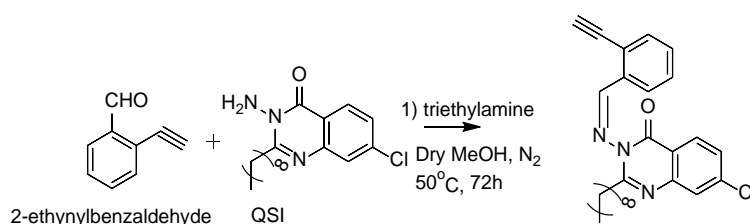


Supporting Information

Dual Bioresponsive Antibiotic and Quorum Sense Inhibitor Combination Nanoparticles for Treatment of *Pseudomonas aeruginosa* Biofilms *In Vitro* and *Ex Vivo*.

Nishant Singh,^a Manuel Romero,^b Alessandra Travanut,^a Patricia Monteiro,^a Elena Jordana-Lluch,^{b,c} Kim R. Hardie,^b Paul Williams,^b Morgan Alexander^a and Cameron Alexander.^{a*}

Scheme S1- Synthesis of 2-ethynylbenzaldehyde+QSI hydrazone -



$^1\text{H NMR}$ (CDCl_3 , 400MHz), δ (ppm)- 1H (9.55, s), 2H (8.26-8.24, d), 1H (7.72-7.73, d), 1H (7.67-7.62, dd), 2H, (7.60-7.50, p), 1H (7.44-7.41, dd), 1H (3.43, s), 2H (2.98-2.94, t), 2H (1.86-1.79, p), 2H (1.59, s), 2H (1.45-1.40, s), 8H (1.40-1.25, m), 3H (0.90-0.57, t).

$^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ - 165.1, 158.24, 158.2, 147.4, 140.4, 134.3, 133.4, 131.9, 129.2, 128.8, 126.9, 126.7, 126.1, 124.4, 119.9, 84.0, 79.9, 35.2, 31.8, 29.4, 29.3, 29.3, 29.2, 26.7, 22.6, 14.1.

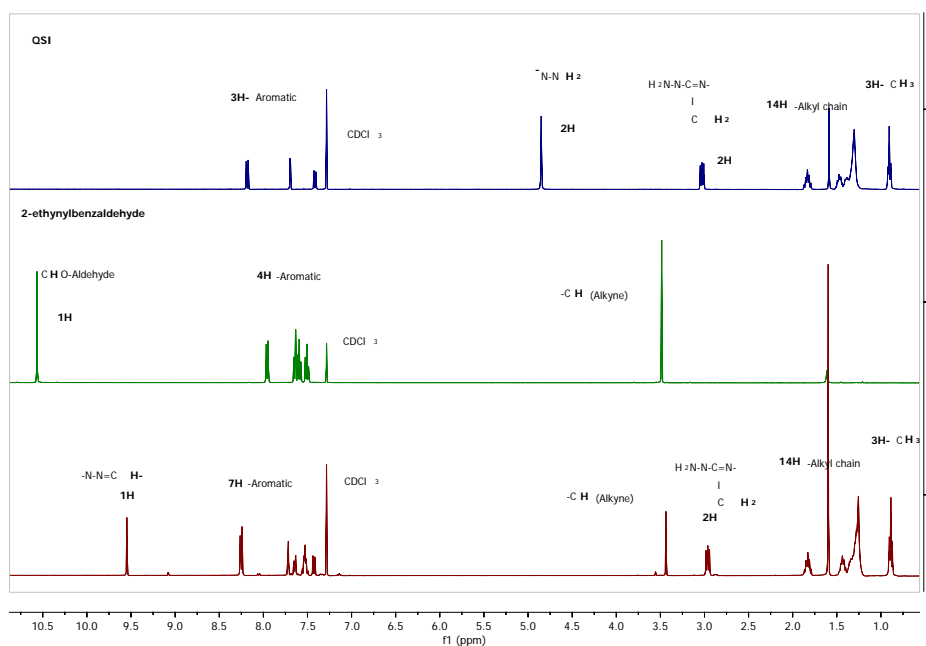


Figure S1- $^1\text{H NMR}$ (CDCl_3 , 400MHz) spectra comparison of ACNQ QSI drug in blue, 2-ethynylbenzaldehyde in green and the QSI+2-ethynylbenzaldehyde product in red.

