Supporting Information

Anti-adhesive and biocidal properties of newly synthesized dicephalic amine-containing cationic surfactants

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1. Materials for the surfactant's synthesis and their spectroscopic spectra

1-bromododecane (97 %), 1-bromotetradecane (97 %), 1-bromohexadecane (97 %), diethanolamine (98 %), phosphorus tribromide (97 %), and trimethylamine solution (wt. 31-35 %) in ethanol were purchased from Sigma-Aldrich, while anhydrous potassium carbonate (99 %) was purchased from Chempur. Isopropanol, acetonitrile, diethyl ether, dichloromethane and ethyl acetate were supplied by Avantor Performance Materials. The chemical structures of the studied dicephalic cationic surfactants -2,2'-(alkylmino)bis(*N*,*N*,*N*-trimethyletanammonium) dibromindes (abbreviated as C_n-D_NNMe_3Br; n=12, 14, 16) - were confirmed by ^{1}H NMR and ^{13}C NMR spectra ([1], for which the spectra are shown in Fig. S1).

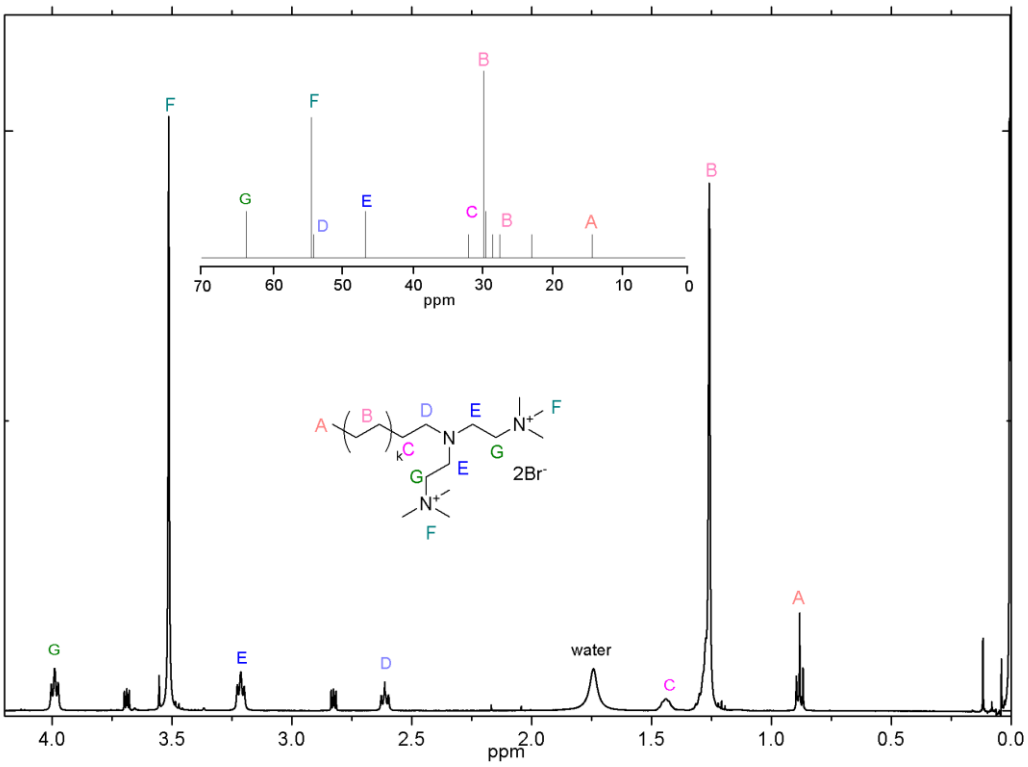


Fig. S1. ^{1}H and ^{13}C NMR spectra for the studied C_n-$D_N$$NMe_3Br$ compounds.

