Electronic Supplementary Information

for

Supramolecular color-tunable photoluminescent materials based on a chromophore cascade as security inks with dual encryption

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1. General methods and materials

All the reagents and solvents were commercially available and used as received unless otherwise
specified purification. Thioflavin T (ThT) and nile red (NiR) were purchased from Sigma-Aldrich.
5,11,17,23,29-pentacarboxylic acid-31,32,33,34,35-n-dodecyloxy calix[5]arene (CA), amphiphilic β-
cyclodextrin (CD), 4-(dodecyloxy)benzamido-terminated methoxy poly(ethylene glycol) (PEG-C12)
and 2-(4-phenylboronic acid)-1-pyrenemethamide (PB) were synthesized and purified according to the
literature procedures.\[1,2,3,4\] The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer
solution (10 mM) of pH 8.0 was prepared by dissolving 2.383 g of HEPES in approx. 900 ml of
double-distilled water. Titrate to pH 8.0 at the lab temperature of 25 °C with NaOH and make up
volume to 1000 ml with double-distilled water. The pH value of the buffer solution was then verified
on a pH-meter calibrated with two standard buffer solutions.

Preparation of the CD/CA co-assembly: CD and CA were dissolved in chloroform at a
concentration of 1.0 mM, respectively. A mixed chloroform solution of CD and CA (1:1 molar ratio)
was dried by slow rotary evaporation to yield a thin film in a glass vial. Residual organic solvent was
removed under high vacuum. HEPES buffer (10 mM, pH 8.0) was added and the solution was
sonicated at 50 °C for 2 h to make the CD/CA co-assembly.

UV-Vis spectra were recorded in 1 mL low-volume disposable PMMA cuvettes (Brand GmbH &
CO KG, Wertheim) on a JASCO V-650 double-beam spectrophotometer (JASCO Labor- and
Datentechnik GmbH, Gross-Umstadt) at 25 °C. Steady-state fluorescence spectra were recorded in 1
mL low-volume disposable PMMA cuvettes (Brand GmbH & CO KG, Wertheim) on a JASCO FP
6500 and a conventional quartz cell (light path 10 mm) on a Varian Cary Eclipse equipped with a
Varian Cary single-cell peltier accessory to control temperature, all the peaks caused by second-order
transmission have been eliminated by derivation of original peaks. Fluorescence lifetimes were
recorded on a FluoTime300 spectrometer from PicoQuant equipped with two emission
monochromators (Czerny-Turner, selectable gratings blazed at 500 nm with 2.7 nm/mm dispersion and
1200 grooves/mm, or blazed at 1250 nm with 5.4 nm/mm dispersion and 600 grooves/mm), Glan-
Thompson polarizers for emissions, PMA Hybrid 40 (transit time spread FWHM<120 ps, 300-720 nm)
as detector and used in TCSPC mode by a PicoHarp 300 (minimum base resolution of 4 ps). Lifetime
analysis was performed using the commercial FluoFit software. The quality of the fit was assessed by
minimizing the reduced chi-squared function (χ²) and visual inspection of the weighted residuals and
their autocorrelation. Dynamic light scattering (DLS) measurements were examined on a laser light
scattering spectrometer (BI-200SM) equipped with a digital correlator (Turbo Corr.) at 636 nm at a
scattering angle of 90°. Scanning electron microscopy (SEM) images were recorded on a Hitachi S-3500N scanning electron microscope. The samples for SEM measurements were prepared by dropping the solution onto a coverslip, followed by evaporating the liquid in air. Differential scanning calorimetry (DSC) measurements were carried out on a DSC Q100 (TA Instruments). The co-assembly solutions (50 µL) were sealed in pressure-resistant DSC cups and scanned against a reference cup of HEPES buffer (pH = 8.0, 10 mM). The samples were equilibrated at 5 °C, then heated to 80 °C at 1 °C/min. After cooling the sample to room temperature, the measurements were repeated at least five times. The molecular packing model was performed by molecular dynamic simulation using MMFF94 force field.

