RAFT-derived antimicrobial polymethacrylates: Elucidating the impact of end-groups on activity and cytotoxicity

Thomas D. Michl[a,f], Katherine E. S. Locock[b,f], Natalie E. Stevens[c], John D. Hayball[c], Krasimir Vasilev[d], Almar Postma[b], Yue Qu[e], Ana Traven[e], Matthias Haeussler[b], Laurence Meagher[b], Hans J. Griesser[d]

[a]Ian Wark Research Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
email: mictd002@mymail.unisa.edu.au

[b]CSIRO Materials Science and Engineering, Bayview Avenue, Clayton, VIC 3168, Australia

[c]Sansom Institute, School of Pharmacy and Medical Sciences, University of South Australia, City East, SA 5000, Australia

[d]Mawson Institute, University of South Australia, Mawson Lakes, SA 5095, Australia

[e]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia,

[f]these two authors contributed equally

Supporting Information

Contents

Polymer synthesis and Characterization

Synthesis of Amine Polymers

Reversible addition−fragmentation chain transfer (RAFT) polymerization of 2-AEMA and MMA was performed in DMSO at 70 °C for 18 h using 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA1), 2-cyanopropan-2-yl dodecyl carbonotrithioate (CTA2) or 4-cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA3) as the RAFT agent and AIBN as the radical initiator.

PA1

2-AEMA (4.64 g, 28 mmol), MMA (1.20 g, 12 mmol), AIBN (98 mg 0.6 mmol), and 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA1, 807 mg, 2 mmol) were dissolved in DMSO (6 mL) in a 50 mL Schlenk flask. The reaction was subject to three freeze−evacuate−thaw cycles under high vacuum (10^{-3} Torr) before being heated to 70 °C for 16 h. The crude product was first diluted with MeOH before being precipitated three times into ether, collected each time by centrifugation. All traces of solvent were removed under high vacuum to give PA1 as a yellow powder (5.66 g, 85% yield).

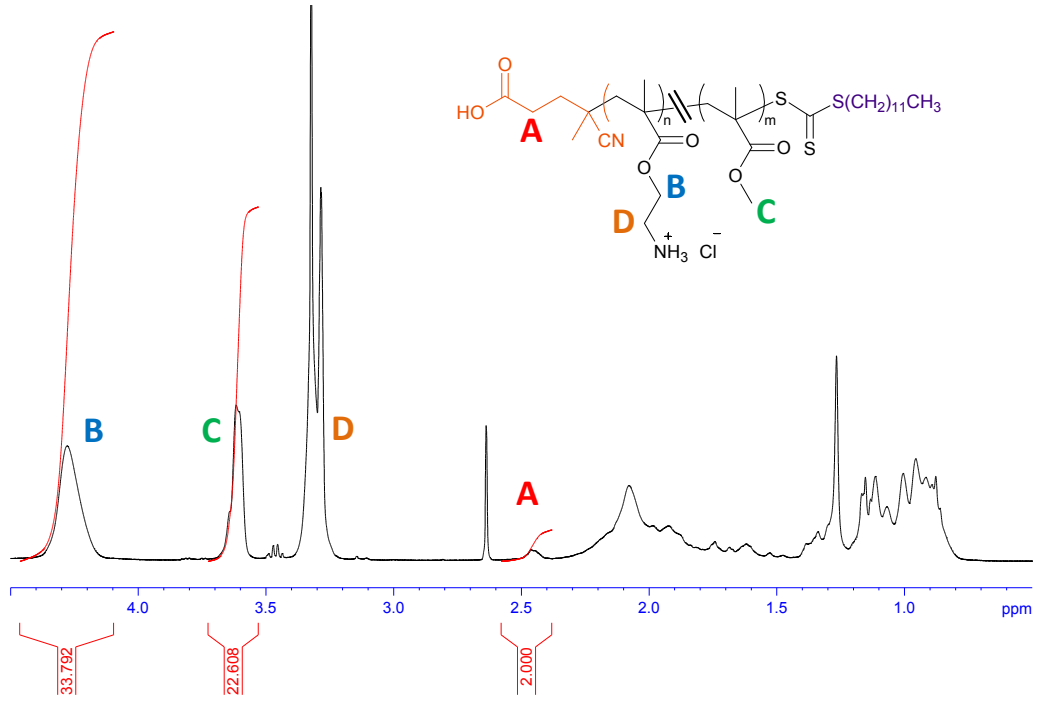
PA2

2-AEMA (2.32 g, 14 mmol), MMA (601 mg, 6 mmol), AIBN (98 mg 0.6 mmol), and 4-cyano-4-[(ethylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA3, 263 mg, 1 mmol) were dissolved in DMSO (6 mL) in a 50 mL Schlenk flask. The reaction was subject to three freeze−evacuate−thaw cycles under high vacuum (10^{-3} Torr) before being heated to 70 °C for 16 h. The crude product was first diluted with MeOH before being precipitated three times into ether, collected each time by centrifugation. All traces of solvent were removed under high vacuum to give PA1 as a yellow powder (2.80 g, 77% yield).

