Supplementary Information

Ultralong Purely Organic Aqueous Phosphorescence Supramolecular Polymer for Targeted Tumor Cell Imaging

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Supplementary Discussion

Synthesis of BrBP–NH_2.

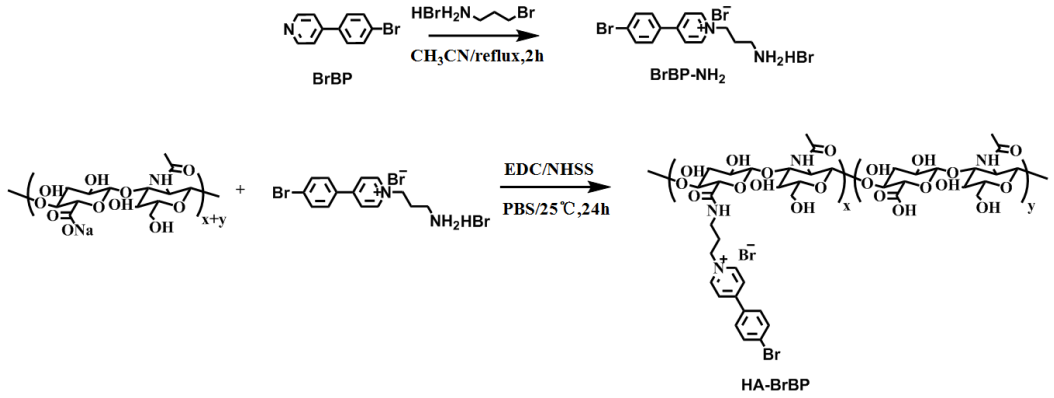
3–Bromopropan–1–amine hydrobromide (0.23 g, 1.00 mmol) was added to a solution of 4–(4–bromophenyl)pyridine (0.47 g, 2.00 mmol) in acetonitrile (50 ml). The solution was heated under reflux for 2 h, during which time a large amount of precipitate formed. The reaction mixture was allowed to cool to room temperature and then filtered, and the obtained solid was washed thoroughly with acetonitrile to afford BrBP–NH_2 as a pale yellow solid (0.15 g, 67%). ^{1}H NMR (400 MHz, D_2O, 25 °C) δ 8.88 (d, *J* = 7.0 Hz, 2H), 8.35 (d, *J* = 6.9 Hz, 2H), 7.88–7.82 (m, 4H), 4.75–4.71 (m, 2H), 3.20–3.14 (m, 2H), 2.53–2.40 (m, 2H). ^{13}C NMR (100 MHz, D_2O, 25 °C) δ 156.09, 144.22, 132.78, 131.35, 129.66, 126.69, 125.21, 57.66, 36.17, 28.32.

HRMS (ESI) for $C_{14}H_{16}Br_2N_2$: calcd. $[M–HBr–Br]^+$: 291.05, found: 291.05.

Synthesis of HA–BrBP.

1–Ethyl–3–(3–dimethylaminopropyl)carbodiimide (1.68 g, 0.875 mmol) and *N*–hydroxysulfosuccinimide sodium salt (1.9 g, 0.875 mmol) were added to a solution of sodium hyaluronate (*Mw* = 250,000, 1.00 g, 0.53 μmol) in phosphate buffered saline (0.1 M, pH 7.2, 30 mL). The mixture was stirred at 25 ℃ for 30 min, and then BrBP–NH_2 (1.14 g, 0.25 mmol) in phosphate buffered saline (10 mL) was added. After stirring at room temperature for 24 h, the solution was dialyzed against excess deionized water for 5 days. Freeze–drying afforded HA–BrBP as a white powder. ^{1}H NMR (400 MHz, D_2O, TMS) δ 1.98 (s, 3 H, H of methyl group of HA), 2.84–4.60 (m, H of HA and the alkyl chain of BrBP–NH_2), 8.84, 8.32, 7.81 (H of the benzene ring of BrBP–NH_2). Using the single–point method and the integrated peak area of the benzene ring and the HA backbone in the NMR spectrum, we determined the degree of substitution to be 3.5%.

a



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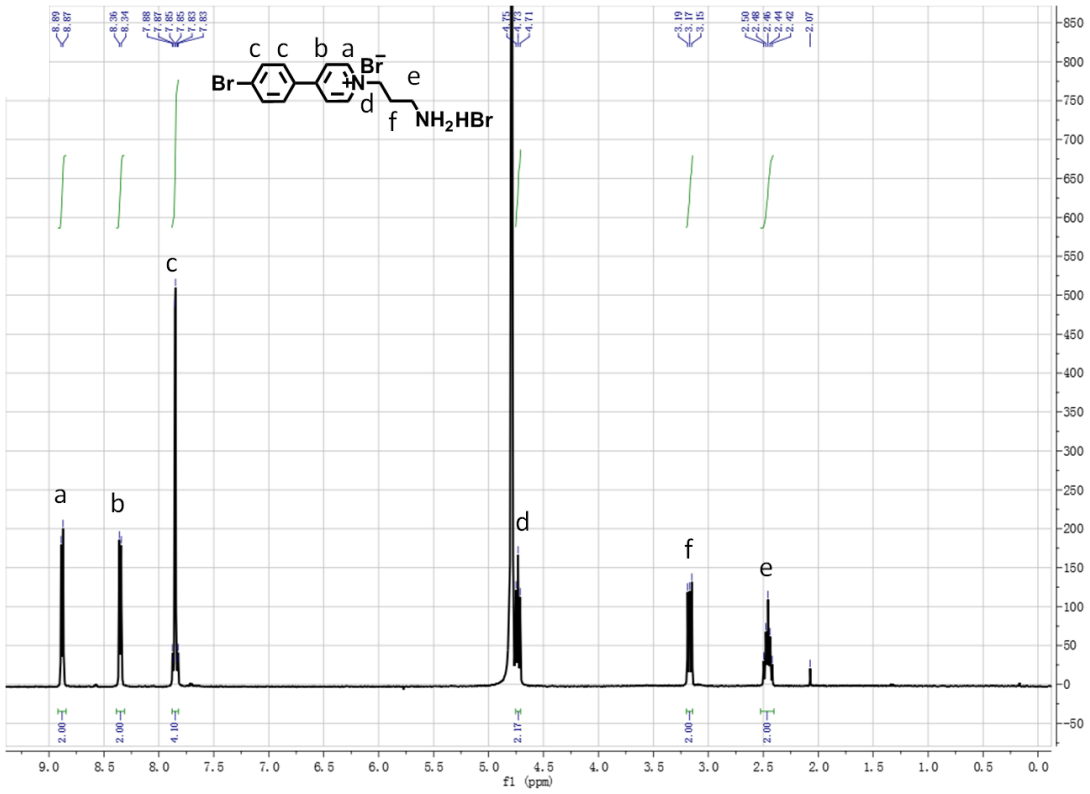
c c b a
Br
Br⁻
N⁺
e
d
f
NH2HBr
a
b
c
d
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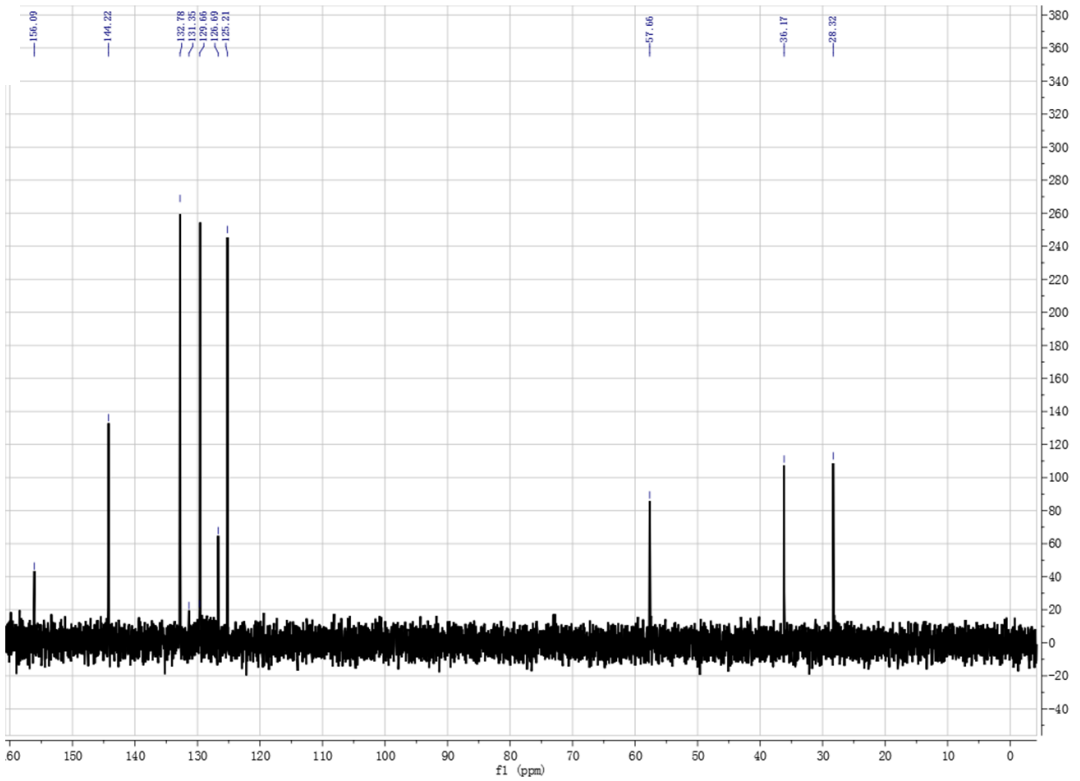
f
f1 (ppm)