2. **Determination of the CMC (Critical Micelle Concentration) values from surface tension measurements.**

The CMC values are taken as the molar concentrations at the intersection of two linear parts of the relationship γ = f(logc) above and below the discontinuity (Fig. S2). The analyzed surfactant solutions were prepared directly before measurements utilizing dilution method (concentration of stock solution – 0.1 M or 0.01 M). The equilibrium surface tension measurements were performed on DSA25 Expert Goniometer (Krüss, Germany), equipped with Peltier controlled temperature chamber, humidity control as well as ADVANCE Software for data collection and analysis, utilizing the pendant drop shape analysis method. Each solution was measured at least triplicate and an average of results with relative error less than 1 % have been taken for surface tension determination by fitting to Young – Laplace equation. All surface tension measurements were performed at 295 K and relative humidity 75 – 90 %.

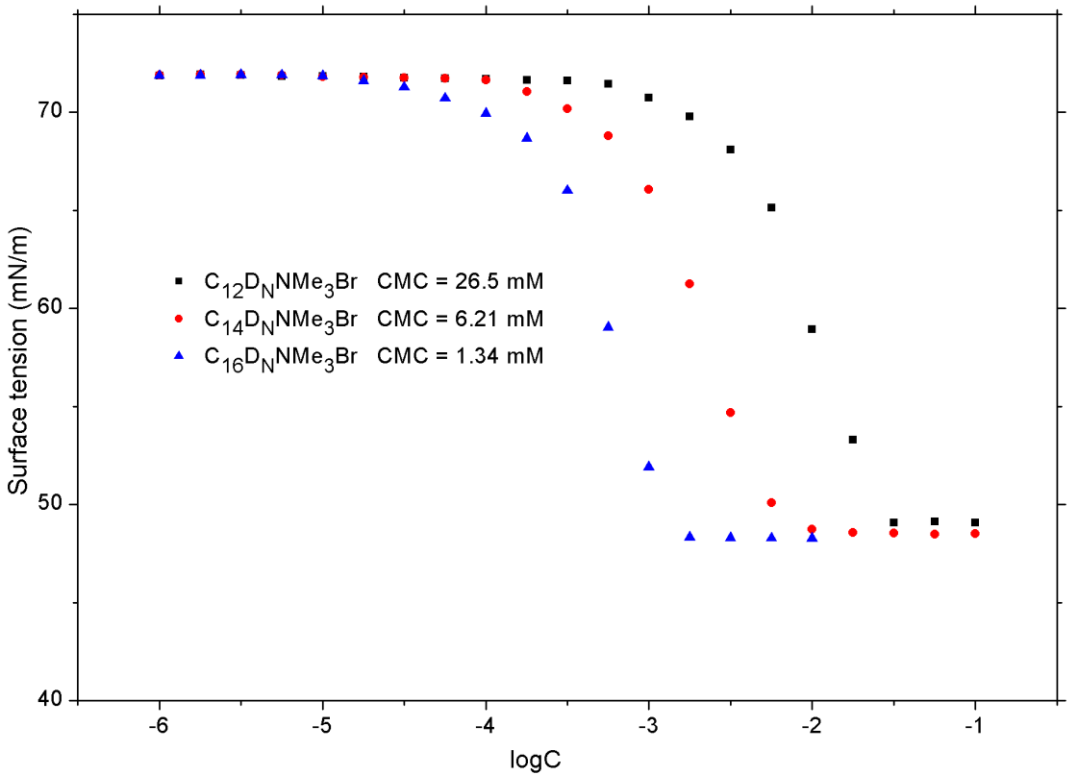


Fig. S2. Surface tension isotherms for the studied C_n-D_NNMe_3Br compounds with the determined CMC values.

