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When Self-assembly Meets Topology: An Enhanced Micellar Stability

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Experimental Section

Materials

Corannulene (COR) was kindly provided by Prof. Jay Siegel from Tianjin University. 3-Bromoperylene (PER-Br) was purchased from Shanghai Bide Phamatech Ltd. (Shanghai, China). Methoxy-poly(ethylene glycol) alkyne (mPEG-Alkyne, Mw = 1000 Da) was sourced from XingJiaFeng Science & Technology Development Co., Ltd. (Shenzhen, China). Bis(triphenylphosphine)palladium(II) dichloride, bromine and ferric chloride ($FeCl_3$) were obtained from InnoChem Science & Technology Co., Ltd (Beijing, China). Copper(I) iodide (CuI), magnesium sulfate ($MgSO_4$) and sodium thiosulfate ($Na_2S_2O_3$) were obtained from Heowns Biochemical Technology Co., Ltd (Tianjin, China). Triethylamine (TEA), tetrahydrofuran (THF), *N, N*-dimethylformamide (DMF), ethanol and acetonitrile (ACN) was purchased from Concord Technology Co., Ltd. (Tianjin, China). 1,2-dichloroethane was purchased from Jingchun Reagent Co., Ltd (Shanghai, China). Dichloromethane (DCM), methanol, ethyl acetate, and petroleum ether were acquired from Jiangtian Chemicals (Tianjin, China). Deuterated chloroform was obtained from Jinouxiang Science & Technology Co., Ltd. (Beijing, China). Certified fetal bovine serum was purchased from (Biolnd, Cromwell, USA).

Instruments

Nuclear magnetic resonance analysis (^{1}H NMR and ^{13}C NMR) was carried out on a Bruker AVANCE III (400 MHz or 600 MHz) NMR spectrometer with deuterated chloroform as the solvent. High resolution mass spectrometry (HRMS) analysis was performed on a Bruker quadrupole time-of-flight mass spectrometer at 25°C; the mobile phase was acetonitrile (0.4 mL/min) and the injection volume was 0.2 μL. The absorption spectra of micelles were obtained by an Agilent Cary 60 ultraviolet-visible spectrophotometer. The fluorescence analysis was conducted by a FLS980 fluorescence spectrometer (Edinburgh Instruments Ltd.) Dynamic light scattering was performed on Zetasizer Nano ZS (Malvern instruments Ltd).

Synthesis of Cor-Br

Cor (0.50 g, 2.0 mmol) and $FeCl_3$ (32.50 mg, 0.20 mmol) was dissolved in 3 mL DCM at -78°C. Bromine (0.38 g, 2.4 mmol) was also dissolved in 20 mL DCM (20 mL); then the bromine solution was carefully transferred to the above Cor solution. The mixture was maintained at ambient temperature with constant stirring under nitrogen protection. After 6 h, a saturated aqueous solution of $Na_2S_2O_3$ was added to the mixture, followed by water washing in triplicate, $MgSO_4$ treatment, filtration and DCM removal under vacuum. The crude product was purified by flash column chromatography with petroleum ether as the eluent to get Cor-Br (yield: 57.3%). ^{1}H NMR (400 MHz, Chloroform-*d*, ppm): 8.02 (s, 1H), 7.93 (d, 1H), 7.86 (d, 1H), 7.82–7.77 (m, 5H), 7.70 (d, 1H).

