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# Laurdan and Prodan as Polarity-Sensitive Fluorescent Membrane Probes

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The steady-state and dynamic fluorescence spectral properties of 2-dimethylamino-6-lauroyl-naphthalene (LAURDAN) and several other naphthalene derivatives are summarized to illustrate their sensitivity to the polarity of the environment. Results obtained both in solvents of different polarity and in phospholipid vesicles in two phase states are presented. The emission red shift observed in polar solvents and in the phospholipid liquid-crystalline phase is explained on the basis of dipolar relaxation of solvent molecules surrounding the fluorescent naphthalene moiety of these probes. In phospholipid environments, experimental evidence is shown that excludes the intramolecular relative reorientation of the dimethylamino and carbonyl groups in the naphthalene and the reorientation of the entire fluorescent moiety. The solvent dipolar relaxation observed for LAURDAN and PRODAN in phospholipid bilayers has been attributed to a small number of water molecules present at the membrane interface. A comparison between LAURDAN emission in phospholipid vesicles prepared in D<sub>2</sub>O and in H<sub>2</sub>O is also presented. The definition and the derivation of the generalized polarization function are also discussed.

**KEY WORDS:** Dipolar relaxation; generalized polarization; oxygen-quenching; time-resolved spectra; water.

## NAPHTHALENE DERIVATIVES FOR THE STUDY OF SOLVENT DIPOLAR RELAXATION

The two naphthalene derivatives, 6-lauroyl and 6-propionyl-2-dimethylamino naphthalene, LAURDAN and PRODAN, respectively (Fig. 1), were first designed and synthesized by Gregorio Weber [1,2] to study the phenomenon of dipolar relaxation. The fluorescent naphthalene moiety of these probes possesses a dipole moment due to a partial charge separation between the 2-dimethylamino and the 6-carbonyl residues. This dipole moment increases upon excitation and may cause reorientation of the solvent dipoles. The energy required for solvent reorientation decreases the probe's excited

state energy, which is reflected in a continuous red shift of the probe's steady-state emission spectrum. A schematic diagram of this relaxation process is given in Fig. 2. Of course the solvent dipolar relaxation phenomenon can be observed only in polar solvents. A bluer emission is observed in apolar solvents, while a redder emission is observed in polar solvents. LAURDAN sensitivity to solvent polarity can be illustrated by the linear Lippert plot [3] and by the increasing red shift observed in LAURDAN's emission spectrum with increasing solvent polarity. For instance, LAURDAN's emission maximum in dodecane is  $\approx 380$  nm, in dimethylsulfoxide  $\approx 460$  nm, and in methanol  $\approx 490$  nm [4].

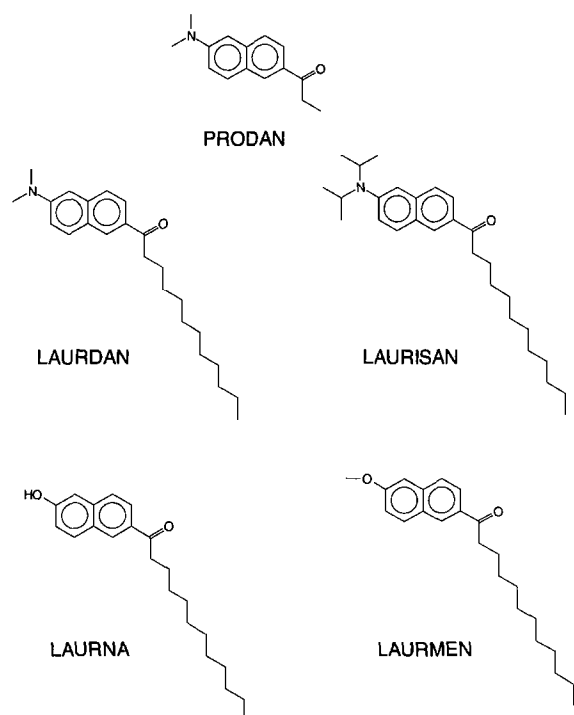
## DIPOLAR RELAXATION OBSERVED IN PHOSPHOLIPID BILAYERS

The emission maxima of LAURDAN and PRODAN in phospholipid bilayers depend upon the phase

