

# Polymeric Nanoparticles Active against Dual-Species Bacterial Biofilms

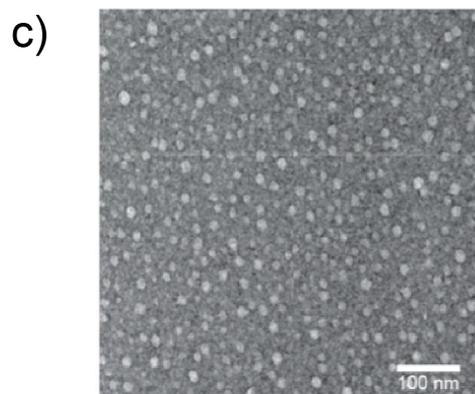
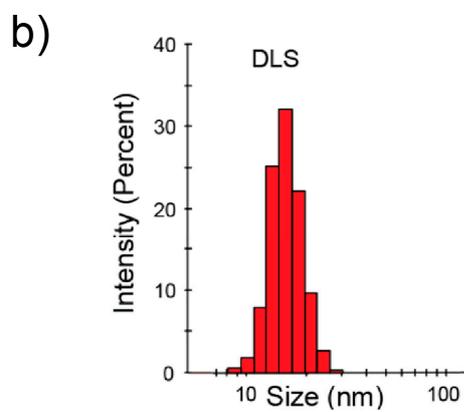
