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Supporting Information

for

Highly Efficient Discriminating Cancer Cells Based on in Situ Activated Phosphorescence Energy Transfer for Targeted Cells Imaging

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Materials and Instruments.

The molecular weights of HAR and HAR2 are about 550 and 49 kDa, respectively. All chemicals were commercially available reagent grade and used without further purification, unless otherwise noted. NMR spectra were recorded on Bruker 400 MHz instrument in D_2O, and chemical shifts were recorded in parts per million (ppm). High resolution mass (HRMS) spectra were performed on Varian 7.0T FTMS with ESI or MALDI source. TEM images were acquired by a high-resolution transmission electron microscope (Philips Tecnai G2 20S-TWIN microscope) operating at an accelerating voltage of 200 keV. The samples were prepared by placing a drop of solution onto a carbon-coated copper grid and air-dried. The morphological information was directly obtained from the fresh TEM samples without staining. UV-Vis absorption and transmission spectra were recorded on a Shimadzu UV-3600 spectrophotometer in a quartz cell (light path 10 mm) at 25 °C with a PTC-348WI temperature controller. Dynamic light scattering (DLS) was recorded on BI-200SM (Brookhaven Company) at 25 °C. Photoluminescence spectra was measured on an FLS980 instrument (Edinburg Instruments, Livingstone, UK). Fluorescence spectra and phosphorescence spectra (delayed by 0.2 ms) were recorded in a conventional quartz cell (light path, 10 mm) on a Varian Cary Eclipse spectrophotometer equipped with a Varian Cary single-cell Peltier accessory to control the temperature. Absolute phosphorescence and fluorescence quantum yields and lifetime by means of time-correlated single photon counting were measured on FS5 instrument (Edinburg Instruments, Livingstone, UK). The zeta potentials were determined on a NanoBrook 173Plus at 25 °C. Confocal florescence imaging was recorded with Olympus FV1000.

Cell viability assay

To investigate the toxicity of BPPY⊂CB[8]@HAR, BPPY⊂CB[8] and HAR to the

A549 and 293T cells, the cells were cultured in 96-well plates in corresponding medium containing 10% FBS for 24 h, and then the corresponding samples were added into the wells. The cells were further cultured for 24 h, and then cell viability was examined using the CCK-8 assay kit (NCM Biotech, China).

Confocal laser imaging

Cell lines (A549 and 293T cells) were obtained from the Cell Resource Center of China Academy of Medical Science (Beijing, China). The A549 and 293T cells were treated with BPPY⊂CB[8] for 8 h and followed by HAR for another 16 h ([BPPY] = 2[CB[8] 20 μM, [HAR] = 20 μM). The cells were observed by confocal microscopy after washing with PBS 3 times.

To observe the intracellular distribution of supramolecular assembly, the A549 cells were treated with for 24 h. The cells were washed with PBS and then incubated with Lyso-Tracker Blue for another 1 hour. The stained cells were then observed by confocal microscopy after washing with PBS 3 times.

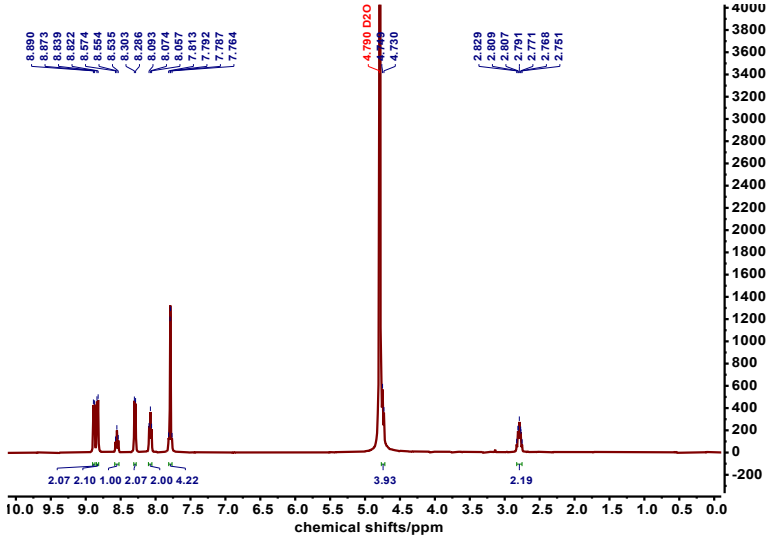

Figure S1. ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of BPPY.

