

## *-Supplementary Information-*

### **The non-bilayer lipid MGDG and the major light-harvesting complex (LHCII) promote membrane stacking in supported lipid bilayers**

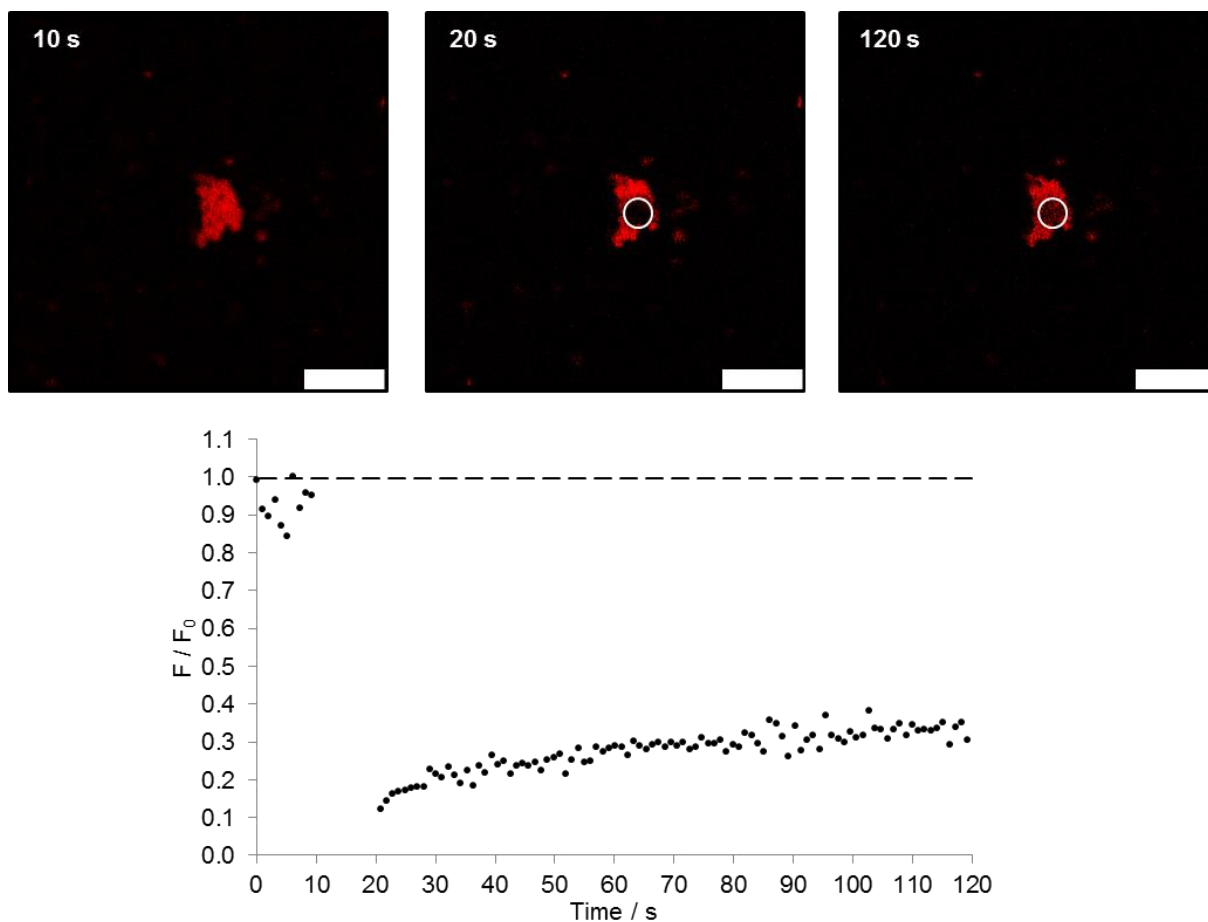
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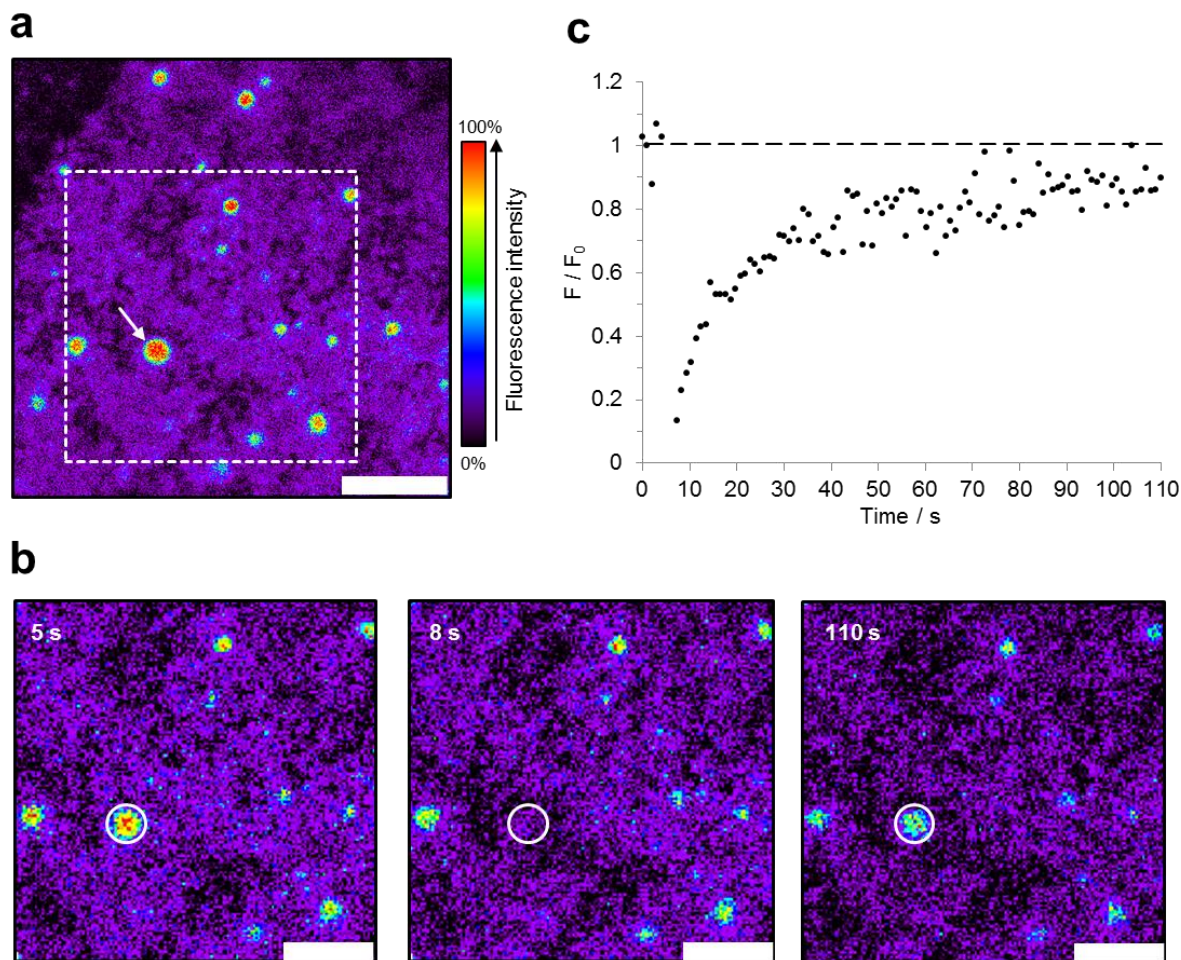
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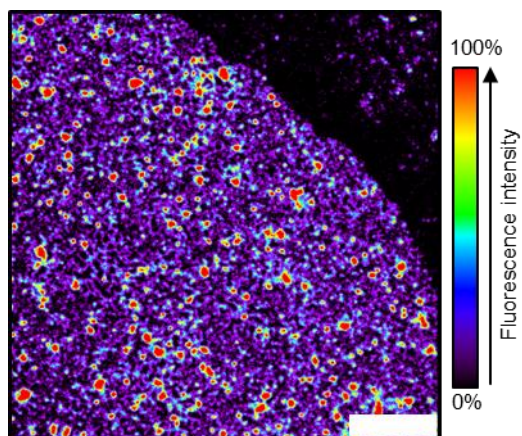