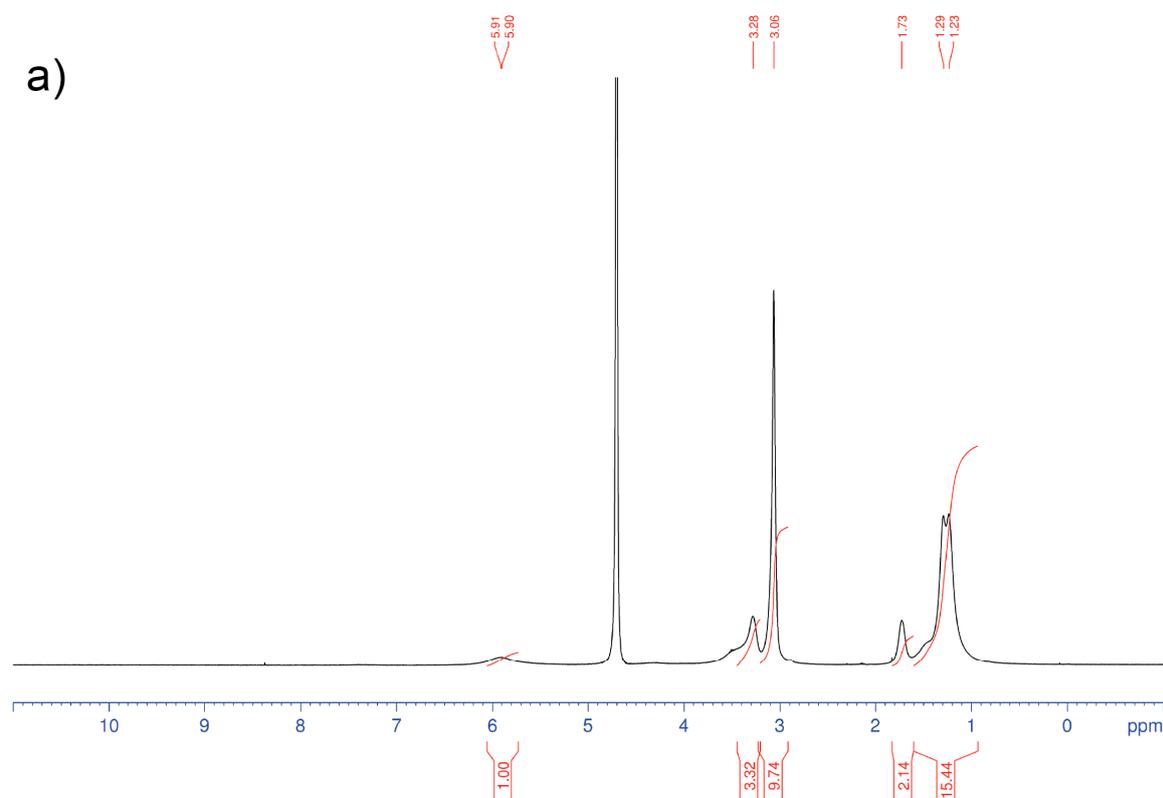
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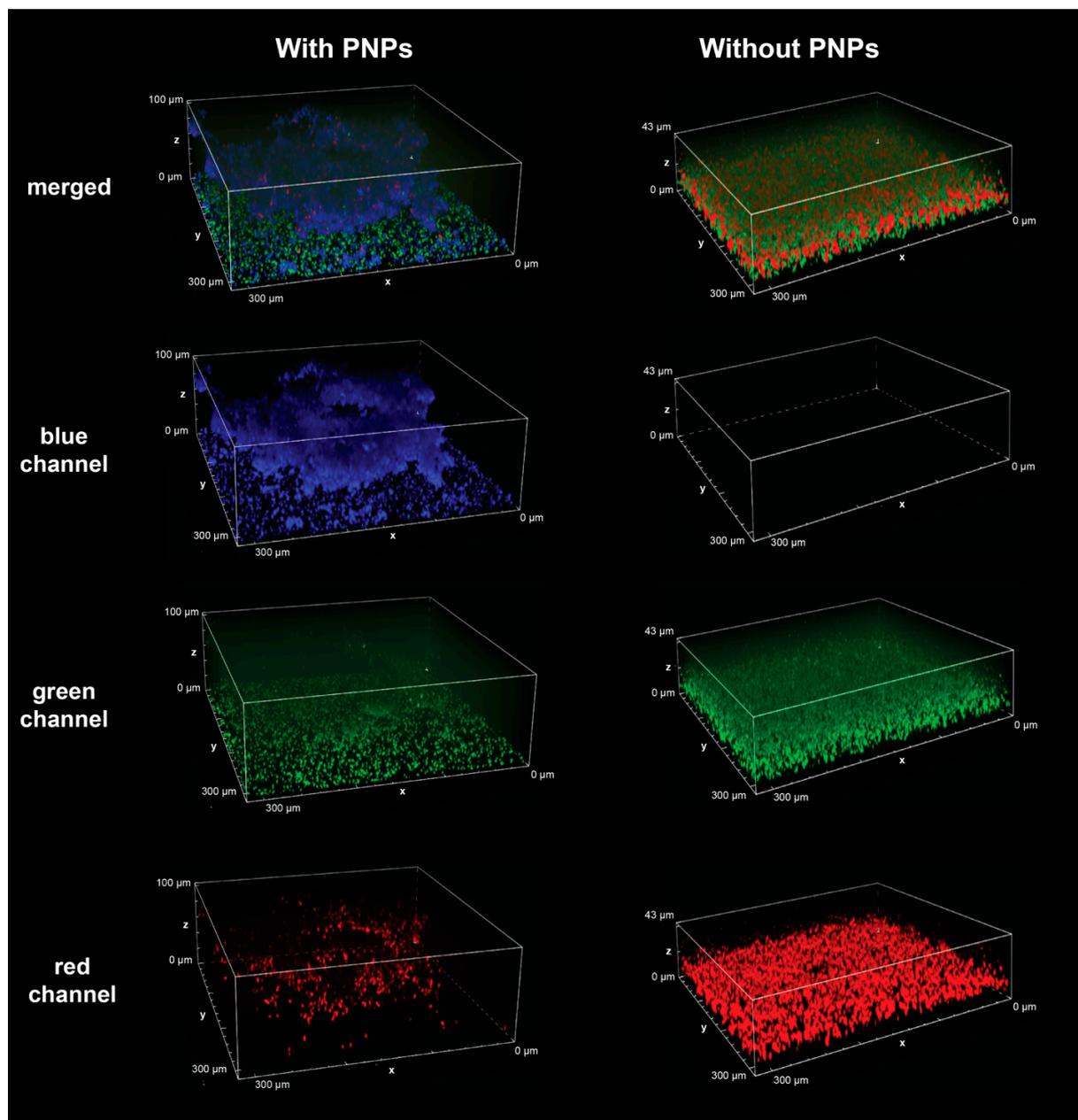
