Supporting information: Fast biofilm penetration and anti-PAO1 activity of nebulized azithromycin in nanoarchaeosomes

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S.1 Small angle x ray scattering (SAXS) measurements

Data analysis was evaluated according to:

$$I(q) \propto k\,(w_1 P_{Uni}(q) + w_2 P_{MLV}(q)) \quad \text{(equation 1)}$$

Where I(q) is (scattered intensity), k is related to the experimental setup and must be the same for a set of scattering curves measured with the same experimental set up. In equation 1, w_1 and w_2 are the weight in the scattering curve of the uni- and multilamellar structures, respectively. Using this approach, it is possible to describe that:

$$P_{Uni}(q) = \frac{2\pi P_t(q)}{q^2} \text{ and } P_{Mult}(q) = \frac{2\pi P_t(q)}{q^2} S_{mult}(q) \quad \text{(equation 2)}$$

where $P_t(q)$ is the infinity bilayer form factor and can be modelled considering that the membranes are composed by three distinct regions: the polar head group, the paraffinic (CH_2) chains and the inner most methyl group (CH_3) region. In this scenario, each region has its own electron density and thickness. Thus, it is possible to write $P_t(q)$ as [1-2]:

$$P_t(q) = \left\{\frac{2}{q}\left\{\Delta\rho_{CH_3} sin(qR_{CH_3}) + \Delta\rho_{par}\left(sin\left(q\left(R_{CH_3} + R_{par}\right)\right) - sin(qR_{CH_3})\right) + \Delta\rho_{pol}\left(sin\left(q\left(R_{CH_3} + R_{par} + R_{pol}\right)\right) - sin\left(q\left(R_{CH_3} + R_{par}\right)\right)\right)\right\}\right\}$$

(equation 3)

where $\Delta\rho_{CH3} = \rho_{CH3} - \rho_{sol}$; $\Delta\rho_{pol} = \rho_{pol} - \rho_{sol}$ and $\Delta\rho_{par} = \rho_{par} - \rho_{sol}$. The lipid bilayer thickness in this model is $2(R_{pol} + R_{par} + R_{CH3})$. During the fitting procedure, some of these parameters were allowed to vary within a narrow range: R_{CH3} (1.5 Å < R_{CH3} < 3.5 Å), ρ_{CH3} (0.15 e/Å^3 < ρ_{CH3} < 0.20 e/Å^3) and ρ_{par} (0.25 e/Å^3 < ρ_{par} < 0.30 e/Å^3), in accordance with data from the literature [1,3,4]

The other Pt(q) parameters could vary in a corresponding broader range. All SAXS curves were analysed with the global fitting procedure using GENFIT software [5].

For the multilamellar vesicles (MLV) the Modified Caillé Theory (MCT) [2,6] was used to calculate S(q) in equation 1. This model considers the bending of the membrane and the fluctuations in the space between bilayers by a statistical approach. For that, a disorder parameter $\eta_{Caillé}$ is added to the equation, that can be written as [6,7]:

$$S(q) = N + 2\left\{\sum_{n=1}^{N-1}\left[(N-n)\cos(nqd)e^{-0.5772\eta_{Caillé}\left(\frac{qd}{2\pi}\right)^2}(\pi n)^{-\eta\left(\frac{qd}{2\pi}\right)^2}\right]\right\} \quad \text{(equation 4)}$$

where N is the number of stacked bilayers, d is the repetitions distance (or the centers of two consecutive bilayers) and $\eta_{Caillé}$ is described as [6]:

$$\eta_{Caillé} = \frac{\pi k_B T}{2d^2\sqrt{KB}} \quad \text{(equation 5)}$$

where K is bending modulus of the bilayers and B is the bulk modulus for compression. During the fitting process, N and η and *d* were allowed to vary as well.

S.2 Activity of AZ-nanovesicles against *S. aureus*

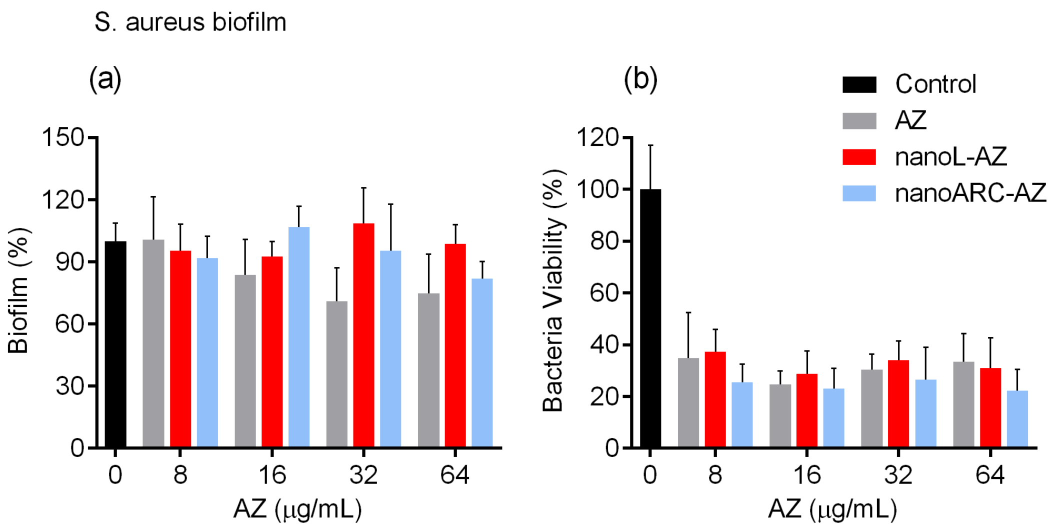

Figure S.2 Disruption of preformed biofilm (a) and antibacterial activity (b) of *S. aureus* upon 24 h incubation with AZ or AZ-nanovesicles.

S.3 Activity of AZ-nanovesicles against PAO1: virulence factors, effect of mucins and biofilm penetration

The number of nanovesicles per milliliter of suspension was calculated with equation 6[8].

$$N_{nv} = \frac{M_{lipid}\, N_A}{1000\, N_{tot}} \quad \text{(equation 6)}$$

N_A is the Avogadro number and it is equal to 6.02E23. M_{lipid} is the molar concentration of lipid. The mean MW used for lipids was 10^3 daltons. N_{tot} is the total number of lipids per nanovesicle and was calculated with equation 7.

$$N_{tot} = 17.69\left[\left(\frac{d}{2}\right)^2 + \left(\frac{d}{2} - 5\right)^2\right] \quad \text{(equation 7)}$$

d is the mean size of nanovesicles. The mean size used for nanovesicles was 150 nm.

S.4 References

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