Electronic Supplementary Material (ESI) for RSC Applied Polymers.

Supplementary information

Triblock copolymer micelles enhance solubility, permeability and activity of a quorum sensing inhibitor against Pseudomonas aeruginosa biofilms

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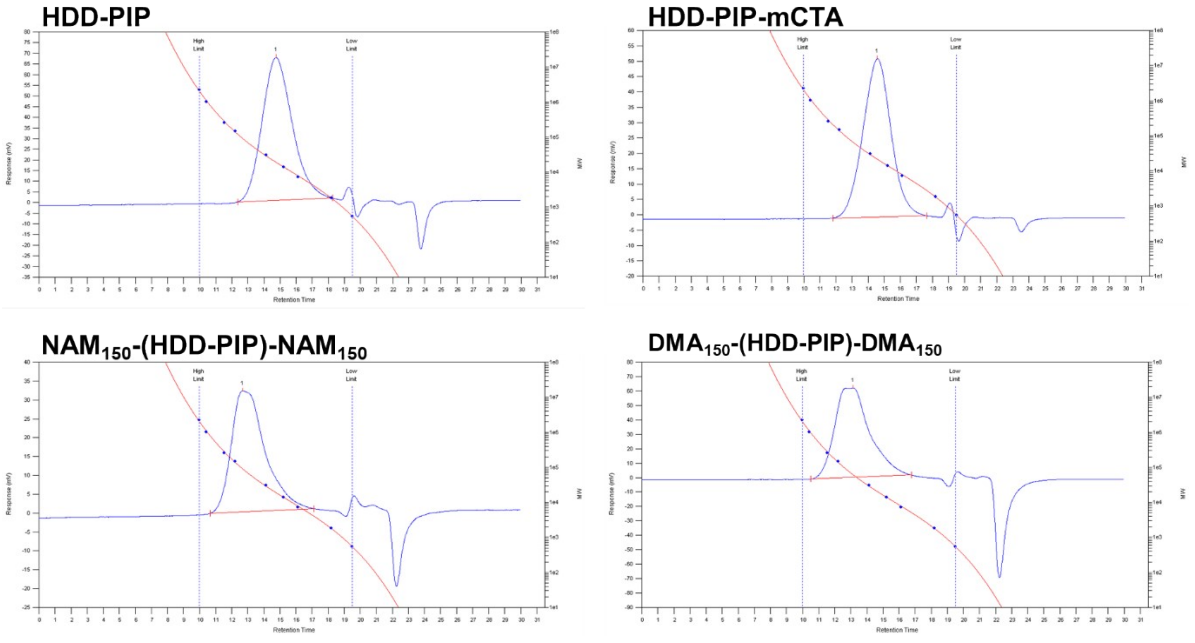
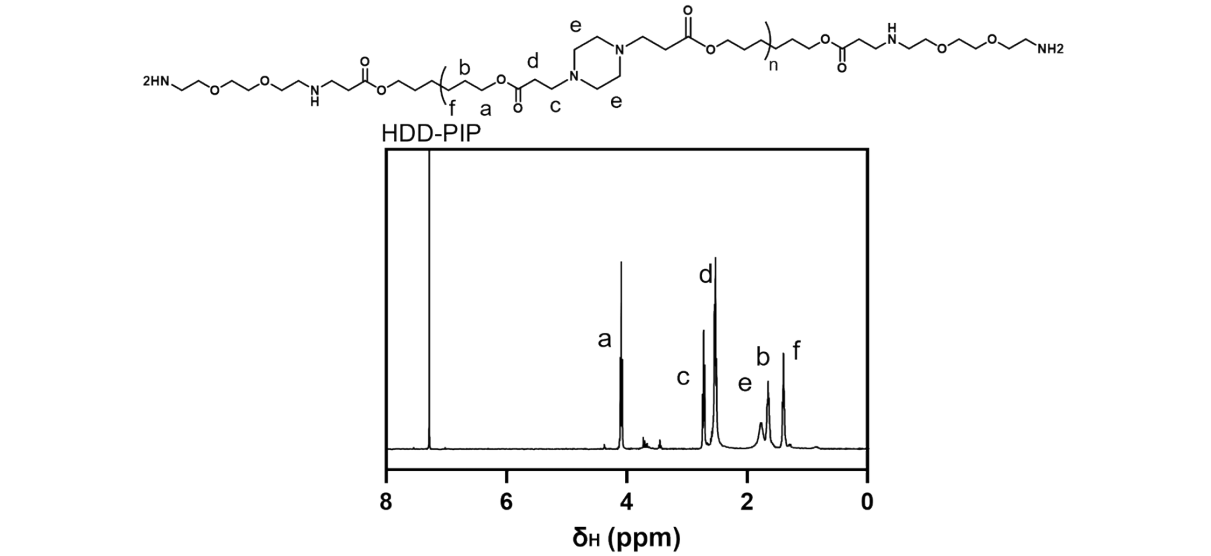


