Modulating photochemical reactions in Langmuir monolayers of *Escherichia coli* lipid extract with the binding mechanisms of eosin decyl ester and toluidine blue-O photosensitizers

Lucas G. Moreira[1], Alexandre M. Almeida Jr.[1], Tyler Nield[1,2], Sabrina A. Camacho[1,3] and Pedro H.B. Aoki[1]

[1] School of Sciences, Humanities and Languages, São Paulo State University (UNESP), Assis, SP, 19806-900, Brazil

[2] Faculty of Engineering, University of Victoria, Victoria, BC, V8P 5C2, Canada

[3] IFSC, São Carlos Institute of Physics, University of São Paulo (USP), São Carlos, SP 13566-590, Brazil

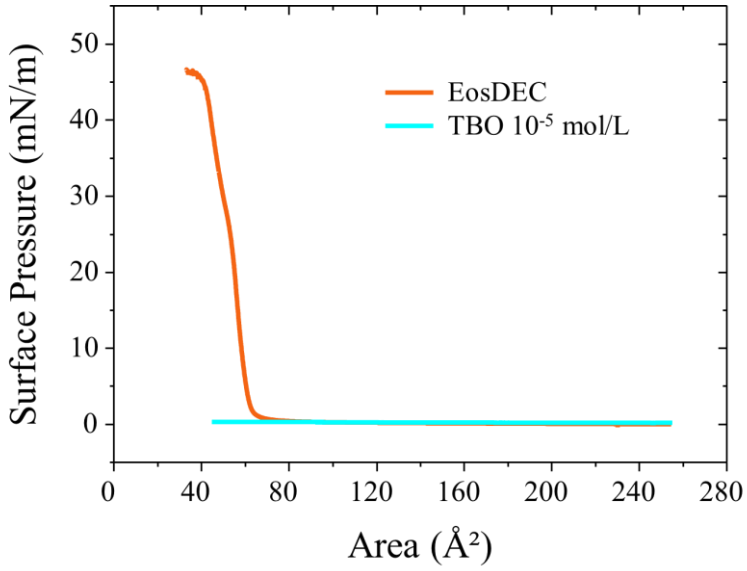

Fig. S1. Surface pressure (mN/m) *versus* mean molecular area (Å²) of neat EosDEC and TBO photosensitizers.

Fig. S2a and b shows how the in-plane elasticity of neat *E. coli* lipid extract monolayer is affected upon EosDEC and TBO incorporation, respectively. The in-plane elasticity, or surface compressional modulus (C_s^{-1}), was calculated from the π-A isotherms of Fig. 2. In the case of EosDEC, the maximum values of elasticity decreased from 113 to 93 mN/m and 87 mN/m considering *E. coli*:EosDEC at 1:10 and 1:5, respectively. The TBO incorporation also decreased the monolayer elasticity from 113 to 84 mN/m. These modifications occur at 30 mN/m (insets), which is relevant from the biological point of view since this surface pressure is believed to correspond to the lateral pressure of cell membranes.

