Supporting Information for:

Synthesis, properties, and antibacterial activity of polyphosphonium semi-interpenetrating networks

Tyler J. Cuthbert[#], Tristan D. Harrison[#], Paul J. Ragogna[#], and Elizabeth R. Gillies[#$]*

[#]Department of Chemistry and Center for Advanced Materials and Biomaterials Research (CAMBR), The University of Western Ontario, 1151 Richmond St., London, Canada N6A 5B7

[$]Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond St., London, Canada N6A 5B9

Table of Contents

1. Experimental procedures for monomer synthesis……S2-S3
2. NMR spectra of monomers and polymers……S4-S11
3. DSC data for polymers……..S12
4. Images of the UV curing and SIPN formation set-up……..S13
5. Representative ATR-FTIR spectra before and after curing……S14
6. DSC data for SIPNs……S14
7. SEM-EDX analyses of phosphorus content……S15
8. UV-vis spectra of SIPN washings……...S16
9. Raw data for antibacterial tests……...S17-S18
10. Zone of inhibition test results……..……S19
11. References……..……..S19

Synthesis of triethyl(4-vinylbenzyl)phosphonium chloride (Et-P)

Triethylphosphine (5.46 g, 46.2 mmol) and 4-vinylbenzyl chloride (6.87 g, 45.0 mmol) were dissolved in CH_3CN (15 mL) under an N_2 atmosphere in a pressure tube and stirred at 80 °C for 16 hours. The solvent was then removed *in vacuo*. The resulting solid was dissolved in minimal CH_2Cl_2 (10 mL) and precipitated in Et_2O (500 mL). The precipitate was filtered, washed with Et_2O, and dried *in vacuo* yielding a white powder (13.2 g, 93%). Spectral data agreed with those previously reported.[1] A ^1H NMR spectrum is included for comparison with those of the polymers.

Synthesis of tributyl(4-vinylbenzyl)phosphonium chloride (Bu-P)

Tributylphosphine (12.1 g, 59.9 mmol) and 4-vinylbenzyl chloride (8.68 g, 56.9 mmol) were dissolved in CH_3CN (50 mL) under an N_2 atmosphere in a pressure tube and stirred at 80 °C for 16 hours. The solvent was then removed *in vacuo*. The resulting oil was dissolved in a minimal amount of CH_2Cl_2 (5 mL) and then precipitated in cold Et_2O (500 mL). The precipitate was filtered, washed with Et_2O, and dried *in vacuo* yielding a white powder (17.1 g, 85%). Spectral data agreed with those previously reported.[1] A ^1H NMR spectrum is included for comparison with those of the polymers.

Synthesis of trioctyl(4-vinylbenzyl)phosphonium chloride (O-P)

Trioctylphosphine (10.3 g, 19.7 mmol) and 4-vinylbenzyl chloride (4.07 g, 26.6 mmol) were dissolved in CH_3CN (30 mL) under an N_2 atmosphere in a pressure tube and stirred at 80 °C for 24 hours. The solvent was then removed *in vacuo.* The resulting solid was dissolved in minimal CH_2Cl_2 (3 mL) and precipitated in Et_2O (500 mL). The precipitate

was filtered, washed with Et_2O, and dried *in vacuo*, yielding a white powder (9.61 g, 68 %). Spectral data agreed with those previously reported.[1] A ^1H NMR spectrum is included for comparison with those of the polymers.

Zone of inhibition test for leaching

An even lawn of *S. aureus* was scratch plated onto an agar plate from a 10^6 CFU/mL suspension of the bacteria. SIPNs were cut with a razor blade into squares of approximate dimensions of 1 cm x 1 cm. The SIPN was then placed face down on the agar and incubated for 24 hours at 37 °C and 75% humidity. Surfaces had no zone of inhibition (a ring or space from the edge of the SIPN surface where no bacteria are present) indicating a surface that does not leach an active amount of biocide.

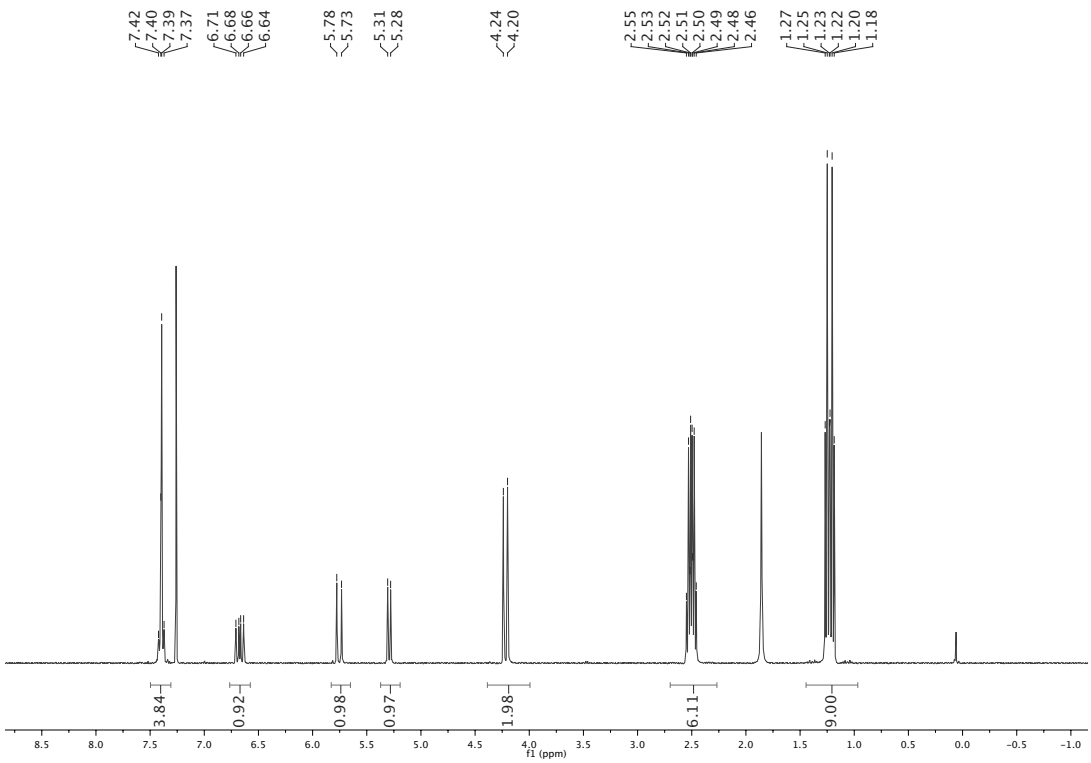

Figure S1. ^{1}H NMR spectrum of **Et-P** (400 MHz, D_2O).

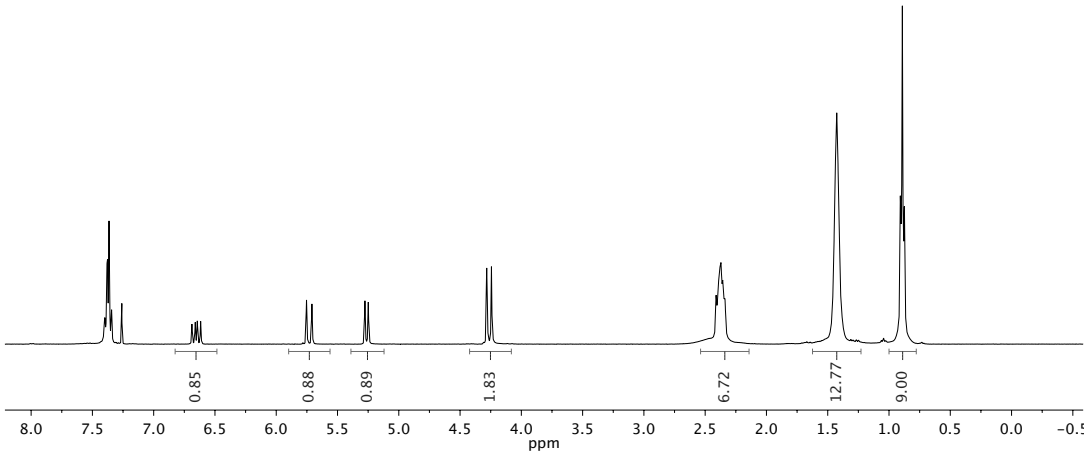

Figure S2. ^{1}H NMR spectrum of **Bu-P** (400 MHz, $CDCl_3$).