PA3

2-AEMA (2.32 g, 14 mmol), MMA (601 mg, 6 mmol), AIBN (98 mg 0.6 mmol), and 2-cyanopropan-2-yl dodecyl carbonotrithioate (CTA2, 263 mg, 1 mmol) were dissolved in DMSO (6 mL) in a 50 mL Schlenk flask. The reaction was subject to three freeze−evacuate−thaw cycles under high vacuum (10^{-3} Torr) before being heated to 70 °C for 16 h. The crude product was first diluted with MeOH before being precipitated three times into ether, collected each time by centrifugation. All traces of solvent were removed under high vacuum to give PA1 as a yellow powder (3.14 g, 96% yield).

Representative ^{1}H NMR for Amine Polymers



Synthesis of Guanidine Polymers PG1-PG3

A post polymerization guanylation method was used to convert amine polymers PA1, PA2 and PA3 to the corresponding guanidine functionalized polymers PG1, PG2 and PG3.

PG1

To a solution of PA1 (4 g, 1 mmol) in anhydrous methanol (50 mL), was added 1*H*-pyrazole-1-carboxamidine hydrochloride (3.78 g, 26 mmol) and *N,N*-diisopropylethylamine base (6.34 g, 49 mmol), which equated to 1.5 and 3 equivalents to the number of amine units per polymer chain. The reaction was heated at 55 °C overnight under nitrogen positive pressure. Solvent was removed in vacuo and the polymer purified by precipitation from methanol−acetone three times to obtain PG1 as a slight yellow powder in quantitative yield.

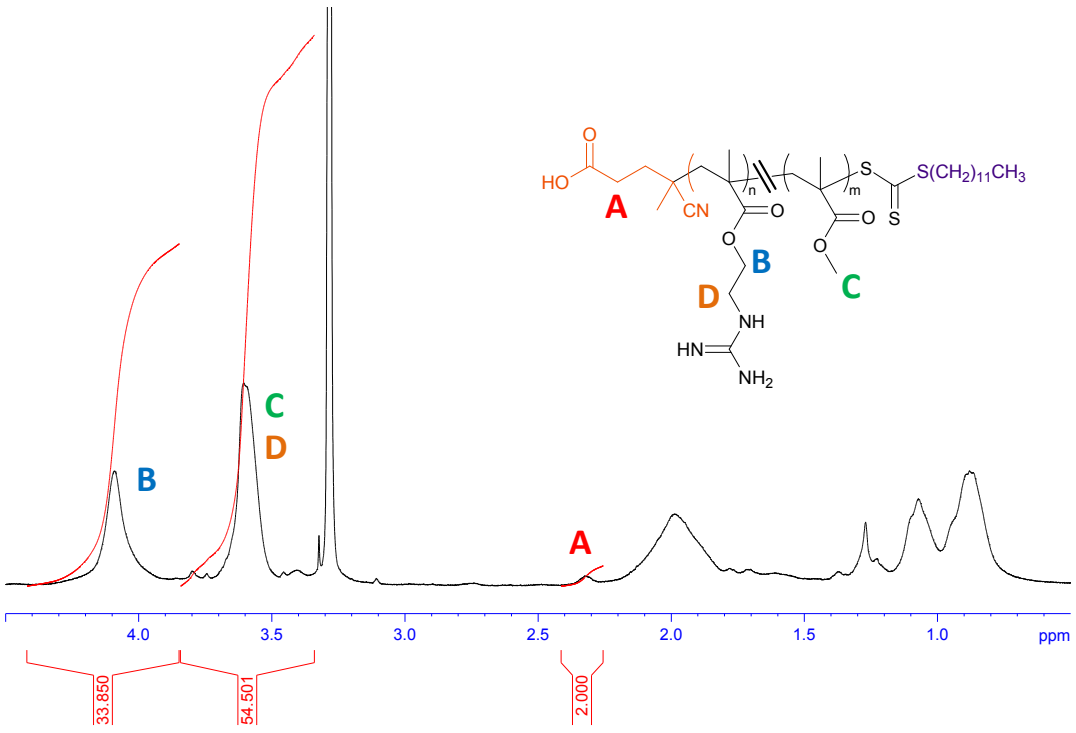
PG2

To a solution of PA2 (2 g, 0.5 mmol) in anhydrous methanol (25 mL), was added 1*H*-pyrazole-1-carboxamidine hydrochloride (1.76 g, 12 mmol) and *N,N*-diisopropylethylamine base (3.10 g, 24 mmol), which equated to 1.5 and 3 equivalents to the number of amine units per polymer chain. The reaction was heated at 55 °C overnight under nitrogen positive pressure. Solvent was removed in vacuo and the polymer purified by precipitation from methanol−acetone three times to obtain PG2 as a slight yellow powder in quantitative yield.

