Electronic Supporting Information

A Supramolecular Miktoarm Star Polymer Based on Porphyrin Metal Complexation in water

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Materials

All solvents and basic materials were commercially available (Sigma-Aldrich) and used as received, unless otherwise stated. Dichloromethane (DCM) and dimethylformamide (DMF) were dried in a solvent purification system (JC Meyer) before use as reaction solvents. Milli-Q Water (18.2 MΩ/cm) was generated using a Millipore Milli-Q academic water purification system. The buffer solutions for UV-Vis titration were potassium tetraoxalate dihydrate (50 mM, pH= 1.68), sodium carbonate and sodium bicarbonate (25 mM, pH=10.01) in Milli-Q water. Azobisisobutyronitrile (AIBN, 98%, Aldrich) was recrystallized twice from methanol prior to use. Methyl-2-(*n*-butyltrithiocarbonyl)propanoate (MBTTCP) was prepared according to the established procedures.[1] Poly(ethylene glycol) monomethyl ether (average Mn= 2000, Sigma Aldrich) was characterized by size exclusion chromatography with dimethylacetamide as eluent (M_n = 2400, PDI = 1.06) and ^{1}H NMR spectroscopy using $CDCl_3$ as solvent (DP=48). 5, 10, 15, 20-tetra(4-acetylphenyl)porphyrin ($H_2TPP(OAc)_4$) was synthesis according to a literature procedure.[2]

Analytical Techniques

^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer. Samples were dissolved in $CDCl_3$ or D_2O. Chemical shifts are expressed in ppm by comparison with the signal of TMS used as an internal standard.

Size-exclusion chromatography (SEC) was performed on an Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-Pump, a 1260 automatic liquid sampler, a thermostatted column compartment, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). Analyses were performed on a PPS Gram30 column in series with a PPS Gram 1000 column at 50 C. DMA containing 50 mM of LiCl was used as an eluent at a flow rate of 0.6 mL/min. The SEC traces were analysed using the Agilent Chemstation software with the GPC add on. Molar mass and PDI values were calculated against PMMA standards.

Gas chromatography(GC) was performed on 7890A from Agilent Technologies with an Agilent J&W Advanced Capillary GC column (30 m, 0.320 mm and 0.25 μm). Injections were performed with an Agilent Technologies 7693 auto sampler. Detection was done with a FID detector. Injector and detector temperatures were kept constant at 250 and 280 ^{o}C, respectively. The column was initially set at 50 ^{o}C, followed by two heating stages: from 50 ^{o}C to 100 ^{o}C with a rate of 20 ^{o}C/min and from 100 ^{o}C to 300 ^{o}C with a rate of 40 ^{o}C/min. and then held at this temperature for 0.5 minutes. Conversion was determined based on the integration of monomer peaks using DMA as internal standard.

MALDI-TOF mass spectra were acquired with a Voyager DE-STR (PerSeptive Biosystem) using a simultaneous delay extraction procedure (20 kV applied after 233 ns with a potential gradient of 2545 V/mm and a wire volgage of 200 V) and detection in reflection mode. The instrument was equipped with a nitrogen laser (emission at 337 nm for 3 ns) and a flash AD converter (time base 2 ns). Trans-2-[3-(4-t-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) was used as matrix

UV/Vis spectra were recorded on a Varian Cary 300 Bio UV/VIS spectrophotometer equipped with a Cary temperature and stir control.

Fluorescence measurement for job plot were carried out on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies) equipped with a Varian Cary Temperature Controller. The emission spectra resulting from excitation by a 428.5 nm laser were monitored from 500 -700 nm, and the slit width was kept at 5 nm during the measurements.

Job plots (continuous variation method)

The stoichiometry of the self-assembly was determined via Job's method of continuous variation.[3] A stock solution was prepared for each complementary recognitions motif dissolved in Milli-Q water in a 5 mL around bottom flask. The appropriate amount from the stock solution was

transferred to the UV-Visible cuvette or fluorescence cuvette in which the total concentration of the recognition motifs was kept constant at 5.05 μM. The molar fraction of the motifs was varied between 0 and 1. The changes in absorption intensity were multiplied by the molar fraction and plotted *vs*. molar fraction to construct the Job plot.

UV-Vis spectrophotometric titration experiment

UV-Visible titration was performed by adding solutions containing the Py-PmDEGA polymer to a solution of the ZnTPP$(PEG)_4$ in a 1 cm path quartz cuvette by using microliter syringes. In all cases the ZnTPP$(PEG)_4$ was present in the Py-PmDEGA solution at the same concentration as that in the cuvette to avoid dilution effects. Mili-Q water (18.2 mΩ/cm) was used as solvent for UV-Visible titration. UV-Visible scanning conditions were as follows: Scanning rate =300 nm/min, bandwidth = 0.5 nm, response time = 0.1 s, accumulations = 1 scan.

Isothermal titration calorimetry (ITC) experiment

ITC experiments were performed at 20 ^{o}C using a nano-ITC titriation calorimeter from TA Instruments with a standard sample cell volume of 1 mL, following standard procedures. A 250 μL injection syringe was used with stirring at 400 rpm. Host molecules were dissolved in Milli-Q water and the solutions were degassed gently under vacuum before use. Each titration comprised an initial 2 μL preinjection followed by 24 × 10 μL injections of Py-PmDEGA (14.28 mM) into Zn-TPP$(PEG)_4$ solution (1.49 mM). Control experiments with identical injections of Py-PmDEGA into water alone were used to correct titration data.