c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129.66
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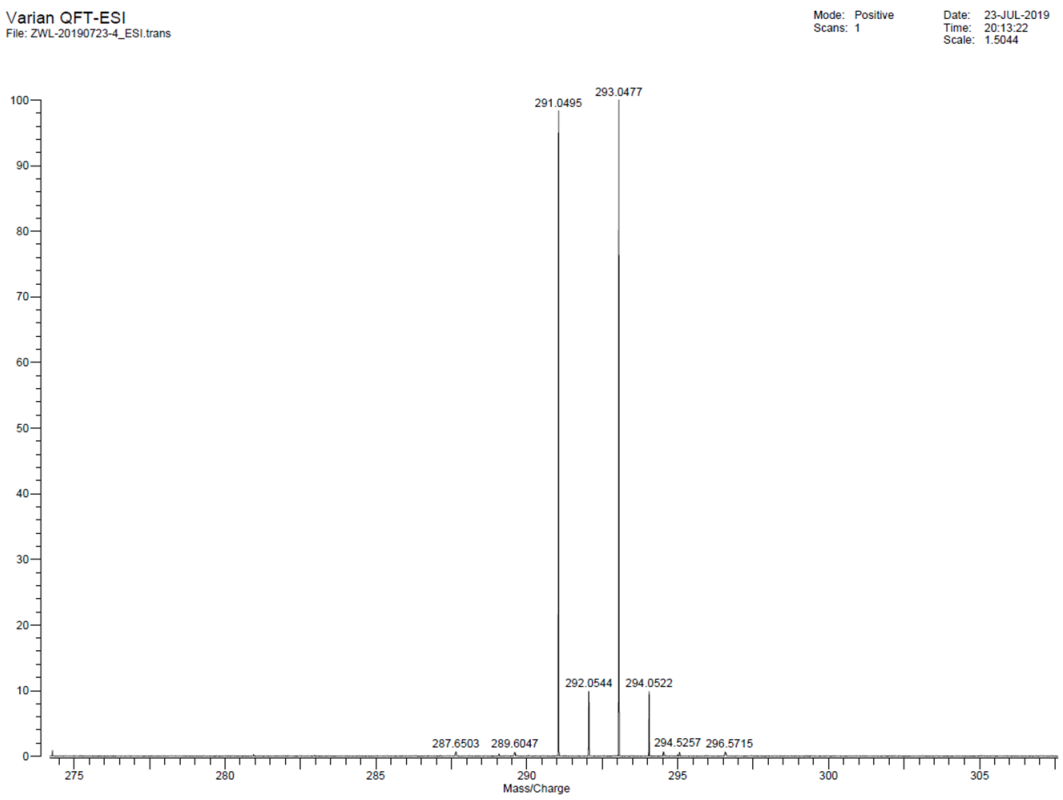
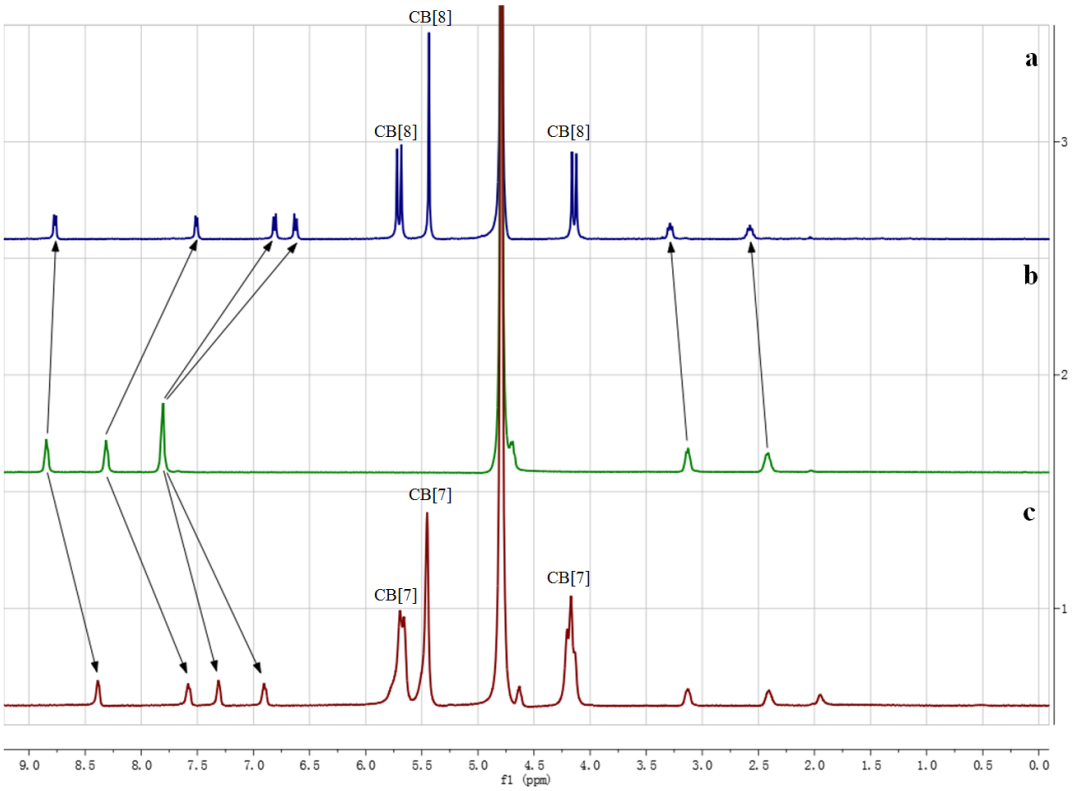
36.17
28.32
f1 (ppm)

d

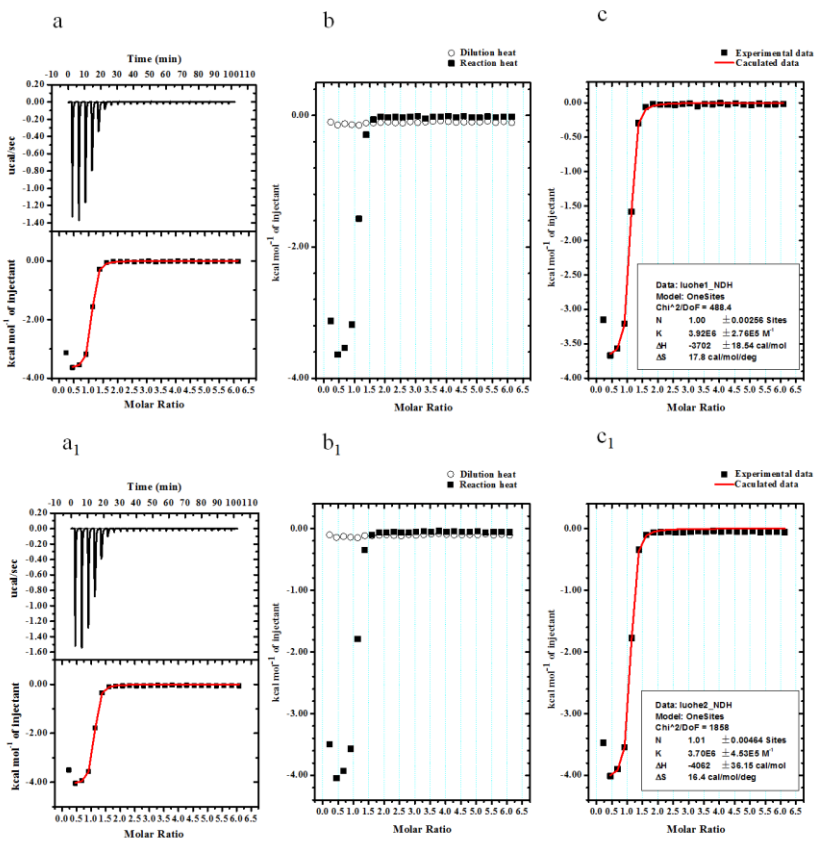


Supplementary Figure 1. Characterization of BrBP–NH_2. (**a**) Synthetic route of BrBP–NH_2; (**b**) ^{1}H NMR spectrum of BrBP–NH_2 (400 MHz, D_2O, 25 ℃); (**c**) ^{13}C NMR spectrum of BrBP–NH_2 (100 MHz, DMSO–d_6, 25 ℃); (**d**) ESI mass spectrum of BrBP–NH_2.



Supplementary Figure 2. ^{1}H NMR spectra of CB[8]/BrBP–NH_2, BrBP–NH_2, and CB[7]/BrBP–NH_2. ^{1}H NMR spectrum (D_2O, 400 MHz, 298 K) of (**a**) CB[8]/BrBP–NH_2 complex, (**b**) BrBP–NH_2 and (**c**) CB[7]/BrBP–NH_2 complex.

