Ammonia uptake by transmembrane pH gradient poly(isoprene)-*block*-poly(ethylene glycol) polymersomes

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Supplementary Information

Supplementary Figures

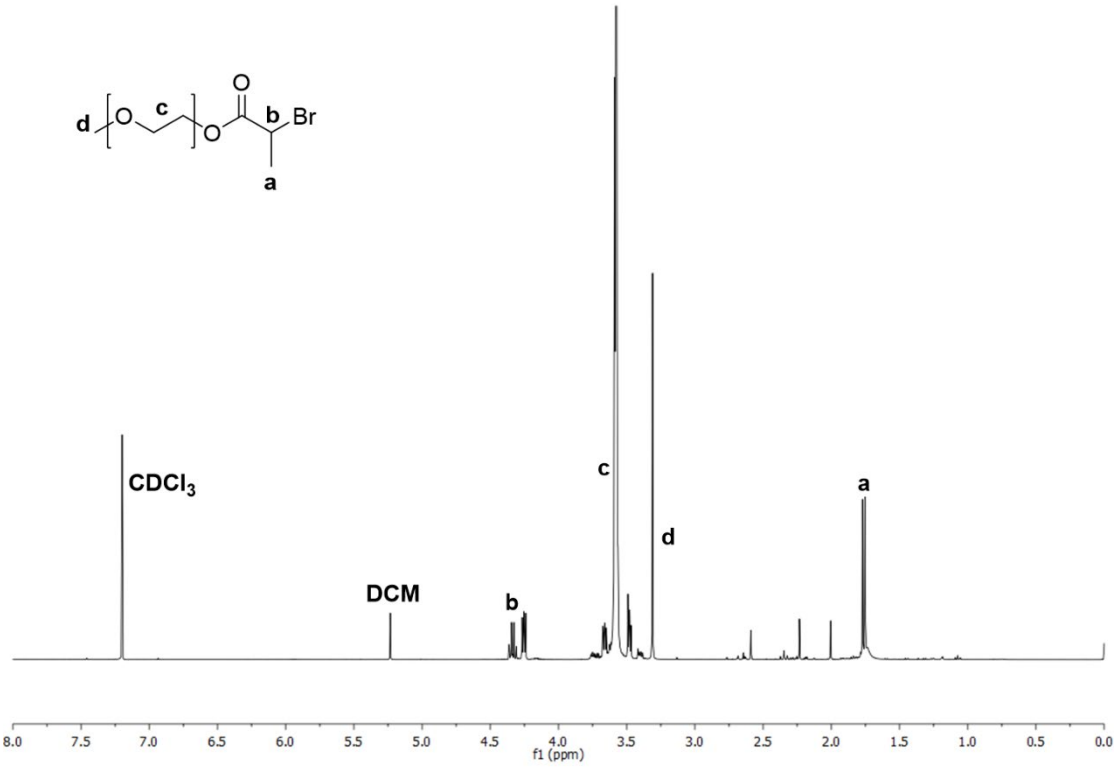

Figure S1. ^{1}H NMR spectrum of PEG_{700}-Br recorded in $CDCl_3$.

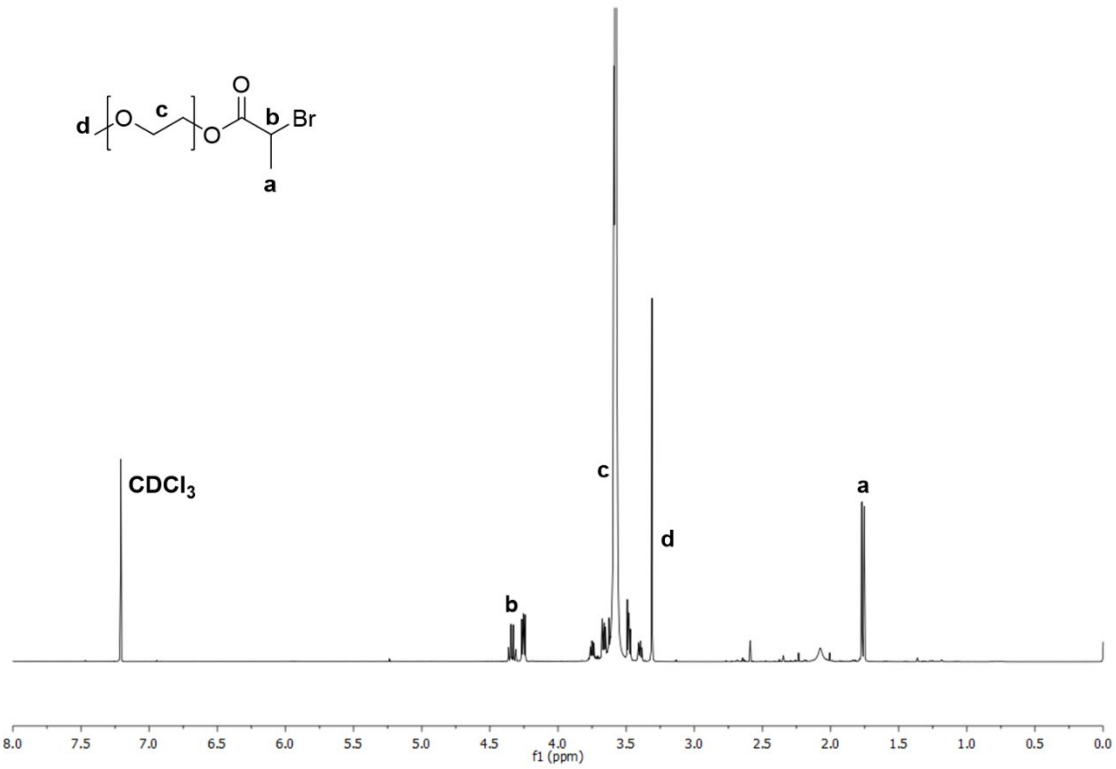

Figure S2. ^{1}H NMR spectrum of PEG_{2000}-Br recorded in $CDCl_3$.

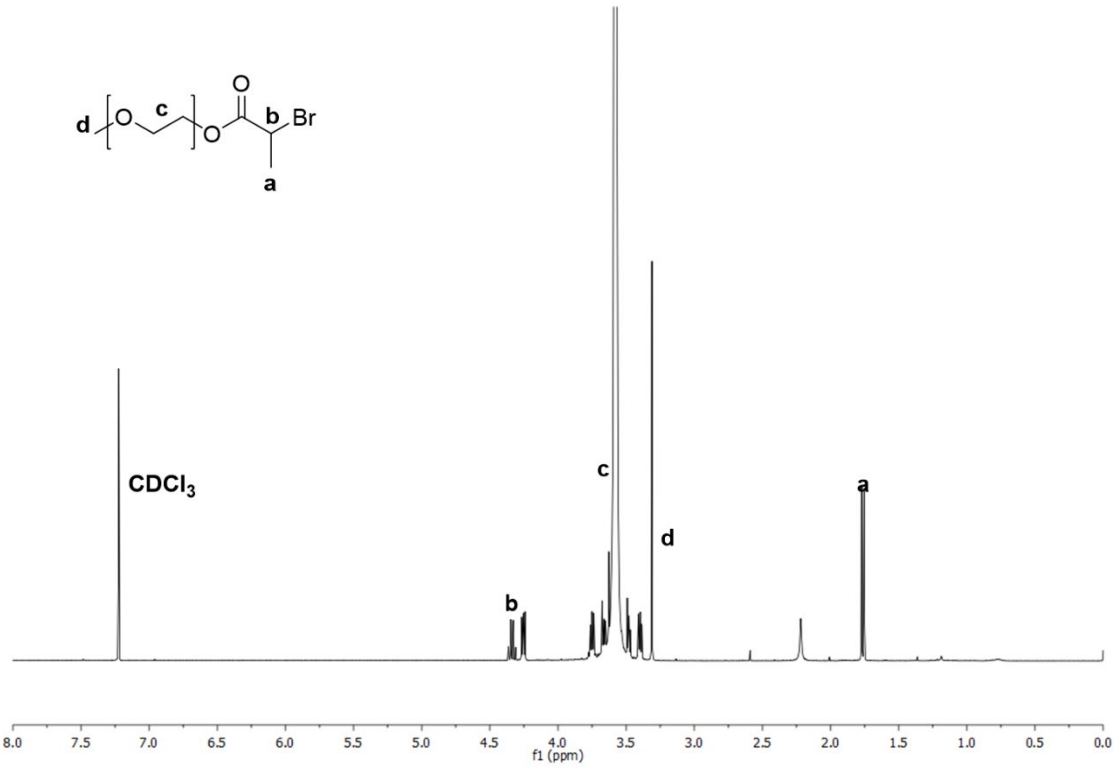

Figure S3. ^{1}H NMR spectrum of PEG_{5000}-Br recorded in $CDCl_3$.