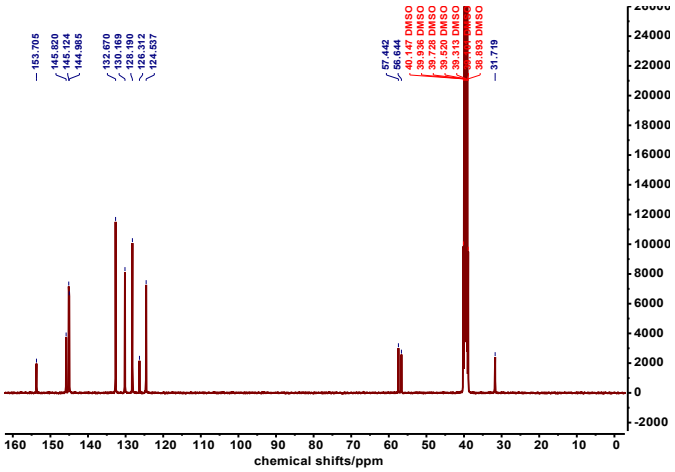

Figure S2. ^{13}C NMR spectra (101 MHz, d_6-DMSO, 298 K) of BPPY.

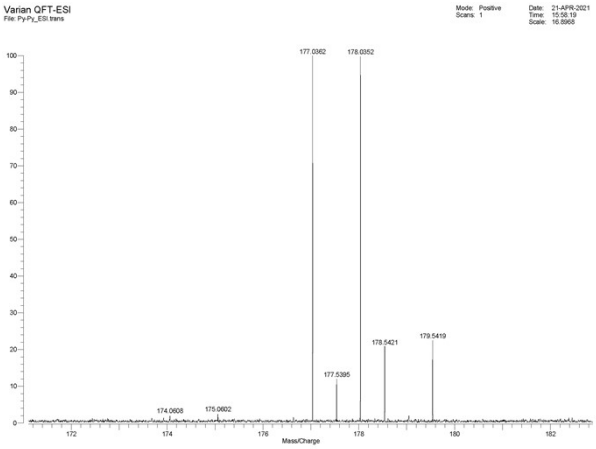

Figure S3. ESI-HRMS spectrum of BPPY.

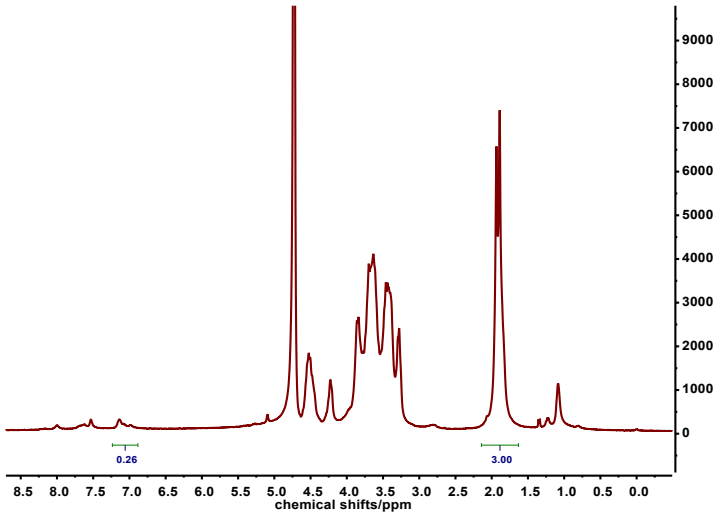

Figure S4. ^{1}H NMR spectrum (400 MHz, D_2O, 298K) of HAR. According to the integral area, rhodamine B has a degree of modification of 5.2 %.