Synthesis of mPEG-Cor

The synthesis of mPEG-Cor employed previous reports with minor modification.[S1,S2] Briefly, mPEG-Alkyne (120.0 mg, 0.12 mmol), $PdCl_2(PPh_3)_2$ (3.3 mg, 0.005 mmol), and CuI (2.0 mg, 0.011 mmol) were added to a mixture of 1,2-dichloroethane (1 mL) and triethylamine (2 mL). Then COR-Br (32.9 mg, 0.10 mmol) was supplemented dropwise to the above solution. The reaction was maintained at 80°C under nitrogen atmosphere. After 24 h, the crude product was purified by column chromatography with a mixture of ethyl acetate and methanol (9:1, v/v) as the eluent to get mPEG-Cor (yield: 70.8%). ^{1}H NMR (600 MHz, Chloroform-*d*, ppm): 8.02 (d, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.85 (d, 1H, Ar-H), 7.83–7.77 (m, 5H, Ar-H), 7.74 (d, 1H, Ar-H), 4.60 (s, 2H, -C≡CCH_2), 3.90–3.52 (m, 87H, -CH_2CH_2O), 3.38 (s, 3H, CH_3). ^{13}C NMR (150 MHz, Chloroform-*d*, ppm): 136.07, 135.62, 135.30, 135.08, 131.58, 131.19, 131.10, 130.93, 130.90, 130.17 (2C), 127.63, 127.47, 127.44, 127.40, 127.13, 126.61 (2C), 125.95, 88.80, 84.49, 71.89, 70.63, 70.49, 69.26, 59.40, 59.04. ESI-HRMS *m/z* calc. for $C_{24}H_{14}O(C_2H_4O)_n$ $[M + Na]^+$: 1309.6704 (n = 22); found 1309.6701 (n = 22).

Synthesis of mPEG-Per

Per-Br (80.0 mg, 0.24 mmol) and mPEG-Alkyne (200.0 mg, 0.2 mmol) were dissolved in a mixture of anhydrous DMF (2 mL) and triethylamine (4 mL). Afterwards, $PdCl_2(PPh_3)_2$ (7.2 mg, 0.01mmol) and CuI (1.0 mg, 0.005 mmol) were added to the above solution. The reaction was kept at 120°C with the presence of nitrogen protection. After 24 h, the products were purified by column chromatography with mixed ethyl acetate and methanol (9:1, v/v) as the eluent to obtain mPEG-Per (yield: 69.4%). ^{1}H NMR (400 MHz, Chloroform-*d*, ppm): 8.19-8.04 (m, 4H, Ar-H), 8.01 (d, 1H, Ar-H), 7.65–7.53 (m, 3H, Ar-H), 7.47 (t, 1H, Ar-H), 7.39 (td, 2H, Ar-H), 4.52 (s, 2H, -C≡CCH_2), 3.90-3.39 (m, 89H, -CH_2CH_2O), 3.29 (s, 3H, CH_3). ^{13}C NMR

(100 MHz, Chloroform-*d*, ppm): 134.58, 134.47, 131.81, 131.31, 131.24, 130.76, 130.44, 128.35, 128.28, 128.00, 127.21, 126.60, 126.5, 125.91, 120.86 (2C), 120.61, 120.54, 119.51, 119.42, 90.96, 84.65, 72.50, 71.84, 70.48, 70.23, 69.20, 61.52, 58.94. ESI-HRMS *m/z* calc. for $C_{24}H_{16}O(C_2H_4O)_n$ $[M + Na]^+$: 1311.6861 (n = 22); found 1311.6673 (n = 22).

Micelles generation and characterization

mPEG-Cor and mPEG-Per micelles were prepared by a typical dialysis method.[S3] In brief, a fixed amount of mPEG-Cor or mPEG-Per were dissolved in THF before dialyzed against water (molecular weight cut-off/MWCO: 500 Da). The generation of hybrid micelles employed the same method with the feeding of mPEG-Cor and mPEG-Per at different ratios (75:25, 50:50, and 25:75, w/w). The micelles were subject to UV-vis and fluorescence analysis at pre-defined concentration (10 or 100 μM). The corresponding Cor and Per in ACN was used as the control. The hydrodynamic diameters of micelles were obtained by dynamic light scattering at ambient temperature. Micelle stability was characterized using CMC as the indicator. As both Cor and Per display intrinsic fluorescence, an external probe was not employed. A series of micelles ranging from 1 μg/mL to 100 μg/mL were prepared. The CMC of mPEG-Cor was determined via plotting Cor's maximum fluorescence over the mPEG-Cor concentration (E_x = 263 nm/slit = 2 nm, E_m = 450-600 nm/slit = 5 nm). The CMC of mPEG-Per and hybrid micelles utilized the maximum fluorescence of Per (E_x = 256 nm/slit = 2 nm, E_m = 450-600 nm/ slit = 5 nm). The inflexion point was defined as the CMC (n = 3).

