



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/151343/>

Version: Accepted Version

---

**Article:**

Wood, W.H.J., Barnett, S.F.H., Flannery, S. et al. (2019) Dynamic thylakoid stacking is regulated by LHCII phosphorylation but not its interaction with PSI. *Plant Physiology*, 180 (4). pp. 2152-2166. ISSN: 0032-0889

<https://doi.org/10.1104/pp.19.00503>

---

© 2019 American Society of Plant Biologists. This is an author-produced version of a paper subsequently published in *Plant Physiology*. Uploaded in accordance with the publisher's self-archiving policy.

**Reuse**

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

1 **Short title**

2 LHCII phosphorylation regulates thylakoid stacking

3

4 **Dynamic thylakoid stacking is regulated by LHCII**  
5 **phosphorylation but not its interaction with**  
6 **photosystem I**

7 William H. J. Wood+, Samuel F. H. Barnett+, Sarah Flannery, C. Neil Hunter and Matthew P. Johnson\*

8 Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield, S10 2TN, United Kingdom.

9 + = These authors contributed equally. \*Corresponding author [matt.johnson@sheffield.ac.uk](mailto:matt.johnson@sheffield.ac.uk)

10

11 **One-sentence summary**

12 Phosphorylation of light harvesting complex II controls chloroplast thylakoid membrane  
13 organization in response to changing light intensity.

14 **Author contributions**

15 W.H.J.W. and S.F.H.B. performed the 3D-SIM, EM experiments and data analysis. S. F. performed the  
16 gel electrophoresis and immunoblotting. M.P.J performed the fluorescence analysis. The work was  
17 conceived and written by M.P.J and C.N.H. All authors discussed the results and commented upon  
18 the manuscript.

19

20 **Abstract**

21 Grana stacking in plant chloroplast thylakoid membranes dynamically responds to the light  
22 environment. These dynamics have been linked to regulation of the relative antenna sizes of  
23 photosystems I and II (PSI and PSII) (state transitions), the PSII repair cycle, and the regulation of  
24 photosynthetic electron transfer. Here, we used 3D structured illumination microscopy (3D-SIM), a  
25 subdiffraction-resolution fluorescence imaging technique, to investigate the light-intensity  
26 dependence, kinetics, reversibility, and regulation of dynamic thylakoid stacking in spinach

27 (*Spinacia oleracea*) and *Arabidopsis* (*Arabidopsis thaliana*). Low-intensity white light (150  $\mu\text{mol}$   
28 photons  $\text{m}^{-2} \text{s}^{-1}$ ) behaved similarly to light preferentially exciting PSII (660 nm), causing a reduction  
29 in grana diameter and an increased number of grana per chloroplast. By contrast, high-intensity  
30 white light (1000  $\mu\text{mol}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ), darkness, and light preferentially exciting PSI (730 nm)  
31 reversed these changes. These dynamics occurred with a half-time of 7-8 minutes and were  
32 accompanied by state transitions. Consistent with this, the dynamics were dependent on STN7  
33 (light harvesting complex II (LHCII) kinase) and TAP38 (LHCII phosphatase), which are required for  
34 state transitions, but were unaffected by the absence of STN8 (PSII kinase) or PBCP (PSII  
35 phosphatase). Unlike state transitions, however, thylakoid stacking dynamics did not rely on the  
36 presence of the LHCI and PSAL phospho-LHCII binding sites on PSI. Since oligomerization of  
37 thylakoid curvature protein (CURT1A) was unaffected by the absence of STN7 or TAP38, we  
38 conclude that the primary determinant of dynamic thylakoid stacking is LHCII phosphorylation.

## 39 Introduction

40 The chloroplast thylakoid membrane is the site of the light reactions of photosynthesis,  
41 which use solar energy to drive coupled electron and proton transfer reactions, generating NADPH  
42 and ATP for CO<sub>2</sub> fixation in the surrounding stroma. The thylakoid is spatially divided into the  
43 stacked grana regions and the interconnecting unstacked stromal lamellae regions, and encloses an  
44 aqueous space known as the lumen (Paolillo, 1970; Mustardy and Garab, 2003). The grana are  
45 comprised of stacks of circular disc membranes with an average size of ~0.4-0.5 μm, with the  
46 stromal lamellae membranes forming elongated sacs that wrap helically around the grana stacks  
47 (Shimoni et al., 2005; Daum et al., 2010; Austin and Staehelin, 2011; Engel et al., 2015; Kowalewska  
48 et al., 2016). The photosynthetic complexes that perform the light reactions are heterogeneously  
49 distributed amongst these two regions with photosystem II (PSII) and its associated light harvesting  
50 complex (LHCII) predominantly located in the grana stacks, while the ATP synthase and  
51 photosystem I (PSI) are found in the stromal lamellae (Andersson and Anderson, 1980). In contrast,  
52 cytochrome *b<sub>6</sub>f* (*cytb<sub>6</sub>f*) is found in both thylakoid domains (Cox and Andersson 1981; Albertsson et  
53 al., 1994; 2001; Johnson et al. 2014). Grana stacking is dependent on the presence of positively  
54 charged counterions to screen the net negative charge on the membrane, with experiments  
55 showing that thylakoids unstack *in vitro* in low-salt media and then spontaneously restack at Mg<sup>2+</sup>  
56 concentrations >1-2 mM or K<sup>+</sup> concentrations >100 mM, typical of those in the stroma (Staehelin,  
57 1976). The formation of grana is a highly cooperative process in which cations promote lateral  
58 interactions between PSII and LHCII complexes, leading to formation of domains containing PSII-  
59 LHCII supercomplexes and loosely attached LHCII trimers. Attractive van der Waals and electrostatic  
60 interactions between the flat stromal surfaces of PSII-LHCII macrodomains in neighbouring  
61 membranes then lead to the formation of the characteristic stacked grana structure (Barber, 1982;  
62 Chow et al., 1982; Day et al., 1984; Chow et al., 1991; Kiss et al., 2008). The bulky stroma-

63 protruding domains on PSI and ATP synthase ensure that these complexes are excluded from the  
64 stacked regions, partitioning into the surrounding stromal lamellae.