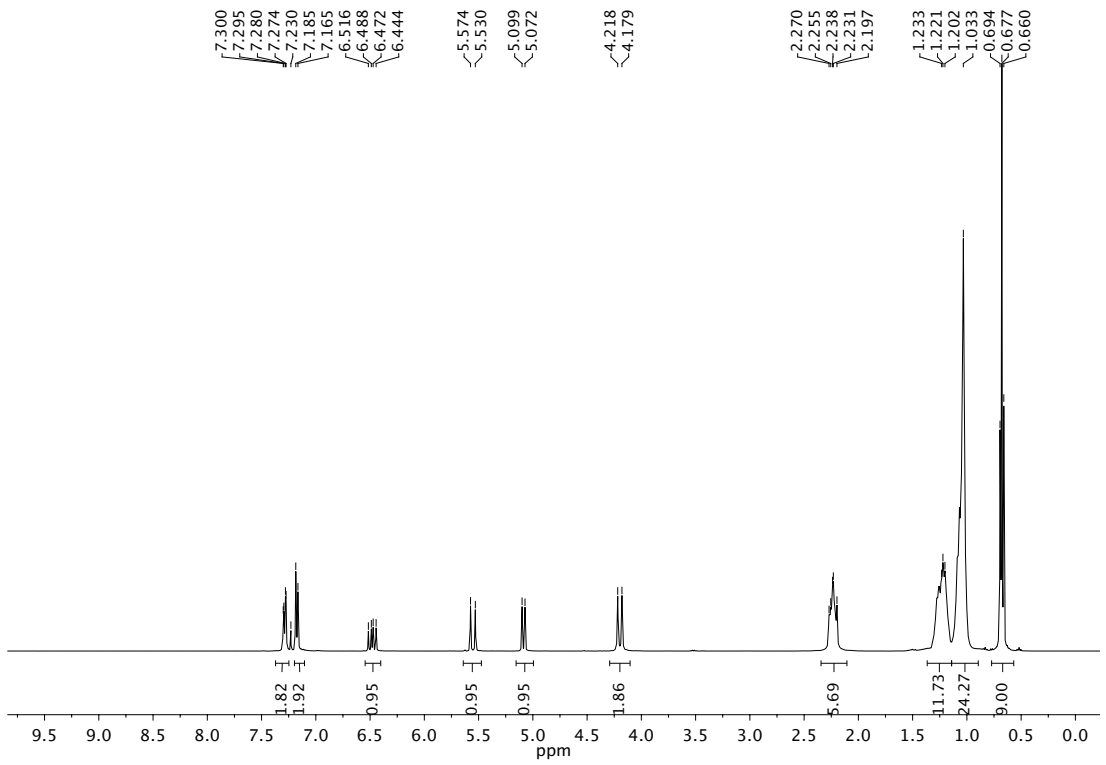

Figure S3. ^{1}H NMR spectrum of **O-P** (400 MHz, $CDCl_3$).

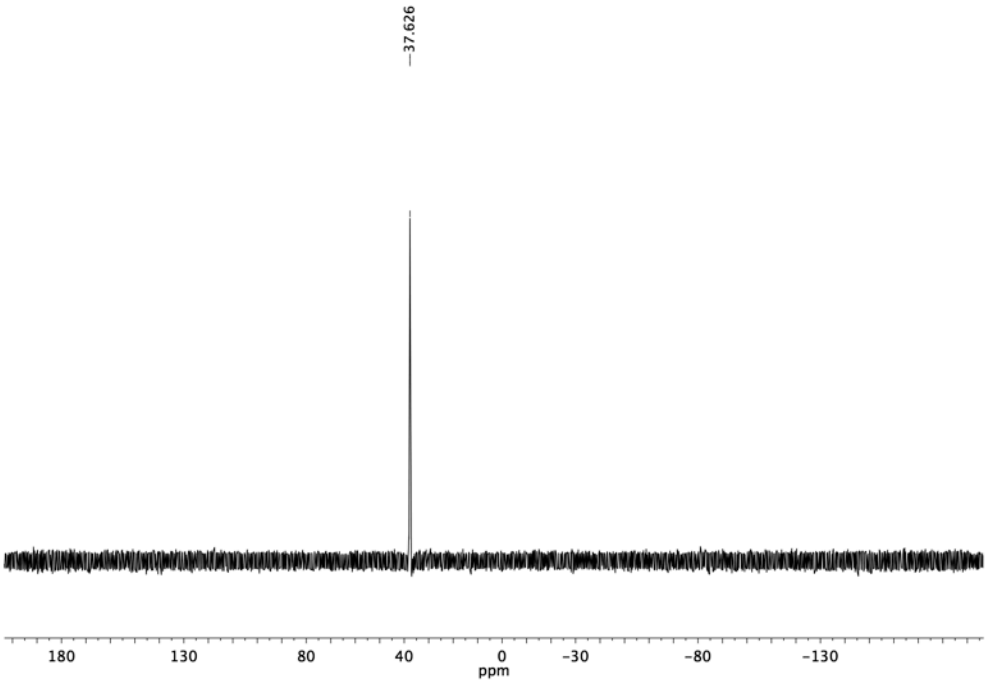

Figure S4. $^{31}P\{^{1}H\}$ NMR spectrum of **P(Et-P)-10k** (162 MHz, $CDCl_3$).

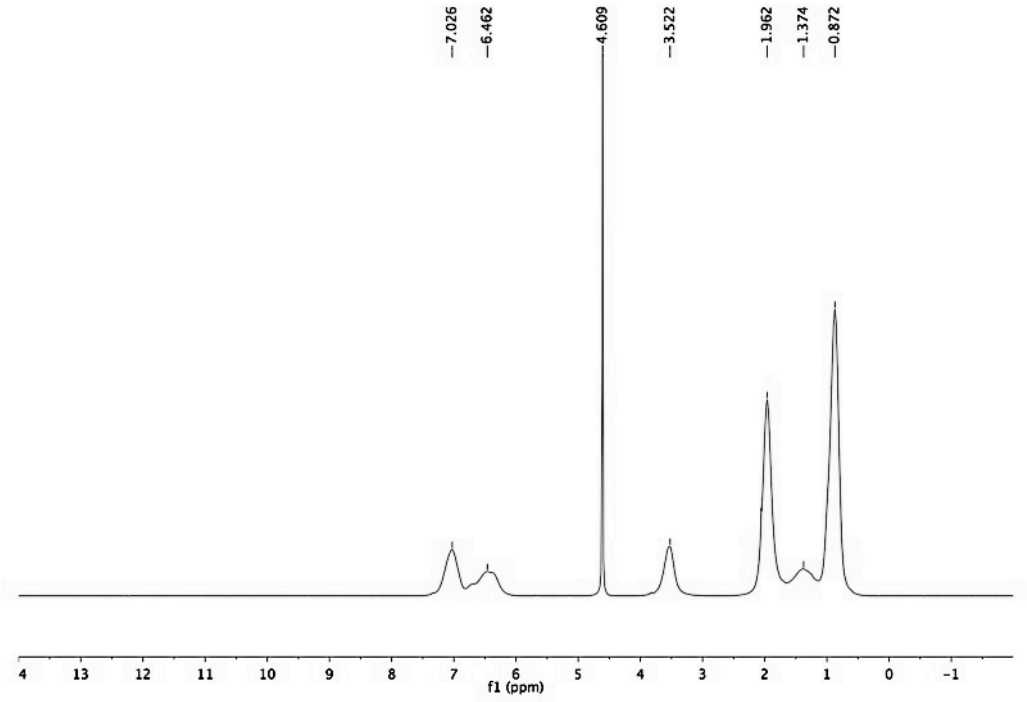

Figure S5. ^{1}H NMR spectrum of **P(Et-P)-10k** (400 MHz, $CDCl_3$).