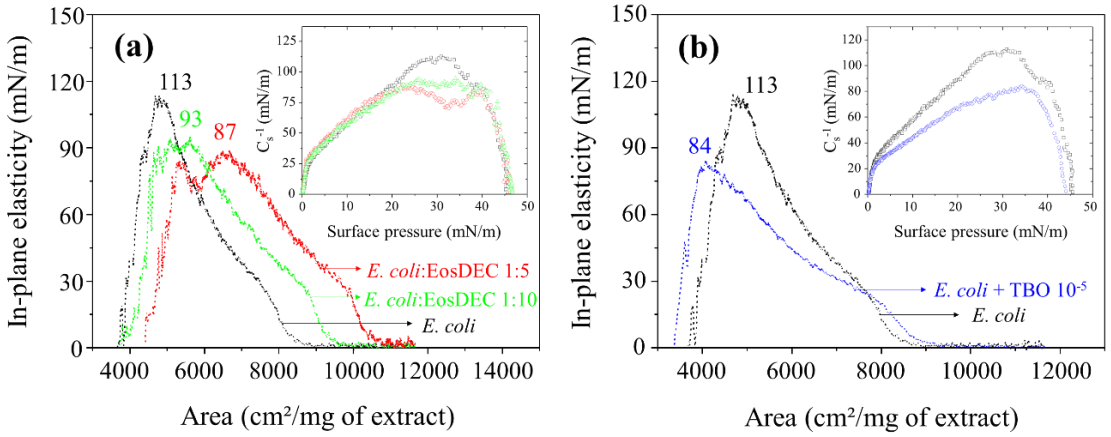

Fig. S2. In-plane elasticity (C_s^{-1}) for neat *E. coli* lipid extract monolayers and for the PSs incorporation with (a) EosDEC (1:5 and 1:10) and (b) TBO (10^{-5} mol/L). The equation $C_s^{-1} = -A\,(d\pi/dA)$ was used as function of area (cm²/mg of extract) and the surface pressure (insets).

Langmuir-Schaefer (LS) films of *E. coli* lipid extract, EosDEC, *E. coli*:EosDEC (10:1 *v*/*v*) and *E. coli* + TBO subphase (10^{-5} mol/L) were grown at a constant surface pressure of 30 mN/m. The deposition of the LS films was monitored every 4 layers up to 60 layers using UV-Vis absorption spectroscopy, the results of which are presented in Fig. S3. The LS film of *E. coli* lipid extract exhibits three bands in the near UV range, at 200 nm, 227 nm and at 364 nm (Figure S3a). Similar absorption bands were identified by Van den Berg et al. [5] characterizing *E. coli* HCP (HCP, named 'prismane protein'), a gene that encodes the hybrid-cluster protein in *E. coli*. A linear growth is observed following the absorbance at 200 nm as the number of deposited layers increases (inset – Fig. S3a; R^2 = 0.9693), suggesting that controlled amounts of material were deposited per layer.

Fig. S3b displays the absorption spectrum for EosDEC LS film with bands located at 201, 230, 252, 298, 402, 507 and 543 nm, attributed to electronic transitions of EosDEC molecule. Particularly, the bands at 507 and 543 nm, are assigned to dimeric and monomeric forms of EosDEC, respectively. A trimeric form appears at 402 nm, due the high concentration used for EosDEC (1 mM) [6,7]. The EosDEC dimeric form in chloroform solution (Fig. S4a) at 455 nm, displaced to 507 nm in the LS film. The monomeric form in chloroform at 479 nm and 514 nm (Fig. S4a) is combined in a single band and displaced to 543 nm in the LS film. Such changes indicate the formation of J-aggregates in the LS film, with the dipoles of molecules in a head-to-tail arrangement [7,8]. Other three well defined bands of the chloroform solution located at 244, 285 and 323 nm are assigned to the π-π^* transitions in the aromatic rings [9], which is blue-shifted at EosDEC LS film to 201 nm, 230-252 nm (splitted) and 298 nm, respectively. The inset exhibits the linear growth of the LS film at 200 nm (R^2 = 0.9986) and at 543 nm (R^2 = 0.9948), indicating similar amounts of material per each deposited layer.

The monomeric (507 nm) and dimeric (543 nm) absorption bands of the EosDEC LS film is also observed in the *E. coli*:EosDEC mixed LS film (Fig. S3c), confirming the deposition of EosDEC. A smaller peak at 396 nm confirms a trimeric form. The π-π* transitions from the aromatic rings are located at 198, 259 and 313 nm. The main modification is related to the dimeric band at 507 nm, which is slightly lower in the absorption spectrum than the EosDEC LS film, suggesting the presence of less aggregates. In fact, EosDEC have demonstrated a tendency in form highly packed monolayers favored by the π-π interactions between the xanthene rings [10]. With respect to the monitored absorbance *versus* the number of layers, a linear growth was also noted at 200 nm (R^2 = 0.9490) and at 543 nm (R^2 = 0.9759). Such behavior suggests that, independently of the monitored absorbance, approximated amounts of material were deposited per layer (inset – Fig. S3c).

