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Phasor Plots and Spectral Phasor Analysis of Laurdan and Prodan for Membrane Heterogeneity Studies: New Frontiers in Membrane Biophysics

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Since its introduction by Weber [1], fluorophores in the PRODAN series have contributed to our understanding of hydration and packing in biological membranes. Here we apply methods based on lifetime determinations and phasor plots as well as steady-state measurements using the spectral phasor approach, for analysis of the behavior of LAURDAN and PRODAN in vesicles. The lifetime Phasor approach (Jameson et al., [2]) uses a plot of $M \cdot \sin(\Phi)$ versus $M \cdot \cos(\Phi)$, where M is the modulation ratio and Φ is the phase angle taken from frequency domain fluorometry. With Spectral phasors, introduced by Fereidouni et al [3], the steady-state fluorescence spectrum is Fourier transformed, resulting in two coordinates in x and y used for a scatter plot (Spectral phasor). The temporal Phasor approach shows significant improvement compared with older methods as regards discrimination of the effects of temperature, cholesterol content and drug addition, in our membrane model systems. This approach is very convenient for characterization of complex systems wherein lifetime heterogeneity and relaxation processes are present.

The Spectral phasor approach is a very useful method for characterization of subtle changes in membrane hydration and packing.

The major advantage of both methods is that they provide a model-less approach, which is relevant to complex studies on native systems, where endogenous fluorescence can introduce undesired mistakes. Examples of the application of both methods to membrane systems will be given.

[1] Weber et al, Biochemistry, 1979. [2] Jameson et al, Appl. Spectrosc. Rev., 1991. [3] Fereidouni et al, Opt. Express, 2012.