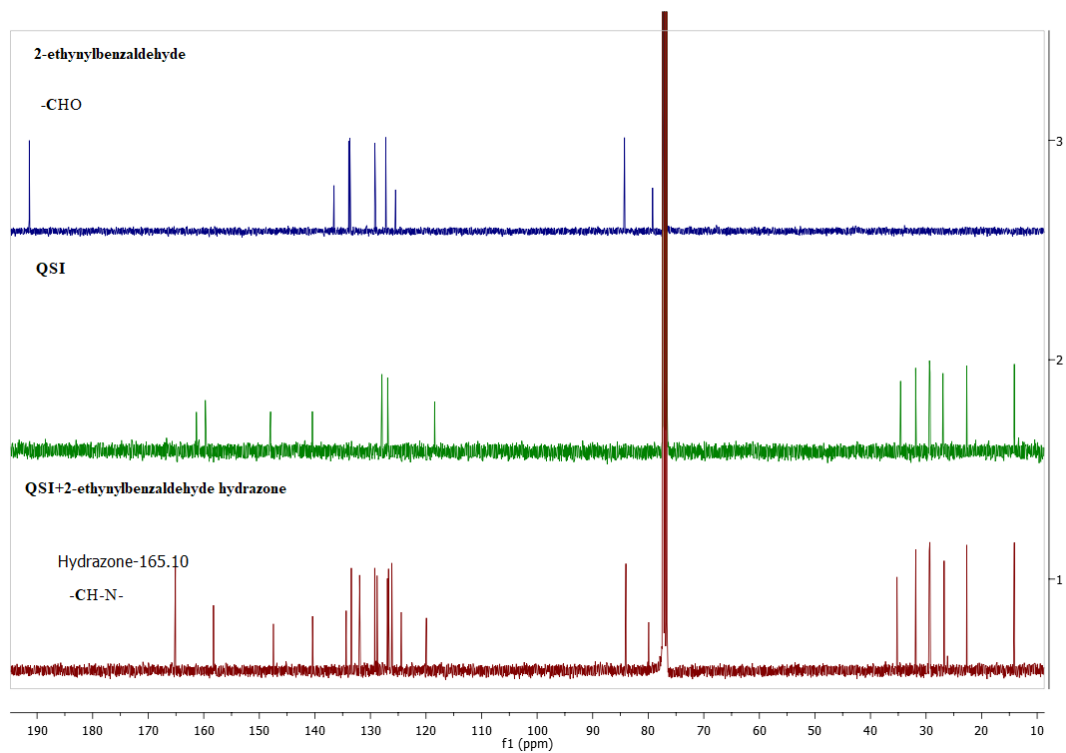
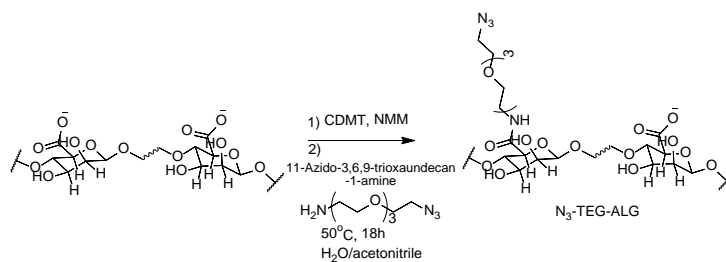


Figure S2- ^{13}C NMR (CDCl_3 , 101MHz) spectra comparison of 2-ethynylbenzaldehyde in blue, QSI drug in green, and the QSI+2-ethynylbenzaldehyde product in red.

Scheme S2- N_3 -TEG-ALG



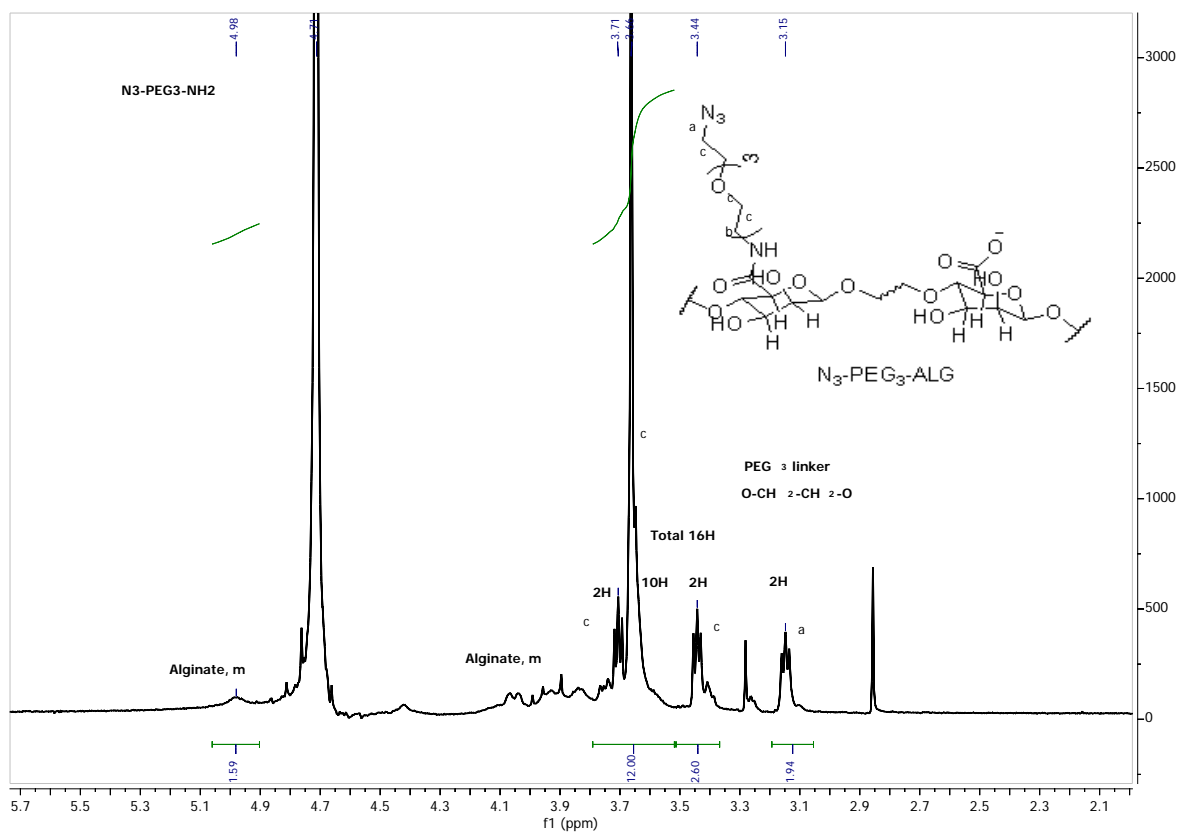
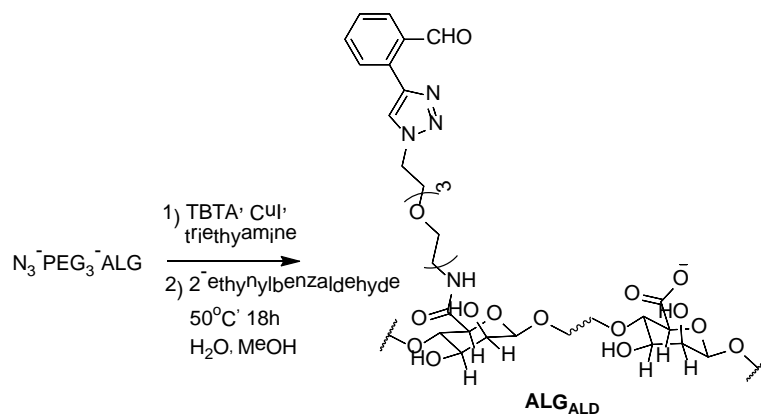


Figure S3 ¹H NMR of N₃-TEG-ALG in D₂O at 25 C (400MHz).