Diffusion ordered spectroscopy (DOSY) NMR experiment

DOSY NMR experiments were performed on a 400 MHz Bruker Avance II spectrometer equipped with a broadband ^{1}H decoupling probe (PABBO) using double stimulated echo, 2 spoil gradients and alternative phase cycle provided by Garreth Morris at a temperature of 298.2 K. Proton pulse lengths were determined to be 10.12 μs and bipolar gradient of δ=2.5 ms length were incremented from G=2.588 G/cm to 49.163 G/cm in 64 steps. 16 scans with 12 k complex data points were recorded for each increment with 16 dummy scans per experiment. The diffusion delay Δ was set to 1000 μs. Processing was achieved using TopSpin 3.2 with the Dynamic Center 2.0.4. After zero filling to 24 k points and apodization using an exponential window function with an additional linewidth of 0.1 Hz, 1D increment spectra were Fourier transformed and the Signal decay due to Gradients was fitted using

$$f(G) = I_0 \cdot e^{\left(-\gamma_H^2 \cdot G^2 \cdot \delta^2 \cdot \left(\Delta - \frac{\delta}{3}\right)\right) \cdot D}$$

with the proton gyromagnetic ratio γ_H and the full signal intensity I_0. Corresponding diffusion coefficients D of the polymer signals and the solvent are the result of the fitting procedure and are plotted against chemical shifts.

Turbidity measurements

The turbidity measurements were performed on UV-Vis spectrophotometer at a wavelength of 700 nm. The concentration of all the sample were 5 mg/mL. The transmittance was measured during at least two controlled cooling/heating cycles with a cooling/heating rate of 0.5 °C under stirring.

Synthesis and characterizations

Synthesis of α-methoxy-ω-toluenesulfonyl-PEG (PEG-TOS)

A solution of poly(ethylene glycol) methyl ether (4.0 g, 2.0 mmol) in 30 mL THF was added to a 7.5 mL aqueous solution of NaOH (1.4 g, 36 mmol). The resulting mixture was cooled in an ice bath. A solution of *p*-toluenesulfonyl chloride (4.4 g, 23 mmol) in 6 mL THF was added. The reaction solution was stirred at room temperature overnight. The solution was extracted with CH_2Cl_2 two times and the organic phase was combined and washed tree times with water. The organic phase was dried over $MgSO_4$, filtered and concentrated in vacuo. Then, precipitation in cold diethyl ether yielded a white solid (3.6 g, 83.5 %). ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): 2.38 (s, $\underline{CH_3}$-C_6H_5), 3.37 (s, $\underline{CH_3}$-O), 3.51-3.72 (m, -$\underline{CH_2}\underline{CH_2}$-O), 3.81 (t, -$\underline{CH_2}CH_2$-TOS, 4.09 (t, -$CH_2$-$\underline{CH_2}$-TOS), 7.28 (d, arom), 7.73 (d, arom).

Synthesis of 5, 10, 15, 20-tetra(4-hydroxyphenyl)porphyrin ($H_2TPP(OH)_4$)

In a 10 mL flask, 0.8 g $H_2TPP(OAc)_4$ (*ca.* 0.95 mmol) was dissolved in 6 mL of a EtOH/H_2O (1:3) solution. Then 1 mL concentrated HCl was added and the mixture solution was refluxed for 2 hours. After cooling to room temperature, the reaction solution was diluted with H_2O (40 mL), neutralized with 5% NaOH until the color of the green solution turned dark red and pH was 7.5. The mixture was stirred vigorously for 1 hour, then ethyl acetate was added, and the mixture was stirred for another 1.5 hours. the organic layer was separated, washed twice with water, dried over anhydrous $MgSO_4$, filtered, and the solvent was removed at reduced pressure. Chromatography (silica,

toluene/ethyl acetate = 2:1) was employed to isolate the pure product (506 mg, 79% yield). ^{1}H NMR (400 MHz, acetone-d_6, δ/ppm): -2.69 (s, N-H pyrrole protons), 7.30(d, C-H phenyl protons in β-position with respect to the phenolic oxygen), 8.07(d, C-H phenyl protons γ to the phenolic oxygen), 8.87 (s, O-H phenolic protons), 8.93(s, C-H pyrrole protons).

Synthesis of H_2TPP(PEG)$_4$

Firstly, PEG-TOS was melted at 80 ^{o}C in vacuum for 2 hours before use to removed traces of moisture. Then, a mixture of [H_2TPP(OH)$_4$] (100 mg, 0.147 mmol) and PEG-TOS (2528 mg, 1.179 mmol) was dissolved in 12 mL of dimethylformamide. To this solution, potassium carbonate (164 mg, 1.179 mmol) was added and the solution was stirred at 80 ^{o}C for 48 hours. After cooling to room temperature, the solution was poured into water and extracted three times with CH_2Cl_2. The combined organic phase was washed with water three times and then with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by precipitation into CH_2Cl_2/diethyl ether (4/45 v/v) from CH_2Cl_2 and column chromatography on silica gel using CH_2Cl_2/C_2H_5OH/$N(C_2H_5)_3$ (50:1:0.5) as eluent to yield the desired product (H_2TPP(PEG)$_4$ (1.1 g, 80.6%). UV-Vis (H_2O) λ_{max} = 421 nm. ^{1}H NMR (400 MHz, $CDCl_3$, δ/ppm): -2.83 (*s*, N-H pyrrole protons), 3.31 (s, O-CH3), 3.46-3.82 (m, OCH_2CH_2O), 3.99 (*t*, O-CH_2CH_2OPh), 4.37 (*t*, O-CH_2CH_2OPh), 7.24(*d*, C-H phenyl protons in β-position with respect to the phenolic oxygen), 8.05(*d*, C-H phenyl protons γ to the phenolic oxygen), 8.78 (s, C-H pyrrole protons).

