

Supporting Information.

Nuclear magnetic resonance spectroscopy of 2-vinylpyridine copolymers. The NMR spectra of the 2VP-based copolymers is largely similar to that of the 4VP-based copolymers as reported in previous publications¹, but substantially different reactivity was observed. The NMR spectrum of synthesized P2VP is displayed in Fig. 1. In this spectrum two major groups of peaks can be seen: the first group consists of the peaks between 1.0 and 2.5 ppm, which correspond to the aliphatic protons labeled as “1” in Figs. S1 and S2. The second group consists of the peaks between 6.0 and 9.0 ppm, corresponding to the aromatic hydrogens labeled as “2” in Figs. S1 and S2, excepting the small peak at 7.271 ppm which corresponds to non-deuterated chloroform and can be disregarded. The NMR spectrum of synthesized PPEGMA 1100 is displayed in Fig. S3, and is representative of all PEGMA monomers used. The peaks between 0.0 and 1.0 ppm correspond to the small amount of aliphatic hydrogens present in the polymer backbone labeled as “1” in Figs. S3 and S4, while the large peaks between 3.0 and 4.0 ppm correspond with the protons of the poly(ethylene glycol) (PEG) chain, labeled as “2” in Figs. S3 and S4. Therefore, it is possible to determine the copolymer composition of the materials by comparing the relative peak weights of the three regions that should appear: an aliphatic region between 0.0 and 2.5 ppm, a PEG region between 3.0 and 4.0 ppm, and an aromatic region between 6.0 and 9.0 ppm (again excluding the chloroform peak at 7.271 ppm), as can be seen in the sample spectrum shown in Fig. S5. The composition of the final copolymers can be determined through the calculation of the ratio of these groups. These peaks are labeled in Fig. S5 as “1”, “2”, and “3” for the aliphatic, aromatic, and PEG regions, respectively.

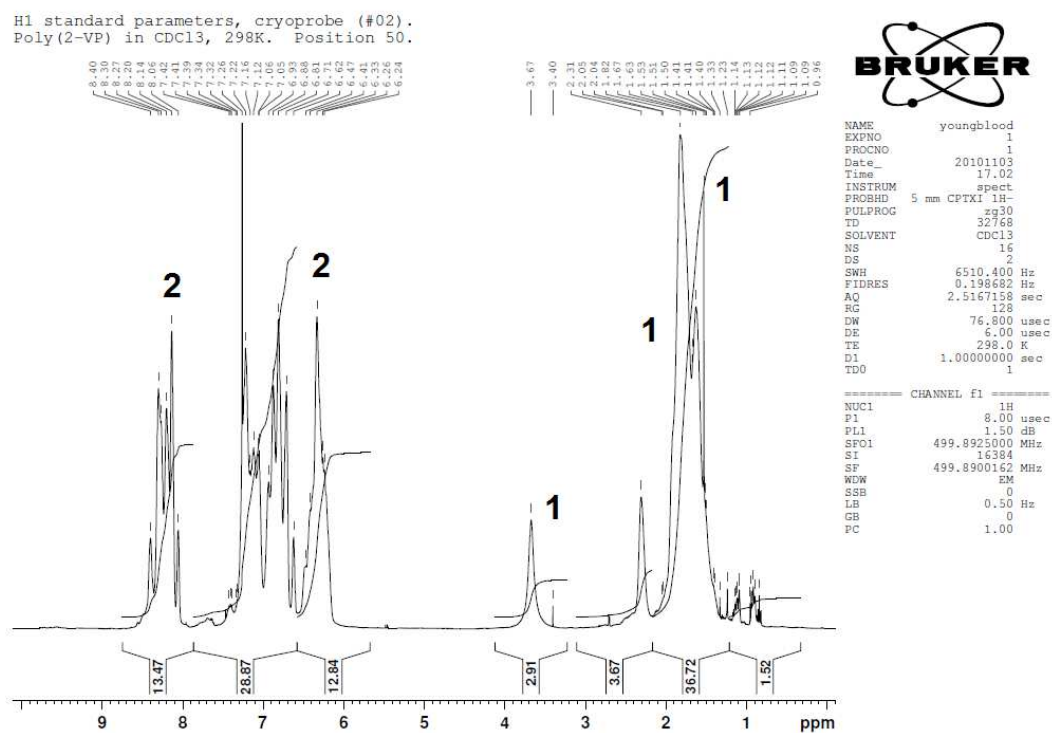


Figure S1. ¹H NMR spectrum of P2VP.