Micelle dilution-induced fluorescence variation

A range of aqueous stock solution of five types of micelles were prepared and diluted with different portion of ACN (up to 100% ACN) to maintain a constant eventual micelle concentration of 100 μg/mL. The fluorescence spectra of mPEG-Cor micelles were obtained (E_x = 263 nm/slit = 2.5 nm, E_m = 300-600 nm/slit = 5 nm). The spectra of hybrid micelles (HM-1 and HM-2) employed the fluorescence of Per (E_x = 256 nm/slit = 2.5 nm, E_m = 300-600 nm/slit = 5 nm). Similarly, the spectra of mPEG-Per micelles and hybrid micelles (HM-3) were generated at a narrower slit condition (E_x = 256 nm/slit = 2.5 nm, E_m = 300-600 nm/slit = 2.5 nm). The extent of fluorescence variation upon ACN dilution was a reflection of micelle stability. At the same dilution conditions, the higher the degree of fluorescence increase, the lower the micelle stability.

Micelle stability in serum-containing medium

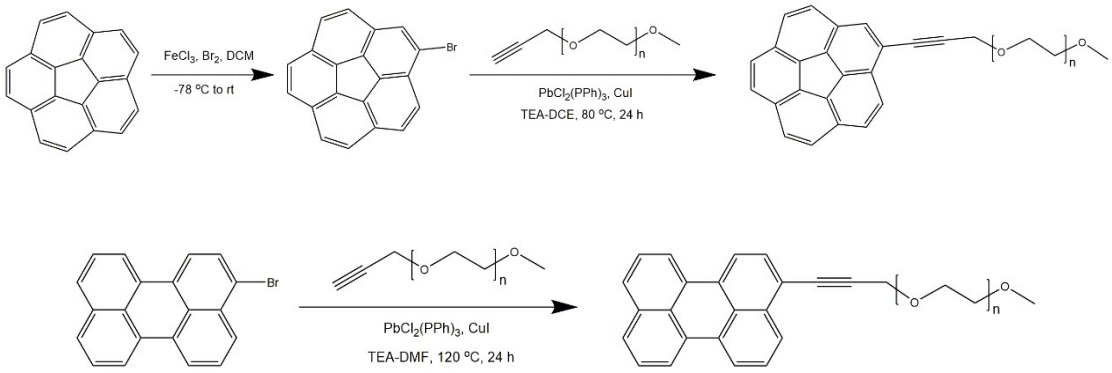
Based on a previously published protocol with minor modification, we assessed the stability of mPEG-Cor and mPEG-Per micelles in serum-containing medium.[S4] In brief, mPEG-Cor or mPEG-Per micellar solution in water (1 mM) was diluted with FBS to reach a final concentration of 100 μM. The temperature was maintained at 37°C. The emission spectra of both types of conjugate micelles were recorded at 0 h, 0.5 h, 1 h, 2 h, 3 h, and 4 h (E_x = 270 nm/slit = 2.5 nm, E_m = 350-600 nm/slit = 5 nm). A preliminary test was made to ensure no fluorescence inference from FBS at the above conditions. At different time points, the fluorescence (F_t) at 450 nm (mPEG-Cor) or 500 nm (mPEG-Per) was divided by the corresponding fluorescence intensity at 0 h (F_0); the ratio was plotted against time. A higher slope indicates inferior micelle stability.

Statistical analysis

The hydrodynamic diameters and CMC of five types of micelles were statistically analyzed by Analysis of Variance (ANOVA) with appropriate post-hoc analysis. The threshold P value was set at 0.01.