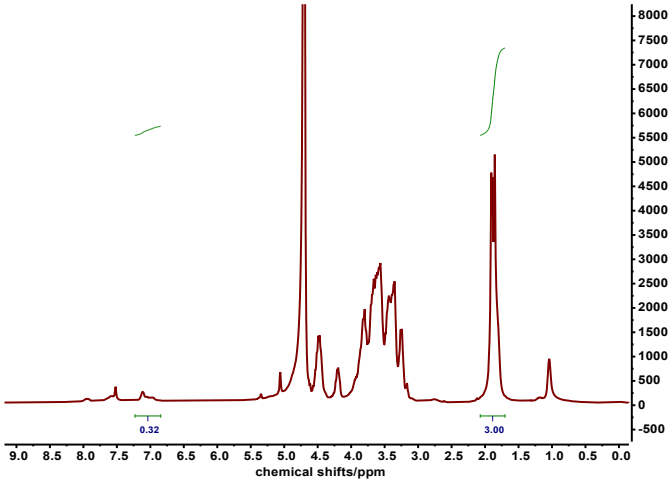

Figure S5. ^{1}H NMR spectrum (400 MHz, D_2O, 298K) of HAR2. According to the integral area, rhodamine B has a degree of modification of 6.4 %.

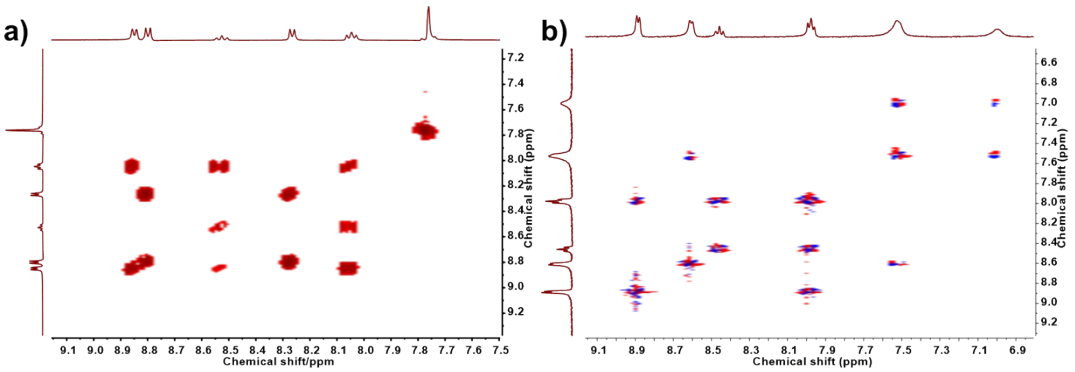

Figure S6. ^{1}H-^{1}H COSY spectrum (400 MHz, D_2O, 298 K) of BPPY and BPPY⊂CB[7].

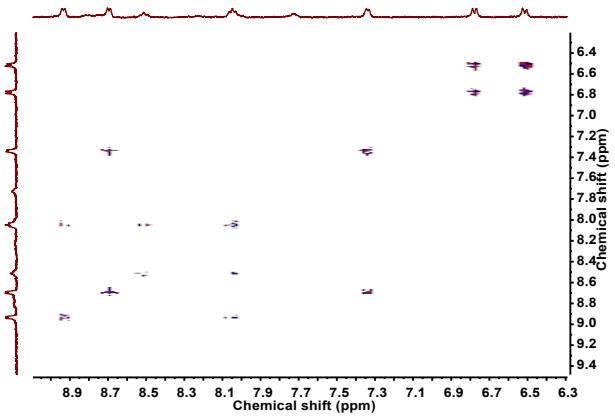

Figure S7. ^{1}H-^{1}H COSY spectrum (400 MHz, D_2O, 298 K) of BPPY⊂CB[8].