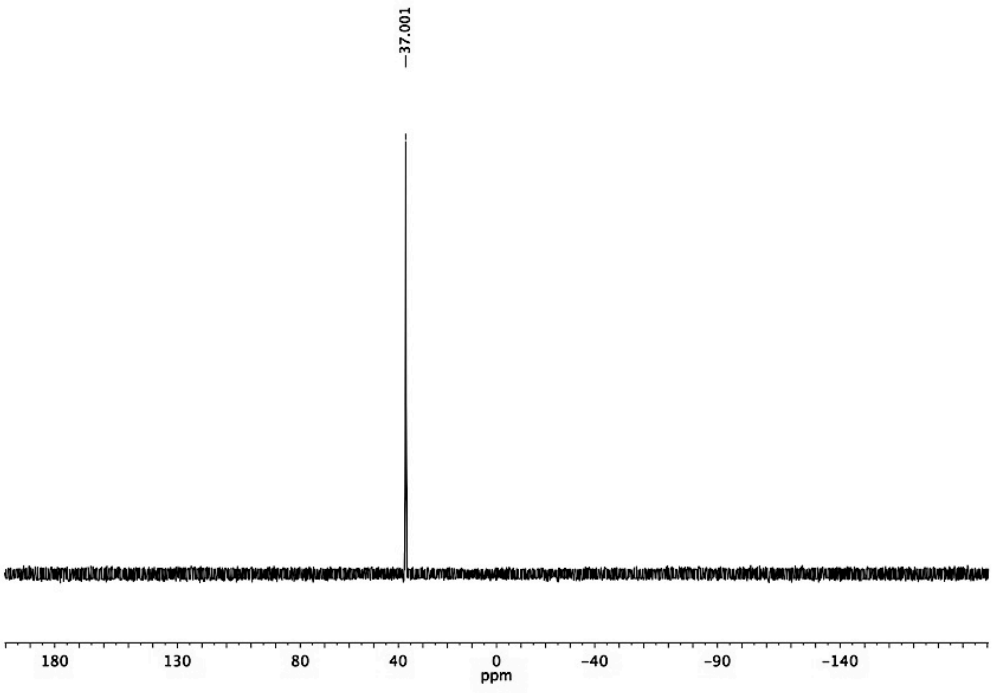

Figure S6. ^{31}P{^{1}H} NMR spectrum of **P(Et-P)-40k** (162 MHz, $CDCl_3$).

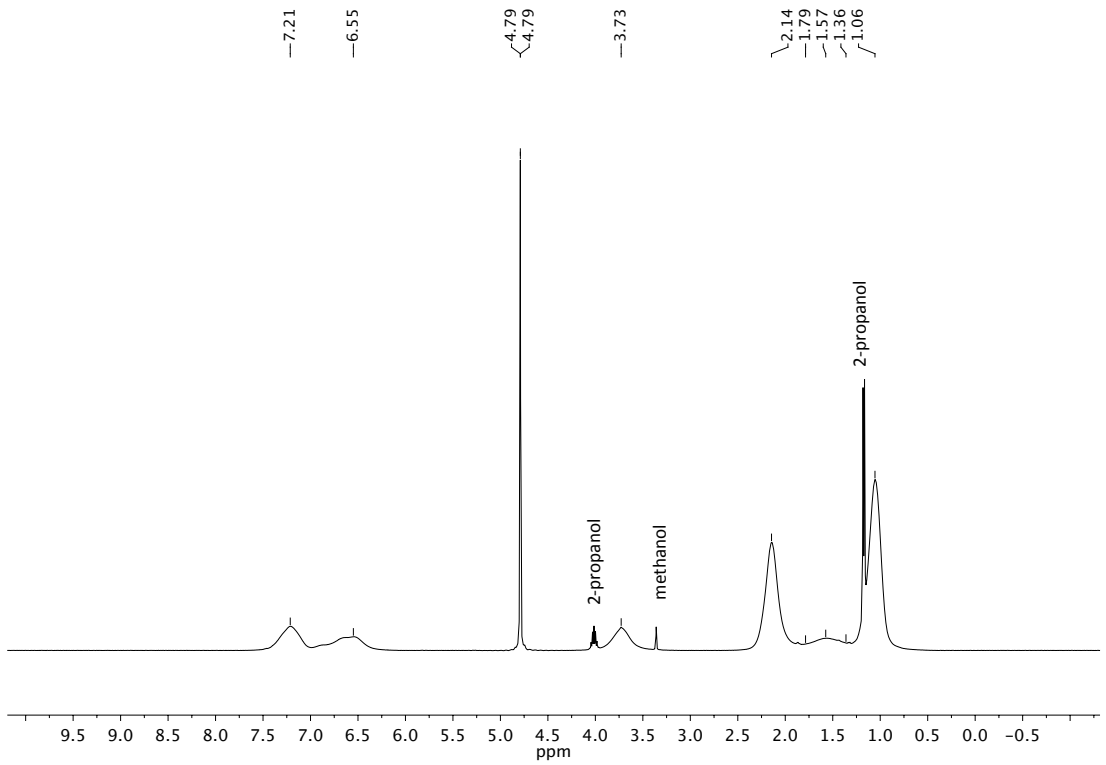

Figure S7. ^{1}H NMR spectrum of **P(Et-P)-40k** (400 MHz, $CDCl_3$).

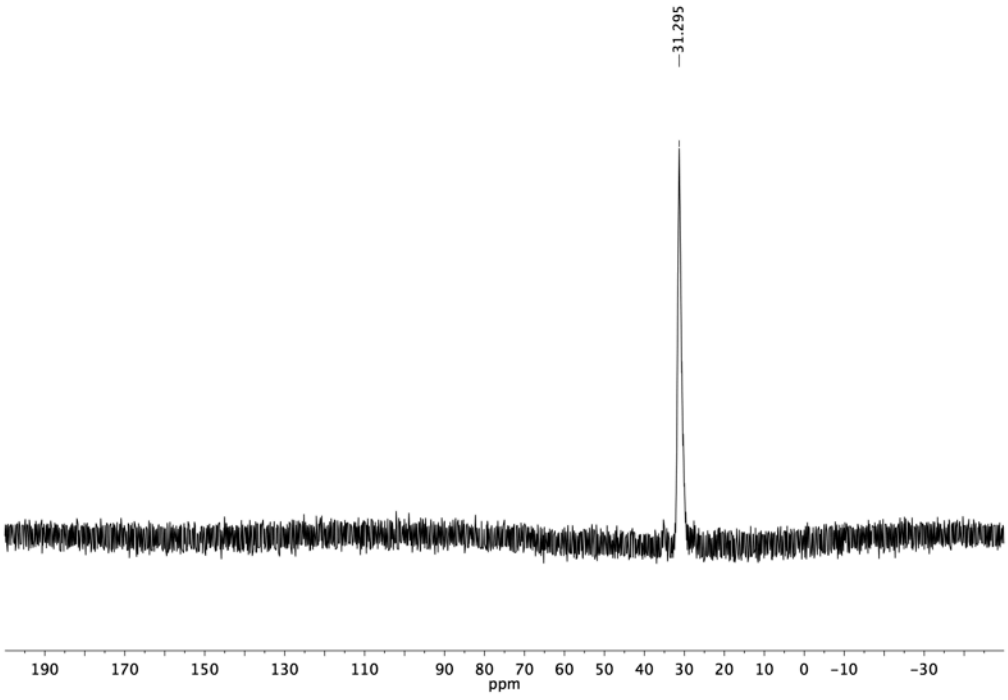

Figure S8. $^{31}P\{^{1}H\}$ NMR spectrum of **P(Bu-P)-10k** (161 MHz, $CDCl_3$).

