Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Polypeptide Vesicles with Densely Packed Multilayer Membranes

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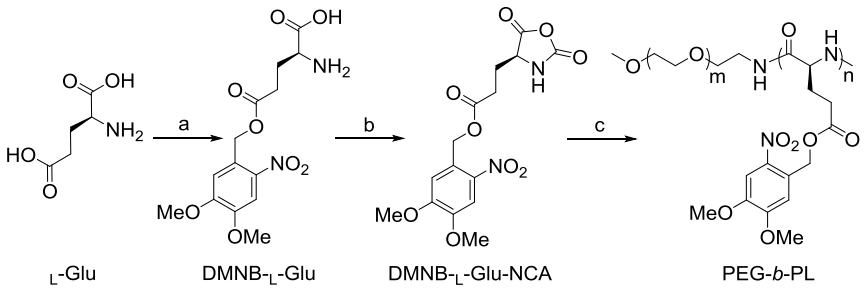

Scheme S1. Synthetic route of amphiphilic rod-coil diblock copolymer PEG-*b*-PL. *a*) $_{L}$-Glutamic acid copper (II) complex, 4,5-dimethoxy-2-nitrobenzyl bromide, *N*,*N*,*N'*,*N'*-tetramethylguanidine, DMF, H_2O, 40 ^{o}C, 24 h. *b*) Phosgene, THF, 50 ^{o}C, 2 h. *c*) mPEG-NH_2, DMF, rt, 24 h.

Table S1. Synthesis of PEG-*b*-PL, PEG-*b*-PD and PEG-*b*-PDL via mPEG-NH_2 initiated ring-opening polymerization of *N*-carboxyanhydrides (NCAs). [a]

Copolymer	Initiator	M [b]	M/I [c]	M_n(M_n*) (kDa) [d,e]	M_w/M_n [e]	Composition [f]
PEG_{1k}-*b*-PL_{10}	$mPEG_{1k}$-NH_2	L	10/1	4.4 (4.2)	1.12	PEG_{22}-*b*-PL_{10}
PEG_{1k}-*b*-PL_{20}	$mPEG_{1k}$-NH_2	L	20/1	7.3 (7.5)	1.10	PEG_{22}-*b*-PL_{22}
PEG_{1k}-*b*-PL_{40}	$mPEG_{1k}$-NH_2	L	40/1	13.7 (14.0)	1.10	PEG_{22}-*b*-PL_{44}
PEG_{5k}-*b*-PL_{10}	$mPEG_{5k}$-NH_2	L	10/1	8.4 (8.2)	1.14	PEG_{113}-*b*-PL_{11}
PEG_{5k}-*b*-PL_{20}	$mPEG_{5k}$-NH_2	L	20/1	12.0 (11.5)	1.07	PEG_{113}-*b*-PL_{23}
PEG_{5k}-*b*-PL_{40}	$mPEG_{5k}$-NH_2	L	40/1	18.6 (18.0)	1.06	PEG_{113}-*b*-PL_{39}
PEG_{2k}-*b*-PL_{20}	$mPEG_{2k}$-NH_2	L	20/1	8.5 (8.5)	1.11	PEG_{44}-*b*-PL_{20}
PEG_{2k}-*b*-PL_{40}	$mPEG_{2k}$-NH_2	L	40/1	15.4 (15.0)	1.07	PEG_{44}-*b*-PL_{38}
PEG_{1k}-*b*-PD_{20}	$mPEG_{1k}$-NH_2	D	20/1	7.6 (7.5)	1.14	PEG_{22}-*b*-PD_{22}
PEG_{1k}-*b*-PDL_{20}	$mPEG_{1k}$-NH_2	L/D	(10+10)/1	7.6 (7.5)	1.06	PEG_{22}-*b*-PDL_{22}
PEG_{5k}-*b*-PD_{20}	$mPEG_{5k}$-NH_2	D	20/1	11.9 (11.5)	1.11	PEG_{113}-*b*-PD_{25}
PEG_{5k}-*b*-PDL_{20}	$mPEG_{5k}$-NH_2	L/D	(10+10)/1	11.8 (11.5)	1.03	PEG_{113}-*b*-PDL_{23}