A


a
b
c
Time (min)
ucal/sec
kcal mol^{-1} of injectant
Molar Ratio
Dilution heat
Reaction heat
Experimental data
Caculated data
Data: luohe1_NDH
Model: OneSites
Chi^2/DoF = 488.4
N 1.00 ±0.00256 Sites
K 3.92E6 ±2.76E5 M^{-1}
ΔH -3702 ±18.54 cal/mol
ΔS 17.8 cal/mol/deg
a$_1$
b$_1$
c$_1$
Data: luohe2_NDH
Model: OneSites
Chi^2/DoF = 1858
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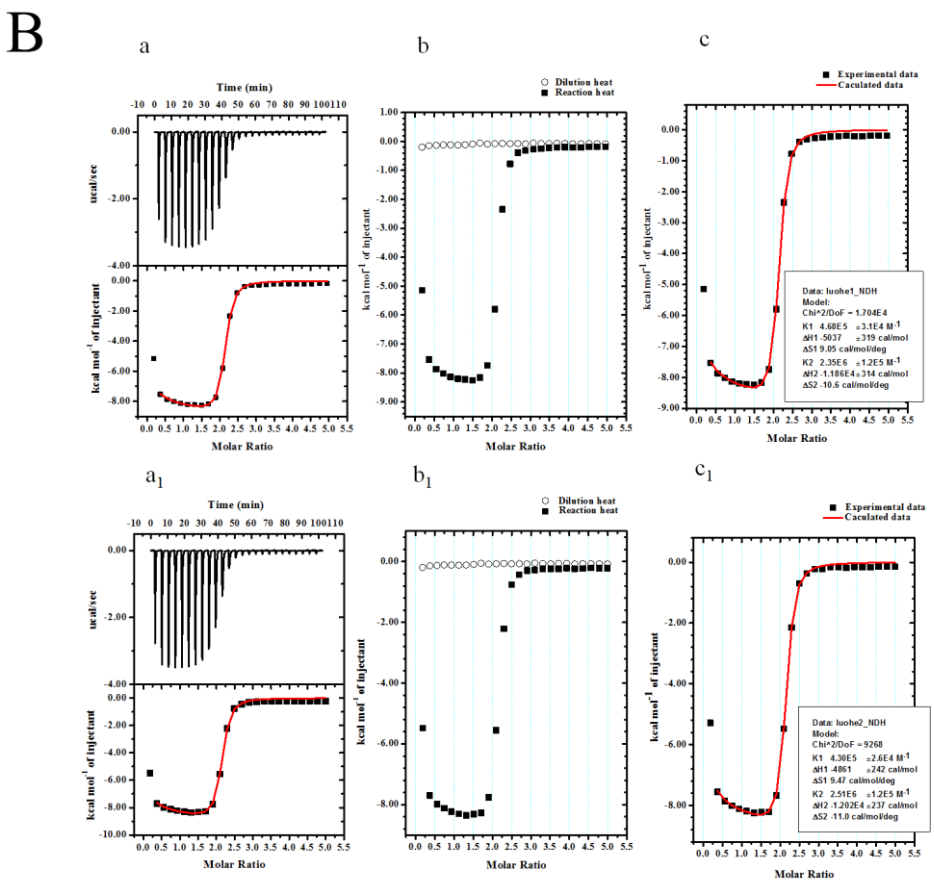
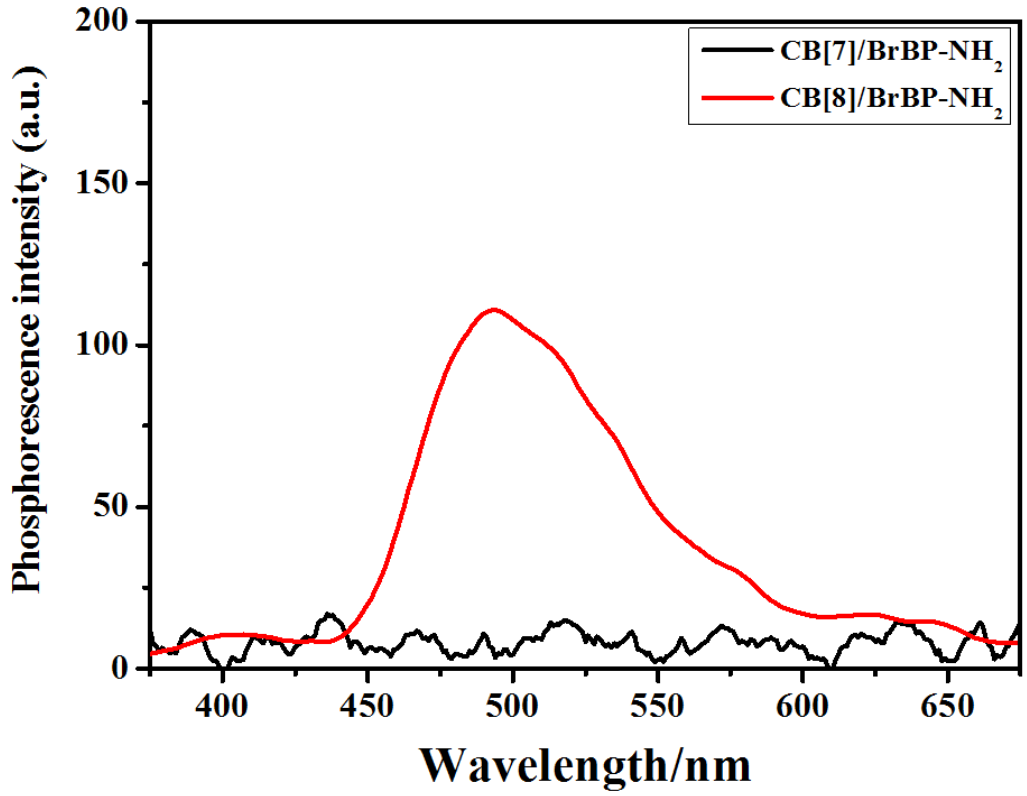
ΔH -4062 ±36.15 cal/mol
ΔS 16.4 cal/mol/deg

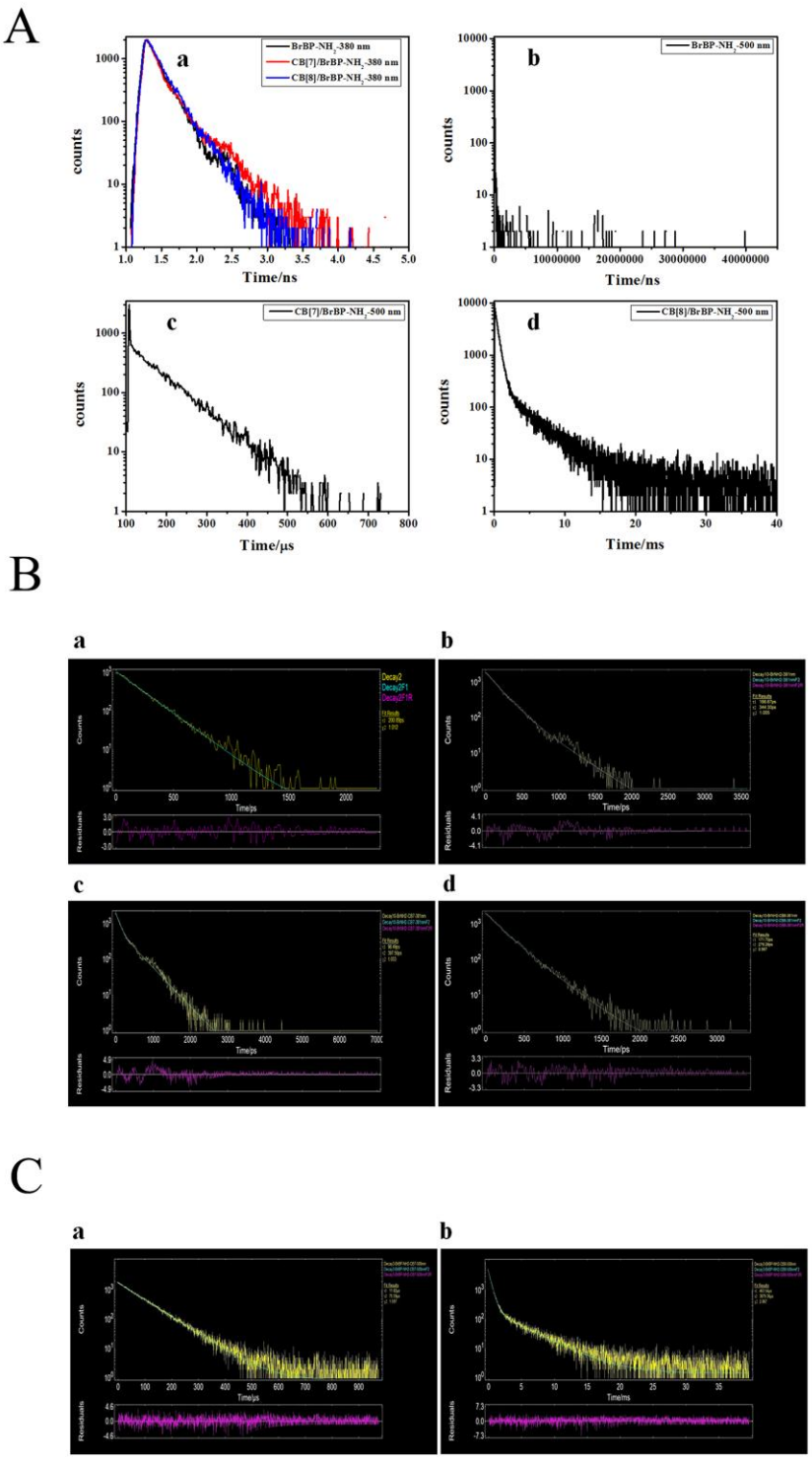


Supplementary Figure 3. ITC data for CB[7]/BrBP–NH_2 and CB[8]/BrBP–NH_2 complexation. (**A**) Calorimetric titrations in aqueous solution for sequential 25 injections (10 μL per injection) of BrBP–NH_2 solution (1.25 mM) injecting into CB[7] solution (0.048 mM): (a) raw data and apparent reaction heat; (b) heat effects of the dilution and of the complexation reaction; (c) "Net" heat effects fitted using the "one set of binding sites" model. The thermodynamic data in CB[7]/BrBP–NH_2 complexation were obtained as K_S = (3.81 ± 0.22) × 10^6 M^{-1}, $-\Delta H$ = (1.63 ± 0.15) × 10^4 J·mol^{-1}, $T\Delta S$ = (2.13 ± 0.18) × 10^4 J·mol^{-1}, and $-\Delta G$ = (3.76 ± 0.01) × 10^4 J·mol^{-1}, respectively; (**B**) Calorimetric titrations in aqueous solution for sequential 25 injections (10 μL per injection) of BrBP–NH_2 solution (1.28 mM) injecting into CB[8] solution (0.04 mM): (a) raw data and apparent reaction heat; (b) heat effects of the

dilution and of the complexation reaction; (c) "Net" heat effects fitted using the "1:2 sequential binding sites" model. The thermodynamic data in CB[8]/BrBP–NH_2 complexation were obtained as $K_{S,1}$ = (4.49 ± 0.19) × 10^5 M^{-1} , $K_{S,2}$ = (2.43 ± 0.08) × 10^6 M^{-1} , –ΔH = (7.07 ± 0.003) × 10^4 $J{\cdot}mol^{-1}$, TΔS = (1.92 ± 0.01) × 10^3 $J{\cdot}mol^{-1}$, and – ΔG = (6.87 ± 0.005) × 10^4 $J{\cdot}mol^{-1}$, respectively.