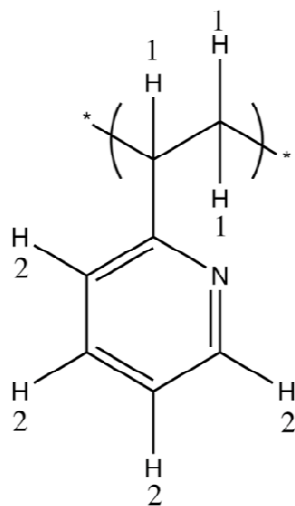


Figure S2. Hydrogen atom positions on P2VP.

H1 standard parameters, cryoprobe (#02).
Poly(PEGMA 1100) in CDCl₃, 298K. Position 49.

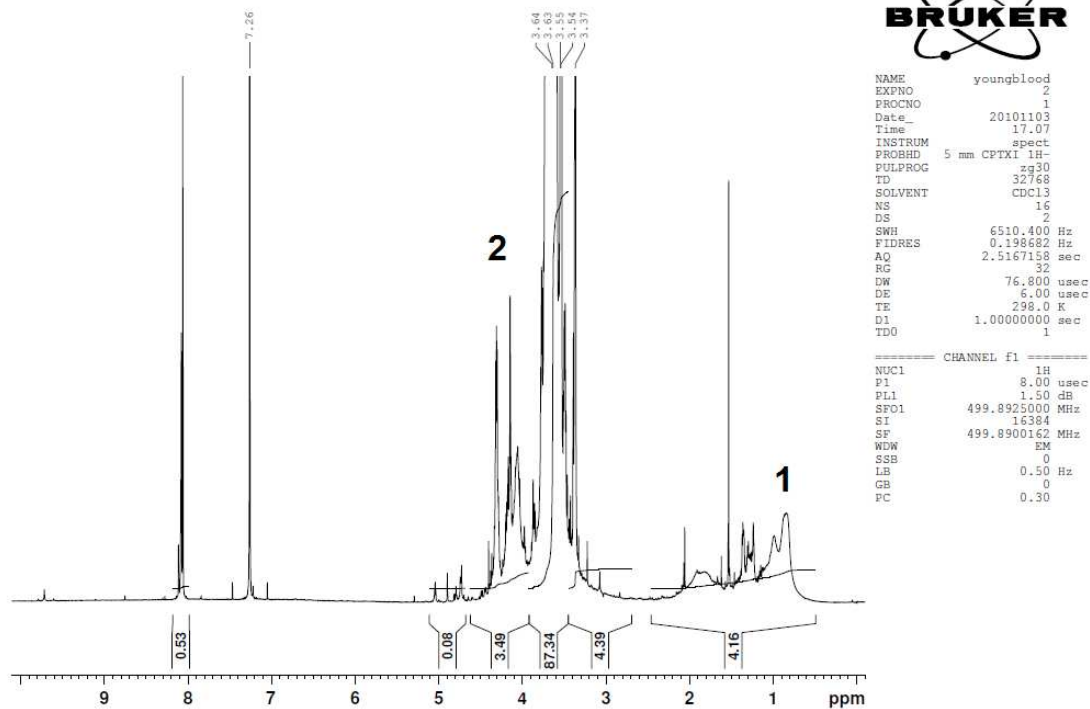


Figure S3. ¹H NMR spectrum of PPEGMA 1100.