References

S1. B. S. Kim, D. J. Hong, J. Bae and M. Lee, *J. Am. Chem. Soc*., 2005, **127**, 16333.
S2. M. C. Stuparu, *Angew. Chem. Int. Ed*., 2013, **52**, 7786.
S3. R. Yang, S. Zhang, D. Kong, X. Gao, Y. Zhao and Z. Wang, *Pharm. Res*., 2012, **29**, 3512.
S4. J. Lu, S. C. Owen and M. S. Shoichet, *Macromolecules*, 2011, **44**, 6002.



Scheme S1. Synthetic route of 1-bromocorannulene, methoxy poly(ethylene glycol)-corannulene and methoxy poly(ethylene glycol)-perylene.

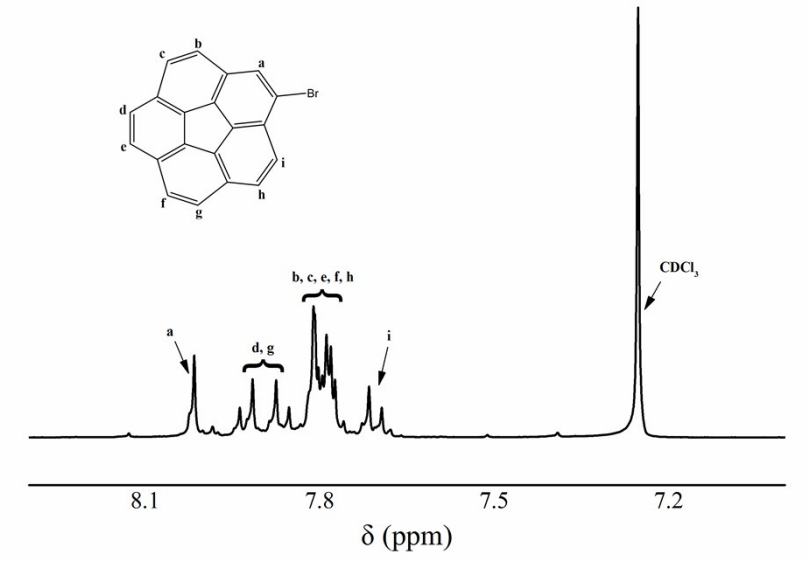


Figure S1. ^{1}H NMR spectrum of 1-bromocorannulene (Chloroform-d).

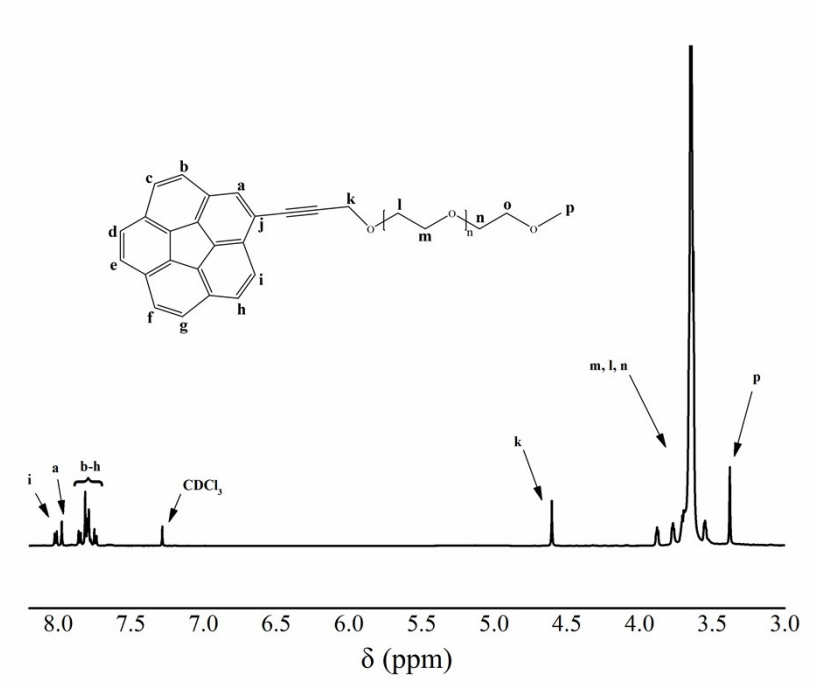


Figure S2. [1]H NMR spectrum of methoxy poly(ethylene glycol)-corannulene (Chloroform-*d*).