Scheme S3- ALG_{ALD}



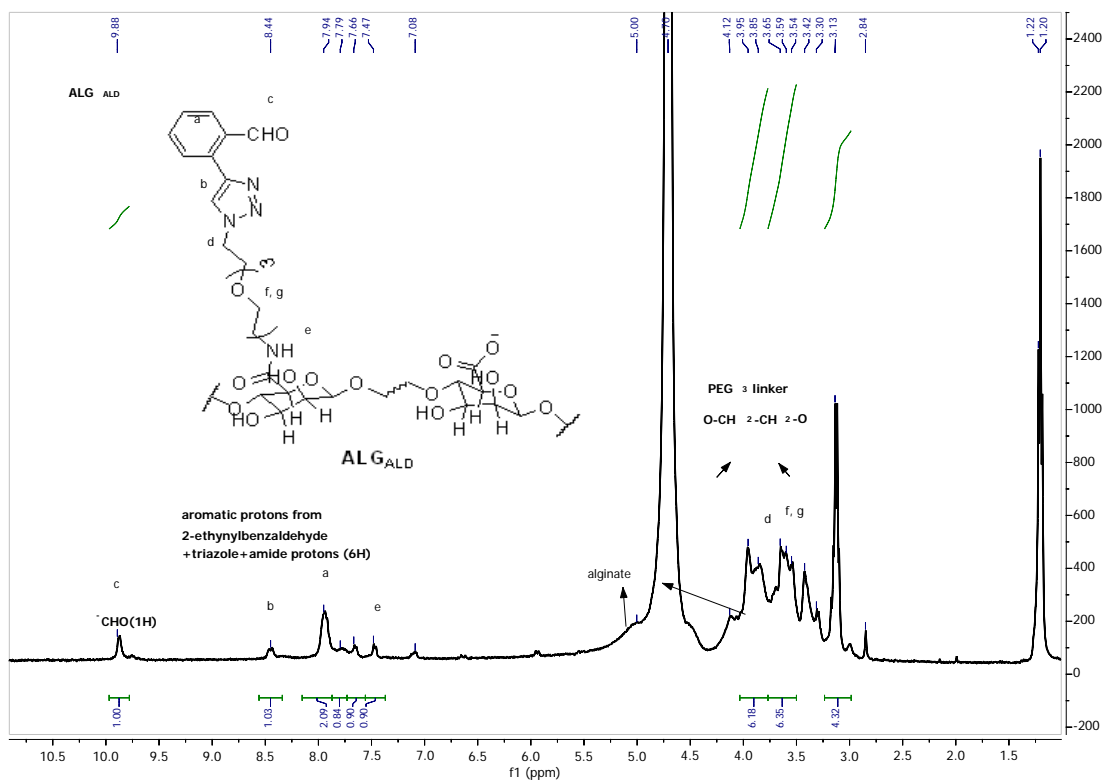


Figure S4-¹H NMR of ALG_{ALD} in D₂O at 25 C (400MHz).