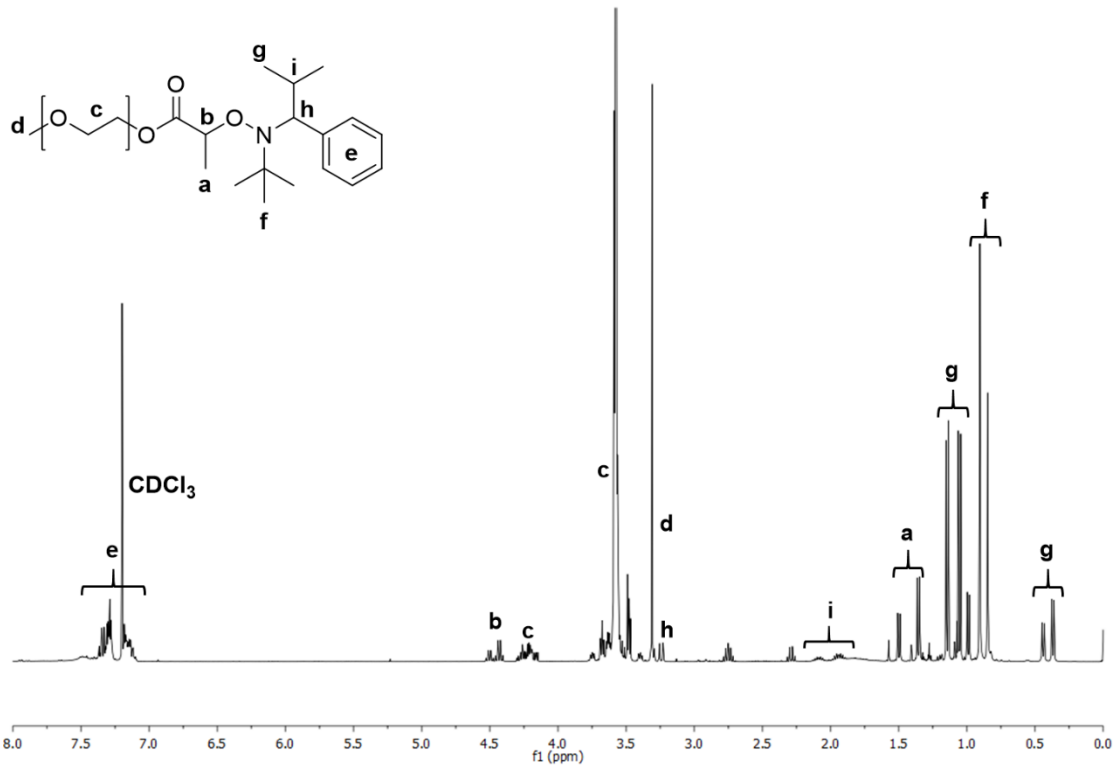

Figure S4. ^{1}H NMR spectrum of PEG_{700}-TIPNO recorded in $CDCl_3$.

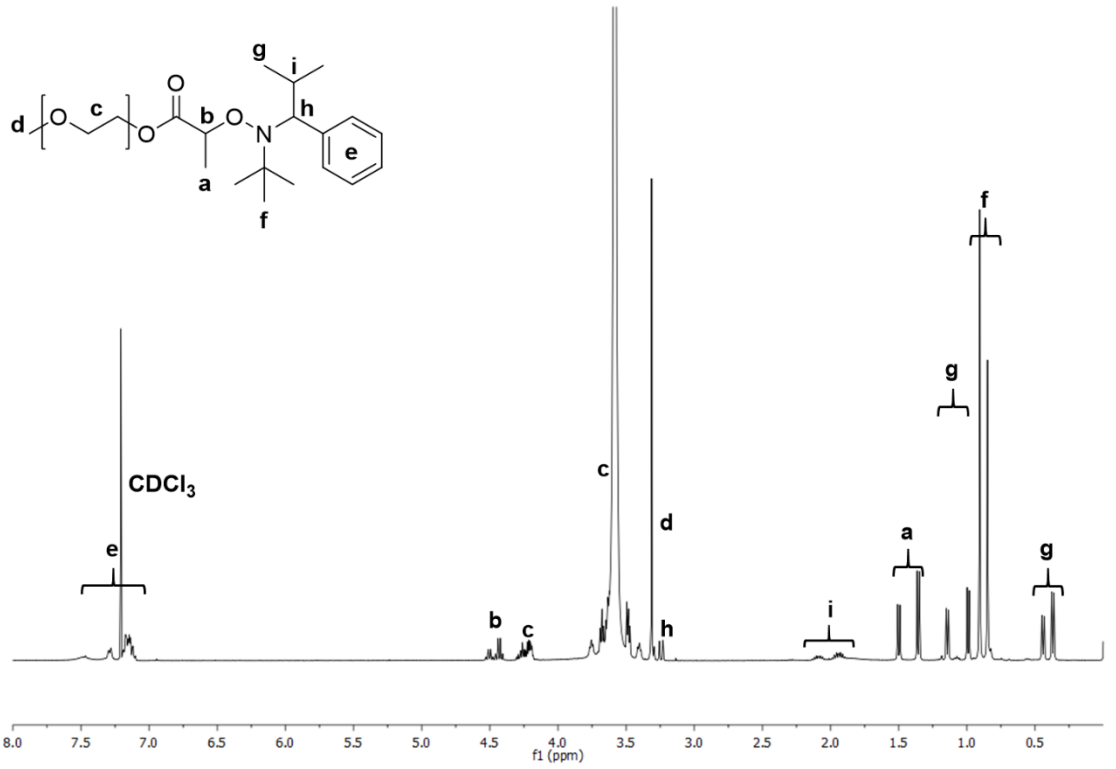

Figure S5. ^{1}H NMR spectrum of PEG_{2000}-TIPNO recorded in $CDCl_3$.

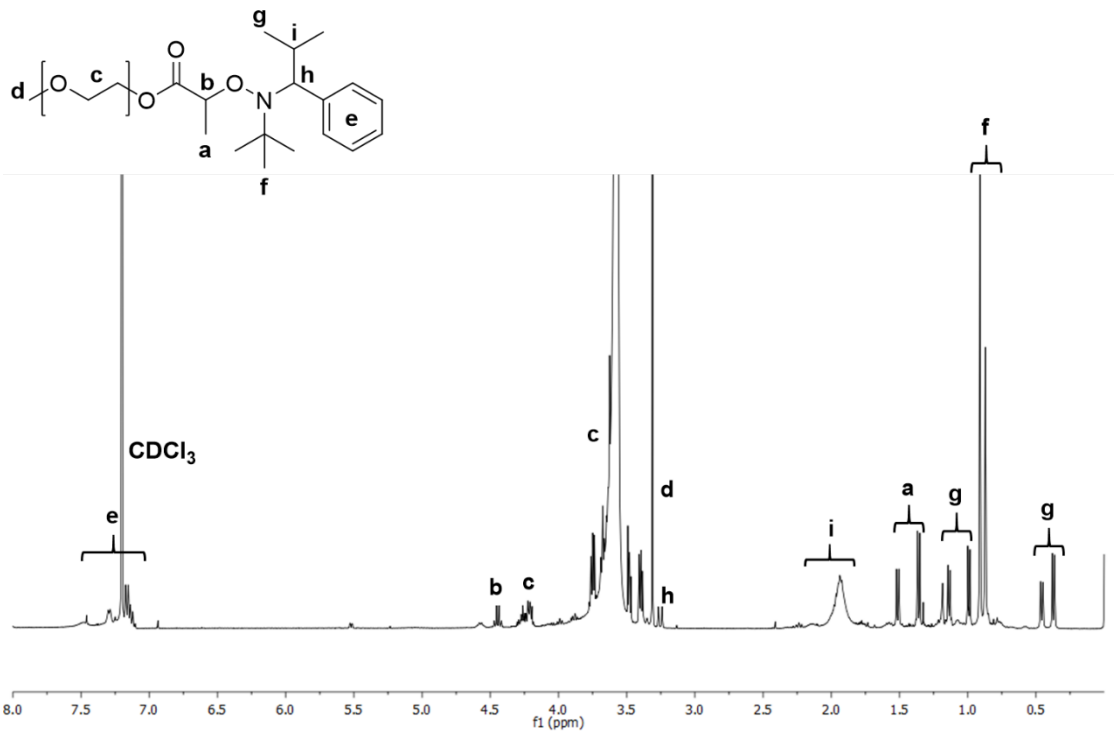

Figure S6. ^{1}H NMR spectrum of PEG_{5000}-TIPNO recorded in $CDCl_3$.

