SUPPLEMENTARY INFORMATION

LIPID POLYMORPHISM OF PLANT THYLAKOID MEMBRANES. THE DYNAMIC EXCHANGE MODEL – FACTS AND HYPOTHESES

Győző GARAB[1,2], Kinga BÖDE[1,2], Ondřej DLOUHÝ[1], Zoltán NÁSZTOR[3], Václav KARLICKÝ[1], András DÉR[3] and Vladimír ŠPUNDA[1]

[1]Department of Physics, Faculty of Science, University of Ostrava, Ostrava, Czech Republic
[2]Institute of Plant Biology, HUN-REN Biological Research Centre, Szeged, Hungary
[3]Institute of Biophysics, HUN-REN Biological Research Centre, Szeged, Hungary

E-mail: garab.gyozo@brc.hu
ORCID: 0000-0002-3869-9959

Fingerprinting lipid polymorphism in model systems and in thylakoid membranes

In model systems, the phase behavior of lipids can readily be determined by different well-established techniques: most commonly by freeze fracture electron microscopy (FF-EM), small-angle X-ray or neutron scattering (diffraction) (SAXS or SANS, respectively), and ^{31}P-NMR spectroscopy (see e.g. Cullis and de Kruijff 1979, Gounaris et al. 1983, Lipowsky and Sackmann 1995, Páli et al. 2003, Pabst et al. 2010, Quinn 2012, Demé et al. 2014, Pabst et al. 2014, Pan et al. 2015, Tyler et al. 2015). As illustrated in Figure S1 for the case of SANS (neutron diffraction), these techniques 'recognize' the long-range order packing of lipid molecules. Compared to scattering techniques, FF-EM enables the visualization of individual fractured membrane faces, including the inner hydrophobic regions, offering detailed insights into the spatial organization of non-lamellar structures. An additional, widely used technique, differential scanning calorimetry (DSC) is ideally suited to characterize the thermotropic phase behavior of hydrated lipid dispersions (Lewis et al. 2007).

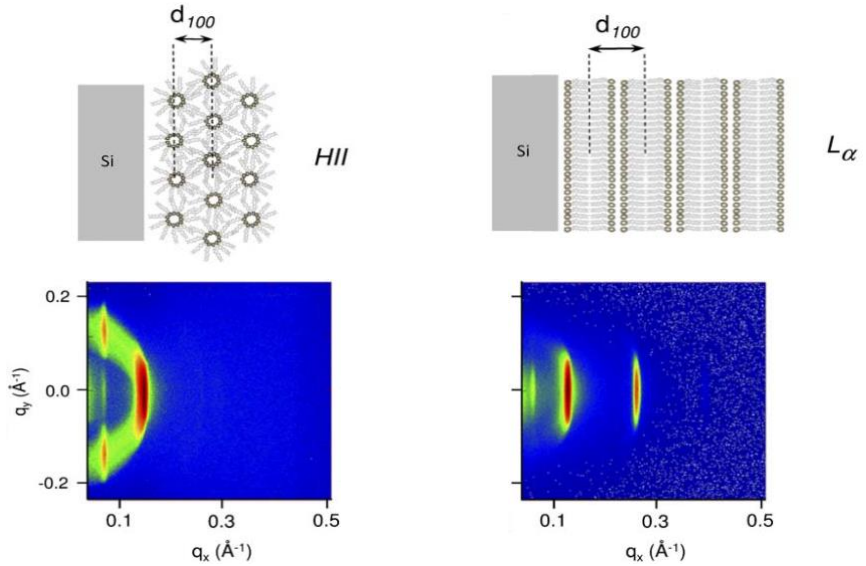

Figure S1: Two-dimensional neutron diffraction pattern of fully hydrated MGDG (left panel) and DGDG (right panel) dispersions – also showing (above) the schemes of lipid structures. (For further details, see (Demé et al. 2014); this figure is modified from this publication.)