3. The theoretical determination of Krafft points

The determination of T_K for novel surfactants comprises an appropriate group increment method, developed and described in [1]. This method is based on a statistical analysis of 227 anionic, 46 cationic and 27 zwitterionic surfactants, followed by distinguishing of appropriate chemical groups and their additive (positive or negative) increments of values into total T_K of the whole surfactant molecule. The theoretical T_K value comprise an algebraical sum of all chemical groups' increments (for repeating fragments their number is multiplied by their single increment). The appropriate data and calculations are given below:

Chemical group	-CH_2-	-CH_3	-N<	-N^+Me_3	T_K
Increment	6.415°C	-15.521°C	7.304°C	-55.768°C	
C_{12}-D_NNMe_3Br	13	1	1	2	-36.358°C
C_{14}-D_NNMe_3Br	15	1	1	2	-23.528°C
C_{16}-D_NNMe_3Br	17	1	1	2	-10.698°C

4. Determination of wetting properties and surface free energy of polystyrene and stainless steel

Knowledge about interfacial free energy interactions is necessary for understanding and modeling many surfaces and interface processes. Surface free energy is an important physicochemical property of a solid surface in biological science for the thermodynamic description of adsorption of surfactants and microorganisms on the surface. Based on this parameter, information about the mechanism of aggregation, biofouling and biofilms can be obtained [3, 4]. The study of the adsorption of dicephalic surfactants (C_n-D_NNMe_3Br; n=12, 14 and 16) on the solid surfaces was done to find out whether it reduces adhesion of *S. epidermidis*, *P. aeruginosa* and *C. albicans* cells. It started by determining the wettability behavior as deduced from the contact angle measurements of probe liquids on polystyrene and stainless steel. To get further insight into the surface properties determination of their surface free energies is needed. The average values of the advancing contact angles of water, formamide, and diiodomethane on the polystyrene and stainless steel are presented in Table 1.

Table S1. Average values of the contact angles of water (W), formamide (F), and diiodomethane (D) on the surfaces of stainless steel and polystyrene.

Solid	θ_W	θ_F	θ_D
	Degree		
Polystyrene	86.6±1.9	64.6±1.4	17.3±3.1
Stainless steel	80.0±1.4	61.2±2.2	50.2±1.4

The contact angles of water and formamide are relatively high, slightly higher on the polystyrene than on the stainless steel. It indicates their rather weak wetting by polar liquids and quite hydrophobic properties of their surfaces. In case of polar water and formamide, the type and magnitude of interactions at the solid-liquid interface differ than those of nonpolar diiodomethane, which interacts mainly through the London dispersion forces. On the stainless steel, the contact angle of diiodomethane is significantly higher than on polystyrene. Similar values of contact angles of water and diiodomethane on polystyrene were obtained by Zhang et al. [5]. However, after measuring the contact angle of diiodomethane, a dulling of the polystyrene surface was observed, which indicates some dissolution of the polymer surface. Shimizu and Demarquette [6] obtained a higher contact angle of diiodomethane on polystyrene,

similarly to the contact angles of water and formamide. It means that the sample origin can be important as for its surface properties.

The methods used most frequently for the surface free energy determination rely on wetting contact angle measurements [7]. The surface free energy and its components of the polystyrene and the stainless-steel was determined applying two different theoretical approaches to the interface tension and the Young's equation [8]. Namely, the Owens and Wendt method (O-W) [8] and the Lifshitz-van der Waals/acid-base (LW-AB) model proposed by van Oss et al. [9, 10]. According to the O-W method [9] it is possible to calculate γ_S^d and γ_S^n components from the contact angle of two liquids of which at least one is polar from the following equations:

$$\gamma_L(cos\theta + 1) = 2\sqrt{\gamma_S^d \gamma_L^d} + 2\sqrt{\gamma_S^n \gamma_L^n} \quad (1)$$

where *L* refers to the liquid and *S* to the solid.

