Chloroplast membrane lipid remodeling protects against dehydration by limiting membrane fusion and distortion

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2021

Chng, C., Wang, K., Ma, W., Hsia, K. J. & Huang, C. (2021). Chloroplast membrane lipid remodeling protects against dehydration by limiting membrane fusion and distortion. Plant Physiology, 188(1), 526-539. https://dx.doi.org/10.1093/plphys/kiab512

https://hdl.handle.net/10356/153324

https://doi.org/10.1093/plphys/kiab512

Supplemental Information

Chloroplast membrane lipid remodeling protects against dehydration by limiting membrane fusion and distortion

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Supplemental Note S1: Derivation of the relationship between hydration level and fraction of membrane area occupied by membrane stalks

We consider two bilayer membranes of area A_0 initially separated by a layer of water of thickness d_0. The number of water molecules in the water layer is given by,

$$N_w = \frac{A_0 d_0}{v_0} \quad \text{(S1)}$$

where v_0 is the effective volume occupied by each water molecule. Under standard laboratory conditions of temperature (T = 298 K) and pressure (P = 1 atm), v_0 = 0.03 nm^3. The number of lipid molecules facing the water layer is given by

$$N_L = \frac{2A_0}{a_0} \quad \text{(S2)}$$

where a_0 is the average area/lipid over the two leaflets. The hydration level is defined as the ratio of water molecules in the water layer to the number of lipid molecules facing the water layer: $h = N_W/N_L$. Combining Eqns. (S1) and (S2) leads to a relationship between the initial water layer thickness and the hydration level, which is presented as Eqn. (3) in the main text.

When stalks form, the migration of lipids into the stalks causes a reduction of the membrane area from A_0 to A (Fig. 4C). Due to the conservation of water volume, the thickness of the water layer in the non-stalk regions increases from d_0 to d. We postulate that, after d reaches a critical value d_c, no further stalks will form. Hence, in the steady state with no further formation of stalks, $d = d_c$ and the volume of the water layer can be estimated as: $V = (A - A_s)d_c$, where A_s is the total cross-sectional membrane area occupied by stalks in the inter-membrane region. Assuming conservation of water in the inter-membrane region gives:

$$(A - A_s)d_c = A_0 d_0 \quad \text{(S3)}$$

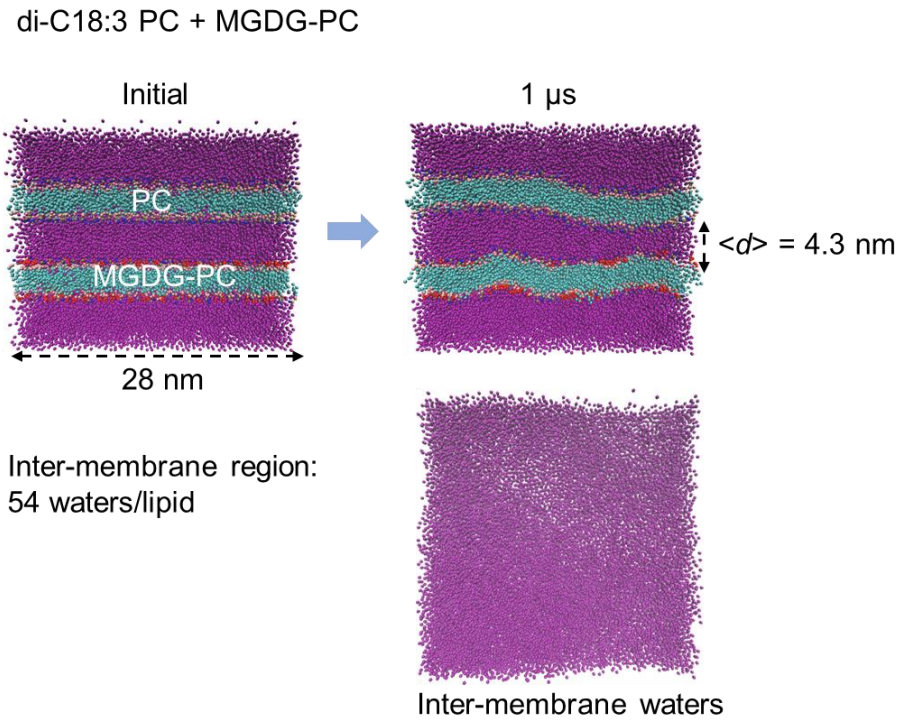
Rearranging Eqn. (S3) leads to Eqn. (4) in the main text.

