Highly Fluorescent Cucurbit[8]uril-Perylenemonoimide Host-Guest Complexes As Efficient Fluorescent Probes for N-Terminal Phenylalanine

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1. Experimental Section

1.1. General methods

Unless otherwise stated, all solvents and chemicals were purchased from Sigma Aldrich and used without further purification. Cucurbit[7]uril and Cucurbit[8]uril were purchased Strem Chemicals Inc. (Newburyport, MA) and dried at 110 °C for 24 hours before use. Amino acids: Glycine, Phenylalanine, Tyrosine and Tryptophan were purchased from Sigma Aldrich. Peptides: Phe-Gly-Gly was purchased from Sigma Aldrich and Gly-Phe-Gly, Gly-Gly-Phe were obtained from Chem-Impex International, Inc. 

$^1$H- and $^{13}$C-NMR spectra were recorded on either a Varian 400 or 500 MHz spectrometer in CDCl$_3$, DMSO-d$_6$, CF$_3$COOD, or D$_2$O. MALDI-TOF spectra were recorded on a Bruker Autoflex3 Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS).

UV-vis spectra were recorded with a dual-beam Perkin Elmer Lambda 950 Spectrophotometer using UV-WIN Lab version 5.1.5 software. Fluorescence spectra were acquired using a Jobin - Yvon Horiba Fluorolog 3-222 Fluorescence Spectrophotometer. 1-cm or 0.5-cm quartz cuvettes were used for both UV-vis and fluorescence studies. The binding constant of CB[8]:PMI-1 as determined by fluorescence titration were calculated using a 1:1 binding model in the Origin 9.1 program.$^1$

Quantum yields were determined using another water-soluble perylene monoimide as a standard (QY=0.50 in methanol)$^2$.

Lifetime measurements were performed at 25°C using a Fluorolog-3 Fluorometer equipped with a NanoLED-492, FluoroHub TCSPC, a single photon detection cooled photocathode TBX-05, and Datastation DAS6 Foundation Software. All studies were performed without purging the solutions with inert gas to remove oxygen.
1.2 Synthesis Overview

1.2.1. Synthesis of PMI-1

Figure S1: Synthesis of PMI-1.

1.3. Synthetic Procedures and Characterization

Synthesis of 3

A mixture of monopotassium salt 2 (It was synthesized using a previous reported procedure without further characterization.\textsuperscript{3}) (5.00 g, 11.0 mmol) and N, N-Dimethylthlenediamine (3.93 g, 44.0 mmol) was dissolved in 200 mL water. The mixture was stirred at room temperature for 4 hours. Acetone (800 mL) was added to induce precipitation and the resulting red solid was filtered and dried under vacuum at 80 °C to give (4.84 g, 9.0 mmole, 85% yield) of 3. \(^1\)H NMR (400 MHz, CF\(_3\)COOD, 25 °C) \(\delta\) 8.84-8.52 (m, 8H), 4.95 (br, 2H), 4.02 (br, 2H), 3.42 (s, 6H) ppm.
Synthesis of 4

Compound 3 (2.00 g, 3.8 mmol) and KOH (1.90 g, 34.0 mmol) were dissolved in 40 mL water in a Teflon cup. The Teflon cup was placed in a stainless-steel reaction vessel with a lead?. The closed reaction vessel was heated in a sand bath at 220 °C for 12 hours. The reaction vessel was cooled to room temperature and the resulting solid was collected and washed with excess water using centrifugation. A dark-red solid was dried under vacuum at 110 °C. The resulting crude product was dissolved in chloroform and purified using an alumina column to yield 3 (0.89 g, 2.3 mmol, 60%) 4. 1H NMR (Varian 400 MHz, CDCl₃) δ 8.39 (d, J = 6.8 Hz, 2H), 8.24 (d, J = 6 Hz, 2H), 8.17 (d, J = 6.4 Hz, 2H), 7.82 (d, J = 6.4 Hz, 2H), 7.54 (t, J =6.4 Hz, 2H), 4.36 (t, J = 5.6 Hz, 2H), 2.76 (t, J = 4 Hz, 2H), 2.46 (s, 6H); 13C NMR (100 MHz, DMSO-d₆) δ 167.8, 138.5, 135.6, 131.7, 128.6, 126.9, 124.2, 63.9, 62.3, 53.1, 37.1, 9.6; ESI+: m/z = 393.34 [M+H]⁺ (calc’d. 393.16 for C₂₆H₂₁N₂O₂).