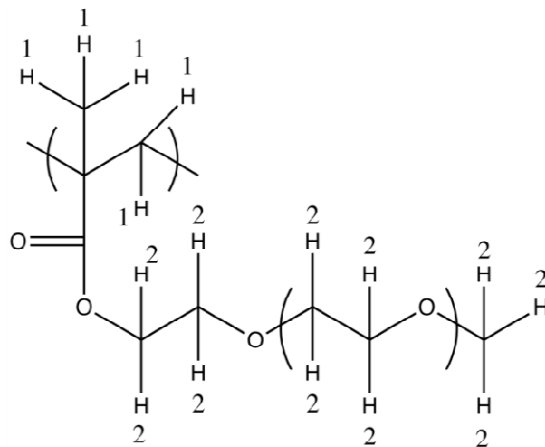


Figure S4. Hydrogen atom positions on PPEGMA *n*.

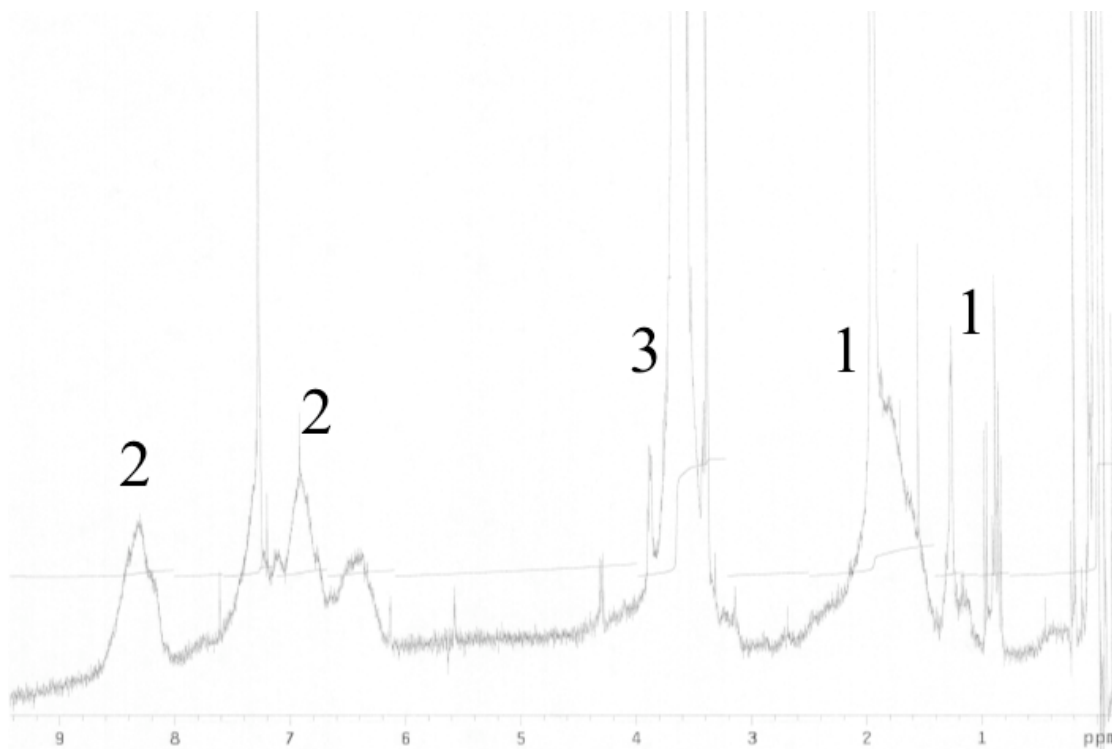


Figure S5. ^1H NMR spectrum of P(2VP-co-PEGMA 1100) copolymer containing 80 mol% 2VP and 20 mol% PEGMA 1100.