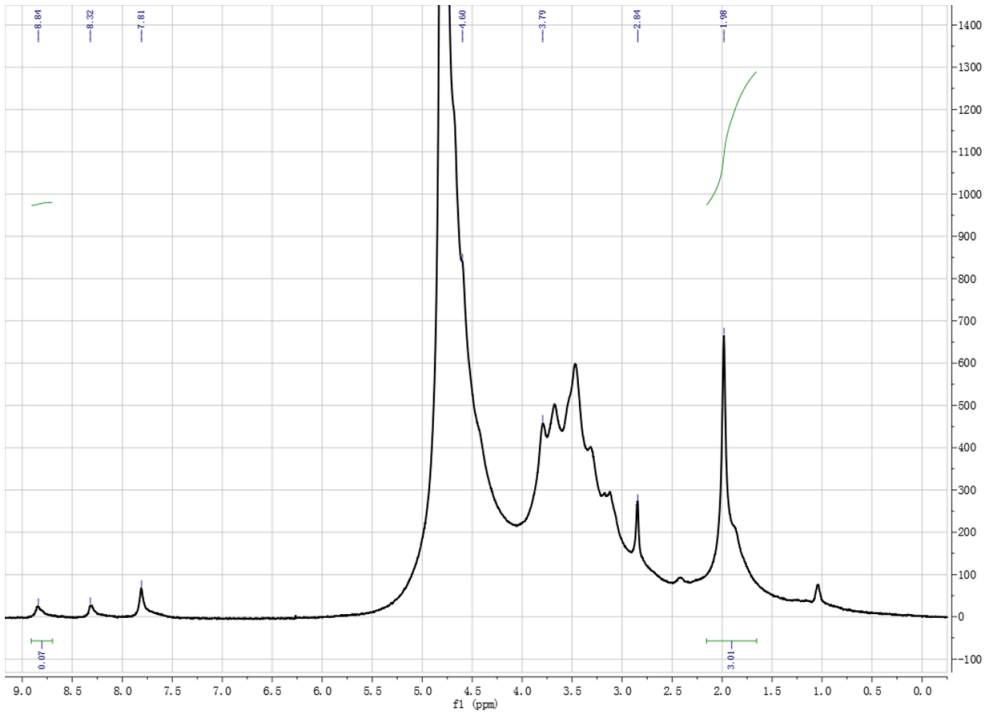


Supplementary Figure 4. Phosphorescence contrast spectra (delayed 0.2 ms) of CB[7]/BrBP–NH_2 and CB[8]/BrBP–NH_2. The phosphorescence spectra (delayed by 0.2 ms) of CB[7]/BrBP–NH_2 (black) and CB[8]/BrBP–NH_2 (red) (BrBP–NH_2 = 0.5 mM, CB[7] = 0.5 mM, CB[8] = 0.25 mM) in water (25 ℃, λ_{ex} = 320 nm) (Ex. Slit = 5 nm, Em. Slit = 5 nm).

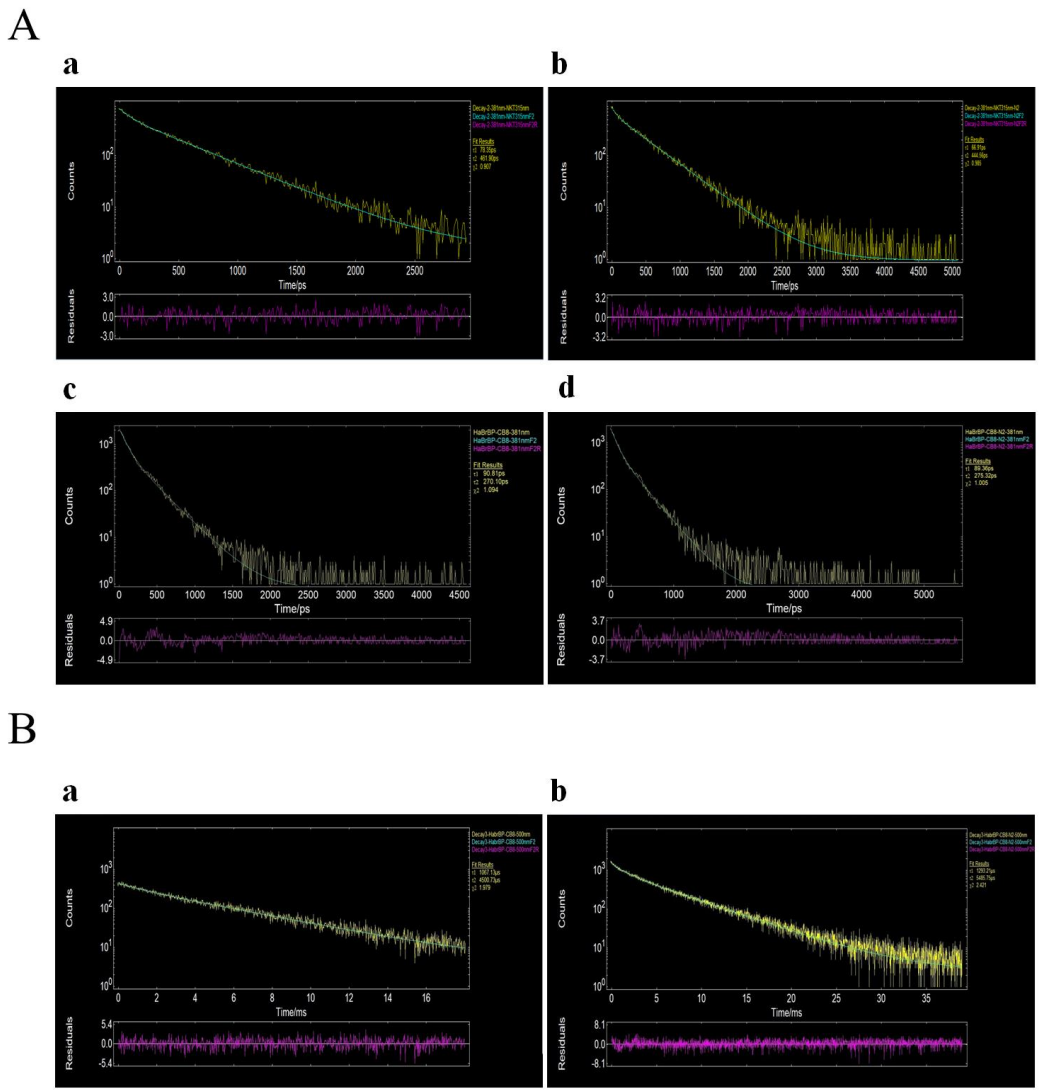


Supplementary Figure 5. Fluorescence and phosphorescence lifetime contrast curves for CB[7]/BrBP–NH_2 and CB[8]/BrBP–NH_2. (**A**) (a) Fluorescence decay curves of BrBP–NH_2, CB[7]/BrBP–NH_2 and CB[8]/BrBP–NH_2 at 380 nm at 298 K. Phosphorescence decay curves

of (b) BrBP–NH_2; (c) CB[7]/BrBP–NH_2 and (d) CB[8]/BrBP–NH_2 at 500 nm at 298 K. (BrBP–NH_2 = 0.5 mM, CB[7] = 0.5 mM, CB[8] = 0.25 mM); (**B**) Fluorescence lifetime decay fitting curve of (a) HA–BrBP (b) BrBP–NH_2, (c) CB[7]/BrBP–NH_2 and (d) CB[8]/BrBP–NH_2 measured for 380 nm at 298 K. (BrBP–NH_2 = 0.5 mM, CB[7] = 0.5 mM, CB[8]=0.25 mM); (**C**) Phosphorescence lifetime decay fitting curve of (a) CB[7]/BrBP–NH_2 (b) CB[8]/BrBP–NH_2 measured for 500 nm at 298 K**.** (BrBP–NH_2 = 0.5 mM, CB[7] = 0.5 mM, CB[8] = 0.25 mM).



Supplementary Figure 6. ^{1}H NMR spectrum of HA–BrBP. ^{1}H NMR spectrum of HA–BrBP in D_2O at 298 K.



Supplementary Figure 7. Fluorescence and phosphorescence lifetime decay fitting curves for CB[7]/HA–BrBP and CB[8]/HA–BrBP. (**A**) Fluorescence lifetime decay fitting curve of (a) CB[7]/HA–BrBP, (b) CB[7]/HA–BrBP/N_2, (c) CB[8]/HA–BrBP and (d) CB[8]/HA–BrBP/N_2 measured for 380 nm at 298 K. ([BrBP] = 0.5 mM, [CB7] = 0.5 mM, [CB8] = 0.25 mM); (**B**) Phosphorescence lifetime decay fitting curve of (a) CB[8]/HA–BrBP (b) CB[8]/HA–BrBP/N_2 measured for 500 nm at 298 K. ([BrBP] = 0.5 mM, [CB8] = 0.25 mM).

Supplementary Table 1. RTP lifetimes reported in the literature.