In complex systems, such as TMs, which are densely packed with proteins, the same arsenal of biophysical techniques can be employed only with severe limitations and the detection of non-bilayer phases is less straightforward than in lipid model systems. This is because, long-range order arrangement of the lipid molecules is hindered by the membrane-embedded proteins. Thus, domains containing lipid molecules with non-bilayer packing order usually cannot be detected by small angle scattering techniques. In fact, in TMs, SAXS and SANS carry information mainly on the periodic order and repeat distances of multilamellar membrane systems (Dlouhý et al. 2021, Nagy and Garab 2021). Although, using FF-EM, H_{II} phases have been observed in TM preparations (see main text), but their relation to functional TMs have not been clarified. In DSC thermograms of TMs, the thermotropic phase transitions of membranes, are dominated by reorganizations and denaturation of proteins and protein assemblies (Dobrikova et al. 2003), which evidently mask the thermally induced lipid phase transitions (cf. Harańczyk et al. 1995, Krumova et al. 2008a).

In contrast, molecular spectroscopic techniques, while being less sensitive (or insensitive) to the long-range order of the lipid molecules, provide information about the physico-chemical environments and molecular motions in lipid-containing domains with different lipid packing orders. In particular, ^{31}P-NMR spectroscopy, which is based on different motional abilities of bulk phospholipid molecules has been shown to be capable of detecting different lipid phases contained in artificial lipid assemblies and native membranes (Cullis and de Kruijff 1979, Harańczyk et al. 1995, Krumova et al. 2008a, Afonso et al. 2016). Lipophilic fluorescent probes, such as the merocyanine 540 (MC540), also carry valuable information about different molecular environments of lipids. The spectral features of MC540 depend on the local dielectric constant (Lelkes and Miller 1980) and its emission spectra and fluorescence lifetimes are sensitive to variations in the packing and spacing of the lipid molecules (Williamson et al. 1983, Yu and Hui 1992, Stillwell et al. 1993). By this means, MC540 is well suited to characterize the heterogeneity in the molecular environments of lipid molecules and thus, indirectly, their polymorphic phase behavior. Indeed, steady-state and time-resolved fluorescence spectroscopy of MC540 in TMs revealed pronounced variations in the different fluorescence lifetime components upon elevating the temperature (Krumova et al. 2008b), an effect which strongly depended on the MGDG/DGDG ratio of the sample (Krumova et al. 2010) (Figure S2).

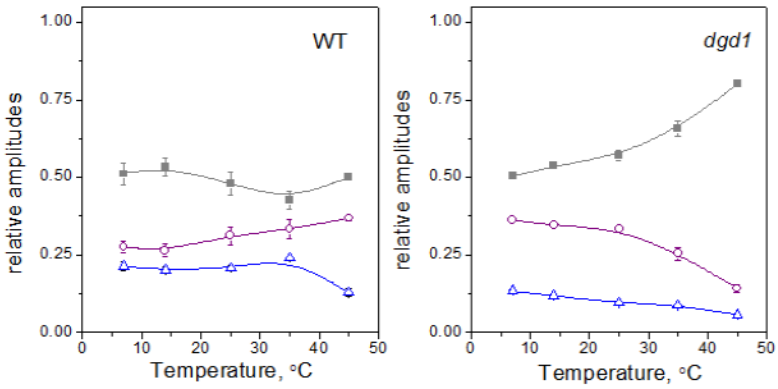

Figure S2: Temperature dependence of the three different lifetime components of MC540 in wild-type and DGDG deficient (*dgd1*) *Arabidopsis* TMs. These investigations revealed different temperature dependences of the different lipid phases / different physico-chemical environments of lipid molecules in the WT and mutant TMs. (For further details, see (Krumova et al. 2010); the figure is based on data of this publication.)

Reversible changes were also observed upon the addition of 2 M sucrose to isolated MC540 stained TMs (Kotakis et al. 2018). In general, it must be noted, however, that the currently available data do not allow us for assigning the fluorescence spectral and/or lifetime components of MC540 to lipid phases detected by ^{31}P-NMR spectroscopy; i.e. MC540 cannot be used for fingerprinting of individual lipid phases. Nevertheless, it is proposed that MC540 and other lipophilic dyes can be used to reveal further details of the heterogenous lipid environments in TMs.

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