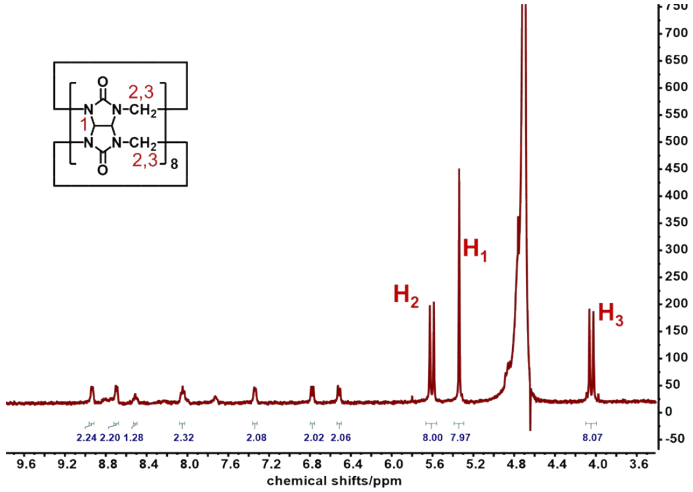

Figure S8. ^{1}H NMR spectra (400 MHz, D_2O, 298K) of BPPY⊂CB[8]. The binding ratio of BPPY and CB[8] can be calculated as 2:1 via the integral area.

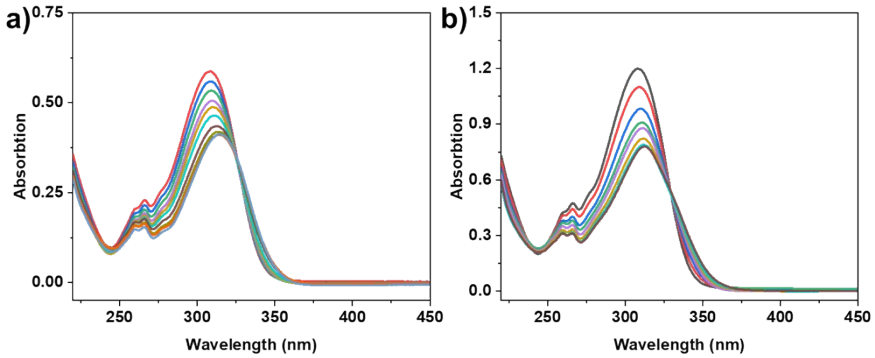

Figure S9. UV-vis absorbtion spectra of BPPY with addition of (a) CB[7] or CB[8] in PBS solution.

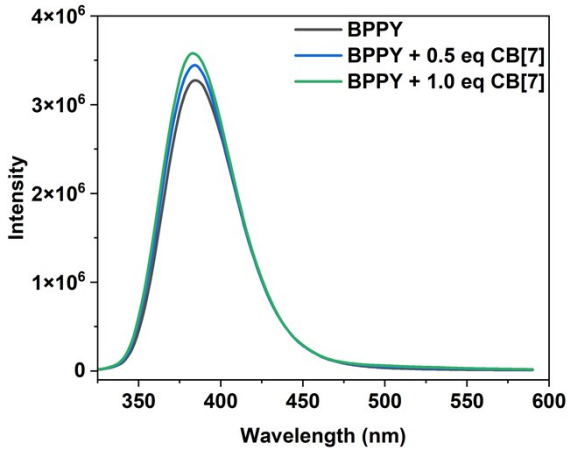

Figure S10. Photoluminescence spectra of BPPY ([BPPY] = 20 μM) upon the addition of CB[7] in PBS solution.