The composition of 2VP and PEGMA 1100 in the copolymers can be determined through the following relationships as determined by the area of the peaks on the NMR spectra:

$$\frac{\text{Aliphatic}}{\text{Aromatic}} = \frac{3X + (5(1 - X))}{4X}$$

$$\frac{\text{Aliphatic}}{\text{PEG}} = \frac{3X + (5(1 - X))}{(7 + 4n)(1 - X)}$$

$$\frac{\text{PEG}}{\text{Aromatic}} = \frac{(4n + 7)(1 - X)}{4X}$$

Where n is the number of PEG groups in the monomer (n equals 4.15 for PEGMA 300, 8.24 for PEGMA 475, and 22.0 for PEGMA 1100, as determined by Allison et al.²) and X

is the percentage of 4VP monomer in the copolymer, which can be solved for to determine:

$$X = \frac{5Aromatic}{4Aliphatic + 2Aromatic}$$

$$X = \frac{7Aliphatic - 5PEG + 4nAliphatic}{7Aliphatic - 2PEG + 4nAliphatic}$$

$$X = \frac{7Aromatic + 4nAromatic}{3Aromatic + 1PEG + 1nAromatic}$$

Of these, for the 2VP and PEGMA 1100 copolymers, only the aliphatic-aromatic relationship provided sensible results, which are displayed in Tables S1 and S2. As can be seen in this table, the 2VP compositions of the polymer are substantially higher than the feed ratios, indicating a substantially higher reactivity of the 2VP. As these copolymers are quite far from their monomer feed ratios, they will be referred to instead by their effective copolymer compositions as detailed in Tables S3 and S4. Due to these anomalous results, as well as the anomalous results found in the GPC assay and solubility testing, an additional copolymer of 2VP and PEGMA *n* was synthesized using a shorter chain length PEGMA monomer (PEGMA 300 replacing PEGMA 1100) to obtain a set of copolymers with a more typical composition profile, molecular weight, and solubility.

Table S1. Compositions of 2VP and PEGMA 1100 copolymers as determined by relative peak heights in NMR.

Feed Mol% 2VP	PEG	Aromatic	Mol% 2VP
25	104.82	5.14	53.8
50	2604.41	234.81	68.2
75	1906.61	330.89	80.5
90	670.03	353.8	92.6

100	0	448.53	100
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Table S2. Relationship between feed 2VP percentage and realized 2VP percentage, rounded to the nearest 5% percent for clarity, of the 2VP and PEGMA 1100 copolymers.

Feed mol% 2VP	Effective 2VP mol%	Effective 2VP wt%
25	55	10
50	70	17
75	80	28
90	95	57
100	100	100

Table S3. Compositions of 2VP and PEGMA 300 copolymers as determined by relative peak heights in NMR.

Feed % 2VP	Aliphatic	Aromatic	PEG	2VP Comp. %
0	213.98	0	995.22	0
5	283.23	40.69	980.74	19.7
10	241.52	60.89	843.41	29.9
25	406.10	143.09	1374.88	38.0
50	148.61	106.05	322.8	66.0
75	410.09	400.54	555.74	81.0
90	847.20	951.26	474.49	92.2
100	466.62	599.14	0	100

Table S4. Relationship between feed 2VP percentage and realized 2VP percentage, rounded to the nearest 5% percent for clarity, of the 2VP and PEGMA 300 copolymers.

Feed mol% 2VP	Effective 2VP mol%	Effective 2VP wt%
0	0	0
5	20	9
10	30	14
25	40	19
50	65	42
75	80	61
90	90	81
100	100	100

The results of the 2VP and PEGMA 300 copolymers were much improved, although the 2VP monomer still showed substantially greater reactivity than the PEGMA monomer, as displayed in Tables S3 and S4. All three equations gave results within several percentage points of each other in the high PEG regime, although the Aliphatic-PEG relationship gave inconsistent results in the low PEG regime. The PEG-Aromatic relationship gave the most consistent results over all concentrations, perhaps due to the lack of overlapping protons as in the aliphatic region. As these copolymers are also quite far from their monomer feed ratios, they will be referred to instead by their effective copolymer compositions as detailed in Table S4.

From these results, the reactivity ratios of the 2VP and PEGMA n comonomers were characterized, using the Finemann-Ross and Kelen-Tudos methods. The results of these analyses are displayed in Table S5. The low reactivity ratios of both PEGMA n monomers with itself, coupled with the somewhat higher reactivity of 2VP with itself, indicates a polymer that will deviate somewhat from true randomness, as we have seen in the experimental results. For this system, the 2VP monomers are much more likely to be followed by another 2VP monomer as opposed to a PEGMA n monomer, tending towards long blocks of 2VP. The polymer will not be blocky, however, as the PEGMA n monomers have a low reactivity with themselves. The net polymer structure is therefore long series of 2VP sections with the occasional PEGMA n monomer interspersed in the chain.