Synthesis of PMI-1

In a round bottom flask, compound 4 (100 mg, 0.25 mmol) in toluene (5 mL) was added to ethyl 1-bromoethanol (0.5g, 0.004 mole). The resulting mixture was stirred at room 110 °C for 48 hrs. The reaction mixture was precipitated out with ethyl acetate and collected using a centrifuge. The resulting product was dissolved in water and filtered using a fine porosity fritted glass filter. The filtrate was collected and dried under vacuum. (97 mg, 0.18 mmol, 75%). 1H NMR (Varian 400 MHz, DMSO-d₆) δ 8.65-8.63 (m, 4H), 8.42 (t, J = 6 Hz, 2H), 8.07 (d, J = 6.4 Hz, 2H), 7.71 (t, J = 6 Hz, 2H), 5.35 (t, J = 3.6 Hz, 2H), 4.48 (t, J = 5.6 Hz, 2H), 3.93 (br, 2H), 3.68 (t, J = 6.8 Hz, 6H), 3.59 (t, J = 4.8 Hz, 2H), 3.31 (s, 3H); 13C NMR (100 MHz, CF₃COOD) δ 167.8, 138.5, 135.6, 131.7, 128.6, 126.9, 124.2, 63.9, 62.3, 53.1, 37.1, 9.6; MALDI-TOF: m/z = 437.20 [M+H]⁺ (calc’d. 437.19 for C₂₈H₂₅N₂O₃).
1.4. NMR and Mass spectra

**Figure S2.** $^1$H NMR of 4 in CDCl$_3$.

**Figure S3.** $^1$H NMR of PMI-1 in DMSO-d$_6$. 
Figure S4. $^{13}$C NMR of 4 in DMSO-d$_6$.

Figure S5. $^{13}$C NMR of PDI PMI-1 in CF$_3$COOD.
Figure S6. $^1$H-NMR of PMI-1 (0.1 mM) (bottom) and CB[8]-PMI-1 (0.5 mM) (top) at 50 °C.

Figure S7. ESI mass of CB[8]:PMI-1 in H$_2$O with 10% (v/v) MeOH. (The observed mass is 1766.21 that is equal to the calculated value.)
1.6 UV-vis spectra, fluorescence spectra, fluorescence titration, and fluorescence decay

**Figure S8.** (left) Fluorescence emission spectra and (right) titration curve (Fluorescence intensity vs. the concentration of CB[7]) of PMI-1 (2.0x10^{-5} M) in water after the addition of 0, 1.0, 2.0, 3.0, 4.0, 5.0 equivalents of CB[7]. Fluorescence emission spectra were collected when samples were excited at 490 nm.

**Figure S9.** Fluorescence decay of PMI-1 (2x10^{-5} M) and CB[8]: PMI-1 in Water.
Figure S10. Fluorescence titration experiment for the binding of PMI-1 to CB[8] at 0, 1, 2, 40, 60, 80, 100, 120, and 140 μM. The fluorescence intensity at 570 nm were plotted against the concentration CB[8]. The red line represents the best fits of the data to a 1:1 binding.

Figure S11. Fluorescence titration experiment for the binding of PDI-1 to CB[8] at 0, 1, 2, 40, 60, and 80 μM. The fluorescence intensity at 570 nm was plotted against the concentration CB[8]. The red line represents the best fits of the data to a 1:1 binding.
Figure S12. UV-vis absorbance spectra of CB[8]:PMI-1 (2x10^{-5} M) in the absence and presence of 1.0 equivalent MV in water.