Synthesis of ZnTPP(PEG)$_4$

To a well stirred solution of H_2TPP(PEG)$_4$ (14.5 mg, 0.002 mmol) in 24 mL CH_2Cl_2/CH_3OH (3/1 v/v) was added 4.0 mg $Zn(OAc)_2 \cdot 2H_2O$ (0.018 mmol). The mixture was refluxed for 3 h. After the solution cooled, it was diluted by CH_2Cl_2, washed three times with water, dried over $MgSO_4$ and concentrated to give 13 mg product (90%). UV-Vis (H_2O) λ_{max} = 428 nm. ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 3.31 (s, O-CH_3), 3.46-3.82 (m, OCH_2CH_2O), 3.99 (t, O-CH_2CH_2OPh), 4.37 (t, O-CH_2CH_2OPh), 7.24(d, C-H phenyl protons in β-position with respect to the phenolic oxygen), 8.05(d, C-H phenyl protons γ to the phenolic oxygen), 8.78 (s, C-H pyrrole protons).

Synthesis of di(ethylene glycol) methyl ether acrylate (mDEGA)

Diethylene glycol monomethyl ether (87.72 g, 0.73 mol) and trimethylamine (74.36 g, 0.73 mol) were dissolved in 400 mL dichloromethane. To this stirred solution, acryloyl chloride (73.85 g, 0.82 mol) was added dropwise at 0 °C. After stirring for 10 hours at room temperature, the precipitated salt was removed by filtration. The filtrate was washed with aqueous $NaHCO_3$ solution, and distilled water. Then the solution was dried over $MgSO_4$, followed by filtration. The solvent (DCM) was evaporated under reduced pressure at room temperature. Then 113.7 g (89.5%) product was collected by reduced-pressure distillation in presence of hydroquinone as inhibitor. ^{1}H NMR (300 MHz, $CDCl_3$, δ/ppm): 3.36 (s, 3H, O$\underline{CH_3}$), 3.49-3.78 (m, 6H, -$\underline{CH_2}$O$\underline{CH_2}\underline{CH_2}$-OCH$_3$), 4.30 (t, 2H, -C(O)O$\underline{CH_2}$-), 5.81 (dd, 1H, -$\underline{CH}$CHC(O)-, *trans*-position with respect to carbonyl group), 6.07-6.19 (m, 1H, CH$_2\underline{CH}$C(O)-), 6.37-6.43 (m, 1H, -$\underline{CH}$CHC(O)-, cis-position with respect to carbonyl group).

Synthesis of Poly(mDEGA) (PmDEGA)

mDEGA (1045 mg, 6 mmol), MBTTCP (25 mg, 0.1 mmol) and AIBN (0.99 mg, 0.006 mmol) were dissolved in toluene/DMA solvent mixture (3/2 *vol*) in a Schlenk flask. The concentration of monomer was fixed at 2 M. After four freeze-pump-thaw cycles, the flask was filled with argon, immersed in a preheated oil bath of 70 °C and stirred for 2 hours. The polymerization was stopped by cooling the solution in an ice bath. After the solution was cooled down to room temperature, the polymers were precipitated in ice-cold diethyl ether/hexane (80/20). The crude polymer was dissolved in dichloromethane and precipitated again in ice-cold diethyl ether/hexane (80/20). This procedure was repeated three times. The polymer finally was dried under reduced pressure at room temperature. Conversion of the monomers was analyzed by GC with DMA as internal standard. SEC was used to evaluate number average molecular weight (M_n) and Polydispersity index (Đ) of the obtained polymers. (see the kinetic data from Figure S6)

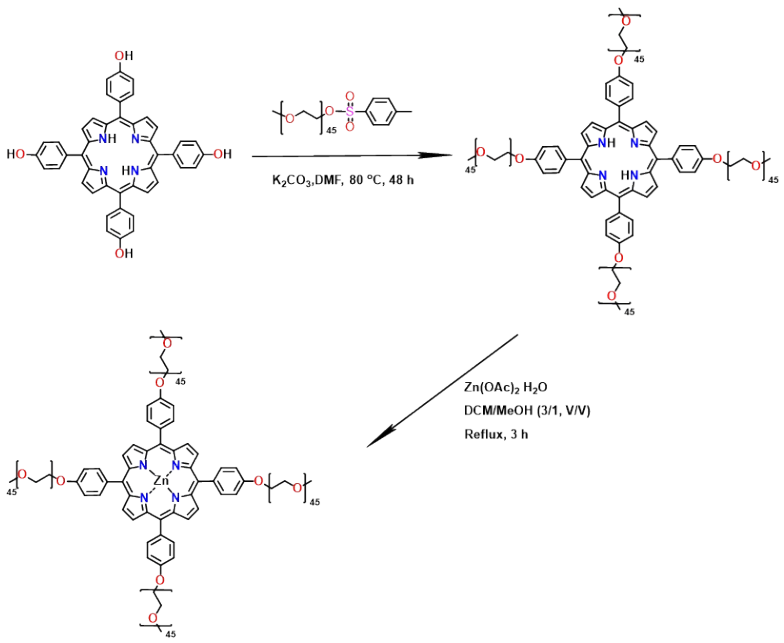
Synthesis of *N*-(pyridin-4-ylmethyl) acrylamide (NP4MAM)