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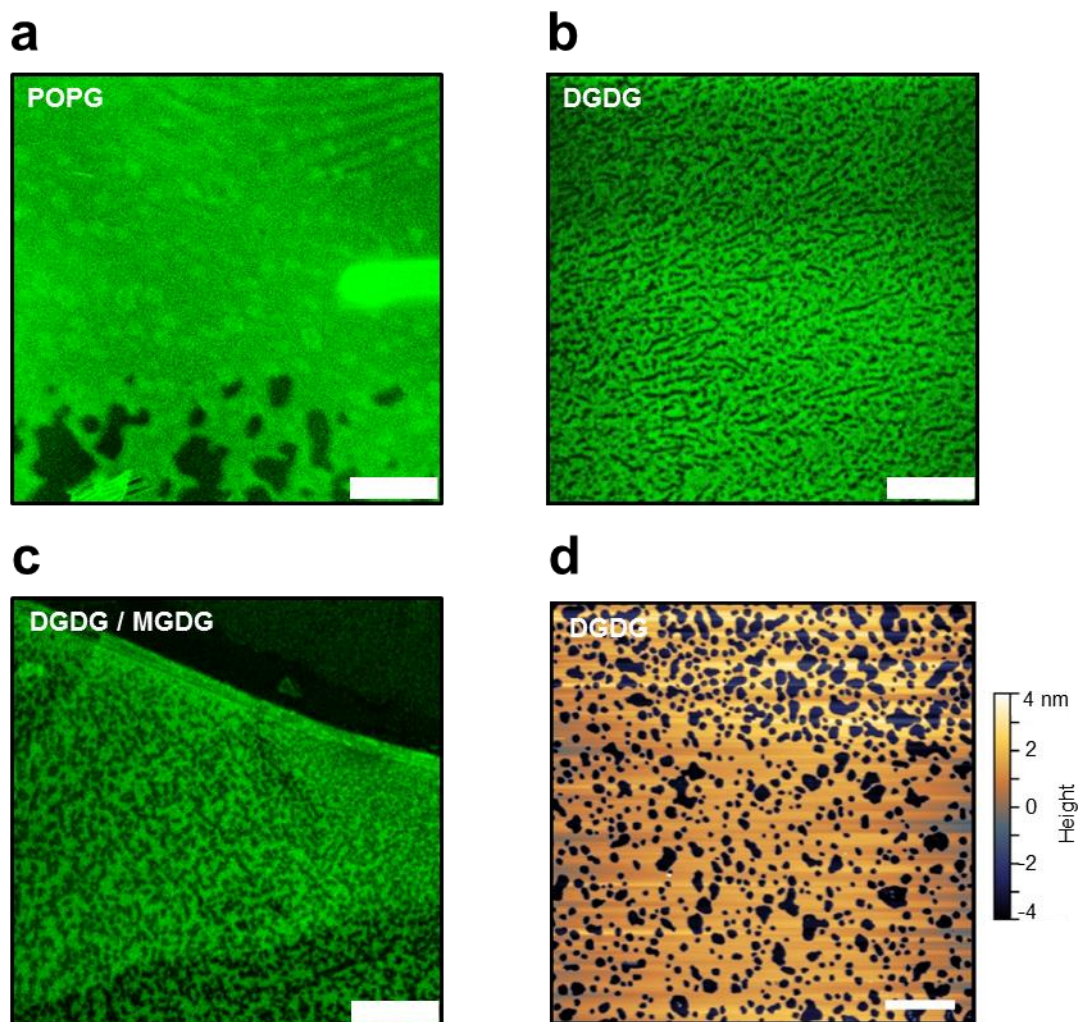
**Figure S1** FRAP experiment on an isolated membrane patch formed by spreading LHCII-containing SUVs at POPG/DGDG = 1:1 on APTES-coated coverglass. A series of fluorescence micrographs (**top**) before (10 s), immediately after (20 s), and 2 min after bleaching (120 s) shows bleaching within the region of interest (white circle). As monitored by the time-resolved relative fluorescence intensity  $F/F_0$  (**bottom**), almost no fluorescence recovery occurs after bleaching since the membrane patch is not surrounded by a supported bilayer membrane. Scale bars: 10  $\mu\text{m}$ .