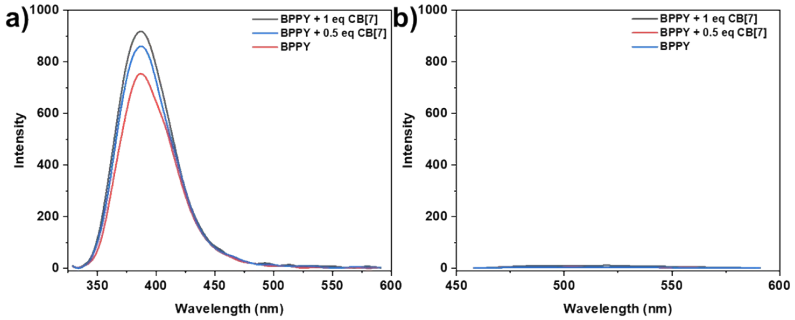

Figure S11. (a) Fluorescence spectra and (b) time-lapse photoluminescence spectra of BPPY (10 μM) and CB[7] at concentrations of 0, 5 and 10 μM.

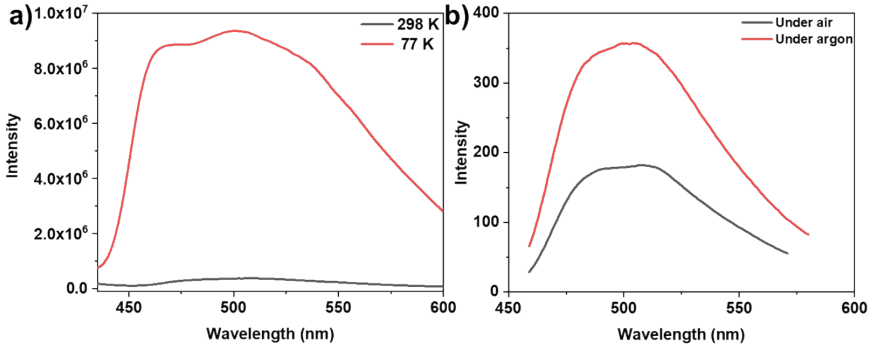

Figure S12. (a) Photoluminescence spectra of BPPY⊂CB[8] at low temperature; (b) Time-lapse photoluminescence spectra (delayed by 0.2 ms) of BPPY⊂CB[8] under argon atmosphere.

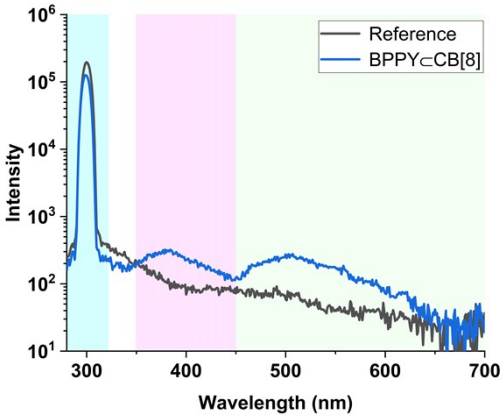

Figure S13. The quantum yield of BPPY⊂CB[8] ([BPPY] = 2[CB[8]] = 10 μM).

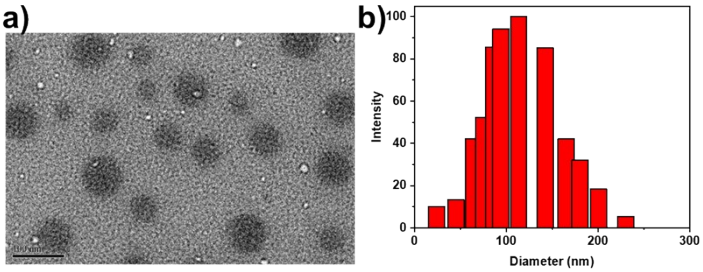

Figure S14. (a) TEM image and (b) DLS experiment of BPPY⊂CB[8]@HAR2.