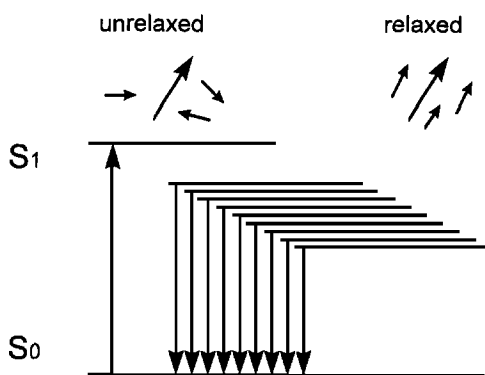
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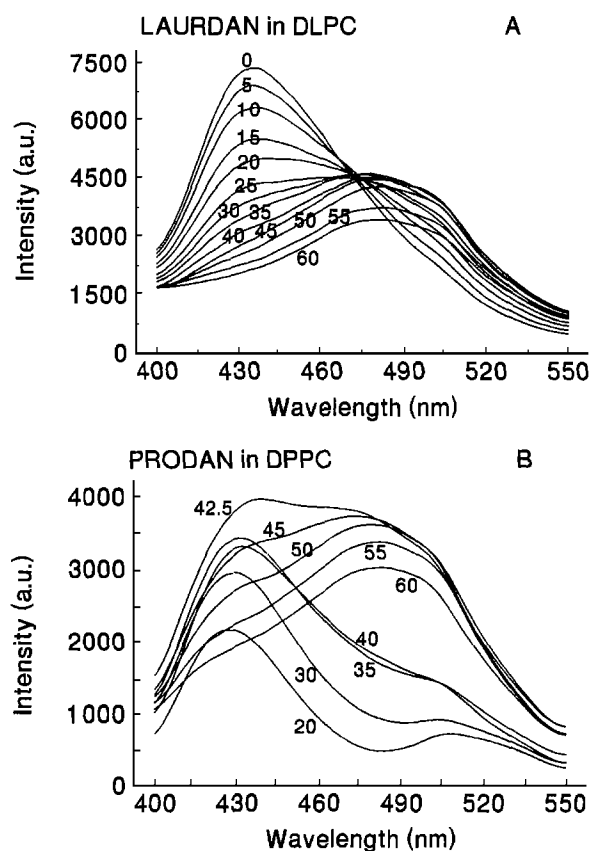


**Fig. 1.** Chemical structures of 2-dimethylamino-6-lauroyl-naphthalene (LAURDAN), 2-dimethylamino-6-propionynaphthalene (PRODAN), 2-diisopropylamino-6-lauroyl-naphthalene (LAURISAN), 2-hydroxy-6-lauroyl-naphthalene (LAURNA), and 2-methoxy-6-lauroyl-naphthalene (LAURMEN). PRODAN and LAURDAN are from Molecular Probes Inc., Eugene, OR. LAURISAN, LAURNA, and LAURMEN were a generous gift of Gregorio Weber, synthesized following the procedure described in Ref. 1.



**Fig. 2.** Schematics of the ground ( $S_0$ ) and excited-state ( $S_1$ ) energy levels in the presence of the solvent dipolar relaxation.  $S_1$  decreases in energy as solvent dipolar relaxation proceeds.

state of the phospholipids, being blue in the gel (maximum emission  $\approx 440$  nm) and green in the liquid crystalline phase (maximum emission  $\approx 490$  nm) (Fig. 3). Moreover, in gel phase phospholipids, neither probe ex-



**Fig. 3.** Emission spectra of (A) LAURDAN in dilauroyl-phosphatidylcholine (DLPC) vesicles as a function of temperature, from 0 to 60°C, and (B) PRODAN in dipalmitoyl-phosphatidylcholine (DPPC) vesicles as a function of temperature, from 20 to 60°C. DPPC and DLPC (Avanti Polar Lipids, Inc., Alabaster AL) multilamellar vesicles were prepared and labeled using standard procedures [3]. The spectra were acquired using a GREG 200 fluorometer (ISS Inc., Champaign, IL), equipped with photon counting electronic (P01, ISS Inc.), with a xenon arc lamp as the light source, 8-nm excitation and emission band-passes, and continuous mild stirring of the samples. The cell holder was thermostated at  $\pm 0.1^\circ\text{C}$  using a circulating water bath.

hibits a temperature-dependent shift of the emission maximum. Instead, at temperatures above the phospholipid phase transition, a continuous red shift of the emission is observed, to a limiting green emission at the highest temperatures, with a maximum at about 490 nm (Fig. 3). This shift of the emission spectrum has been attributed to dipolar relaxation processes occurring in the phospholipid liquid-crystalline phase but not in the gel phase.

To investigate the molecular entities in the phospholipid bilayer that reorient around LAURDAN and PRODAN excited-state dipoles, experiments have been performed in bilayers with different polar head compo-