The LS film of *E. coli* on TBO subphase (10^{-5} mol/L) displays absorption bands at 243, 276, 518, 579 and 654 nm (Fig. S3d), assigned to the absorption modes of the deposited TBO. The UV-Vis absorption spectrum of TBO water solution (Fig. S4b) presents maximum absorption at 632 nm and a shoulder at 595 nm assigned to the monomeric and dimeric TBO species, respectively [11,12]. The monomer band is red-shifted to 654 nm while the dimer band is splitted and blue-shifted to 518 nm and 579 nm [13]. Such behavior indicates the presence of both J- and H-aggregates in the LS film, with dipoles arranged head-to-tail and parallel to each other [8]. In addition, the monitored absorbance at 230 nm (R^2 = 0.9305) and 579 nm (R^2 = 0.9873) presented a linear growth, suggesting that similar amounts of material were transferred to the substrate per layer (inset – Fig. S3d).

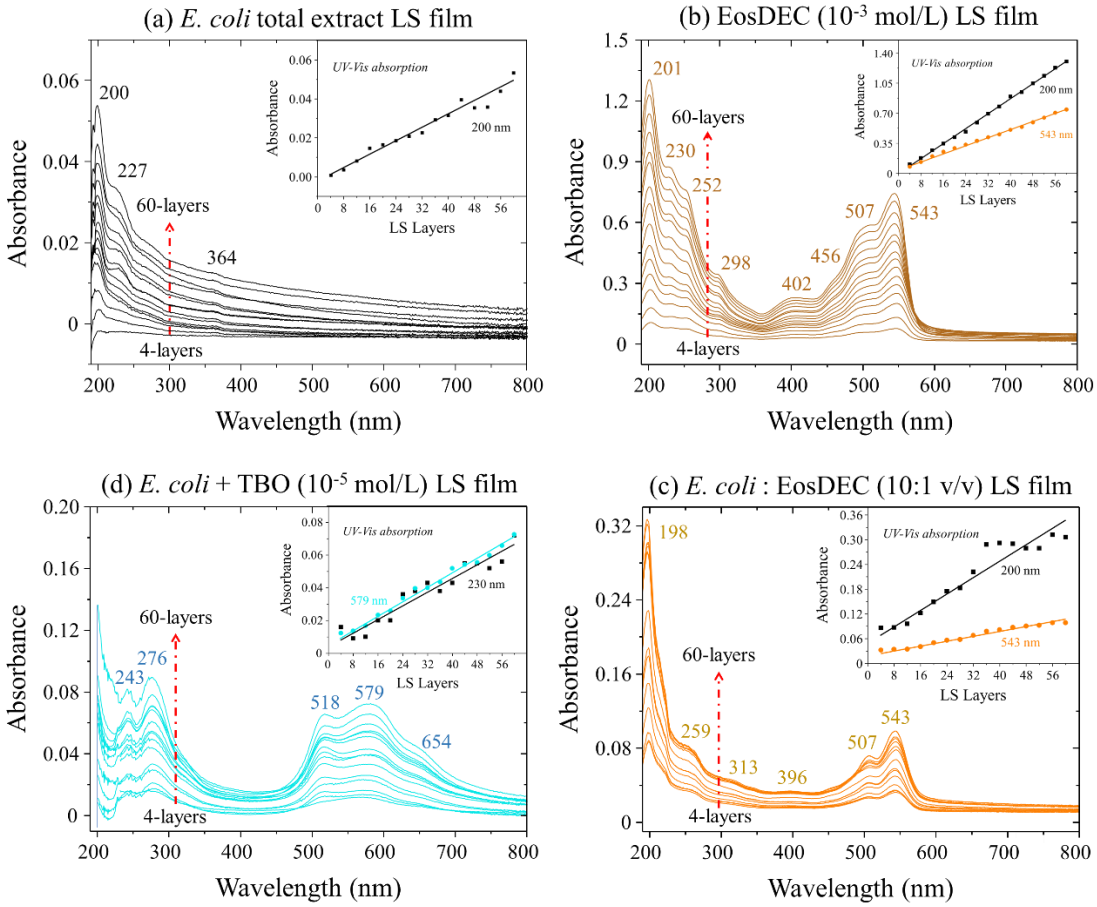