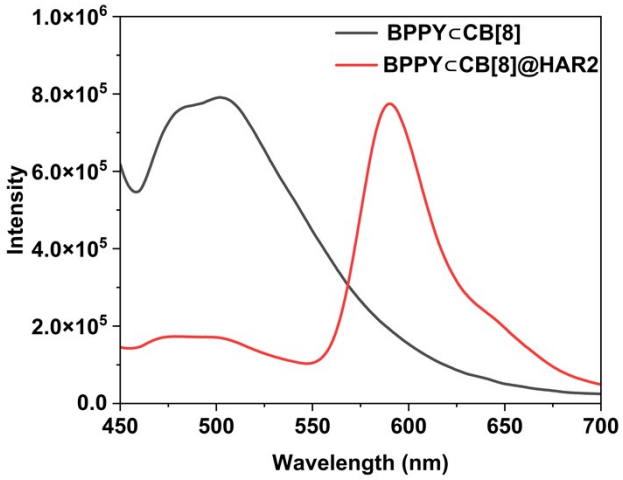

Figure S15. Photoluminescent spectrum of BPPY⊂CB[8] in the presence of HAR2 ([BPPY] = 2CB[8] = [HAR2] = 10 μM). The energy transfer efficiency from BPPY⊂CB[8] to HAR2 was 78 %.

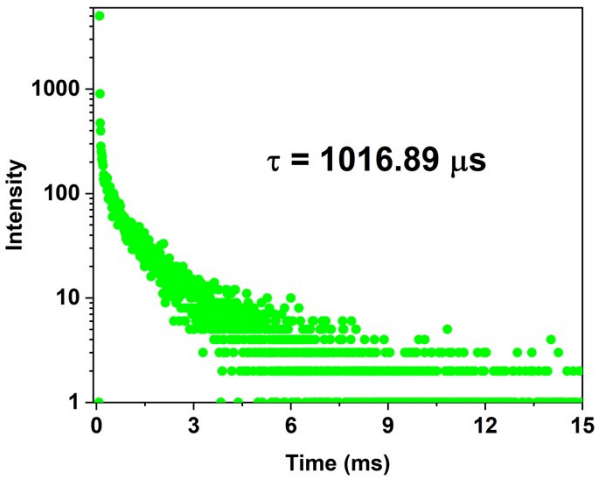

Figure S16. Time resolved photoluminescence decay curves of BPPY⊂CB[8]@HA at 505 nm ([BPPY] = 2[CB[8]] = 10 μM).

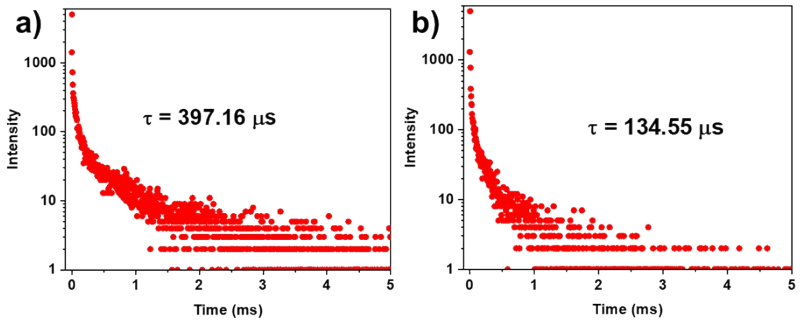

Figure S17. Time resolved photoluminescence decay curves of BPPY⊂CB[8]@HAR at (a) 505 nm and (b) 595 nm ([BPPY] = 2[CB[8]] = [HAR] = 10 μM).

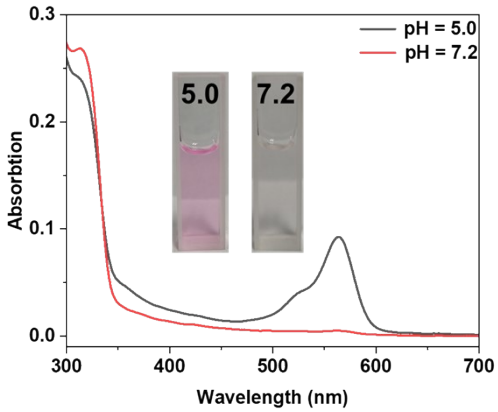

Figure S18. UV-vis absorbtion spectra of HAR at pH 5.0 and pH 7.2. The inserted images were digital photos of HAR at pH 5.0 and pH 7.2.