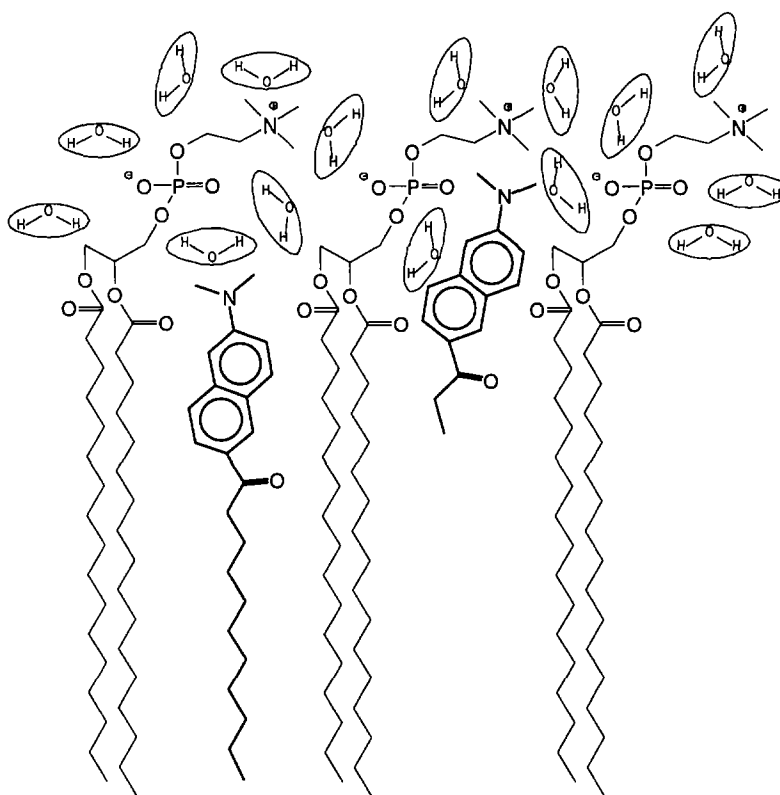


Fig. 4. Schematic representation of LAURDAN and PRODAN locations in the phospholipid bilayer.

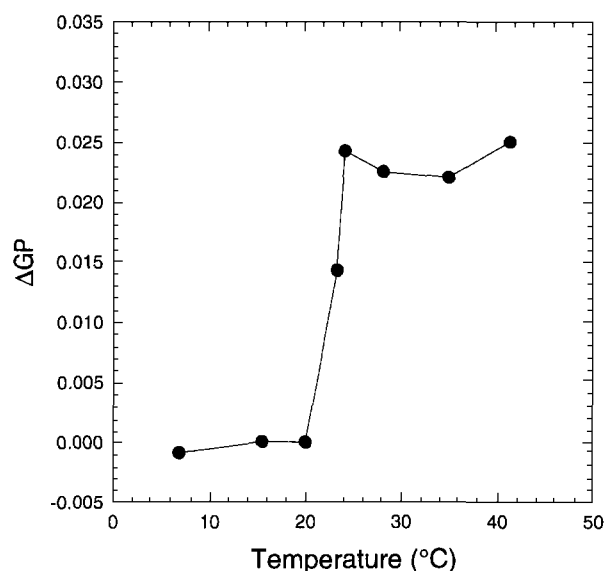
sition and charge, i.e., different phospholipids at pH values between 4.0 and 10.0. The red spectral shift has been found to be independent of the polar head residue and of its charge. Instead, this shift depends only on the phase state of the bilayer [4]. Thus, the origin of the dipolar relaxation observed in phospholipids has been attributed to a few water molecules present in the bilayer at the level of the glycerol backbone, where the fluorescent moiety of LAURDAN and PRODAN resides (Fig. 4). The concentration and the molecular dynamics of these water molecules change in the two phospholipid phase states, i.e., the water reorientation along the probes excited-state dipole occurs only in the liquid-crystalline phase. In the tightly packed gel phase phospholipid bilayers, red shift of the emission cannot be observed. Instead, the continuous red shift of the emission observed in the liquid-crystalline phase with increasing temperature is due both to the increased concentration of water in the bilayer and to its increased mobility.

Experiments have also been performed using  $D_2O$  instead of  $H_2O$  as a solvent for the phospholipid vesicles preparation. Due to the slower dynamics of  $D_2O$  com-

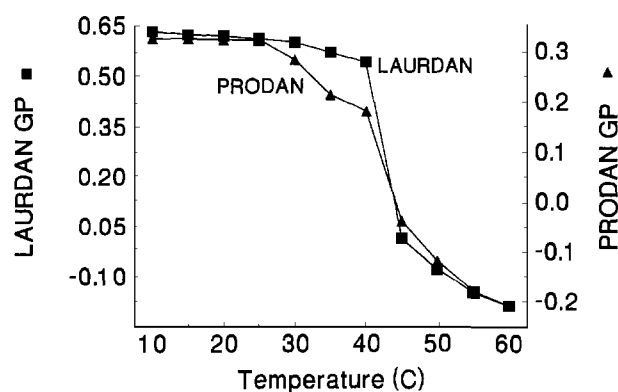
pared to  $H_2O$ , bluer emission spectra were observed when the vesicles were prepared in  $D_2O$ . In Fig. 5 this blue shift is reported as the difference between the GP ( $\Delta GP$ ) values measured in dimyristoyl-phosphatidylcholine (DMPC) vesicles prepared using  $D_2O$  and  $H_2O$  at different temperatures (the definition and discussion of the GP value are given below). The  $\Delta GP$  increases at the transition temperature and reaches the maximum value in the liquid-crystalline state. We conclude that the origin of the dipolar relaxation observed during the phospholipid transition and in the liquid-crystalline phase is actually due to water molecules surrounding the naphthalene fluorescence moiety as proposed previously [4]. Similar results have been obtained with LAURDAN in deuterated methanol (not shown).

#### COMPARISON BETWEEN LAURDAN AND PRODAN

In phospholipid bilayers LAURDAN is tightly anchored in the hydrophobic core by the cooperative van



**Fig. 5.** Difference between the GP values ( $\Delta GP$ ) measured in dimyristoyl-phosphatidylcholine (DMPC) vesicles prepared in  $D_2O$  and in  $H_2O$ . The GP values were calculated following Eq. (1) from emission spectra. The spectra were acquired using a PCI photon counting spectrofluorometer (ISS Inc., Champaign, IL), with a xenon arc lamp as the light source, with 2-nm excitation and emission bandpasses. The cell holder was thermostated at  $\pm 0.1^\circ C$  using a circulating water bath. Excitation at 360 nm.



