Artificial Phospholipid Membrane Formation on Tin Oxide Surfaces

Inga Gabriunaite, Anastasija Aleksandrovič, Aušra Valiūnienė

Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko Str. 24, LT- 03225, Vilnius, Lithuania. E-mail: inga.gabriunaite@chf.vu.lt

INTRODUCTION. Biological lipid membrane mimicking systems are convenient subject for investigating interactions between enzymes, toxins, other proteins and biological membranes. Such systems were designed as membranes on solid supports. They are physically stable enough and they could be investigated using different surface sensitive methods, such as various electrochemical methods, surface plasmon resonance or atomic force microscopy. So far, such system was designed on gold substrate with alkanethiol self-assembled monolayers (SAM) and widely investigated [1]. Even though such system shows great properties, but it has some flaws. Gold is expensive material and to fabricate thin films one needs expensive devices. Additionally, atoms on the Au surface are mobile, so Au–SH bond is mobile across the substrate. After being in a contact with aqueous solution, SAM reassembles into islands and it is no longer uniform. This leads to a system which could be used only one time [2]. In this study, we tested inexpensive and commercially available fluorine doped tin oxide (FTO) for more robust membrane formation (fig. 1). It was functionalized with octadecyltrichlorosilane (OTS) and methyltrichlorosilane (MTS) SAM. OTS-MTS were mixed at different molar ratios to produce anchoring SAM for bilayer lipid membrane (BLM) formation via vesicle fusion method. Vesicle solution contained 60 mol% 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 40 mol% cholesterol (Chol) while total lipid concentration was 1.5 mM. To investigate SAM and membrane, contact angle (CA), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) methods were used. From recorded EI spectra presented in Cole – Cole and Bode plot it is possible to make assumptions about surface coverage with SAM, calculate number of defects [3]. Membrane biocompatibility was tested using three different toxins: melittin (Mel), α-hemolysin (αhl) and phospholipase A2 (PLA2).

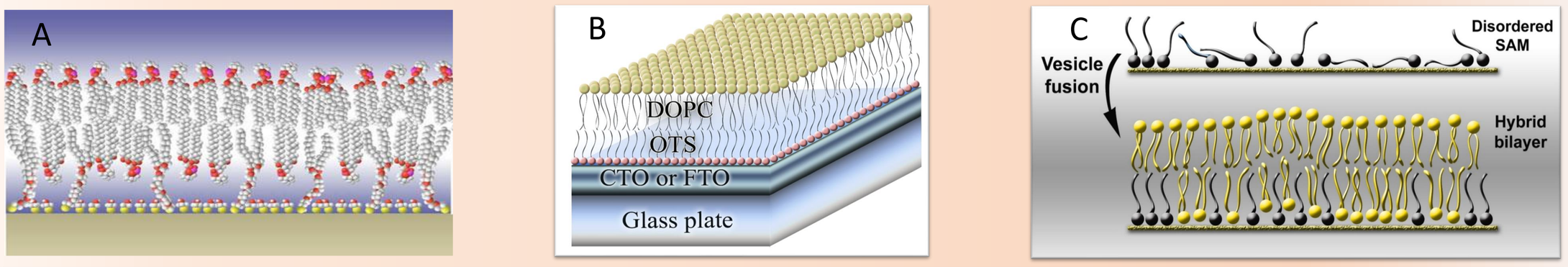

Fig. 1. A – Tethered bilayer lipid membrane model [4], B – hybrid bilayer lipid membrane model, C – hybrid bilayer formation on disordered SAM

DISCUSSION. Contact angle (Fig. 2) and EIS (Fig. 3) data show, that after silanization surface is covered with hydrophobic and dielectric layer. After phospholipid membrane formation, thicker dielectric layer was formed. The importance of tethered membrane is showed in Fig. 5, where the interaction of melittin with phospholipid membrane formed on mixed OTS:MTS 8:2 SAM (Fig. 5 C,D), showed the appearance of defects, while interaction of melittin with BLM formed on 100% OTS SAM showed negligible difference (Fig. 5 A, B). Next, larger toxin α-hemolysin (αhl) was tested (Fig. 6). Yet, it did not penetrate the membrane. Phospholipase A2 disrupted the outer leaflet of the membrane. The level of disruption depended on used PLA2 concentration (Fig. 4).