65 Grana structure has been found to be remarkably plastic on the timescale of minutes, with  
66 the diameter, number of layers and number of grana per chloroplast varying with light intensity and  
67 spectral quality (Rozak et al., 2002). In both spinach (*Spinacia oleracea*) and *Arabidopsis*  
68 (*Arabidopsis thaliana*), illumination in low light conditions ( $< 300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) consistently  
69 leads to a decrease in grana diameter and number of membrane layers per stack, and an increase in  
70 the number of grana per chloroplast compared to those under darkness, shade ( $< 20 \mu\text{mol photons}$   
71  $\text{m}^{-2} \text{s}^{-1}$ ), and far-red ( $> 700 \text{ nm}$ ) illumination conditions (Kyle et al., 1983; Rozak et al., 2002;  
72 Chaurtzman et al., 2008; Anderson et al., 2012; Pietrzykowska et al., 2014; Wood et al., 2018; Iwai  
73 et al., 2018). In contrast, reports detailing the effect of high-intensity light on grana size are more  
74 mixed. In spinach leaves, Rozak et al., (2002) reported that grana diameter and layers per stack  
75 increased at  $1500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  compared to low-light illumination, while a similar increase  
76 in stacking was seen in *Arabidopsis* leaves at  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  by Fristedt et al., (2009) and  
77 Schumann et al., (2017). At variance with these reports, Khatoon et al., (2009) found that grana  
78 stacking was reduced in isolated spinach thylakoids after treatment with  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$   
79 light, while Herbstova et al., (2012) and Puthiyaveetil et al., (2014) found  $1500\text{-}2000 \mu\text{mol photons}$   
80  $\text{m}^{-2} \text{s}^{-1}$  light reduced the grana diameter in *Arabidopsis* protoplasts and leaves. The functional  
81 significance of dynamic thylakoid stacking is still debated but several ideas have been advanced.  
82 Since grana prevent the 'spillover' of excitation energy through spatial separation of PSII and LHCII  
83 from the longer-wavelength energy trap PSI (Trissl and Wilhelm 1993), a change in grana size may  
84 facilitate regulation of the relative antenna sizes of the two photosystems via exchange of LHCII,  
85 the 'state transition' (Allen 2003; Bellafiore et al., 2005). Changes in grana size could also regulate  
86 the accessibility of photo-damaged PSII to the repair machinery that resides in the stromal lamellae

87 (Goral et al., 2010; Herbstova et al., 2012; Theis and Schroda, 2016). Finally, changes in grana size  
88 may affect the distribution of electron transfer complexes and the diffusion of mobile electron  
89 carriers between the two thylakoid domains, which may regulate the partition of electrons  
90 between photosynthetic linear and cyclic electron transfer pathways (LET and CET) (Vallon et al.,  
91 1991; Albertsson 2001; Wood et al., 2018; Johnson 2018).

92         Currently, two major views regarding the physical mechanism behind dynamic thylakoid  
93 stacking exist in the literature. In the first view, phosphorylation of LHCII and PSII weakens the  
94 lateral interactions between these complexes and promotes those with PSI at the PSAL/H/O (Lunde  
95 et al., 2000; Kouril et al., 2005; Pan et al., 2018) and LHCA1-4 subunits (Benson et al., 2015; Sathish-  
96 Yadav et al., 2017), causing a decrease in grana size (State II), with dephosphorylation promoting  
97 LHCII-LHCII and PSII-LHCII interactions and stacking (State I) (Kyle et al., 1983; Chaurtzman et al.,  
98 2008; Pietrzykowska et al., 2014; Wood et al., 2018). Since stacking is sensitive to the surface  
99 charge density on complexes within the grana, the additional negative charges introduced by  
100 phosphorylation alter the delicate balance of forces (Barber 1982; Puthiaveetil et al., 2017).  
101 Consistent with this view, mutants lacking the LHCII-specific TAP38 (PPH1) and PSII-specific PBCP  
102 phosphatases show constitutively reduced grana size compared to wild-type *Arabidopsis* (Samol et  
103 al., 2012; Armbruster et al., 2013; Iwai et al., 2018), while mutants lacking the STN8 and STN7  
104 kinases have constitutively larger grana (Fristedt et al., 2009; Herbstova et al., 2012; Samol et al.,  
105 2012; Armbruster et al., 2013; Iwai et al., 2018). An alternative view that has recently emerged is  
106 that thylakoid stacking dynamics may instead be regulated by the degree of oligomerization of the  
107 thylakoid curvature protein family (CURT1A-D), which induce membrane bending in the grana  
108 margins at the edges of the grana discs (Armbruster et al., 2013). The *curt1abcd* mutant showed  
109 extremely large (~1.3 µm) diameter pseudo-grana composed of only a few layers and lacking clearly  
110 defined margins, while plants overexpressing CURT1A (*oeCURT1A*) showed grana with a narrower

111 diameter ( $\sim 0.3 \mu\text{m}$ ) and with more layers than the wild type (Armbruster et al., 2013; Pribil et al.,  
112 2018). CURT1 oligomerization decreased as plants transitioned from low light to high light (Pribil et  
113 al., 2018). It was suggested that CURT1 oligomerization state and hence grana size may be  
114 regulated by post-translational modification e.g. phosphorylation by either STN7 or STN8 (Pribil et  
115 al., 2014).