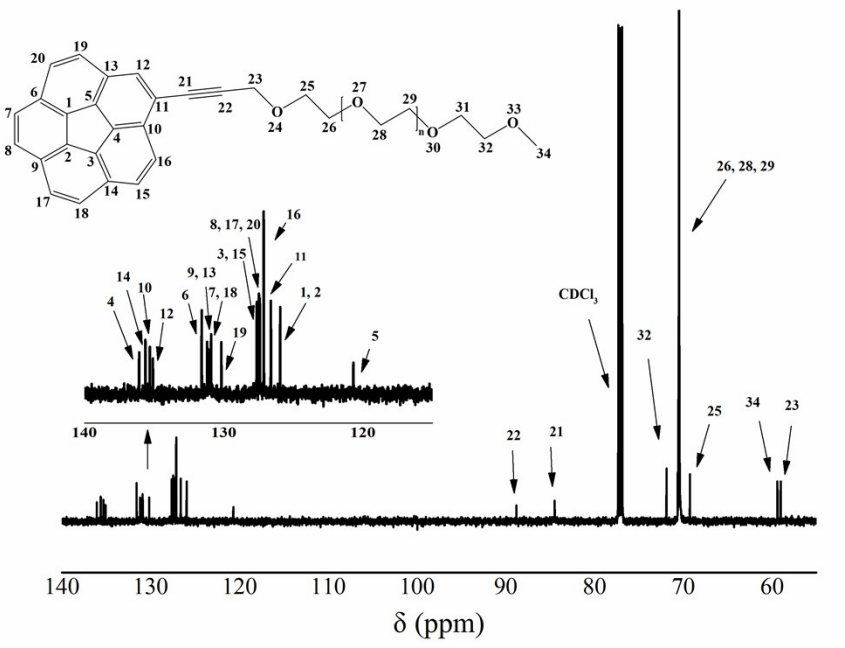


Figure S3. [13]C NMR spectrum of methoxy poly(ethylene glycol)-corannulene (Chloroform-*d*).

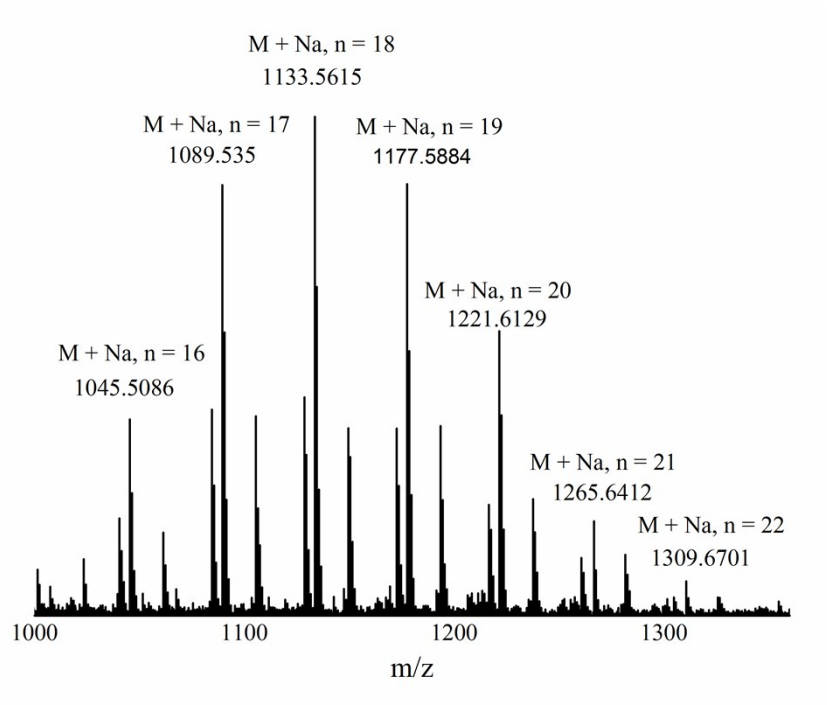


Figure S4. HRMS spectrum of methoxy poly(ethylene glycol)-corannulene.

