Supplementary Information

Engineering the Morphology of Mn(II)-Chelated Hybrid Polyion Nanocomplexes via Tryptophan-Directed Assembly of Double-Hydrophilic Block-Copolymers for Enhanced Magnetic Relaxation

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Experimental Section

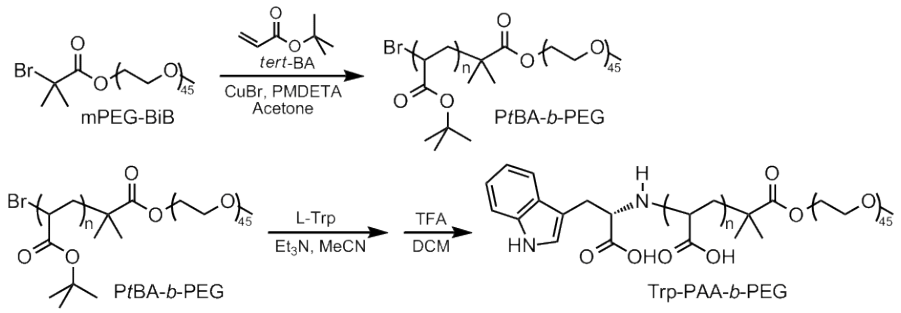
Syntheses of mPEG-modified Macroinitiator (mPEG-BIB). mPEG-modified macroinitiator was prepared using a modified literature procedure.[S1] mPEG (2.0 g, 1.0 mmol), TEA (2.81 mL, 20 mmol) and anhydrous THF (24 mL) was added to 50-mL dry Schlenk flask equipped with a magnetic stir-bar. After the reaction mixture was cooled in an ice-water bath, 2-bromoisobutyryl bromide (0.50 mL, 4.0 mmol) dissolved in 0.7 mL anhydrous THF was added dropwise to the reaction flask for about 2 h at 0 °C under an inert condition. The reaction mixture was then kept at 0 °C for an additional 1 h. Next, the flask was placed in a 40 °C water bath and stirred for 3 h, then lowered to 30 °C and stirred for 3 days during which mPEG-modified macroinitiator was formed. After the reaction mixture was concentrated under reduced pressure and extracted with chloroform/DI water, the product was precipitated by adding excess amount of cold diethyl ether. The precipitated product was collected by filtration and dried under vacuum for 24 h.

Syntheses of Poly(*tert*-butyl acrylate)-*b*-Poly(ethylene glycol) (P*t*BA-*b*-PEG). P*t*BA-*b*-PEG block copolymers were prepared using a modified literature procedure (Scheme S1).[S1, S2] mPEG-BIB macroinitiator (1.0 g, 0.466 mmol) and CuBr (0.067 g, 0.466 mmol) were added to a 50-mL dry Schlenk flask equipped with a magnetic stir-bar. The flask was sealed with a rubber septum, degassed and back-filled with nitrogen three times. To the reaction flask, anhydrous acetone (5 mL), PMDETA (0.12 mL, 0.559 mmol), and *t*BA (5.8 mL, 39.6 mmol), which was purified by passing through an alkaline aluminum oxide column, were subsequently added *via* syringe under inert condition. Next, the solution was stirred until the Cu complex was formed, which can be easily recognized by a color change from colorless/turbid to a greenish clear solution. After complex formation, the mixture was degassed by three cycles of freeze-pump-thaw. Then, the flask was placed in an oil bath and polymerization was carried out at 60 °C. After the predetermined reaction time, the polymerization was quenched by placing the reaction flask in an ice bath. After the evaporation of acetone under reduced pressure, the reaction product was purified through extraction using 1:3-Chloroform/IPA solution followed by passing through basic alumina to remove the copper catalysts. After removing the moisture from the oil layer, the product was dried under a vacuum and obtained as a brown product.

