Electronic Supplementary Information

Electrochemical impedance spectroscopy and fluorescence lifetime imaging of lipid mixtures self-assembled on mercury

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To interpret the measured impedance spectra, it is necessary to compare them with the electrical response of an equivalent circuit simulating the system under investigation. As a rule, an equivalent circuit is assembled from resistors and capacitors representing the electrically dominant components of a system. In particular, a metal-supported self-assembled mono- or multilayer can be regarded as consisting of a series of slabs with different dielectric properties. When ions flow across each slab, they give rise to an ionic current $\boldsymbol{J}_{ion}=\sigma\boldsymbol{E}$, where $\boldsymbol{E}$ is the electric field and σ is the conductivity. Ions may also accumulate at the boundary between contiguous dielectric slabs, causing a discontinuity in the electric displacement vector $\boldsymbol{D}=\varepsilon\boldsymbol{E}$, where ε is the dielectric constant. Under a.c. conditions, the accumulation of ions at the boundary of the dielectric slabs varies in time, and so does the electric displacement vector, giving rise to a capacitive current $J_c= \mathrm{d}\boldsymbol{D}/\mathrm{d}t$. The total current is, therefore, given by the sum of the ionic current and of the capacitive current. In this respect, each dielectric slab can be simulated by a resistance, accounting for the ionic current, with in parallel a capacitance, accounting for the capacitive current, namely by an RC mesh. Accordingly, the impedance spectrum of a self-assembled layer can be simulated by a series of RC meshes.

The impedance Z of a single RC mesh is clearly given by:

$$Z^{-1} = R^{-1} + \mathrm{i}\omega C \tag{1}$$

where i is the imaginary unit and ω is the angular frequency. Rearranging terms, the in-phase component, Z', of the impedance and its quadrature component, Z'', can be written:

$$Z' = R\big/\left(1+\omega^2R^2C^2\right) \quad (a); \qquad Z'' = Z'\,\omega RC \quad (b) \tag{2}$$

Eliminating ωRC from Eqs. (2a) and (2b) we get:

$$Z''^2 + Z'^2 - RZ' = 0 \quad \rightarrow \quad \left(Z' - R/2\right)^2 + Z''^2 = (R/2)^2 \tag{3}$$

On a Z'' vs. Z' plot, called Nyquist plot, Eq.(3) yields a semicircle of diameter R and center of coordinates (R/2,0). Noting that the maximum of this semicircle is characterized by the equality of Z' and Z'', from Eq. 2a it follows that the angular frequency ω at this maximum equals the reciprocal of the time constant RC of the mesh. In the presence of a series of RC meshes, their time constants may be close enough to cause the corresponding semicircles to overlap partially. In this case, if the mesh of highest time constant has also the highest resistance, R_4, as is often the case, then the Nyquist plot of the whole impedance spectrum exhibits a single well formed semicircle, R_4 in diameter. The semicircles of the remaining meshes are compressed in a very narrow area close to the origin of the Z'' vs. Z' plot, and can be visualized only by enlarging this area. Therefore, the Nyquist plot of the whole spectrum can be conveniently employed if one is interested in estimating the resistance and capacity of the dielectric slab of highest time constant. Figure 1SI shows the Nyquist plots for three mercury-supported lipid monolayers consisting of (10:90), (50:50) and (90:10) DOPC/PSM mixtures, as well as the fits of a series of four RC meshes to the corresponding impedance spectra. In the present case, displaying the impedance spectra on the Nyquist plot emphasizes the contribution from the R_4C_4 mesh, ascribable to the hydrocarbon tail region.

