The distribution analysis of parameters of the membrane lipid phase state

Nurminsky V.N.[1], Bukin Yu.S.[2], Ozolina N.V.[1]*

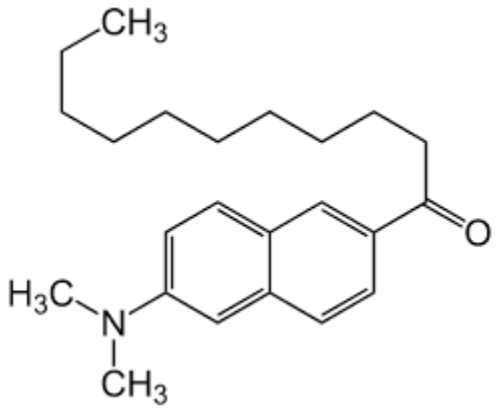
[1]Siberian Institute of Plant Physiology and Biochemistry, SB RAS, Irkutsk, Russia

[2]Limnological Institute, SB RAS, Irkutsk, Russia

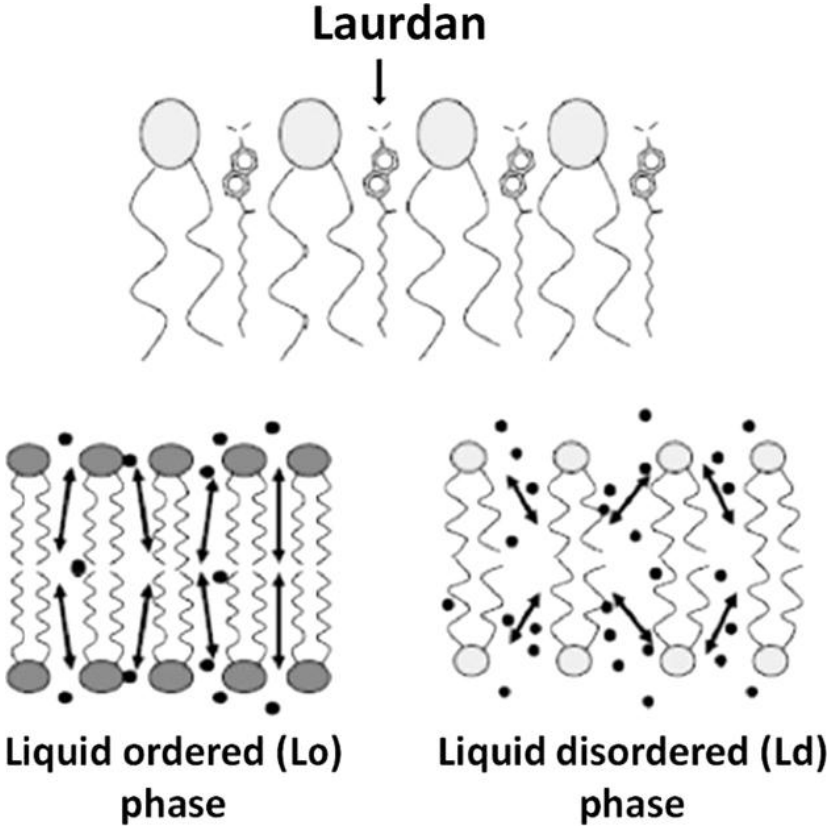
*e-mail: cell@sifibr.irk.ru

Objectives of the investigation:

(1) elaboration of a technique for analysis of the data related to the packing density (phase state, lipid order) for the membrane lipid phase;
(2) conduct a probation of this technique on the objects of raft structures of mitochondrial membranes in halophytes *Salicornia perennans* Willd., *Halocnemum strobilaceum* Bieb. and *Artemisia santonica* L.



Formula of laurdan



Schematic representation of Laurdan and of its localization (black points) within the phospholipid bilayer in the Lo and Ld phases (from Bessa et al., 2018).

Materials and methods:

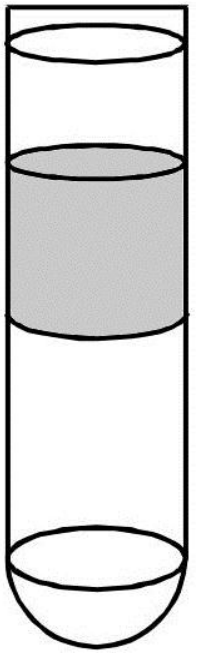
Analysis of the ordering of lipids in the membrane rafts of mitochondrial membranes in halophytes *Salicornia perennans* Willd., *Halocnemum strobilaceum* Bieb. and *Artemisia santonica* L. has been conducted.