Figure S1: DMF-SEC chromatograms of prepared polymers.



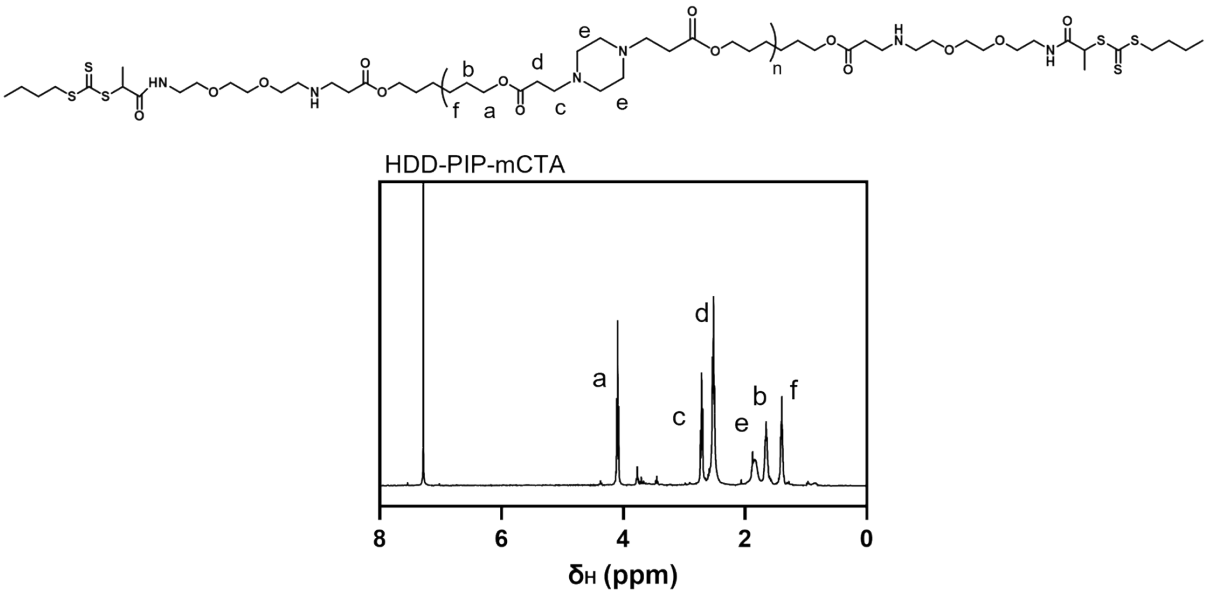


Figure S2: ^{1}H NMR spectra of polymers in chloroform-d recorded at 25°C with a Bruker NMR400 MHz.