Table S5. Reactivity ratio determination for the copolymers of 2VP and PEGMA *n*.

	2VP-co-PEGMA 1100			2VP-co-PEGMA 300		
	<i>r</i> ₁	<i>r</i> ₂	<i>r</i> ₁ <i>r</i> ₂	<i>r</i> ₁	<i>r</i> ₂	<i>r</i> ₁ <i>r</i> ₂
Finemann-Ross	1.293	0.194	0.251	1.236	0.249	0.307
Kelen-Tudos	1.211	0.068	0.083	1.165	0.214	0.250

The quaternization process was identical to those used in previously published works^{1, 2} and the precise details of their compositions are given in those works, but a brief summary is provided here. As compared with the unquaternized material, the quaternized 2VP feature an increased number of protons in the aliphatic range due to the presence of the additional hydrogen atoms on the hexyl group used in quaternization (See Figs. 2 and S6). These additional aliphatic protons can be seen in the strong difference shown in the quaternized P(2VP)-HB shown in Fig. S7 versus the unquaternized P(2VP) shown in Fig. S1. This also extends to the copolymers of 2VP, as seen in Fig. S8. It is possible to determine the extent of quaternization by adjusting the previous equation to consider the effects of the additional aliphatic protons added by the hexyl group:

$$\frac{Aliphatic}{Aromatic} = \frac{14X + (6(1 - X))}{6X}$$

$$\frac{Aliphatic}{PEG} = \frac{14X + (6(1 - X))}{(7 + 4n)(1 - X)}$$

The relationship between the PEG and aromatic groups will not change, which provides us with a useful verification for the results: if these results differ widely from the previous results, some unintended reaction has taken place. The above equations can be solved to determine:

$$X = \frac{3Aromatic}{3Aliphatic - 4Aromatic}$$

$$X = \frac{7Aliphatic - 6PEG + 4nAliphatic}{7Aliphatic + 8PEG + 4nAliphatic}$$

If we compare the NMR spectra with these equations, the degree of quaternization can be observed. If the calculated mol% 2VP compositions are approximately equal that of the unquaternized sample, it can be presumed that the copolymer is fully quaternized. The results of this approach to the copolymers of 2VP and PEGMA n are shown in Tables S6 and S7, and show that there is reasonably complete quaternization of the copolymers.

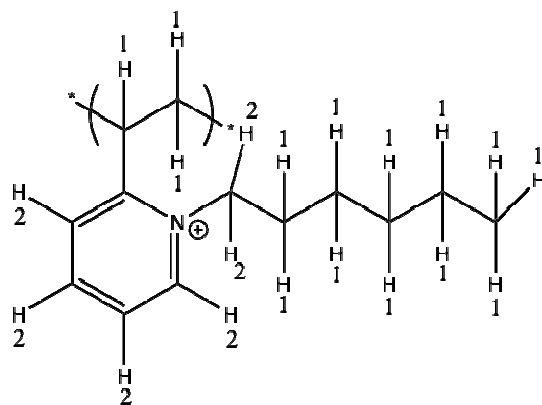


Figure S6. Hydrogen atom positions on P2VP-HB.