Figure S13. Fluorescence spectra of PMI-1 (2x10^{-5} M) and CB[8]:PMI-1 (1:1) in the absence and presence of 0-12 equivalent of Tryptophan in water.
**Figure S14.** UV-vis absorbance spectra of PMI-1 \((2 \times 10^{-5} \text{ M})\) and CB[8]:PMI-1 \((1:1)\) in the absence and presence of 1.0 equivalent of Tryptophan in water.

**Figure S15.** Fluorescence spectra of PMI-1 \((2 \times 10^{-5} \text{ M})\) and CB[8]:PMI-1 \((1:1)\) in the absence and presence of 1.0 equivalent of Tyrosine in water.
Figure S16. UV-vis absorbance spectra of PMI-1 (2x10^{-5} M) and CB[8]:PMI-1 (1:1) in the absence and presence of 1.0 equivalent of Tyrosine in water.

Figure S17. Fluorescence spectra of PMI-1 (2x10^{-5} M) and CB[8]:PMI-1 (1:1) in the absence and presence of 1.0 equivalent of phenylalanine in water.
Figure S18. UV-vis absorbance spectra of PMI-1 ($2 \times 10^{-5}$ M) and CB[8]:PMI-1 (1:1) in the absence and presence of 1.0 equivalent of phenylalanine in water.
Figure S19. $^1$H-NMR (in D$_2$O) spectra at 25 °C. (a) PGG (1.0 mM), (b) CB[8] (0.5 mM) with 2.0 equivalent PGG (1.0 mM), (c) CB[8]:PMI-1 (1:1) in the presence of 2.0 equivalent PGG (1.0 mM), and (d) PMI-1 (0.5 mM). Aromatic protons of Phe unit of PGG shifted upfield after adding 0.5 eq CB[8].
Figure S20. $^1$H-NMR (in D$_2$O) spectra at 25 °C. (top) GPG (1.0 mM), (bottom) CB[8] (0.5 mM) with 2.0 equivalent GPG (1.0 mM). Aromatic protons of GPG at 7.2-7.4 ppm broadened and shifted upfield to 6.8-7.2 ppm after adding CB[8].

Figure S21. $^1$H-NMR (in D$_2$O) spectra at 25 °C. (top) PGG and GPG (0.5 mM), (bottom) CB[8] (0.5 mM) with 1.0 equivalent PGG and GPG (0.5 mM). Aromatic protons of PGG and GPG at 7.2-7.4 ppm broadened and shifted in two set of peaks (broad peak at 7.2 ppm and a set of 3 peaks at 5.8-6.6 ppm) after adding CB[8].
Figure S22. ESI mass of CB[8]:PGG (1:2) in H$_2$O with 10% (v/v) MeOH and 5% (v/v) HCOOH (The observed mass is 943.6 that is equal to the calculated M$^2+$ value of CB[8]:PGG in 1:2 ratio).

Figure S23. ESI mass of CB[8]:GPG (1:2) in H$_2$O with 10% (v/v) MeOH and 5% (v/v) HCOOH (The observed mass is 943.8 that is equal to the M$^2+$ value of CB[8]:GPG in 1:2 ratio).
Figure S24. ESI mass of CB[8]:GGP (1:2) in H$_2$O with 10% (v/v) MeOH and 5% (v/v) HCOOH (The observed mass 945.2 is equal to the M$^2$ value of CB[8]:GGP in 1:2 ratio).

Figure S25. Partial $^1$H-NMR (in D$_2$O) spectra at 25 ºC. (top) PMI-1 and Phenylalanine (0.5 mM), (bottom) CB[8] (0.5 mM) with 1.0 equivalent PMI-1 and Phenylalanine (0.5 mM). Aromatic protons of Phe and PMI-1 at 6.6-7.4 ppm broadened after adding CB[8].
Figure S26. Fluorescence spectra CB[8]:PMI-1 (2x10^{-5} M) (1:1) in the absence and presence of PGG and GPG mixture in water.

Figure S27. A Job plot of guest PGG with CB8. The UV-vis spectra were collected from 230 to 380 nm for all samples (The total concentration of guest and host was fixed at 100 μM) and the absorbance at 257 nm (λ_{max}) was used in the calculation.
References