The model LW-AB [10, 11] allows for the determination of two components; the Lifshitz-van der Waals γ_S^{LW} and acid-base γ_S^{AB}, which consists of two parameters: the electron-acceptor γ_S^+ and electron-donor γ_S^-. For this purpose, the following relationship was used:

$$\gamma_L(cos\theta + 1) = 2\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + 2\sqrt{\gamma_S^- \gamma_L^+} + 2\sqrt{\gamma_S^+ \gamma_L^-} \quad (2)$$

Moreover, total surface free energy of a solid can be calculated based on the advancing contact angle by using the Neumann et al. approach [8], which is represented by the following equation:

$$cos\,\theta = 2\sqrt{\frac{\gamma_S}{\gamma_L}}\, exp\left[-\beta\left(\gamma_L - \gamma_S\right)^2\right] - 1 \quad (3)$$

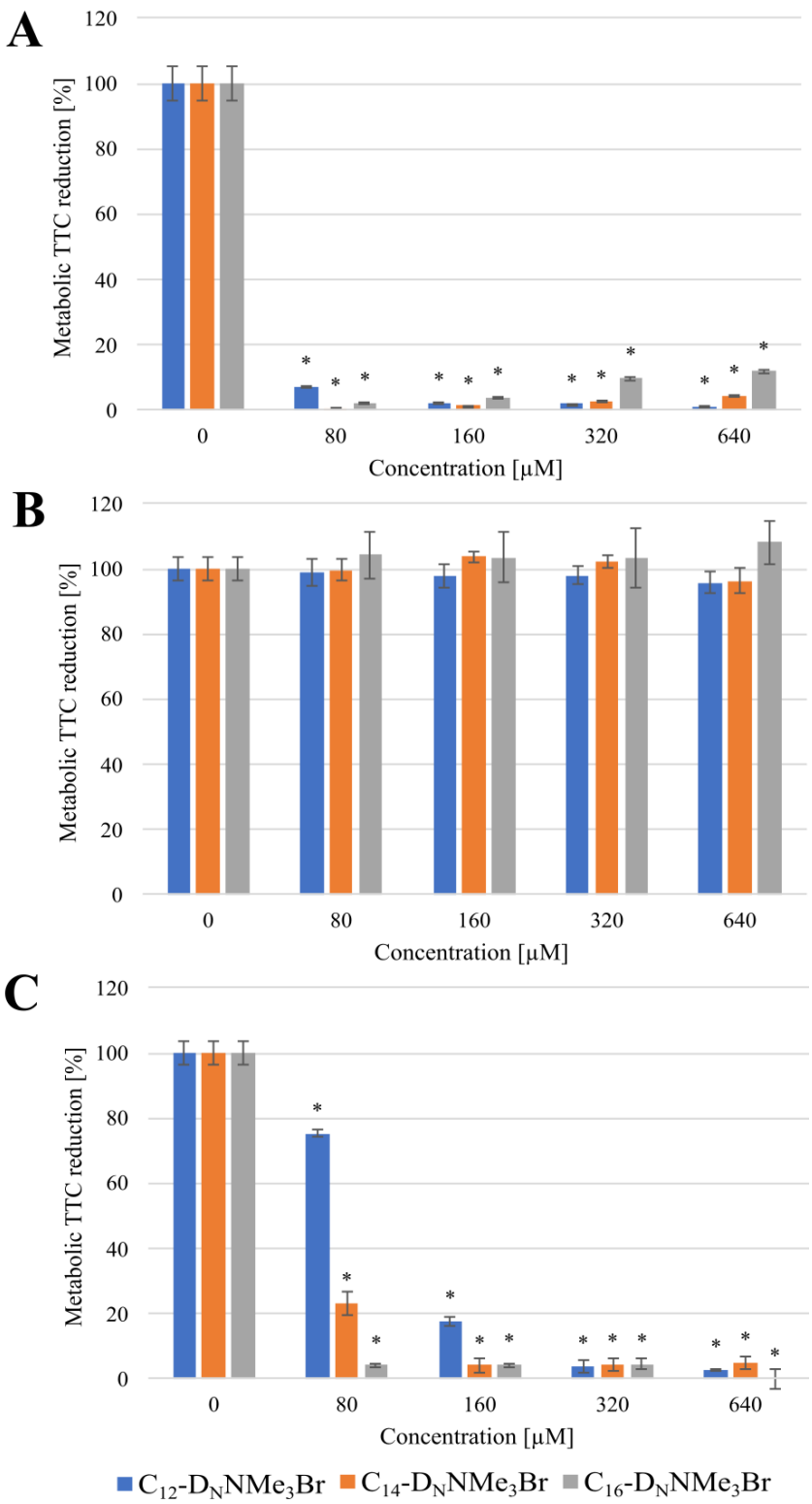
where β is the constant equal to 0.000115 $(m^2/mJ)^2$, which does not depend on the kind of solid and liquid.