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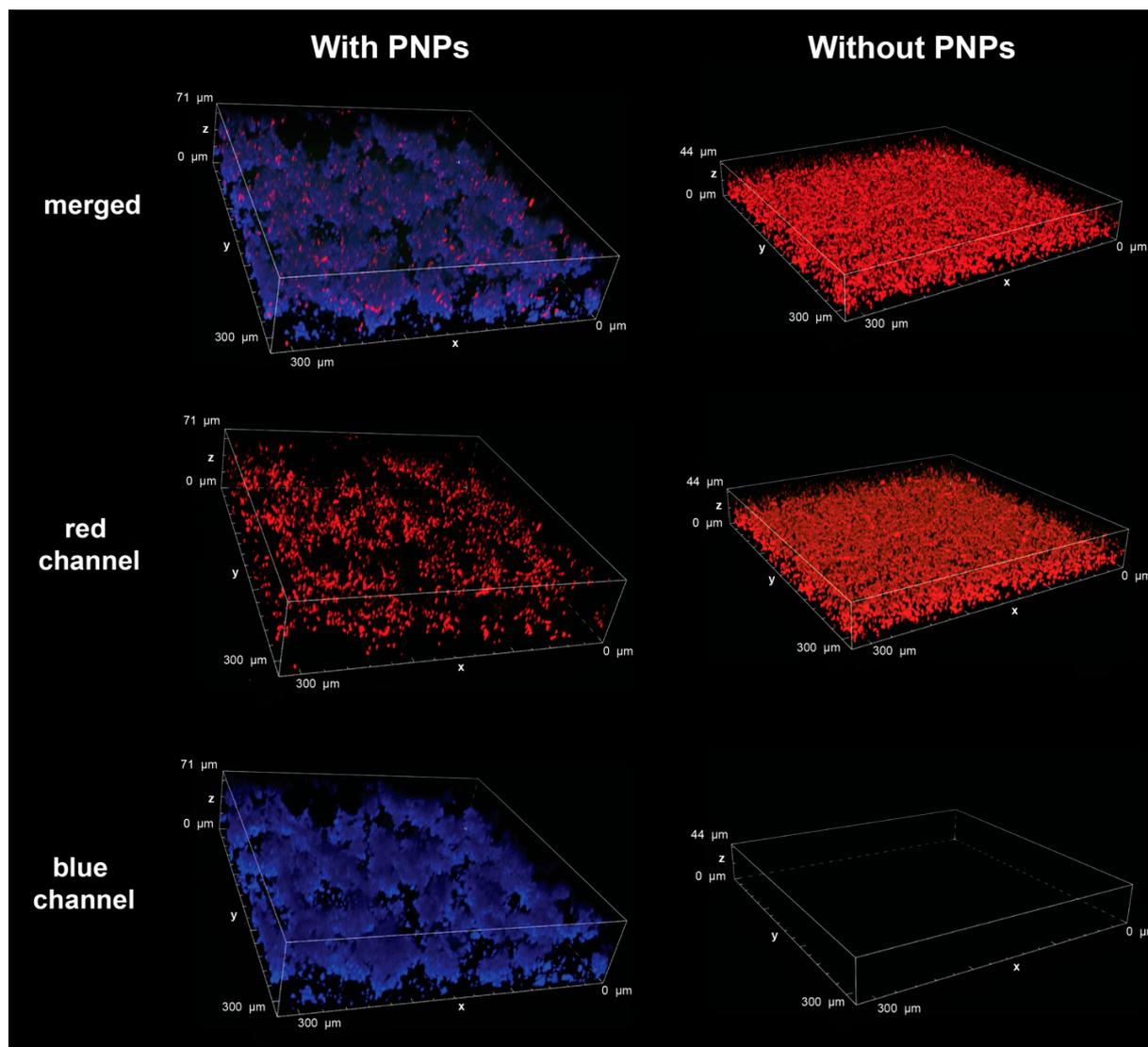
† These authors contributed equally to this work.