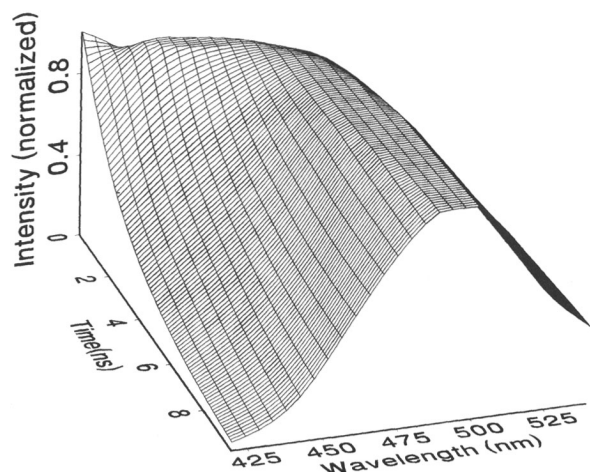
**Fig. 6.** LAURDAN and PRODAN GP values measured in DPPC vesicles as a function of temperature following Eq. (1). Excitation wavelength was 340 nm. Measurements were performed as described in the legend to Fig. 3.

der Waals interactions between the lauric acid tail and the lipid hydrocarbon chains, with its fluorescent moiety residing at the level of the phospholipid glycerol backbone [5]. Being virtually insoluble in water, LAURDAN emission originates entirely from probes within the phospholipid environment [6]. PRODAN, however, with

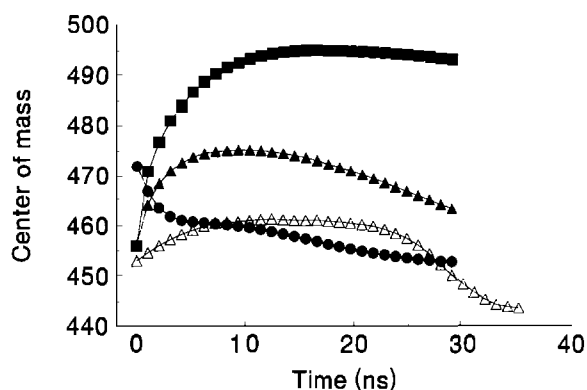
its shorter propionyl tail, is more loosely anchored to the bilayer [7] and is also water soluble. PRODAN shows an appreciable fluorescence in water, with a maximum at about 515 nm (Fig. 3B). Relative to LAURDAN, PRODAN is located closer to the aqueous surface of the bilayer, in a more polar environment and is able to sense the more freely rotating water dipoles (Fig. 4). Since PRODAN also partitions in the aqueous environment, where its emission maximum is near 515 nm, and since its partitioning in the bilayer decreases when the membrane is tightly packed in the gel phase, the PRODAN emission spectra reported in Fig. 3B show some peculiarities: (1) the band at about 515 nm is particularly clear at low temperatures; (2) its total emission intensity increases with temperature in the phospholipid transition (for DPPC at temperatures  $\geq 40^\circ C$ ); and (3) when PRODAN spectral red shift is used to monitor the phospholipid phase transition, with increasing temperature we first observe a blue emission in the gel phase, followed by a progressive red shift starting at temperatures corresponding to the phospholipid polar-head pretransition. Instead, when LAURDAN's spectral shift is used for the same purpose, with increasing temperature we observe a red emission shift only at temperatures corresponding to the main acyl chain transition. By observing LAURDAN's and PRODAN's GP values (see below for the derivation of the GP function) in DPPC vesicles as a function of temperature, we can clearly detect the polar-head pretransition only in the case of PRODAN (Fig. 6).

#### TIME-RESOLVED EMISSION SPECTRA

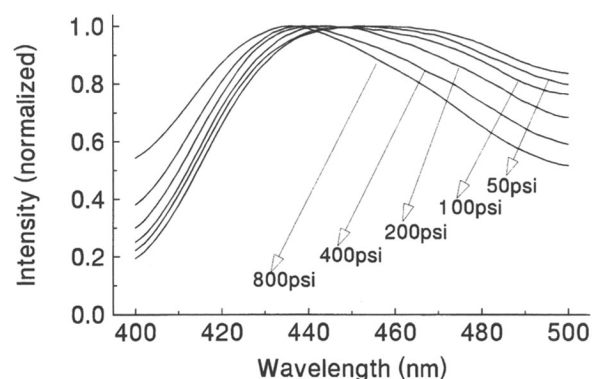
In addition to the observation of the steady-state emission spectral shift, a dynamic determination of the dipolar relaxation rate can be achieved by the measurement of time-resolved emission spectra. We observed that in a polar environment those probe molecules with a bluer emission spectrum have a shorter lifetime value than the molecules emitting with a redder emission. In other words, if we look at the emission spectrum as a function of time after excitation we observe a continuous time-dependent red spectral shift (Fig. 7) [3,8]. This red shift of the emission spectrum is particularly clear when we plot the emission center of mass as a function of the time after excitation (Fig. 8). Of course, this time-dependent red spectral shift can be observed only in a polar environment and only when the dynamics of the solvent dipoles is on the same time scale as the probe's lifetime. Consistent with the steady-state observations, the time-resolved spectral shifts also depend on the composition