[a] Polymerizations were conducted at room temperature. Monomer conversions were all above 99%. [b] Monomer: L = DMNB-$_L$-Glu-NCA, D = DMNB-$_D$-Glu-NCA. [c] Monomer to initiator molar ratio. [d] Obtained molecular weight (theoretical molecular weight). [e] Determined by GPC. [f] Determined by ^{1}H NMR.

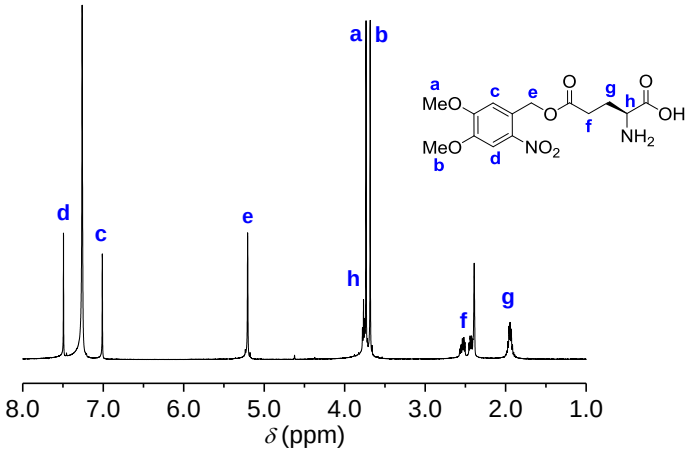

Fig. S1 ^{1}H NMR spectrum of DMNB-$_{L}$-Glu in DMSO-d_6/D_2O-DCl (9:1, v/v).

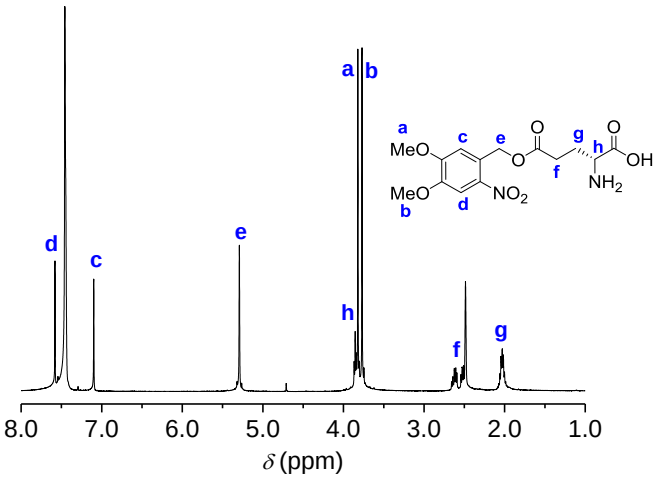

Fig. S2 ^{1}H NMR spectrum of DMNB-$_{D}$-Glu in DMSO-d_6/D_2O-DCl (9:1, v/v).