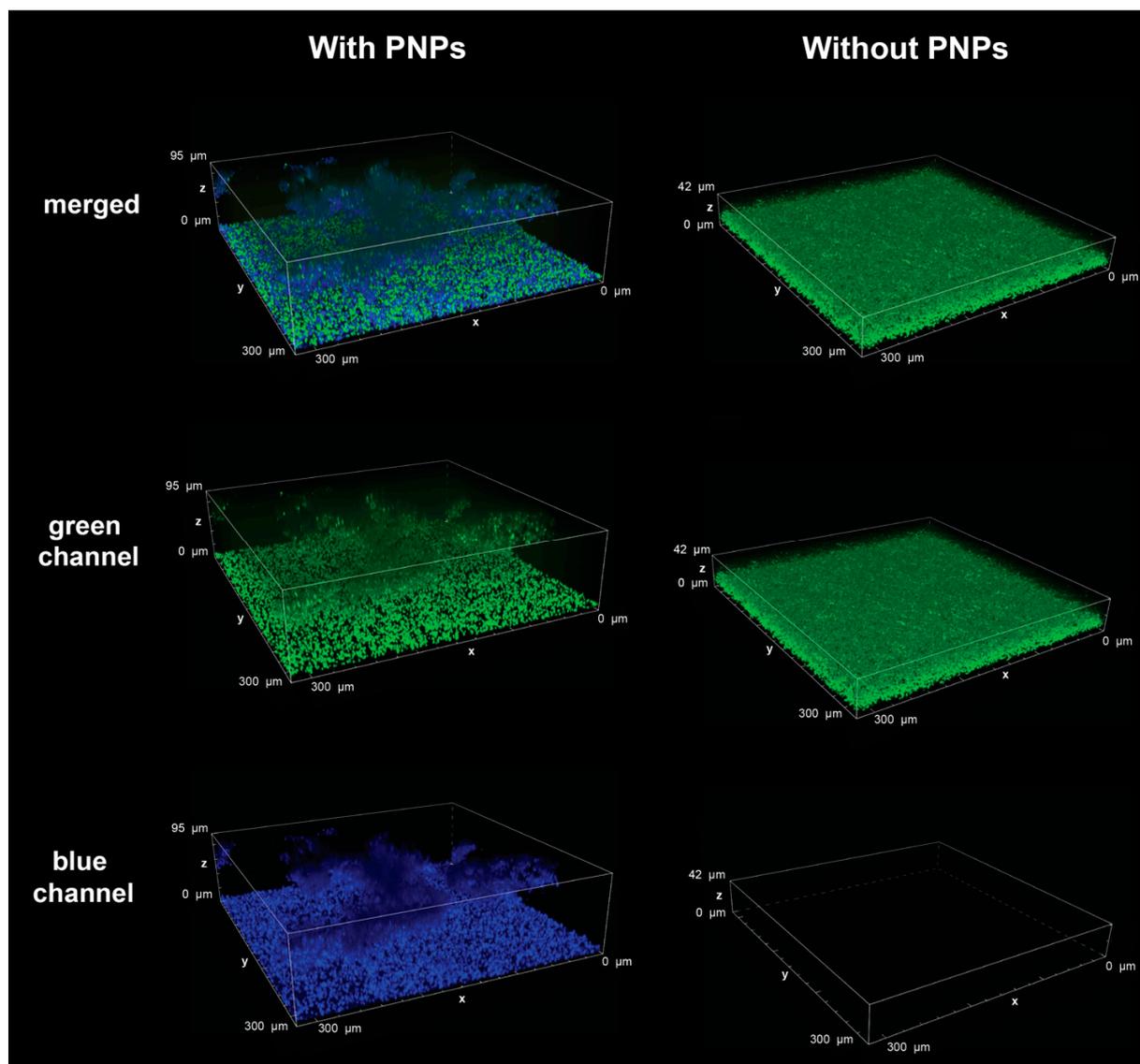
**Figure S1.** a)  $^1\text{H}$  NMR spectrum of PONI-C11-TMA. The polymer self-assembles into cationic polymeric nanoparticles (PNPs) characterized by b) dynamic light scattering (DLS) measurement and c) transmission electron microscopy (TEM) imaging.



