### Supporting Information for

# Conformational Changes as Driving Force for Phase Recognition: The Case of Laurdan

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## Thermostatistics, quantum chemical analysis and the transition state dipole moments of both conformers

The geometries of the probes have been also optimized by means of density functional theory (DFT) along with the B3LYP functional<sup>1</sup> and cc-pVDZ basis set,<sup>2</sup> their frequencies have been calculated and the Gibbs free energy differences between the structures have been obtained at temperatures varying from 0 to 480 K in steps of 20 K making use of a continuum model for diethyl ether (epsilon of 4.24), which is commonly used to mimic the environment of the lipid bilayer membrane.<sup>3,4</sup> These calculations have been performed by means of the Gaussian 09 package of programs.<sup>5</sup>

Making use of the so-called Duan model,<sup>6</sup> the barrier amounts to a Gibb's free energy difference ( $\Delta$ G) of 8.5 kcal/mol at 298 K. Towards higher temperatures, this value increases almost linearly up to 9.1 kcal/mol. On the other side, below 100 K the rotational barrier rather stagnates around 8.1 kcal/mol. The differences in Gibb's free energy between the optimized forms of both conformers, with e.g. 1.0 kcal/mol reached at 350 K, give finally access to a calculation of the abundances (see Figure S1).





**Figure S1:** (top)  $\Delta G$  energy difference for Conf-II and the barrier in between the two conformers in diethyl ether which is described by a continuum model. Conf-I is used as zero value reference; (bottom) the abundances of Conf-I and Conf-II calculated by means of Boltzmann statistics along the investigated temperature range up till 480 K.

Laurdan is often used in Generalized Polarization spectroscopy measurements, which are based upon differences in emission intensities obtained at two wavelengths. It is inherently coupled to the response of the environment to the excitation process. Using the same theories as here above, the state dipole moment of Conf-I amounts to 7.7 Debye and 15.7 Debye in the ground and first excited state, respectively. For Conf-II, dipole moments are a bit lower and amount to 6.9 Debye and 14.8 Debye, respectively. In both cases, it is a locally excited state with mainly a HOMO to LUMO assignment. For the CAM-B3LYP functional, the obtained quantities are slightly smaller, but the same qualitative results are obtained (See Table S1). Experimentally, for Prodan, a value of 5.2 Debye is obtained for the ground state, while for the excited state values of 9.6 and 10.2 Debye are obtained, dependent on the solvent of 1,4-dioxane and benzene, respectively.<sup>7</sup> Thanks to these changes in state dipole moments, the neighboring tissues and solvent molecules undergo strong relaxation, which might however not be completed within the limited window of one lifetime of an excited Laurdan conformer.

		GS	$\mathbf{S1}^{b}$
CAM-B3LYP	Conf-I	7.1 D	13.7 D
	Conf-II	6.3 D	12.9 D
B3LYP <sup>c</sup>	Conf-I	7.7 D	15.7 D
	Conf-II	6.9 D	14.8 D

Table S1: Ground- and excited state dipole moments for Conf-I and Conf-II of Laurdan.<sup>a</sup>

<sup>*a*</sup> The values are given in Debye.

<sup>*b*</sup> For both functionals and conformers, the S1 is a local excitation from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). For B3LYP, the so-called  $\Lambda$ -overlap parameter of Peach *et al.*<sup>8</sup> amounts to 0.62 and 0.66 for Conf-I and Conf-II, respectively. For CAM-B3LYP, this parameter is 0.85 and 0.68 for both conformers, respectively. In all these cases,  $\Lambda$  is found to be considerably larger than the critical value of 0.30; the S1 excited state can therefore be qualified as a locally excited state rather than a Charge Transfer state, for which Time Dependent Density Functional Theory (TDDFT) calculations have to be handled with extreme care.

 $^{c}$  In the GS, the distance between the amino-N and the carbonyl-C amounts to 8.0 Å; the distance between the carbonyl-C and the end of the stretched tail amounts to 14.1 Å.



**Figure S2.** Mass density profile along the z axis for the two conformers in DPPC (So) while considering either the full probe (dashed lines) or only the head of the probe (solid lines). The bilayer center is set at zero. Of particular interest is the maximum of the mass density of Conf-I at z=0: at lower position of the head group, which is stiff and flat and quite perpendicularly oriented with respect to the membrane surface, the tail folds itself in the middle. It can also be seen that the tails are able to reach the other leaflet.



**Figure S3.** Number of hydrogen bonds of which evidence has been found in the MD simulations along the simulation time (given in nano seconds). Above, the results are given for Conf-I; below, they are given for Conf-II. Two cut-off distances have been used: 3.5 Å and 5 Å characterizing the moderate and weak electrostatic interactions, respectively.