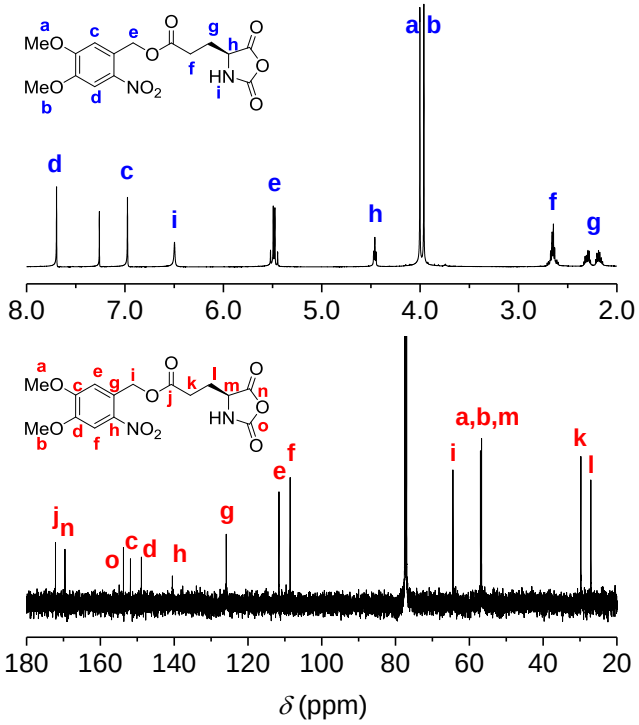

Fig. S3 ^{1}H and ^{13}C NMR spectra of DMNB-$_{L}$-Glu-NCA in $CDCl_3$.

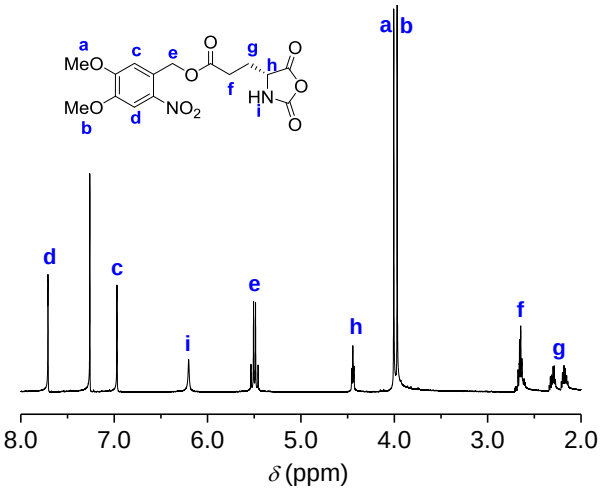

Fig. S4 ^{1}H NMR spectrum of DMNB-$_{D}$-Glu-NCA in $CDCl_3$.

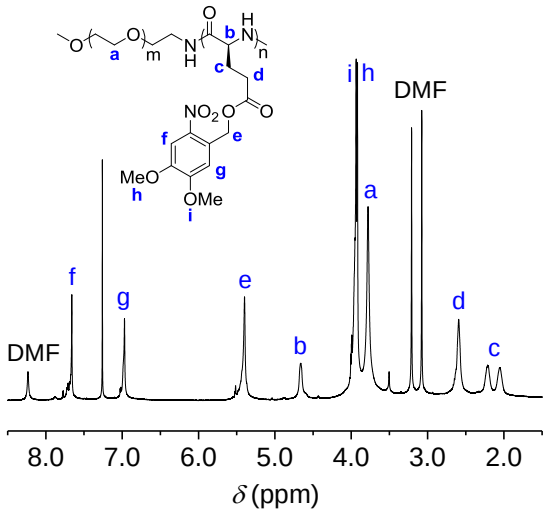

Fig. S5 ^{1}H NMR spectrum of PEG_{1k}-*b*-PL_{20} in $CDCl_3$/TFA-*d* (85:15, v/v). The block composition was calculated by the integral ratio of PEG methylene protons (peak ***a***) to DMNB dimethoxy protons (peak ***i*** and ***h***).

