Multidimensional Spectral Phasors of LAURDAN's Excitation–Emission Matrices: The Ultimate Sensor for Lipid Phases?

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DOI: 10.1021/jacs.4c03443

Este artículo ha sido embargado por la editorial hasta el 2025-06-15.

Abstract

The impact of lipid diversity on the lateral organization of biological membranes remains a topic of debate. While the existence of domains in lamellar membranes is well-established, the nonlamellar phases occurring in biological systems are less explored due to technical constraints. Here, we present the measurement of the excitation–emission matrices (EEM) of LAURDAN in several lipid structures. LAURDAN is a fluorescence probe widely used for characterizing lipid assemblies. The EEMs were analyzed by multidimensional spectral phasors (MdSP), an approach that seizes information from both the excitation and emission spectra.
We developed a computer algorithm to construct EEM data based on a model for LAURDAN's photophysics. The MdSP calculated from the simulated EEMs reveals that all feasible possibilities lie inside a universal triangle in the phasor's plot. We use this triangle to propose a ternary representation for the phasors, allowing a better assessment of LAURDAN's surroundings in terms of hydration, water mobility, and local electronic environment. Building upon this foundation, we constructed a theoretical "phase map" that can assess both lamellar and nonlamellar membranes. We thoroughly validated this theory using well-known lipid mixtures under different phase-state conditions and enzymatically generated systems. Our results confirm that the use of MdSP is a powerful tool for obtaining quantitative information on both lamellar and nonlamellar structures. This study not only advances our understanding of the impact of lipid diversity on membrane organization but also provides a robust and general framework for the assessment of fluorescence properties that can be further extendedto other probes.