Using the LW-AB approach [10, 11] and the components and parameters of the surface tension of water (γ_W^{LW}=26.85; γ_W^+=γ_W^-=22.975 mN/m), formamide (γ_F^{LW}=39; γ_F^+=3.74; γ_F^-=24.1 mN/m) and diiodomethane (γ_D=50.8 mN/m) [12] the components of surface free energy of stainless steel were determined. They amount to: γ_S^{LW}=34.94; γ_S^+=0.16; γ_S^-=4.52; γ_S^{AB}= 3.2 and γ_S=36.62 mJ/m^2. These data indicate that stainless steel is a weak bipolar solid, which is in good agreement with the results obtained by Szymczyk et al. [13]. In the case of polystyrene, which is one of most extensively used polymer material in cell culture applications, it was not possible to determine the surface free energy components using the LW-AB method because of the chemical interaction of diiodomethane with were seen on the surface.

Next, based on the contact angles of two liquids and the surface tension components of water (γ_W=72.8; γ_W^d=26.85; γ_W^n=49.95) and formamide (γ_F=58; γ_F^d=39; γ_F^n=19) [12] and applying the O-W method [9] the following surface free energy component were determined for steel (γ_S=37.37; γ_S^d=34.94; γ_S^n=2.43 mJ/m^2) and for polystyrene (γ_S=31.95; γ_S^d=29.8; γ_S^n=2.15 mJ/m^2).

The calculations conducted by Jańczuk [14] show that for many solids their total surface free energy determined from the contact angle of formamide and using Neumann equation (Eq. 3) [15] are close to those obtained by different approaches. The values of γ_S of stainless steel and polystyrene are 36.7 and 33.2 mJ/m^2, respectively, and are in a good agreement with data obtained from other methods. It was found that polystyrene and stainless differ in their surface properties. As expected, polystyrene is a low-energy hydrophobic solid and a very low polarity [16, 17], while stainless steel is a weakly polar solid [13].

5. Biofilm-oriented antiseptic test (BOAT)



6.

Fig. S3 Metabolic reduction of TTC in biofilms of A – *S. epidermidis* ATCC 35984, B – *P. aerugin osa* PAO1, C – *C. albicans* ATCC 10231, treated with tested surfactants – C_{12}-D_NNMe_3Br, C_{14}-D_NNMe_3Br, C_{16}-D_NNMe_3Br, (BOAT – biofilm-oriented antiseptics test). * – p-value < 0,05.