116 In this study, we used 3D-structured illumination microscopy (3D-SIM) and a range of  
117 supporting biochemical and spectroscopic methods to investigate the light-intensity dependence,  
118 speed and reversibility of the stacking changes and their dependence on CURT oligomerization, and  
119 PSII and LHCII phosphorylation. 3D-SIM offers numerous advantages over the conventional method  
120 of using resin-exchanged, thin-section electron microscopy (EM) to investigate thylakoid  
121 ultrastructure, allowing us to follow the dynamic structural changes *in situ* using chlorophyll  
122 fluorescence with sub-diffraction ( $\sim 120 \text{ nm } x, y$  and  $\sim 320 \text{ nm } z$ ) resolution, an eightfold volumetric  
123 improvement compared to standard confocal imaging (Wood et al., 2018; Iwai et al., 2018). The  
124 application of 3D-SIM to a series of kinase and phosphatase mutants of *Arabidopsis* showed that  
125 phosphorylation of LHCII is the major factor regulating dynamic thylakoid stacking.

126

## 127 RESULTS

### 128 Low-intensity white light induces State II; high-intensity white light induces State I.

129 Spinach plants grown at a light intensity of  $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  under white LED bulbs with  
130 the spectrum shown in Fig. 1A were subjected to one of five different light conditions to assess  
131 their effect on state transitions, thylakoid protein phosphorylation and thylakoid macrostructural  
132 organization. The conditions were 1 hour of either (i)  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  730 nm light  
133 preferentially exciting PSI to provoke State I (PSI light), (ii)  $20 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  660 nm light  
134 preferentially exciting PSII to provoke State II (PSII light), (iii) darkness (dark), (iv)  $150 \mu\text{mol photons}$

135  $\text{m}^{-2} \text{s}^{-1}$  white LED light (low light) or (v)  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  white LED light (high light). The  
136 spectra of the PSII and PSI lights are also shown in Fig. 1A. We measured the effect of these five  
137 different treatments on the low-temperature (77K) fluorescence emission ratio of PSI and PSII (Fig.  
138 1B,C), an indicator of the relative antenna size of each photosystem. The ratio of the PSII  
139 fluorescence band at 685 nm relative to the PSI band at 735 nm increased in the order PSII light >  
140 low light > high light > dark > PSI light (Fig 1B and C, statistical analysis in Supplemental Table 1).  
141 Phospho-staining of the thylakoid proteins isolated from each set of plants revealed that  
142 phosphorylation of the LHCII subunits Lhcb1 and 2 was strongest in the PSII light > low light > high  
143 light > dark > PSI light. In contrast, the phosphorylation of the PSII D1 and D2 subunits followed a  
144 different pattern and decreased in the order PSII light > high light > low light > dark > PSI light (Fig.  
145 1D). Summing the total intensity of all the phospho-protein bands in each sample, with PSII light set  
146 to 100%, gave values of 32% for PSI light, 48% for darkness, 73% for low light and 59% for high light.  
147 Thylakoids from each set of plants were also subjected to digitonin-solubilisation, and the  
148 complexes released from the stromal lamellae region were separated by Blue-Native (BN) PAGE  
149 (Fig. 1E). The most obvious difference was in the PSI-LHCI-LHCII supercomplex band that was  
150 strongest in PSII light and lowest in PSI light. The PSI-LHCI-LHCII supercomplex was only present at a  
151 very low level in the dark and in high light, but was present at a much higher level in low light (Fig.  
152 1E). The only other major difference was a slight increase in the level of free LHCII trimers  
153 recovered in PSII light compared to the other treatments. The results of the biochemical analysis  
154 therefore reveal a consistent picture; low-intensity white light induces a condition similar to State  
155 II, with the phosphorylation of LHCII, appearance of the PSI-LHCI-LHCII supercomplex and decrease  
156 in the PSII/PSI fluorescence emission ratio. In contrast, high-intensity white light and darkness give  
157 a condition more similar to State I, with decreased LHCII phosphorylation and PSI-LHCI-LHCII  
158 supercomplex levels and increased PSII/PSI fluorescence emission ratio. PSII light appears to induce

159 both slightly stronger LHCII phosphorylation and PSII phosphorylation relative to low light, and this  
160 may explain the extra LHCII in the stromal lamellae and the decreased PSII/PSI fluorescence  
161 emission ratio (Fig. 1B-E).

162

163 **Low-intensity white light reduces grana size; high intensity white light increases grana size.**

164 Having defined the biochemical state induced in spinach plants by the five light treatments we next  
165 used 3D-SIM to image the native chlorophyll fluorescence of chloroplasts in spinach. For each light  
166 treatment, a representative chloroplast is shown rendered in 3-dimensions (Fig. 2A), as a z-  
167 projection (Fig. 2B) and as series of 125 nm z-slices through the chloroplast from top to bottom (Fig.  
168 2C). From the images, clear differences in the size and number of bright fluorescent spots, which  
169 correspond to grana, can be observed in the different samples. For each light condition we  
170 quantified the full-width half-maximum (FWHM) of the chlorophyll fluorescence signal from each  
171 granum, which provides a measure of the average granum diameter (Fig. 2D). The diameter was  
172 largest in high light, which had a mean FWHM of  $0.58 \pm 0.12 \mu\text{m}$ , and smallest in PSII light, which  
173 had a mean FWHM of  $0.36 \pm 0.06 \mu\text{m}$ . In summary, the grana diameters followed the pattern high  
174 light > dark = PSI light > low light = PSII light (Fig 2D, statistical analysis in Supplemental Table 2).  
175 Differences were also observed in the number of grana per chloroplast, which was highest in PSII  
176 light (with a mean of  $69 \pm 5$  grana per chloroplast) and lowest in dark (with a mean of  $47 \pm 6$  grana  
177 per chloroplast). Overall, PSII light = low light > dark = PSI light = high light (Fig. 2E, statistical  
178 analysis in Supplemental Table 3). Therefore, the SIM analysis revealed that low-intensity white  
179 light induces an ultrastructural organization of the thylakoid membranes that closely resembles  
180 State II, with a reduction in grana diameter and an increase in the number of grana per chloroplast.  
181 In contrast, high-intensity white light and darkness induce an ultrastructural state similar to State I  
182 with an increased grana diameter and fewer grana per chloroplast.