Syntheses of Tryptophan-modified Poly(acrylic acid)-*b*-Poly(ethylene glycol) (Trp-PAA-*b*-PEG). Trp-modification of P*t*BA-*b*-PEG block copolymers was performed according to a modified literature procedure (Scheme S1).[S3] L-Trp (0.060 g, 0.322 mmol), TEA (0.04 mL, 0.322 mmol), and acetonitrile (18 mL) were added to a 25-mL two-neck round-bottom flask equipped with a magnetic stir-bar. Stir for 1 h and add P*t*BA-*b*-PEG block copolymers (2.0 g, 0.266 mmol). After the flask was placed in an oil bath with a condenser, the reaction mixture was refluxed at 60 °C. The reaction product was then concentrated under reduced pressure and purified through extraction using chloroform/brine solution (Fig. S1).

For acid-mediated deprotection of *tert*-butyl groups, the polymers product (3.26 g), DCM (15 mL), and TFA (3.7 mL, 99 %) was added to a 50-mL round-bottom flask equipped with a magnetic stir-bar,

which was sealed by a rubber septum with a needle inserted for ventilation, followed by stirring for 120 h at room temperature. After DCM was evaporated under vacuum, the crude product was neutralized with aqueous NaOH (10 mL, 15%) and dialyzed (MWCO = 3.5–5 kDa) using 80% EtOH in D.I. water with gradual decrease of EtOH for 7 days and finally dialyzing with D.I. water. After dialysis, the product was dried by lyophilization and obtained as a brown product (Fig. S2).



Scheme S1. Synthesis of Trp-PAA-*b*-PEG block-copolymers via atom-transfer radical polymerization (ATRP) and end-group modification with L-Trp.

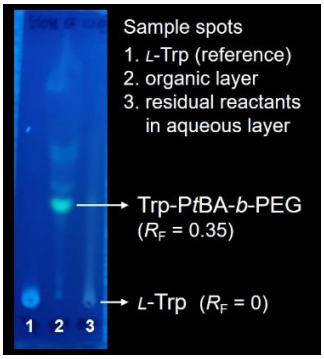


Fig. S1 TLC photograph image of Trp-ligation products after extraction. TLC was developed using an ethanol/chloroform (1:10, vol/vol) mixture as the mobile phase.

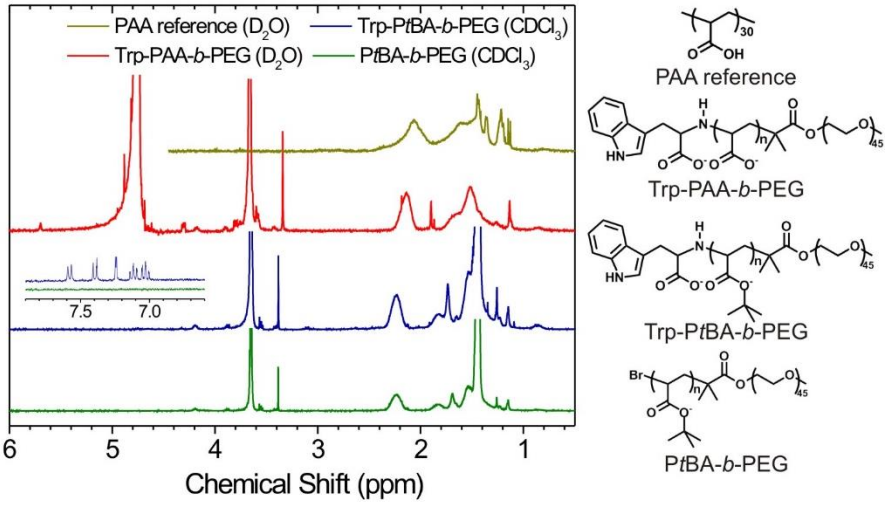
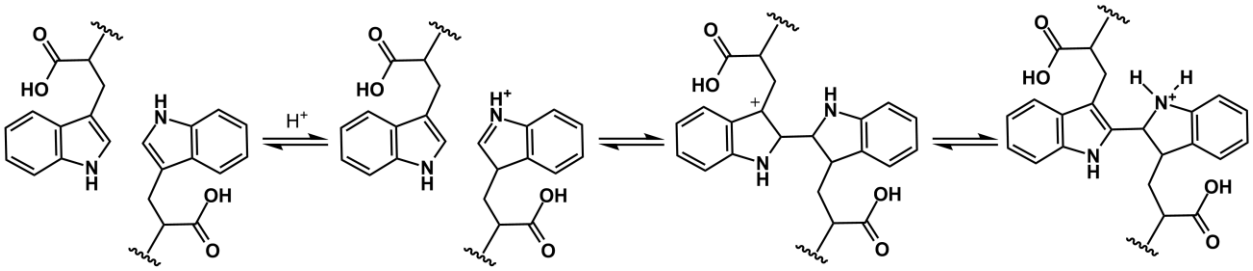


