Supporting Information

Water-induced Self-assembly of Amphiphilic Perylene Bisimide Dyad into Vesicles, Fibers, Coils and Rings

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1. Materials and Methods

**General:** All commercially available reagents and solvents were of reagent grade and used without further purification. Column chromatography was performed using 63–210 µm silica gel. The solvents for the preparation of assemblies were all spectral grade and used without further purification. $^1$H and $^{13}$C NMR spectra were recorded on JEOL JNM-ECA500 NMR spectrometers and chemical shifts are reported in ppm ($\delta$) with the signal of TMS as internal standard. ESI-MS spectra were measured on an Exactive (Thermo Scientific). UV/vis absorption spectra were recorded on a JASCO V660 spectrophotometer. Time-lapse UV/vis absorption spectra were recorded on a JASCO FS-110 spectrophotometer. JASCO V660 and JASCO FS-110 spectrometers were equipped with Peltier device temperature-control unit. UV/vis absorption spectra of solid samples were recorded on JASCO MSV-370 microspectrophotometers. Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano S (Malvern Instruments) equipped with a 4.0 mW He-Ne laser (633 nm). AFM images were acquired under ambient conditions using a Multimode 8 Nanoscope V (Bruker AXS) in Peak Force Tapping mode (cantilever: MPP-21100-10; spring constant = 3 N/m; frequency = 75 kHz) or in Scanasyst mode (cantilever: SCANASYST-AIR; spring constant = 0.4 N/m; frequency = 70 kHz). The samples were prepared by spin-coating the solutions onto freshly cleaved highly oriented pyrolytic graphite (HOPG). TEM images were acquired on JEM-2100F (JEOL) at acceleration voltage at 120 kV. Samples were prepared by spin-coating assembly solutions onto carbon-coated STEM Cu grid (SHR-C075) and dried under vacuum for 24 h. SEM images were acquired on JEOL JSM-6335F field emission scanning electron microscopy and JSM-6510 scanning electron microscopy. Samples were drop-cast or spin-coated on silicon or HOPG substrates, dried under vacuum for 12 h, and sputtered with Pt using JFC-1600 (JEOL) Auto Fine Coater before observation. Confocal fluorescence images were recorded on a OLYMPUS FV1000D IX81 (excitation wavelength: 543 nm) or OLYMPUS FV3000 with FV-OSR (excitation wavelength: 561 nm) and the emission was monitored from 560–660 nm. Samples were drop-cast on glass substrate and dried under vacuum for 12 h. FT/IR spectra were recorded on JASCO FT/IR-4100 spectrometer.

**Synchrotron X-ray Diffraction Analysis:** X-ray diffraction experiments were carried out on the BL45XU beamline at SPring-8 (Hyogo, Japan) using an imaging plate area detector (R-AXIS IV++) (Rigaku). The scattering vector, $q = 4\pi\sin\theta/\lambda$, and the position of the incident X-ray beam on the detector were calibrated using several orders of layer reflections from silver behenate ($d = 58.380 \text{ Å}$), where $2\theta$ and $\lambda$ refer to the scattering angle and wavelength of X-ray beam (1.00 Å), respectively. The sample-to-detector distance was 0.40 m. The obtained diffraction patterns were integrated along the Debye-Scherrer ring to afford one-dimensional intensity data using the FIT2D software (http://www.esrf.eu/computing/scientific/FIT2D/).
**Microflow Experiments:** Microfluidic devices were purchased from IMT (Institute of Microchemical Technology Co., Ltd.) as custom-made products. The microchannel was designed to feature a depth of 45 µm and a width of 100 µm; the distance from the cross-point to outlet was 75 mm, giving a total channel length of 95 mm. For a flow rate of 100 µL/min, the residence time of the injected solution in the channel would be approximately 60 ms.

A THF solution of $3 (c = 1.0 \times 10^{-4} \text{ M})$ was injected from a central stream and squeezed between two streams of water. The flow rates of the central and side solutions were adjusted (syringe pump) to 60 and 30 µL/min, respectively. Thus, the total flow rate after mixing these solutions at the crossing point was 120 µL/min. Final concentration of 3 was $5.0 \times 10^{-5} \text{ M}$ and solvent composition was THF/water = 50/50 (v/v). The resultant eluted solutions were directly casted on a silicon substrate through a Teflon capillary (inner diameter: 260 µm).