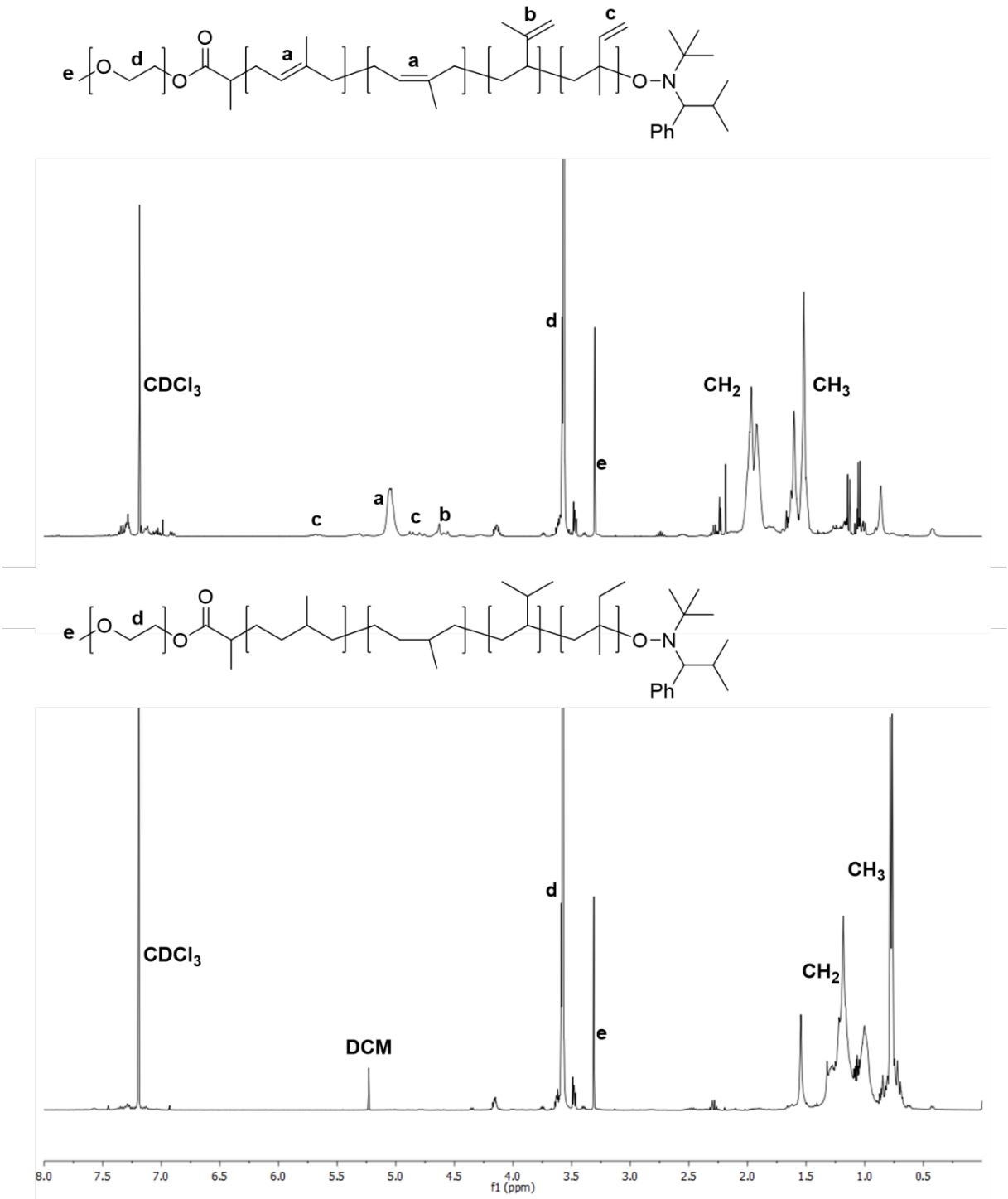

Figure S7. ^{1}H NMR spectra recorded in $CDCl_3$ of PI-*b*PEG_{700} polymer (PI/PEG (*w/w*) of 1.62) before (top) and after (bottom) the hydrogenation reaction.

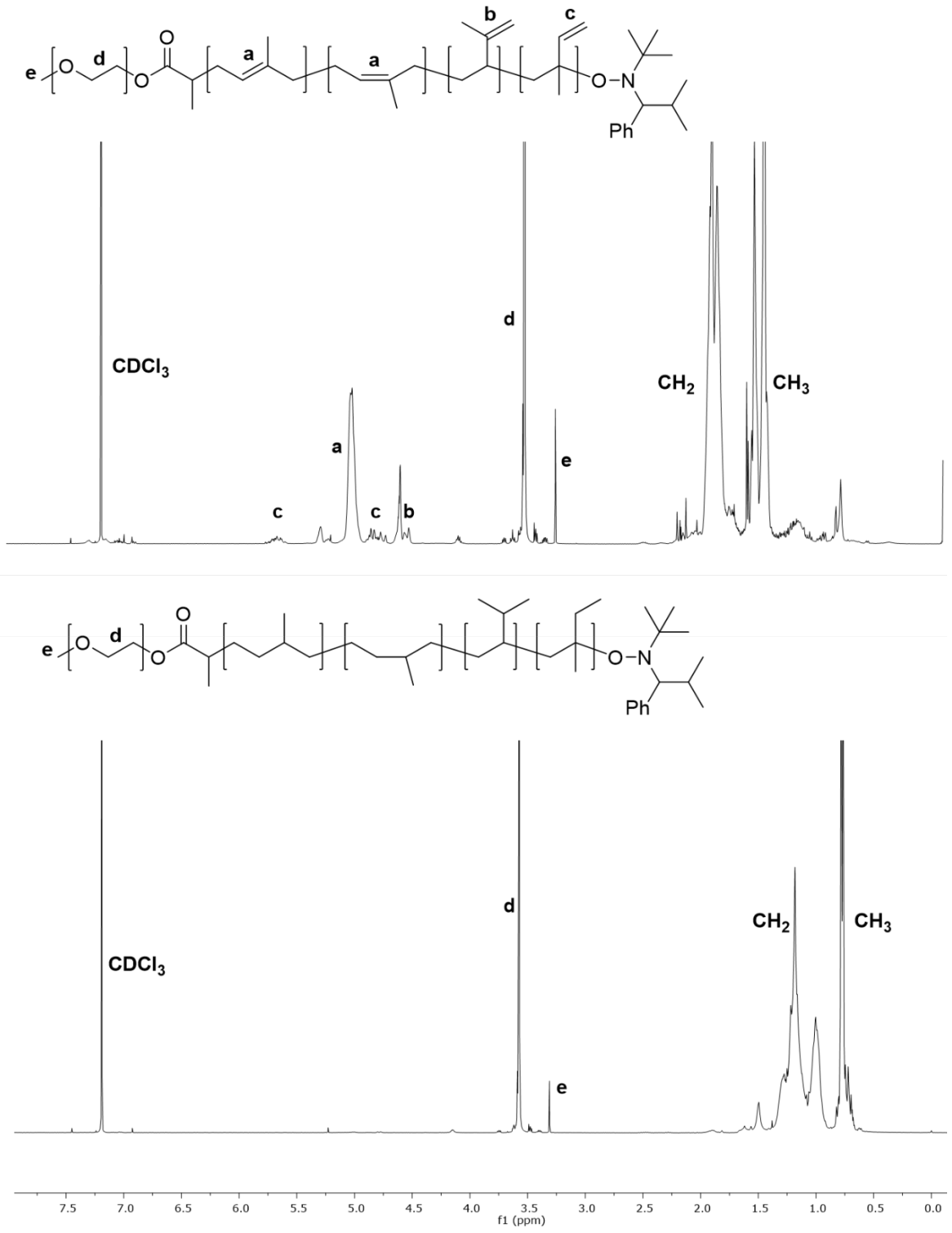

Figure S8. ^{1}H NMR spectra recorded in $CDCl_3$ of PI-*b*-PEG_{2000} polymer (PI/PEG (*w/w*) ratio of 3.62) before (top) and after (bottom) the hydrogenation reaction.