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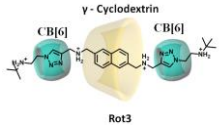
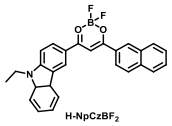
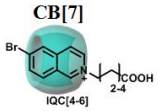
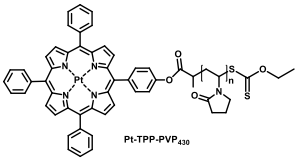
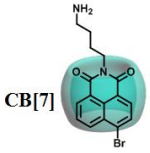
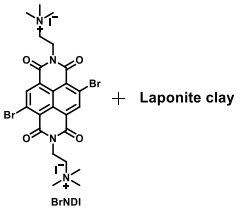
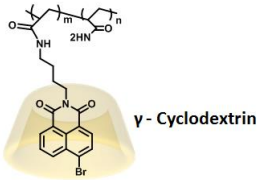
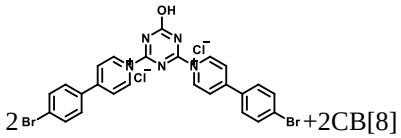
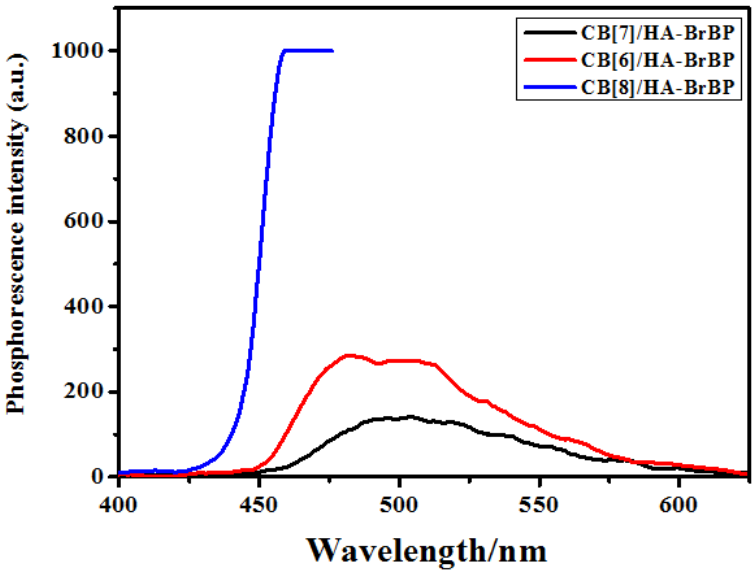
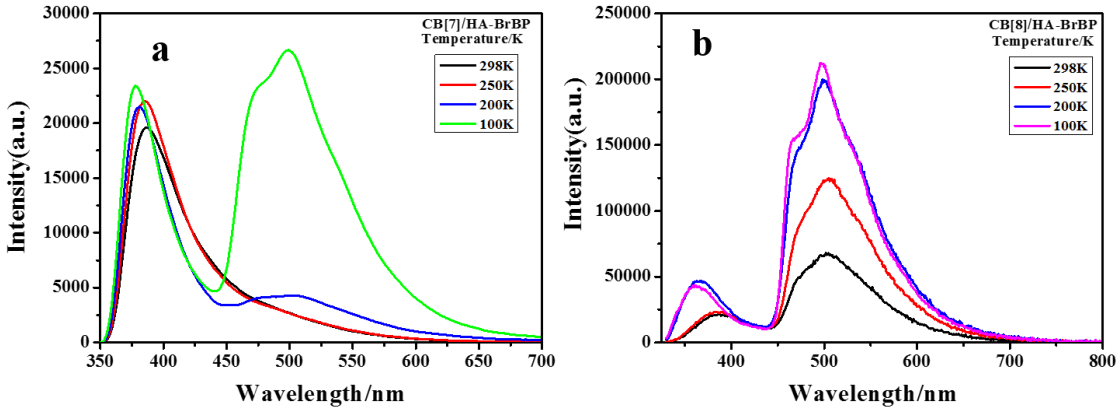
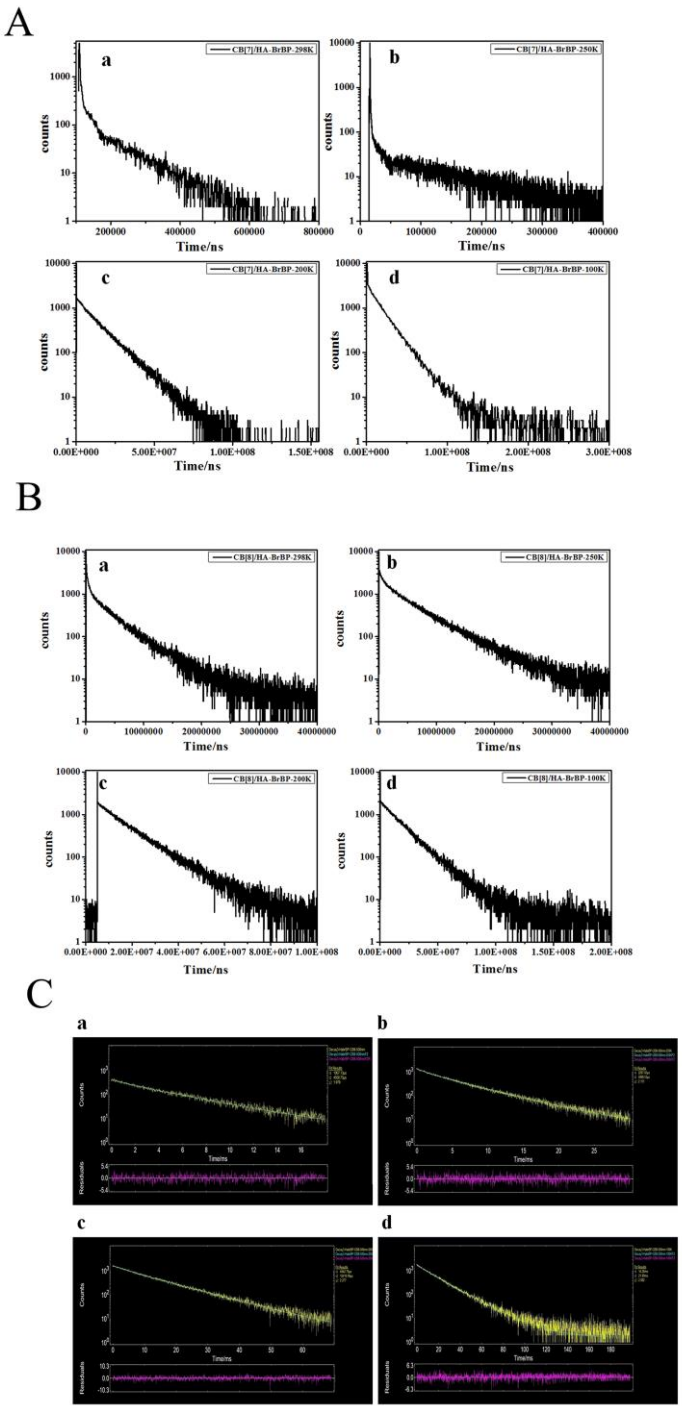
	2.5 ms	Ref.12
	29.0 μs	Ref.24
	127.38 μs	Ref.28
	7.96 μs	Ref.22
	400 μs	Ref.30
	385 μs	Ref.6
	320 μs	Ref.29
	190 μs	Ref.31



Supplementary Figure 8. Effect of complexation with CB[6], CB[7] and CB[8] on the spectra of HA–BrBP. The phosphorescence spectra of CB[6]/HA–BrBP, CB[7]/HA–BrBP and CB[8]/HA–BrBP at 298 K in aqueous solution (delayed by 0.2 ms, Ex. Slit = 10 nm, Em. Slit = 10 nm). ([BrBP] = 0.5 mM, [CB[6]] = 0.5 mM, [CB[7]] = 0.5 mM, [CB[8]] = 0.25 mM).

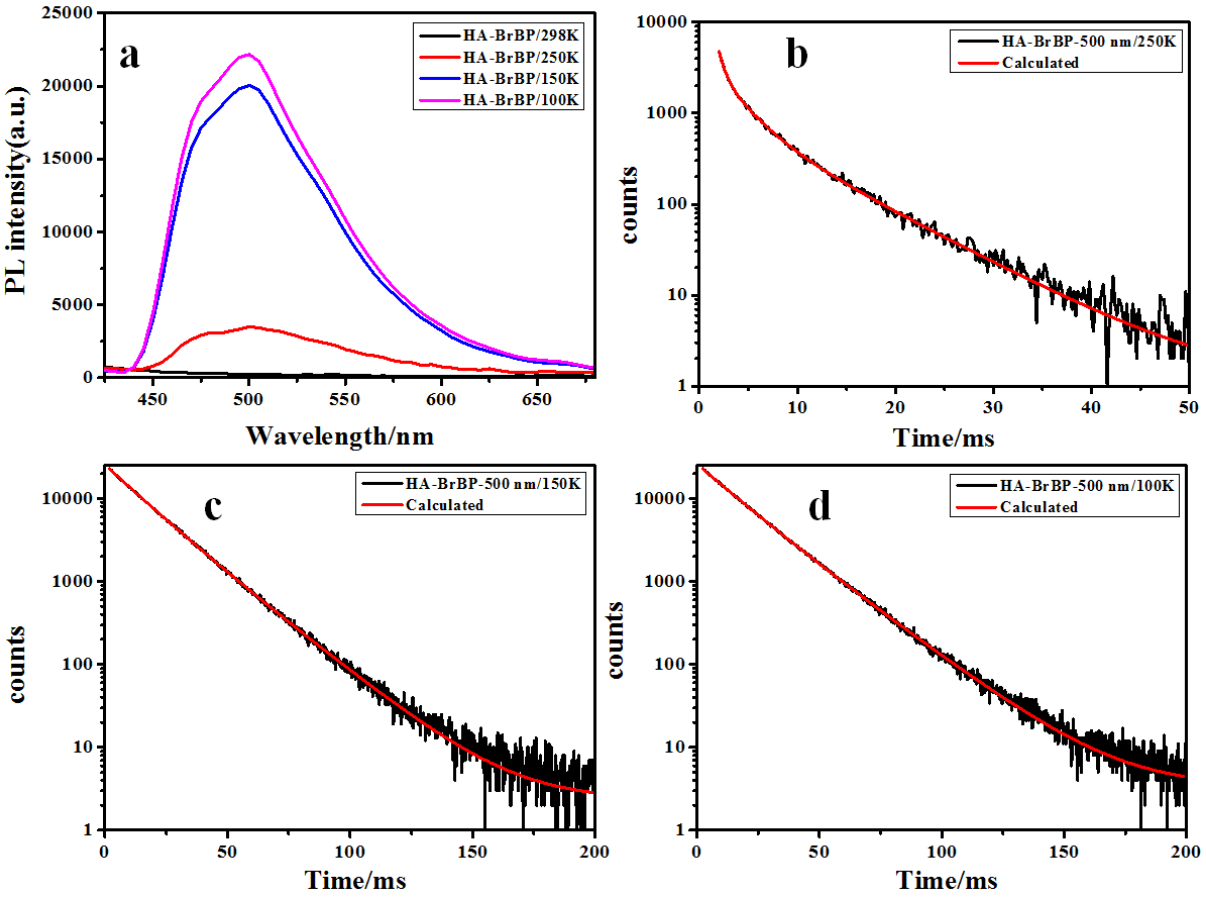


Supplementary Figure 9. Temperature effect on the photoluminescence spectra of CB[7, 8]/HA–BrBP. Prompt photoluminescence spectra of (a) CB[7]/HA–BrBP and (b) CB[8]/HA–BrBP in the temperature range from 298 K to 100 K in aqueous solution.