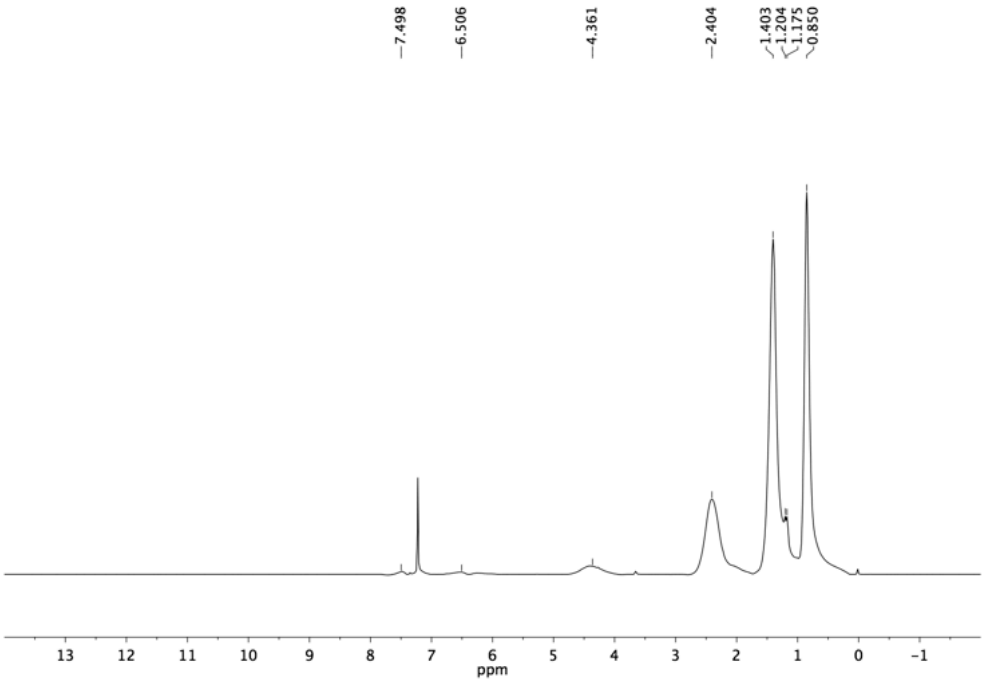

Figure S9. ^{1}H NMR spectrum of **P(Bu-P)-10k** (400 MHz, $CDCl_3$).

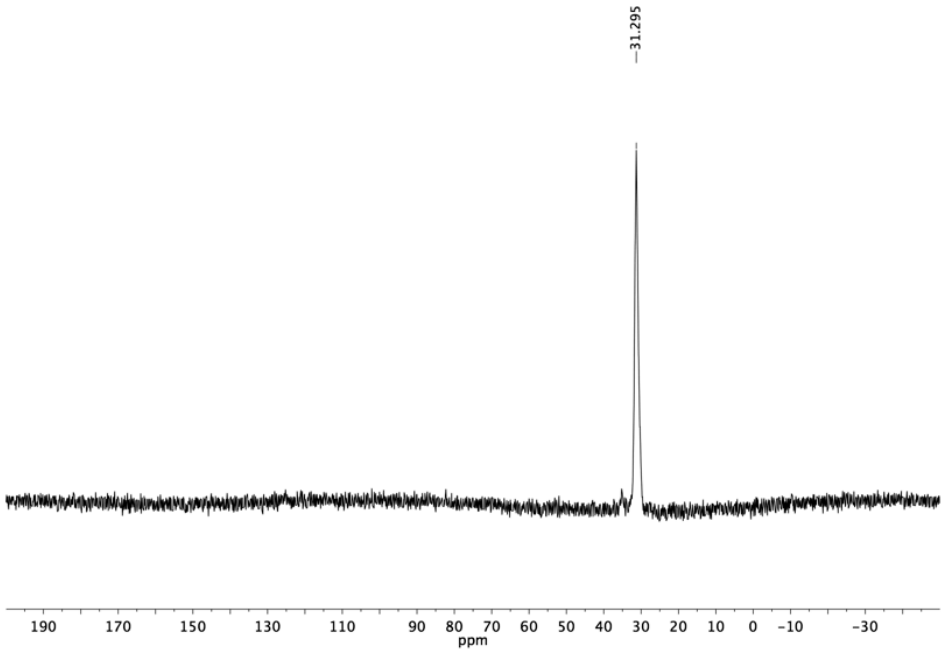

Figure S10. $^{31}P\{^{1}H\}$ NMR spectrum of **P(Bu-P)-40k** (162 MHz, $CDCl_3$).

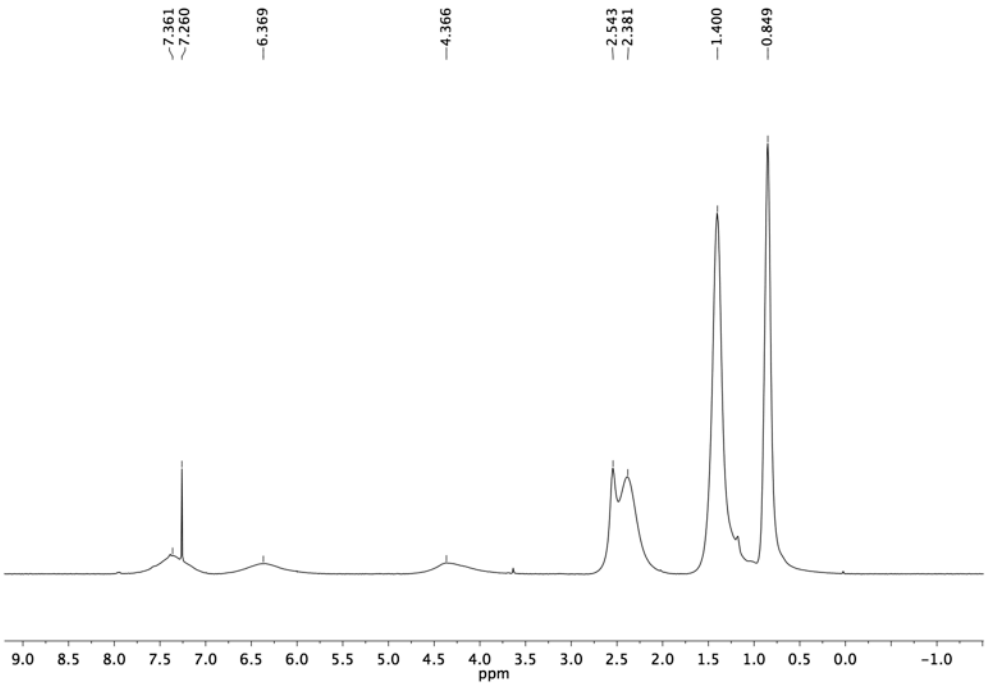

Figure S11. ^{1}H NMR spectrum of **P(Bu-P)-40k** (400 MHz, $CDCl_3$).

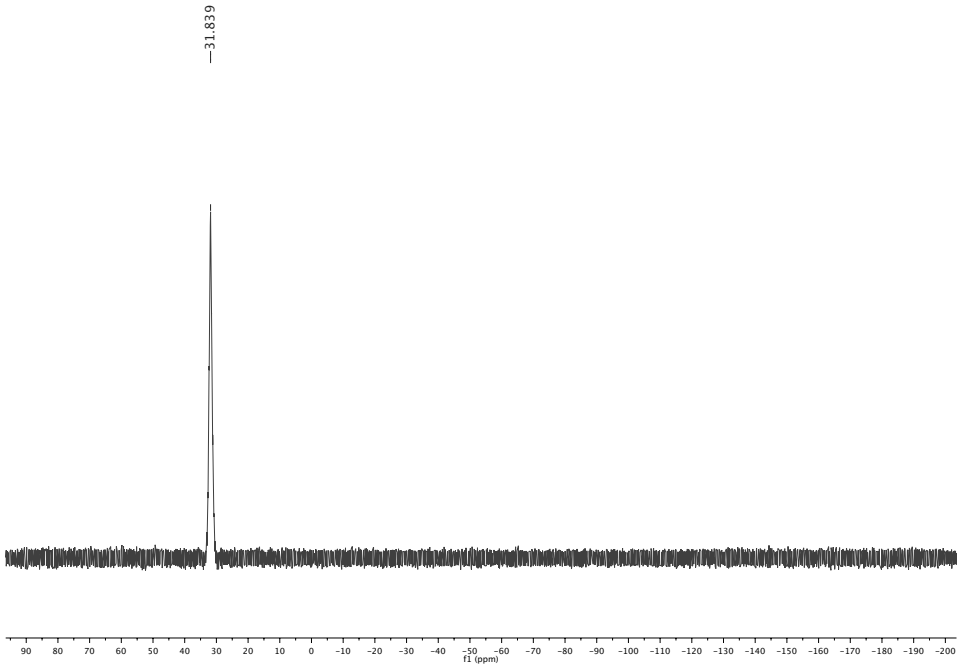

Figure S12. $^{31}P\{^{1}H\}$ NMR spectrum of **P(O-P)-10k** (162 MHz, $CDCl_3$).