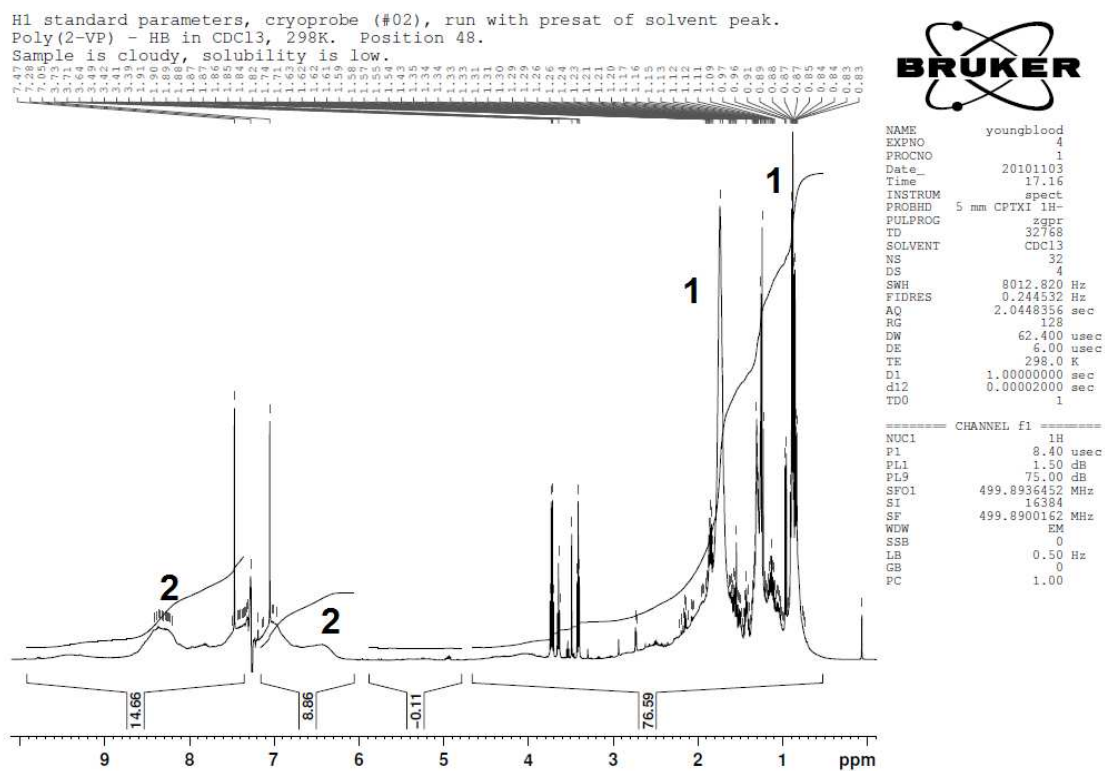


Figure S7. ¹H NMR spectrum of P(2VP)-HB.

H1 standard parameters, cryoprobe (#02).
Copolymer 75/25 (green liquid) in CDCl₃, 298K. Position 47.