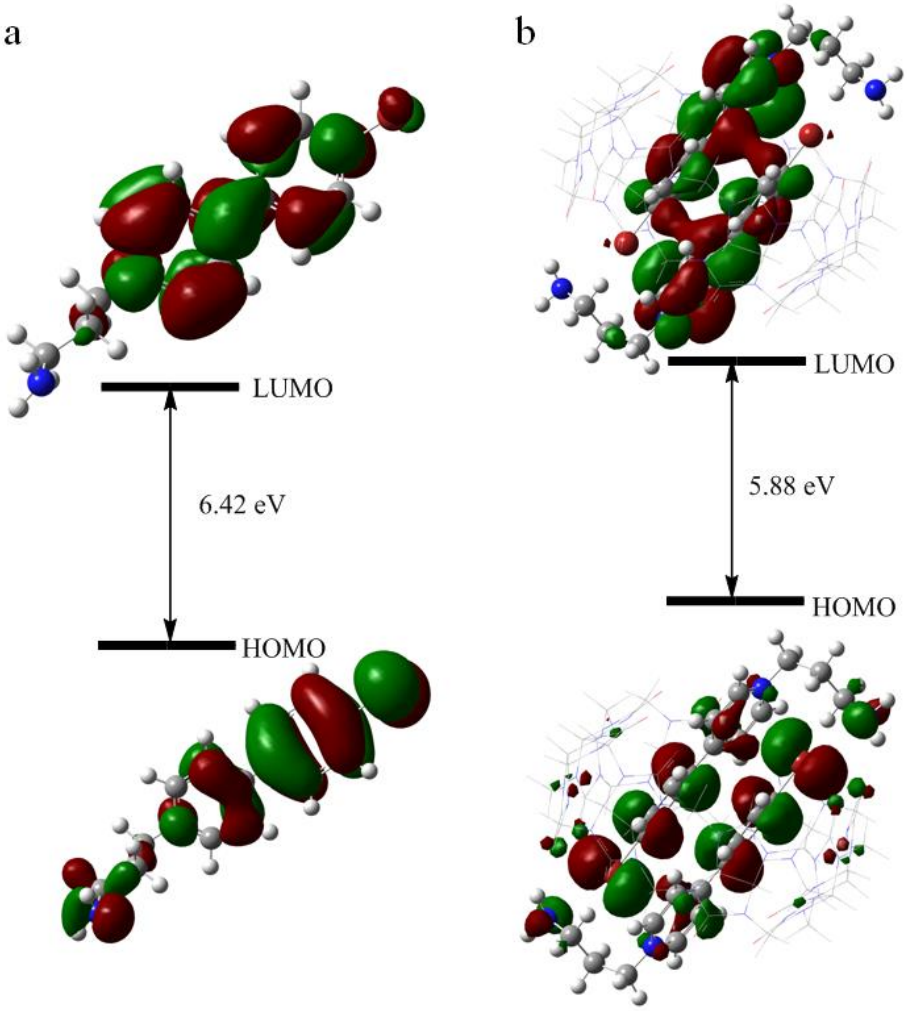


Supplementary Figure 10. Variable–temperature phosphorescence lifetime contrast curves for CB[7]/HA–BrBP and CB[8]/HA–BrBP. (**A**) The Phosphorescence lifetime decay curve of CB[7]/HA–BrBP for 500 nm from (a) 298 K; (b) 250 K; (c) 200 K to (d) 100 K. ([BrBP] = 0.5 mM, [CB7] = 0.5 mM); (**B**) The Phosphorescence lifetime decay fitting curve of CB[8]/HA–BrBP for 500 nm from (a) 298 K; (b) 250 K; (c) 200 K to (d) 100 K.

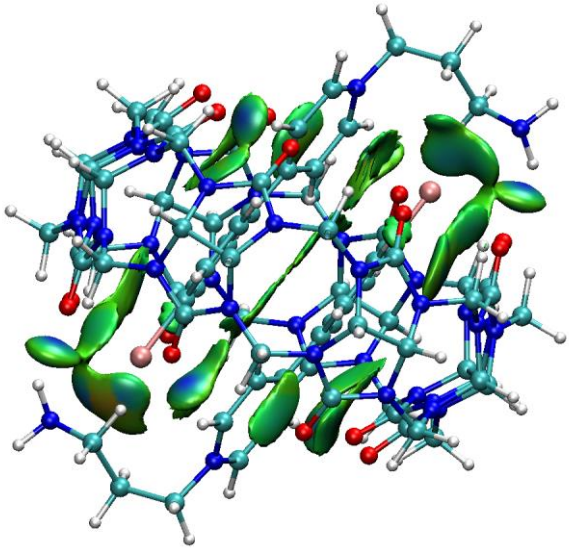
([BrBP] = 0.5 mM, [CB8] = 0.25 mM); (**C**) The Phosphorescence lifetime decay fitting curve of CB[8]/HA–BrBP for 500 nm from (a)298 K; (b) 250 K; (c) 200 K to (d) 100 K. ([BrBP] = 0.5 mM, [CB8] = 0.25 mM).



Supplementary Figure 11. Temperature effect on the photoluminescence spectra and lifetime curves of HA–BrBP. (**a**) Prompt photoluminescence spectra of HA–BrBP in the temperature range from 298 K to 100 K in aqueous solution; The Phosphorescence lifetime decay fitting curve of HA–BrBP for at 500 nm from (b) 250 K; (c) 150 K to (d) 100 K. ([BrBP] = 0.5 mM).

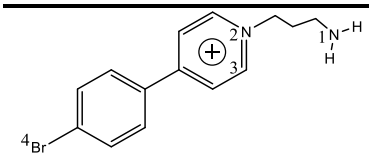


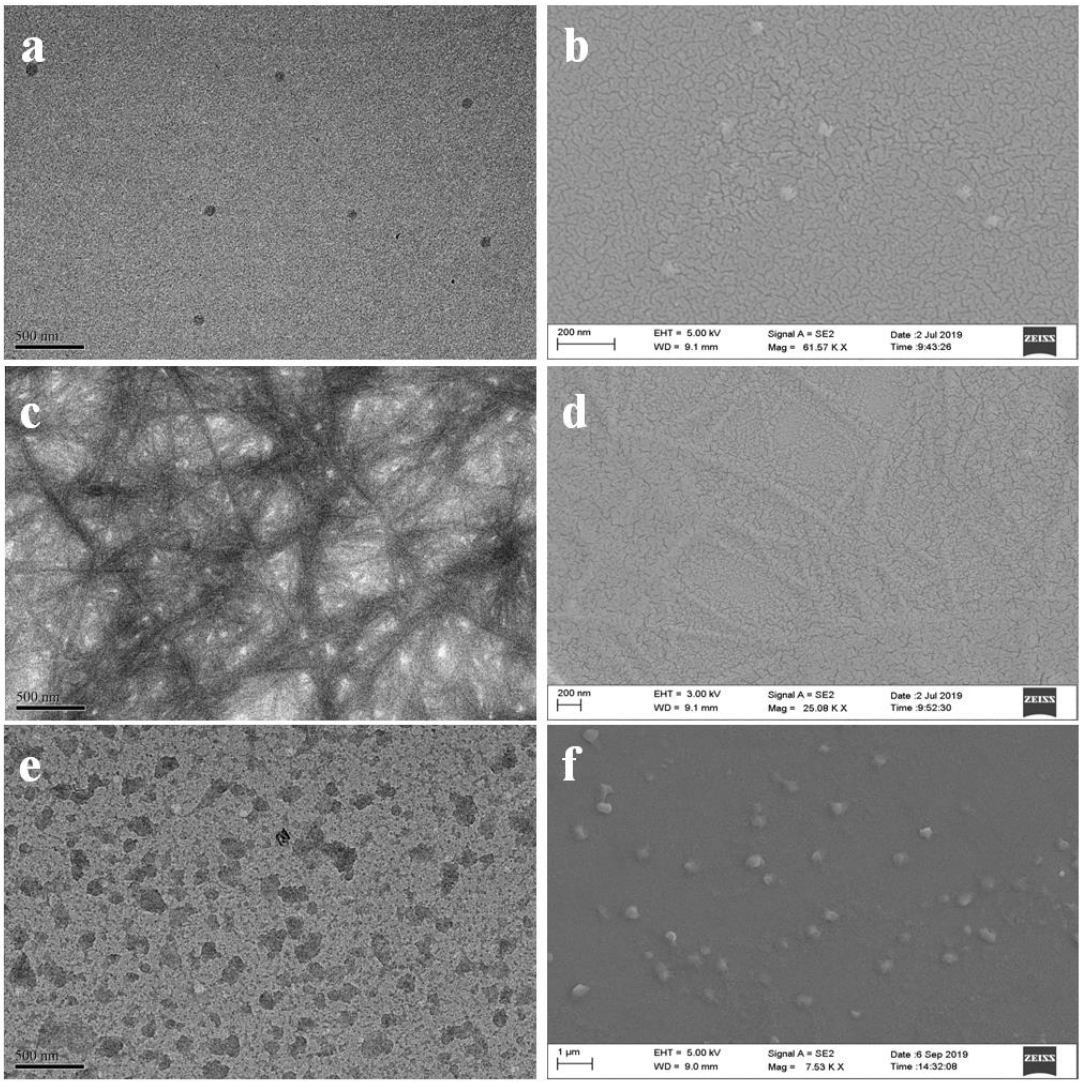
Supplementary Figure 12. Frontier molecular orbitals and energy gaps. (**a**) FMOs and energy gaps of the single guest (SPE= –3226.540604 a.u.); (**b**) FMOs and energy gaps of the CB[8]/BrBP–NH_2 (SPE= –11267.065376 a.u.).



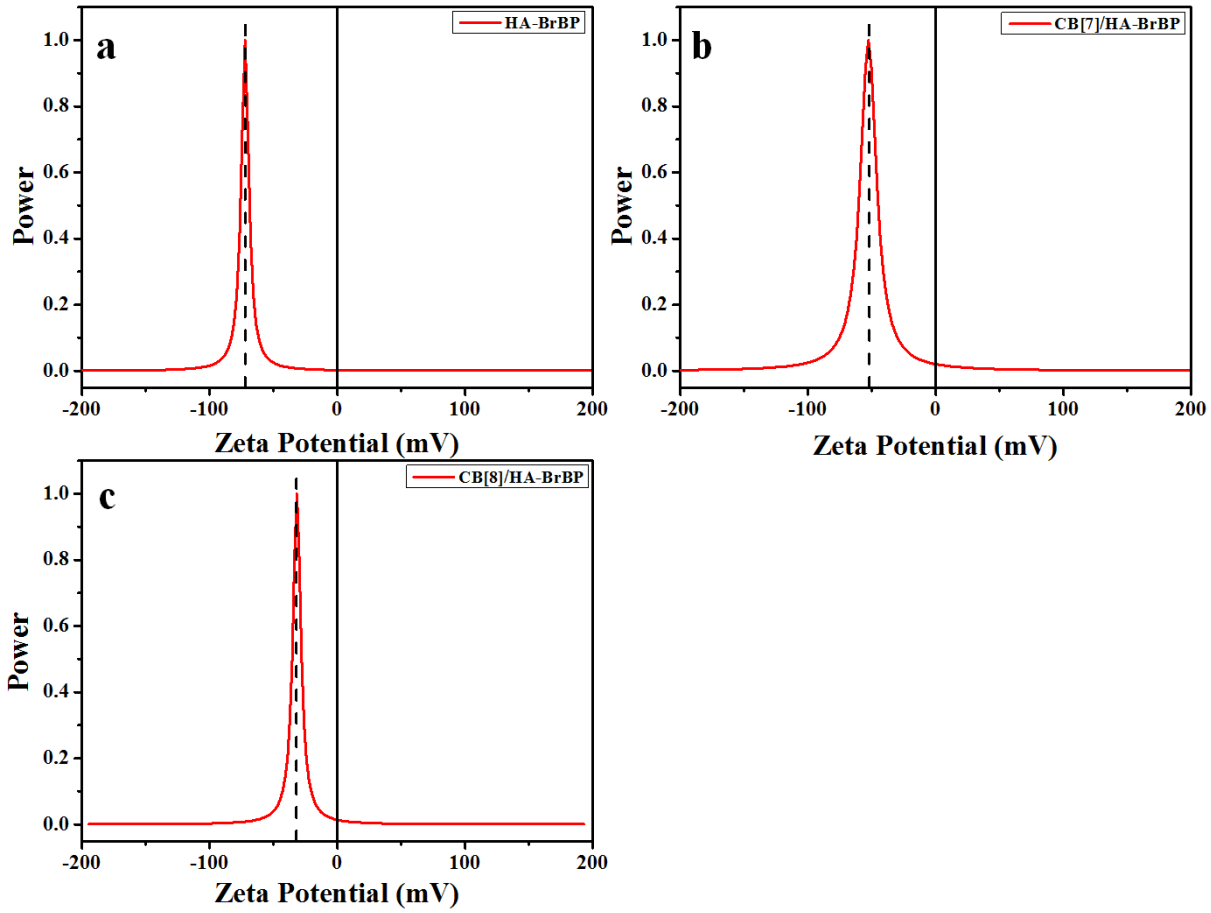
Supplementary Figure 13. The non-covalent interaction (NCI) analysis. The non-covalent interaction (NCI) analysis of two same guest molecules and CB[8].(Isosurface value=0.01. The green, blue, and red regions represent the weak, strong, and repulsive interactions, respectively.)

