Electronic Supplementary Information

Regulating Morphologies and Near-infrared Photothermal Conversion of Perylene Bisimide by Sequence-Dependent Peptide Self-Assembly

*Lingyun Cui, Yang Jiao, Anhe Wang, Luyang Zhao, Qianqian Dong, Xuehai Yan, Shuo Bai**

E-mail: baishuo@ipe.ac.cn

Materials: All chemicals and materials used in this work were of analytical grade without further purification. All the chemicals were purchased from Sigma-Aldrich with the exceptions of 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), which were purchased from Novabiochem. The phosphate buffer solution (PBS, pH 8) was prepared by adding $NaH_2PO_4 \cdot H_2O$ (94 mg) and $Na_2HPO_4 \cdot 7H_2O$ (2.5 g) into 100 mL water. The water applied in all experiments was purified in a Milli-Q plus 185-purification system with a resistivity higher than 18.2 MΩ.

Characterization and instrumentation. JAS.C.O V-660 spectrophotometer (scanning speed of 400 $nm \cdot min^{-1}$) was used to measure the spectra of UV-Vis in the range from 300 to 700 nm. Data of emission fluorescence were recorded by a JAS.C.O FP-6500 spectrofluorometer, which measured the light orthogonally to the

excitation light with bandwidth and data pitch of 3 nm. The FTIR (Fourier Transform Infrared Spectroscopy) data was obtained from a Bruker Vertex 70 spectrometer with a resolution of 1 cm^{-1}. To prepare the samples for FTIR, peptide aggregates were placed in the space formed by two CaF_2 plates (size 2 mm) taken a polytetrafluoroethylene (PTFE, 50 μm) as spacer.

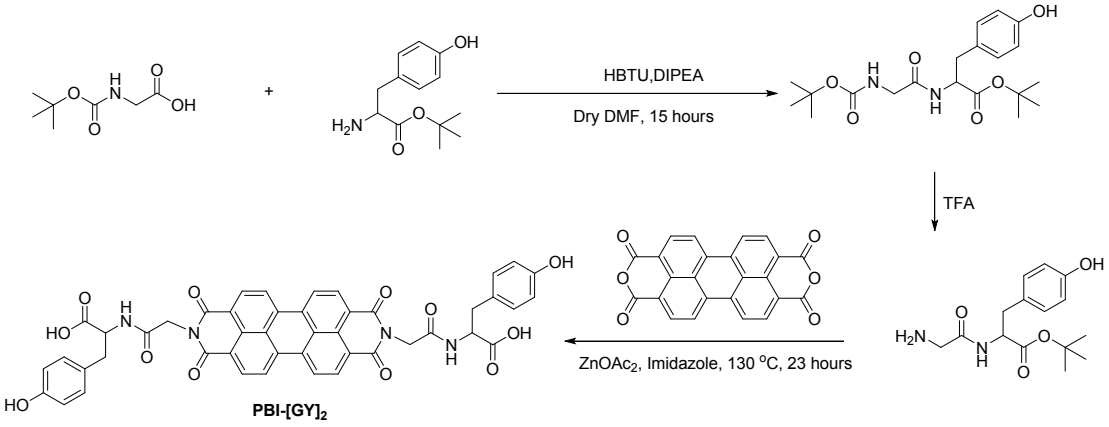
For the AFM (Atomic Force Microscopy) measurement, Veeco diINNOVA Scanning Probe Microscope (VEECO/BRUKER, Santa Barbara, CA, USA) were performed in tapping mode under ambient conditions with 512 x 512 pixels resolution. 20 μl of samples were dropped on a freshly cleaved mica sheet (G250-2 Mica sheets 1" x 1" x 0.006"; Agar Scientific Ltd, Essex, UK) which was placed on an AFM stage and kept in a dust free environment overnight. For a typical AFM operation, the parameters were set as integral and proportional gains 0.3 and 0.5 respectively, tapping frequency 308 kHz, set point 0.5-0.8 V and scanning speed 1.0 Hz.

Density-functional-theory (DFT) simulation. The B3LYP level of theory with the Gaussian 09 computer code was run in all computations, which is a widely used density functional in quantum chemistry and get great success in solving chemical problems. The 6-31G (d,p) basis set has been used for geometry optimization. Frequency calculations were performed to verify the minima nature of reactant and product. Different amount of PBI-$[GY]_2$ molecules and solvent molecules have been chosen. THF molecules could only be the proton acceptor in the hydrogen bond and occupy the proton donors in PBI molecules. Water molecules could be both proton donor and acceptor forming hydrogen bond network.