**Fig. 7.** Normalized PRODAN time-resolved emission spectra in DLPC vesicles at 25°C. Fluorescence lifetime measurements were performed using a K2 phase fluorometer (ISS Inc.), with a xenon arc lamp as the light source. Excitation was at 360 nm. An additional polarizer was inserted in the excitation light path, with an angle of 35° with respect to the vertical. A solution of 2,2'-*p*-phenylene-bis-(5-phenyl)oxazole (POPOP) in ethanol was used as the reference (lifetime = 1.35 ns). During measurements, samples were continuously stirred. The sample compartment was kept at  $25 \pm 0.1^\circ\text{C}$  by a circulating water bath. The emission was observed through a series of seven interference filters, from 420 to 540 nm, with a 10-nm bandwidth and at 20-nm intervals. Phase and modulation data were acquired at each emission wavelength using a set of 10 frequencies, logarithmically spaced from 25 to 250 MHz. To obtain the time-resolved spectra, data were analyzed using the Globals Unlimited software (Laboratory for Fluorescence Dynamics, University of Illinois at Urbana-Champaign).



**Fig. 8.** Variation of the center of mass of the time-resolved emission spectra of PRODAN in vesicles composed of DLPC (■), of DPPC (●), and of an equimolar mixture of the two phospholipids (▲). Also reported is the variation of the center of mass of LAURDAN time-resolved emission spectra in the equimolar mixture of the two phospholipids (△). Measurements at 25°C, performed as described in the legend to Fig. 7.

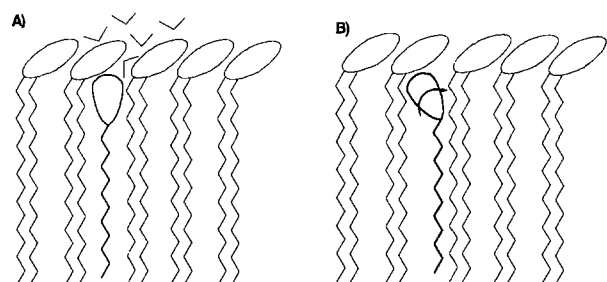


**Fig. 9.** LAURDAN emission spectra in DLPC vesicles, at 20°C, at different oxygen pressures. The spectra were obtained using a photon counting fluorimeter, Model GREG PC (ISS Inc., Champaign IL), equipped with a xenon arc lamp and an oxygen pressure bomb, described by Lakowicz and Weber [27], thermostated at  $20 \pm 1^\circ\text{C}$  by a circulating water bath. A steel needle slowly bubbled oxygen into the bottom of the cuvette for rapid equilibration. After the application of each oxygen pressure, the sample was allowed to equilibrate for 20 min in the dark. During experiments samples were continuously stirred. The measurements in the absence of oxygen were performed under  $\text{N}_2$  atmosphere. To minimize the time of illumination under high oxygen pressure, the emission spectra were collected from 400 to 500 nm in about 20 s per spectrum. Excitation and emission bandpasses were 8 nm.

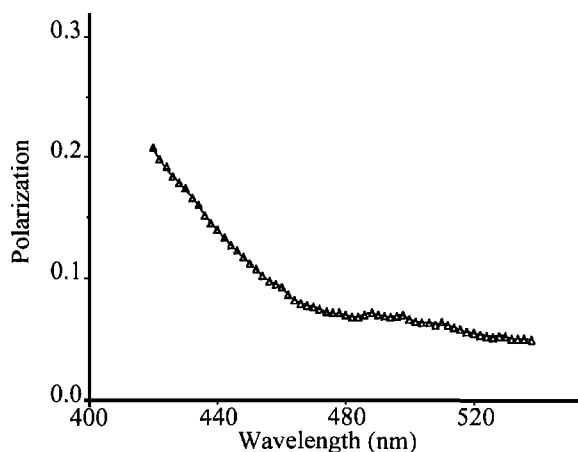
of the phospholipid vesicles [8]. Time-resolved emission spectra have been acquired for vesicles composed of mixtures of DPPC and DLPC at various relative concentrations and the extent of the red shift of the emission has been found to be related to the phase composition of the vesicles [8].