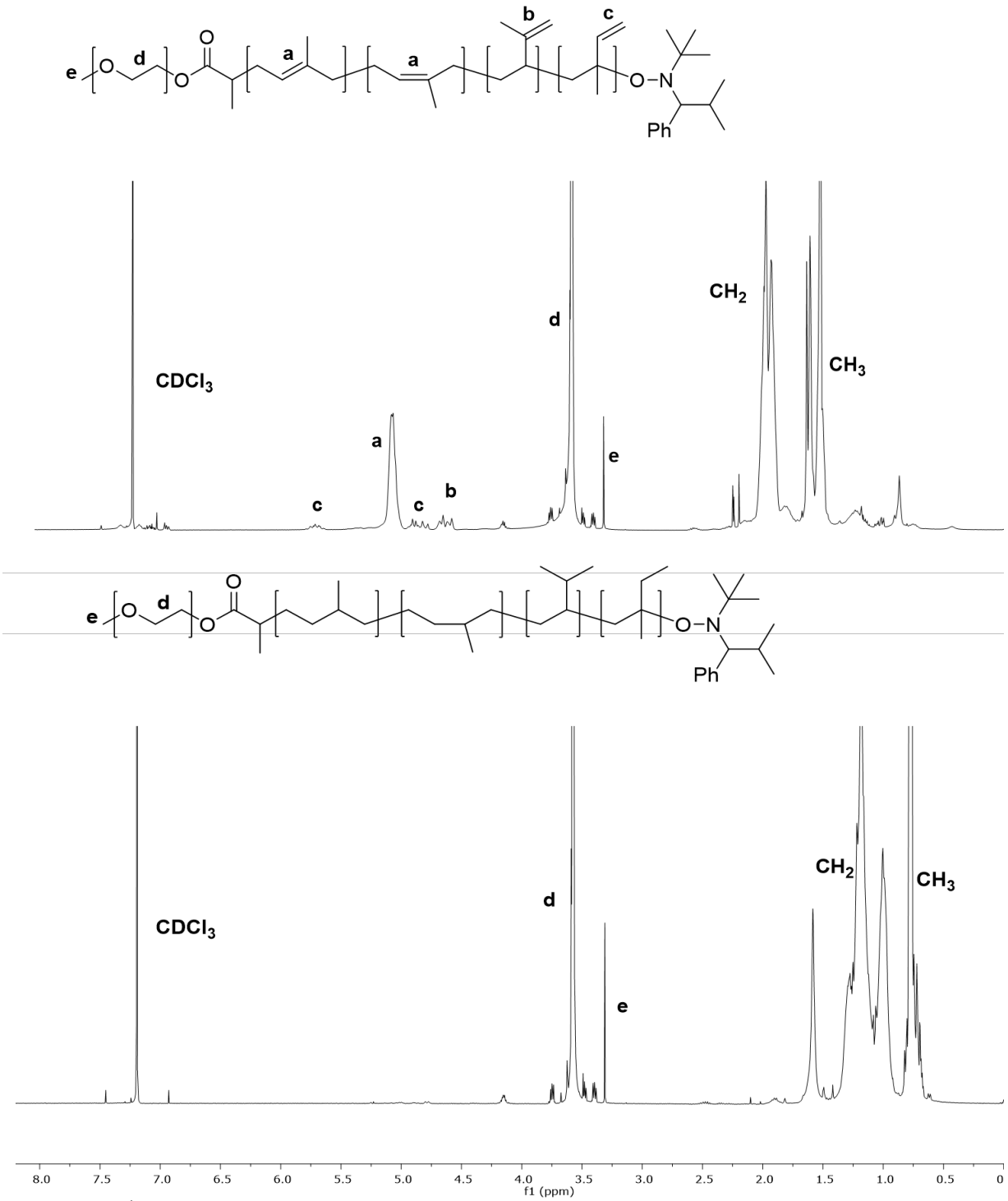

Figure S9. ^{1}H NMR spectra recorded in $CDCl_3$ of PI-*b*-PEG_{5000} polymer (PI/PEG (*w/w*) ratio of 0.77) before (top) and after (bottom) the hydrogenation reaction recorded in $CDCl_3$.

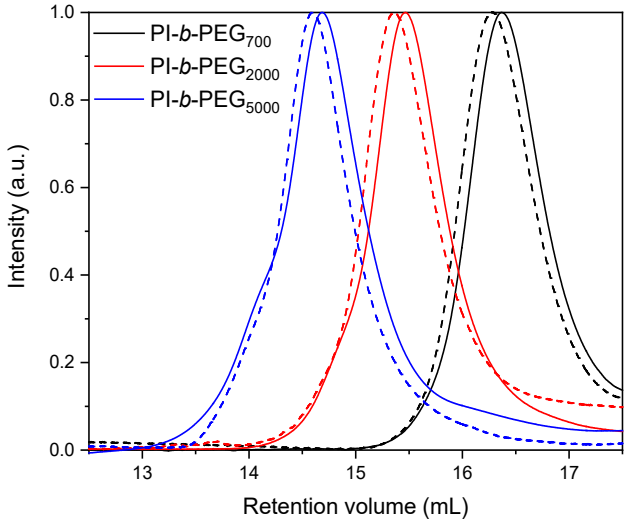

Figure S10. SEC traces of PI-*b*-PEG polymers of all three libraries before (straight lines) and after (dashed lines) hydrogenation. Recorded at 35 °C in THF and measured against PMMA standards.

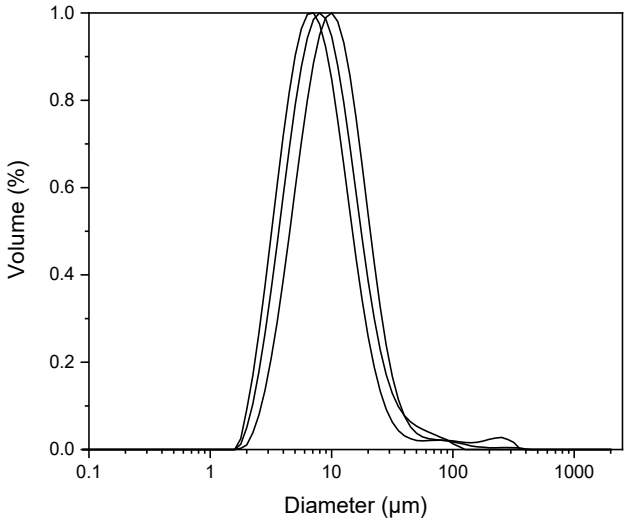

Figure S11. Size distribution of PI-*b*-PEG_{2000} polymer (PI/PEG (*w/w*) 1.99) by LD (n = 3).

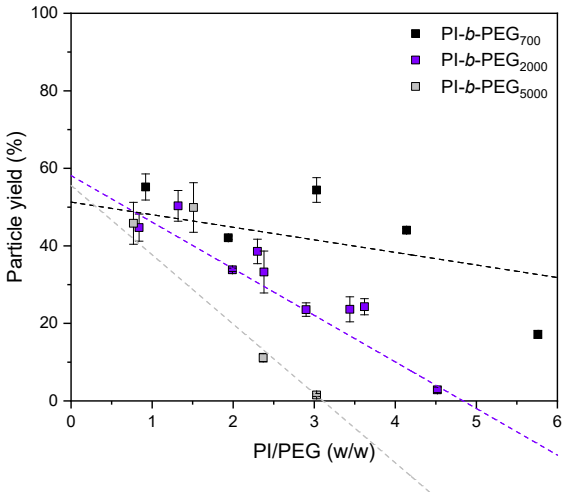

Figure S12. Particle yield of PI-*b*-PEG polymer assemblies with different PI/PEG (*w/w*) ratios. Particles were prepared using an emulsification process with an initial polymer feed of 30 mg (mean ± SD, n = 3).

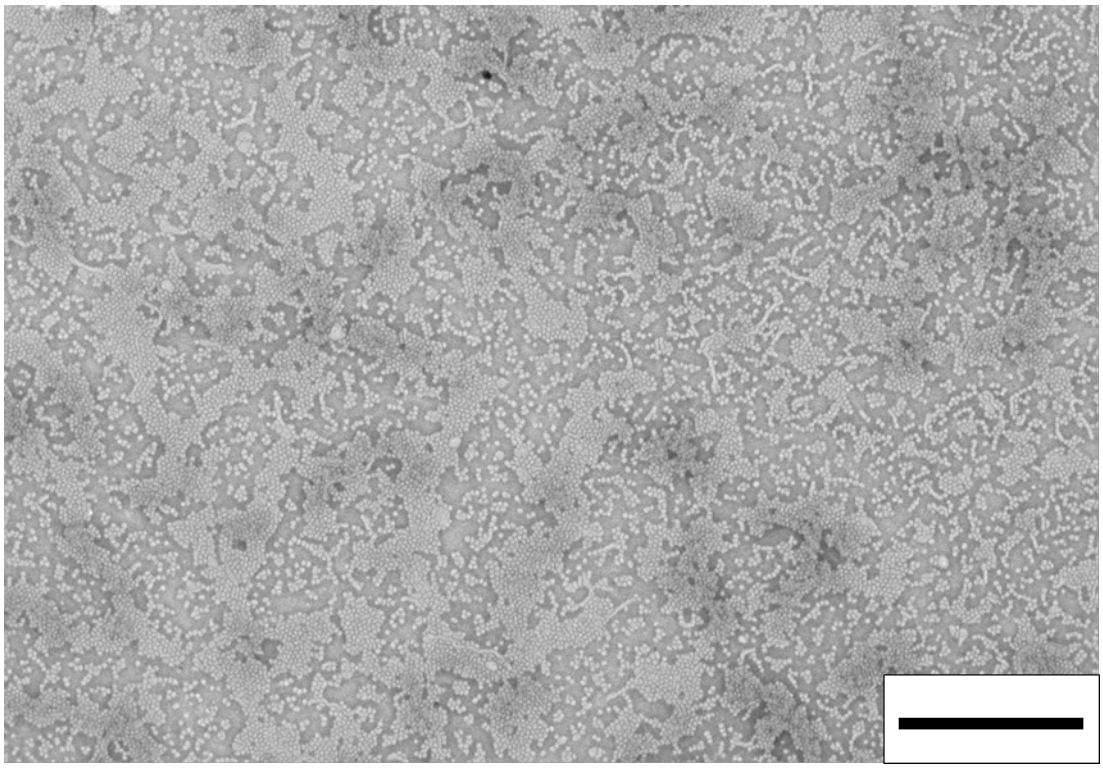

Figure S13. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 0.84) after self-assembly in phosphate buffer at pH 6.8 (scale bar: 1000 nm).