4-aminomethyl pyridine (1.07 g, 9.85 mmol) and trimethylamine (0.99 g, 9.85 mmol) were dissolved in 20 mL dichloromethane. To this stirred solution, acryloyl chloride (0.98 g, 10.83 mmol) was added dropwise at 0 °C. The reaction solution was stirred over night at room temperature. The reaction solution was washed twice with saturated aqueous $NaHCO_3$ solution, and three times with water. Then, the solvent (DCM) was evaporated under reduced pressure at room temperature. The crude product was dissolved in distilled water. The pure product was extracted from the aqueous solution by ethyl acetate and then finally it was obtained by evaporation

at 40 °C. (1.4 g, 87.6%) ^{1}H NMR (300 MHz $CDCl_3$, δ/ppm): 4.55 (d, J = 6.2 Hz, 2H, -NH$\underline{CH_2}$-), 5.73 (dd, J = 10.2, 1.4 Hz, 1H, -$\underline{CH}$CHC(O)- trans-position with respect to carbonyl group), 6.02 (br, 1H, -NH-), 6.12-6.21 (m, 1H, $CH_2\underline{CH}$C(O)-), 6.37 (dd, J = 17.0, 1.4 Hz, 1H, -$\underline{CH}$CHC(O)- cis-position with respect to carbonyl group), 7.21 (d, J = 4.4 Hz, 2H, α-pyridine proton), 8.56 (d, J = 6.1 Hz, 2H, β-pyridine proton).

Synthesis of Pyridine functionalized PmDEGA (Py-PmDEGA)

The solutions PmDEGA (280 mg, 0.033 mmol) in 1.5 mL DMF, $NH_2NH_2{\cdot}H_2O$ (50 mg) in 0.5 mL DMF and NP4MAM (100 mg) in 0.5 mL DMF was placed into three Schlenk vials, respectively. 0.5 mL aqueous solution of sodium ascorbate (3.3 mg, 0.0165 mmol) as reducing agent was added into the PmDEGA solution. All of the three solution was degassed four times by freeze-vacuum-thaw cycles. $NH_2NH_2{\cdot}H_2O$ solution (83 μL, 0.165 mmol) was added into the PmDEGA solution under argon atmosphere. The reaction solution was stirred for 1 hour at 30 °C under argon atmosphere. During this period, the originally yellow solution became colorless. The NP4MAM solution (269 μL, 0.332 mmol) was added to the reaction mixture which was stirred at 30 °C for a further 12 hours. The polymer was recovered and purified by two repeated re-precipitation from DCM to hexane/diethyl ether (80:20 v/v) and a following PD-10 gel chromatography. The obtained end-functionalized polymer was confirmed by UV-Vis and SEC. (see the data in Figure S8 & S7)



Scheme S1. The synthetic route of ZnTPP(PEG)$_4$.

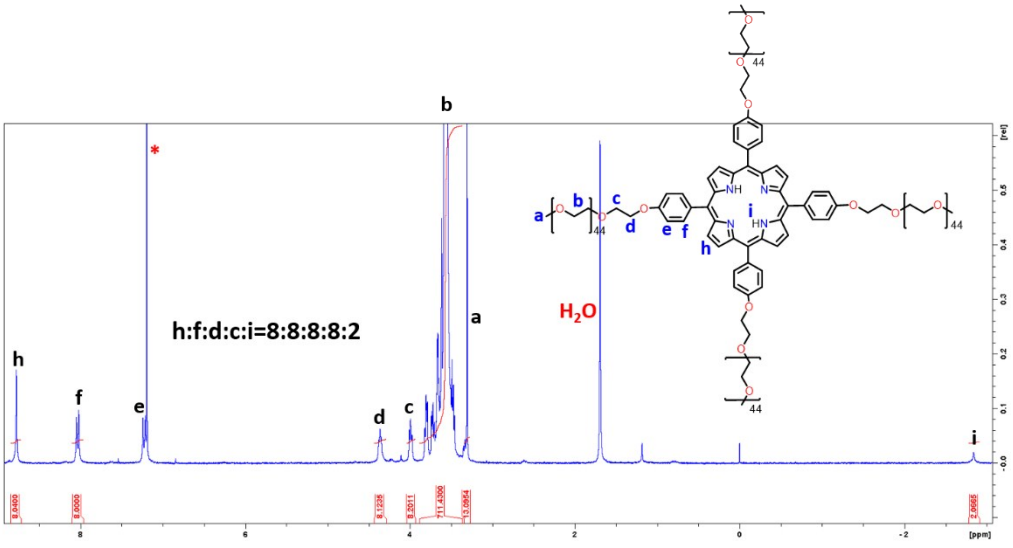


Figure S1. The ^{1}H NMR spectrum of H_2TPP(PEG)$_4$ recorded in $CDCl_3$ at 25°C.

