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Visualization of chlorophyll-protein complexes in thylakoid and artificial membranes

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Thylakoid membranes in chloroplasts of higher plants are sophisticated assemblies of chlorophyll-protein complexes (CP), lipids and free carotenoids, organized into two distinct domains: grana arranged in stacks of appressed membranes and non-appressed membranes consisting of stroma thylakoids and margins of granal stacks. CP complexes are organized hierarchically and spatially segregated in supercomplexes and megacomplexes. The LHCII-PSII supercomplexes are present in grana regions while LHCI-PSI supercomplexes are localized in unstacked thylakoids [1]. The lipids phase of thylakoids is formed mainly by monogalactosyldiacylglycerol (50% of the total lipid content), digalactosyldiacylglycerol (~30%) and sulfoquinovosyldiacylglycerol (~5-12%) as well as phosphatidylglycerol (~5-12%). These lipids not only form the bilayer structure of membranes but also stabilize the CP complexes and play an important role in forming uniquely curved thylakoids structure [2].

Spatial network of thylakoid membranes is investigated by electron microscopy (EM) and electron tomography (ET) [3]. Previously, based on confocal laser scanning microscopy (CLSM) images of chlorophyll (Chl) fluorescence, attributed mainly to LHCII-PSII [4,5], we constructed three-dimensional (3D) computer models of the whole chloroplasts in nearly intact state (*in situ*) [1]. We also presented 3D models of chloroplasts with different thylakoid stacking [6].

Now we present the improved 3D models of Mg^{2+}-dependent thylakoid stacking based on high numbers of CLSM images. As shown in Figure 1A at low magnesium ions concentration the Chl fluorescence exhibits wide dispersion within chloroplasts. Furthermore the 3D models revealed that grana stacks are unfolded and LHCII-PSII migrated to stroma thylakoids (Figure 1B-D). At stacked conditions Chl fluorescence is condensed (Figure 1E) and grana regions are clearly separated and merge only at the edge of stacks (Figure 1F-H). Furthermore, the distribution of PSI and PSII in thylakoids is analyzed by specific excitation of chlorophyll *a* and *b* species as well as by selective detection of fluorescence emission from both photosystems.

The arrangements of CP complexes in relations to lipid phase is present in LHCII-containing model membranes. Red fluorescence is attributed to LHCII (Figure 2A), while the green fluorescence is related to lipid phase (Figure 2B). As shown in Figure 2C (co-localization of fluorescence emissions) the LHCII complexes are incorporated in lipid multilayer similarly to native thylakoid membranes.

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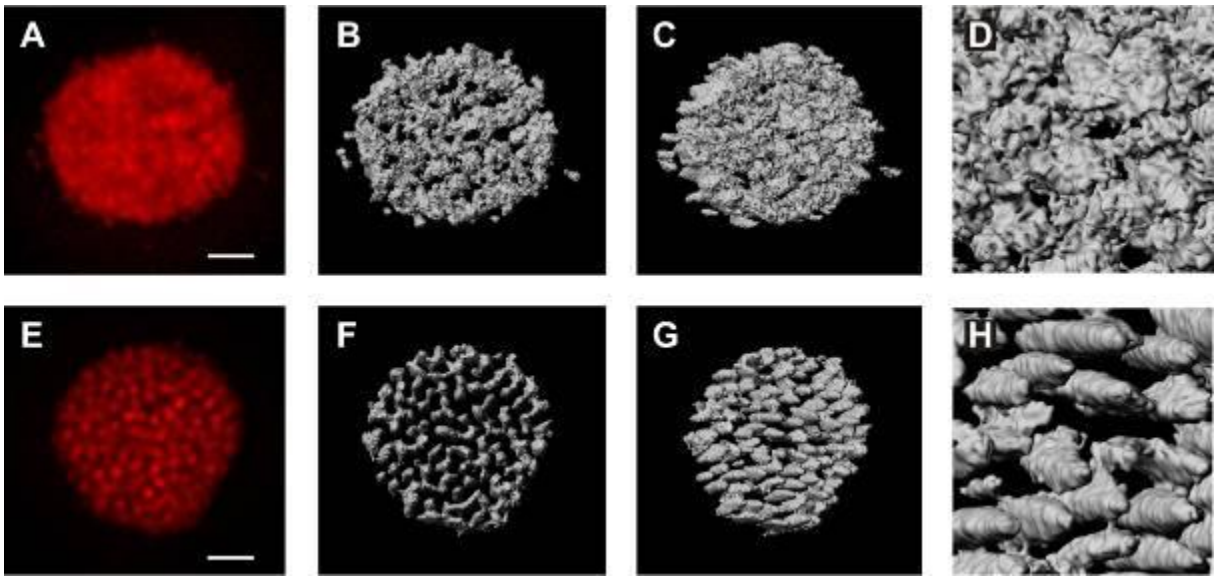

Figure 1. CLSM images (A, E) and computer models (B- D, E-G) of pea intact chloroplast incubated without (A-D) and in presence of 6 mM $MgCl_2$ (E-H). Scale bar = 2 µm

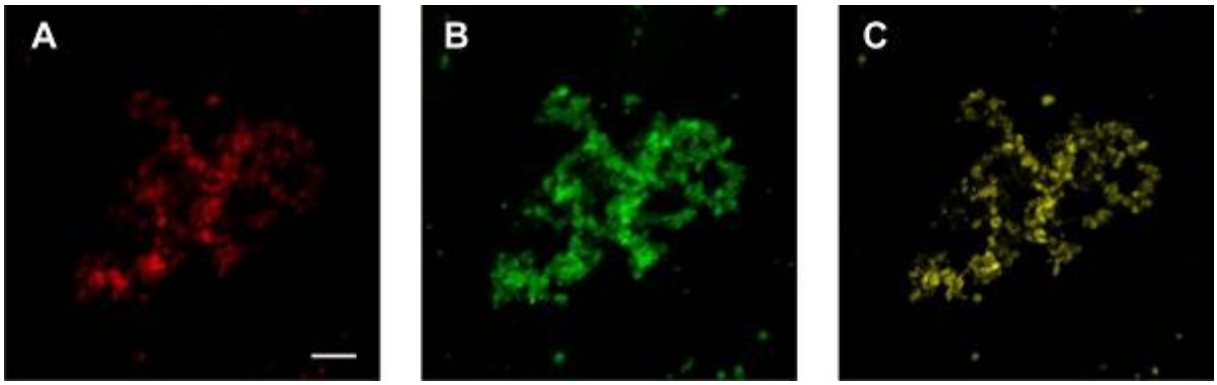

Figure 2. CLSM image of LHCII-containing model membranes stained with 3,3′-dioctadecyloxacarbocyanine perchlorate ($DiOC_{18}(3)$) membrane lipid dye. Red fluorescence of LHCII-bound chlorophylls (A), green fluorescence of $DiOC_{18}(3)$ in membranes (B) and co-localization analysis (C). Bar = 5 µm.