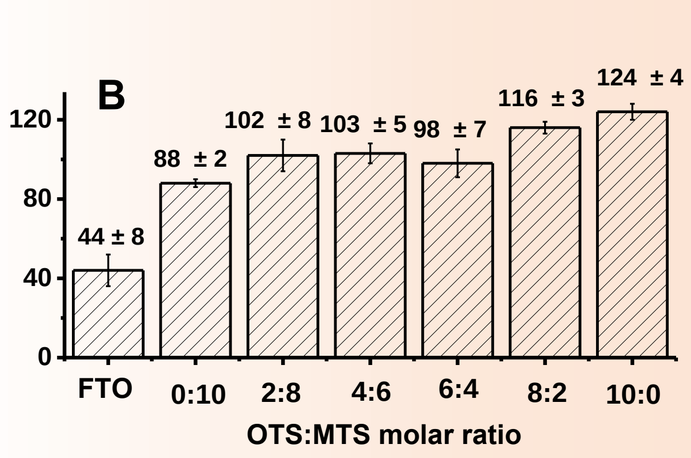

Fig. 2. Contact angles of 5 µl water droplet on pristine and silanized FTO with different OTS:MTS molar ratios.

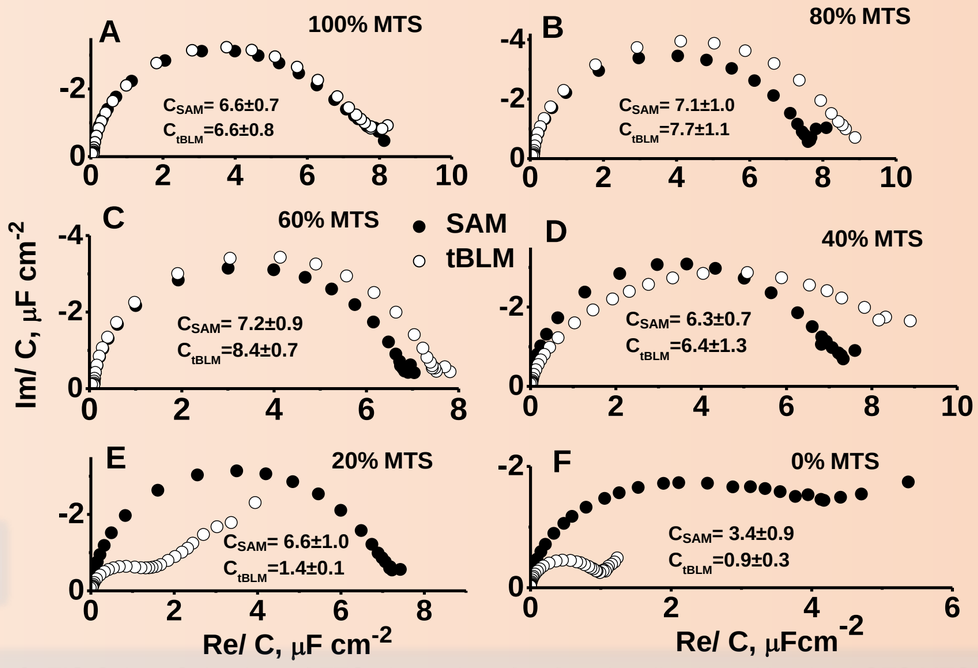

Fig. 3. EIS Cole – Cole plots of FTO electrodes after functionalization with mixed OTS:MTS SAMs (filled circles) and after 1 h incubation of SAMs in DOPC:Chol(40%) vesicle solution (open circles). Molar ratios of OTS:VTS in silanization solutions: A – 0:10, B – 2:8, C – 4:6, D – 6:4, E – 8:2, F – 10:0. Insets – values of complex capacitances after SAM and tBLM formation in µF cm^{-2} units. Bias potential 0 V *vs* Ag/AgCl, NaCl$_{sat}$ electrode.