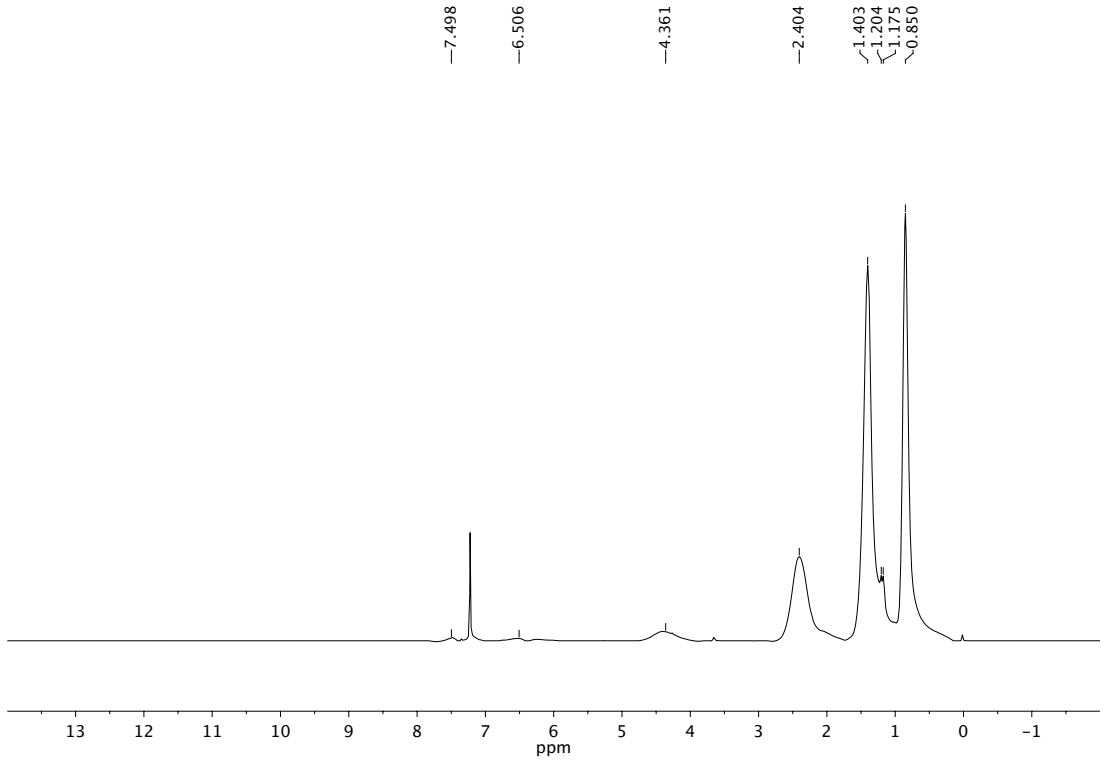

Figure S13. ^{1}H NMR spectrum of **P(O-P)-10k** (400 MHz, $CDCl_3$).

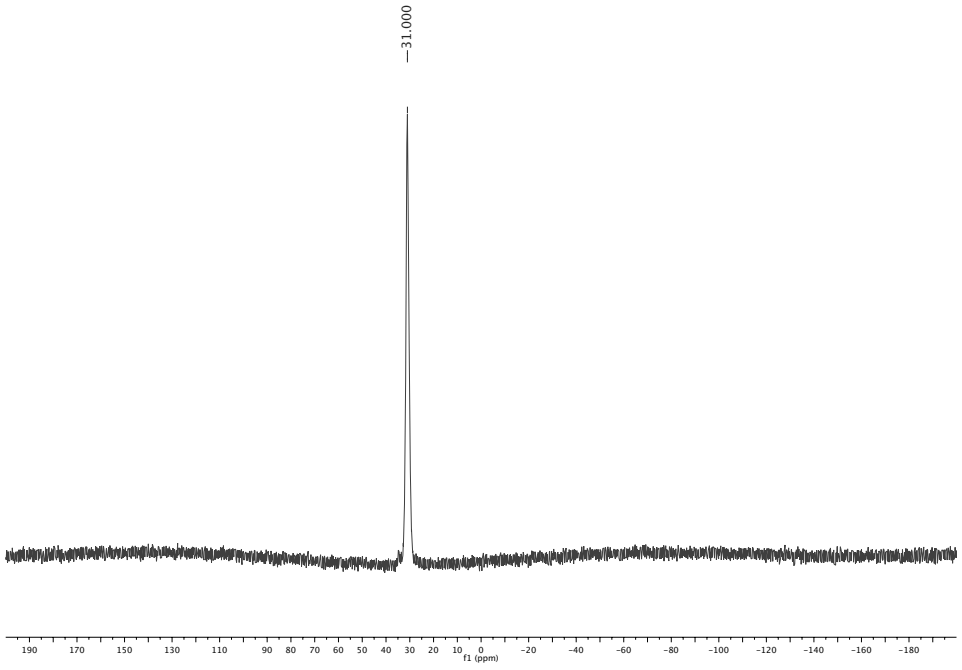

Figure S14. $^{31}P\{^{1}H\}$ NMR spectrum of **P(O-P)-40k** (162 MHz, $CDCl_3$).

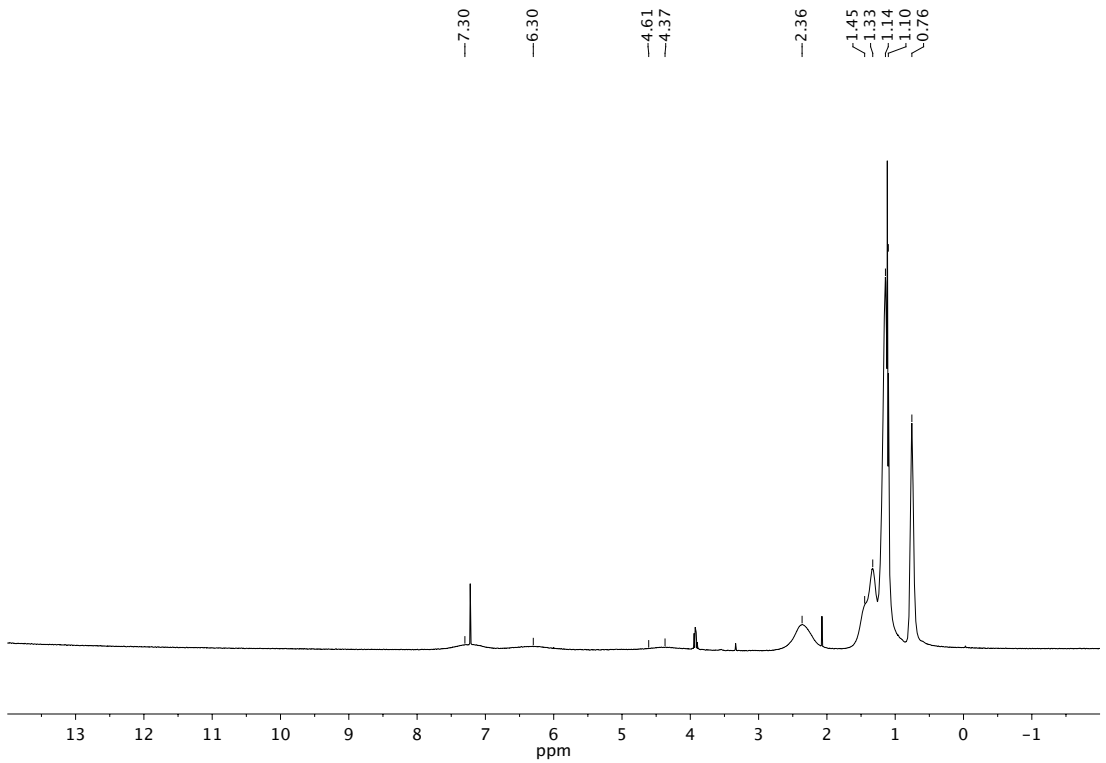

Figure S15. ^{1}H NMR spectrum of **P(O-P)-40k** (400 MHz, $CDCl_3$).