Ink-writing tests were performed as follows: The aqueous solutions of the CD/CA co-assembly with three chromophores as fluorescent inks were taken by pipette (Thermo, Scientific) and written on the ordinary filter paper. After drying in air, the written papers were photographed or cut into appropriate size and inserted into a conventional quartz cell (light path 10 mm) for solid-state fluorescence measurement.

2. Chemical structures of the employed compounds

![Chemical structures of CA, CD, PEG-C12, ThT, PB and NiR.](image)

Figure S1. Chemical structures of CA, CD, PEG-C12, ThT, PB and NiR.
3. Characterization of the CD/CA co-assembly

Figure S2. (a) DLS data, (b) Zeta potential, and (c) SEM image of the CD/CA co-assembly in 10 mM HEPES buffer (pH = 8.0, [CA] = 100 μM, [CD] = 100 μM). (d) DSC heating curve for phase transition temperature measurement in 10 mM HEPES buffer (pH = 8.0, [CA] = 2 mM, [CD] = 2 mM).

Figure S3. (a) DLS data and (b) Zeta potential of the CD assembly in 10 mM HEPES buffer (pH = 8.0, [CD] = 100 μM); (c) DLS data of the CD/CA co-assembly in 10 mM HEPES buffer (pH = 8.0, [CA] = 100 μM, [CD] = 100 μM) standing for 13 days.
Due to their characteristic amphiphilic features, CA and CD form co-assemblies in aqueous solution. It should be mentioned that the alone CA cannot be hydrated well in HEPES buffer (10 mM pH = 8.0), but can be done when mixing with CD, indirectly proving the formation of co-assemblies. Direct evidences came from the DLS and Zeta potential measurements. The averaged diameter of the CD/CA co-assembly is about 140 nm (Figure S2a), bigger than that of the CD assembly (about 112 nm) (Figure S3a). The CD/CA co-assembly gives a Zeta potential of about −60.6 mV (Figure S2b), much more negative than free CD (−11.6 mV) (Figure S3b), since CA possesses negatively charged carboxyl groups at its upper rim. SEM image of the CD/CA co-assembly shows the spherical-like morphology with comparable size to DLS results (Figure S2c). DSC results show the phase transition temperature (only exited for vesicles rather than micelles) of 16.1 °C for the CD/CA co-assembly (Figure S2d), much higher than the previously reported CD vesicle whose phase transition temperature is too low to be measured.[5] These combined results indicate that CA and CD co-assembled into the vesicular aggregation.

4. Host-guest complexation and complexation-induced fluorescence enhancement

Figure S4. a) Host-guest fluorescence titration of PB (1 µM) with the CD/CA co-assembly in HEPES buffer (10 mM, pH = 8.0), λex = 326 nm, inset showing the curve fitting data at λem = 377 nm; b) Fluorescence spectra of PB (10 µM), PB (10 µM) with PEG-C12 (20 µM) and CA (50 µM), PB (10 µM) with CD (50 µM) and CA (50 µM) in HEPES buffer (10 mM, pH = 8.0) , λex = 326 nm.
Figure S5. a) Host-guest fluorescence titration of ThT (5 µM) with the CD/CA co-assembly in HEPES buffer (10 mM, pH = 8.0), $\lambda_{ex} = 430$ nm, inset showing the curve fitting data at $\lambda_{em} = 500$ nm; b) Fluorescence spectra of ThT (10 µM) with CD (100 µM), CD (100 µM) and CA (100 µM) in HEPES buffer (10 mM, pH = 8.0), $\lambda_{ex} = 430$ nm.