183 We next used thin-section EM to confirm the differences observed by SIM in chloroplast  
184 ultrastructure between plants treated with dark or low- or high-intensity white light. Three  
185 representative chloroplasts are shown for dark, low light, and high light (Fig. 3A). The measured  
186 grana diameter decreased by around 12% in low light compared to high light and darkness (Fig. 3B,  
187 statistical analysis in Supplemental Table 4). Using EM also allowed us to count the number of  
188 membrane layers per grana stack, which is beyond the resolution of SIM (Iwai et al., 2018). The  
189 mean number of membrane layers per grana stack was decreased from  $9.7 \pm 1$  in dark to  $7.6 \pm 1$  in  
190 low light (Fig. 3C, statistical analysis in Supplemental Table 5). In high light the number of  
191 membrane layers per stack was increased, compared to both dark and low light, with a mean value  
192 of  $13.6 \pm 2$  (Fig. 3C). We also quantified the total length of grana membranes, excluding the PSI-  
193 containing grana end membranes, as a percentage of the total length of grana + end membrane +  
194 stromal lamellae membranes in the EM images (Fig. 3D). The percentage of grana membrane was  
195 lower in low light compared to that in dark, while the percentage in high light was higher than that  
196 under either of these conditions (Fig. 3D, statistical analysis in Supplemental Table 6). The EM  
197 results were therefore consistent with the SIM analysis, with low-intensity white light leading to a  
198 decrease in grana size. Furthermore in low light there was an overall reduction in grana area  
199 compared to that in dark and high light, showing that the increased number of grana per  
200 chloroplast was not sufficient to compensate for the decrease in grana diameter. The EM also  
201 supported the SIM in showing that high-intensity white light induces an ultrastructural state more  
202 akin to State I. Interestingly, the EM suggested a slightly more extreme increase in stacking in high  
203 light compared to darkness.

204

205 **Changes in grana size are rapidly reversible with half times of around 10 minutes.**

206 We next sought to understand how rapidly the thylakoid stacking dynamics can occur and whether  
207 they are reversible on a short-timescale. Using SIM we quantified the change in the grana diameter  
208 and the number of grana per chloroplast as spinach plants transition from dark to low intensity  
209 white light, 0, 5, 10 and 60 minutes after the transfer. A single exponential fit of the data gave a  
210 half-time for the change of 8.8 minutes (Fig. 4A). The increase in the number of grana per  
211 chloroplast followed similar kinetics, implying the changes in diameter and increased number of  
212 grana per chloroplast are closely linked (Fig. 4B). Next we quantified the change in the grana  
213 diameter and the number of grana per chloroplast as spinach plants transition from low-intensity to  
214 high-intensity white light, 0, 5, 10 and 60 minutes after the transfer. The grana diameter increased  
215 with a half-time of 6.5 minutes (Fig. 4C), while the number of grana per chloroplast also decreased  
216 on a similar timescale (Fig. 4D). Finally, we quantified the change in the grana diameter and the  
217 number of grana per chloroplast as spinach plants transition from high-intensity to low-intensity  
218 white light, 0, 5, 10 and 60 minutes after the transfer. Here the grana diameter decreased and the  
219 number of grana stacks per chloroplast increased (Fig. 4E and F), with a half-time of 7.0 minutes. In  
220 the above studies the plants were adapted to each condition for 1 hour prior to the changing the  
221 light intensity, whereas in Fig. 4G we took a different approach to quantifying the change in grana  
222 diameter, with 20 minutes low light followed by 20 minutes high light to see if significant rapid and  
223 reversible changes can be observed. The results confirmed that dynamic thylakoid stacking is  
224 rapidly reversible on the timescale of minutes. We then tested how quickly the PSII/PSI low-  
225 temperature (77K) fluorescence emission ratio changed in comparison to the changes in grana  
226 stacking. As the plants transitioned from dark to low intensity white light, the PSII/PSI ratio  
227 decreased with a half-time of 1.1 minutes (Fig. 4H). Switching the plants to high intensity white light  
228 then increased the PSII/PSI emission ratio to a similar value to that seen in the dark with a half-time  
229 of 3.5 minutes (Fig. 4H). The thylakoid stacking dynamics therefore appear to occur on a slightly

230 slower timescale than the changes in photosystem antenna size associated with the state  
231 transition.

232

### 233 **Thylakoid stacking dynamics are STN7- and TAP38-dependent, but STN8- and PBCP-independent**

234 Next, we investigated the dependence of the thylakoid stacking dynamics on LHCII and PSII

235 phosphorylation in the *stn7*, *tap38*, *stn8* and *pbcp* mutants compared to wild-type Arabidopsis.