Supplementary Table 2. Some key Mulliken charge of single molecule and assembly in water at M06-2X-GD3/6-311G(d,p) level.

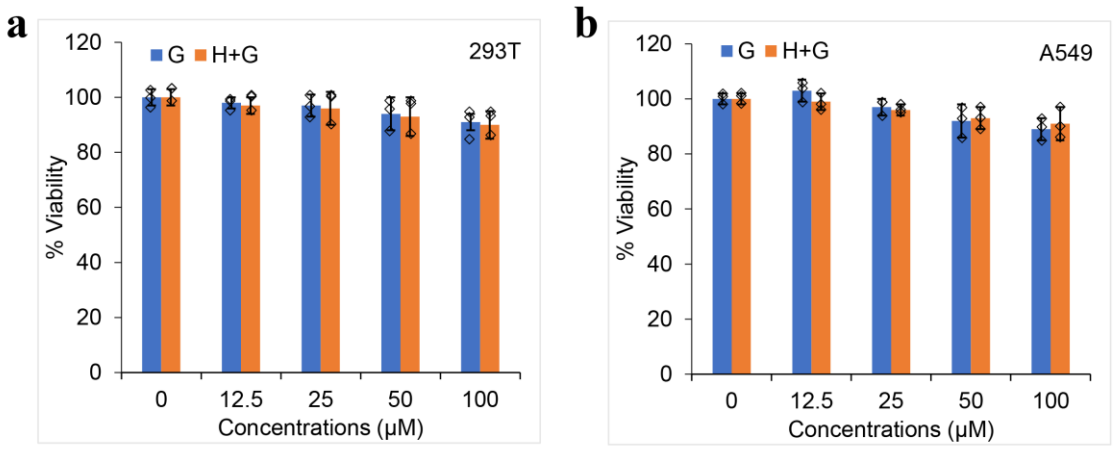
	Number-Atom	Charge in single molecule	Charge in assembly
	1-N	-0.585	-0.558
	2-N	-0.393	-0.360
	3-C	0.223	0.175
	4-Br	0.003	0.063



Supplementary Figure 14. Transmission and scanning electron microscopy images. TEM image of (**a**) HA–BrBP, (**c**) CB[7]/HA–BrBP, (**e**) CB[8]/HA–BrBP and SEM image of (**b**) HA–BrBP, (**d**) CB[7]/HA–BrBP, (**f**) CB[8]/HA–BrBP.

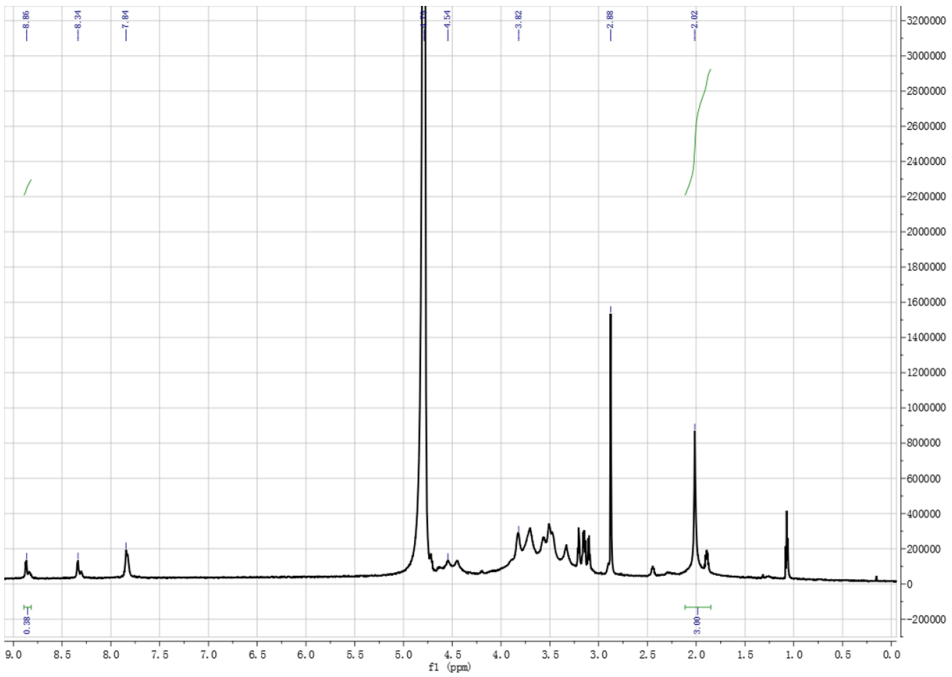


Supplementary Figure 15. Zeta potential data. Zeta potential results of (**a**) HA–BrBP (**b**) CB[7]/HA–BrBP and (**c**) CB[8]/HA–BrBP.



Supplementary Figure 16. Cytotoxicity assay results for HA–BrBP and CB[8]/HA–BrBP. Relative cell viability of HA–BrBP(G) and CB[8]/HA–BrBP(H+G) with different concentrations([BrBP]= 0 μM, 12.5 μM, 25 μM, 50 μM, 100 μM) at 25 ℃ in (**a**) 293T cells, (**b**) A549 cells. n = 3 independent experiments, with the bar data indicating mean ± SD. Noting that there is no significant difference between the G group and the H+G group under each concentration for both 293T and A549 cells ($P < 0.05$).

a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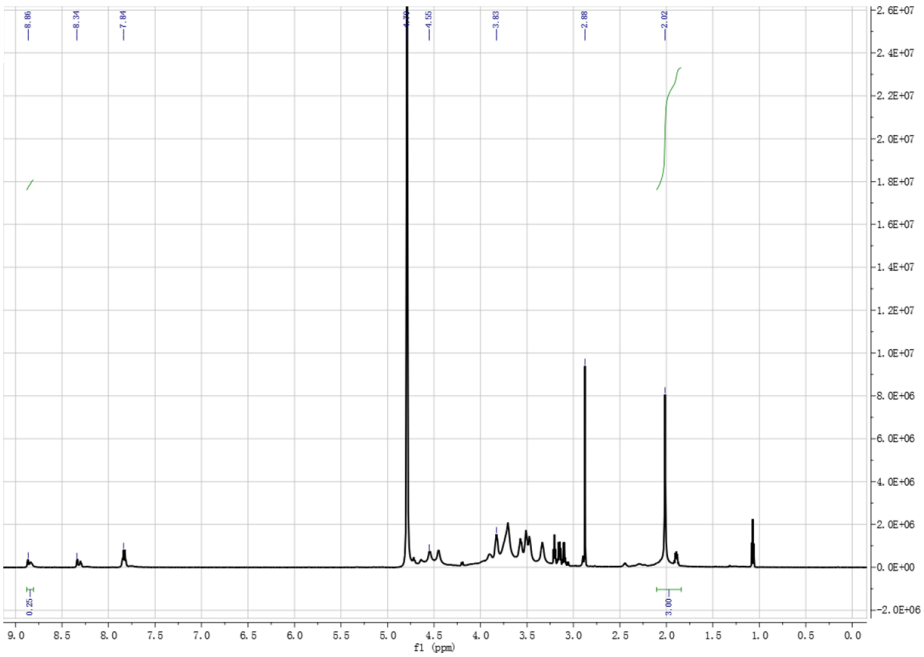
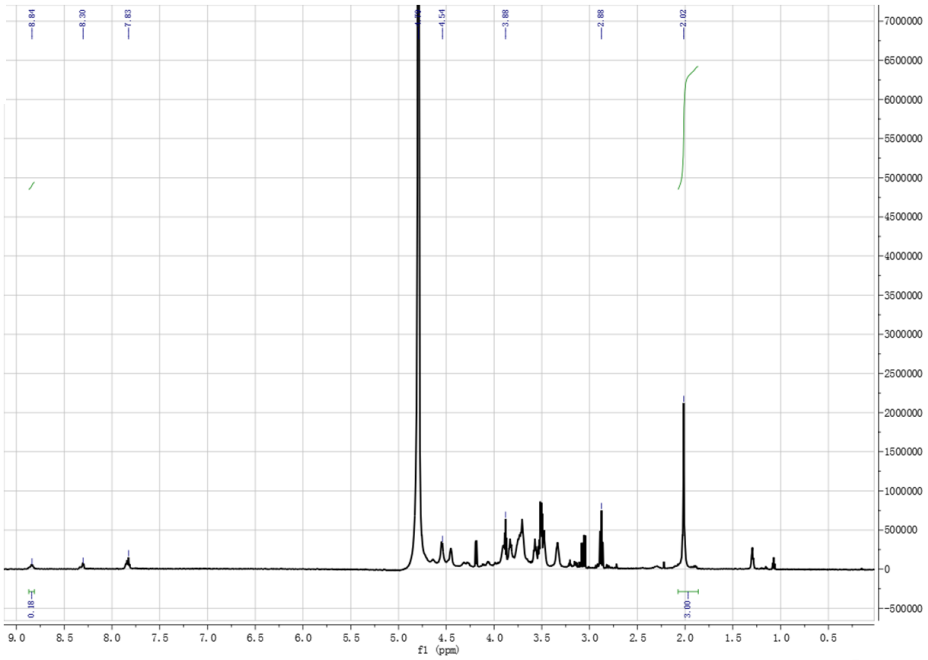
2.88
2.02
0.38
3.00
f1 (ppm)