Fig. S3. UV–Vis absorption spectra of LS films deposited on quartz substrates. The film growth was monitored every 4 layers up to 60 layers for (a) *E. coli* lipid extract, (b) neat EosDEC, (c) *E. coli*:EosDEC (10:1 *v*/*v*) and (d) *E. coli* on TBO subphase (10^{-5} mol/L). The insets show the absorbance at a fixed wavelength (±200 nm, 543 nm and 579 nm) for each LS layer deposited from the different films.

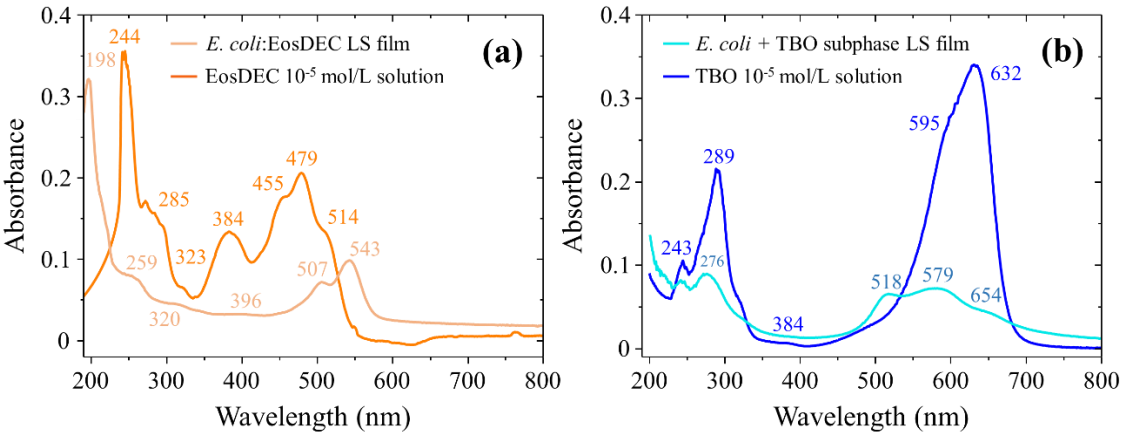

Fig. S4. UV–Vis absorption spectra for the LS film (60 layers) of (a) *E. coli*:EosDEC (10:1 *v*/*v*) and (b) *E. coli* + TBO subphase (10^{-5} mol/L). The spectra of EosDEC chloroform solution and TBO aqueous solution (both at 10^{-5} mol/L) are given as reference.

Upon irradiation, no statistical significance was observed for the *E. coli*:EosDEC (5:1 *v*/*v*) film (Fig. S5). The highly aggregated state of EosDEC molecules at the monolayer interface, which is caused by π-π interactions between the xanthene rings [2], may decrease the $^1O_2$ quantum yield owing to self-quenching and may be a reasonable explanation for the lower photodynamic efficiency.