236 Using SIM we compared the chloroplast ultrastructure in plants adapted to 1 hour of dark, 1 hour

237 of low intensity white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), or 1 hour of high intensity white light ( $1000$

238  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) using the LED source shown in Fig. 1A (Fig. 5A, statistical analysis in

239 Supplemental Table 7). In the wild-type the grana diameter decreased in low light compared to that

240 in dark and high light conditions (Fig. 5B), as observed in spinach. In contrast, in the *stn7* mutant,

241 which lacks the LHCII kinase, the grana diameter was not significantly different between the three

242 conditions (Fig. 5C). Similarly in the *tap38* mutant, which lacks the LHCII phosphatase, the grana

243 diameter changed very little between dark and low light, but was larger in high light (Fig. 5D). In the

244 *stn8* mutant, which lacks the PSII kinase, the grana diameter was significantly smaller in low light

245 compared to that in dark and high light conditions (Fig. 5E), as in the wild-type. In line with this, in

246 the *pbcp* mutant, which lacks the PSII phosphatase, the grana diameter was also significantly

247 smaller in low light compared to that in dark and high light conditions (Fig. 5F). Indeed, phospho-

248 protein staining of thylakoid proteins separated by SDS-PAGE from wild-type, *stn7* and *tap38*

249 confirmed that although PSII phosphorylation is upregulated under high light this does not

250 compensate for the decrease in total protein phosphorylation arising from reduced LHCII

251 phosphorylation (Fig. 5G). Summing the total intensity of the phospho-protein bands in each

252 sample, with wild-type low light set to 100%, gave values of 71% for wild-type high light, 93% for

253 *tap38* low light and 95% for *tap38* high light, 56% for *stn7* low light and 54% for *stn7* high light. To

254 facilitate comparison of the actual difference in grana diameters in each condition between each  
255 mutant and the wild-type we constructed the heat maps shown in Fig. 5H, I and J. Grana diameter  
256 in the dark was clearly smaller in the two phosphatase mutants *tap38* and *pbcp* than in the wild-  
257 type, consistent with previous studies (Samol et al., 2012; Armbruster et al., 2013; Iwai et al., 2018)  
258 (Fig. 5H). In contrast, the grana diameter in the *stn7* mutant was clearly larger than that in all the  
259 other mutants in low light, with the wild-type showing smaller grana like *tap38* and *pbcp* (Fig. 5I). In  
260 high light, the *tap38* mutant stood out with significantly smaller grana size, while grana sizes of the  
261 wild-type and *stn7* were more similar (Fig. 5J). These results indicate that the thylakoid stacking  
262 dynamics depend principally on the activity of STN7 and TAP38 rather than that of STN8 and PBCP.

263

#### 264 **CURT oligomerization state is unaffected in the *stn7* and *tap38* mutants**

265 It was previously suggested that the oligomerization state of CURT1 proteins could regulate  
266 thylakoid stacking dynamics and that their post-translational modification by phosphorylation  
267 might be involved (Pribil et al., 2014; Pribil et al., 2018). In principle our results might be explained  
268 if STN7/ TAP38 also regulated CURT1 oligomerization. We tested this idea by assessing changes in  
269 the oligomerization state of CURT1A in wild-type, *stn7* and *tap38* plants in dark, low light and high  
270 light. Thylakoid proteins were solubilized in digitonin and separated by BN-PAGE (Fig. 6A). In the  
271 wild-type, the major difference between the different light conditions was in the amount of the PSI-  
272 LHCI-LHCII supercomplex band, which increased in low light and was reduced in dark and high light  
273 conditions. In *stn7* in contrast the PSI-LHCI-LHCII supercomplex band was absent in all conditions,  
274 while in *tap38* it was constitutively present in all conditions though at a higher level in low light and  
275 high light compared to the dark (Fig. 6A). Immunoblotting against the CURT1A protein revealed the  
276 presence of both monomeric and oligomeric forms of the protein throughout the native gel in wild-  
277 type, *stn7* and *tap38*, and in all conditions (Fig. 6B). A slight decrease in CURT1A oligomerization

278 level was observed in low light in the wild-type compared to darkness and high light. However,  
279 since no major differences in CURT1A oligomerization levels were observed between the mutants,  
280 this process is either not involved in dynamic thylakoid stacking or is insufficient in the absence of  
281 concomitant changes in LHCII phosphorylation.

282         Given that CURT1 oligomerization changes alone are insufficient for dynamic thylakoid  
283 stacking, it was interesting to test if changes in LHCII phosphorylation alone are sufficient. To this  
284 end we used SIM to investigate chloroplast thylakoid ultrastructure in the *curt1abcd* and *oeCURT1A*  
285 mutants adapted to 1 hour of dark, 1 hour of low intensity white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ),  
286 or 1 hour of high intensity white light ( $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) using the LED source shown in  
287 Fig. 1A (Fig 6C). As expected, much larger pseudo-grana were observed in the *curt1abcd* mutant  
288 compared to the wild-type, with an average diameter of  $1.35 \pm 0.38 \mu\text{m}$ , while in the *oeCURT1A* the  
289 grana diameter was just  $0.3 \pm 0.05 \mu\text{m}$  in the dark. The extremely small grana in the *oeCURT1A* had  
290 significantly smaller diameter in low light compared to high light and dark (Fig.6D, statistical  
291 analysis in Supplemental Table 7). Given the lack of regular grana structure in the *curt1abcd* plants  
292 we were unable to undertake a robust quantitative analysis of grana FWHM for this mutant, but  
293 qualitatively in low light the pseudo-grana structures became even less well defined compared to  
294 those in dark and high light, suggesting that LHCII phosphorylation, which is induced normally in  
295 this mutant (Armbruster et al., 2013), leads to an almost complete unpacking of the remaining  
296 appression (Fig. 6C).