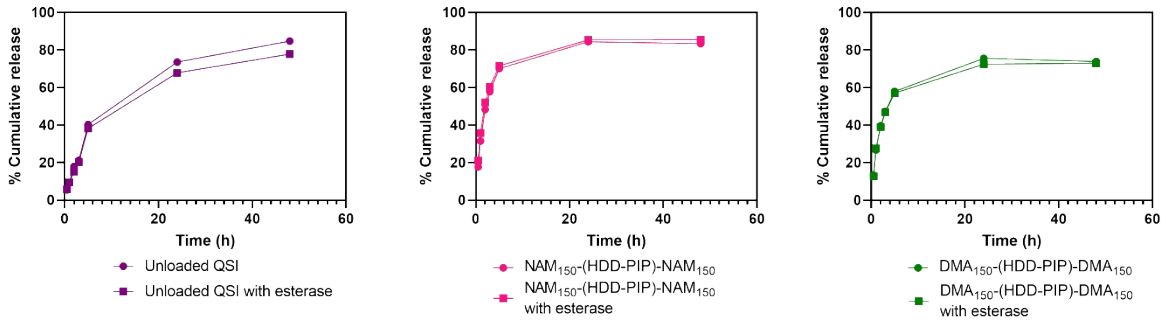


Figure S3: QSI release from NAM_{150}-(HDD-PIP)-NAM_{150} (pink) and DMA_{150}-(HDD-PIP)-DMA_{150} (green) particles and unloaded QSI (purple) in phosphate buffer pH 7.4, containing

0.01% Tween 20, at 37°C, across 48 h, with and without esterase presence samples analysed by HPLC.

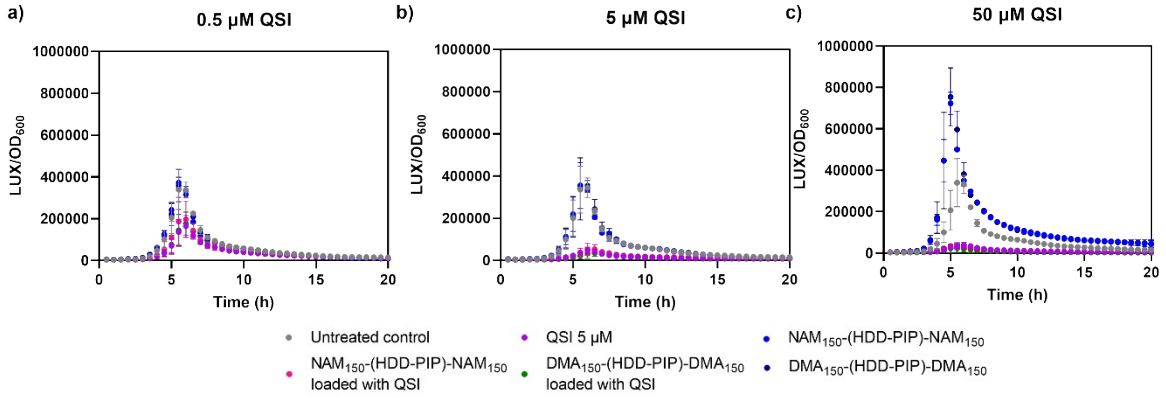


Figure S4: Effect of QSI and QSI loaded polymers on a P_{pqsA}-*lux* transcriptional fusion, which reports the PQS-dependent activation of the *pqs* operon mediated by PqsR. **a)** QSI concentrations at 0.5 µM QSI and non-loaded particles at corresponding concentrations; **b)** QSI concentrations at 5 µM QSI and non-loaded particles at corresponding concentrations; **c)** QSI concentrations at 50 µM QSI and non-loaded particles at corresponding concentrations. Values given are averages from three different cultures for QSI activity testing and two different cultures for the testing of non-loaded polymeric particles and correspond to the relative light units normalized to culture density (Lux/OD_{600}) over time (20 h).

