EFFECTIVE DEFECT DENSITY AS A PREDICTIVE METRIC FORREPRODUCIBLE TOXIN DETECTION IN TETHERED BILAYER MEMBRANES

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Tethered bilayer membranes (tBLMs) serve as mimetic platforms to study membrane-related phenomena, such as ions membrane-protein interactions. They consist of a solid substrate supporting a lipid bilayer, that is attached through molecular anchors. The anchors – such as polymers or self assembled silane or thiol monolayers – create a several nanometer aqueous reservoir that preserves bilayer fluidity and prevents direct contact between the substrate and membrane components, including transmembrane proteins commonly examined with tBLMs. The solid support provides stability and enables characterization by various methods. Conductive substrates, in particular, allow electrochemical analysis, with electrochemical impedance spectroscopy (EIS) offering a rapid, non invasive way to extract structural information. This makes EIS a promising tool for developing biosensors for pore forming toxins, which contribute to numerous diseases. One example is α hemolysin (αHL), a toxin implicated in sepsis, pneumonia, and related conditions. Although widely used, EIS analysis of tBLMs presents major challenges because the system's geometry introduces interpretive ambiguity. Ion movement in the submembrane space is restricted despite immersion in bulk electrolyte, and these conditions are not reliably captured by equivalent circuit models that assign fixed physical meaning to each element. In addition, poor tBLM reproducibility – arising from substrate roughness, stochastic anchor distribution, and variability in bilayer formation – leads to inconsistent responses to membrane active species. This variability, combined with the limitations of equivalent circuit models, complicates efforts to link initial membrane properties to their changes upon exposure to pore forming toxins during biosensor development. In this work we use a tBLM that consists of fluorine doped tin oxide (FTO) with a self-assembled monolayer of silane (the mixture of allyltrichlorosilane (ATS) and trichloro(3-(octadecylthio)propyl)silane (TOPS)) and a phospholipid bilayer made from 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) and cholesterol. We analyze EIS data before and after incubation with αHL by employing a mathematical algorithm that represents the system as a probability distribution of defect density, $P(N_{def})$. Since the algorithm requires setting the resistance of the submembrane reservoir (ρ_{sub}), which cannot be unambiguously determined from EIS data because it scales co-linearly with the defect density, we introduce the concept of effective defect density N_{def}^{eff}, which is as close to the real physical parameter of defect density as the estimate of the value of (ρ_{sub}) is. While not providing the actual defect density values with certainty, N_{def}^{eff} scales linearly with the rate of α-hemolysin pore formation. As a result, the design of αHL biosensor whose response is predictable from the properties of the assembled tBLM is possible.

Keywords: Biosensors, tethered bilayer lipid membranes, pore-forming toxins, electrochemical impedance spectroscopy, alpha-hemolysin