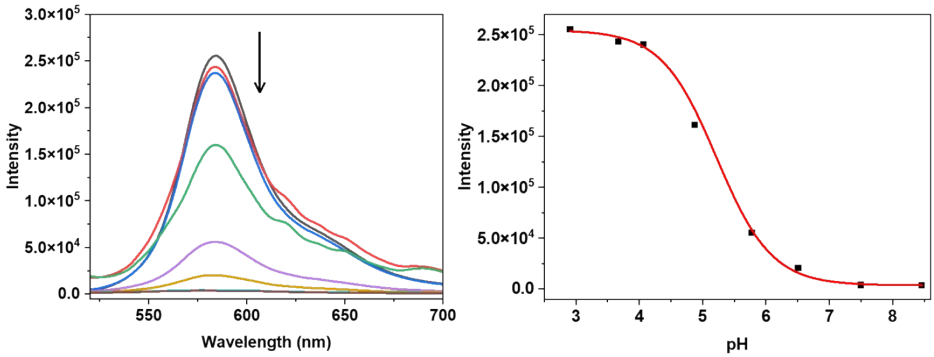

Figure S19. Photoluminescent spectrum of BPPY⊂CB[8]@ HAR2 at different pH and the analysis of fluorescence intensity changes as a function of pH at 580 nm ([BPPY] = 2CB[8] = [HAR2] = 10 μM).

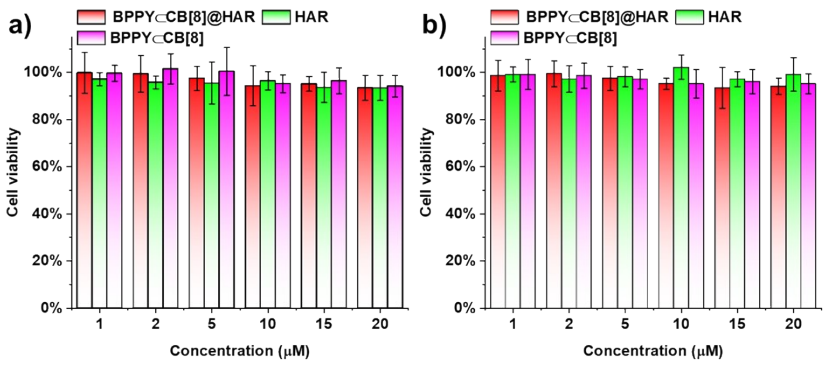

Figure S20. Cell viability of (a) A549, (b) 293T cells incubated with BPPY⊂CB[8]@HAR, HAR and BPPY⊂CB[8] at different concentration.

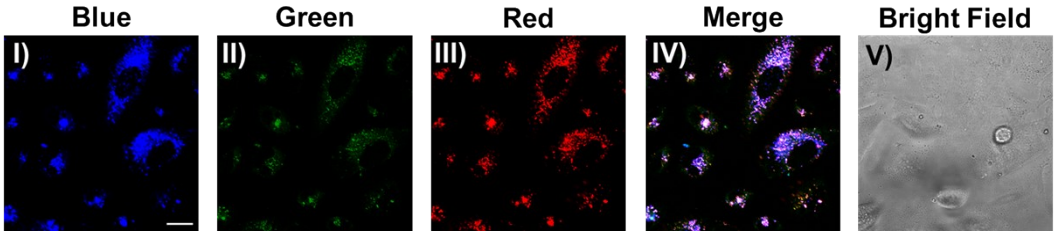

Figure S21. Confocal fluorescence images of living A549 cells incubated with BPPY⊂CB[8] and HAR successively (scale bar = 20 μm).