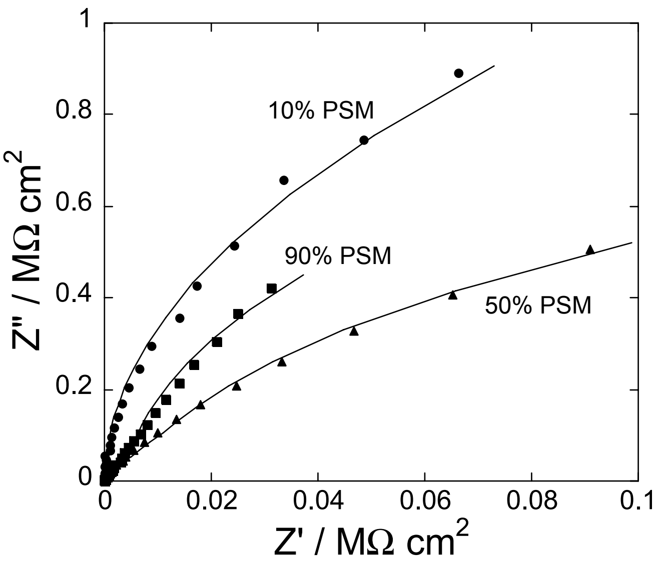

Figure 1SI - Nyquist plots for three mercury-supported DOPC/PSM monolayers containing 10, 50 and 90 mol% PSM. The solid curves are fits of a series of four RC meshes to the impedance spectra. The resistance and capacity values of the RC mesh ascribed to the lipid bilayer moiety are: 7.5 MΩ cm^2, 1.8 μF cm^{-2} for 10 mol% PSM, 3.5 MΩ cm^2, 2.8 μF cm^{-2} for 50 mol% PSM, and 7.0 MΩ cm^2, 3.6 μF cm^{-2} for 90 mol% PSM.

To better visualize all semicircles, we have found it convenient to plot impedance spectra on a ωZ' vs. ωZ'' plot. Henceforth, this plot will be briefly referred to as a "M plot", since ωZ' and ωZ'' are the components of the modulus function M. Even this plot yields a semicircle for a single RC mesh. Thus, if we multiply both members of Eq. 3 by ω^2 and we combine the resulting equation with Eq. 2b, after simple passages we obtain:

$$\omega^2 Z''^2 + \omega^2 Z'^2 - \omega Z''/C = 0 \quad \rightarrow \quad \left(\omega Z'' - \frac{1}{2C}\right)^2 + (\omega Z')^2 = \left(\frac{1}{2C}\right)^2 \qquad (4)$$

This is the equation of a semicircle of diameter C^{-1} and center of coordinates ($2/C$,0) on a ωZ' vs. ωZ" plot. Moreover, ω at the maximum of the semicircle is again equal to the reciprocal of the time constant RC of the mesh. While ω decreases along the positive direction of the abscissas on a Z" vs. Z' plot, it increases on a M plot. Therefore, for a series of RC meshes, the last semicircle on the M plot is characterized by the lowest time constant. This is, unavoidably, the semicircle simulating the solution that baths the self-assembled film. The capacity of the solution interposed between the working and the counter electrode is only of a few nF cm^{-2}, and it is often regarded as negligibly small; in this case, the impedance of the solution is simulated by a pure resistance. As a matter of fact, the radius of the semicircle simulating the solution is not infinitely large, and its curvature is often clearly visible. The solution mesh does not depend on the structure of the self-assembled layer and its semicircle can be excluded, at least partially, from the M plot in order to evidence the contribution from all other meshes. The M plot permits a straightforward comparison between an experimental impedance spectrum and the corresponding fitting to a series of RC meshes. In this respect, it differs from the Bode plot, which is often almost featureless. Figure 2SI shows the M plot for a DPTL monolayer tethered to mercury, with a lipid monolayer of composition DOPC/PSM/Chol (59:15:26) on top.

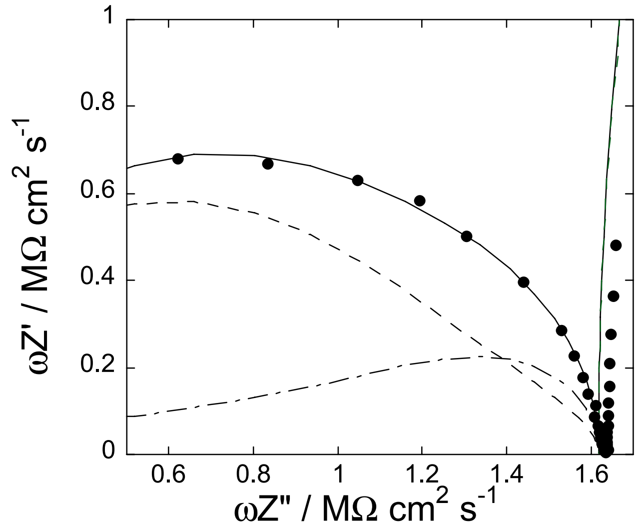

Figure 2SI – ωZ' vs. ωZ" plot for a mercury supported DPTL/(59 mol% DOPC:15 mol% PSM: 26 mol% Chol) bilayer in aqueous 0.1 M KCl. The solid curve is the best fit of a series of three RC meshes to the experimental impedance spectrum. The dashed curve is the ωZ' contribution from the lipid bilayer moiety, the dashed-point curve that from the tetraethyleneoxy moiety.