Upon complexation with the CD/CA co-assembly, the fluorescence of PB was enhanced for 5 times, that of ThT was enhanced for 285 times. However, no appreciable enhancement was observed when adding CA to PB, and adding CD to ThT (Figures S4b and S5b). These results imply that PB was selectively encapsulated into the cavity of CD, while ThT was selectively encapsulated into the cavity of CA. Herein, we should mention that PEG-C12 was used to solubilize the CA assembly (free CA cannot be hydrated well as mentioned above), to perform the fluorescence measurement between PB and CA (Figure S4b).
5. Energy transfer (ET) between dyes

**Figure S6.** Fluorescence spectra of a) PB/ThT (10/2 μM) and ThT (16 μM), $\lambda_{ex}=326$ nm; b) PB/NiR (3/6 μM) and NiR (6 μM), $\lambda_{ex}=326$ nm; c) ThT/NiR (50/0.5 μM) and NiR (6 μM), $\lambda_{ex}=430$ nm in the CD/CA co-assembly ($[\text{CD}]=[\text{CA}]=50$ μM) in HEPES buffer (pH = 8.0, 10 mM).
Figure S7. The nonlinear fluorescence changes of a) 10 µM PB upon gradual addition of ThT (0, 2, 4, 6, 8, 10, 12, 14, 16 µM), $\lambda_{ex} = 326$ nm, $\lambda_{em} = 377$ nm; b) 50 µM ThT upon gradual addition of NiR (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 µM), $\lambda_{ex} = 430$ nm, $\lambda_{em} = 500$ nm; c) 10 µM PB upon gradual addition of NiR (0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 µM), $\lambda_{ex} = 326$ nm, $\lambda_{em} = 377$ nm. All experiments were performed in the presence of the CD/CA co-assembly ($[\text{CD}] = [\text{CA}] = 50$ µM) in HEPES buffer (10 mM, pH = 8.0).

Figure S8. (a) Fluorescence spectra of 10 µM PB, 2 µM ThT and 0.5 µM NiR in the CD/CA co-assembly ($[\text{CD}] = [\text{CA}] = 50$ µM) in HEPES buffer (10 mM, pH = 8.0) upon excitation with different wavelengths (326, 346, 366, 386, 406, 426, 446, 466, 486, 506, 526 nm). (b) showing the enlarged fluorescence spectra from 450 to 700 nm.
Figure S9. Fluorescence decay profiles of a) 10 µM PB and 10 µM PB with 6 µM NiR, $\lambda_{ex} = 310$ nm, $\lambda_{detection} = 378$ nm; b) 10 µM PB with 16 µM ThT, $\lambda_{ex} = 310$ nm, $\lambda_{detection} = 378$ nm; c) 16 µM ThT, $\lambda_{ex} = 410$ nm, $\lambda_{detection} = 500$ nm; d) 16 µM ThT with 6 µM NiR, $\lambda_{ex} = 410$ nm, $\lambda_{detection} = 500$ nm in the CD/CA co-assembly ([CD] = [CA] = 50 µM) in HEPES buffer (10 mM, pH = 8.0). Note: The value of $\tau_{Av}$ is an amplitude-weighted average lifetime.\cite{6}
Figure S10. Distance and orientation estimates for donors and acceptors in the CD/CA co-assembly. CD is shown in grey, CA in black, ThT in green, PB in blue and NiR in red.
6. Ink-writing tests

Figure S11. Fluorescence spectra of 20 µM PB, 32 µM ThT and 1 µM NiR in the CD/CA co-assembly ([CD] = [CA] = 100 µM) in HEPES buffer (10 mM, pH = 8.0, black line) and in paper (red line), $\lambda_{ex} = 326$ nm, inset showing the photographs under 365 nm UV light.

Figure S12. Stability test of the fluorescent inks: photographs of the written paper a) kept in dark for 50 days; b) sprayed by water; c) irradiated for 2 h under UV light (365 nm, 14.6 mJ/cm²); d) photobleaching measurements of the fluorescent inks in HEPES solution, irradiated by 365 nm (blue line), 460 nm (red line), 520 nm (black line), $\lambda_{em} = 615$ nm.
7. References