Fig. S2 ^{1}H NMR spectra of P*t*BA-*b*-PEG, Trp-P*t*BA-*b*-PEG, and Trp-PAA-*b*-PEG. Also shown is ^{1}H NMR spectrum of commercially available PAA reference for comparison. Inset shows the Trp peaks from Trp-P*t*BA-*b*-PEG.



Scheme S2. Possible mechanism of Trp dimerization under acidic condition.[S4]

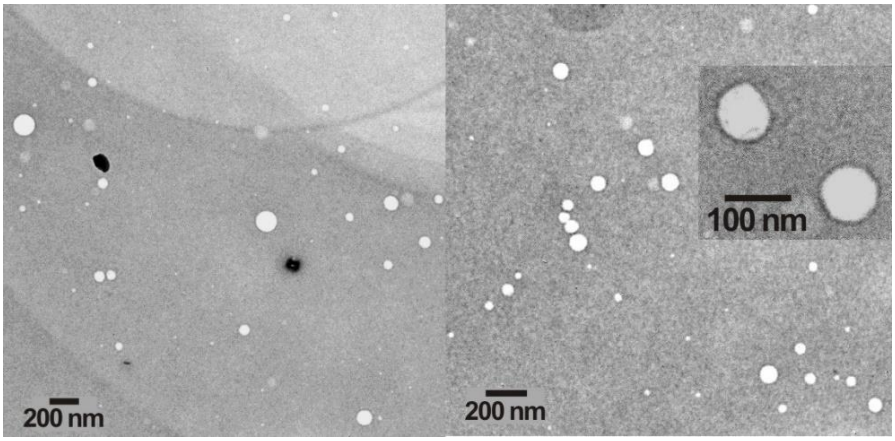


Fig. S3 TEM images of Trp-PAA-*b*-PEG vesicles. Images were obtained without staining.

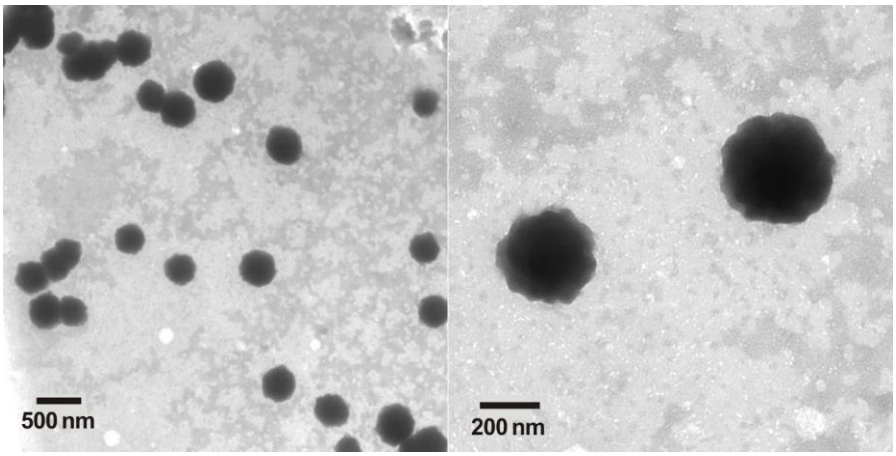


Fig. S4 TEM image of negatively stained Trp-PAA-*b*-PEG vesicles with 1.0% phosphotungstic acid.