Supplemental Note S2: Stalk formation facilitates the lipid exchange between the two bilayers

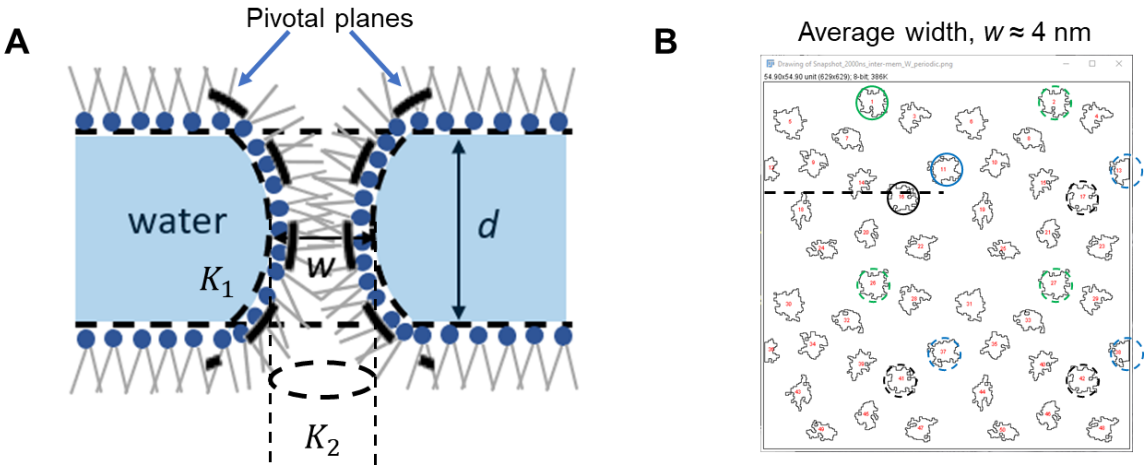
We have observed the mixing of monogalactosyl-diacylglycerol (MGDG) and digalactosyl-diacylglycerol (DGDG) lipids from the lower membrane into the upper membrane upon the formation of inter-membrane stalks. To quantify the migration of MGDG and DGDG lipids, we have monitored the evolution of the fractional change of MGDG and DGDG in the upper bilayer (Supplemental Fig. S5). Before membrane stalks are formed, galactolipids are absent in the upper bilayer. In the MGDG-containing system at low hydration levels of 13 and 15 waters/lipid, MGDG lipids start migrating into the upper bilayer almost immediately due to the formation of a large amount of membrane stalks and reaches a steady-state fraction (relative to the number of MGDG in the upper leaflet of the lower bilayer before stalk formation) of about 47% after

just 0.25 μs (Supplemental Fig. S5A). This result suggests that about half of the MGDG in the upper leaflet of the lower bilayer has migrated into the upper bilayer. At the higher hydration level of 17 waters/lipid, the migration of MGDG lipids starts after 0.2 μs but reaches a similar fraction at 1 μs. At the even higher hydration level of 24 waters/lipid, migration starts only after 0.8 μs due to delayed stalk formation. The rate of inter-membrane lipid migration is closely related to the kinetics of stalk formation, with stalks forming earlier at the lower hydration levels. In contrast, the migration of DGDG lipids in the DGDG-containing system occurs at a relatively slower rate (Supplemental Fig. S5B). At the lowest hydration level of 12 waters/lipid, the migration of DGDG lipids reaches a similar steady state value of 50% as for MGDG-containing systems but took twice as long time. At higher hydration levels, the rate of migration is much slower and the steady-state fraction of DGDG decreases with increase in the hydration level.