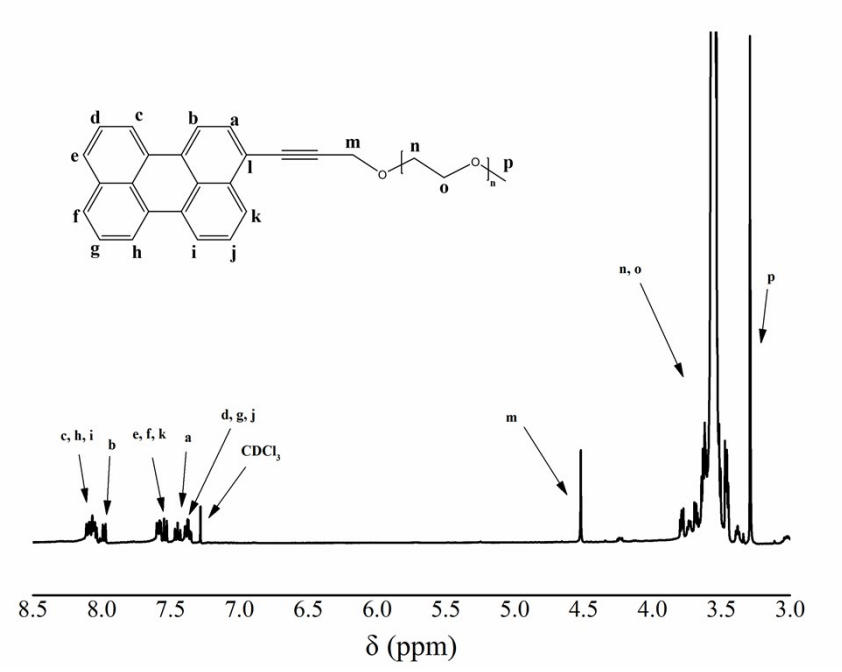


Figure S5. ^{1}H NMR spectrum of methoxy poly(ethylene glycol)-perylene (Chloroform-*d*).

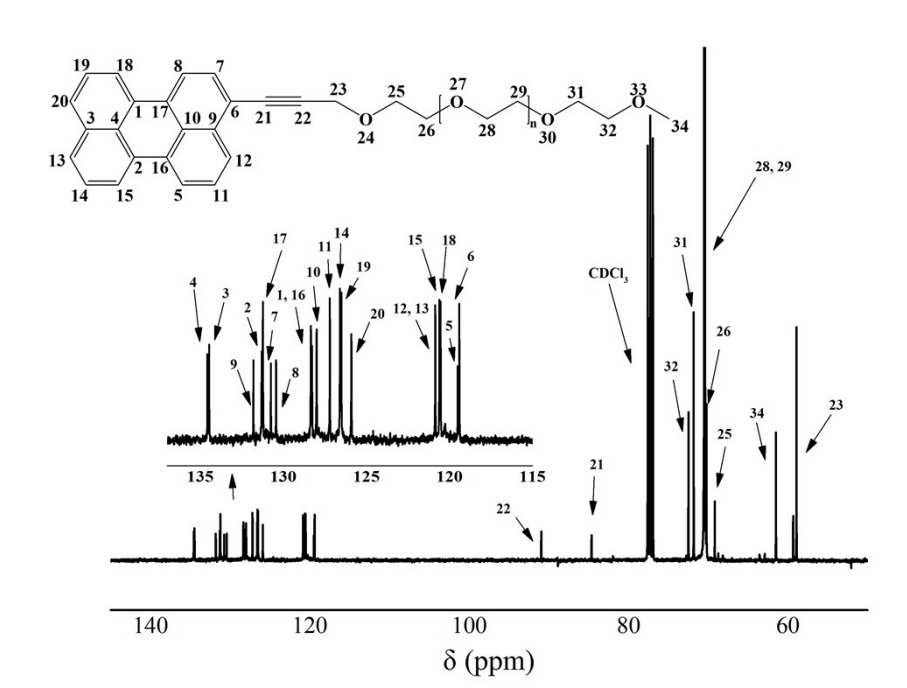


Figure S6. ^{13}C NMR spectrum of methoxy poly(ethylene glycol)-perylene (Chloroform-*d*).