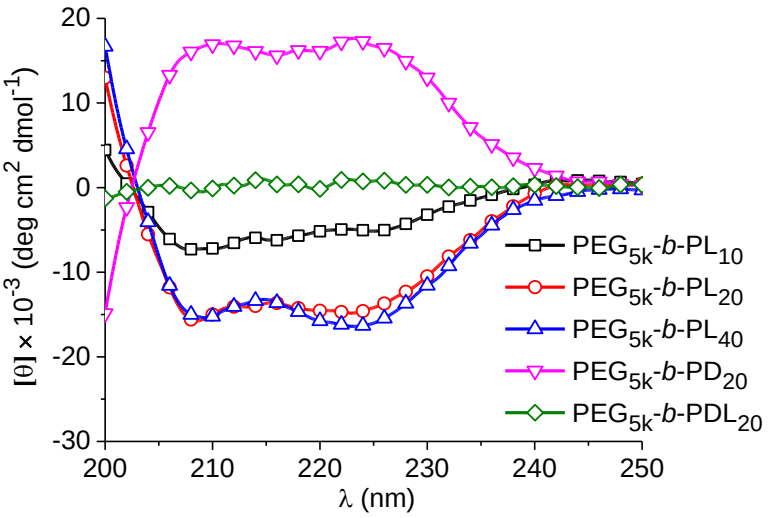

Fig. S6 CD spectra of PEG_{5k}-*b*-PL_{10}, PEG_{5k}-*b*-PL_{20}, PEG_{5k}-*b*-PL_{40}, PEG_{5k}-*b*-PD_{20}, and PEG_{5k}-*b*-PDL_{20} in aqueous solution at pH = 7. PEG_{1k} based copolymers aqueous suspension did not show strong CD signals because the polypeptide block was insoluble in water. Therefore, PEG_{5k} based water soluble copolymers were used instead to study the secondary structure of the copolymers.