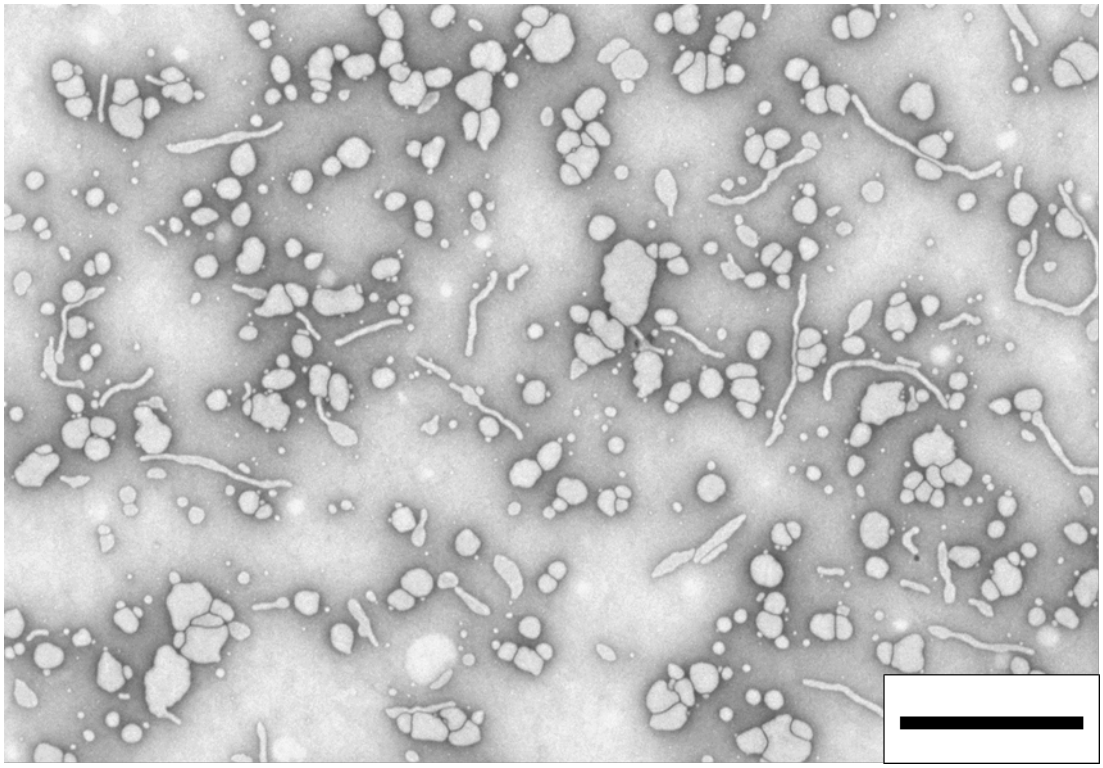

Figure S14. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 1.32) after self-assembly in phosphate buffer at pH 6.8 (scale bar: 1000 nm).

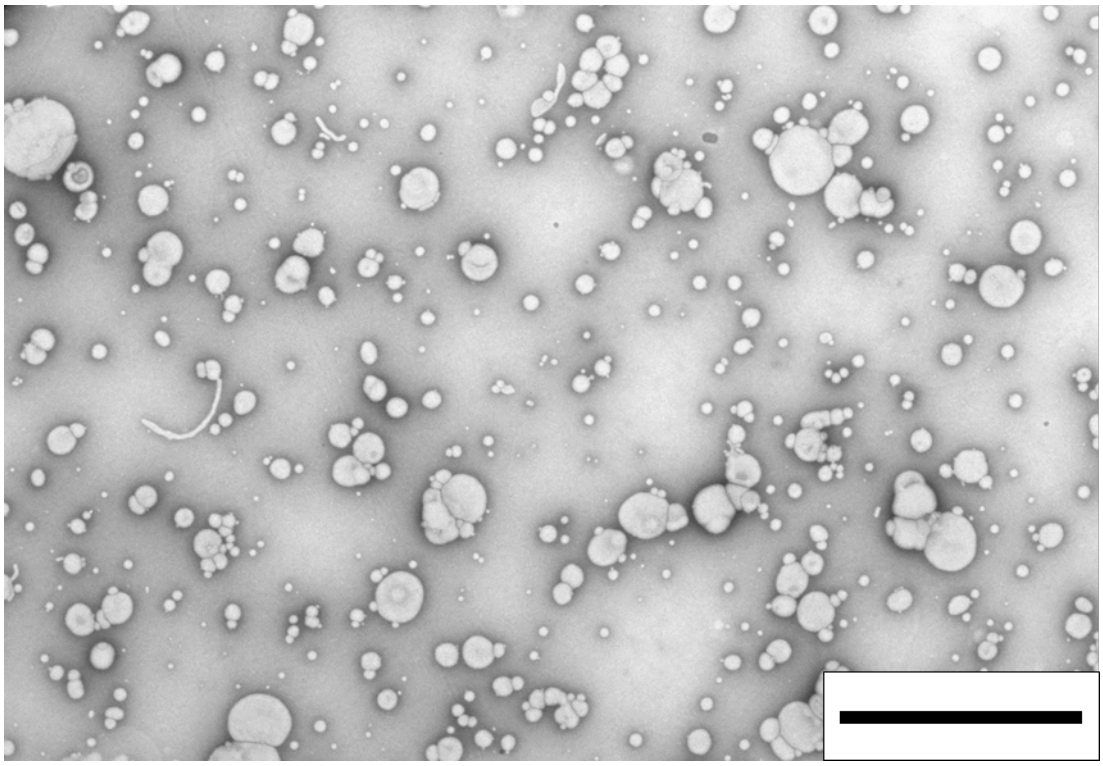

Figure S15. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 1.99) after self-assembly in phosphate buffer at pH 6.8 (Scale bar: 2000 nm).

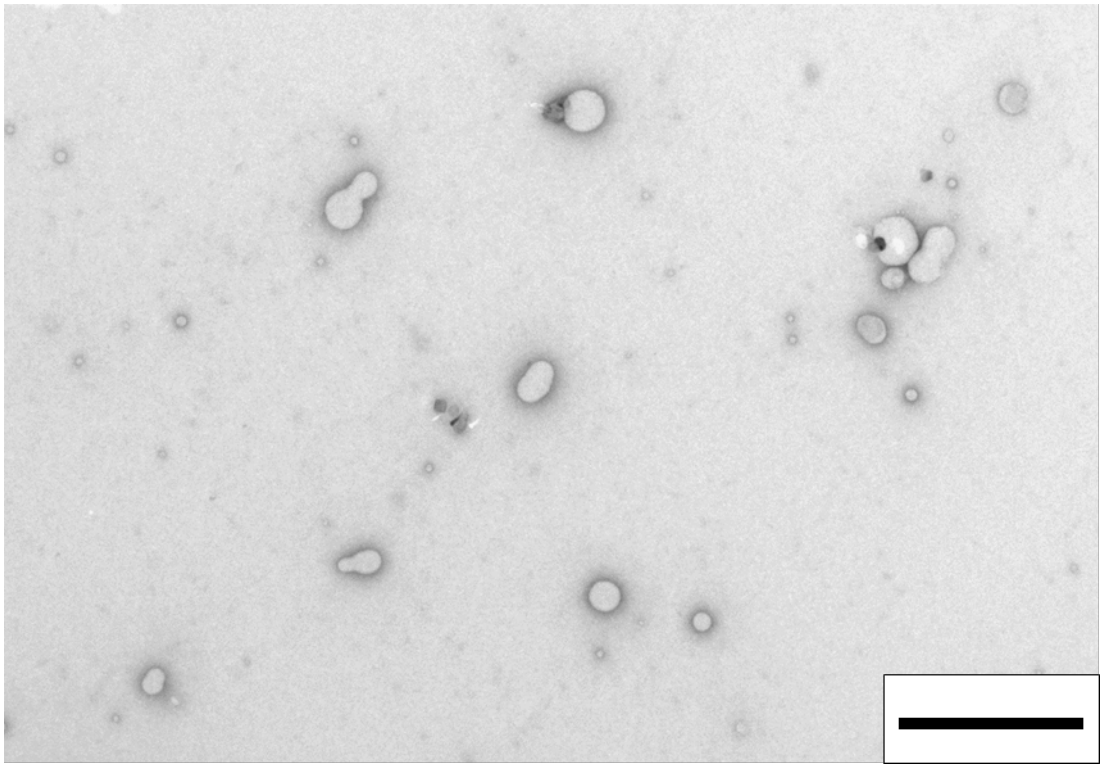

Figure S16. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 2.30) after self-assembly in phosphate buffer at pH 6.8 (scale bar: 1000 nm).