PG3

To a solution of PA2 (2 g, 0.5 mmol) in anhydrous methanol (25 mL), was added 1*H*-pyrazole-1-carboxamidine hydrochloride (1.87 g, 13 mmol) and *N,N*-diisopropylethylamine base (3.30 g, 26 mmol), which equated to 1.5 and 3 equivalents to the number of amine units per polymer chain. The reaction was heated at 55 °C overnight under nitrogen positive pressure. Solvent was removed in vacuo and the polymer purified by precipitation from methanol−acetone three times to obtain PG1 as a slight yellow powder in quantitative yield.

Representative ^{1}H NMR for Guanidine Polymers



Radical Reduction Removal of RAFT End-Groups to give PA4 and PG4

A radical induced reduction method was used to convert PA1 and PG1 into the corresponding proton terminated PA4 and PG4. A representative procedure is given below.

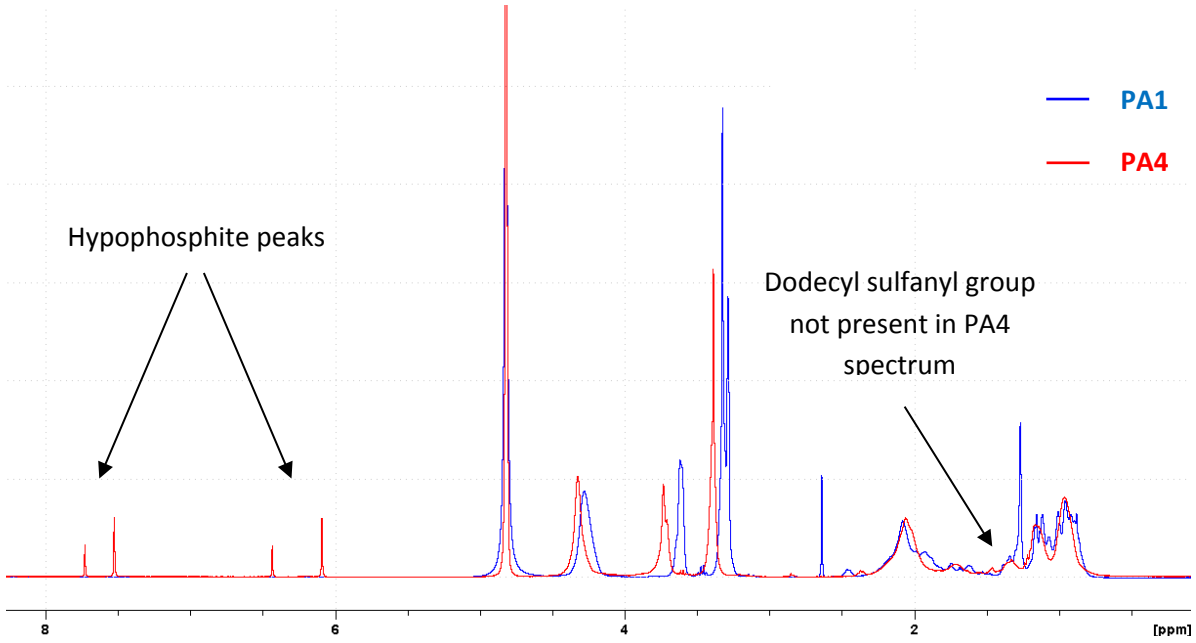
PA4

To a solution of PA1 (600 mg, 0.15 mmol) in DMSO (5 mL) was added Vazo-88 (13 mg, 0.075 mmol, 0.5 eq) and EPHP (367 mg, 1.5 mmol, 10 eq) in a 50 mL Schlenk flask. The reaction underwent three high vacuum (10^{-3} Torr) freeze-evacuation-thaw cycles before being heated to 100°C for 16 h. The product was isolated as the hypophosphite salt via three precipitations from methanol-acetone followed by high vacuum to remove trace solvent. This gave PA4 as a white powder (421 mg, 76% yield). The complete removal of RAFT end-groups was confirmed using UV-Vis and ^{1}H NMR analysis (see below for relevant spectra).