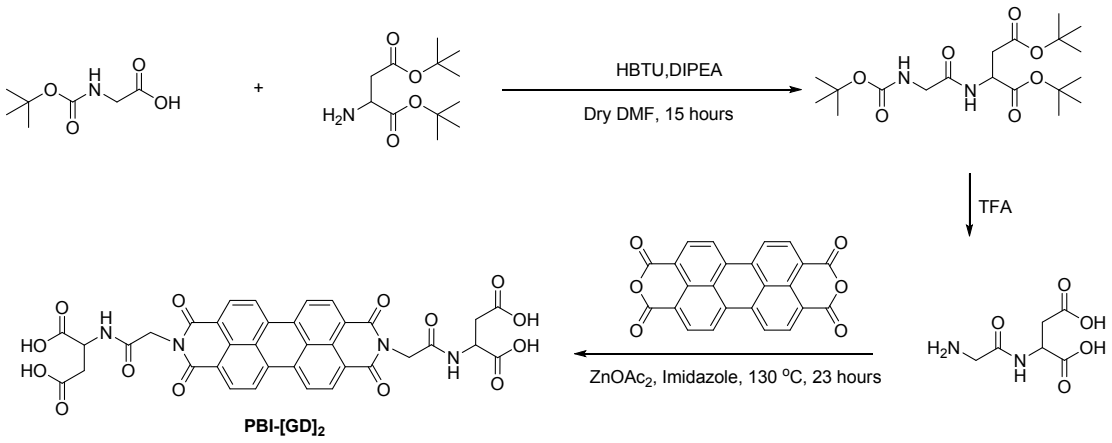
All of these structures were optimized with explicit solvent model in periodical boxes of 40Å×40Å×40Å employing COMPASS force field. Amorphous Cell, Discovery, and Blends modules in Materials Studio software (Accelrys Inc.) was used to calculate coordination numbers and binding energies considering the inter- and intra-molecular interactions (electronic, van der waals, and hydrogen bonding et al.). The densities of the boxes were set at 1.0000 g mL^{-1} for aqueous solvent and 0.8892 g mL^{-1} for the THF solvent. In the Figures, solvent molecules are omitted, except for the close contact ones direct linked to PBI-$[GY]_2$ molecules via hydrogen bonds.

Synthesis of PBI-$[GY]_2$ and PBI-$[GD]_2$ [1]

PBI-$[GY]_2$:



PBI-[GD]$_2$:



Scheme S1 General synthetic scheme for the synthesis of PBI-[GY]$_2$ and PBI-[GD]$_2$.

Synthesis of PBI-[GY]$_2$:

Boc-Glycine (0.81 g, 4.64 mmol), L-Tyrosine tert-butyl ester (1.0 g, 4.21 mmol) and 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1.91g, 5.02 mmol) were mixed together in 10 mL dry DMF. Then, diisopropylethylamine (1.93 mL, 10.52 mmol) was added to this solution and the mixture was stirred overnight under nitrogen atmosphere. After reaction, the product was extracted by ethyl acetate (75 mL) after successive wash with 25 mL of 1 N $NaHCO_3$ and 25 mL of 1 N hydrochloric acid, and then dried over $MgSO_4$. After evaporation of the solvent, the compound was purified by column chromatography on silica gel using dichloromethane/methanol (96:4) as eluent (1.3 g, yield: 71 %).

After purification, the Boc group of N-terminus and tert-butyl group of C-terminus was removed by one step reaction using trifluoroacetic acid (TFA, 5 mL) in dry dichloromethane for 24 hours. The excess TFA was removed by high vacuum pump and the product was washed thoroughly for 3 times with diethyl ether to get pure GY dipeptide (0.76 g, yield: 97%).

Finally, the dipeptide was attached at the both ends of Perylene-3,4,9,10-tetracarboxylic dianhydride. 0.3 g (0.76 mmol) of Perylene-3,4,9,10-tetracarboxylic dianhydride was mixed with 0.73 g (3.06 mmol) of GY dipeptide, 10 g of imidazole as a solvent and 0.145 g (0.79 mmol) of Zinc acetate. The mixture was heated at 130

^{o}C for 24 hours under nitrogen atmosphere. The product was cooled down and imidazole dissolved in 1 N hydrochloride acid filtered in the product. The residue was collected and dissolved in 1 N NaOH, filtered, and precipitated out by adding 1 N HCl. This process was repeated for 4 times to give pure PBI-$[GY]_2$. The final yield of the product was 0.25 g.