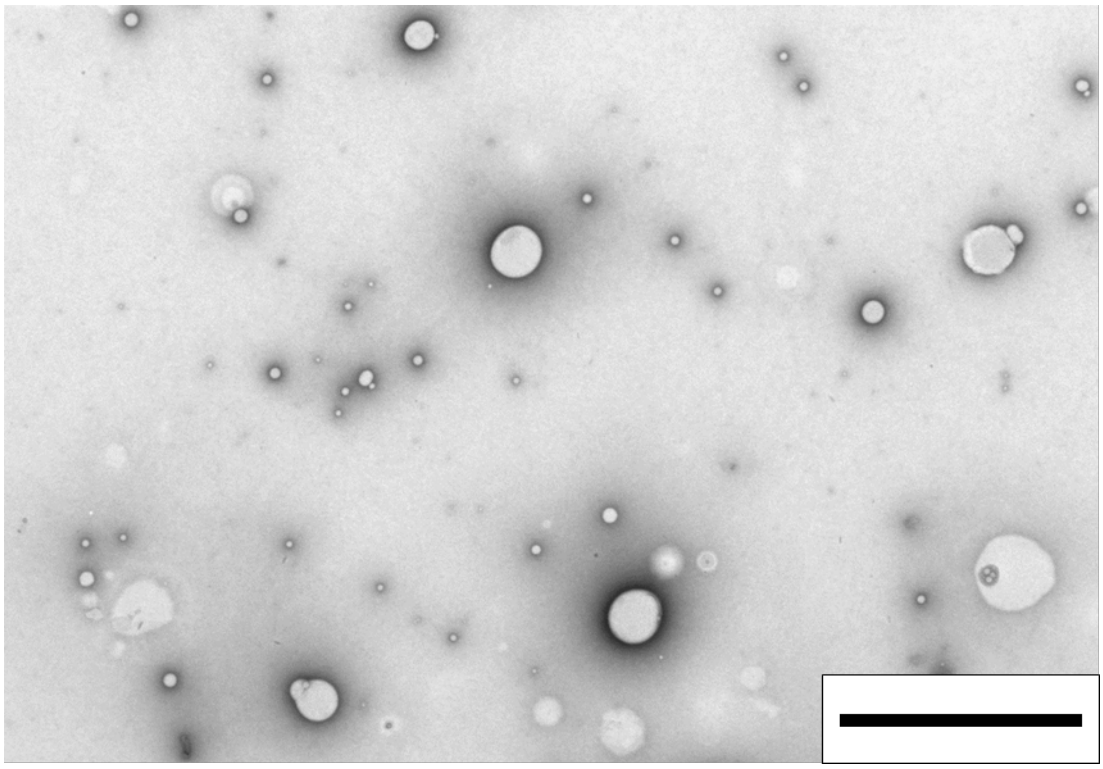

Figure S17. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 2.90) after self-assembly in phosphate buffer at pH 6.8 (scale bar: 2000 nm).

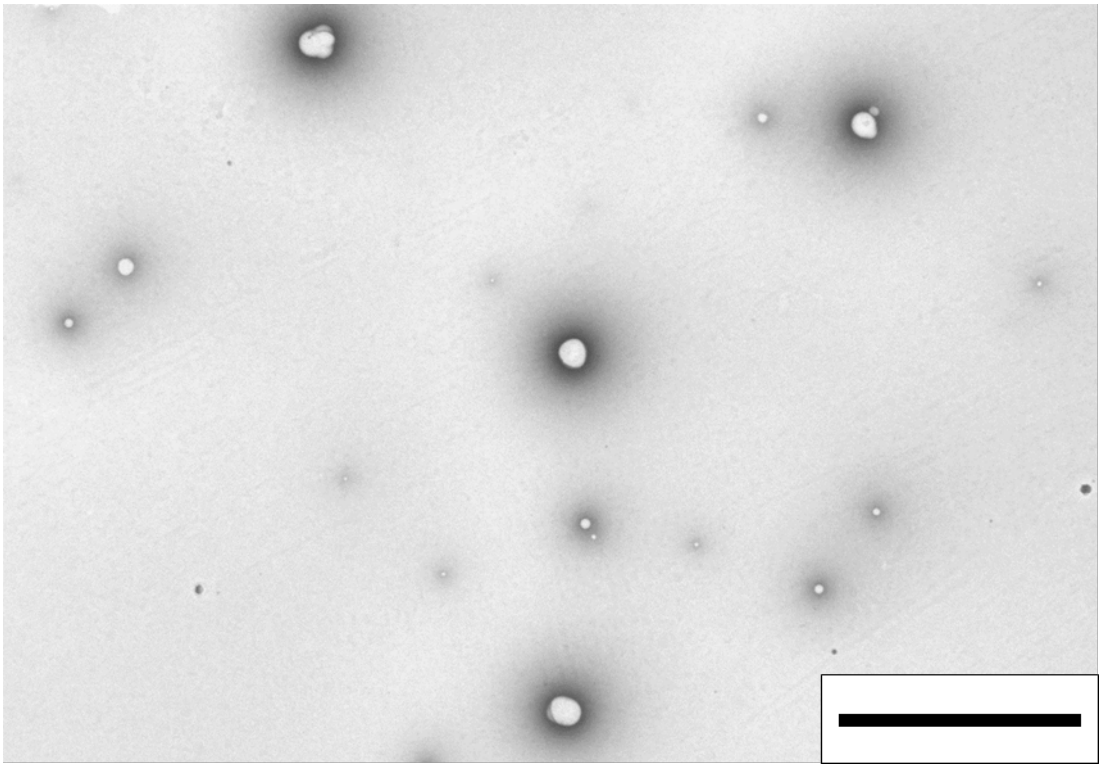

Figure S18. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 3.62) after self-assembly in phosphate buffer at pH 6.8 (scale bar: 2000 nm).

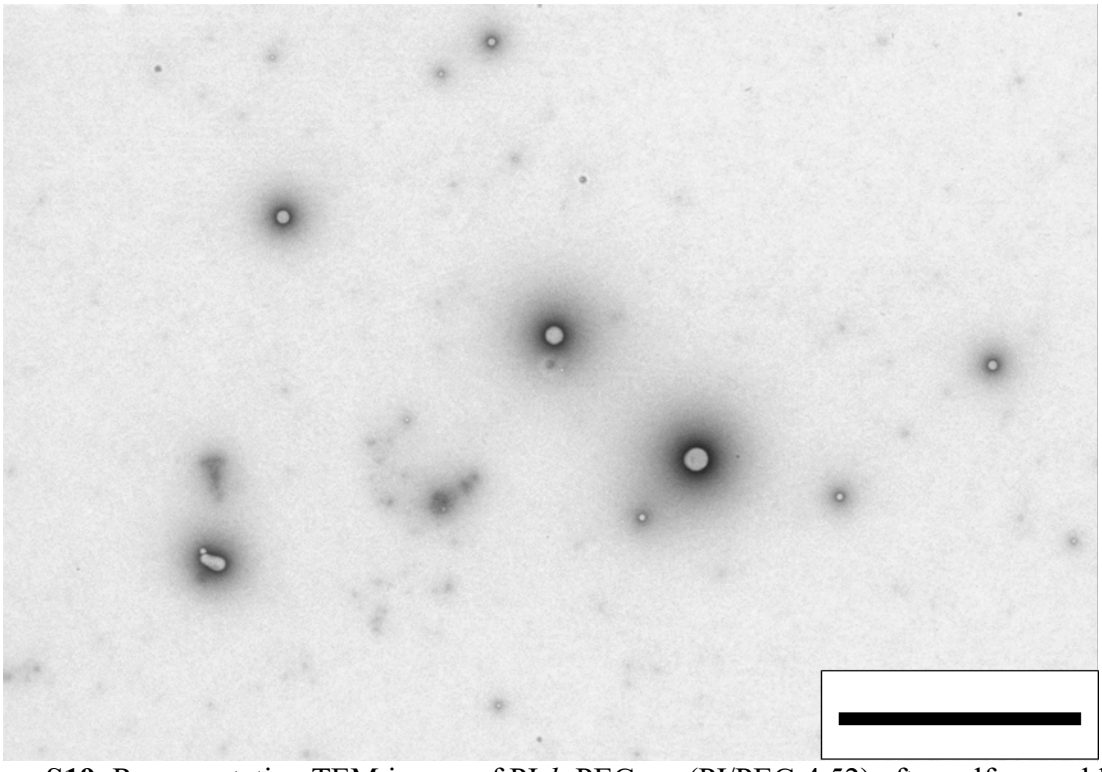

Figure S19. Representative TEM image of PI-*b*-PEG_{2000} (PI/PEG 4.52) after self-assembly in phosphate buffer at pH 6.8 (scale bar: 2000 nm).