## OXYGEN-QUENCHING EXPERIMENTS

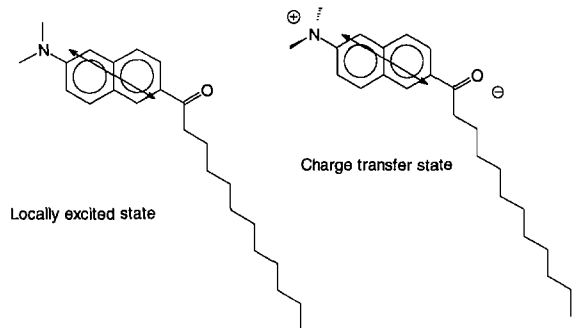
The occurrence of probe molecules with a longer lifetime, i.e., surrounded by relaxed solvent dipoles, in the red part of the emission spectrum can also be shown by oxygen quenching experiments performed in the phospholipid liquid-crystalline phase. In this phospholipid phase, oxygen has a higher probability to quench the longer lifetime LAURDAN molecules. Increasing oxygen pressures have been applied to phospholipid bilayers labeled with LAURDAN in the liquid crystalline phase (DLPC at 20°C). The emission spectra have been acquired at variable oxygen pressures from 50 to 800 psi [9]. We observe a continuous preferential decrease of the intensity at longer wavelengths (long-lived LAURDAN molecules) with increasing oxygen pressure (Fig. 9). When the spectra at the various oxygen concentra-



**Fig. 10.** Model of dipolar relaxation due to the reorientation of water molecules (A) and to the reorientation of the naphthalene moiety (B).



**Fig. 11.** Polarization emission spectra of LAURDAN in DLPC vesicles, at 20°C, obtained with the instrumentation described in the legend to Fig. 3. Excitation at 340 nm.



**Fig. 12.** Model of the LAURDAN locally excited state and charge transfer excited state.

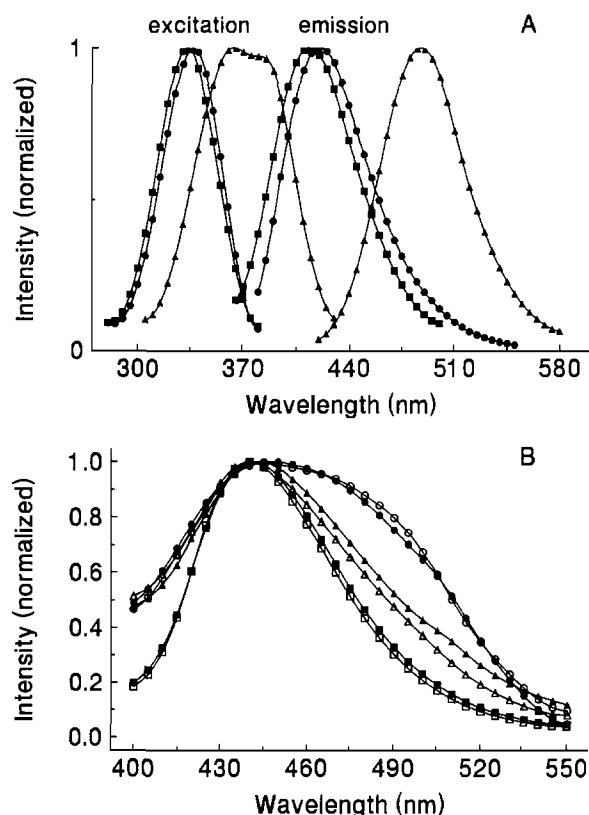
tions are normalized to their total intensity, we could not observe an isosbestic point. This observation rules out the possibility that only two molecular species of the probe are responsible for the emission red shift, as discussed further below.

## WATER RELAXATION VERSUS PROBE REORIENTATION

An explanation of the emission red shift alternative to the reorientation of a few water molecules present at the bilayer interface (Fig. 10A) can be hypothesized. The fluorescent naphthalene residue could reorient with respect to its molecular axis (Fig. 10B). Indeed, in the latter case, these different orientations of the molecule dipole should be observed in the polarization emission spectrum. The extent of spectral shift and the angle of rotation should be correlated. LAURDAN's polarization emission spectrum has been measured in phospholipids in the liquid-crystalline phase (Fig. 11) [3]. The data show an initial decrease of the polarization, up to about 460 nm, and then a constant value. If the spectral shift and the angle of rotation were correlated, then the polarization decrease should also continue at longer emission wavelengths. Instead, the decrease in polarization at shorter wavelengths and its constant value at longer wavelengths can be completely explained on the basis of the dependence of the emission wavelength on the lifetime (Figs. 7 and 8). Moreover, in the case sketched in Fig. 10B, the reorientation of the probe fluorescent moiety should be sensitive to the charge in the polar head residue, which, instead, was not observed [4].

## INTRAMOLECULAR REORIENTATION AND DIPOLAR RELAXATION

Recently, a reinterpretation of LAURDAN's spectroscopic behavior in phospholipids has been proposed, based on measurements performed on the probe in ethanol [10] and on previous theoretical calculations reported on PRODAN crystals [11]. The proposed model involves the relative reorientation of the dimethylamino and carbonyl residues, giving rise to two states, the locally excited state and the charge transfer state [10], the first with a blue emission and the second with a red-shifted emission. Of these two relative orientation, only the charge transfer state can give rise to the reorientation of the surrounding solvent dipoles (Fig. 12). Following Viard *et al.* [10], the locally excited state should predominate in apolar solvents and in polar solvents at very low temperatures. In our measurements performed in apolar solvents, such as dodecane and cyclohexane, we did observe a relevant blue shift of LAURDAN emission [4]. In agreement with the hypothesis of the locally excited state, in these apolar solvents, LAURDAN's quantum yield is very low and its lifetime is very short, about 0.1 ns. However, we do not have evidence of this locally