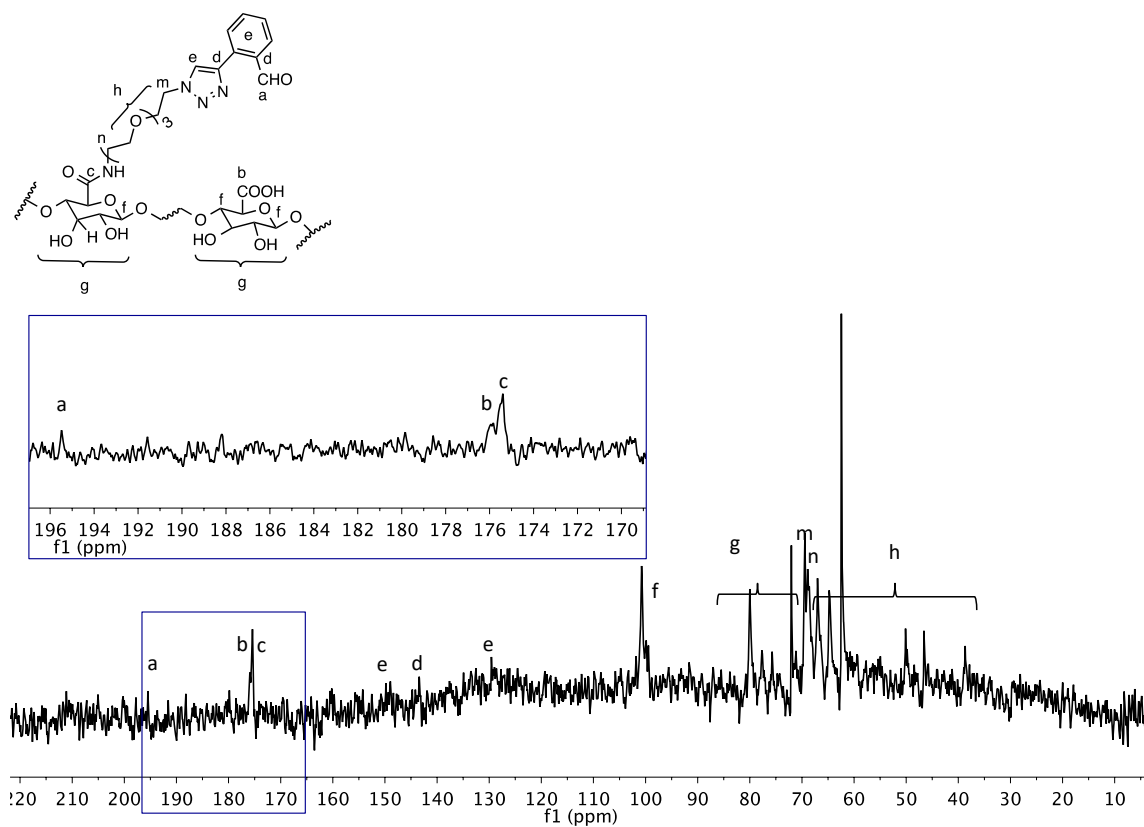


Figure S5-¹³C NMR of ALG_{ALD} in DMF-d₇ at 25 C (120MHz).

Scheme S4- Synthesis of ALG_{QSI}-

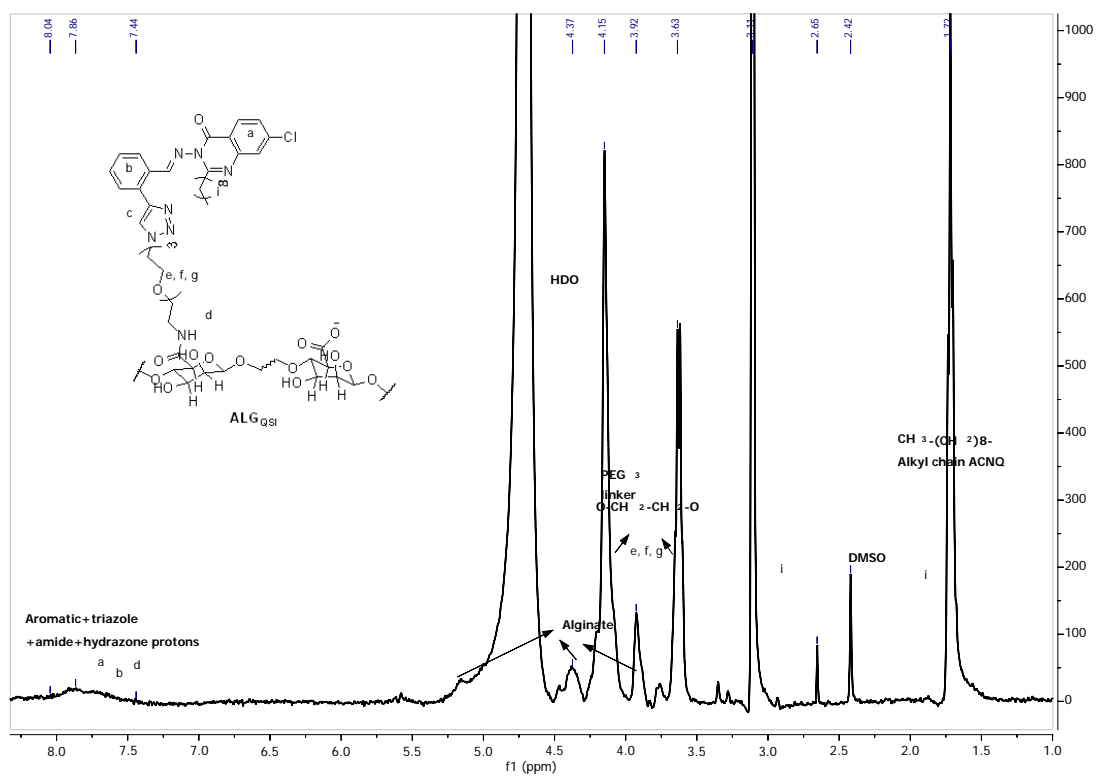
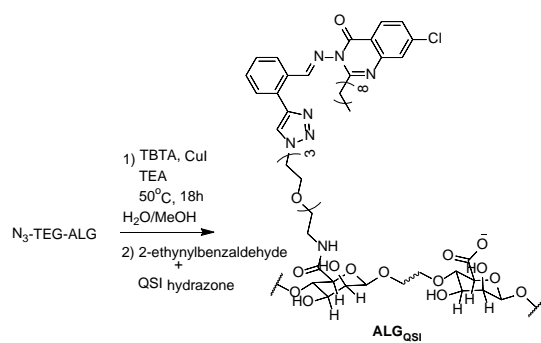


Figure S6- ¹H NMR of ALG_{QSI} in D₂O+DMSO-d₆ t 70 °C (400MHz).