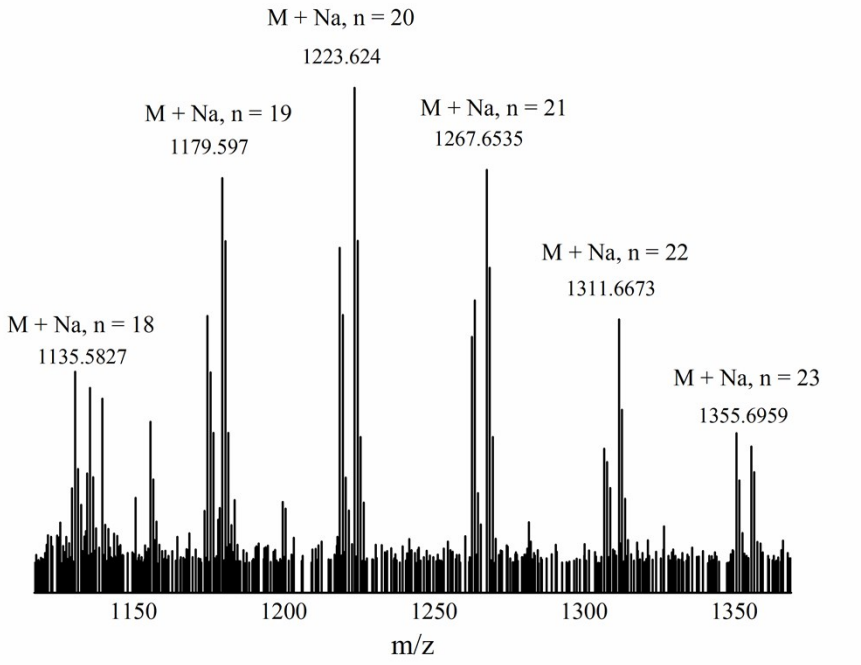


Figure S7. HRMS spectrum of methoxy poly(ethylene glycol)-perylene.

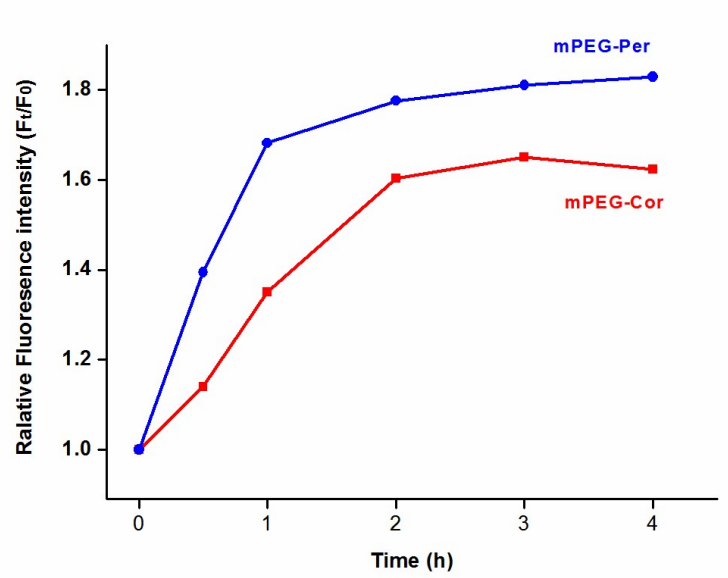


Figure S8. Stability of mPEG-Cor and mPEG-Per micelles (100 μM) in the mixture of water and fetal bovine serum (1:9,v/v). Data are presented as the variation of relative peak fluorescence against time.