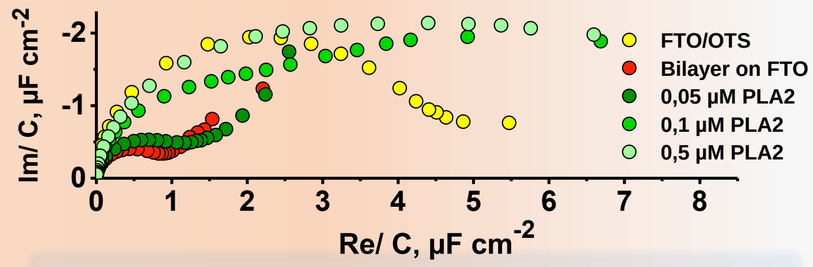

Fig. 4. EI spectra recorded using different concentration of phospholipase A2 (PLA2) to disrupt DOPC:Chol (6:4) bilayer membrane.

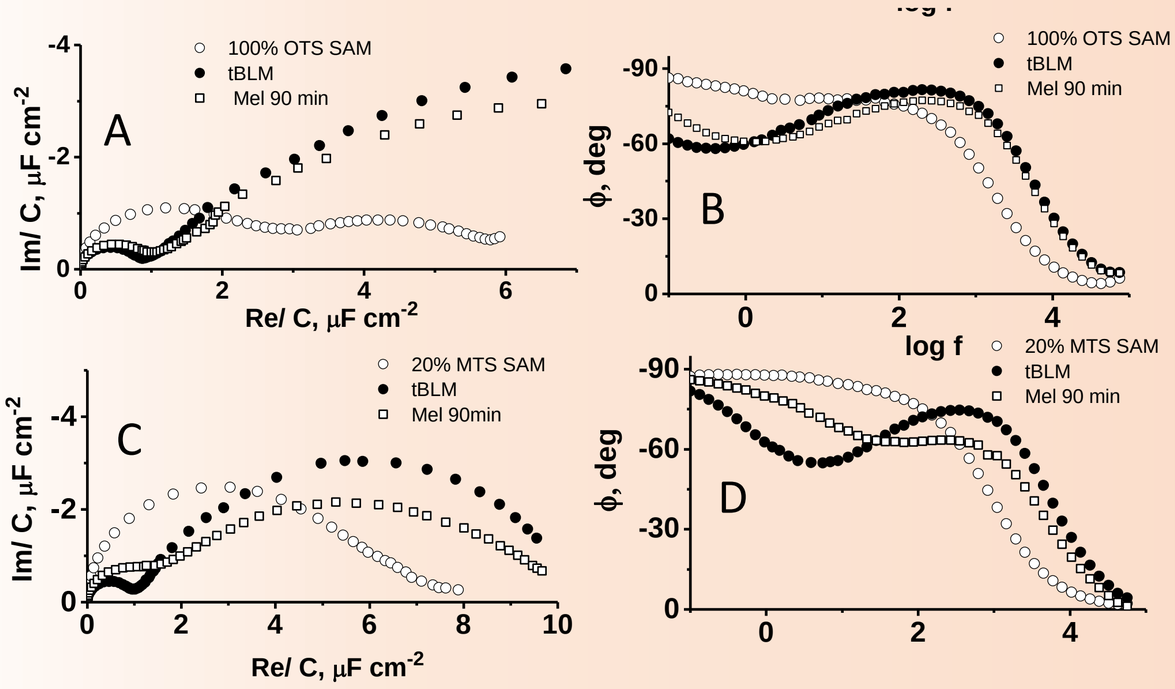

Fig. 5. EI spectra in Cole-Cole (A, C) and Bode (B, D) plots recorded using FTO electrode: A and B - after OTS:MTS SAM formation at 10:0 molar ratio, after DOPC:Chol(40%) bilayer membrane formation and after interaction with 100 mM melittin; C and D - after OTS:MTS SAM formation at 8:2 molar ratio, after DOPC:Chol(40%) bilayer membrane formation and after interaction with 100 mM melittin.