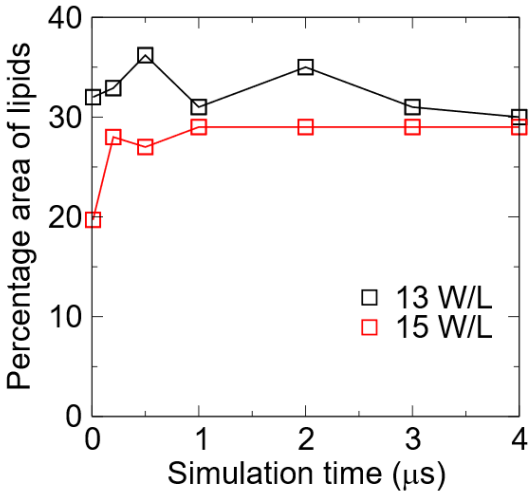
Supplemental Figures:



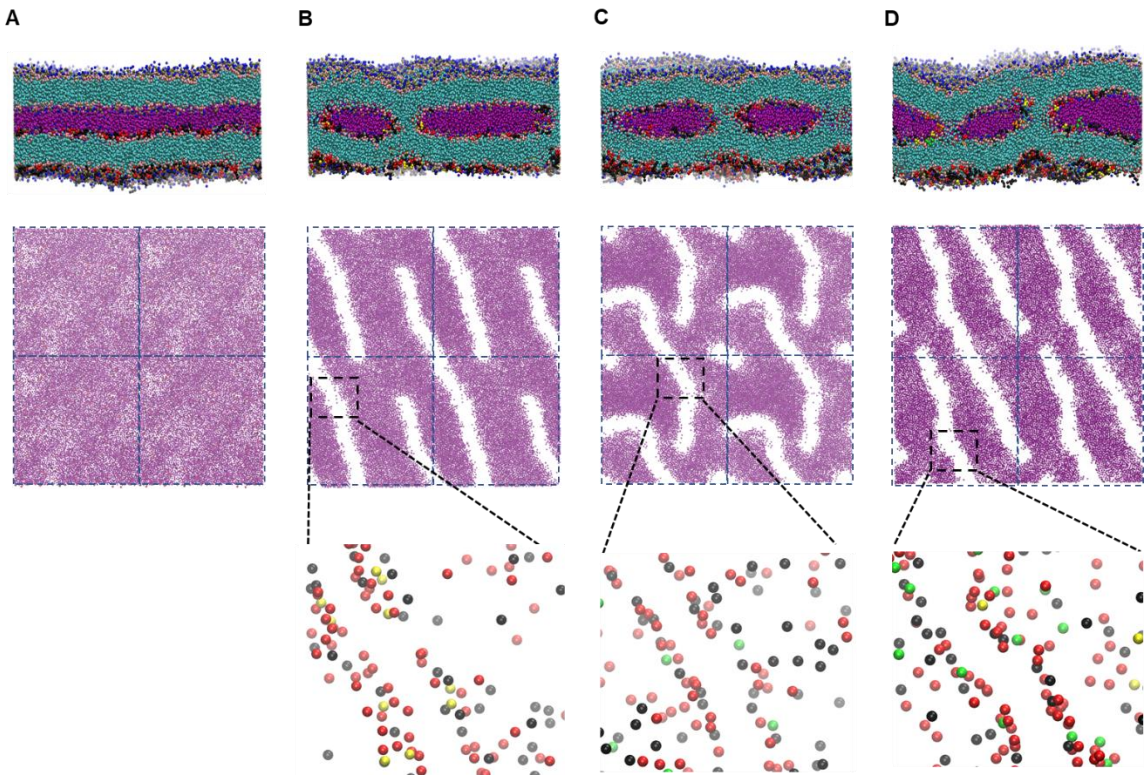
Supplemental Figure S1. Molecular dynamics simulation of a bi-lamellar membrane system comprising of a phosphatidylcholine (PC) upper bilayer membrane and a mixed monogalactosyl-diacylglycerol (MGDG)-PC (50:50) lower lipid bilayer. The two bilayers are separated by an initial distance of 4 nm. Both the PC and MGDG lipids have C18:3 fatty acid tails. The 4 nm separation is shown here to be sufficiently large to prevent membrane fusion from occurring across the gap within the simulation time of 1 μs. The bottom right figure shows that the inter-membrane water layer is not punctuated by membrane stalks.