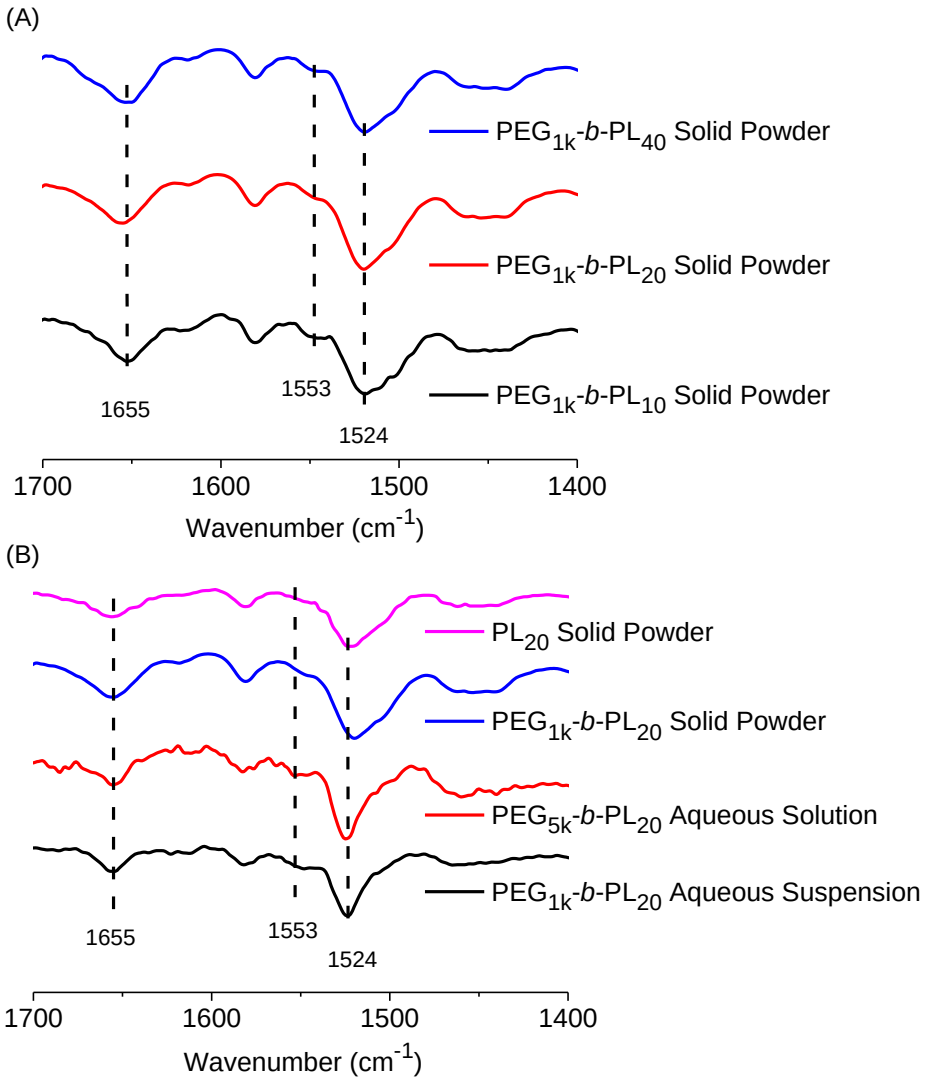

Fig. S7 Attenuated total reflectance (ATR)-FTIR spectra of PEG-*b*-PL diblock copolymers in solid, solution, or assembled states. (A) FTIR spectra of PEG_{1k}-*b*-PL solid powders; (B) FTIR spectra of homopolypeptide PL_{20}, PEG_{1k}-*b*-PL_{20} solid powder, water-soluble PEG_{5k}-*b*-PL_{20} aqueous solution, and PEG_{1k}-*b*-PL_{20} vesicle aqueous suspension. Amide I band (1655 cm^{-1}) and amide II band (1553 cm^{-1}) in all samples indicate α-helical conformation, the strong peak at 1524 cm^{-1} generates from nitrobenzene vibration.

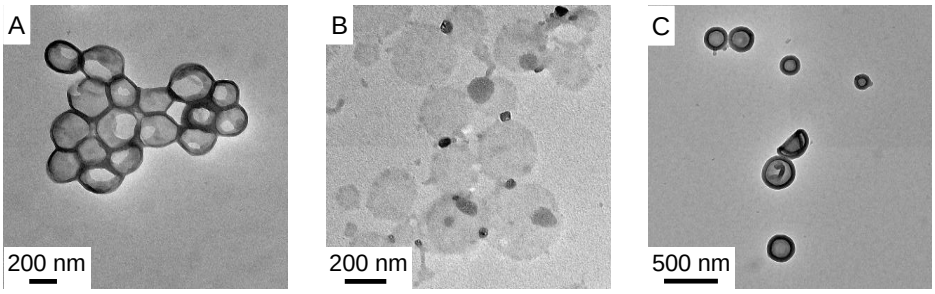

Fig. S8 Regular TEM contrast comparison of PEG_{1k}-*b*-PL_{20} (A) and PEG_{2k}-*b*-PL_{40} (B) assemblies. Regular TEM image of PEG_{5k}-*b*-polystyrene $(PSt)_{990}$ polymersome (C) was included for comparison.[1] PEG_{1k}-*b*-PL_{20} assembly, with much shorter copolymer amphiphile used, showed similar contrast (membrane thickness) with PEG_{5k}-*b*-PSt_{990} polymersome. PEG_{2k}-*b*-PL_{40} exhibited much lower contrast compared with PEG_{1k}-*b*-PL_{20} and PEG_{5k}-*b*-PSt_{990} vesicles, indicating thinner membrane.

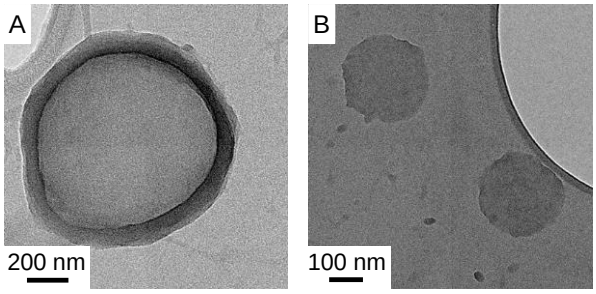

Fig. S9 Cryogenic TEM images of PEG_{1k}-*b*-PL_{20} (A) and PEG_{2k}-*b*-PL_{40} (B) assemblies.