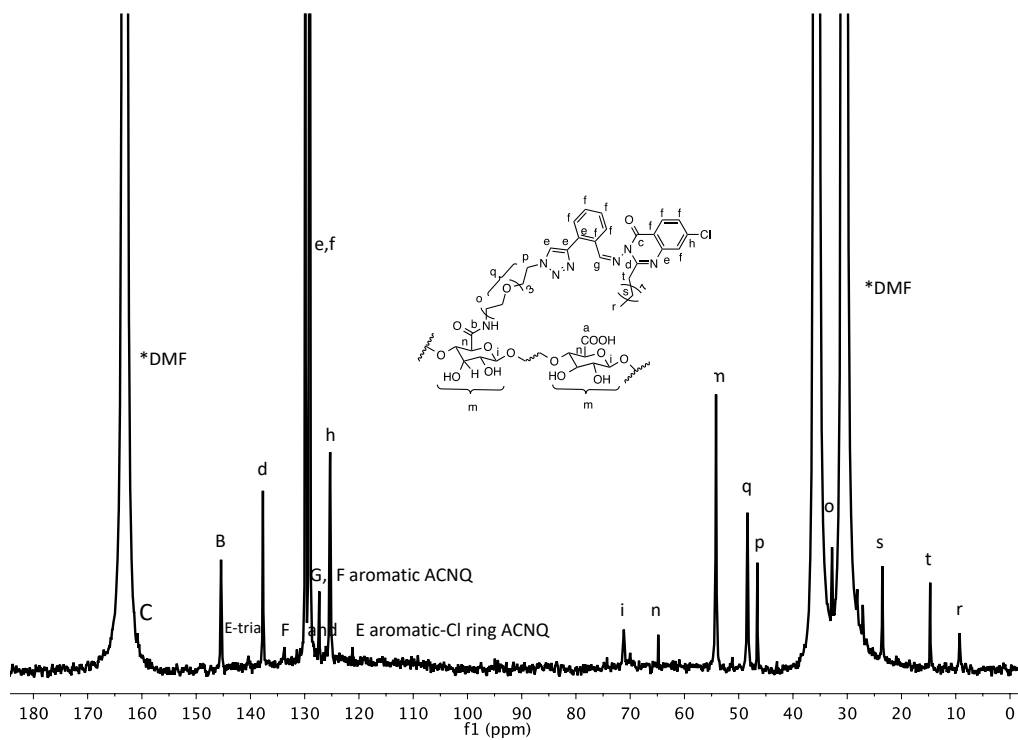


Figure S7- ^{13}C NMR of ALG_{QS1} in DMF-d_7 at 25°C (120MHz).

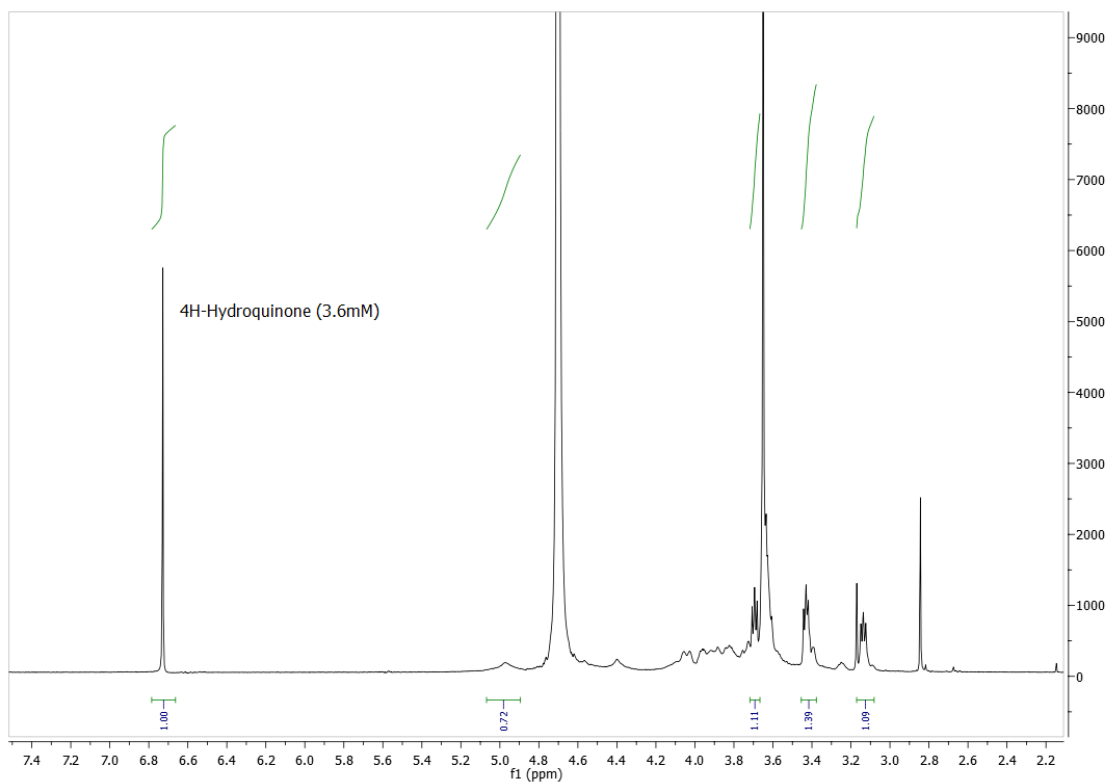


Figure S8- ^1H NMR spectra of $4\text{mg N}_3\text{-TEG-ALG} / 700\mu\text{L D}_2\text{O}$ along with a known concentration of an internal standard (hydroquinone, 3.6 Mm).

Ciprofloxacin calibration

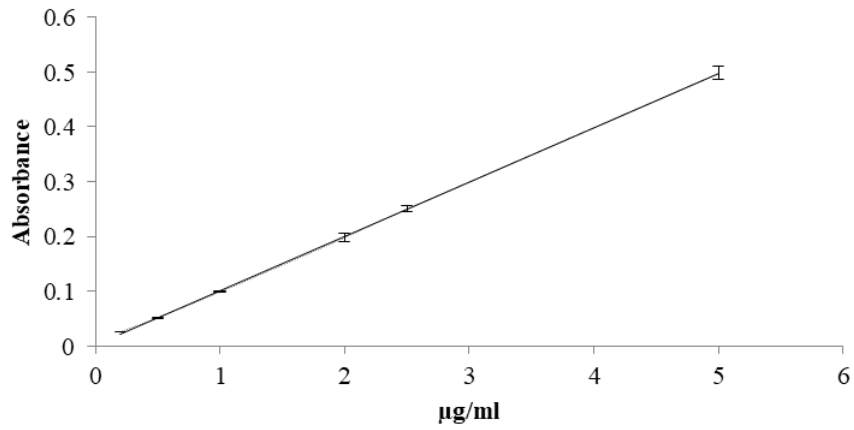


Figure S9- Calibration curve (275nm) obtained for different concentrations of Ciprofloxacin in PBS (stock solution made in 0.1 N HCl followed by dilution in PBS).