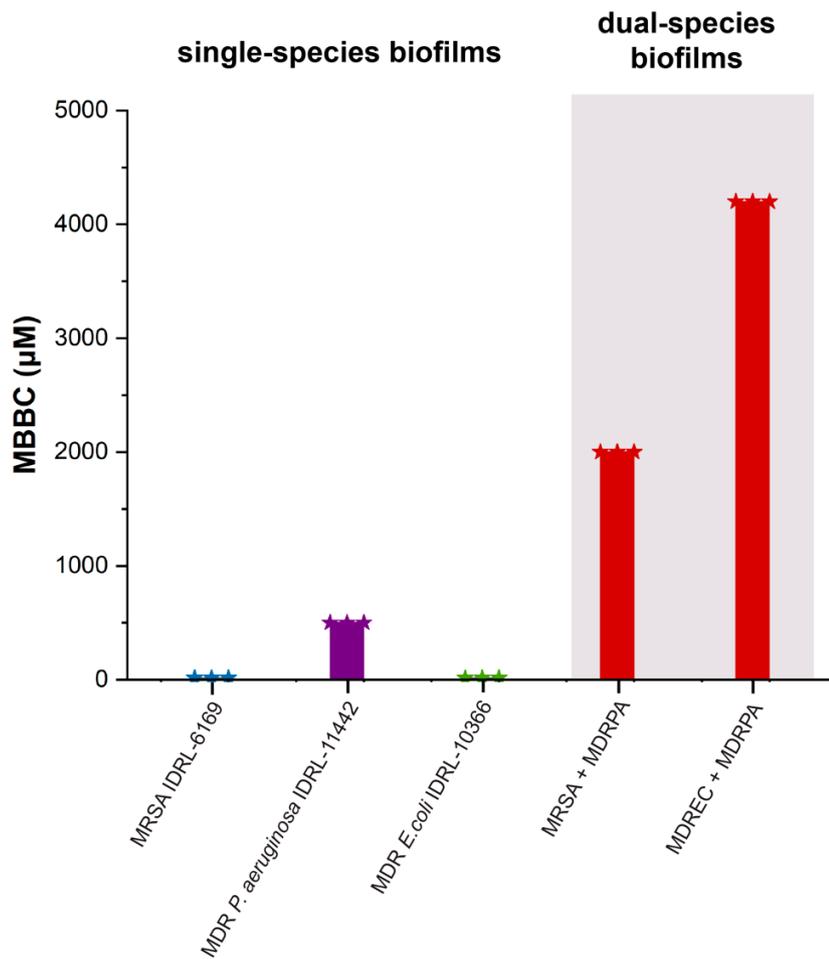
**Figure S2.** Representative 3D views of confocal image stacks of 4-day old dual-species biofilm of DsRed-expressing *Escherichia coli* (red channel) and GFP-expressing methicillin-resistant *Staphylococcus aureus* (green channel), coumarin blue-tagged PNPs (blue channel), and their overlay after treating the biofilms for 1 h with 1  $\mu$ M coumarin blue-tagged PNPs in M9 media. The biofilms were imaged immediately without washing. Untreated biofilm, serving as the negative control, was prepared similarly without treatment with PNPs.



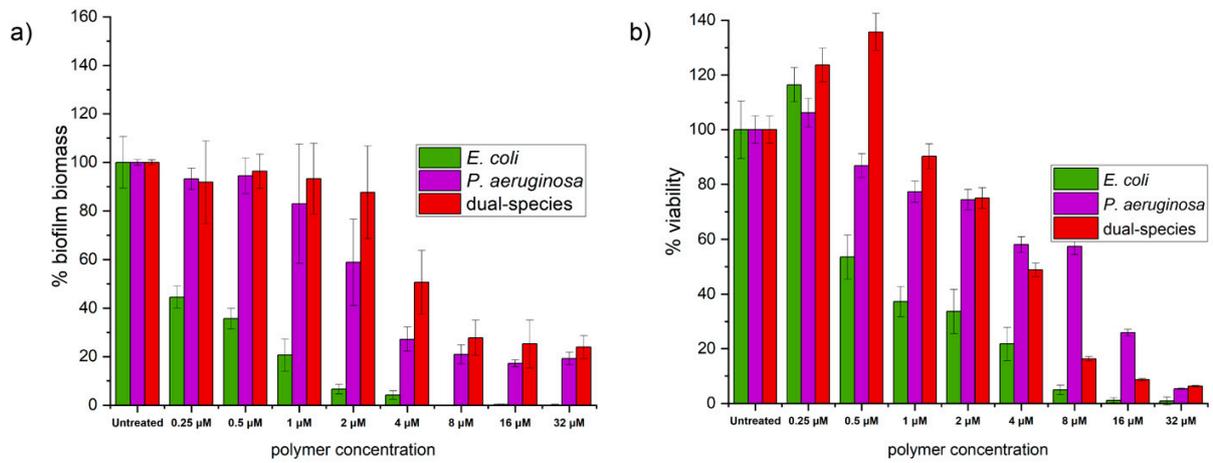
**Figure S3.** Representative 3D views of confocal image stacks of 4-day old biofilm of DsRed-expressing *Escherichia coli* (red channel) and coumarin blue-tagged PNPs (blue channel), and their overlay after treating the biofilms for 1 h with 1  $\mu\text{M}$  coumarin blue-tagged PNPs in M9 media. The biofilms were imaged immediately without washing. Untreated biofilm, serving as the negative control, was prepared similarly without treatment with PNPs.