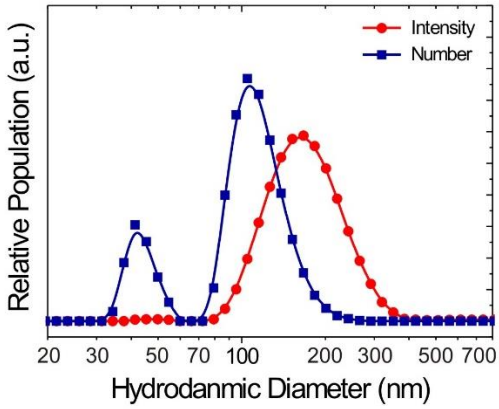


Fig. S5 DLS distribution of SEC eluates at the retention volume of 19–21 mL. SEC-DLS analysis indicates partial disassembly of vesicles under high-pressure chromatographic conditions during SEC measurements.

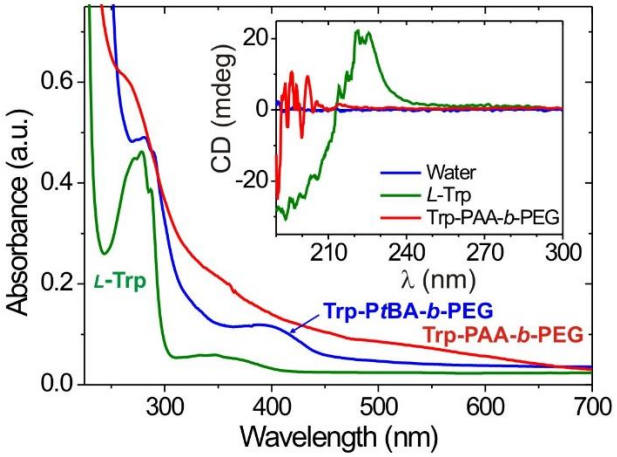
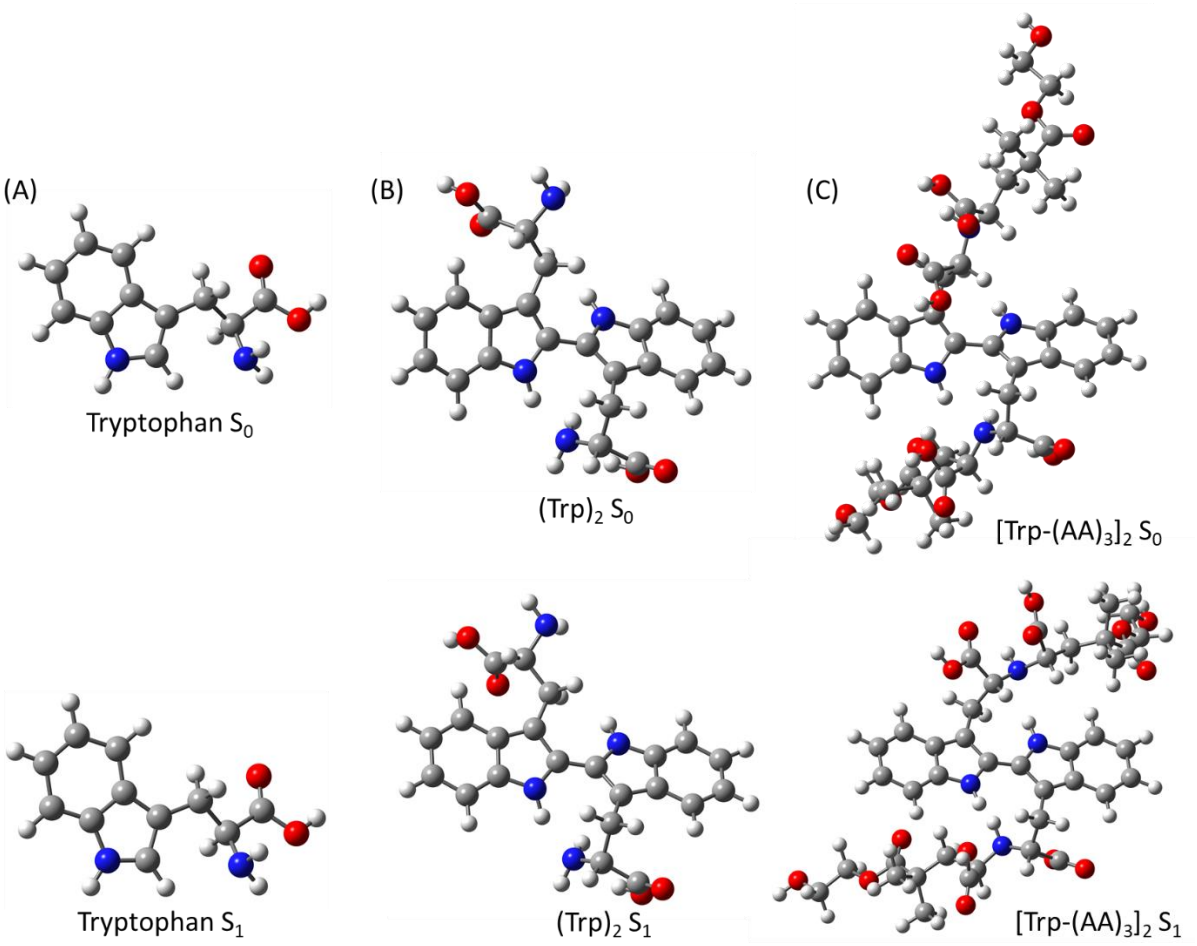