^{1}H NMR (DMSO-d_6, 90 ^{o}C, 400 MHz) δ 2.87-2.96 (m, 4H, CH_2 in tyrosine moiety), 4.45-4.47 (m, 2H, CH in tyrosine moiety), 4.74 (s, 4H, N-CH_2), 6.68 (d, 4H, CH at orthoposition of OH group of tyrosine, J = 8.8 Hz), 7.03 (d, 4H, CH at meta position of OH group of tyrosine, J = 8.8 Hz), 8.19 (d, 2H, CONH, J = 7.4 Hz), 8.40-8.42 (m, 4H, perylene aromatic CH), 8.67-8.69 (m, 4H, perylene aromatic CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 35.8 (Tyr CH_2), 47.6 (NCH_2), 53.4 (Tyr chiral CH), 107.4 (Ar CH), 115.05 (Ar CH), 122.1 (Ar CH), 122.8 (Ar Cq), 127.2 (Ar Cq), 130.1 (Ar CH), 134.0 (Ar Cq), 134.5 (Ar Cq), 139.1 (Ar Cq), 156.5 (Ar Cq), 170.2 (C=O), 172.7 (C=O), 179.8 (C=O).

ESI-MS: m/z: calculated for $C_{46}H_{32}N_4O_{12}$: 832.20; found: 831.0 [M -H].

Synthesis of PBI-$[GD]_2$:

Boc-Glycine (1.1 g, 6.28 mmol), H-Asp(Otbu)-Otbu·HCl (1.6 g, 5.71 mmol) and HBTU (2.6 g, 5.02 mmol) were mixed together in 15 mL dry DMF. Then 2.6 mL (14.27 mmol) of diisopropylethylamine was added to this solution and the mixture was stirred overnight under nitrogen atmosphere. After reaction, the product was extracted by 100 mL of ethyl acetate after successive wash with 30 mL of 1 N $NaHCO_3$ and 30 mL of 1 N hydrochloric acid, and then dried over $MgSO_4$. After evaporation of the solvent, the compound was purified by column chromatography on silica gel using dichloromethane/ methanol (96:5) as eluent (1.9 g, yield:76 %).

After purification the Boc group of N-terminus and both the tert-butyl group of C-terminus was removed by one step reaction using trifluoroacetic acid (TFA, 8 mL) in dry dichloromethane for 24 hours. The excess TFA was removed by high vacuum pump and the product was washed thoroughly for 3 times with diethyl ether to get pure GD dipeptide (0.8 g, yield: 97%).

Finally, the dipeptide was attached at the both ends of Perylene-3,4,9,10-

tetracarboxylic dianhydride 0.35 g (0.89 mmol) of Perylene-3,4,9,10-tetracarboxylic dianhydride was taken with 0.75 g (3.94 mmol) of GD dipeptide, 10 g of imidazole as a solvent and 0.179 (0.97 mmol) of Zinc acetate. This mixture was heated at 130 ^{o}C for 24 hours under nitrogen atmosphere. The product was cooled down and imidazole dissolved in 1 N hydrochloride acid filtered in the product. The residue was collected and dissolved it in 1 N NaOH, filtered, and precipitated out by adding 1 N HCl. This process was repeated for four times to get pure PBI-$[GD]_2$. The final yield of the product was 0.26 g.

^{1}H NMR (DMSO-d_6, 90 ^{o}C, 400 MHz) δ 2.66-2.75 (m, 4H, CH_2 in aspartic acid moiety), 4.64-4.75 (m, 6H, CH in aspartic acid moiety and N-CH_2), 8.27-8.45 (m, 10H, perylene aromatic CH and CONH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 33.2 (Asp CH2), 47.3 (NCH_2), 51.2 (Asp chiral CH), 115.5 (Ar CH), 122.1 (Ar Cq), 127.7 (Ar Cq), 130.3 (Ar CH), 134.5 (Ar Cq), 134.8 (Ar Cq), 139.3 (Ar Cq), 156.5 (Ar Cq), 170.8 (C=O), 172.1 (C=O), 179.3 (C=O), 180.1 (C=O).

ESI-MS: m/z: calculated for $C_{36}H_{24}N_4O_{14}$: 736.13; found: 759.14 [M +Na].