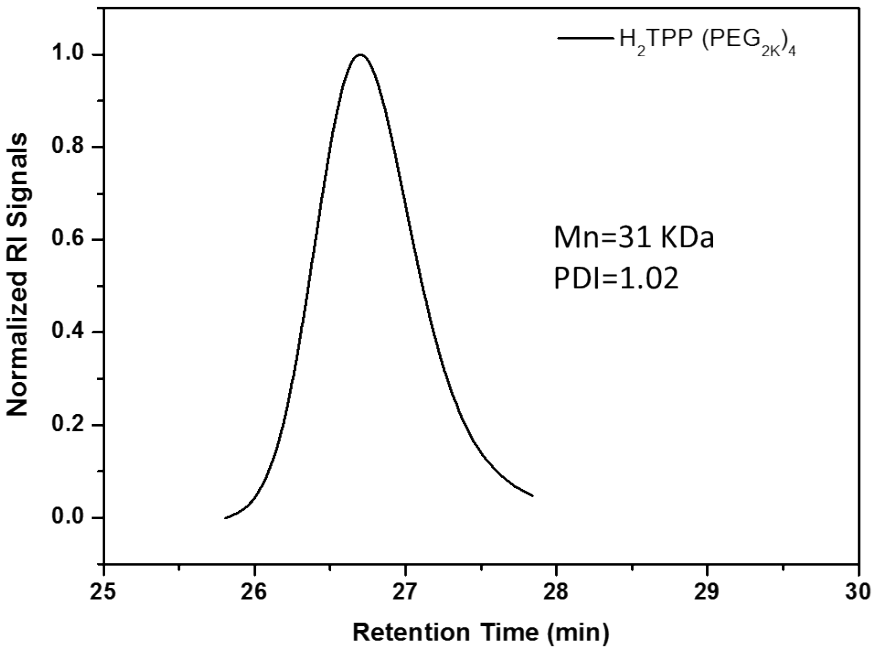


Figure S2. SEC trace of metal-free star polymer $H_2TPP(PEG)_4$.

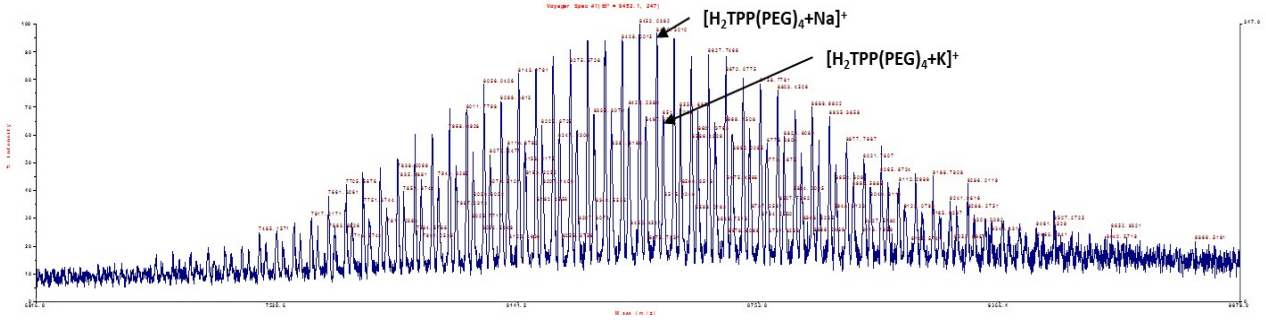


Figure S3. MALDI-TOF MS spectrum of metal-free star polymer $H_2TPP(PEG)_4$.

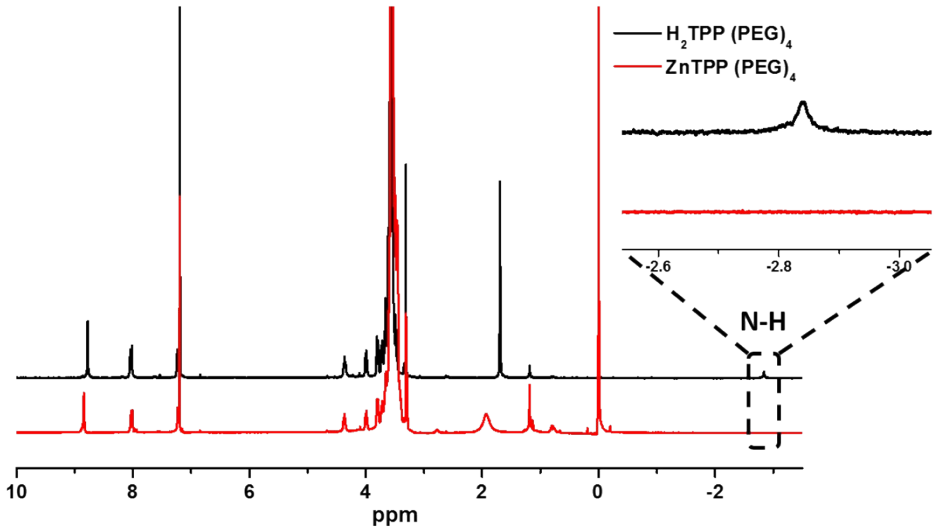


Figure S4. ^{1}H NMR spectra of $H_2TPP(PEG)_4$ and $ZnTPP(PEG)_4$, recorded in $CDCl_3$ at 25°C.