6. Biofilm visualization

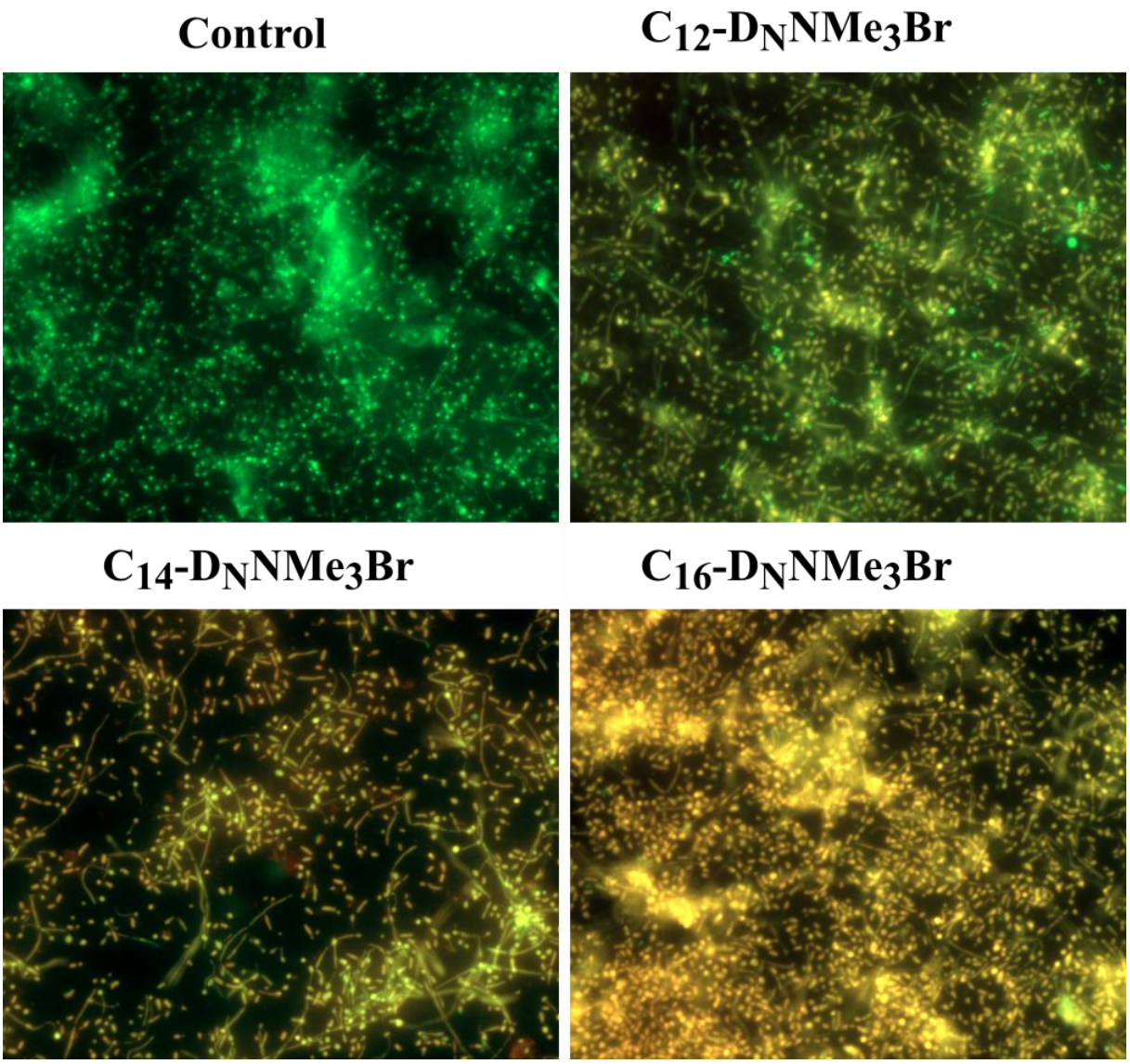


Fig. S4 Visualization of *C. albicans* biofilm formed on a polystyrene surface under control conditions and under the influence of selected surfactants. Syto 9 (green fluorescence of live and dead cells), PI (red fluorescence of cells with damaged cell membranes or dead), combined image (Syto 9 + PI) – yellow/orange color of cells with compromised cell membrane integrity).