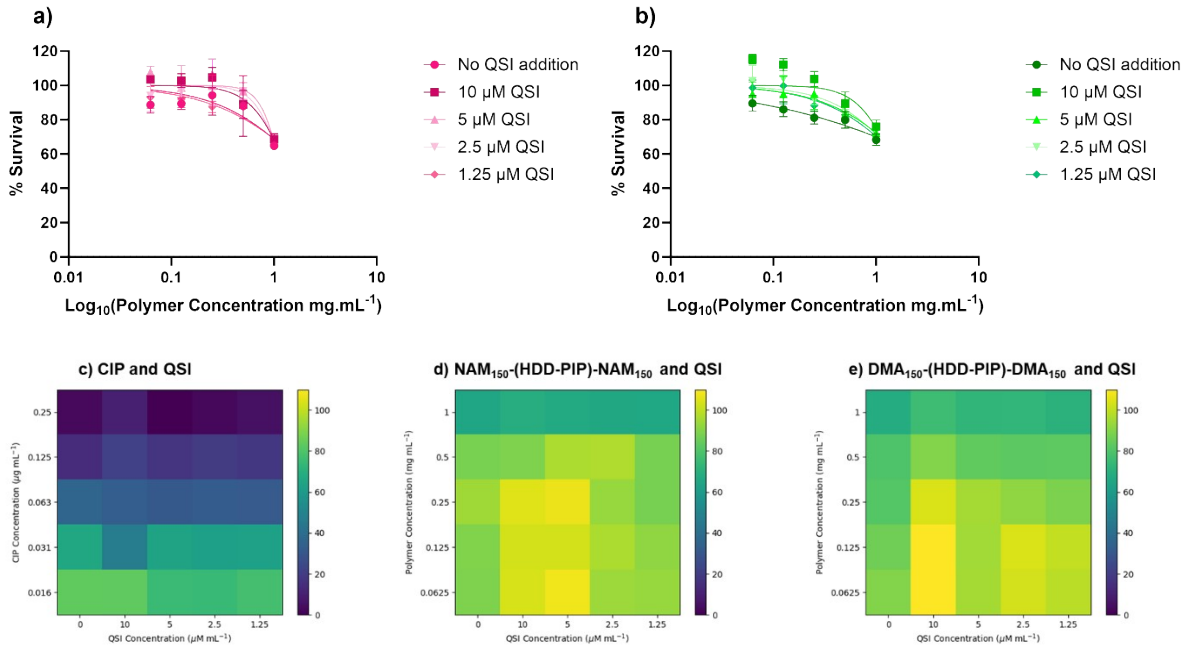


Figure S5: Antimicrobial activity of polymeric particles with and without QSI61 addition. **a)** Concentration curves of NAM_{150}-(HDD-PIP)-NAM_{150} (pink) with and without QSI61 addition against planktonic *P. aeruginosa*; **b)** Concentration-activity curves of DMA_{150}-(HDD-PIP)-DMA_{150} (green) with and without QSI61 addition against planktonic *P. aeruginosa*. Heat maps to show effects of combinations of **c)**, CIP +QSI, **d**, NAM_{150}-(HDD-PIP)-NAM_{150} and QSI, and **e**) DMA_{150}-(HDD-PIP)-DMA_{150} and QSI.

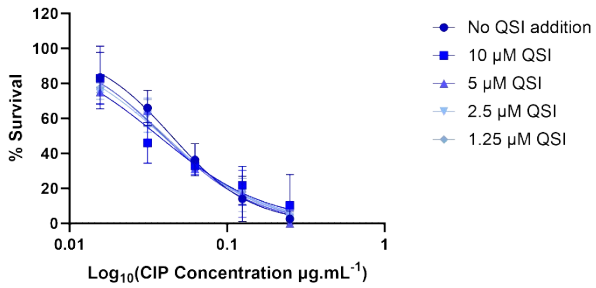


Figure S6: CIP concentration-activity curves with and without QSI61 addition against planktonic *P. aeruginosa*.