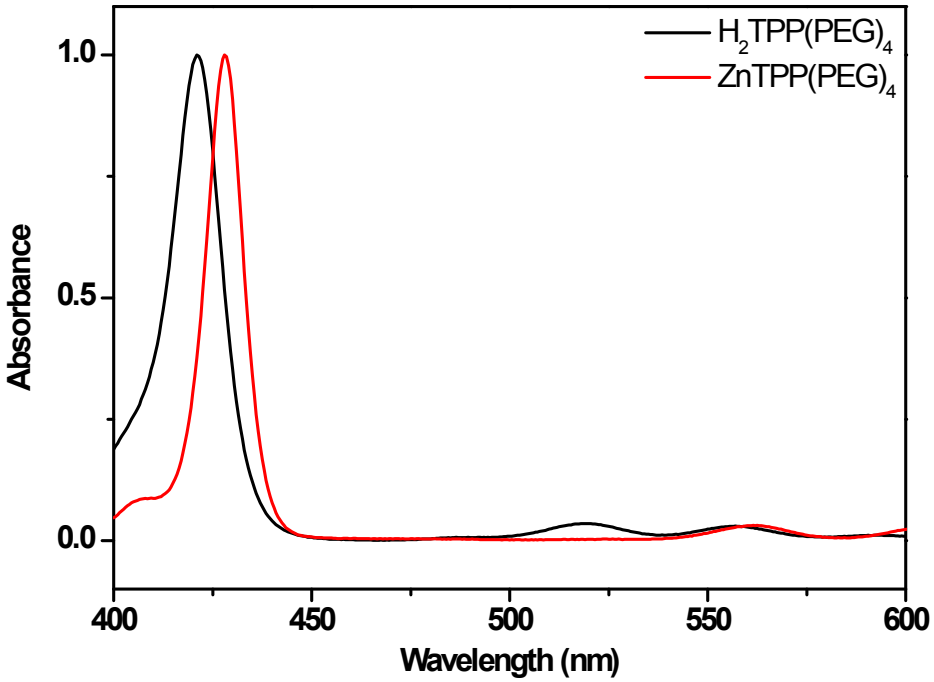


Figure S5. UV-Vis spectra of $H_2TPP(PEG)_4$ and $ZnTPP(PEG)_4$ in aqueous solution.

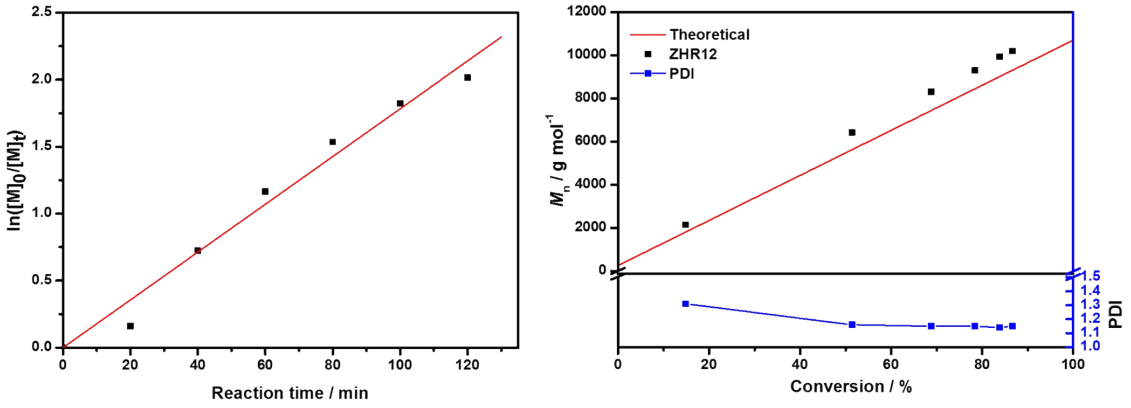


Figure S6. The kinetic data for the polymerization of mDEGA.

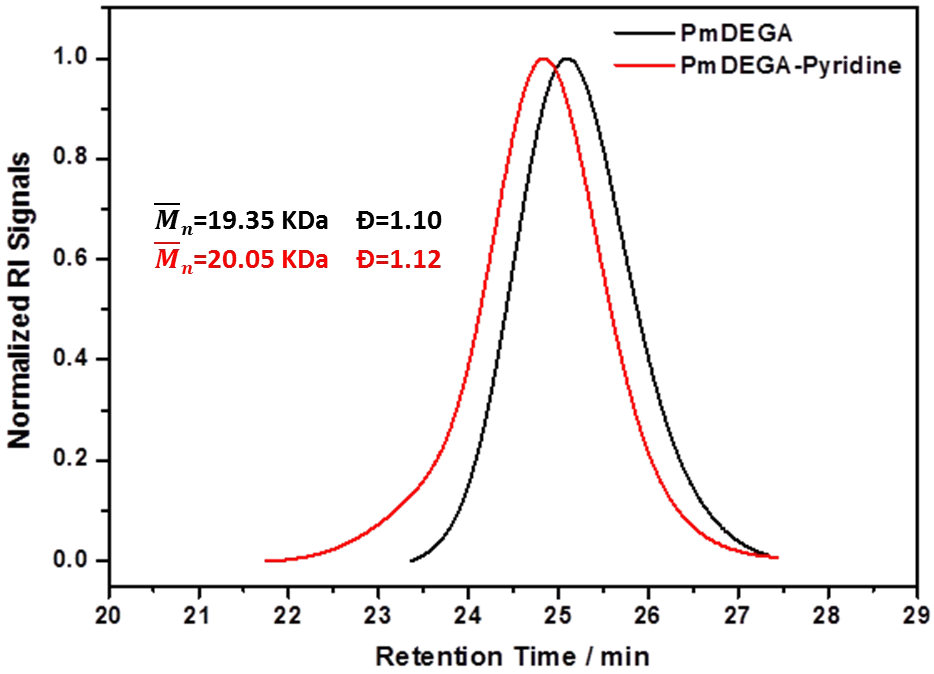


Figure S7. SEC traces of PmDEGA and pyridine end-functionalized PmDEGA.