**Calculation of $\alpha_{agg}$:** Normalized molar fraction of aggregates ($\alpha_{agg}$) were calculated from molar extinction coefficient at 520 nm in concentration- and temperature-dependent UV/vis absorption spectra, based on eqn S1, in which $\varepsilon$ is the molar extinction coefficient at a given concentration or temperature, and $\varepsilon_{min}$ is the molar extinction coefficient of fully aggregated $3 (c = 2.0 \times 10^{-3} \text{ M})$ in THF at 20°C. On the other hand, for $\varepsilon_{max}$ obtainable from the fully monomeric state, we used the molar extinction coefficient of fully monomeric $3 (c = 1.0 \times 10^{-6} \text{ M})$ in THF at 60°C ($I_{0.0}/I_{0.1}$ ratio = 1.5) for concentration-dependent analysis that of fully monomeric $3 (c = 1.0 \times 10^{-5} \text{ M})$ in chlorobenzene at 20°C ($I_{0.0}/I_{0.1}$ ratio = 1.6) for temperature-dependent analysis, because PBI dyad 3 cannot be completely molecularly dissolved state ($\alpha_{agg} = 0$) under experimental conditions.

$$\alpha_{agg} = \frac{\varepsilon_{max} - \varepsilon}{\varepsilon_{max} - \varepsilon_{min}}$$  \hspace{1cm} (eqn S1)

**Synthesis and Characterization:** Amphiphilic PBI dyad 3 and its precursor 5 were synthesized according to Scheme S1. Compound $4^{S1}$, $6^{S2}$, $7^{S3}$, and $8^{S4}$ were synthesized according to the procedures reported previously.
Scheme S1. Synthesis of amphiphilic PBI dyad 3. Reagents and conditions: i) K₂CO₃, KI, DMF, 80°C; ii) Zn(OAc)₂, imidazole, 130°C.

**Compound 5**

A mixture of compound 4 (300 mg, 0.52 mmol), K₂CO₃ (72 mg, 0.52 mmol), and KI (10 mg, 0.060 mmol) in dry DMF (30 mL) was stirred for 30 min at 80°C under N₂ atmosphere. To this mixture, 1,7-dibromoheptane (1.35 g, 5.23 mmol) was added and the mixture was stirred overnight at 80°C. The reaction mixture was cooled to r.t. and evaporated to dryness. The resulting solid was purified by column chromatography (silica gel, eluent: CHCl₃) to give compound 5 as a red solid (214 mg, 55%). 

**1H NMR (500 MHz, CDCl₃):** δ = 8.64–8.56 (m, 8H, perylene- H), 5.22–5.15 (m, 1H, CH), 4.21–4.18 (m, 2H, BrCH₂), 3.41 (t, J = 6.9 Hz, 2H, NCH₂), 2.29–2.22 (m, 2H, BrCH₂CH₂), 1.91–1.84 (m, 4H, CH₂), 1.80–1.74 (m, 2H, NCH₂CH₂), 1.53–1.21 (m, 22H, CH₂), 0.83 (t, J = 7.0 Hz, 6H, CH₃);

**13C NMR (125 MHz, CDCl₃):** δ = 163.04, 134.26, 133.94, 131.03, 129.33, 129.03, 126.05, 125.99, 122.91, 122.83, 122.73, 54.84, 40.48, 33.94, 32.73, 32.37, 31.77, 29.26, 28.47, 28.07, 27.95, 26.98, 26.92, 22.61, 14.07; HRMS (ESI) m/z calcd. for C₄₄H₄₀O₄N₂BrCl 783.2559 [M+Cl⁻], found 783.2584.
Chart S1. $^1$H NMR spectrum of 5 in CDCl$_3$ at 296 K.
Chart S2. $^{13}$C NMR spectrum of 5 in CDCl$_3$ at 294 K.

Chart S3. HRMS (ESI) m/z calcd. for C$_{44}$H$_{49}$O$_{4}$N$_{2}$BrCl of 5 (chloride ion adduct).
Compound 3
A mixture of compound 5 (80 mg, 0.11 mmol), 8 (71 mg, 0.11 mmol), K$_2$CO$_3$ (18 mg, 0.13 mmol), and KI (9 mg, 0.054 mmol) in dry DMF (12 mL) was stirred for 5 h at 80°C under N$_2$ atmosphere. The reaction mixture was cooled to r.t. and evaporated to dryness. The resulting solid was purified by column chromatography (silica gel, eluent: CHCl$_3$/methanol = 49:1). This compound was further purified by preparative gel permeation chromatography (eluent: CHCl$_3$) to give compound 3 as red solid (69 mg, 47%).