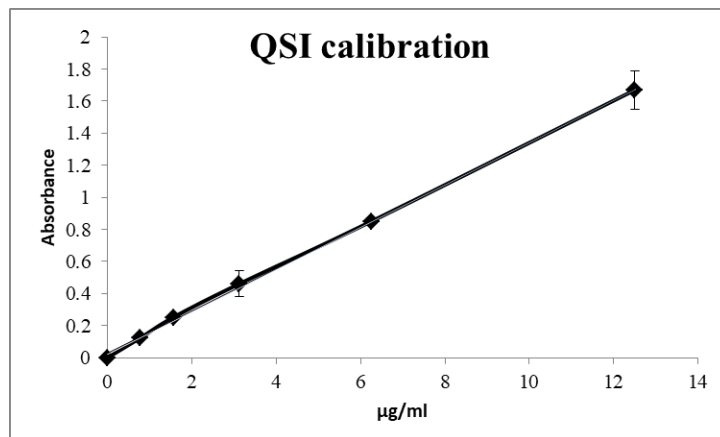


Figure S10- Calibration curve (240nm) obtained for different concentrations of QSI drug in PBS (stock solution made in methanol followed by dilution in PBS).

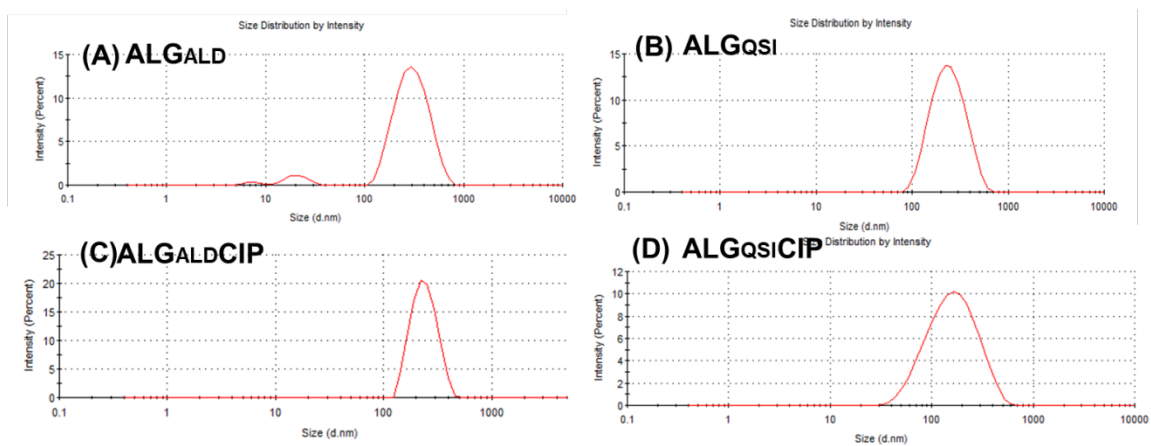


Figure S11- Intensity vs Size distribution of different NPs.

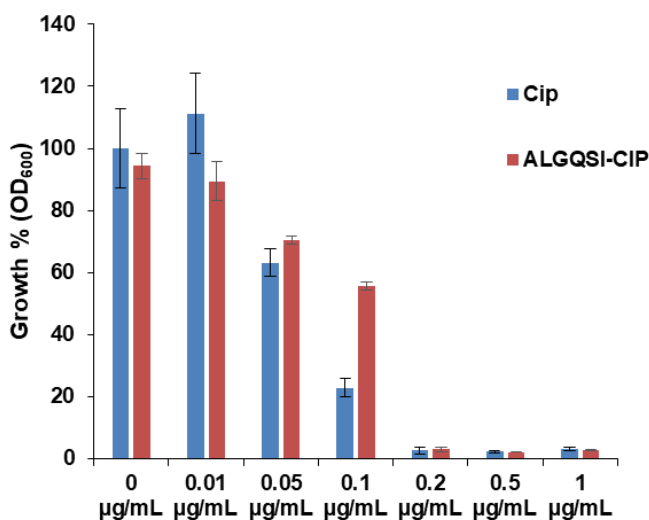


Figure S12- Effect of encapsulated ciprofloxacin on *Pseudomonas aeruginosa* growth. Bactericidal activity of serial concentrations (0.01-1 µg/mL) of free (CIP) or alginate-encapsulated ciprofloxacin (ALG-QSI CIP) were assessed in FAB cultures. Values given are averages from three different cultures ± standard deviation and correspond to the area under the curve derived from plotting single OD₆₀₀ measurements over time (24 h), and as percentage of the corresponding growth obtained in the untreated controls (set at 100%).