**Fig. 13.** (A) Emission and excitation spectra in ethanol at 20°C of LAURDAN ( $\blacktriangle$ ), LAURMEN ( $\blacksquare$ ), and LAURNA ( $\bullet$ ). LAURDAN fixed excitation and emission wavelengths were 350 and 490 nm for emission and excitation spectra, respectively. LAURMEN and LAURNA fixed excitation and emission wavelengths were 340 and 420 nm, for emission and excitation spectra, respectively. (B) Emission spectra in phospholipids of LAURDAN (open symbols) and LAURISAN (filled symbols) in vesicles composed of DLPC ( $\bullet$ ,  $\circ$ ), of DPPC ( $\blacksquare$ ,  $\square$ ), and of an equimolar mixture of the two phospholipids ( $\blacktriangle$ ,  $\triangle$ ). Measurements at 25°C performed as reported in the legend to the Fig. 3.

excited state when LAURDAN (as PRODAN) is inserted in phospholipid vesicles. In bilayers, (1) the quantum yield of both probes is quite high; (2) their lifetime value ranges between 4 and 6 ns, depending on the phospholipid phase state; and (3) an isosbestic point indicative of a transition between two conformations is not observed (Fig. 3), such as reported for LAURDAN's spectra in ethanol at low temperatures [10].

Several LAURDAN derivatives were synthesized by Gregorio Weber, with the purpose of (1) ascertaining that a partial charge separation in the naphthalene moiety is necessary for the emission red shift, and (2) studying the effect of the intramolecular reorientation. In Fig. 13A we report the excitation and emission spectra in ethanol

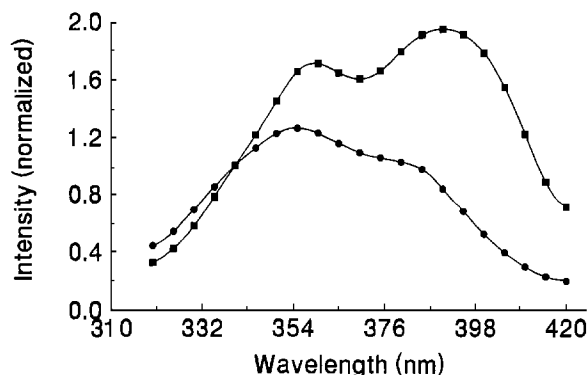
of LAURDAN together with those of 2-hydroxy-6-lauroylnaphthalene (LAURNA) and of 2-methoxy-6-lauroylnaphthalene (LAURMEN). We can clearly observe that when the partial positive charge of the dimethylamino residue is substituted with an hydroxy or methoxy residue, the emission spectra are blue-shifted (maximum at about 420 nm) with respect to LAURDAN's emission (maximum at about 490 nm), demonstrating that the charge transfer state cannot be formed. In Fig. 13B we report the emission spectra in phospholipids of LAURDAN and of 2-isopropyl-6-lauroylnaphthalene (LAURISAN). The LAURDAN and LAURISAN spectra almost overlap, proving that, in LAURISAN, the steric hindrance to an intramolecular reorientation due to the isopropyl residue has no effect on the red shift observed in the liquid-crystalline phase. These results show that the molecular entity responsible for the dipolar relaxation cannot be the fluorophore itself.

#### GROUND-STATE CONFORMATIONS

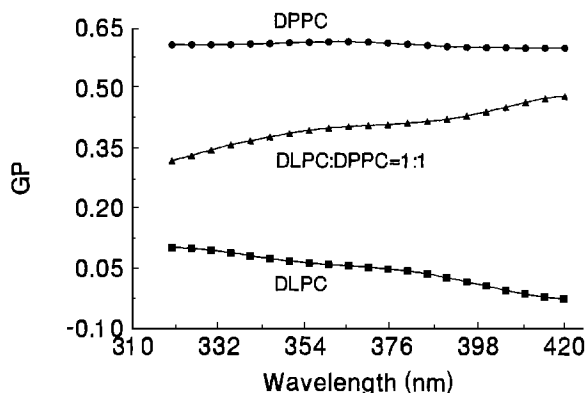
In addition to the emission spectral features of these naphthalene probes, their excitation spectra also show the existence of different excitation states. In polar solvents, the excitation spectra of LAURDAN and PRODAN are composed of at least two bands, centered at about 350 and 390 nm. The red excitation band is particularly intense in gel phase phospholipids. Using LAURMEN and LAURNA, we were able to show that, in polar solvents, the excitation spectra of these two probes do not show the second red excitation band. In Fig. 13A the excitation spectra of LAURMEN and LAURNA in ethanol are reported, together with the excitation spectra of LAURDAN (and PRODAN). The presence of a partial charge separation due to the dimethylamino residue of LAURDAN appears necessary for obtaining a second red excitation band in polar solvent. The second red excitation band at 390 nm has been interpreted as due to the absorption by LAURDAN molecules stabilized in a ground-state  $L\alpha$  conformation [3] by surrounding dipoles.