Fig. S6 UV-vis absorption spectra of Trp-PAA-*b*-PEG (red), Trp-P*t*BA-*b*-PEG precursor (blue), and *L*-Trp (green). (Inset) Circular dichroism (CD) spectra of Trp-PAA-*b*-PEG and *L*-Trp.



Excited states	Tryptophan		$(Trp)_2$		$[Trp\text{-}(AA)_3]_2$	
	Excitation energies (eV)	Oscillator strengths	Excitation energies (eV)	Oscillator strengths	Excitation energies (eV)	Oscillator strengths
T_1	3.40	–	2.81	–	2.82	–
T_2	4.24	–	3.35	–	3.35	–
T_3	4.72	–	3.83	–	3.84	–
S_1	4.89	0.0920	3.99	0.8856	3.99	0.8057
T_4	4.92	–	4.21	–	4.21	–
T_5	4.95	–	4.44	–	4.44	–
T_6	5.06	–	4.50	–	4.47	–
S_2	5.06	0.0416	4.58	0.0588	4.57	0.0545
S_3	5.22	0.0008	4.70	0.0976	4.69	0.1114
T_7	5.86	–	4.76	–	4.73	–

Fig. S7 Theoretical calculation results of geometry optimization performed using MN15/6-31G(d,p) and their solvent (water) effects using integral equation formalism polarizable continuum model (IEFPCM).[S5] (A) Tryptophan, (B) Trp dimer, and (C) Trp-$(AA)_3$ dimer. Excitation energies of optimized S_0 structures are listed at bottom table.

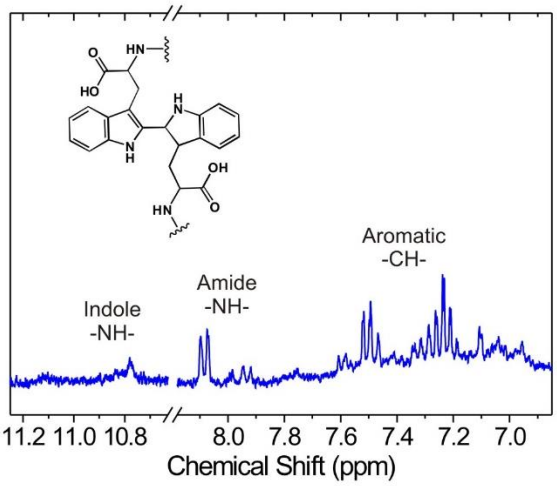


Fig. S8 ^{1}H NMR spectrum of Trp-PAA-*b*-PEG dimer in DMSO-d_6 with a possible peak assignment.[S6]