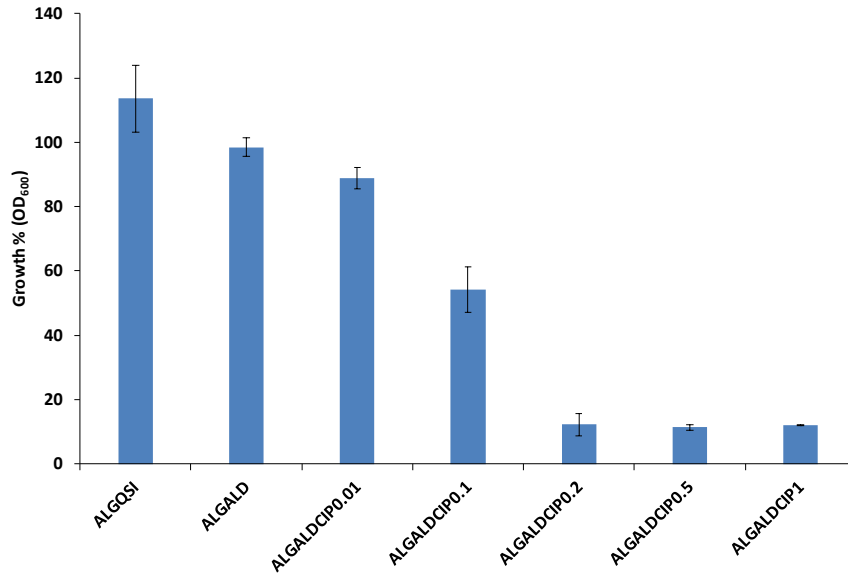


Figure S13. Effect of encapsulated ciprofloxacin on *Pseudomonas aeruginosa* growth. Bactericidal activity of serial concentrations (0.1-1 $\mu\text{g}/\text{mL}$) of alginate-encapsulated ciprofloxacin (ALGALDCIP) were assessed in FAB cultures. No antimicrobial activity was detected in cultures supplemented with ALGALD and ALQSI NPs over 24 hours at concentration 85 $\mu\text{g}/\text{mL}$ (equivalent to the highest ALGALDCIP concentration). Values given are averages from three different cultures \pm standard deviation and correspond to the area under the curve derived from plotting single OD600 measurements over time (24 h), and as percentage of the corresponding growth obtained in the untreated controls (set at 100%).

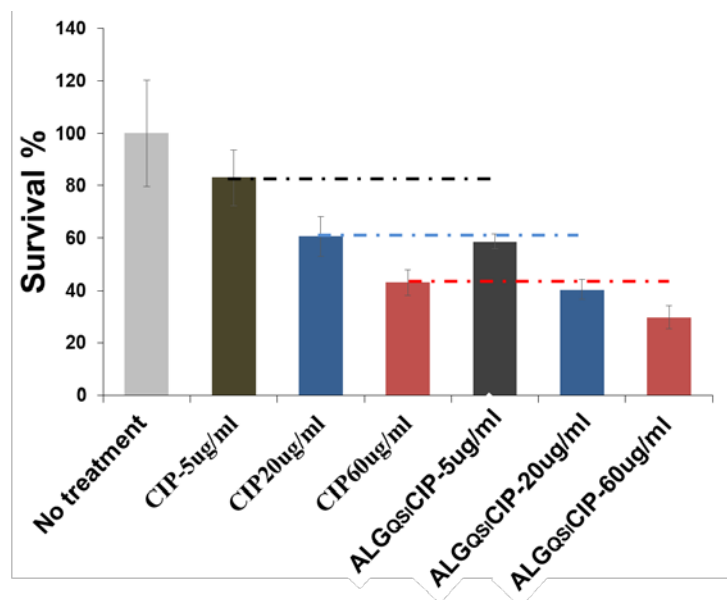


Figure S14- Comparison between the effect of ciprofloxacin and NP mediated delivery of QSI+ciprofloxacin on biofilm viability for different concentrations. The QSI+CIP system demonstrated improved performance in reducing biofilm survival with respect to the same antibiotic concentrations when delivered alone. The value of $\mu\text{g}/\text{mL}$ in the graph refers to the amount/volume of ciprofloxacin in the system.