**Figure S2** FRAP experiment on a membrane stack formed by spreading LHCII-containing SUVs at DGDG/MGDG = 2:1 on mica. **(a)** The presence of MGDG in DGDG-SLBs provokes the formation of membrane stacks with high fluorescence signals (red intensity) on the flat lipid bilayer (purple intensity); see also **Fig. 6c**. **(b)** A series of fluorescence micrographs within the dotted section in **(a)** before (5 s), immediately after (8 s), and 110 s after bleaching shows complete bleaching of such a membrane stack (arrow in **(a)**). **(c)** Almost full recovery (~90%) within the region of interest (white circle) as monitored by the time-resolved relative fluorescence intensity  $F/F_0$  demonstrates that the different layers of the stack are connected with one another and fully connected to the surrounding bilayer. Scale bars: **(a)** = 5  $\mu\text{m}$ , **(b)** = 3  $\mu\text{m}$ .



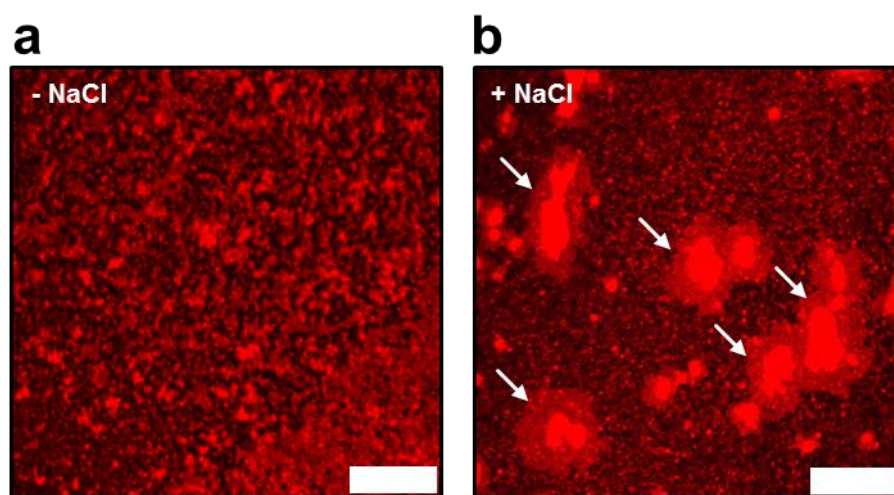
**Figure S3** Fluorescence micrograph of an SLB with several high-intensity stacks formed by spreading LHCII-containing DGDG/MGDG = 2:1-SUVs on mica. For MGDG-containing SLBs up to ~20% of the membrane stacks exceed the fluorescence emission detection limit (red intensity; see **Fig. 6c**) within a certain section of the sample. Scale bar: 10  $\mu\text{m}$ .



**Figure S4** Fluorescence micrographs (**a-c**) and AFM topograph (**d**) of SLBs formed by spreading SUVs containing TopFluor-GC at different thylakoid lipid compositions on mica. (**a**) POPG vesicles form bilayer areas of complete surface coverage that can be interrupted by gaps of a few  $\mu\text{m}$ . DGDG (**b**) and DGDG/MGDG = 2:1 vesicles (**c**) form partially connected bilayer patches as confirmed by



topographic imaging at 5x magnification for DGDG (**d**). Note that in (**a-d**) there are no membrane stacks detectable on top of the bilayer. Scale bars: (**a-c**) = 10  $\mu\text{m}$ ; (**d**) = 1.5  $\mu\text{m}$ .



**Figure S5** Fluorescence micrographs of SLBs formed by spreading LHCII-containing DGDG-SUVs on mica affected by NaCl. (**a**) In the absence of NaCl partially connected bilayer patches and small membrane stacks (bright spots) are formed (see also **Fig. 6b**). (**b**) Spreading at 200 mM NaCl provokes the formation of stacks of significantly larger diameters (up to 10  $\mu\text{m}$ ) in the center of the bilayer patches (white arrows). Scale bars: 10  $\mu\text{m}$ .