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 8.47 (d, $J = 7.9$ Hz, 2H, perylene-H), 8.43 (d, $J = 7.9$ Hz, 2H, perylene-H), 8.36 (d, $J = 7.9$ Hz, 2H, perylene-H), 8.33 (d, $J = 7.9$ Hz, 2H, perylene-H), 8.25 (d, $J = 8.0$ Hz, 2H, perylene-H), 8.20–8.18 (m, 4H, perylene-H), 8.13 (d, $J = 8.0$ Hz, 2H, perylene-H), 5.68–5.63 (m, 1H, CH), 5.18–5.12 (m, 1H, CH), 4.21 (t, $J = 7.2$ Hz, 2H, OEG-H), 4.18 (dd, $J_1 = 10.6$ Hz, $J_2 = 7.7$ Hz, 2H, CH$_2$), 4.14 (t, $J = 7.5$ Hz, 2H, OEG-H), 3.97 (dd, $J_1 = 10.6$ Hz, $J_2 = 5.9$ Hz, 2H, CH$_2$), 3.76–3.72 (m, 2H, OEG-H), 3.69–3.62 (m, 6H, OEG-H), 3.59–3.57 (m, 4H, OEG-H), 3.46–3.44 (m, 4H, NCH$_2$H), 3.29 (s, 6H, CH$_3$), 2.28–2.21 (m, 2H, NCH$_2$CH$_2$), 1.94–1.87 (m, 2H, NCH$_2$CH$_2$), 1.86–1.76 (m, 4H, CH$_2$), 1.52 (br, 5H, CH$_2$), 1.45–1.27 (m, 17H, CH$_2$), 0.85 (t, $J = 6.9$ Hz, 6H, CH$_3$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 163.28, 162.89, 162.80, 133.88, 133.70, 133.56, 130.79, 130.65, 129.14, 129.01, 128.85, 125.67, 123.26, 123.16, 122.91, 122.53, 122.35, 122.28, 72.06, 70.66, 70.54, 70.52, 69.46, 58.91, 54.99, 52.61, 40.68, 40.59, 32.53, 31.82, 29.61, 29.28, 28.16, 27.11, 26.81, 26.67, 22.63, 14.00; HRMS (ESI) m/z calcd. for C$_{81}$H$_{85}$O$_{14}$N$_4$ 1337.6057 [M+H]$^+$, found 1337.6047.

Chart S4. $^1$H NMR spectrum of 3 in CDCl$_3$ at 333 K.
Chart S5. $^{13}$C NMR spectrum of 3 in CDCl$_3$ at 333 K.

Chart S6. HRMS (ESI) $m/z$ calcd. for C$_{81}$H$_{85}$O$_{14}$N$_4$ of 3.
2. Supporting Figures and Tables

Figure S1. Chemical structure and UV/vis absorption spectrum (in THF, $c = 1.0 \times 10^{-4}$ M) of amphiphilic PBI monoad.

Figure S2. AFM images of 3 in THF at (a) $1.0 \times 10^{-4}$ M and (b) $5.0 \times 10^{-4}$ M.
Figure S3. (a–d) Temperature-dependent UV/vis absorption spectra (20–60 °C) in THF at $c = 5.0 \times 10^{-6} \text{ M}$ (a), $c = 7.0 \times 10^{-6} \text{ M}$ (b), $c = 1.0 \times 10^{-5} \text{ M}$ (c), and $c = 1.2 \times 10^{-5} \text{ M}$ (d). (e–h) Temperature-dependent UV/vis absorption spectra (20–60 °C) in THF/water = 90:10 at $c = 5.0 \times 10^{-6} \text{ M}$ (e), $c = 7.0 \times 10^{-6} \text{ M}$ (f), $c = 1.0 \times 10^{-5} \text{ M}$ (g), and $c = 1.2 \times 10^{-5} \text{ M}$ (h).
Figure S4. (a,b) Temperature dependence (20–60 °C) of the molar fraction of aggregated molecules ($\alpha_{agg}$) estimated from UV/vis absorption spectral changes of 3 in THF (a) and in THF/water mixtures (90:10, v/v) (b) at varying concentrations (red: $c = 5.0 \times 10^{-6}$ M; green: $c = 7.0 \times 10^{-6}$ M; blue: $c = 1.0 \times 10^{-5}$ M; purple: $c = 1.2 \times 10^{-5}$ M). Black dotted curves represent the corresponding fits according to the isodesmic model. (c, d) van’t Hoff plot for 3 in THF (c) and THF/water mixtures (90:10, v/v).
Figure S5. (a) Time-lapse UV/vis absorption spectral changes at time intervals of 20 s for 5 min when water was added into a THF solution of 3 (final THF/water ratio is 50:50 and the final concentration is $1.75 \times 10^{-5}$ M). Arrows indicate the absorption changes with time. (b) Plot of $\varepsilon$ at 494 nm as a function of time.