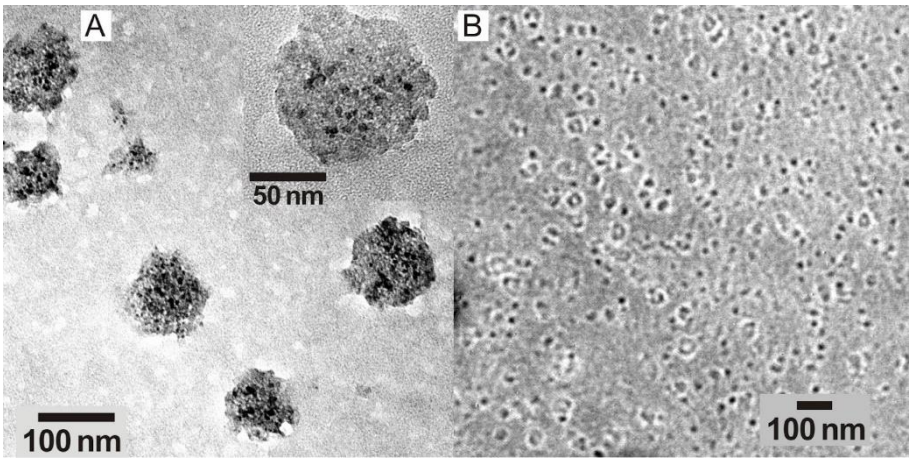


Fig. S9 TEM images of (A) ***v***-Mn and (B) ***m***-Mn. Images were obtained without staining.

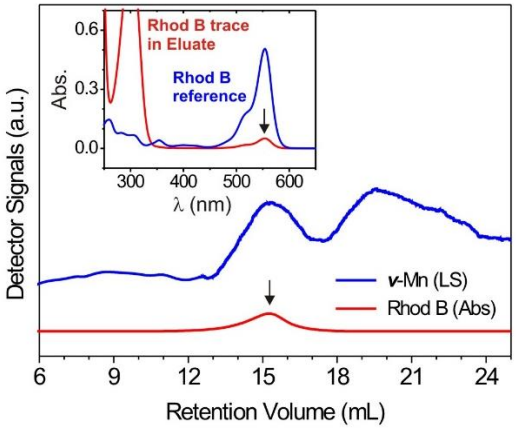


Fig. S10 Aqueous-phase size-exclusion chromatography (SEC) chromatogram of ***v***-Mn containing rhodamine B (Rhod B) as an aqueous-phase tracer. (Inset) UV-vis absorption spectra of eluates at V_R = 15–16 mL and native Rhod B as a reference.

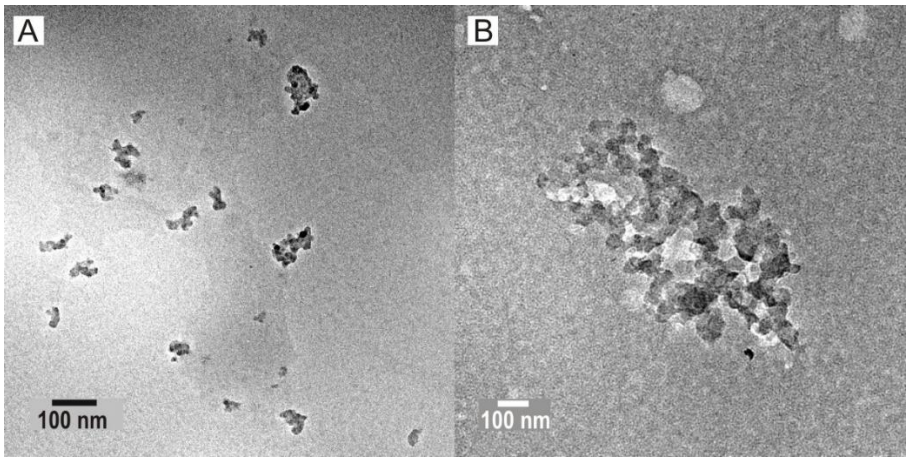


Fig. S11 TEM images of Trp-PAA-*b*-PEG hydrated with an aqueous solution of (A) Co(III) and (B) aquated cisplatin (*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$). Images were obtained without staining.

