCC-1 and CPT@PCC-1 (2.5, 5.0, 10.0 and 15.0 µg/mL DMEM solution) were prepared in cultured medium and added to the wells. After incubation for another 24 hours at 37 °C with 5% CO₂, cell viability was determined by MTT assay.

The viability of different cells was evaluated by the standard MTT assay (**Fig. S39**). The culture medium was carefully removed, and 100 µL of freshly prepared MTT solution (2.5, 5.0, 10.0 and 15.0 µg/mL in culture medium) was added to each well. After incubation at 37 °C for 4 hours, the MTT solution was removed, and 100 µL of DMSO was added to dissolve the formazan crystals. The plate was shaken for 10 min to fully dissolve formazan and homogenize. Absorbance values of the wells were read with a microplate reader at 492 nm (BIO RAD, iMark). The cell viability rate (VR) was calculated from the following equation: $VR = A/A_0 \times 100\%$, where A is the absorbance of the experimental group and A₀ is the absorbance from the cells cultured in serum-supplemented medium without any treatment. All data were obtained from three repeatedly parallel experiments.

Statistical analyses for comparison of free drug (CPT), PCC-1 and CPT@PCC-1 were carried out using Student's t-test (**Table S4**). When *P*-values were 0.05 or less, differences were considered statistically significant.

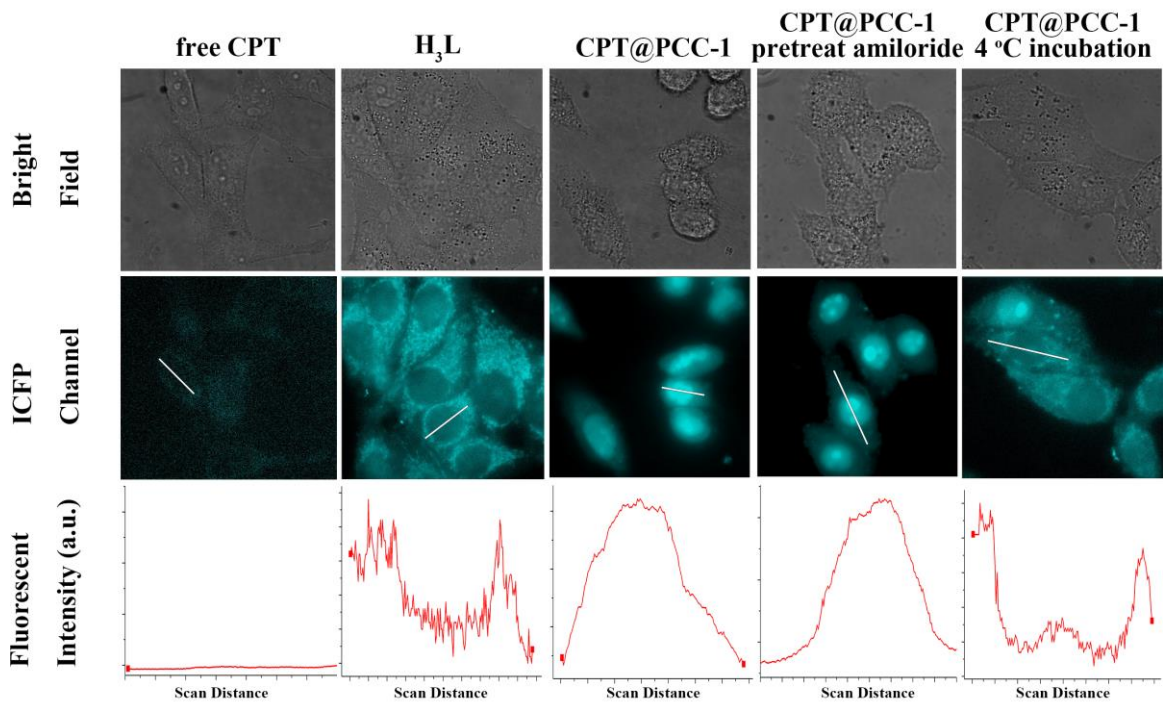


Fig. S37 CLSM images of free CPT, H₃L, CPT@PCC-1 pretreated with amiloride, CPT@PCC-1 incubated at 4 °C with HeLa cells for 6 h at a concentration of 10 $\mu\text{g mL}^{-1}$.

