# SUPPLEMENTARY MATERIALS:

**Quantifying the effects of antibiotic treatment on the extracellular polymer network of antimicrobial resistant and sensitive biofilms using multiple particle tracking.**

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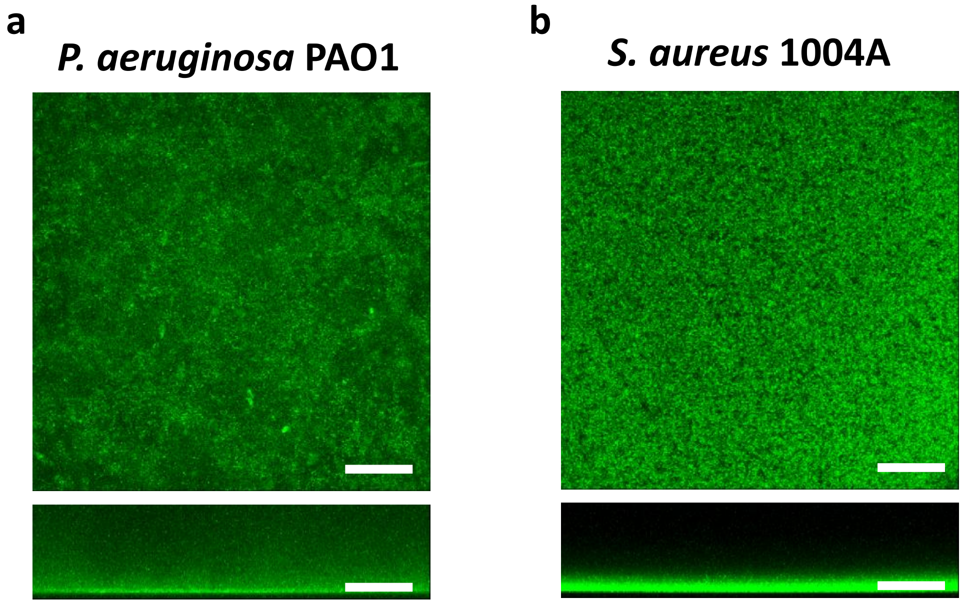
## SUPPLEMENTARY FIGURE




**Supplementary Fig. 1. Comparison *of P. aeruginosa* and *S. aureus* biofilm structures.** CLSM 3D imaging of (a) *P. aeruginosa* PAO1 and (b) *S. aureus* 1004A (MRSA) biofilms grown for 72 h at 37°C in MH broth, using Syto9® staining (Scale bar, 40 µm; n=3).

## SUPPLEMENTARY MATERIALS AND METHODS

### *P. aeruginosa* bacterial strain.

*P. aeruginosa* PAO1 used in this study closely resembles PAO1_Orsay and PAO1_ ATCC 15692 strains, as demonstrated in high-throughput genome resequencing in an unrelated study (European Nucleotide Archive[ENA] project number PRJEB36146 and accession number for the draft genome sequence GCA_902860215).

### Calculation of mean square displacement ‹MSD›, effective diffusion coefficient ‹Deff›, and heterogeneity of particle diffusion

The mean square displacement ‹MSD› was determined as follows:

$$MSD_{(n)} = (X_{\Delta t})^2 + (Y_{\Delta t})^2 \qquad (1)$$

where the distance the nanoparticle (n) moved over a selected time frame (t) in the X-Y trajectory was expressed as a squared displacement (SD).

The effective diffusion coefficient ‹Deff›, of the nanoparticles determined by the following equation:

$$\langle Deff \rangle = \langle MSD \rangle / (4 * \Delta t) \qquad (2)$$

where 4 is a constant relating to the 2-dimensional mode of video capture and Δt is the selected time interval.

Nanoparticle diffusion in water (D°) was calculated by the Stokes–Einstein equation at 37 ºC:

$$D^{\circ} = k_B T / 6\pi\eta r \qquad (3)$$

where $k_B$ is the Boltzmann constant, T is absolute temperature, η is water viscosity, and r is radius of the nanoparticle.

The diffusion of the nanoparticles was also expressed as the parameter, % ratio [Deff]/[D°]. The heterogeneity of particle diffusion was measured by profiling the diffusion coefficients ($\Delta t$= 2 sec) of all individual particles within the entire population (360 particles) from the highest (90th) to the lowest (10th) percentiles in ‹Deff› values.

## Error arising from experimental noise

The error arising from experimental noise (tracking resolution [σ]) was measured for each of the *Fluo*Sphere particles individually, by fixing the particles onto a glass-bottomed imaging dish (MatTek life sciences) with cyanoacrylate-based glue and tracking their movements. Using this set-up, 20 videos were analysed using ImageJ software with Mosaic plugin to independently measure $\sigma^2$ by determining the X- and Y-directional displacement of the particles at the lowest temporal resolution (0.033 frame per second). The value of $\sigma^2$ was subtracted from the MSD measurement at the lowest frame rate to achieve final measurements of MSD and <Deff>. The calculated values of σ ranged between 3.45 to 3.96 nm for each of the *Fluo*spheres (Supplementary Table 1).

**Supplementary Table 1. Tracking resolution (σ) of the *Fluo*Sphere® particles**

| ***Fluo*Spheres®** | **Particle size (nm)** | **σ (nm)** |
|---|---|---|
| -ve carboxylate | 40 | 3.96 |
| -ve carboxylate | 100 | 3.82 |
| -ve carboxylate | 200 | 3.45 |
| -ve carboxylate | 500 | 3.56 |
| +ve amine | 200 | 3.69 |