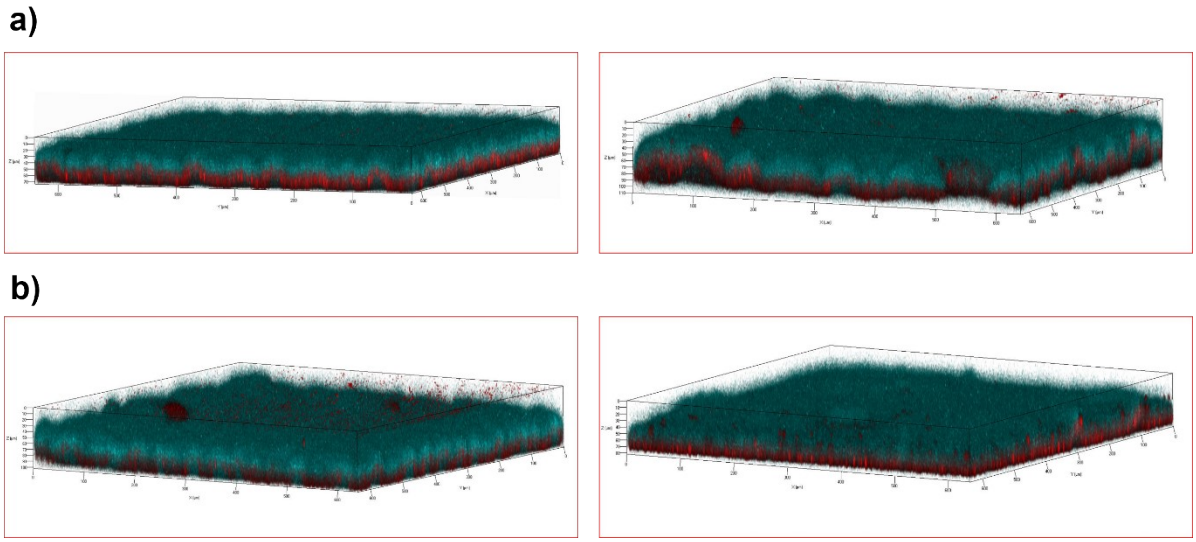


Figure S7: Confocal laser scanning microscopy (CLSM) images the biofilm penetration of rhodamine-tagged DMA_{150}-(HDD-PIP)-DMA_{150} (**a**) and NAM_{150}-(HDD-PIP)-NAM_{150} particles (**b**) (red) in mature *P. aeruginosa* PA01L biofilms (1-day old) stained with SYTO9 (blue) following a 4 h incubation.

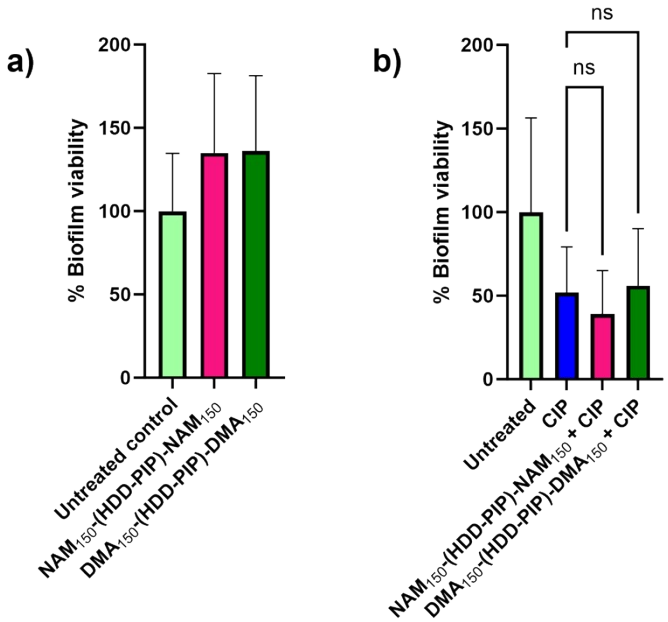


Figure S8: Antimicrobial activity of polymeric particles in *P. aeruginosa* biofilms; **a)** Bar charts showing viability in mature *P. aeruginosa* biofilms, quantified after treatment with NAM_{150}-(HDD-PIP)-NAM_{150} (pink) and DMA_{150}-(HDD-PIP)-DMA_{150} (green) particles with no encapsulated QSI; measurements were performed in duplicate, using biologically independent replicates and the error bars represent the mean ± standard deviation; **b)** Bar charts showing viability in mature *P. aeruginosa* biofilms, quantified after treatment with CIP (60 μg mL^{-1}) and NAM_{150}-(HDD-PIP)-NAM_{150} (pink) and DMA_{150}-(HDD-PIP)-DMA_{150} (green) particles with no encapsulated QSI administered in combination with CIP (60 μg mL^{-1}); measurements are an average of 15 coverslip measurements using one biological replicate and the error bars represent the mean ± standard deviation. Statistical testing was performed with a one-way ANOVA followed by a post-hoc Tukey test to identify individual comparisons. Statistical significance is represented as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.