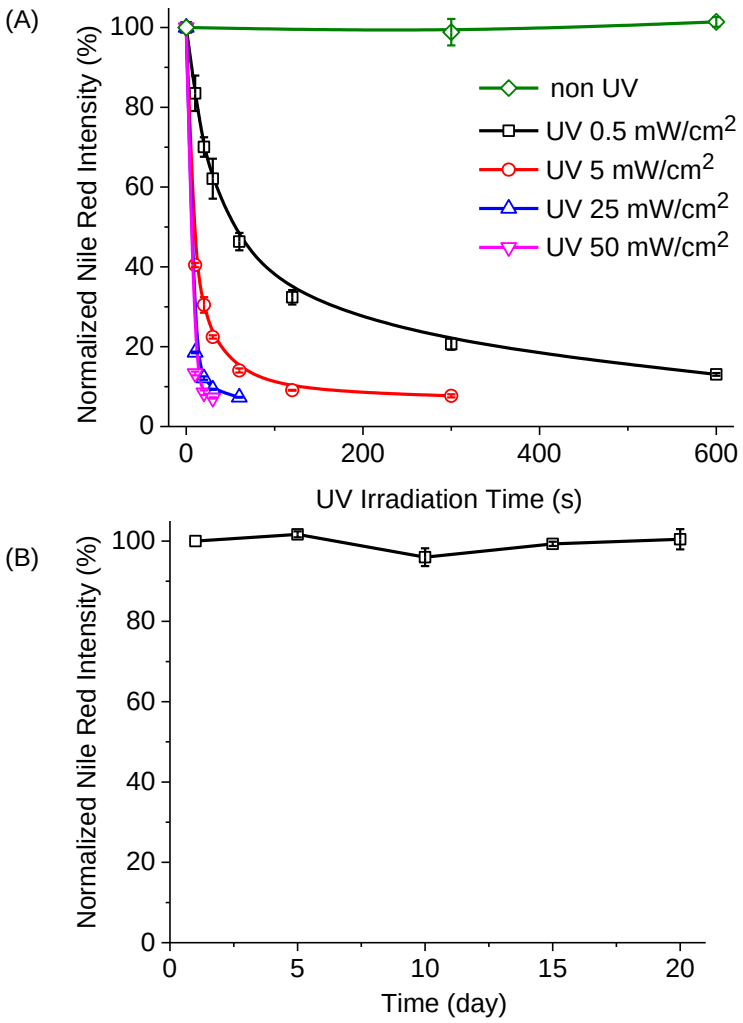

Fig. S10 UV-responsiveness of PEG_{1k}-*b*-PL_{20} vesicle using Nile Red (λ_{ex} = 540 nm, λ_{em} = 620 nm) as an indicator. (A) Normalized fluorescent intensity of Nile Red (0.1 wt%) after different UV irradiation time under different UV intensities (n = 3). (B) Normalized fluorescent intensity of Nile Red under dark. The fluorescent intensity of Nile Red is highly dependent on the hydrophobicity of the environment.[2] Before UV irradiation, PL domain packed as hydrophobic membrane in the aqueous suspension and Nile Red could be loaded in this region due to hydrophobic interaction and showed high fluorescent signal. UV irradiation altered the polypeptide domain into negatively charged hydrophilic poly($_L$-glutamic acid) chain, therefore Nile Red no longer resided in the membrane and precipitated with significant fluorescence decrease.

References

1. C.-Q. Huang and C.-Y. Pan, *Polymer*, 2010, **51**, 5115-5121.

2. A. P. Goodwin, J. L. Mynar, Y. Ma, G. R. Fleming and J. M. J. Fréchet, *J. Am. Chem. Soc.*, 2005, **127**, 9952-9953.