297

298 **Thylakoid stacking dynamics do not require the presence of specific phospho-LHCII binding sites**  
299 **on PSI.**

300 The common dependence of the stacking dynamics on LHCII phosphorylation leads to the question  
301 of whether they are functionally distinct from state transitions. We investigated this further in the

302 *psaL* and *lhca4* *Arabidopsis* mutants, which are deficient in state transitions (Fig 7A), as previously  
303 reported (Lunde et al., 2000; Benson et al., 2015). In *psaL*, the lack of the phospho-LHCII binding  
304 site on PSI precludes formation of the PSI-LHCI-LHCII supercomplex (Lunde et al., 2000; Kouril et al.,  
305 2005; Pan et al., 2018) that is seen in the wild-type in low light (Fig. 7B). In *lhca4*, the reasons for  
306 the lack of state transitions are less clear; despite the lack of LHCI, a low molecular weight PSI-LHCII  
307 complex is still formed in low light, which disappears in high light (Fig. 7B). It has been suggested  
308 that state transitions also involve digitonin-labile LHCII binding sites on PSI-LHCI provided by the  
309 LHCA subunits, that therefore do not show up on the BN-PAGE analysis (Fig. 7B) (Benson et al.,  
310 2015; Bell et al., 2015; Satish-Yadav et al., 2017). We investigated the chloroplast thylakoid  
311 ultrastructure in the *psaL* and *lhca4* mutants using SIM in plants adapted to 1 hour of dark, 1 hour  
312 of low-intensity white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), or 1 hour of high-intensity white light ( $1000$   
313  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) using the LED source shown in Fig. 1A (Fig. 7C). In both the *psaL* and *lhca4*  
314 mutants, the grana diameter was slightly larger than that in the wild-type in the dark, consistent  
315 with previous reports (Lunde et al., 2003; Bressan et al., 2018) (Fig. 7D and E, Statistical analysis in  
316 Supplemental Table 7). However, in low light, both the *psaL* and *lhca4* mutants showed a clear  
317 decrease in grana diameter with a similar amplitude to that observed in the wild-type, consistent  
318 with the normal levels of LHCII phosphorylation reported in these mutants (Lunde et al., 2000;  
319 Benson et al., 2015); both mutants also showed an increased grana diameter in high light ( $1000$   
320  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Fig. 7B and C). The results therefore suggest that dynamic thylakoid stacking,  
321 unlike state transitions, do not depend upon the presence of specific phospho-LHCII binding sites  
322 on PSI.

323

## 324 **DISCUSSION**

### 325 **Changes in white light intensity induce changes in thylakoid stacking.**

326 There are several reports in the literature of white light dependent changes in thylakoid stacking  
327 (Rozak et al., 2002; Anderson et al., 2012; Herbstova et al., 2012; Schumann et al., 2017; Wood et  
328 al., 2018). Yet the timescales involved, their similarity to changes provoked by monochromatic light  
329 preferentially exciting PSI or PSII and their dependence on various regulatory factors associated  
330 with grana architecture have not been clearly defined. In this study we have shown that in both  
331 spinach and *Arabidopsis* large changes in grana size occur as a result of varying white light intensity.  
332 Low-intensity white light caused a reduction in grana diameter and the number of membrane  
333 layers per grana, while increasing the number of grana per chloroplast. The extents of these  
334 stacking changes were similar to those produced by monochromatic light preferentially exciting PSII  
335 (660 nm), which induces State II. In contrast, high light and darkness reversed these changes  
336 leading to larger diameter grana with more membrane layers but fewer grana per chloroplast,  
337 similar to the effect of PSI light (730 nm) that induces State I. Our results therefore agree with those  
338 of Pietrzykowska et al., (2014) and Iwai et al., (2018) that showed PSII light produces similar  
339 changes in thylakoid stacking to low-intensity white light and differ from the more extreme  
340 unstacking in State II reported in Chaurtzman et al., (2008). This discrepancy likely reflects  
341 differences in the material used, with intact leaves used in the present study, Pietrzykowska et al.,  
342 (2014), and Iwai et al., (2018), while Chaurtzman et al., (2008) used isolated thylakoids, thus  
343 underlining the importance of using intact systems. Nonetheless, we did observe differences in the  
344 pattern of phosphorylation and macromolecular organization induced by varying white light  
345 intensity compared to monochromatic light. Both low-intensity white light and PSII light caused the  
346 appearance of the PSI-LHCI-LHCII supercomplex, however the extent of phosphorylation of both  
347 LHCII and PSII was clearly higher in PSII light despite its lower intensity (Fig. 1D and E). Consistent  
348 with this the amount of LHCII solubilized by digitonin was increased in PSII light compared to the  
349 other conditions indicating more LHCII is found in the stromal lamellae. In contrast, high-intensity