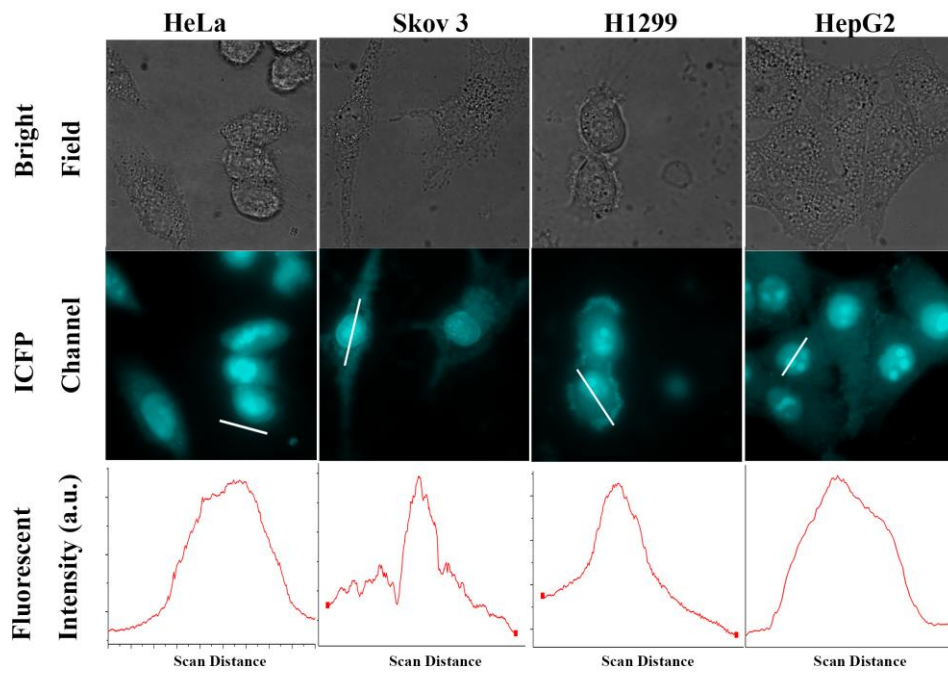


Fig. S38 CLSM images of CPT@PCC-1 incubated at 25 °C with HeLa, Skov 3, H1299, and HepG2 cells for 6 h at a concentration of 10 $\mu\text{g mL}^{-1}$.

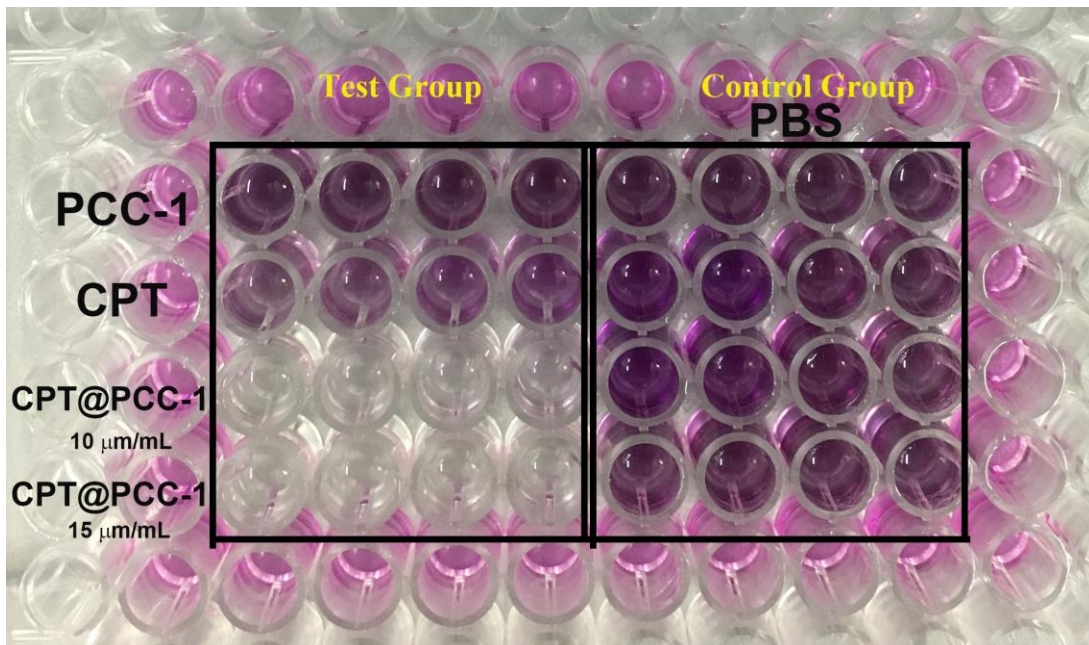


Fig. S39 MTT assay images of PBS, CPT, PCC-1 and CPT@PCC-1 incubated at 25 °C with HeLa cells for 6 h at a concentration of 10 $\mu\text{g mL}^{-1}$. (Purple color indicate the remaining living HeLa cell).

Table S4. MTT assay of cell viability for PCC-1, CPT and CPT@PCC-1 (15.0 $\mu\text{g mL}^{-1}$)

HeLa-PCC-1

Temperature ($^{\circ}\text{C}$)

24.5

490 nm

									Cell viability	error bar
Control	0.8365	0.832	0.8311	0.8397	0.834825				83.5%	0.6%
CPT	0.4492	0.4209	0.4388	0.435	0.538077	0.504178	0.525619	0.521067	52.2%	1.4%
CPT@PCC 10.0	0.0807	0.0797	0.0854	0.0723	0.096667	0.095469	0.102297	0.086605	9.5%	0.6%
CPT@PCC 15.0	0.0723	0.0783	0.0787	0.0764	0.086605	0.093792	0.094271	0.091516	9.2%	0.4%

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