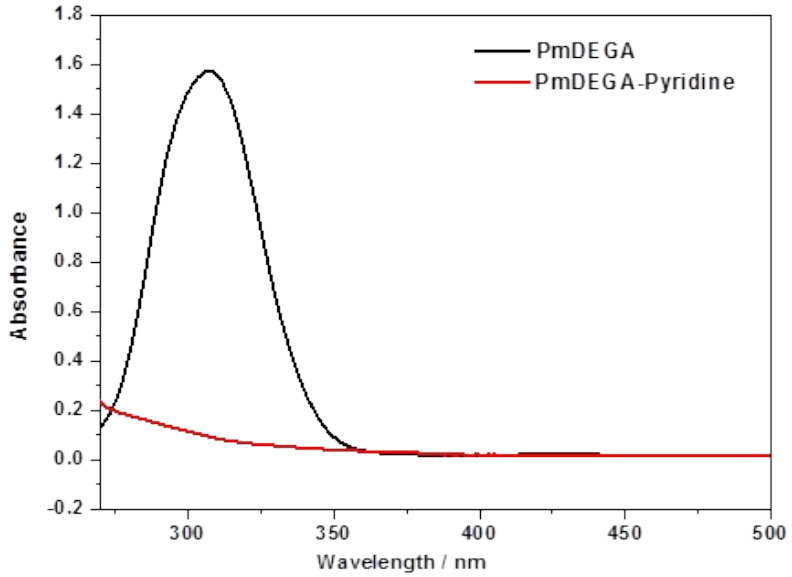


Figure S8. Comparison of the UV-vis absorption spectra of PmDEGA before and after aminolysis of the CTA end-group with hydrazine and modification with pyridine by thiol-ene modification.

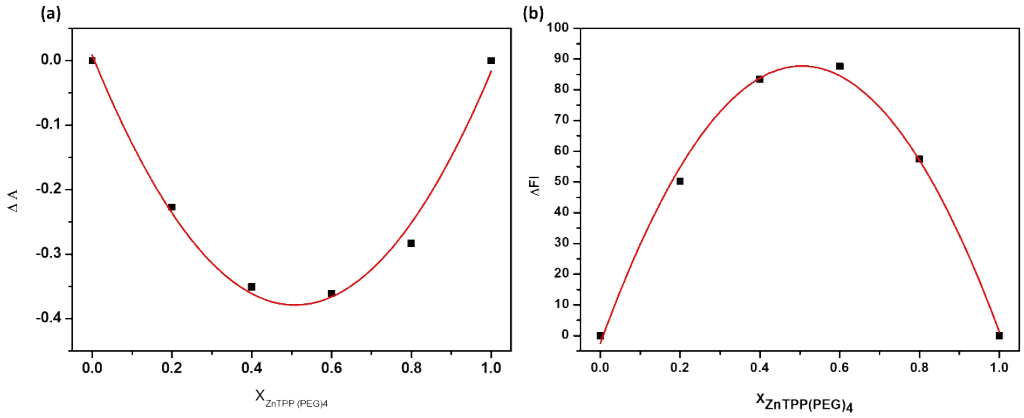


Figure S9. Continuous variation plot (Job plot) of ZnTP(PEG)$_4$ with Py-PmDEGA in Milli-Q water at 25 ^{o}C, a). by UV-Vis spectroscopy; b). by fluorescence spectroscopy, showing a minimum/maximum at 0.5 mole fraction of ZnTPP(PEG)$_4$.

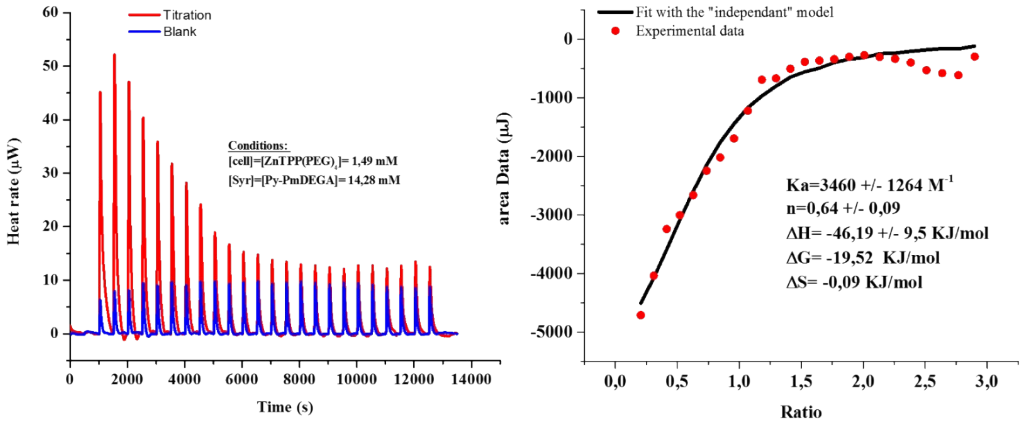


Figure S10. Isothermal titration calorimetry data for the addition of Py-PmDEGA to Zn-Tpp(PEG)$_4$. Record in Milli-Q water at 20 ^{o}C.