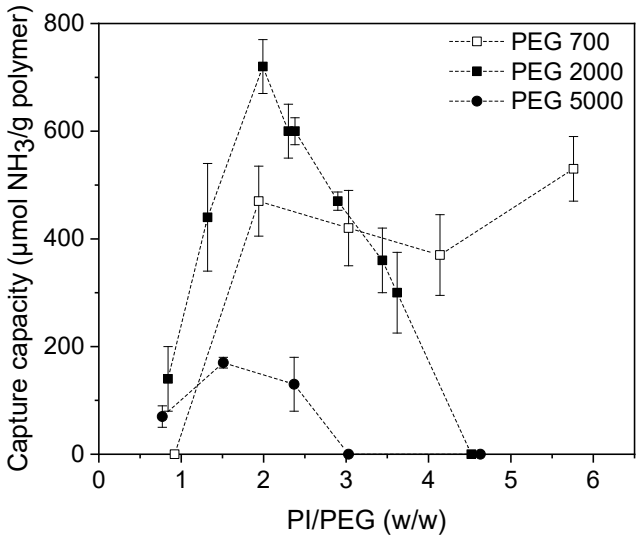

Figure S20. Ammonia capture capacity *vs.* PI/PEG (*w/w*) ratio after 24 h of incubation in phosphate buffer, pH 6.8 (mean ± SD, n = 3).

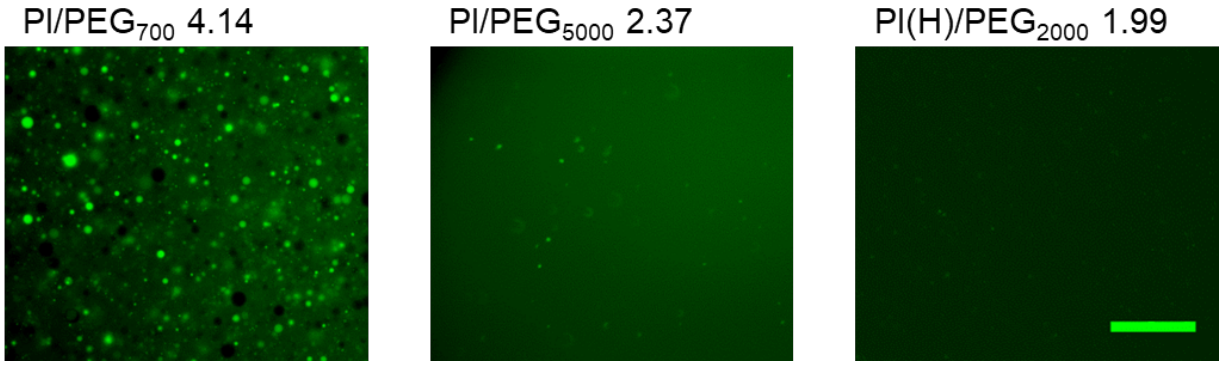

Figure S21. Representative fluorescence microscopy images of PI-*b*-PEG assemblies of PI-*b*-PEG_{700}, PI-*b*-PEG_{5000} and hydrogenated PI-*b*-PEG_{2000}, loaded with pyranine (scale bar: 50 µm).

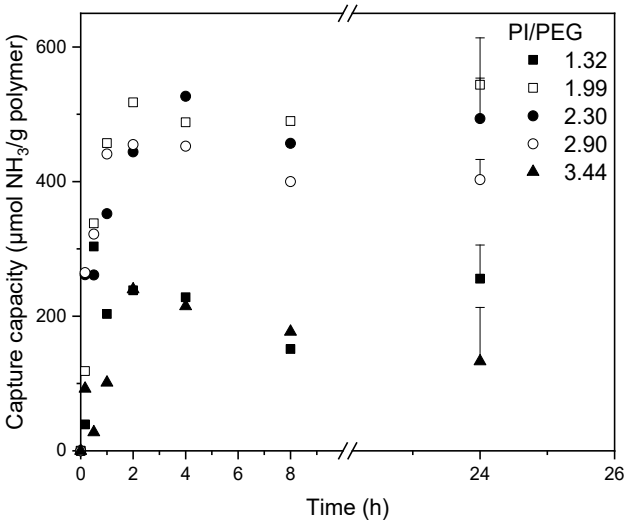

Figure S22. Ammonia capture by vesicles prepared with PI-*b*-PEG_{2000} polymers in simulated intestinal fluids over time (mean + SD, n = 3). Statistics were performed on the area under the capture capacity *vs.* time curve from 0 - 8 h ($AUC_{0\text{-}8h}$) (**Table S10**).

Figure S23. Image of crosslinked PI-*b*-PEG vesicles (PI/PEG 1.99) after resuspension in DCM.

Supplementary tables

Table S1. Microstructure of PI-*b*-PEG amphiphiles synthesized in this study. Molar amount of the different PI isomers present in the PI-*b*-PEG amphiphiles was calculated by ^{1}H NMR spectroscopy. Data presented as % of total PI molar units.

Polymer	PI/PEG [*w/w*]	1,4-PI isomer [%]	1,2-PI isomer [%]	3,4-PI isomer [%]
PI-*b*-PEG $_{700}$	0.92	89	4	7
	1.94	90	4	6
	3.03	88	4	8
	4.14	82	5	13
	5.76	81	5	14
PI-*b*-PEG $_{2000}$	0.84	90	4	6
	1.32	90	4	6
	1.99	86	4	10
	2.30	90	4	6
	2.38	90	4	6
	2.90	90	4	6
	3.44	89	4	7
	3.62	85	5	10
	4.52	90	4	6
PI-*b*-PEG $_{5000}$	0.77	90	4	6
	1.51	90	4	6
	2.37	89	4	7
	3.03	86	4	10
	4.63	88	4	8

Table S2. Hydrogenated PI(H)-*b*-PEG amphiphiles and their respective polymersome ammonia capture capacity after 24 h incubation in phosphate buffer at pH 6.8. Polymersomes were produced using a sonication probe at an amplitude of 5 for 1 min.

	PI/PEG [*w/w*]	Yield [%] [a)]	Ammonia capture capacity [µmol NH_3/g polymer]
PI(H)-*b*-PEG_{700}	1.62	76	-
	3.03	84	168
PI(H)-*b*-PEG_{2000}	0.84	100	-
	1.32	89	160
	1.99	91	85
	2.30	85	22
	3.62	94	*
	4.52	85	*
PI(H)-*b*-PEG_{5000}	0.77	100	*
	2.37	94	*
	3.03	97	*
	4.63	85	*

[a)] Obtained after hydrogenation reaction; - No uptake experiments performed; * No vesicles due to solubility issues of the PI(H)-*b*-PEG.

Table S3. Ammonia capture capacities of PI-*b*-PEG polymersomes after 24 h (mean ± SD, n = 3).

	PI/PEG [w/w]	Ammonia capture capacity [µmol NH_3/g polymer] [a]	Ammonia capture capacity [µmol NH_3/g polymer] [b]
PI-*b*-PEG_{700}	0.92	-	
	1.94	470 ± 70	
	3.03	420 ± 70	
	4.14	370 ± 80	
	5.76	530 ± 60	
PI-*b*-PEG_{2000}	0.84	140 ± 60	-
	1.32	440 ± 100	170 ± 50
	1.99	720 ± 50	540 ± 10
	2.30	600 ± 50	460 ± 120
	2.38	600 ± 25	
	2.90	470 ± 20	410 ± 30
	3.44	360 ± 60	120 ± 80
	3.62	300 ± 80	
	4.52	-	
PI-*b*-PEG_{5000}	0.77	70 ± 20	
	1.51	170 ± 10	
	2.37	130 ± 50	
	3.03	-	
	4.63	-	

[a] Performed in phosphate buffer at pH 6.8; [b] Performed in buffer supplemented with bile salt at pH 6.8; - No capture observed.

Table S4. Statistical data of the *in vitro* ammonia capture experiments in phosphate buffer at pH 6.8 of PI-*b*-PEG_{700} polymersomes. The area under the capture capacity *vs.* time curves from 0-8 h ($AUC_{0\text{-}8h}$) were compared using a one-way ANOVA and subsequent Tukey's multiple comparisons test.