#### Background information to the S<sub>CD</sub> values provided in Figure 5

What is shown are indeed the  $S_{CD}$  values, which are formally derived as

$$S=\frac{3}{2}\left\langle \cos^2\theta\right\rangle -\frac{1}{2},$$

with, in our case,  $\theta$  the angle between the C-H bond vector and the bilayer normal. The brackets mean molecular and temporal ensemble averages, as defined and illustrated in Piggot *et al.*<sup>9</sup> Within a molecular frame, the local *z*-axis can be oriented from C<sub>n-1</sub> to C<sub>n+1</sub>, the *x*-axis can be chosen perpendicular to the *z*-axis (in the H-C<sub>n</sub>-H plane) and the *y*-axis can be taken analogously perpendicular to both *x* and *z* axes to bisect the H-C<sub>n</sub>-H angle. The order parameters can then also be defined as:

$$S = \frac{2}{3}S_{xx} + \frac{1}{3}S_{yy}.$$

where  $S_{xx}$  and  $S_{yy}$  are defined as  $S_{xx} = \frac{3}{2} \langle \cos^2 \theta_x \rangle - \frac{1}{2}$  and  $S_{yy} = \frac{3}{2} \langle \cos^2 \theta_y \rangle - \frac{1}{2}$ , with  $\theta_x$  and  $\theta_y$  the

angle between the *x*- and *y*-axes, respectively, and the membrane normal. It can be remarked that rather the notation  $S_{CD}$  is used than  $S_{CH}$ , since this approach is linked with the quadrupole coupling, which can be experimentally verified using deuterium atoms.<sup>10</sup> In these experiments, only the absolute values of  $S_{CD}$  can be obtained since the sign of the quadrupole coupling is not defined.

Finally, it can be noticed that the deuterium order parameter  $S_{CD}$  should not be confused with the molecular order parameter  $S_{mol} = -2S_{CD}$ .<sup>11</sup>

**Table S2.** Peak position, window and standard deviation for all the calculated  $\gamma$ -angles expressed in degrees for Conf-I and Conf-II embedded in DPPC.

Conf-I	Peak window	Angle max	Standard deviation
γ1	102-127	113.1	2.8
γ5	101-173	134.3	6.1
γ7	92-176	135.7	7.9
γ 11	76-178	137	13
Conf-II	Peak window	Angle max	Standard deviation
γ1	116-143	130.8	2.9
γ 1 γ 5	116-143 86-171	130.8 139	2.9
γ 1 γ 5 γ 7	116-143 86-171 76-174	130.8 139 132	2.9 11 15



**Figure S4.** Tilting (black), torsion (red) and tdm (green) angle distributions of Laurdan in DPPC membrane for Conf-I and Conf-II. For the  $\alpha$ -tilt angle, the angle between the molecular axis (from carbonyl carbon to nitrogen) and the z-axis has been considered. For the  $\beta$ -torsion angle, the angle between the short molecular axis of naphthalene and the z-axis has been described. For the tdm angle, the angle between the transition dipole moment (from carbonyl carbon to  $\epsilon$  carbon) and the z-axis has been considered.

Table S3. Conversion between the nomenclature used in the paper by Parisio et al. (J. Phys.

Chem. B 2011, 115, 9980) and our study.

Our current study	Parisio <i>et al</i> .
Long molecular axis	Molecular z-axis
$\alpha$ - angle between the	$\beta$ - angle between the
normal to the	bilayer normal and
membrane and the	the molecular z-axis
long molecular axis	
$\beta$ - angle between	
positive z-axis of the	
membrane and the	
short molecular	
axis <sup><i>a,b</i></sup>	
$\gamma$ - angle between the	
long molecular axis	
and the tail of the	
probe	
	$\gamma$ - rotation around
	the molecular z-axis <sup><i>a</i></sup>
tdm – angle between	
the transition dipole	
moment of the probe	
and the z-axis of the	
membrane	

<sup>*a*</sup> The difference between angle  $\beta$  (current work) and angle  $\gamma$  (Parisio et al.) amounts to ~20°, which corresponds directly with the angle between long molecular axis and the transition dipole moment of the probe.

<sup>b</sup> The short molecular axis in the current work is oriented perpendicular to the transition dipole moment of the Laurdan probe.



**Figure S5.** Both molecular tilt angle  $\alpha$  (top), torsional angle  $\beta$  (center) and tdm angle (bottom) for both conformers in DPPC have been calculated within a 50 ns time windows, starting from 200 ns. Same time windows are represented by the same color, Conf-I is reported as a solid line, Conf-II as a dotted line. These plots can be seen as a decomposition of Figure S3 (since that figure is an average over 200-400 ns).



**Figure S6.** Dihedral angle distribution between the Laurdan head and the alkyl tail in DPPC. The insert shows the atoms considered for the dihedral analysis.



**Figure S7.** Tilting, torsion and tdm angle distributions of Laurdan in DOPC membrane for Conf-I (red) and Conf-II (green) in their Ground States. During the total trajectory of 300 ns (for which the data in the plots are given in black), a conformational change from Conf I to Conf II is seen at 115 ns; at 260 ns, a change from Conf II to Conf I is observed. The distributions for the total trajectory are given in black.



**Figure S8.** Tilting, torsion and tdm angles distributions of Laurdan in DOPC membrane for Conf-I (red) and Conf-II (green) in their S1 optically excited states. A conformational change from Conf I to Conf II is seen at 15.5 ns. The distributions for the total trajectory of 100 ns are given in black.



**Figure S9** Probability densities for the polar angle theta ( $\theta$ ) for Conf-I and Conf-II in a DPPC (So) membrane.  $\theta$  denotes the polar angle in radians between the vector of the alpha-tilt (left column) or the transition dipole moment (right column) with the z-axis of the membrane.



**Figure S10.** Probability densities for the vector of the  $\alpha$ -tilt, and the transition dipole moment in function of the azimuthal angle  $\varphi$  for Conf-I and Conf-II in DPPC (So) membrane. It denotes the angle in the plane of the membrane in radians. Since the x-axis in the plane of the membrane (to which is referred by the  $\varphi$ -angle) is not uniquely defined, in these plots 0 radians is taken as the midpoint of the sampled angles.

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