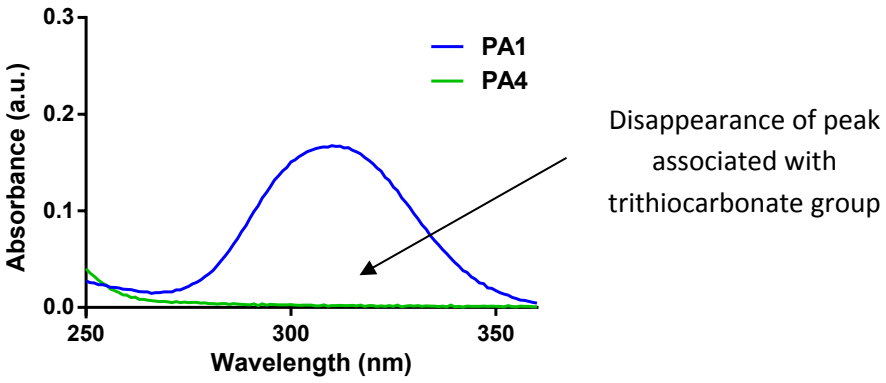
PG4

To a solution of PG1 (600 mg, 0.15 mmol) in DMSO (5 mL) was added Vazo-88 (13 mg, 0.075 mmol, 0.5 eq) and EPHP (367 mg, 1.5 mmol, 10 eq) in a 50 mL Schlenk flask. The reaction underwent three high vacuum (10^{-3} Torr) freeze-evacuation-thaw cycles before being heated to 100°C for 16 h. The product was isolated as the hypophosphite salt via three precipitations from methanol-acetone followed by high vacuum to remove trace solvent. This gave PG4 as a white powder (388 mg, 70% yield). The complete removal of RAFT end-groups was confirmed using UV-Vis and ^{1}H NMR analysis.