Methods:

Preparation of PBI-$[GY]_2$ and PBI-$[GD]_2$ solution : The preparation of 1.0 mM PBI-$[GY]_2$ and PBI-$[GD]_2$ solution: PBI-$[GY]_2$ (8.34 mg, 0.01 mM) and PBI-$[GD]_2$ (7.38 mg, 0.01 mM) were added to 10 mL PBS buffer solution (pH = 8.0), then the solution was carried out by ultrasonic for one hour. The above obtained mother liquor was diluted according to different proportions for various concentrations of the solutions

To optimize the ratio between PBI with $Na_2S_2O_4$, The titration were carried out in standard quartz cuvettes (10 mm path length) sealed with screw-caps equipping with a silicone septum for argon purging with a needle. The PBI-$[GY]_2$ or PBI-$[GD]_2$ (0.15 mM, 2 mL) PBS solution was constantly bubbled for 30 min by nitrogen gas, The sodium dithionite solution (in pH = 8.0, PBS buffer) (7.5 mM, 1mL) was freshly prepared and then injected into the PBI-$[GY]_2$ or PBI-$[GD]_2$ solution with a needle, the solution was stirred, and the absorption spectra were recorded.

The photothermal experiments of PBI-$[GY]_2$ or PBI-$[GD]_2$ were carried out as the

following: First, the PBI-$[GY]_2$ or PBI-$[GD]_2$ free radical solution with different concentration (0.02, 0.15, 1, 10 mM) were prepared, then was irradiated under a 808 nm laser with 1.0 $W \cdot cm^{-2}$ at room temperature (25 °C).

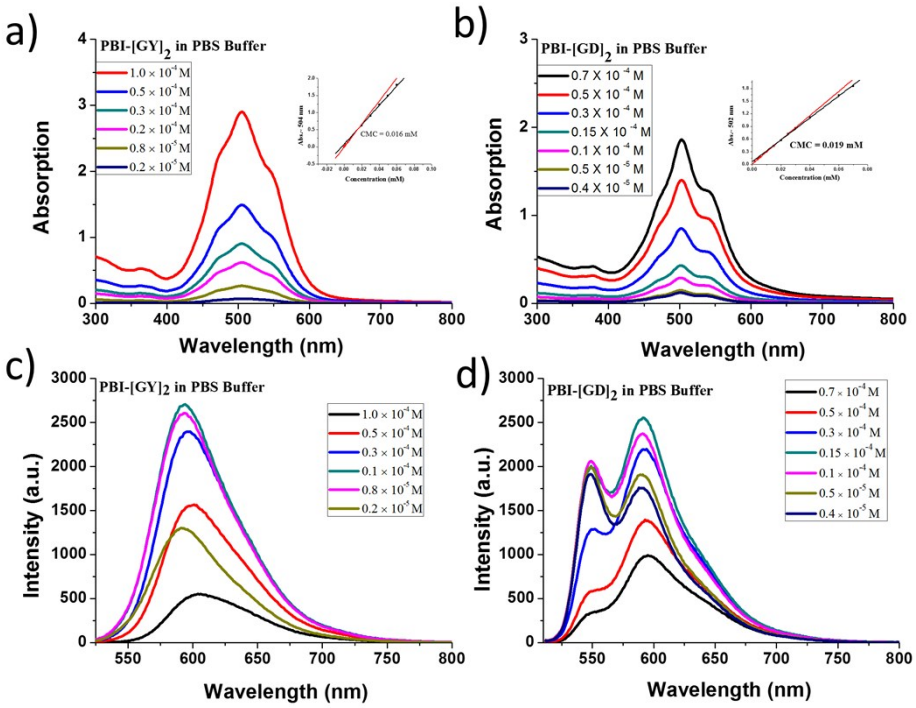


Fig. S1 Concentration-dependent UV−Vis of a) PBI-$[GY]_2$ and b) PBI-$[GD]_2$, and the corresponding fluorescence emission spectra of c) PBI-$[GY]_2$ and d) PBI-$[GD]_2$ b) in PBS buffer solution.