350 white light behaves, from the point of view of the macromolecular organization, more like darkness  
351 or PSI light, with strongly reduced levels of the PSI-LHCI-LHCII supercomplex. This is consistent with  
352 the downregulation of LHCII phosphorylation observed in high light induced by thioredoxin and/or  
353  $\Delta$ pH (Fernyhough et al., 1984; Rintamaki et al., 2000; Mekala et al., 2015). The extent of the state  
354 transition measured as the decrease in the F685/F735 ratio in the 77K fluorescence emission  
355 spectra was largest between the PSII and PSI lights, as expected, while the white light driven  
356 changes fell in between this range. Recently the ability of white light to provoke the state transition  
357 in plants has been questioned since only small changes in F685/F735 ratio were seen (Tikkanen et  
358 al., 2008; 2010; Mekala et al., 2015; Wood et al., 2018). However, the more detailed analysis here  
359 shows that while differences in PSI/PSII antenna size are smaller between low- and high-intensity  
360 white light, compared to the difference between PSI and PSII light, they are still significant  
361 (statistical analysis in Supplemental table 1). This is in line with the relative changes in grana  
362 stacking and levels of PSI-LHCI-LHCII supercomplexes observed between these different conditions.  
363 The imbalance in excitation rates and so the extent of LHCII phosphorylation induced by different  
364 white light levels is obviously dependent on the spectral distribution of the light used to irradiate  
365 the plants. The white light LED source we used here contained far-red wavelengths in the 700-720  
366 nm range but the intensity balance of these wavelengths relative to red and blue was still much less  
367 than natural sunlight. However, since PSI-LHCI-LHCII has been observed under natural sunlight  
368 (Wientjes et al., 2013) and since spectral quality varies with canopy shading and during the diurnal  
369 cycle, the changes in grana stacking reported here are still relevant to the natural situation.

370

### 371 **The primary determinant of thylakoid stacking dynamics is LHCII phosphorylation**

372 PSII and LHCII phosphorylation and CURT1 protein dosage have all been shown to constitutively  
373 affect the size of the stacked grana thylakoid membranes in *Arabidopsis* (Fristedt et al., 2009,

374 Fristedt et al., 2010; Samol et al., 2012; Armbruster et al., 2013) and we observed similar changes  
375 here when comparing dark-adapted plants (Fig. 5). Yet, it was unclear which of these factors  
376 regulates short-term changes in thylakoid stacking brought about by changing white light intensity.  
377 Here we found that dynamic thylakoid stacking depends primarily on the phosphorylation and  
378 dephosphorylation of LHCII by the kinase STN7 and phosphatase TAP38, as outlined in the model in  
379 Fig. 8. Thus, the wild-type shifts its grana diameter from being similar to *stn7* in darkness and high-  
380 intensity light to being similar to *tap38* in low-intensity light, while in these two mutants light-  
381 induced changes are largely absent. In contrast, the light driven changes were still present in *stn8*  
382 and *pbc* mutants, which lack the PSII kinase and phosphatase, respectively. This result is  
383 consistent with the fact that although PSII phosphorylation increases in high light, this is insufficient  
384 to offset the parallel decrease in LHCII phosphorylation (Fig. 1D and 5G). Interestingly, in high light  
385 in spinach the grana diameter was larger than in darkness despite an overall higher level of total  
386 thylakoid protein phosphorylation (Fig. 2D); this effect was absent in wild-type and most  
387 Arabidopsis mutants (Fig. 5B-F), although in *tap38* the grana diameter did increase slightly in high  
388 light (Fig. 5D). A coinciding change of the ionic status of the chloroplast, for instance due to  $H^+/Mg^{2+}$   
389 exchange between the lumen and stroma in high light (Hind et al., 1974) could further promote  
390 stacking, a possibility that warrants future investigation. Perhaps only when plants are placed under  
391 severe photoinhibitory stress is the increased PSII phosphorylation sufficient to offset such ionic  
392 status changes e.g. in light stressed Arabidopsis (Herbstova et al., 2012; Puthiyaveetil et al., 2014).  
393 An alternative explanation could be that in these studies high levels of LHCII and PSII  
394 phosphorylation were seen in tandem, similar to the more extreme PSII light effect seen in our  
395 study (Herbstova et al., 2012; Puthiyaveetil et al., 2014).

396 Recently it was proposed that changes in CURT1 protein oligomerization state could be  
397 responsible for the dynamic short-term changes in thylakoid stacking and that this process could be

398 regulated by phosphorylation via STN7 or STN8 (Pribil et al., 2014; Pribil et al., 2018). Our results  
399 however showed minimal changes in CURT1A oligomerization in wild-type, *stn7* and *tap38* plants as  
400 they transition from dark to low light to high light, despite the large differences in thylakoid  
401 stacking observed between them. Therefore differences in thylakoid stacking between these  
402 mutants is the result of differences in LHCII phosphorylation. Whether changes in LHCII  
403 phosphorylation alone in the absence of CURT1 proteins are sufficient to observe dynamic  
404 thylakoid stacking was somewhat harder to answer since these changes were difficult to robustly  
405 quantify in the *curt1abcd* mutant. Nonetheless, qualitatively low light led to the almost complete  
406 disappearance of the remaining pseudo-grana in this mutant, indicating that LHCII phosphorylation  
407 can still exert an effect.

408

#### 409 **Relationship between thylakoid stacking dynamics and state transitions**

410 The common dependence of the stacking dynamics and state transitions on LHCII phosphorylation  
411 suggested that the two processes may be obligatorily linked. Indeed, it was previously proposed  
412 that unstacking resulting from LHCII-phosphorylation driven repulsion between LHCII and PSII  
413 facilitates the exchange of LHCII between PSII and PSI (Kyle et al., 1983). Later however LHCII  
414 phosphorylation alone was shown to be insufficient for transition to State II with mutants lacking  
415 PSAL and H subunits of PSI deficient in state transitions (Lunde et al., 2000). This is consistent with  
416 the structure of the PSI-LHCI-LHCII supercomplex, which showed specific molecular contacts are  
417 formed between these proteins and LHCB2 (Pan et al., 2018; Allen et al., 2019). Moreover, mutants  
418 lacking LHCI proteins were also unable to undergo full transition to State II (Benson et al., 2015;  
419 Bressan et al., 2018), consistent with the presence of a second digitonin-labile LHCII binding site on  
420 PSI (Bell et al., 2015; Grieco et al., 2015; Sathish-Yadav et al., 2017). Here we investigated a slightly  
421 different scenario - would these state transition mutants still be able to undergo short-term