The solid curve is the best fit of a series of three RC meshes to the experimental impedance spectrum. The calculated contribution to ωZ' from each single RC mesh is plotted against the overall ωZ" quantity, with the exclusion of the contribution from the aqueous solution. The pairs of resistance and capacity values for the three RC meshes, in the order of increasing time constant, are: 3 Ω cm^2, 0.5 μF cm^{-2}; 0.34 MΩ cm^2, 2.2 μF cm^{-2}; 4 MΩ cm^2, 0.86 μF cm^{-2}. The first RC mesh is ascribed to the aqueous solution that baths the tBLM, the second to the tetraethyleneoxy hydrophilic spacer, and the third to the lipid bilayer moiety of the tBLM. In particular, the assignment of the latter RC mesh is dictated by its high resistance and, even more so, by its low capacity, which agrees with that, ~ 0.9 μF cm^{-2}, of a conventional bilayer lipid membrane. This also confirms that the tBLM is characterized by a well-behaved lipid bilayer moiety.

A plot that has been frequently used in the literature to display a spectrum richer in features than the Bode plot is the Y'/ω vs. Y''/ω plot, sometimes called Cole-Cole plot. Here Y' and Y'' are the in-phase and quadrature component of the electrode admittance. However, this plot yields a semicircle for a circuit element consisting of a resistance and a capacity in series, and not for an RC mesh. Thus, the impedance Z of a RC series is given by:

$$Z = R - \mathrm{i}/(\omega C) \tag{5}$$

Noting that Y=1/Z, upon rearranging Eq. 5 we obtain:

$$Y''/\omega = C\Big/\left(\omega^2 R^2 C^2 + 1\right) \quad (a); \quad Y'/\omega = \omega RC\left(Y''/\omega\right) \quad (b) \tag{6}$$

Eliminating ωRC from Eqs. (6a) and (6b) we get:

$$\left(\frac{Y''}{\omega}\right)^2 + \left(\frac{Y'}{\omega}\right)^2 - C\left(\frac{Y''}{\omega}\right) = 0 \quad \rightarrow \quad \left(\frac{Y''}{\omega} - \frac{C}{2}\right)^2 + \left(\frac{Y'}{\omega}\right)^2 = \left(\frac{C}{2}\right)^2 \tag{7}$$

On a Cole-Cole plot, this equation yields a semicircle of diameter C and center of coordinates (C/2,0). Here too, from Eq. 6b it follows that ω at maximum of the semicircle equals $1/RC$. Strictly speaking, a Cole-Cole plot is not suitable to verify the fitting of a series of RC meshes to an experimental impedance spectrum. Thus, the admittance of a single RC mesh is given by:

$$Y = R^{-1} + \mathrm{i}\omega C \quad \rightarrow \quad Y'/\omega = (\omega R)^{-1}; Y''/\omega = C \tag{8}$$

For an RC mesh to yield a semicircle on a Cole-Cole plot, an equation analogous to Eq. 7 should be satisfied:

$$\left(\frac{Y''}{\omega} - x\right)^2 + \left(\frac{Y'}{\omega}\right)^2 = x^2 \quad \rightarrow \quad (C - x)^2 + \left(\frac{1}{\omega R}\right)^2 = x^2 \tag{9}$$

where x is the radius of the semicircle on this plot. Solving this equation yields:

$$x = \frac{C}{2}\left(1 + \frac{1}{\omega^2 R^2 C^2}\right) \tag{10}$$

Consequently, the Cole-Cole plot of a single RC mesh yields a semicircle of diameter C only for ω values high enough to make $\omega^2 R^2 C^2 >> 1$.

An equivalent circuit consisting of a series of RC meshes with relatively close time constants yields calculated semicircles that partially overlap on a M plot. Such an overlapping may resemble a depressed semicircular arc, namely an arc whose center lies below the horizontal axis. Depressed semicircular arcs are also encountered in experimental impedance spectra reported on Cole-Cole or M plots. These arcs are often fitted to an equivalent circuit including a "constant phase element" (CPE), whose empirical impedance function has the form: $Z_{CPE}=A(\mathrm{i}\omega)^{-\alpha}$. This hybrid element reduces to a pure resistance, A, for α=0 and to a pure capacitive impedance, $-\mathrm{i}A/\omega$, for α=1. The CPE finds its justification in a continuous distribution of time constants around a mean.

The relatively well defined structure of a mercury-supported mono- or bilayer does not seem to justify a continuous series of RC meshes for the fitting of its impedance spectra. Therefore, in the present work the following approach was followed. The experimental spectra were fitted to an equivalent circuit consisting of a progressively increasing number of RC meshes in series. The quality of the fitting was monitored on a M plot. Errors less than 10% in the R and C values of the different RC meshes were regarded as acceptable. When the addition of a further RC mesh did not cause a detectable improvement in the agreement between experimental and calculated M plots, the error for the added RC mesh was normally found to be close to 100%. The last added RC mesh was, therefore, discarded from the fitting.