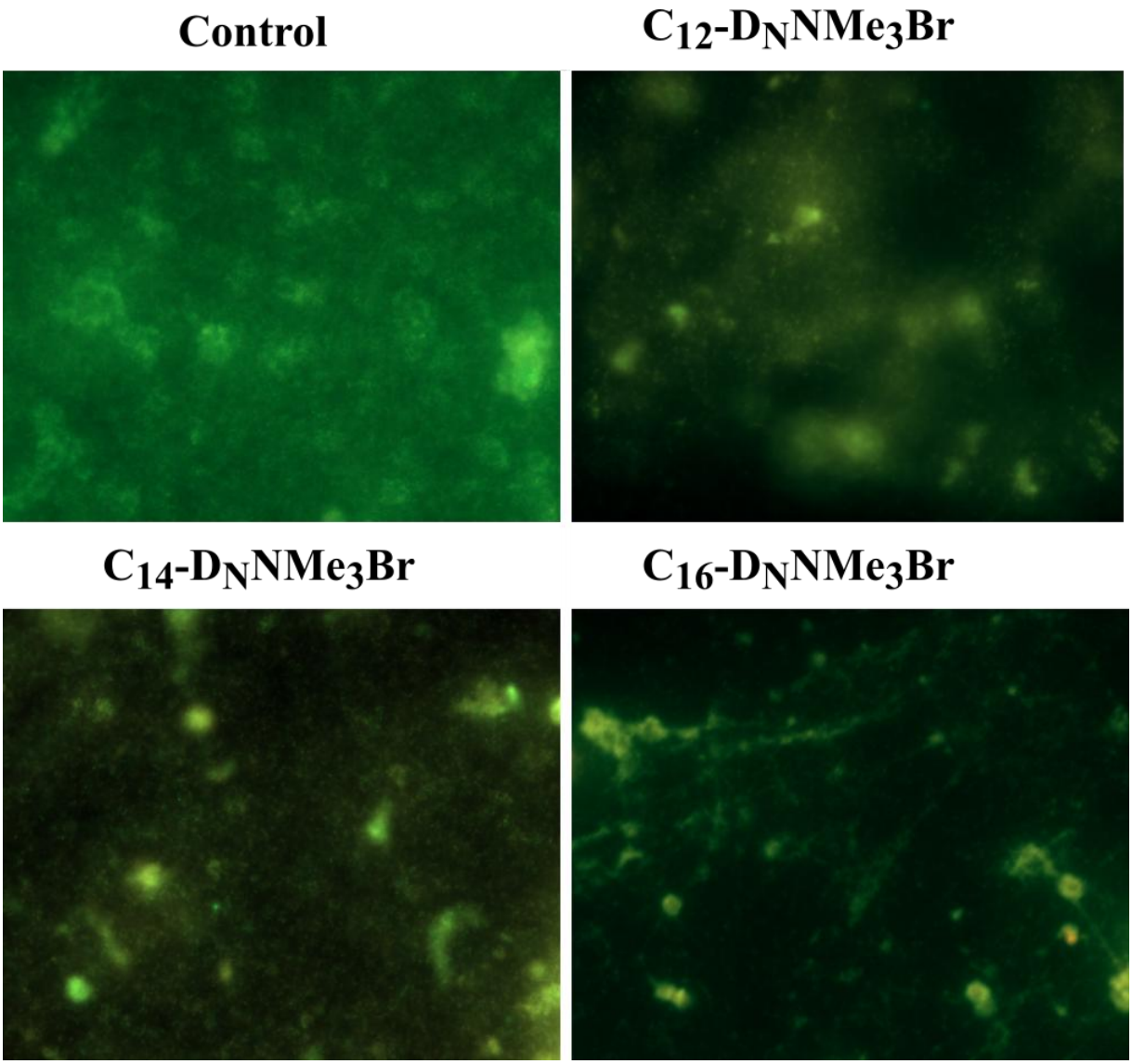


Fig. S5 Visualization of *P. aeruginosa* biofilm formed on a polystyrene surface under control conditions and under the influence of selected surfactants. Syto 9 (green fluorescence of live and dead cells), PI (red fluorescence of cells with damaged cell membranes or dead), combined image (Syto 9 + PI) – yellow/orange color of cells with compromised cell membrane integrity).