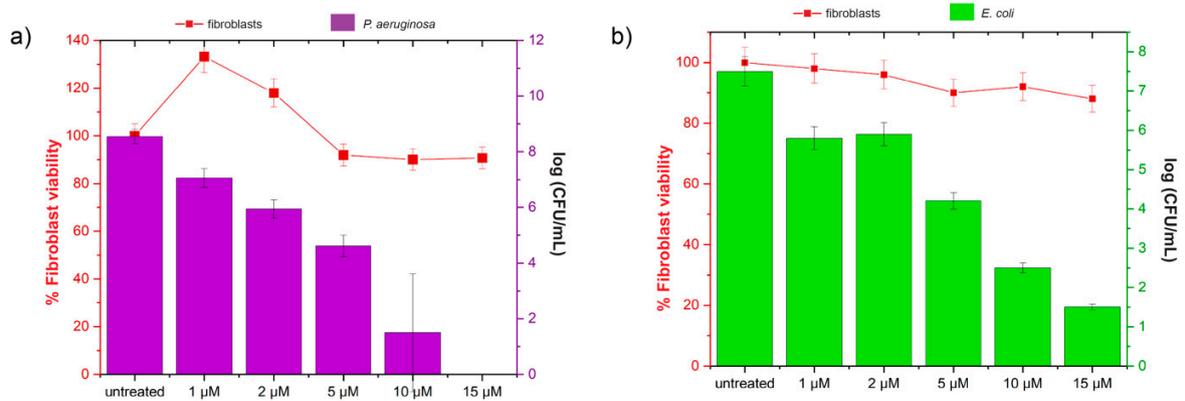
**Figure S4.** Representative 3D views of confocal image stacks of 4-day old biofilm of GFP-expressing methicillin-resistant *Staphylococcus aureus* (green channel) and coumarin blue-tagged PNPs (blue channel), and their overlay after treating the biofilms for 1 h with 1  $\mu\text{M}$  coumarin blue-tagged PNPs in M9 media. The biofilms were imaged immediately without washing. Untreated biofilm, serving as the negative control, was prepared similarly without treatment with PNPs.



**Figure S5.** MBBC values of gentamicin against mono-species [Gram-positive: Methicillin-resistant *Staphylococcus aureus* (MRSA) IDRL-6169; Gram-negative: multi-drug resistant (MDR) *Pseudomonas aeruginosa* IDRL-11442, MDR *Escherichia coli* IDRL-10366] and dual-species biofilms (MRSA + MDR *P. aeruginosa*; MDR *P. aeruginosa* + MDR *E. coli*). Bars represent average value while stars represent individual measurements.



**Figure S6.** a) Biomass and b) bacteria viability of 2-day-old mono- and dual-species biofilms of MDR *Escherichia coli* IDRL-10366 + MDR *Pseudomonas aeruginosa* IDRL-11442 after 3 hours of treatment with PNPs. The data shown are average of triplicates and the error bars indicate the standard deviation.



**Figure S7.** Viability of a) 3T3 fibroblast cells and *Pseudomonas aeruginosa* ATCC-19660 biofilm and b) 3T3 fibroblast cells and *Escherichia coli* DH5 $\alpha$  biofilm dual-species biofilms in the co-culture model after treatment with PNPs. Scatters and lines represent 3T3 fibroblast cell viability. Bars represent log<sub>10</sub> of colony forming units in biofilms. The data are average of triplicates, and the error bars indicate the standard deviations.