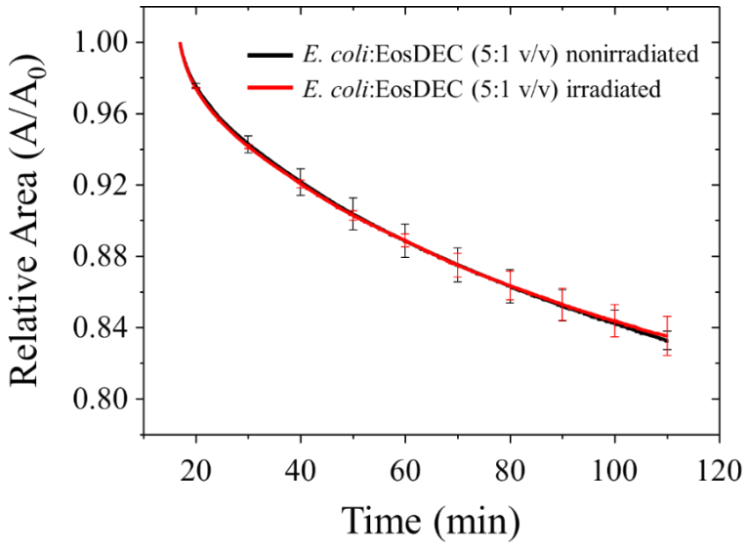

Fig. S5. Changes in relative area (A/A_0) for nonirradiated (black) and irradiated (red) of *E. coli*:EosDEC (5:1 *v*/*v*) mixed monolayer. A_0 is the extrapolated area for the *E. coli:*EosDEC isotherm at 30 mN/m.

References

[1] J. T. Davies, E.K. Rideal, Interfacial Phenomena, 2nd ed., Academic Press, New York, 1963.

[2] D. Marsh, Lateral pressure in membranes, Biochim. Biophys. Acta - Rev. Biomembr. 1286 (1996) 183–223. https://doi.org/10.1016/S0304-4157(96)00009-3.

[3] F. Behroozi, Theory of elasticity in two dimensions and its application to Langmuir−Blodgett films, Langmuir. 12 (1996) 2289–2291. https://doi.org/10.1021/la950636h.

[4] D. Needham, Permeability and stability of lipid bilayers, CRC Press, Boca Raton, FL, 1995.

[5] W. A. M. van den Berg, W.R. Hagen, W.M.A.M. van Dongen, The hybrid-cluster protein ('prismane protein') from Escherichia coli, Eur. J. Biochem. 267 (2000) 666–676. https://doi.org/10.1046/j.1432-1327.2000.01032.x.

[6] Y. R. Suradkar, S.S. Bhagwat, CMC determination of an odd carbon chain surfactant (C13E20) mixed with other surfactants using a spectrophotometric technique, J. Chem. Eng. Data. 51 (2006) 2026–2031. https://doi.org/10.1021/je060064a.

[7] S. De, S. Das, A. Girigoswami, Environmental effects on the aggregation of some xanthene dyes used in lasers, Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 61 (2005) 1821–1833. https://doi.org/10.1016/j.saa.2004.06.054.

[8] M. A. El-Bayoumi, M. Kasha, Energy transfer in hydrogen-bonded N-heterocyclic complexes and their possible role as energy sinks, J. Chem. Phys. 34 (1961) 2181–2182. https://doi.org/10.1063/1.1731841.

[9] A. Alvarez-Martin, S. Trashin, M. Cuykx, A. Covaci, K. De Wael, K. Janssens, Photodegradation mechanisms and kinetics of eosin-Y in oxic and anoxic conditions, Dyes Pigment. 145 (2017) 376–384. https://doi.org/10.1016/j.dyepig.2017.06.031.

[10] L. G. Moreira, A.M. Almeida, S.A. Camacho, B.M. Estevão, O.N. Oliveira, P.H.B. Aoki, Chain cleavage of bioinspired bacterial membranes photoinduced by eosin decyl ester, Langmuir. 36 (2020) 9578–9585. https://doi.org/10.1021/acs.langmuir.0c01600.

[11] M. S. Chan, J.R. Bolton, Structures, reduction potentials and absorption maxima of synthetic dyes of interest in photochemical solar-energy storage studies, Sol. Energy. 24 (1980) 561–574. https://doi.org/10.1016/0038-092X(80)90355-2.

[12] M. Ilanchelian, C. Retna Raj, R. Ramaraj, Spectral studies on the cyclodextrin inclusion complexes of toluidine blue O and meldola's blue in aqueous solution, J. Incl. Phenom. 36 (2000) 9–20. https://doi.org/10.1023/A:1008085829227.

[13] L. D'Ilario, A. Martinelli, Toluidine blue: Aggregation properties and structural aspects, Model. Simul. Mater. Sci. Eng. 14 (2006) 581–595. https://doi.org/10.1088/0965-0393/14/4/003.