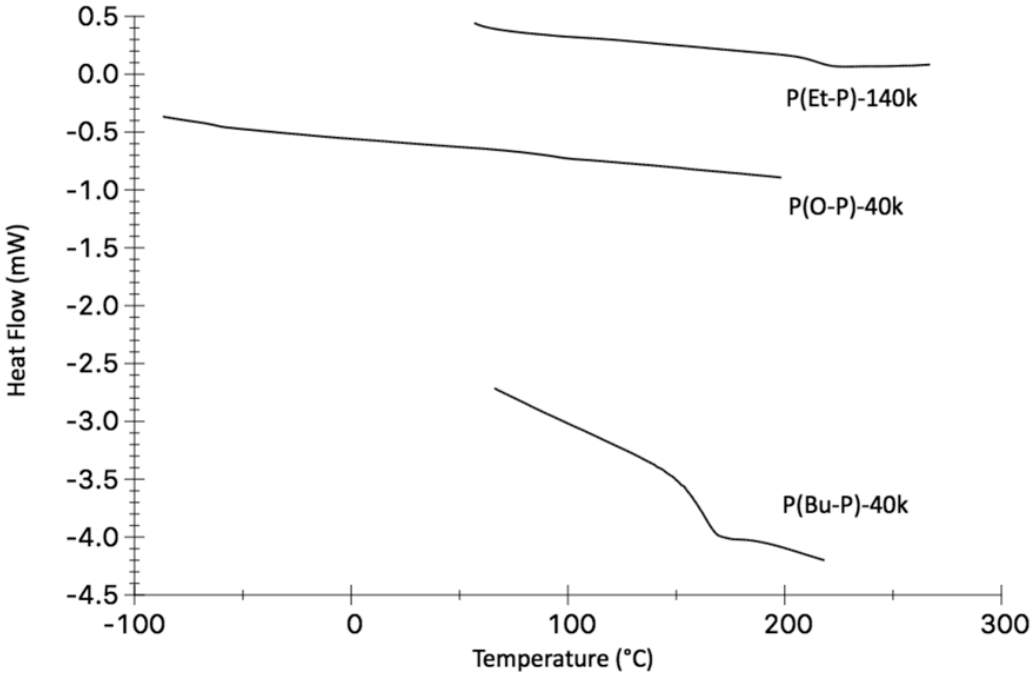

Figure S16. DSC curves for **P(Et-P)-40k**, **P(Bu-P)-40k**, and **P(O-P)-40k** (obtained from the second heating cycle).

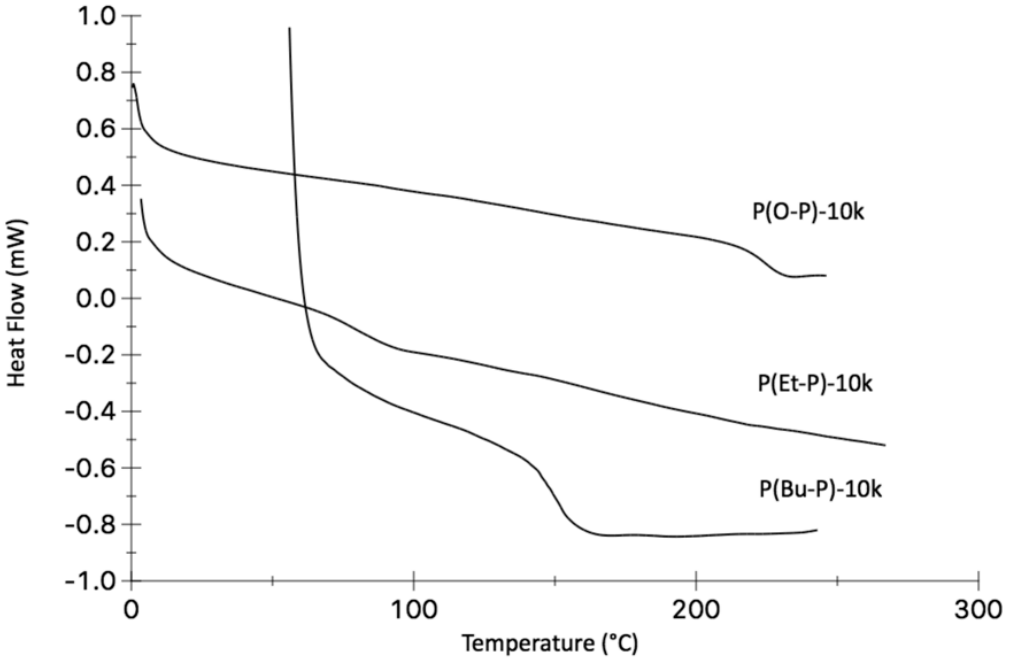

Figure S17. DSC curves for **P(Et-P)-10k**, **P(Bu-P)-10k**, and **P(O-P)-10k** (second heating cycle).

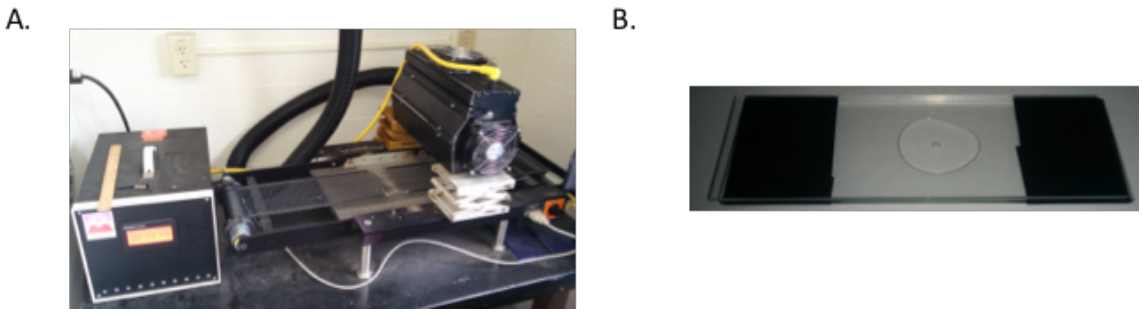

Figure S18. a) Digital photo of the UV-curing belt system b) Digital photograph showing two glass slides with spacers of 170 µm thickness on each side and the SIPN in between.