PI/PEG (*w/w*)	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
0.92 *vs*. 1.94	-4400	****	< 0.0001
0.92 *vs*. 3.03	-2700	****	< 0.0001
0.92 *vs*. 4.14	-2200	***	0.0001
0.92 *vs*. 5.76	-2600	****	< 0.0001
1.94 *vs*. 3.03	1700	**	0.0013
1.94 *vs*. 4.14	2200	***	0.0002
1.94 *vs*. 5.76	1800	***	0.0007
3.03 *vs*. 4.14	500	n.s.	0.4609
3.03 *vs*. 5.76	100	n.s.	0.993
4.14 *vs*. 5.76	-400	n.s.	0.6889

n.s.: not significant.

Table S5. Statistical data of the *in vitro* ammonia capture experiments in phosphate buffer at pH 6.8 of PI-*b*-PEG_{2000} polymersomes. The area under the capture capacity *vs.* time curves from 0-8 h ($AUC_{0\text{-}8h}$) were compared using a one-way ANOVA and subsequent Tukey's multiple comparisons test.

PI/PEG (*w/w*)	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
0.84 *vs*. 1.32	-2900	****	< 0.0001
0.84 *vs*. 1.99	-4600	****	< 0.0001
0.84 *vs*. 2.30	-3300	****	< 0.0001
0.84 *vs*. 2.38	-3600	****	< 0.0001
0.84 *vs*. 2.90	-2500	****	< 0.0001
0.84 *vs*. 3.44	-1400	*	0.0411
0.84 *vs*. 3.62	-900	n.s.	0.2994
0.84 *vs*. 4.52	1200	n.s.	0.0897
1.32 *vs*. 1.99	-1600	*	0.0105
1.32 *vs*. 2.30	-400	n.s.	0.981
1.32 *vs*. 2.38	-600	n.s.	0.7522
1.32 *vs*. 2.90	400	n.s.	0.9666
1.32 *vs*. 3.44	1600	*	0.0121
1.32 *vs*. 3.62	2000	**	0.0012
1.32 *vs*. 4.52	4100	****	< 0.0001
1.99 *vs*. 2.30	1200	n.s.	0.0772

1.99 *vs*. 2.38	1000	n.s.	0.2515

Continuation Table S5

1.99 *vs*. 2.90	2000	**	0.0011
1.99 *vs*. 3.44	3200	****	< 0.0001
1.99 *vs*. 3.62	3600	****	< 0.0001
1.99 *vs*. 4.52	5800	****	< 0.0001
2.30 *vs*. 2.38	-300	n.s.	0.9985
2.30 *vs*. 2.90	800	n.s.	0.5014
2.30 *vs*. 3.44	2000	**	0.0015
2.30 *vs*. 3.62	2400	***	0.0002
2.30 *vs*. 4.52	4500	****	< 0.0001
2.38 *vs*. 2.90	1000	n.s.	0.189
2.38 *vs*. 3.44	2200	***	0.0004
2.38 *vs*. 3.62	2600	****	< 0.0001
2.38 *vs*. 4.52	4800	****	< 0.0001
2.90 *vs*. 3.44	1200	n.s.	0.1059
2.90 *vs*. 3.62	1600	*	0.0117
2.90 *vs*. 4.52	3700	****	< 0.0001
3.44 *vs*. 4.52	400	n.s.	0.9641
3.44 *vs*. 3.62	2600	****	< 0.0001
3.62 *vs*. 4.52	2100	***	0.0006

n.s.: not significant.

Table S6. Statistical data of the *in vitro* ammonia capture experiments in phosphate buffer at pH 6.8 of PI-*b*-PEG_{5000} polymersomes. The area under the capture capacity *vs.* time curves from 0-8 h ($AUC_{0\text{-}8h}$) were compared using a one-way ANOVA and subsequent Tukey's multiple comparisons test.

PI/PEG (*w/w*)	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
0.77 *vs*. 1.51	-600	n.s.	0.0548
0.77 *vs*. 2.37	-800	*	0.0158
0.77 *vs*. 3.03	400	n.s.	0.3742
0.77 *vs*. 4.63	400	n.s.	0.3742
1.51 *vs*. 2.37	-200	n.s.	0.9239
1.51 *vs*. 3.03	1000	**	0.0031
1.51 *vs*. 4.63	1000	**	0.0031
2.37 *vs*. 3.03	1100	**	0.001
2.37 *vs*. 4.63	1100	**	0.001
3.03 *vs*. 4.63	0	n.s.	> 0.9999

n.s.: not significant.

Table S7. Statistical data of the *in vitro* ammonia capture experiments using PI-*b*-PEG polymersomes (PI/PEG 1.99). The area under the capture capacity *vs.* time curves from 0-8 h ($AUC_{0\text{-}8h}$) were compared using an unpaired t-test.

Experiment	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
Phosphate buffer pH 6.8 *vs*. bile salt buffer pH 6.8	-1900	**.	0.0027
No pH gradient *vs.* pH gradient in phosphate buffer	-5500	****	< 0.0001
Hydrogenated polymer *vs*. non-hydrogenated polymer in phosphate buffer	-5800	****	< 0.0001

n.s.: not significant.

Table S8. Statistical data of the *in vitro* ammonia capture experiments using native (Nat) or crosslinked (CL) PI-*b*-PEG_{2000} polymersomes in phosphate buffer (Pho) or colon-mimicking environment (Meta) (PI/PEG 1.99, unless indicated otherwise). For multiple groups ammonia capture values obtained after 24 h were compared using a one-way ANOVA and subsequent Tukey's multiple comparisons test. In case of experiments with only two groups, ammonia capture values obtained after 24 h were compared using an unpaired t-test.

	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
Nat Meta *vs.* Nat Pho	-520	***	0.0005
PI/PEG 2.30, Nat Meta *vs.* Nat Pho	-410	**	0.0014
CL Pho *vs*. CL Meta	380	***	0.0003
CL Pho *vs.* Nat Pho	100	n.s.	0.2909
CL Pho *vs.* Nat Meta	620	****	< 0.0001
CL Meta *vs.* Nat Pho	-280	**	0.0026
CL Meta *vs.* Nat Meta	230	**	0.0087
Nat Pho *vs.* Nat Meta	520	****	< 0.0001

n.s.: not significant.

Table S9. Statistical data of the *in vitro* ammonia capture experiments in phosphate buffer at pH 6.8 of crosslinked polymersomes (PI/PEG_{2000} 1.99) with different concentrations of crosslinker. Ammonia capture capacity values obtained after 24 h were compared using a one-way ANOVA and subsequent Tukey's multiple comparisons test.

Concentration of crosslinker [mM]	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
0 *vs.* 0.5	100	n.s.	0.4543
0 *vs.* 1	190	*	0.0385
0 *vs.* 2	320	***	0.0008
0 *vs.* 5	370	***	0.0002
0 *vs.* 10	340	***	0.0005
0.5 *vs.* 1	90	n.s.	0.5963
0.5 *vs.* 2	220	*	0.0177
0.5 *vs.* 5	260	**	0.0044
0.5 *vs.* 10	240	*	0.0101
1 *vs.* 2	130	n.s.	0.2485
1 *vs.* 5	180	n.s.	0.0666
1 *vs.* 10	150	n.s.	0.1509
2 *vs.* 5	50	n.s.	0.9537
2 *vs.* 10	20	n.s.	0.9993
5 *vs.* 10	-30	n.s.	0.9949

n.s.: not significant.

Table S10. Statistical data of the *in vitro* ammonia capture experiments in simulated intestinal fluids at pH 6.8 of PI-*b*-PEG_{2000} polymersomes. The area under the capture capacity *vs.* time curves from 0-8 h ($AUC_{0\text{-}8h}$) were compared using a one-way ANOVA and subsequent Tukey's multiple comparisons test.

PI/PEG (*w/w*)	Mean difference [µmol g^{-1} h^{-1}]	Significance	p value
0.84 *vs*. 1.32	-2100	*	0.0345
0.84 *vs*. 1.99	-3800	***	0.0003
0.84 *vs*. 2.30	-3700	***	0.0004
0.84 *vs*. 2.90	-3400	***	0.001
0.84 *vs*. 3.44	-1500	n.s.	0.1884
1.32 *vs*. 1.99	-1700	n.s.	0.0934
1.32 *vs*. 2.30	-1600	n.s.	0.1435
1.32 *vs*. 2.90	-1300	n.s.	0.3097
1.32 *vs*. 3.44	600	n.s.	0.8936
1.99 *vs*. 2.30	200	n.s.	0.9998
1.99 *vs*. 2.90	500	n.s.	0.9635
1.99 *vs*. 3.44	2400	*	0.0162
2.30 *vs*. 2.90	300	n.s.	0.9941
2.30 *vs*. 3.44	2200	*	0.0256
2.90 *vs*. 3.44	1900	n.s.	0.0617

n.s.: not significant.