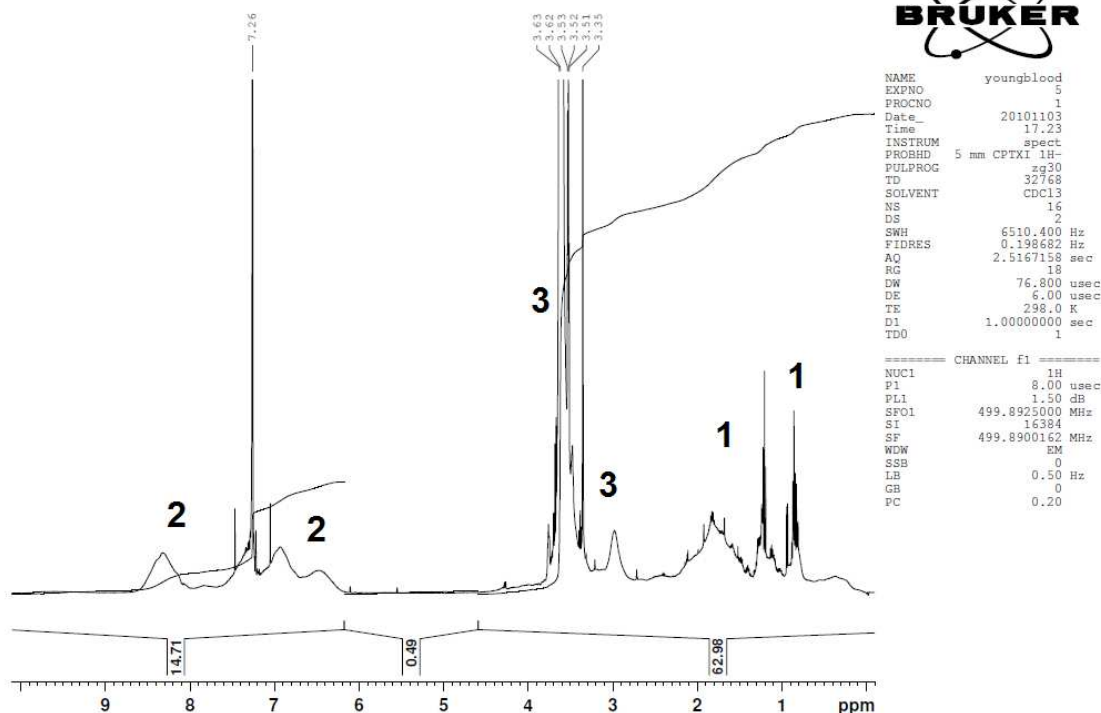


Figure S8. ¹H NMR spectrum of P(2VP-co-PEGMA 1100)-HB copolymer containing 80 mol% 2VP and 20 mol% PEGMA 1100.

Table S6. Estimation of 2VP composition of 2VP and PEGMA 1100 copolymers after quaternization. The PEG/Aro relationship equation is unaffected by the addition of the quaternized group and serves as a control for the results. The results of the Ali/PEG equations show that quaternization is complete or nearly complete.

Mol% 2VP	Aliphatic	Aromatic	PEG	Ali/PEG % 2VP	PEG/Aro % 2VP
55	123.47	36.34	682.34	44.4	55.8
70	640.07	169.35	2178.2	61.0	64.8
80	13.97	11.47	47.23	61.2	85.2
95	1073.98	366.82	677.53	91.2	92.8
100	260.9	99.13	14.76	99.2	99.4

Table S7. Estimation of 2VP composition of 2VP and PEGMA 300 copolymers after quaternization. The PEG/Aro relationship equation is unaffected by the addition of the quaternized group and serves as a control for the results. The results of the Ali/PEG equations show that quaternization is complete or nearly complete.

Mol% 2VP	Aliphatic	Aromatic	PEG	Ali/PEG % 2VP	PEG/Aro % 2VP
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0	213.98	0	995.22	0	0
20	326.11	50.67	1023.59	9.7	22.6
30	366.65	74.23	1025.48	14.8	29.9
40	381.44	142.71	1045.35	15.7	44.6
65	77.41	42.46	139.12	33.7	64.3
80	657.23	416.91	504.66	63.8	83.0
90	363.21	254.44	120.52	82.3	92.6
100	264.65	96.28	26.43	94.2	95.6

Gel permeation chromatography of 2-vinylpyridine copolymers. The copolymers of 2VP and PEGMA 1100, P(2VP-co-PEGMA 1100), showed somewhat lower molecular weights as seen in Table S8, especially for those copolymers with high PEGMA content. Due to these lower molecular weights, some of these polymers (those high in PEGMA content) were liquids in their quaternized state, even after evacuating and heating the samples to 70°C overnight to remove any residual solvent. These copolymers also displayed remarkably low PDIs, below 1.15 in all cases except for the homopolymer of 2VP. This is probably due to a shielding effect caused by the long PEG groups of PEGMA 1100, decreasing the potential for further polymerization. The copolymers of 2VP and PEGMA 300 showed higher molecular weights, ranging from 10,000 to 20,000 g/mol, were consistently solid, and had PDIs keeping more in line with polymers made through this type of free radical polymerization (around 1.4), as seen in Table S9. The PDI of PEGMA 300 was quite high, perhaps due to crosslinking. Further GPC was not completed on the quaternized copolymers due to the potential for the free bromine ions to disrupt the assay. The molecular weights of the quaternized copolymers would be somewhat higher, especially for those copolymers with high 2VP content.