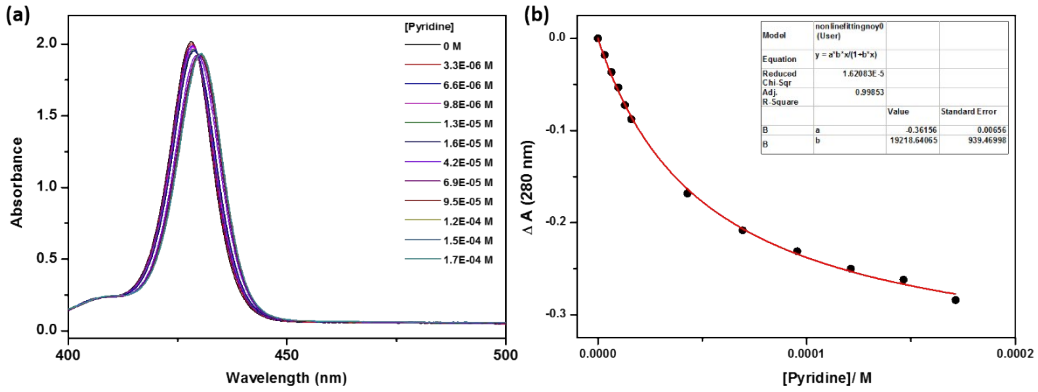


Figure S11. a: UV-Vis titration of Py-PmDEGA to a 5 µM ZnTPP(PEG)$_4$ solution in Milli-Q water at 25 ^{o}C; b: absorbance changes of ZnTPP(PEG)$_4$ at 428 nm upon the addition of Py-PmDEGA; the red solid line is the binding isotherm obtained by the least-squares fit to the experimental data (R^2=0.99853).

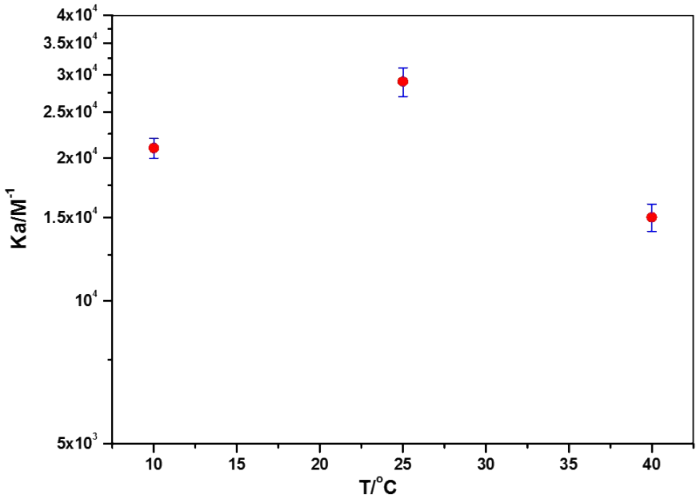


Figure S12. The association constants of Py-PmDEGA and ZnTPP(PEG)$_4$ as function of temperature. All the titration experiments were performed in aqueous solution.

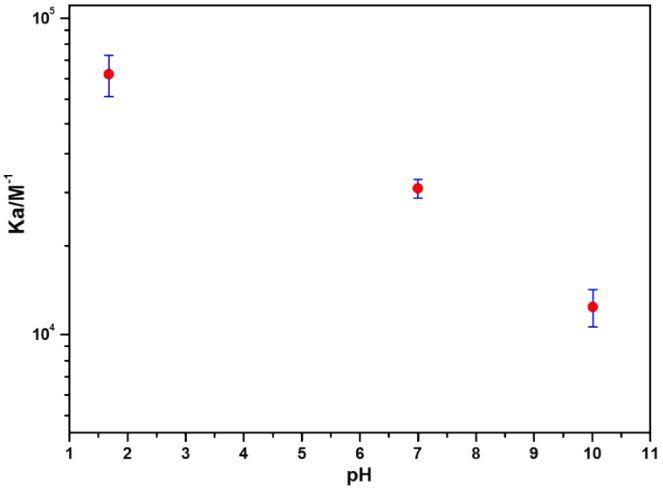


Figure S13. The association constants of Py-PmDEGA and ZnTPP(PEG)$_4$ as function of pH value. All the titration experiments were performed in aqueous solution.

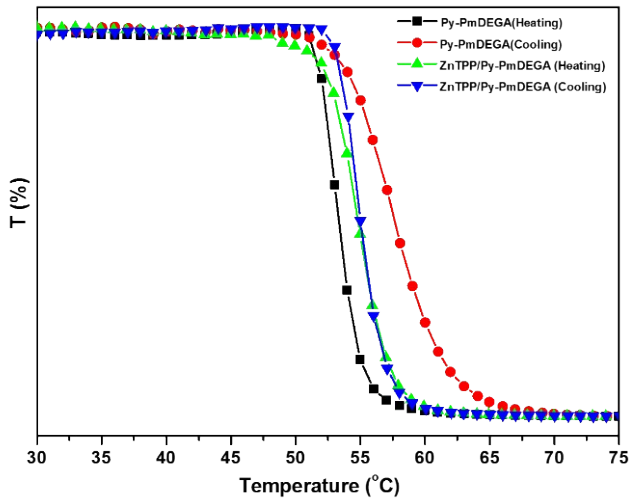


Figure S14. Plots of transmittance as a function of temperature measured for aqueous solutions (5 mg/mL) of Py-PmDEGA and complex Py-PmDEGA@ZnTPP(PEG)$_4$.

Notes and references

1. C. N. Urbani and M. J. Monteiro, *Macromolecules*, 2009, **42**, 3884-3886.
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3. J. S. Renny, L. L. Tomasevich, E. H. Tallmadge and D. B. Collum, *Angew. Chem. Int. Ed.*, 2013, **52**, 11998-12013.