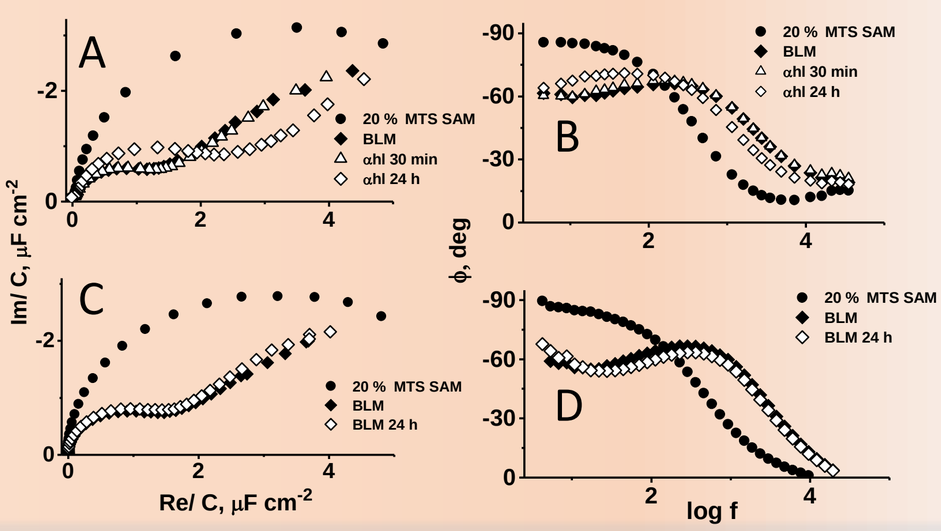

Fig. 6. EI spectra in Cole-Cole (A, C) and Bode (B, D) plots recorded using FTO electrode before (filled circles) and after bilayer membrane formation (filled rhombus) on anchor SAM at 8:2 molar OTS:MTS ratio: A and C - after interaction with 400 mM α-hemolysin; B and D – control experiment after 24 h of vesicle fusion.

CONCLUSIONS. Biologically relevant phospholipid bilayer membrane formation on silane SAM was achieved. Phospholipase A_2, α-hemolysin and melittin disrupted formed membrane. Therefore, such BLM could be applied for investigation of membrane bound proteins, development of biosensors.

References: [1] T. Ragaliauskas, M. Mickevicius, B. Rakovska et al. Fast formation of low-defect-density tethered bilayers by fusion of multilamellar vesicles, Biochimica et Biophysica Acta (BBA) – Biomembranes 1859, 669-679 (2017). **[2]** B. Rakovska, T. Ragaliauskas, M. Mickevicius et al. Structure and Function of the Membrane Anchoring Self-Assembled Monolayers, Langmuir 31, 846-857 (2015). **[3]** G. Valincius, T. Meškauskas, F. Ivanauskas, Electrochemical Impedance Spectroscopy of Tethered Bilayer Membranes, Langmuir 28, 977-990 (2012). **[4]** D. J. McGillivray, G. Valincius, D. J. Vanderah et al. Molecular-scale structural and functional characterization of sparsely tethered bilayer lipid membranes, Biointerphases 2, 21-33 (2007). **[5]** D. J. McGillivray, G. Valincius, F. Heinrich et al. Structure of Functional Staphylococcus aureus a-Hemolysin Channels in Tethered Bilayer Lipid Membranes, Biophysical Journal 96, 1547-1663 (2009). **[6]** I. Gabriunaite , A. Valiuniene, G. Valincius, Formation and properties of phospholipid bilayers on fluorine doped tin oxide electrodes, Electrochimica Acta 283, 1351-1358 (2018). **[7]** A. Valiūnienė, I. Gabriunaite, M. Poderyte et al. Electroporation of a hybrid bilayer membrane by scanning electrochemical microscope, Bioelectrochemistry 136, 107617 (2020).