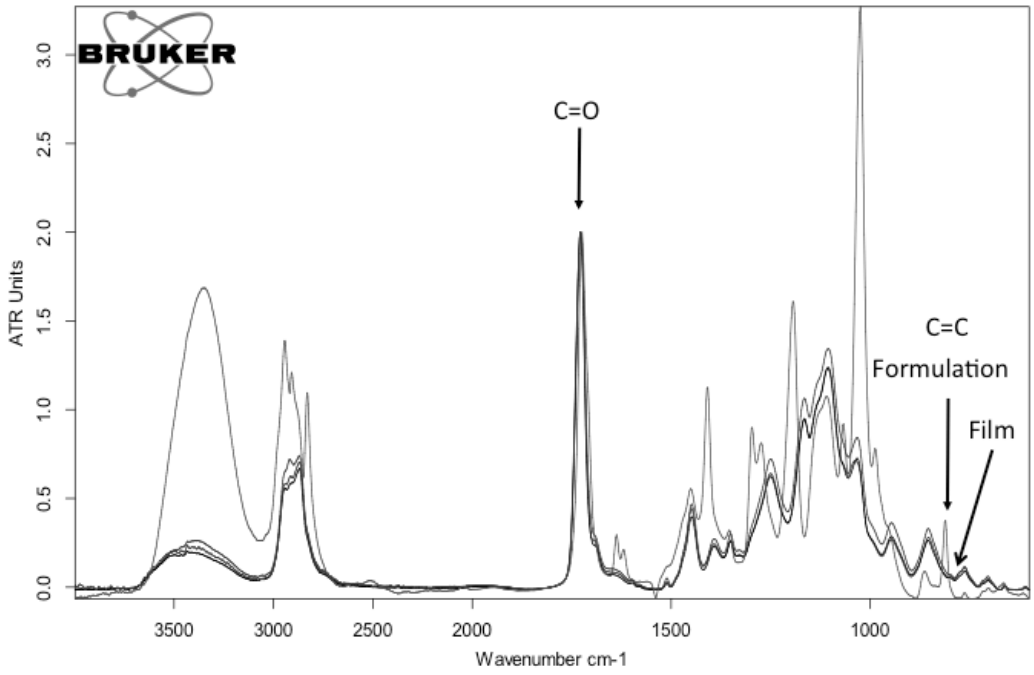

Figure S19. Example ATR-FTIR spectra of a **P(Et-P)-10k 10wt%** formulation and the corresponding cured (unwashed) SIPN, showing the decrease in the intensity of the peak at 810 cm^{-1} corresponding to C=C, in comparison to the internal standard C=O peak at 1720 cm^{-1}.

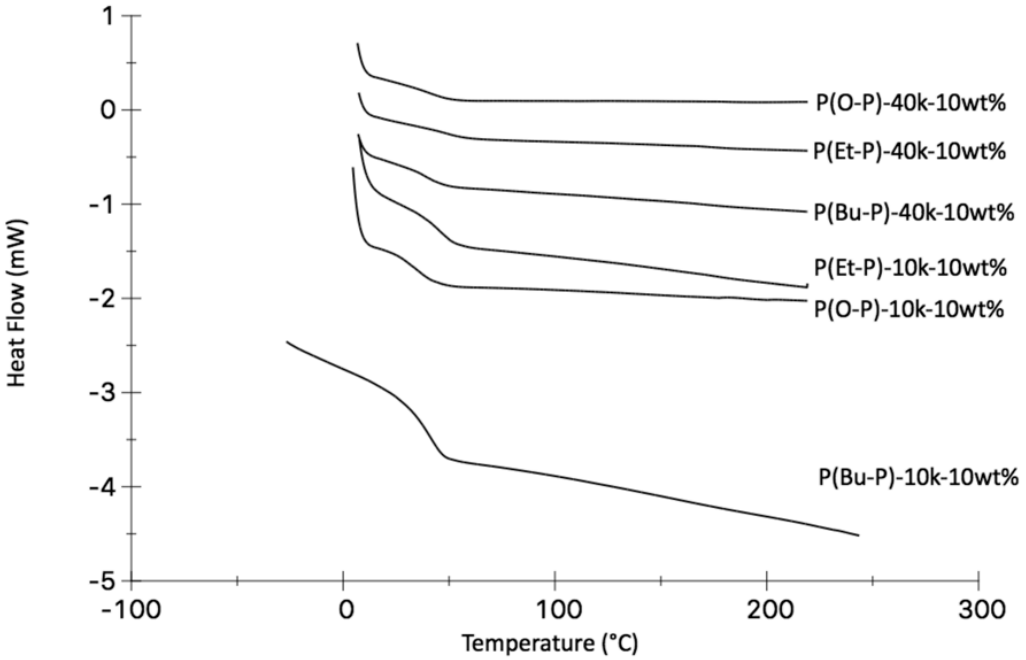

Figure S20. DSC curves for all SIPNs **P(Et-P)-10k/40k**, **P(Bu-P)-10k/40k**, and **P(O-P)10k/40k** at 10 wt% (second heating cycle).

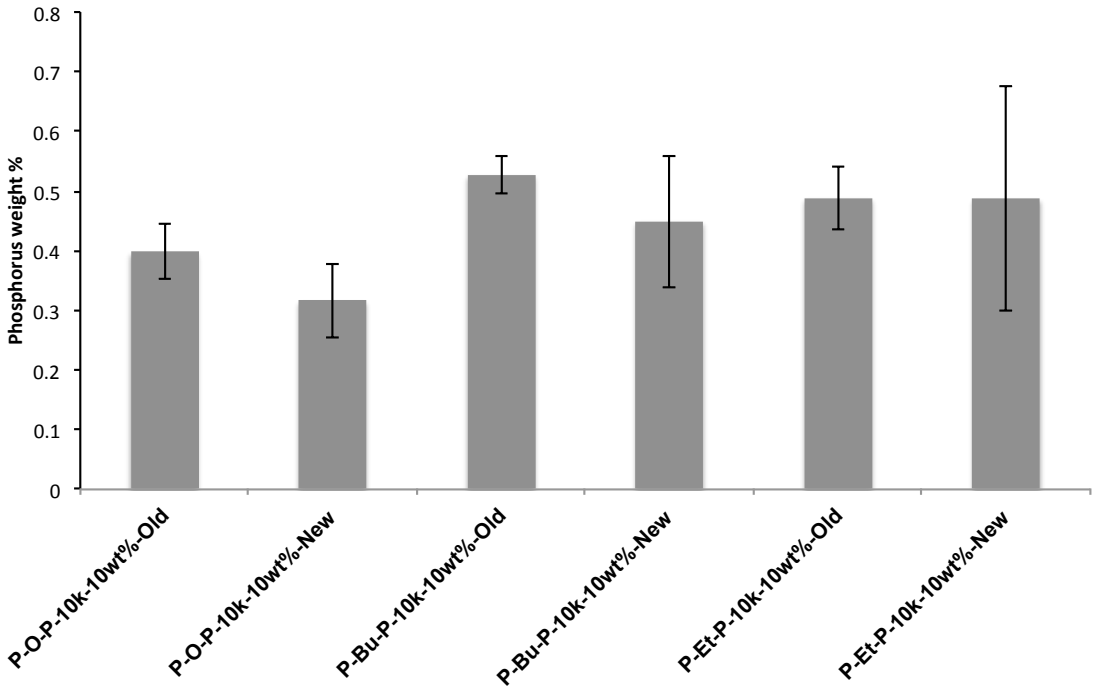

Figure S21. Surface phosphorus weight % analyzed by SEM-EDX on freshly prepared surfaces and aged samples (8 months). 250 μm surfaces were sputter coated with 5 nm of osmium. Samples were imaged at 20kV at a magnification of 4500 at a working distance of 10 μm.