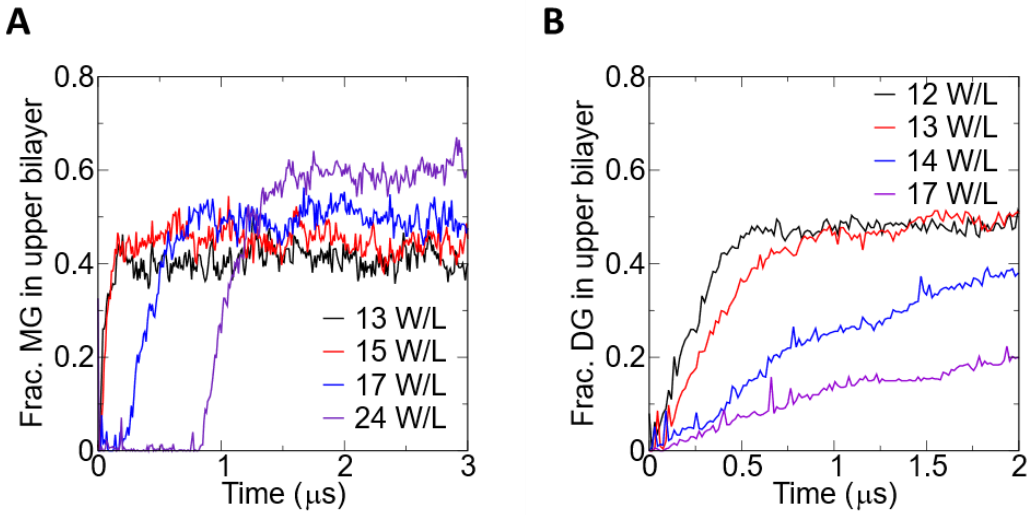
Supplemental Figure S2. Estimation of the principal curvatures for monolayer in rounded stalks formed by monogalactosyl-diacylglycerol (MGDG) or digalactosyl-diacylglycerol (DGDG). A, Schematic illustration of the side-view of a rounded stalk with inter-membrane separation *d* and stalk width at the narrowest (middle) point *w*. Pivotal planes are located at a distance of $l - z_0$ below the head-groups, where l is the length of a lipid and z_0 is the location of the pivotal plane from the bilayer mid-plane. K_1 and K_2 are the two principal curvatures. B, Screenshot of ImageJ window showing identified rounded stalks in the inter-membrane region from the snapshot of the DGDG 12 waters/lipid simulation domain at 2 μs. The domain has been replicated in each direction via periodic boundaries. Three examples of rounded stalks (in solid circles) and their periodic images (in dashed circles) are highlighted.



Supplemental Figure S3. Time evolution of the percentage area of inter-membrane region occupied by lipid stalks for monogalactosyl-diacylglycerol (MGDG)-containing systems at 13 and 15 waters/lipid (W/L).



Supplemental Figure S4. Effect of the minor lipid species on membrane stalk formation in systems containing Arabidopsis wild-type freeze-treated (WT-FT) chloroplast outer envelope membrane model and its variants. A-D, Cross-sectional (*top*) and top (*middle*) views of the coarse-grained bi-lamellar membranes with phosphatidylcholine (PC) lipids in the upper bilayer membrane and Variant 1 (A), Variant 3 (B), Variant 4 (C) or WT-FT (D) bilayer membrane as the lower one (inter-membrane hydration level of about 22 waters/lipid) after 1 μs of molecular dynamics simulation. In the cross-sectional views, water particles above the upper bilayer and below the lower bilayer are omitted for clarity. Head-group particles are colored as follows: monogalactosyl-diacylglycerol (MGDG) in red, digalactosyl-diacylglycerol (DGDG) in black, sulfoquinovosyl diacylglycerols (SQDG) in yellow, tri-galactosyl-diacylglycerol (TGDG) in orange, tetra-galactosyl-diacylglycerol (TeDG) in green, and PC or phosphatidylglycerols (PG) in blue. The glycerol groups of all lipids are shown in pink and the fatty acid tails in cyan. In the top views, only the inter-membrane water molecules are shown, where empty regions (white) are where the two membranes are fused. The system is replicated along both X and Y directions across periodic boundaries to better reveal the long-range patterns of the fused regions. Close-up views (*bottom*) of the top view of the inter-membrane region show the distribution of the lipids in the two leaflets facing the inter-membrane region. Only one of the glycerol particles (GL1) is shown for each lipid, with those of MGDG in red, DGDG in black, SQDG in yellow and PG in green. PC lipids are omitted for clarity purpose.



Supplemental Figure S5. Time evolution of the fraction of (A) monogalactosyl-diacylglycerol (MGDG) and (B) digalactosyl-diacylglycerol (DGDG) lipids in the upper bilayer for MGDG- and DGDG-containing systems, respectively, at different hydration levels indicated by waters/lipid (W/L).