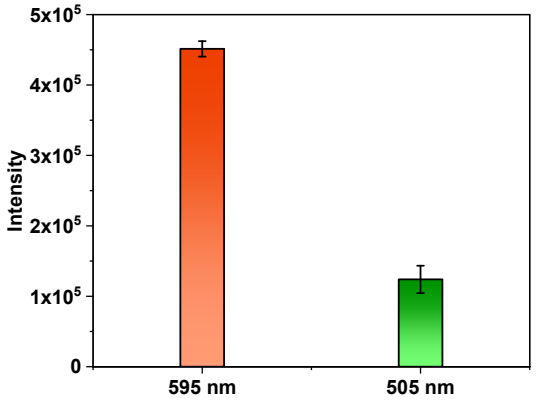

Figure S22. Time-resolved luminescent signal (delayed 50 μs) respectively detected at 595 nm and 505 nm using microplate reader.

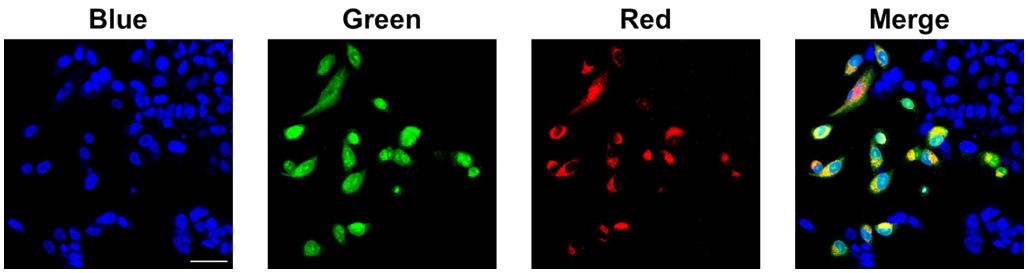

Figure S23. Confocal fluorescence images of living 293T cells and A549 cells in a

same cell culture dish incubated with BPPY⊂CB[8] and HAR successively. The A549 cells was marked by carboxyfluorescein diacetate succinimidyl ester (CFDA SE). Blue channel corresponding to DAPI (excitation = 405 nm, emission = 425-475 nm); green channel corresponding to CFDA SE (excitation = 488 nm, emission = 515-565 nm); red channel corresponding to delayed fluorescence of supramolecular assembly (excitation = 405 nm, emission = 575-675 nm).

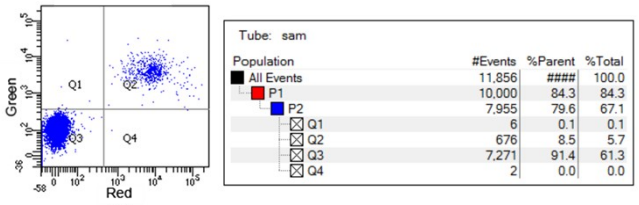

Figure S24. Flow cytometry of living 293T cells and A549 cells in a same cell culture dish incubated with BPPY⊂CB[8] and HAR successively. The A549 cells was marked by carboxyfluorescein diacetate succinimidyl ester (CFDA SE). Green channel corresponding to CFDA SE (excitation = 488 nm, emission = 515-545 nm); red channel corresponding to delayed fluorescence of supramolecular assembly (excitation = 405 nm, emission = 600-620 nm).

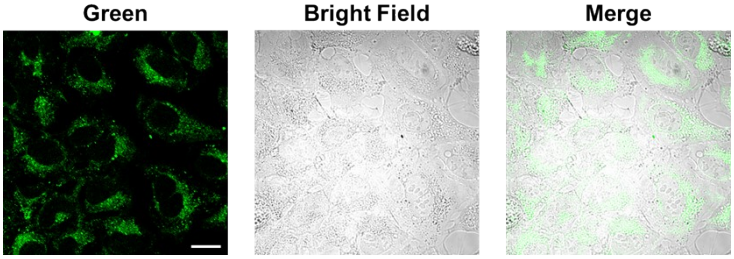

Figure S25. Confocal fluorescence images of living 293T cells incubated with BPPY⊂CB[8] (scale bar = 20 μm).