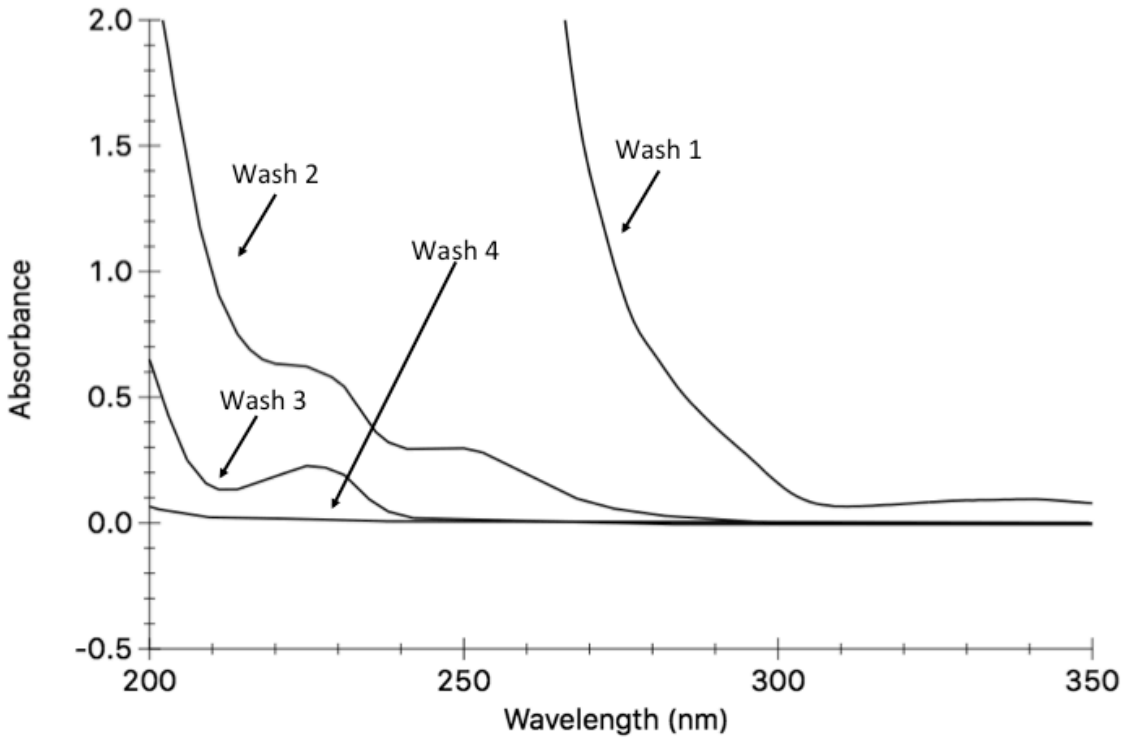

Figure S22. Representative example of the UV-vis spectra of the washings of an SIPN. This confirms that no further polymers or monomers were eluted from the SIPN after the 3rd washing. This was important to ensure that the anti-bacterial efficacy of the SIPNs arose from the activity of the surfaces themselves as opposed to leachable polyphosphoniums. This particular SIPN was **P(Bu-P)-10k** (10 wt%) and the washing solvent was CH_3CN. All other SIPNs exhibited the same behavior.

Table S1. Raw data (bacterial colony counts) from dynamic contact antibacterial test (based on ASTM E2149 13a) against *S. aureus* (ATCC 6538). In this test 2.0 mg of SIPN was incubated with 100 μL of 10^6 CFU/mL (10^5 CFUs total) in 0.3 mM KH_2PO_4. After dilution to 1.0 mL (10^4 CFUs/mL), 100 μL (10^3 CFUs total) were plated.

	S. aureus			
Specimen	CFUs counted	Mean	% Reduction	Std Dev (% std dev)
Control	958			
Control	879	918.50		
P(Et-P)-10k (10 wt%)	0			
P(Et-P)-10k (10 wt%)	0			0.00
P(Et-P)-10k (10 wt%)	0	0.00	100.00	0.00
P(Et-P)-40k (10 wt%)	0			
P(Et-P)-40k (10 wt%)	0			0.00
P(Et-P)-40k (10 wt%)	0	0.00	100.00	0.00
P(Bu-P)-10k (10 wt%)	0			
P(Bu-P)-10k (10 wt%)	0			0.00
P(Bu-P)-10k (10 wt%)	0	0.00	100.00	0.00
P(Bu-P)-40k (10 wt%)	0			
P(Bu-P)-40k (10 wt%)	0			0.00
P(Bu-P)-40k (10 wt%)	0	0.00	100.00	0.00
P(O-P)-10k (10 wt%)	380			
P(O-P)-10k (10 wt%)	461			62.08
P(O-P)-10k (10 wt%)	502	447.67	51.26	(6.76)
P(O-P)-40k (10 wt%)	975			
P(O-P)-40k (10 wt%)	881			47.51
P(O-P)-40k (10 wt%)	916	924.00	-0.60	(5.17)

Table S2. Raw data (bacterial colony counts) from dynamic contact antibacterial test (based on ASTM E2149 13a) against *E. coli* (ATCC 29425). In this test 2.0 mg of SIPN was incubated with 100 μL of 10^5 CFU/mL (10^4 CFUs total) in 0.3 mM KH_2PO_4. After dilution to 1.0 mL (10^3 CFUs/mL), 100 μL (100 CFUs total) were plated.

	E. coli			
Specimen	CFUs counted	Mean	% Reduction	Std Dev (% std dev)
Control	99			
Control	80	89.50		
P(Et-P)-10k (10 wt%)	8			
P(Et-P)-10k (10 wt%)	15			5.57
P(Et-P)-10k (10 wt%)	4	9.00	89.94	(6.22)
P(Et-P)-40k (10 wt%)	30			
P(Et-P)-40k (10 wt%)	34			13.43
P(Et-P)-40k (10 wt%)	9	24.33	72.81	(15.00)
P(Bu-P)-10k (10 wt%)	1			
P(Bu-P)-10k (10 wt%)	2			1.00
P(Bu-P)-10k (10 wt%)	0	1.00	98.88	(1.12)
P(Bu-P)-40k (10 wt%)	9			
P(Bu-P)-40k (10 wt%)	1			4.93
P(Bu-P)-40k (10 wt%)	0	3.33	96.28	(5.51)
P(O-P)-10k (10 wt%)	0			
P(O-P)-10k (10 wt%)	2			1.15
P(O-P)-10k (10 wt%)	0	0.67	99.26	(1.29)
P(O-P)-40k (10 wt%)	95			
P(O-P)-40k (10 wt%)	85			28.73
P(O-P)-40k (10 wt%)	139	106.33	-18.81	(32.10)

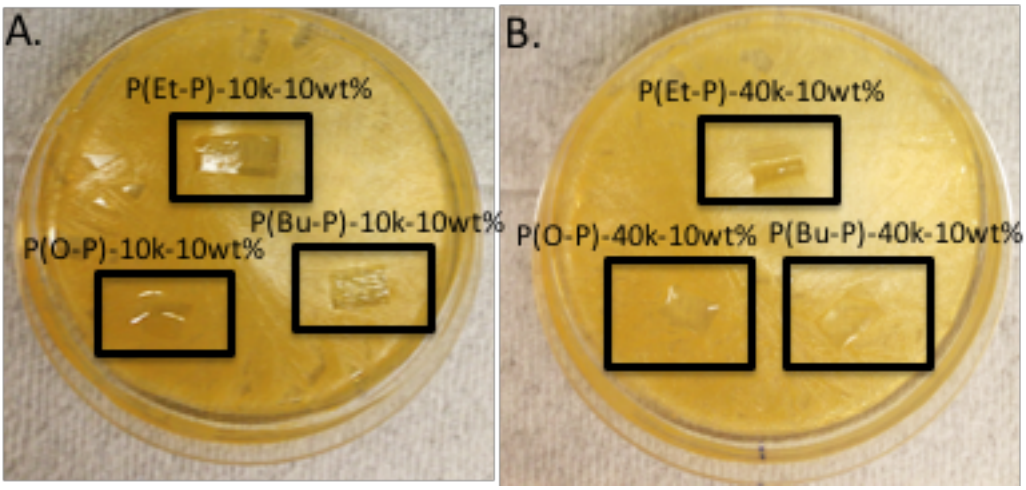

Figure S23. Zone of inhibition test against *S. aureus* using polyphosphonium SIPNs. The SIPNs were cut into squares and placed face down on TSB Agar plates that were scratch plated to create lawns of bacteria. A) **P(Et-P)-10k** (10wt%), **P(-Bu-P)-10k** (10wt%), **P(O-P)-10k** (10wt%); B) **P(Et-P)-40k** (10wt%), **P(Bu-P)-40k** (10wt%), **P(O-P)-40k** (10wt%). No zones of inhibition were observed for any samples, suggesting that the SIPNs do not leach biocide.

References

1. Kanazawa, A.; Ikeda, T.; Endo, T. *J. Polym. Sci. Part A: Polym. Chem.* **1993**, *31*, 335–343.