Confirmation of End Group Removal – Comparison of ^{1}H NMR Spectra



Confirmation of End Group Removal – Comparison of UV Spectra



DLS measurements

PA-series

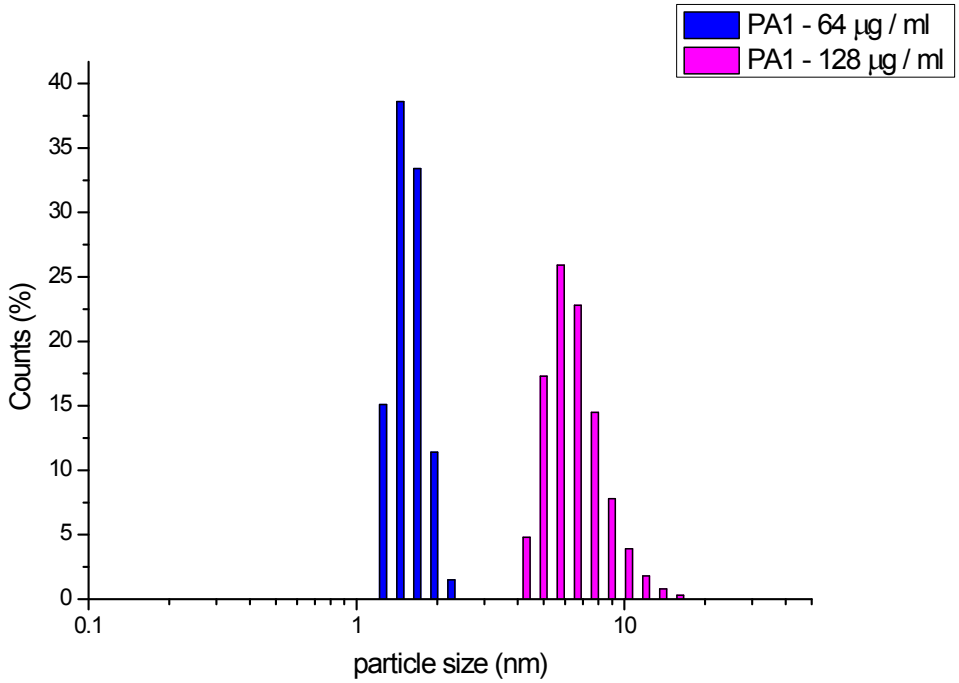


Figure SI1: DLS measurements of PA1

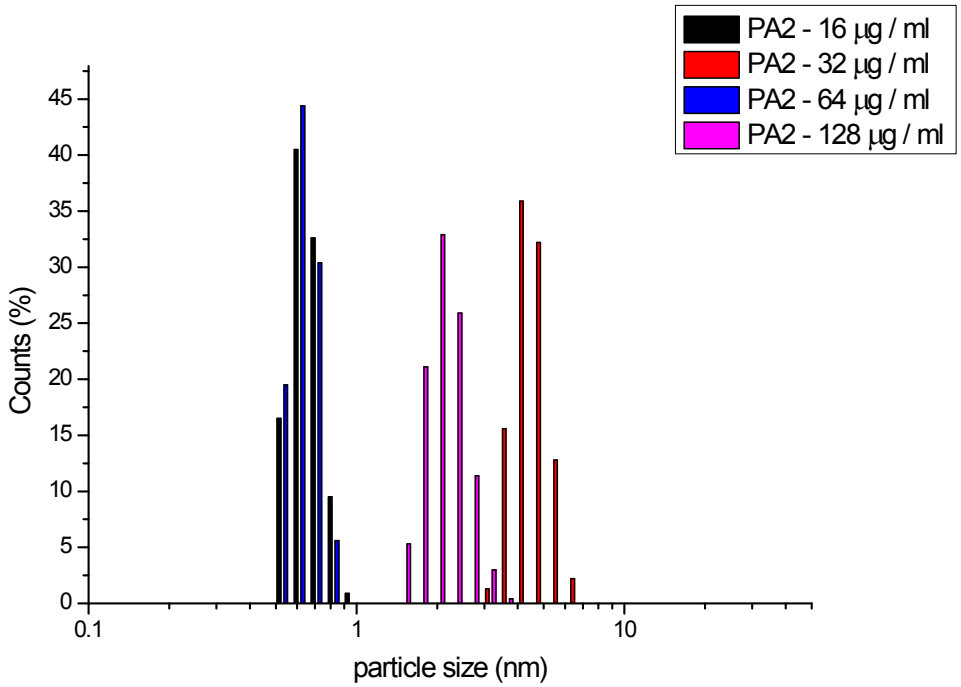


Figure SI2: DLS measurements of PA2

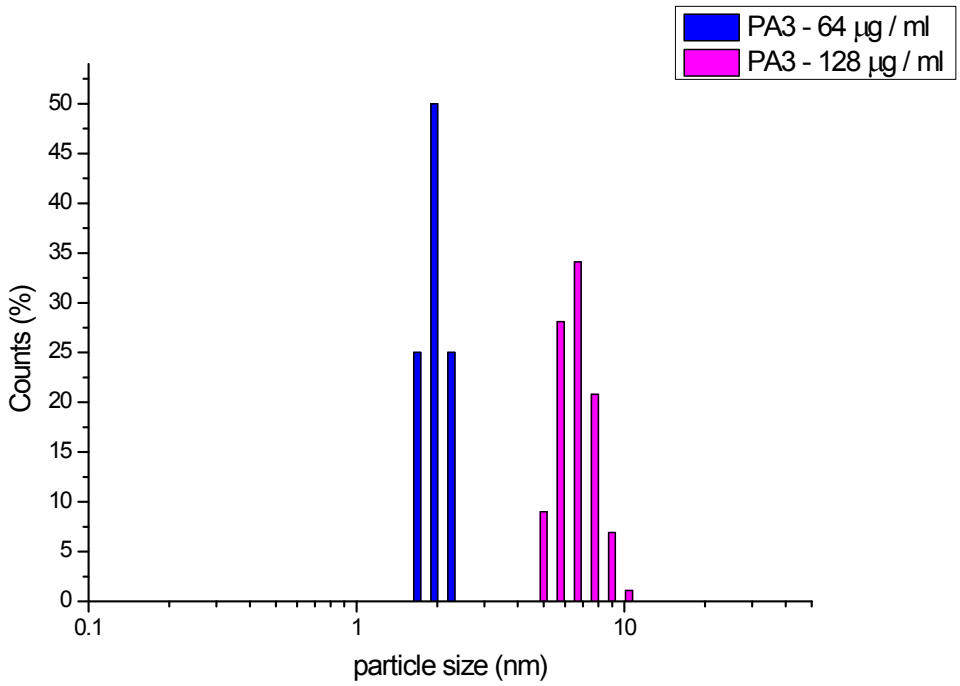


Figure SI3: DLS measurements of PA3

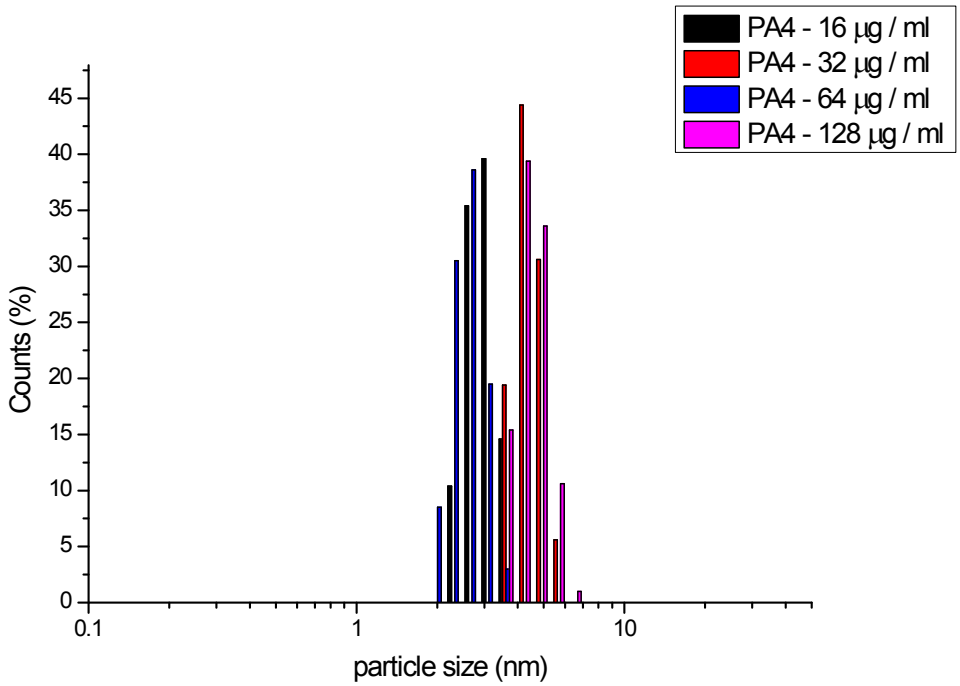


Figure SI4: DLS measurements of PA4

PG-series

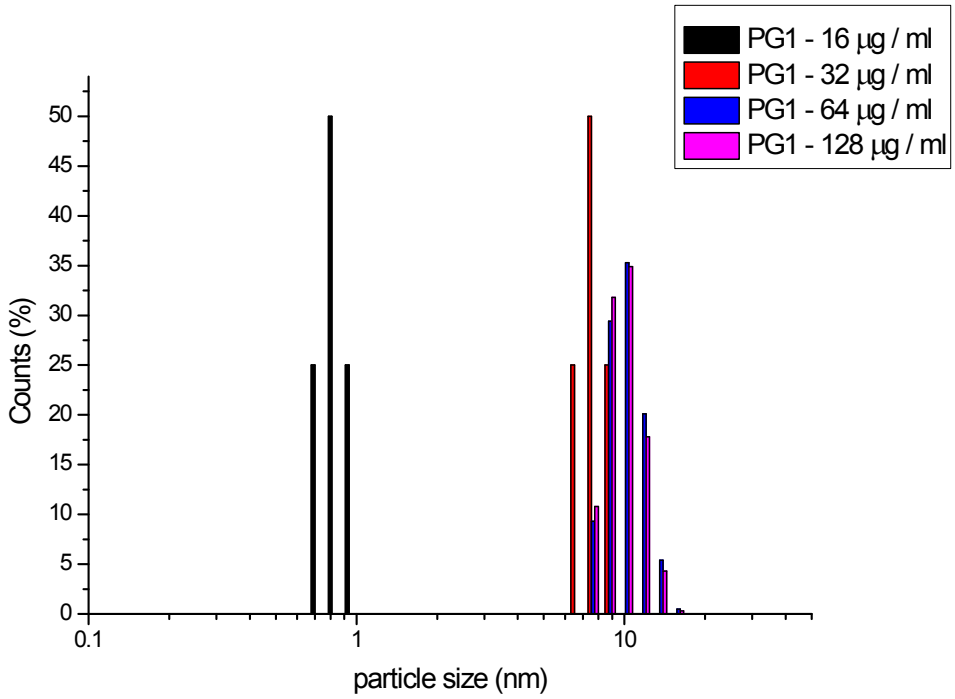


Figure SI5: DLS measurements of PG1