Figure S6. Temperature-dependent UV/vis absorption spectra of vesicular aggregates of 3 ($c = 5.0 \times 10^{-5}$ M) in THF/water 10:90 mixture. (a) Heating and (b) subsequent cooling processes. (c) Plot of the absorbance maxima versus temperature.
**Figure S7.** Temperature-dependent DLS z-average sizes of vesicular aggregates of 3 (c = 5.0 × 10⁻⁵ M) in THF/water 10:90 mixture.

**Figure S8.** (a) CLSM, (b) SEM and (c) AFM images of vesicular aggregates of 3. (d) Cross-section analysis along the yellow line in (c).
Figure S9. IR spectra of vesicular aggregates of 3 \((c = 5.0 \times 10^{-5} \text{ M})\) in THF/water 10:90 mixture (blue line), nanofibers of 3 \((c = 5.0 \times 10^{-5} \text{ M})\) in THF/water 50:50 mixture (red line), and nanocoils of 3 \((c = 5.0 \times 10^{-5} \text{ M})\) in THF/water 10:90 mixture (green line) in (a) C–H stretching vibrational region and in (b) C–H deformation vibrational region.

Figure S10. (a) UV/vis absorption spectra and (b,c) SEM images of vesicular aggregates of 3 \((c = 5.0 \times 10^{-5} \text{ M})\) before and after dialysis of THF from THF/water 10:90 mixture.
Figure S11. Time-dependent UV/vis absorption spectra of 3 ($c = 5.6 \times 10^{-5}$ M) in (a) THF/water 30:70 and (b) THF/water 40:60 prepared by addition of THF into a vesicular solution (THF/water 10:90). Time scale: 1 min to 13 h for (a) and 1 min to 13 min for (b).

Figure S12. Additional SEM images of nanocoils of 3 prepared in a vial.

Figure S13. CLSM image of nanocoils of 3 prepared in a vial.
**Figure S14.** Black spectrum: microscopic absorption spectrum of nanocoils of 3 prepared in a vial. Samples were deposited on quartz substrate. Red spectrum: microscopic absorption spectrum of nanofibers of 3.

**Figure S15.** SEM images of aggregates of 3 formed upon cooling its THF/water 50:50 solution from 80 to 20 °C.
Figure S16. SEM image of fibers formed by standing homogeneous THF/water 50:50 solution of 3 (\(c = 5 \times 10^{-5}\) M) at 60°C overnight. Nanocoils initially formed (Figure S14b) were not observed.

Figure S17. Additional SEM images of nanorings of 3 prepared by the microfluidic device.
**Table S1.** Thermodynamic parameters obtained from isodesmic model fitting of the temperature-dependent UV/vis data of 3 in THF and THF/water 90:10 mixture at different concentrations.

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<th>$\Delta H^{\circ}$/ kJ mol$^{-1}$</th>
<th>$T_m^{[a]}$/ K</th>
<th>$K_{iso}^{[b]}$/ 10$^3$ M$^{-1}$</th>
<th>$DP_n^{[c]}$/ J mol$^{-1}$ K$^{-1}$</th>
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[a] $T_m$ is the melting temperature defined as the temperature in which $\alpha = 0.5$.

[b] $K_{iso}$ is the aggregation constant at 293 K.

[c] $DP_n$ is the number-averaged degree of polymerization at 293 K.
Table S2. Thermodynamic parameters obtained from the van’t Hoff plot of the temperature-dependent UV/vis data of 3 in THF and THF/water 90:10 mixture at different concentrations.

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<th>c / µM</th>
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[a] Values determined at 293 K.

3. Supporting References


