Imaging lipid lateral organization in membranes with C-laurdan in a confocal microscope

Martín M. Dodes Traian1,2, Luis González Flecha2 and Valeria Levi1*

1 Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, CP 1428 Ciudad de Buenos Aires, Argentina

2 Laboratorio de Biofísica Molecular, Instituto de Química y Fisicoquímica Biológicas, Universidad de Buenos Aires, CONICET, Buenos Aires, Argentina

* To whom correspondence should be addressed. E-mail: vlevi12@gmail.com. Phone and fax: 0054-11-4786-3426

SUPPLEMENTAL MATERIAL

Figure I: Laser polarization effects on the intensity of C-laurdan. GUVs composed of DPPC were electroformed at 53 °C. The sample was slowly cooled down and images of the GUVs were acquired at 27 °C as described in the text including in the optical path the Nomarski prism (A). After acquiring the image, the prism was removed and an image of the same field was acquired (B). The polarization axis of the laser is shown with a red arrow in each image. Bar, 5µm
Figure II: Thermotropic behavior of DPPC vesicles studied by C-laurdan at different pH. SUVs of DPPC were prepared as described in Materials and Methods in buffers at pH 5.6 (●) and pH 7.4 (○). The samples were heated in intervals of 0.2 °C and let equilibrate for 90 s at each temperature before acquiring the fluorescence spectrum. The generalized polarization at each temperature was calculated using Eq. 1. The dashed line shows approximately the temperature for DPPC pretransition (gel to ripple).
Figure III: Thermotropic behavior of DPPC:POPC 1:1 followed by C-Laurdan GP analysis. SUVs of DPPC:POPC were prepared as described in Materials and Methods. The sample was heated in intervals of 2 °C and let equilibrate for 120 s at each temperature before acquiring the fluorescence spectrum. GP of C-laurdan at each temperature was calculated using Eq. 1.
Figure IV: Characterization of lipid phases with C-Laurdan generalized polarization in a raft-like lipid system. GUVs of DOPC:SPM:Chol 2:2:1 were electroformed at 62 °C in the presence of C-Laurdan and Rho-DPPE. GUVs were observed in the confocal microscope at 27 °C registering simultaneously the fluorescence of C-laurdan (A, composite image channels 1 and 2 are shown in red and green, respectively) and of Rho-DPPE (B) and GP images were calculated from the C-laurdan images (C). Bar, 10 µm.
Figure V: Effects of methyl-\(\beta\)-cyclodextrin on the fluidity of the cell membrane of *Xenopus laevis* melanophores sensed by C-laurdan. Melanophore cells were incubated with 10 mM methyl-\(\beta\)-cyclodextrin during 15 min. Control and MbCD-treated cells were fixed by incubation with 4 % PFA, washed and labeled with C-laurdan as described before. Images of cells obtained in channel 1 (A, C) and pseudo GP images (B, D) of control (A, B) and MbCD-treated (C,D) cells were obtained as described before. Scale, 30 \(\mu\)m. (E) Representative histograms of the GP at the cell membranes obtained for control (■) and MbCD-treated (○) cells after filtering the GP images with the routine described in Materials and Methods.