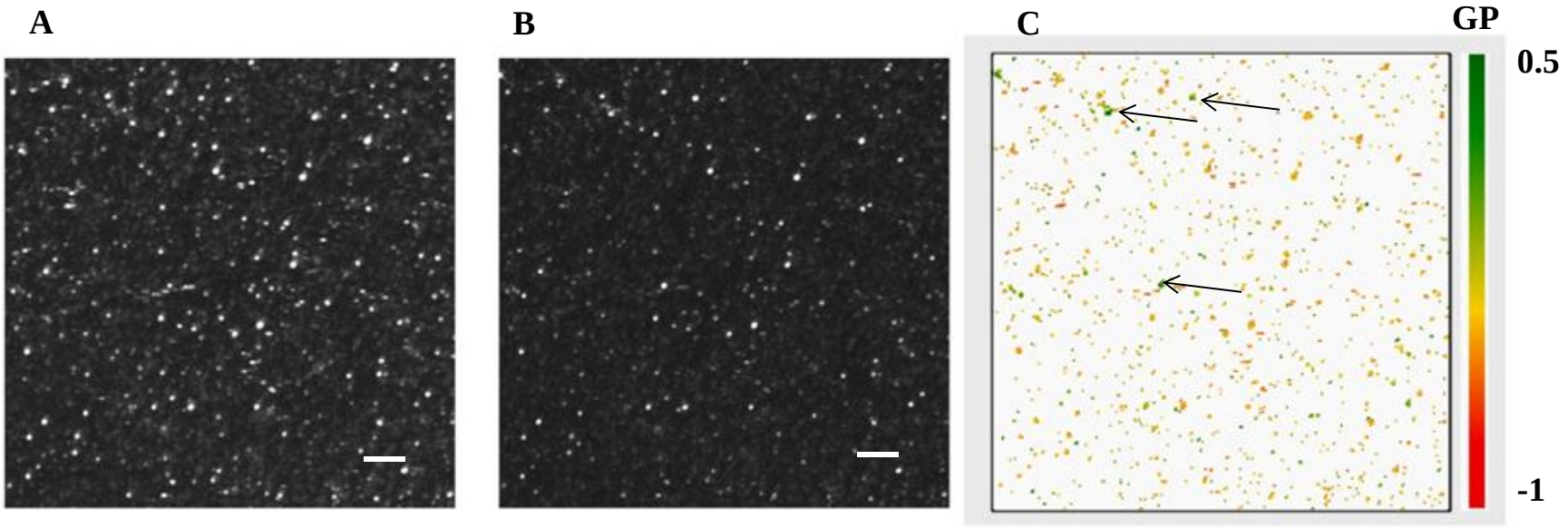
***Salicornia perennans* Willd.**
© Alexander Ebel

***Halocnemum strobilaceum* Bieb.**
© Marina Skotnikova

***Artemisia santonica* L.**
© Tatiana Vinokurova

Table 1. Distribution and subdivision of the membrane material (solubilized with 1% Triton X-100 after its centifuging in the sucrose density gradient (35–25–15–5%) into 8 zones. The opalescence domain is grey.

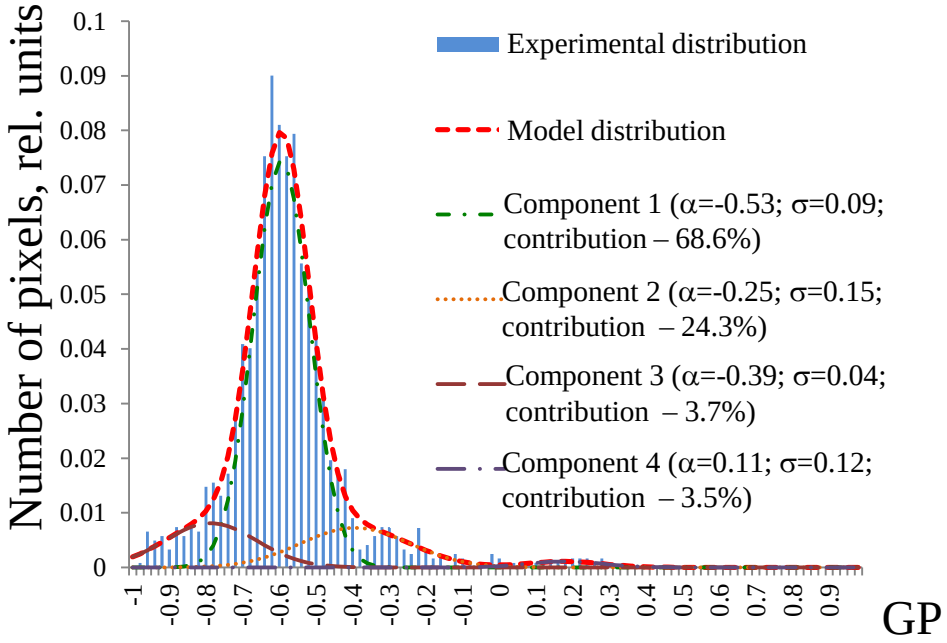
Zone No	Sucrose concentration, %	Density, g/cm^3	Scheme
1	5	1.018	
2			
3	15	1.050	
4			
5	25	1.083	
6			
7	35	1.110	
8			



The confocal microscopic images of the mitochondria membrane material of *A. santonica* in zone 5 (the upper part of the 25% sucrose layer, table 1) of the sucrose density gradient (300×300 pixels), channel 1 (A), channel 2 (B) in cases of registration in wavelength bands from 400 to 460 nm and from 470 to 530 nm respectively (fluorescent probe – laurdan, Iex = 340 nm), the white color spots reflect the fluorescence intensity, scale bar – 10 μm, and the respective GP-image (C), the pseudocoloring reflects the GP-value, the arrows indicate to more densely packed membrane material domains.

$$GP = \frac{I_{(400-460)} - I_{(470-530)}}{I_{(400-460)} + I_{(470-530)}},$$

where I(400-460) and I(470-530) are fluorescence intensities for laurdan in terms of wavelength bands from 400 to 460 nm and from 470 to 530 nm respectively.



The hystogram of the experimental distribution of GP values for the membrane material of mitochondria of *A. santonica* in zone 3 (the upper part of the 15% sucrose layer, table 1) of the sucrose density gradient and the curve of the theoretical multymodal distribution, which represents a superposition of several Gaussian distributions.

Results and conclusion:

A technique for analysis of the data on the packing density (phase state, lipid order) for the membrane lipid phase has been elaborated on the basis of the programming language R.

The data are obtained by measuring the generalized polarization (GP) value of laurdan fluorescence.

Analysis of the density (ordering) data for the membrane lipid phase based on measuring the laurdan GP fluorescence may be used for identifying some more densely packed structures (rafts) in the membrane, in parallel with the analysis of lipids.