Also in phospholipid vesicles, the excitation spectra of LAURDAN and PRODAN are composed of at least two bands. With respect to polar solvents, the red excitation band is particularly intense, especially in gel-phase vesicles (Fig. 14). For LAURDAN in gel-phase phospholipids this red excitation band also constitutes the excitation maximum. In the phospholipid liquid-crystalline phase, the red excitation band is less intense than in the gel phase (Fig. 14). In the presence of the two coexisting phases, the LAURDAN red excitation





**Fig. 14.** Excitation spectra of LAURDAN in DPPC vesicles (■) and in DLPC vesicles (●) at 25°C. To enhance the intensity variation of the red excitation band, the spectra have been normalized at 340 nm. Emission at 440 nm. Measurements were performed as described in the legend to Fig. 3.



**Fig. 15.** LAURDAN excitation GP spectra in phospholipid vesicles, at 20°C, composed of DPPC (top spectrum), DLPC (bottom spectrum), and of the equimolar mixture of the two phospholipids (middle spectrum). For the calculation of the GP values [Eq. (1)] the emission wavelengths were 440 and 490 nm. Measurements were performed as described in the legend to Fig. 3.

band is mainly populated by those probe molecules surrounded by phospholipids in the gel phase [3].

In analogy with the fluorescence polarization, we developed the generalized polarization function (GP) [3]

$$GP = (I_g - I_l)/(I_g + I_l) \quad (1)$$

where  $I_g$  and  $I_l$  are the steady-state intensities at the maximum emission wavelengths in the gel and in the liquid-crystalline phase, respectively, at about 440 and 490 nm. When using different excitation wavelengths, i.e., in the blue or in the red excitation band, the GP value provides a quantitative measurement of what proportion of the initially photoselected LAURDAN molecules are sur-

rounded mainly by the liquid-crystalline or mainly by gel-phase phospholipids. Of course, the GP measurement does not imply the use of polarizers. Due to the solvent dipolar relaxation, the emission spectrum of LAURDAN (and of PRODAN) shows a continuous red shift with temperature, with no isoemissive point (Fig. 3). Thus the GP function also contains information on the dipolar relaxation process. Indeed, the excitation GP spectra (analogous to the excitation polarization spectra) do not show abrupt GP changes as a function of the excitation wavelength (Fig. 14). Instead, we observe either constant GP values (in the gel phase) or a continuous change with a negative (in the case of the homogeneous liquid-crystalline phase) or positive slope (in the case of coexisting domains of the two phases) (Fig. 15). The GP value can be used for the evaluation of the rate and extent of solvent dipolar relaxation processes [8]. The slope of the excitation GP spectrum can be used to ascertain the coexistence of the two phospholipid phases [6]. From the favorable spectroscopic properties of LAURDAN, with its red excitation band populated mainly by molecules stabilized in the  $L\alpha$  conformation by oriented solvent dipoles, and by molecules surrounded by phospholipids in the gel phase, we can easily distinguish between homogeneous and mixed phases. In the case of coexisting phases, LAURDAN molecules surrounded by gel phase will be mainly excited in the red band and will emit with a blue spectrum, i.e., with a high GP value. In this case, by moving the excitation toward the red the GP value increases. In the case of a homogeneous liquid-crystalline phase, the already relaxed LAURDAN molecules in the  $L\alpha$  conformation will emit with a red spectrum, i.e., with a low GP value. In this case, at longer excitation wavelengths the GP value will decrease (Fig. 15)

Since the GP possesses all the properties of the "classical" fluorescence polarization [12], it was used to quantify the relative amount of the two coexisting phospholipid phases [3]. In conjunction with oxygen quenching experiments, the GP value was used to obtain the rate of the dipolar relaxation using an approach similar to the Perrin equation [9].

## CONCLUSIONS: MODEL AND NATURAL MEMBRANE STUDIES

The sensitivity of LAURDAN and PRODAN to the environment polarity has been used in studies of the polarity variations in synthetic bilayers, micelles, and natural membranes [13–16]. The GP function [Eq. (1)] offers the advantages of facile and rapid measurements

of membrane polarity as well as high sensitivity. Among several applications [17–25], the sensitivity of LAURDAN and PRODAN to the bilayer polarity can be used to study structural changes involving the modification of water concentration in the bilayer [7]. Due to their different chemical affinity for the bilayer, i.e., their different location in the bilayer, LAURDAN and PRODAN report on water concentration at different membrane locations. For instance, in bilayers damaged by ionizing radiation, the higher penetration of water at the membrane surface can be monitored by the decrease in the PRODAN GP value, while the relocation of LAURDAN deeper in the bilayer is seen by the increase in the GP value [7].

The recent development of two-photon excitation microscopy, with its inherently reduced photobleaching and phototoxicity, allowed the measurement of LAURDAN GP values in phospholipid vesicle, natural membranes, and whole cells [26]. These microscopy measurements allowed the spatial resolution of the polarity microheterogeneity of synthetic and natural membranes. In particular, a broad distribution of GP values was observed in microscopy measurements of vesicles in the liquid–crystalline phase.

## ACKNOWLEDGMENTS

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