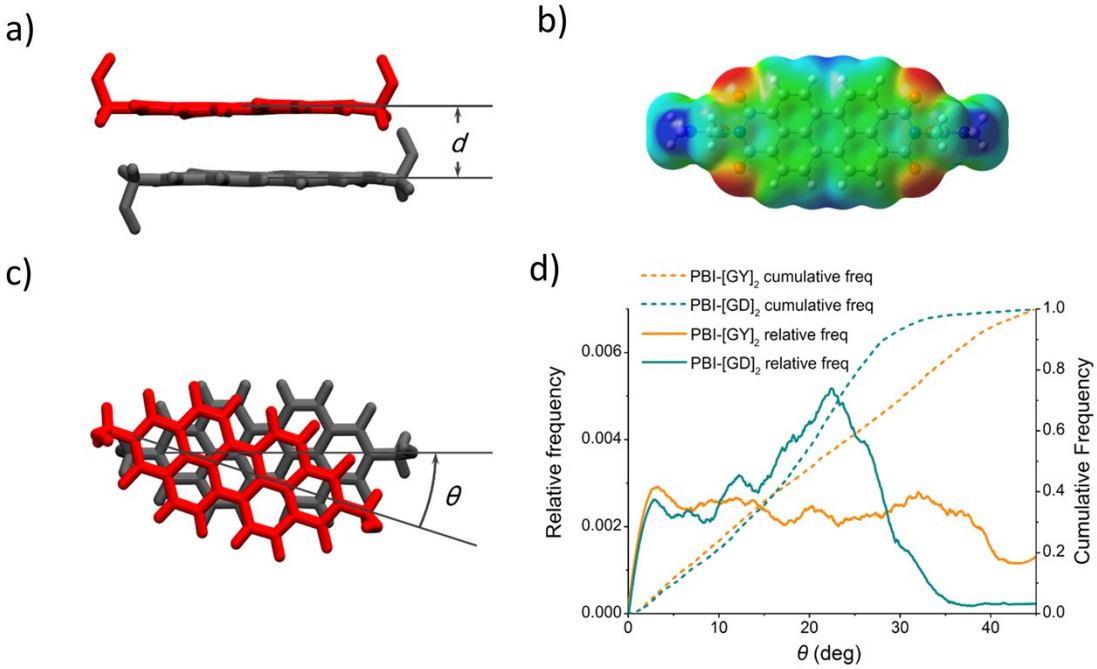


Fig. S2 a) Front view of stacked PBI-G_2 moiety dimers with intermolecular distance *d*; b) Electrostatic potential map of PBI moiety at an iso-value density of 0.0004 a.u. ranging from 0.04 (blue, electron rich) to −0.04 a.u. (red, electron deficient); c) Top view of stacked PBI moiety dimers with intermolecular angle θ; d) θ distribution frequencies (counted by time).

Table S1 Energy difference from start (0 ns) to end (150 ns): the contributions of PBI and terminal amino acid. Coulomb refers to charge interaction, L-J refers to Van der Waals interaction, and ΔE_x (Total) = ΔE_x (Coulomb) + ΔE_x (L-J), where x = PBI or amino-acid. The interaction of each moiety includes that with itself, water, and ions. The unit is kJ·mol^{-1}.

	PBI-[GY]$_2$	PBI-[GD]$_2$
ΔE_{PBI} (Coulomb)	22.3	13.1
ΔE_{PBI} (L-J)	−11.9	−15.3
ΔE_{PBI} (Total)	10.4	−2.2
$\Delta E_{amino\text{-}acid}$ (Coulomb)	13.9	−90.4
$\Delta E_{amino\text{-}acid}$ (L-J)	5.5	6.7
$\Delta E_{amino\text{-}acid}$ (Total)	19.4	−83.8
ΔE (Total)	**29.8**	**-86**

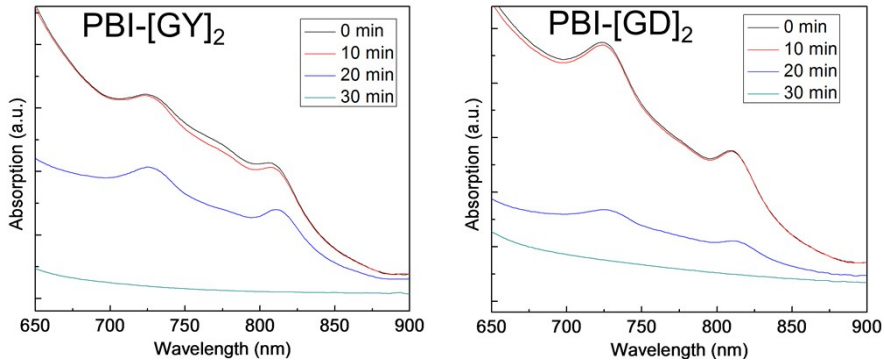


Fig. S3 Uv change of formed PBI radical anions Vs time (the concentration of PBI-$[GY]_2$ and PBI-$[GD]_2$ is 2 mM; the ratio of PBI-$[GY]_2$: $Na_2S_2O_4$ and PBI-$[GD]_2$:$Na_2S_2O_4$ are 1:0.35 and 1:0.6, respectively).