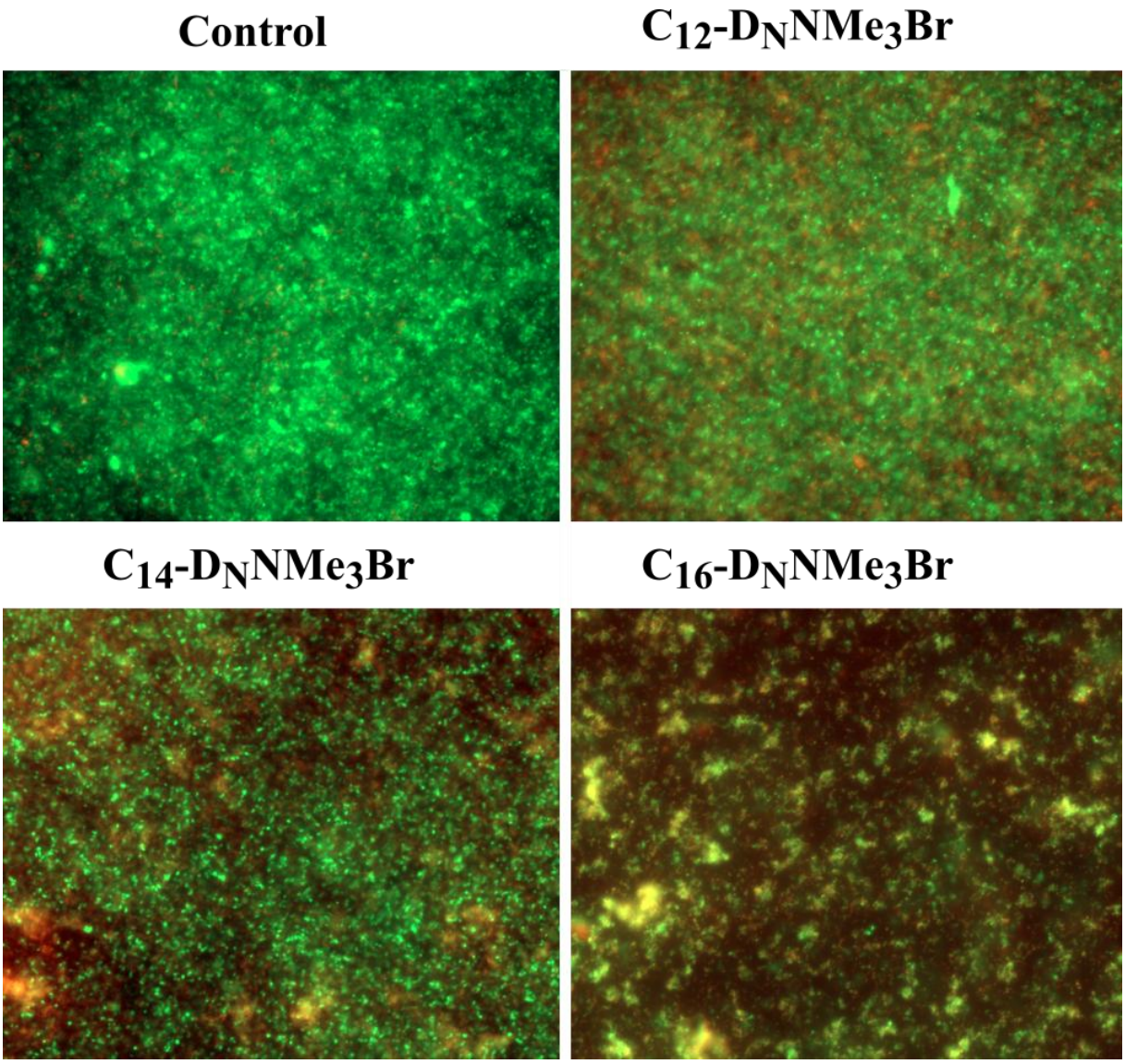


Fig. S6 Visualization of *S. epidermidis* biofilm formed on a polystyrene surface under control conditions and under the influence of selected surfactants. Syto 9 (green fluorescence of live and dead cells), PI (red fluorescence of cells with damaged cell membranes or dead), combined image (Syto 9 + PI) – yellow/orange color of cells with compromised cell membrane integrity).

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