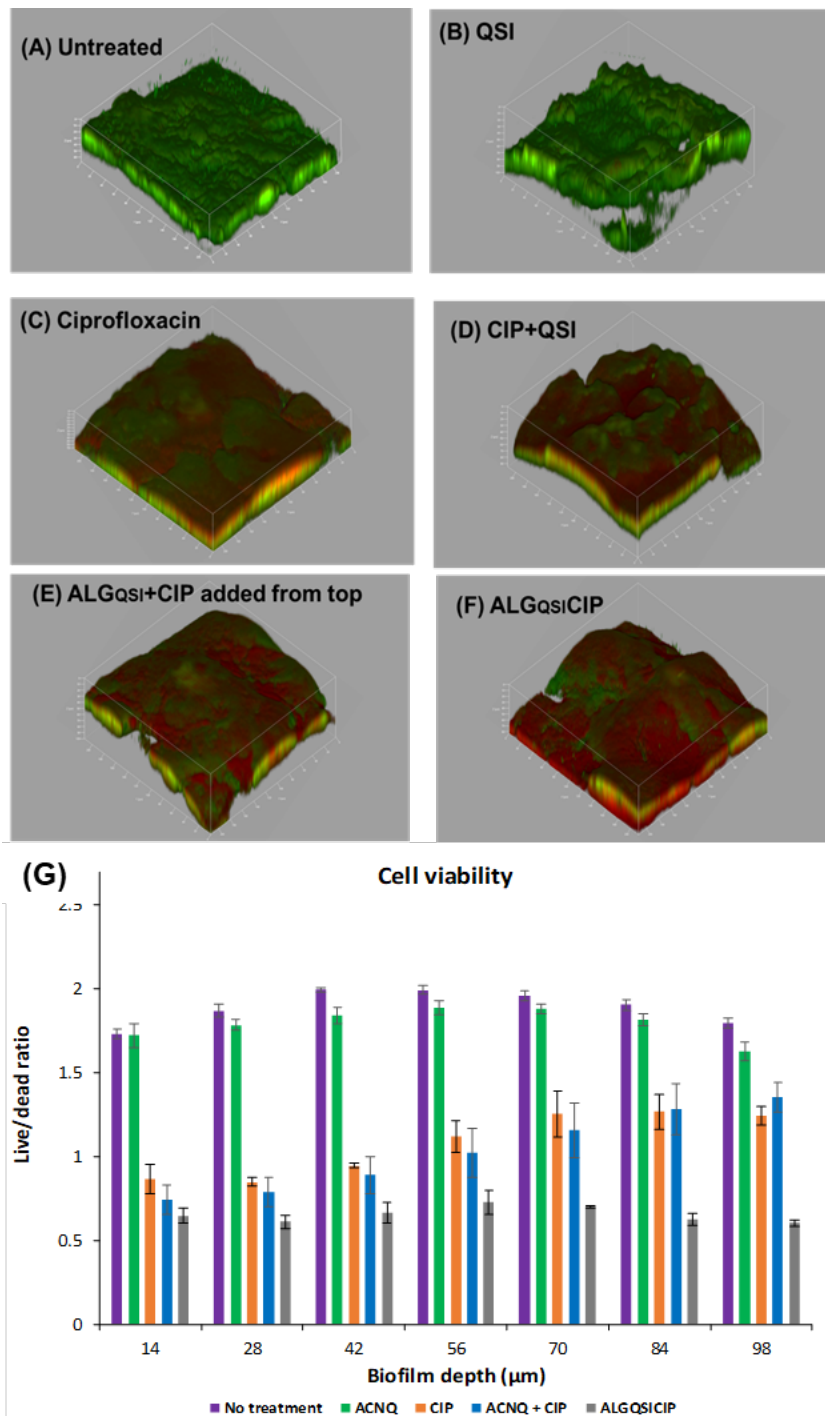


Figure S15- CLSM 3D stacks of biofilms post 24 hour treatment with, (a) untreated biofilm, (b) QSI drug alone, (c) 60μg/mL of ciprofloxacin alone, (d) a combination of QSI(4μg/mL)+CIP(60μg/mL), (e) ALG_{QSI} NPs with ciprofloxacin (60μg/mL) added from top, (f) ALG_{QSI}CIP NPs. The concentration of QSI was ~4μg/mL and CIP was 60 μg/mL in all the cases. The live biofilm is shown in green (Syto9 stain) and the dead in red (PI stain). (g) Bar charts showing live to dead cell ratios at different depths of PAO1-N biofilms quantified after exposure to different conditions for 24 hours. The concentrations of CIP and ACNQ (QSI) were 60μg/mL and 10μg/mL respectively.

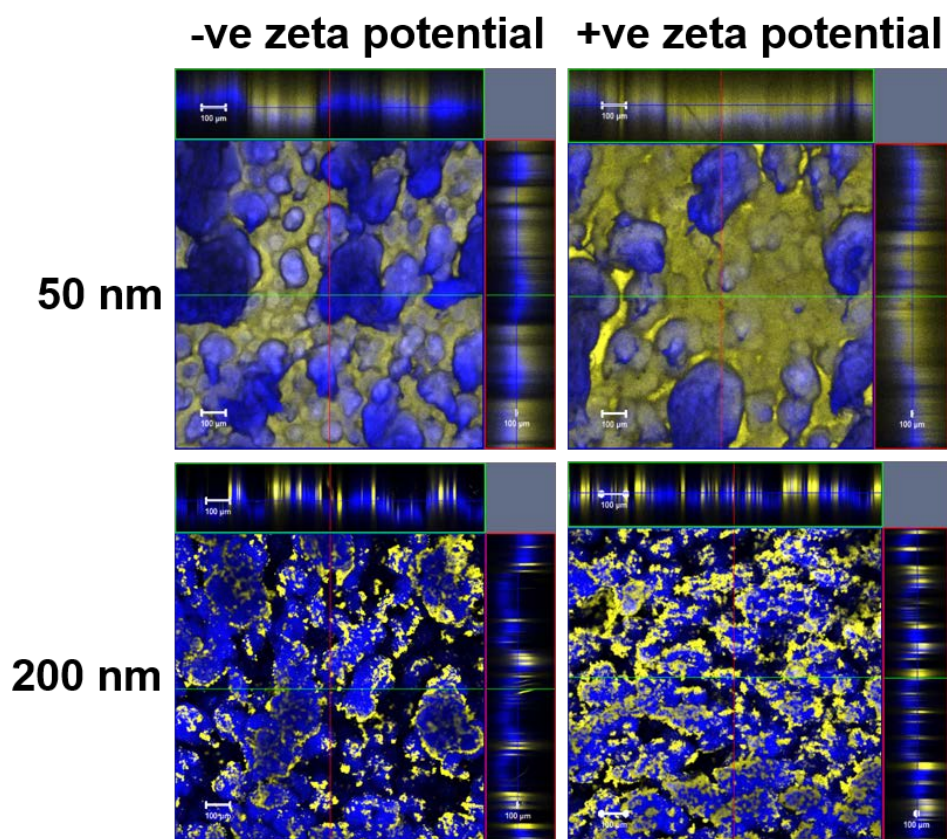


Figure S16- Confocal laser scanning microscopy (CLSM) images showing deficient PAO1-N biofilm penetration of negatively or positively charged polystyrene NPs of 50nm or 200 nm diameter at concentration 1 mg/mL after 24 hours incubation . Top view in the centre and the strips in the top and right side of the figures show the depth profile with extent of NPs (shown in yellow) penetration in the biofilm (shown in blue, SYTO9 stain). Scale bar: 100um..

Dynamic Light Scattering (DLS)-

DLS measurements were performed using a Zetasizer Nano (Malvern Instruments).

	Diameter/nm	Zeta potential/mV
ALG _{ALD}	221	-65.5
ALG _{QSI}	209	-39.7
ALG _{ALD} CIP	242	-42
ALG _{QSI} CIP	179	-44.8
Fluorescent Polystyrene-NH ₂ NPs 50nm (Sigma)	200	48.4
Fluorescent Polystyrene-COOH NPs 50nm' (modified)	200	-32.5
Fluorescent Polystyrene-NH ₂ NPs 200nm (Sigma)	50	53.7
Fluorescent Polystyrene-COOH NPs 200nm (modified)	50	-32.6

Table S1- Diameters and zeta potentials of different NPs used in the study.