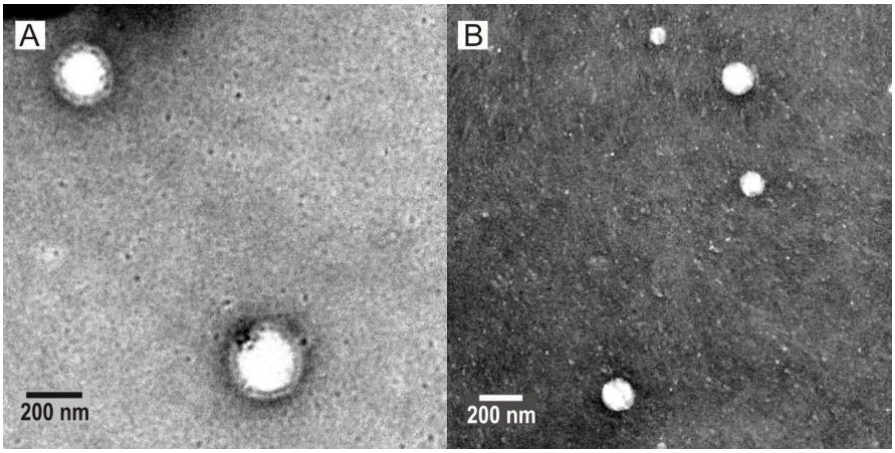


Fig. S12 TEM images of Mn(II)-chelated Trp-PAA-*b*-PEG vesicles redispersed in (A) deionized water and (B) HEPES buffered solution (10 mM, pH 7.4). TEM images revealed size variations of the vesicles in response to the ionic strength of the medium. Images were obtained without staining.

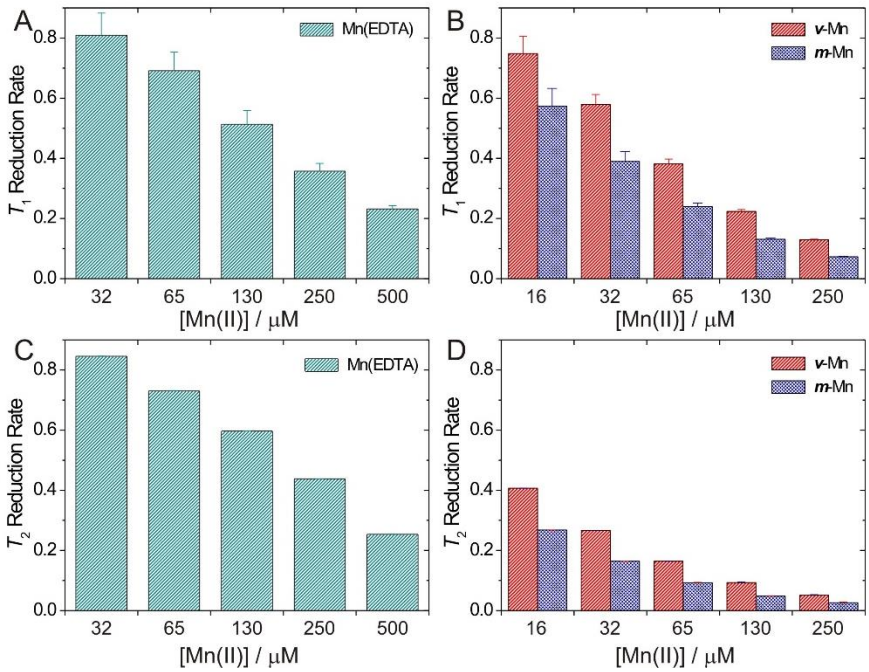


Fig. S13 Mn(II)-dependent relative reduction of (A, B) longitudinal (T_1) and (C, D) transverse (T_2) relaxation time of (A, C) aqueous Mn(EDTA), (B, D) ***v***-Mn, and ***m***-Mn solutions compared to Mn(II)-free medium at pH 7.4 and 25 °C.

References

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S2. E. Seo, S. H. Lee, S. Lee, S. H. Choi, C. J. Hawker and B. S. Kim, *Polym. Chem.*, 2017, **8**, 4528-4537.

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