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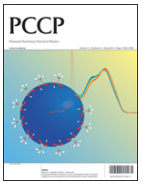

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Optical characterization of Prodan aggregates in water medium †

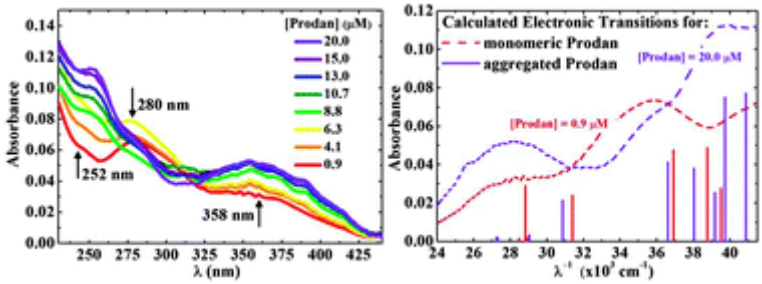
Cíntia C. Vequi-Suplicy,*[a] Kaline Coutinho[a] and M. Teresa Lamy[a]

Author affiliations

Abstract

The fluorescent probe Prodan (2-dimethylamino-6-propionylnaphthalene) has been widely used in biological systems, mainly due to the high sensitivity of its emission spectrum to the medium polarity. Though mostly used as a membrane probe, in lipid dispersions Prodan partitions in water, mainly in the presence of gel-phase bilayers. Here, optical properties of Prodan in aqueous medium are experimentally studied using absorption and emission spectroscopies, and compared with those of the probe in cyclohexane, where it is supposed to be very soluble. In parallel, theoretical calculations of the absorption spectrum of a monomer and aggregated Prodan in water were performed. Moreover, to understand Prodan–water and Prodan–Prodan interactions, solvation free energies of Prodan in water and in liquid Prodan were calculated. A light scattering profile underneath the optical absorption spectrum of Prodan in water clearly indicates the presence of aggregates at very low Prodan concentrations (0.9 μM). Experimental evidence of Prodan aggregation is theoretically supported by solvation free energy calculations, which demonstrate that Prodan molecules interact preferentially with other Prodan molecules than with water molecules. Theoretical calculations for electronic transition energies of monomers and aggregated Prodan in water show that a Prodan optical absorption band at 358 nm is related to the monomeric form of Prodan. This band saturates as Prodan concentration increases, indicating that aggregated Prodan prevails at higher concentrations. The relative increase in Prodan aggregated population is monitored by the increase in an absorption band at higher energies, at around 250 nm, and by the disappearance of a band at around 280 nm.

Surprisingly, it was observed that the fluorescent emission spectrum of Prodan is not sensitive to probe aggregation up to around 15 μM. Hence, Prodan aggregation in water medium, even at very low concentrations, must be considered when using this fluorescent probe in biological systems, having in mind that its fluorescence spectrum is rather insensitive to aggregation.



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