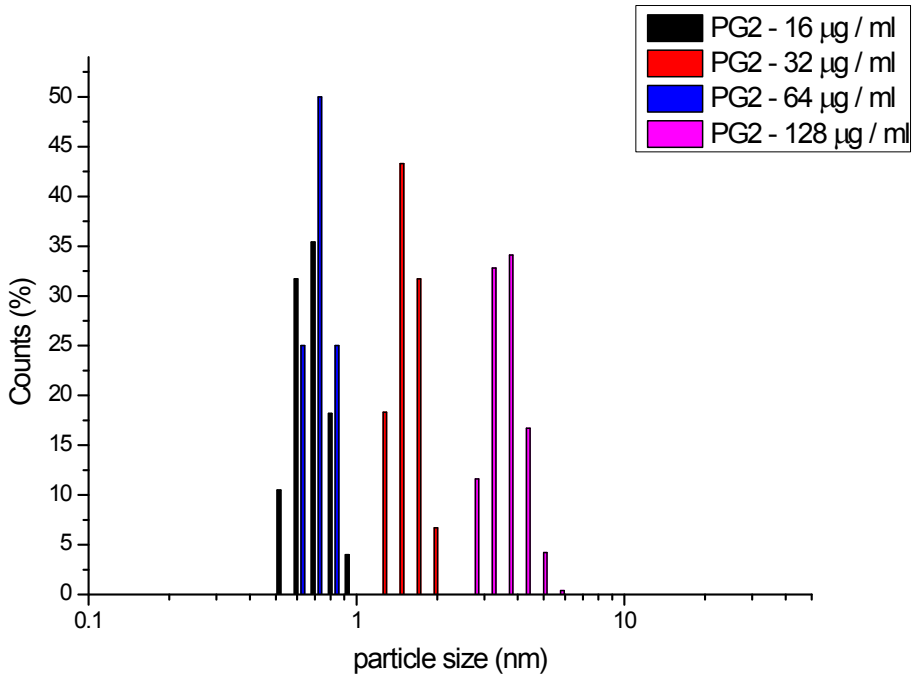


Figure SI6: DLS measurements of PG2

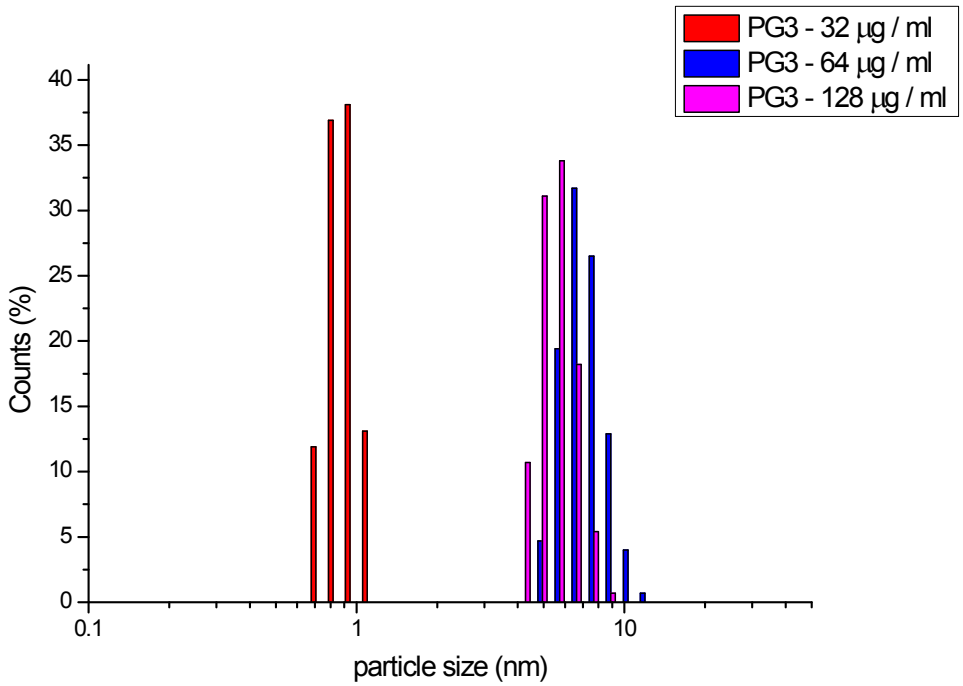


Figure SI7: DLS measurements of PG3

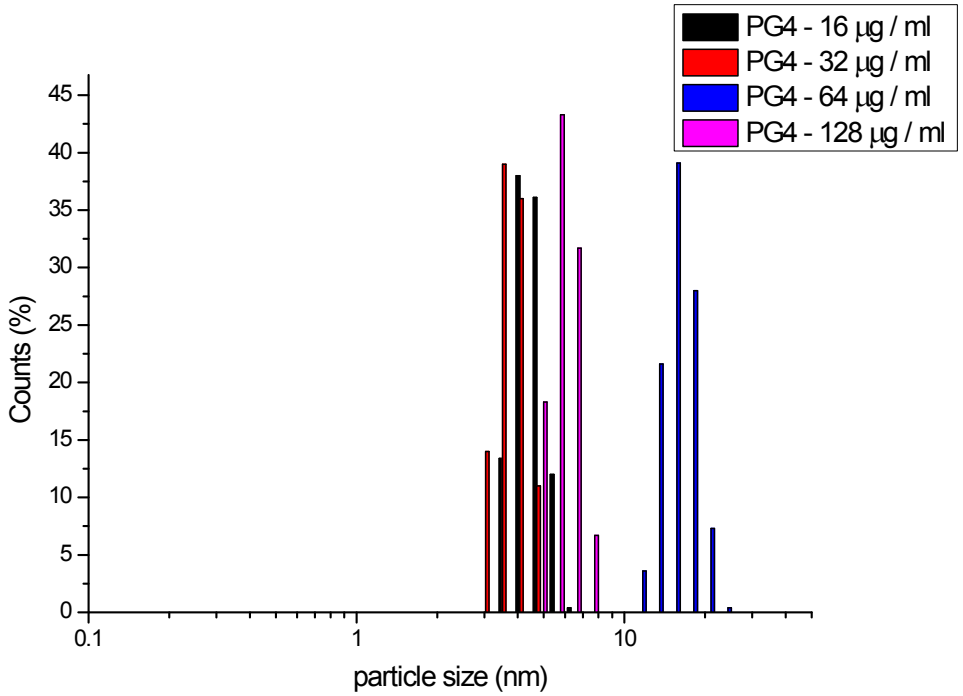


Figure SI8: DLS measurements of PG4

Antibacterial Results

Table SI1. Antimicrobial and haemolytic results

Polymer	VISA	S.epidermidis	C. albicans	Haemolysis (%)[a]
PA1	32	32	32	1.2
PA2	64	32	256	1.2
PA3	32	32	32	26.2
PA4	128	32	128	3.3
PG1	16	16	32	13.4
PG2	32	16	64	10.3
PG3	32	32	128	22.5
PG4	32	32	64	13.4