Table S8. Results of GPC testing performed on P(2VP-co-PEGMA 1100) copolymers to determine average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PDI).

mol% 2VP	M _n	M _w	PDI
55	6,100	6,600	1.07
70	6,900	7,500	1.08
80	7,800	8,400	1.07
95	9,900	11,000	1.13
100	15,000	20,000	1.41

Table S9. Results of GPC testing performed on P(2VP-co-PEGMA 1100) copolymers to determine average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PDI).

mol% 2VP	M _n	M _w	PDI
0	21,000	71,000	3.36
20	13,000	20,000	1.53
30	11,000	17,000	1.53
40	10,000	14,000	1.41
65	10,000	13,000	1.29
80	12,000	16,000	1.36
90	13,000	18,000	1.43
100	13,000	19,000	1.39

Red blood cell hemolysis testing at high polymer concentrations. The results of the hemolysis assays completed on the copolymers of 2VP and PEGMA *n* at a concentration of 5 mg/ml, the highest tested in this assay, are displayed in Figs. S9 and S10, based on the mol% and wt% of 2VP, respectively. For some copolymers at this concentration, the measured hemolysis is in fact much greater than that of the Triton-X control: this is due to the absorption of light by the polymer itself: for some copolymers at 5 mg/ml, this was measured to be as high as 0.4 relative units for the homopolymer P(2VP)-HB. The adsorption of the polymers decreased rapidly with the dissolved concentration, falling to around 0.05 relative units for polymers dissolved at 1 mg/ml, at which point the effect becomes largely irrelevant.

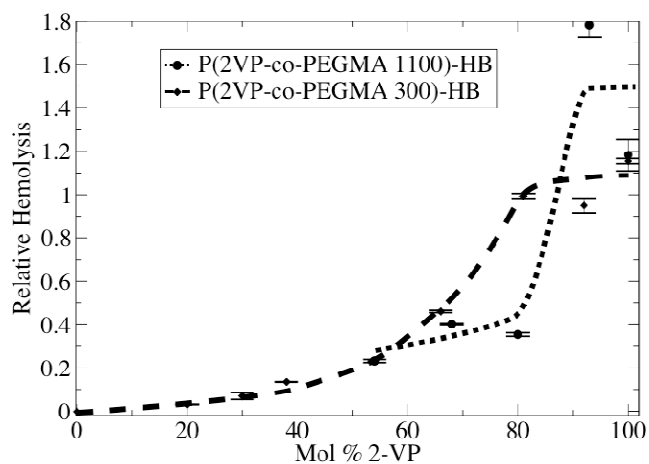


Figure S9. Human red blood cell (RBC) hemolysis of water soluble P(2VP-co-PEGMA)-HB at a concentration of 5 mg/ml as a function of 2VP mol%, relative to the hemolysis induced by a 10 mg/ml Triton-X solution (Hemolysis of Triton-X = 1).

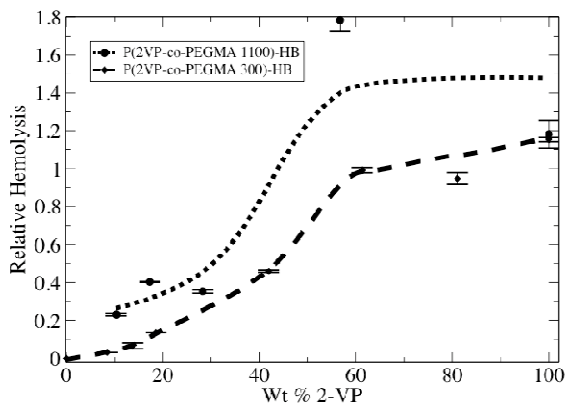


Figure S10. Human red blood cell (RBC) hemolysis of water soluble P(2VP-co-PEGMA)-HB at a concentration of 5 mg/ml as a function of 2VP wt%, relative to the hemolysis induced by a 10 mg/ml Triton-X solution (Hemolysis of Triton-X = 1).

REFERENCES

1. Sellenet, P. H.; Allison, B., et al., *Biomacromolecules*, **2007**, 8, (1), 19-23.
2. Allison, B. C.; Applegate, B. M., et al., *Biomacromolecules*, **2007**, 8, (10), 2995-2999.