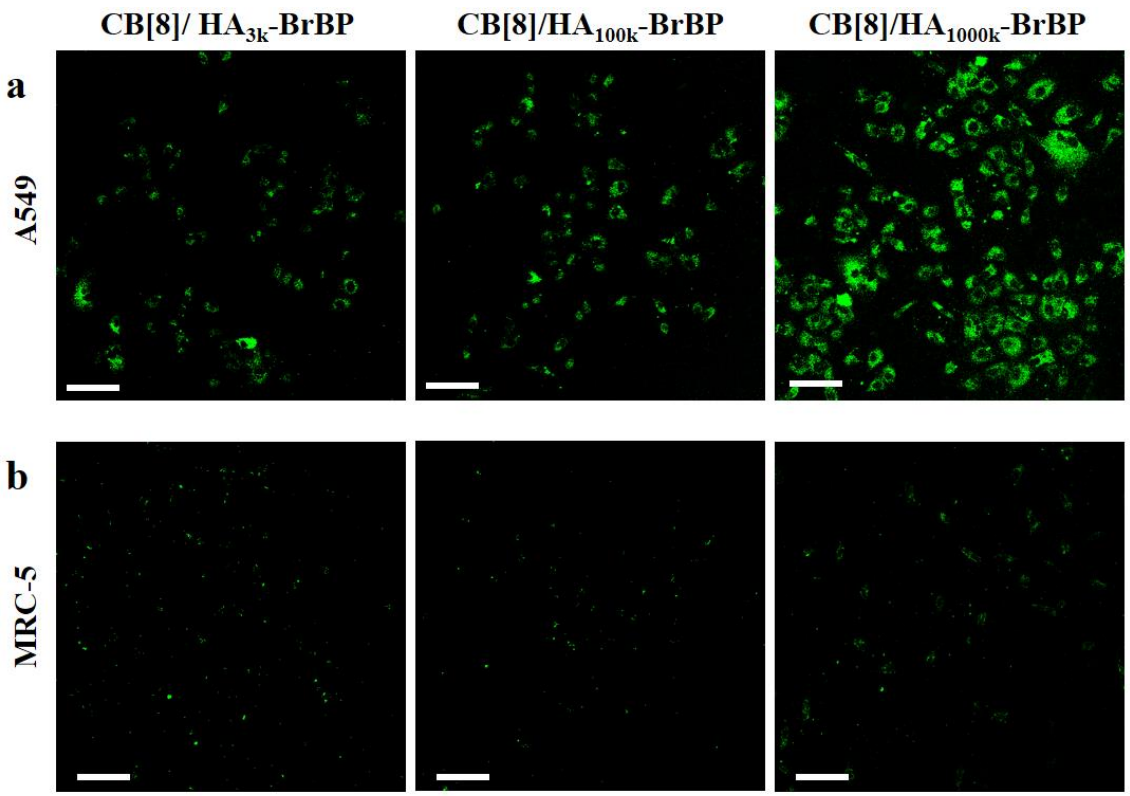
b


8.86
8.34
7.84
4.55
3.83
2.88
2.02
0.21
3.00
f1 (ppm)

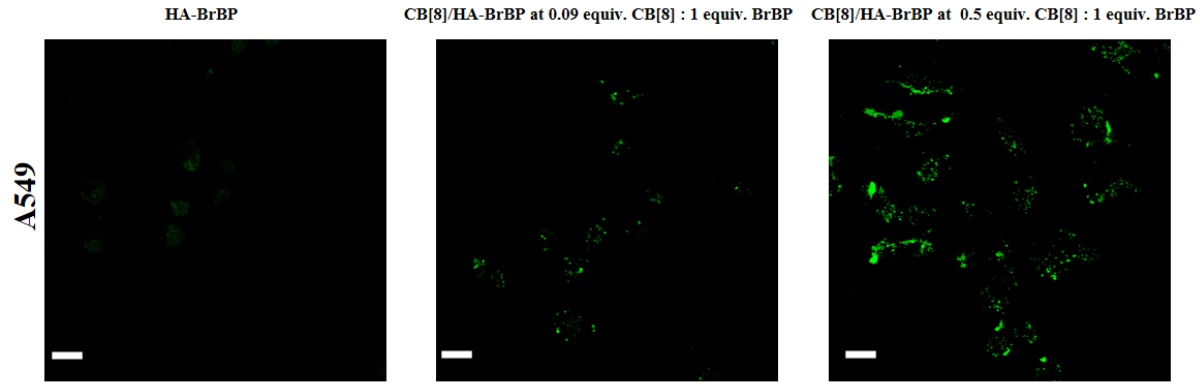
c



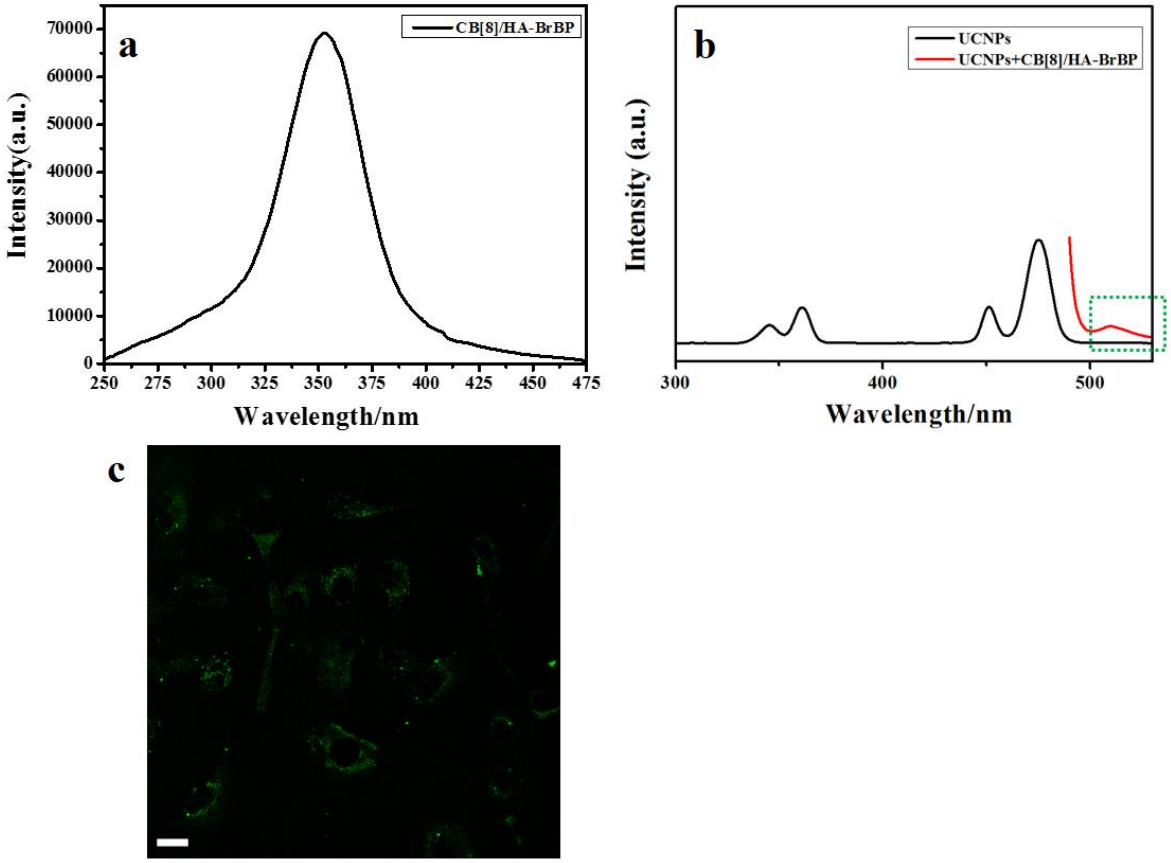
Supplementary Figure 17. ^{1}H NMR spectrum of different molecular weight HA modified BrBP. ^{1}H NMR spectrum of (**a**) HA_{3k}–BrBP (molecular weight of HA for 3k) (**b**) HA_{10w}–BrBP (molecular weight of HA for 100k) and (**c**) HA_{1000k}–BrBP (molecular weight of HA for 1000k) in D_2O at 298 K.



Supplementary Figure 18. Confocal microscopy images of different molecular weight HA modified BrBP. (**a**) A549 cells incubated with HA_{3k}–BrBP, HA_{100k}–BrBP and HA_{1000k}–BrBP. (**b**) MRC–5 cells incubated with HA_{3k}–BrBP, HA_{100k}–BrBP and HA_{1000k}–BrBP ([BrBP] = 25 μM). (Scale bar = 100 μm)



Supplementary Figure 19. Confocal microscopy images of different amount of CB[8] on for the HA–BrBP. A549 cells incubated with HA–BrBP, 0.09 equiv CB[8]/HA–BrBP and 0.5 equiv CB[8]/HA–BrBP ([BrBP] = 25 μM). (Scale bar = 30 μm)



Supplementary Figure 20. The Prompt photoluminescence contrast spectra of UCNPs and UCNPs/CB[8]/HA–BrBP. (**a**) The excitation spectra of the CB[8]/HA–BrBP upon photoirradiation in aqueous solution at 298 K. (**b**) The prompt photoluminescence contrast spectra of UCNPs (black) and UCNPs/CB[8]/HA–BrBP (red) in water (298 K, λ_{ex} = 980 nm). (**c**) Confocal microscopy images of HeLa cells incubated with UCNPs/CB[8]/HA–BrBP (λ_{ex} = 488 nm). UCNPs (7.5 mg/mL, $NaYREF_4$, RE: Yb, Er, Tm, Gd, Mu, Lu) was purchased from Hefei Fluonano Biotech Co., Ltd. (Scale bar = 30 μm)