MIC as measured in µg/mL according to CLSI standards; [a] Haemolysis was determined as the percentage of lysed cells at the MIC concentration of *S.epi*

Haemolysis Results

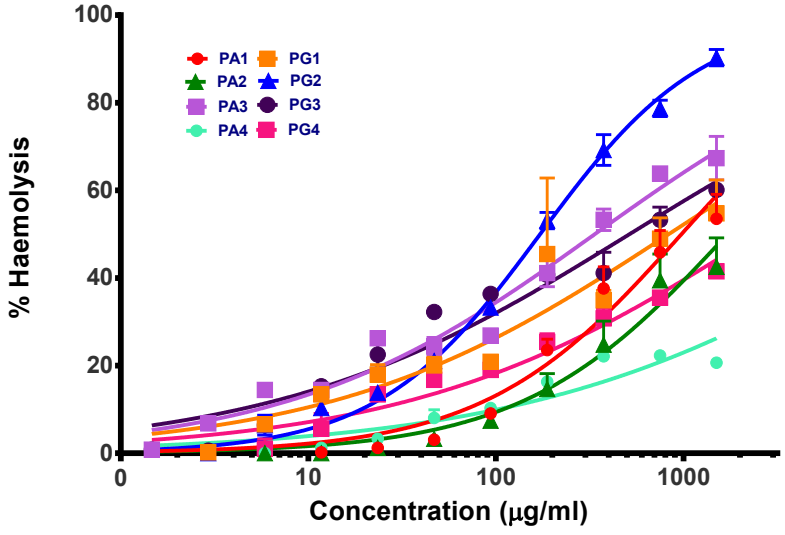


Figure SI9. Haemolysis results for PAI and PGI polymer series.

Haemagglutination Results

Table SI2. Haemagglutination results obtained from PA and PG polymer series.

Polymer	Concentration (μg/mL)										
	1500	**750**	**375**	**187.5**	**93.75**	**46.88**	**23.44**	**11.72**	**5.86**	**2.93**	**1.46**
PA1	++++	++++	++++	+++	+++	++	+	+	+	0	0
PA2	++++	+++	+++	+++	+++	+++	++	++	+	0	0
PA3	++++	++++	++++	+++	+++	++	++	++	++	+	+
PA4	++++	+++	+++	+++	++	++	++	+	0	0	0
PG1	+++	+++	+++	+++	+++	++	++	++	++	++	+
PG2	+++	+++	+++	+++	+++	+++	++	+	0	0	0
PG3	+++	+++	+++	+++	+++	+++	++	++	++	++	+
PG4	+++	+++	+++	+++	+++	++	++	++	++	+	0

Hemagglutination strength: ++++ strong, +++moderate, ++ mild, + weak, 0 no hemagglutination, (L) hemolysis observed

(1) Locock, K. E. S.; Michl, T. D.; Valentin, J. D. P.; Vasilev, K.; Hayball, J. D.; Qu, Y.; Traven, A.; Griesser, H. J.; Meagher, L.; Haeussler, M. *Biomacromolecules* **2013**, *14*, 4021.