The calculation of NIR photothermal conversion efficiency of PBI-$[GY]_2$ and PBI-$[GD]_2$ radical anions.

The efficiency of NIR photothermal conversion was estimated by using the calculation method reported in the literatures.[2-4] According to the energy balance during the process of photothermal conversion, we can get the equation as follows:

$$C_p m\frac{dT}{dt} = hA(T_{max} - T)$$

where m is the total mass of the solution, C_p is the heat capacity of the buffer (can be approximate to water), h is the coefficient of heat transfer, A is the irradiated area, T is the real-time temperature of the solution, and T_{max} is the temperature of the solution when the system reaches the final equilibrium.

Then we defined a dimensionless parameter as the driving force of temperature:

$$\theta = \frac{T - T_s}{T_{max} - T_s}$$

where T_s is the surrounding temperature. By this way we can get a simplified equation as follows for the linear fitting:

$$\ln(1 - \theta) = -\frac{hA}{c_p m} \cdot t$$

Considering that the value of $c_p m$ is constant, the value of hA can be calculated by

the linear fitting based on the data of photothermal experiments. As an example, after reduction 10 mM PBI-$[GD]_2$ solution was irradiated by NIR light and the elevation of temperature was recorded. Through the linear fitting method, we could achieve the value of hA, as shown in Figure S3.

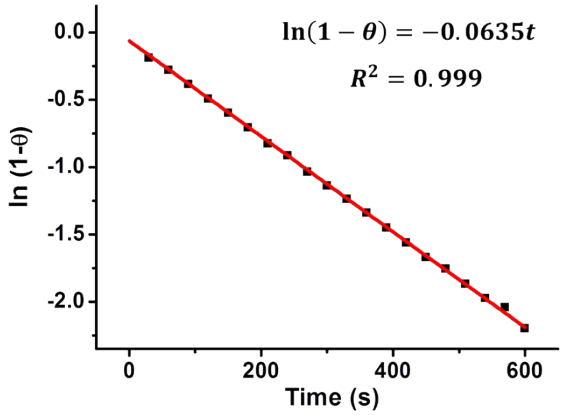


Fig. S4 The calculation of the value of hA by linear fitting of temperature driving force θ to the irradiation time t for the PBI-$[GD]_2$ radical anion (the concentration of PBI-$[GD]_2$ is 10 mM).

Since the photothermal conversion efficiency η is defined as the fraction of the total light energy that is converted to heat, we can calculate it based on the values of T_{max}, T_s and hA by the following relationship:

$$\eta = \frac{Q}{I} = \frac{hA(T_{max} - T_s)}{I}$$

Similarly, the photothermal conversion efficiency η for the other samples could also be calculated, and the photothermal conversion experiments were performed three times for each sample to reduce the random errors.

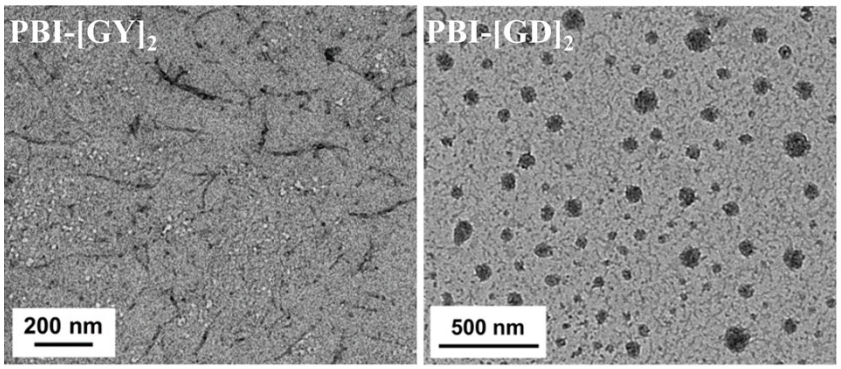


Fig. S5 The TEM topography image of PBI-$[GD]_2$ and PBI-$[GD]_2$ after the reduction by $Na_2S_2O_4$.

References

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