422 dynamic thylakoid stacking? Our data showed that they could (Fig. 7); LHCII phosphorylation alone  
423 is therefore sufficient to drive short-term changes in thylakoid stacking. This disconnect may seem  
424 surprising given the well-known link between grana stacking and spillover between PSI and PSII;  
425 however, it was previously shown that functional association of LHCII with PSII in plants occurs at  
426 low concentrations of  $Mg^{2+}$  cations, below those at which grana stacks form (Kiss et al., 2008).  
427 Moreover, lateral segregation of PSI and PSII has also been observed in cyanobacteria in the  
428 absence of thylakoid stacking (MacGregor-Chatwin et al., 2017). Thus, although grana become  
429 smaller in the *psaL* and *lhca4* mutants in low light the F685/F735 ratio change is suppressed  
430 indicating LHCII remains functionally coupled to PSII, consistent with previous results (Lunde et al.,  
431 2000; Benson et al., 2015).

432

### 433 **The functional significance of thylakoid stacking dynamics**

434 Discrepancies between the kinetics of the state transition and those of the stacking dynamics can  
435 be seen in Fig. 4H. Thus while state transitions, as measured by the change in the F685/F735 ratio,  
436 occur to their full extent in ~3 minutes, thylakoid stacking dynamics occurred with a half-time of ~6-  
437 9 minutes. These timescales for transition between State I and II are consistent with those from  
438 PAM fluorescence studies (Damkjaer et al., 2009). We therefore conclude that changes in  
439 photosystem antenna size can be induced by relatively small changes in thylakoid stacking. Why  
440 then are LHCII, and particularly both LHCb1 and LHCb2, so heavily phosphorylated if small changes  
441 in stacking at the margins are sufficient to achieve state transitions? Could the larger extent of the  
442 stacking changes achieve another function? One possibility is that of PSII repair, with a decrease in  
443 stacking in high light being suggested to facilitate the accessibility of phospho-PSII complexes to  
444 interact with the FTSH protease (Herbstova et al., 2012; Puthiaveetil et al., 2014; Theis and Schroda,  
445 2016). However, since we observe an increase in thylakoid stacking in spinach and Arabidopsis in

446 high light, which would presumably hinder such repair, this seems unlikely to provide an  
447 explanation of our results. An alternative hypothesis is that changes in thylakoid stacking facilitate  
448 regulation of electron transfer pathways, particular the balance between LET and CET (Albertsson  
449 et al., 2001; Wood et al., 2018; Johnson 2018). The changes in grana diameter described here have  
450 been shown to significantly affect the diffusion time of PQH<sub>2</sub> and PC from the grana to stromal  
451 lamellae thylakoids allowing more rapid reduction of PSI, i.e. reduced partition of electron carriers  
452 between domains. Since the rate-limiting step in LET is the diffusion of PQH<sub>2</sub> from PSII to *cytb<sub>6</sub>f* and  
453 its oxidation therein, any change in the diffusion time has the potential to affect the overall rate  
454 significantly (Wood et al., 2018). Consistent with this, more efficient LET has also been observed in  
455 *tap38* Arabidopsis plants (Pribil et al., 2010) which show smaller grana, whereas LET is  
456 compromised in the *curt1abcd* mutant with larger grana (Armbruster et al., 2013). In contrast,  
457 larger more stacked grana were suggested to have the opposite effect, i.e. by greater partition  
458 between grana and stromal lamellae the exchange of PQH<sub>2</sub> between the compartments would be  
459 limited, which may better poise the stromal lamellae PQ pool to accept electrons from ferredoxin  
460 facilitating CET (Wood et al., 2018). Certainly the light intensity-dependence of the dynamic  
461 changes in grana stacking reported here are consistent with the view that they promote LET  
462 optimisation in low light with smaller grana and a requirement for CET in high light and during the  
463 dark to light transition (Joliot and Joliot, 2002; Munekage et al., 2004; Kou et al., 2013) via larger  
464 grana (Wood et al., 2018). It is interesting to note that the short-term grana response strategy of  
465 plants to high light intensity is seemingly the opposite of the long-term acclimation strategy, where  
466 plants grown in full sun are characterized by grana with fewer layers per stack (Anderson, 1986;  
467 Anderson et al., 1988). This dichotomy may be explained by the need for photoprotection in the  
468 short term in high light with larger grana facilitating enhanced photoprotective energy dissipation  
469 via enhanced LHCII-LHCII interactions and CET (Wood et al., 2018). Whereas, in the long term plants

470 decrease the LHCII content and instigate increases in LET and metabolic sink capacity that allow  
471 them to utilize the increased light availability, eventually leading to development of smaller grana.

472

## 473 **MATERIALS AND METHODS**

474

### 475 **Plant growth conditions**

476 Spinach and Arabidopsis plants were grown for 5 weeks in a Conviron plant growth room with a 12  
477 hour photoperiod at a light intensity of  $150 \mu\text{mol photons m}^{-2} \text{sec}^{-1}$  and a day/night temperatures of  
478  $22/18^\circ\text{C}$ , respectively.

479

### 480 **Structured Illumination Microscopy (SIM)**

481 Spinach and Arabidopsis were imaged on a DeltaVision OMX V4 microscope (GE Healthcare)  
482 equipped with the Blaze-3D SIM module and 60x 1.42 oil planapochromat lens. Chlorophyll  
483 fluorescence was excited with a 642 nm laser and the emission was collected through a 683/40 nm  
484 bandpass filter. The structured illumination pattern was projected onto the sample in a series of five  
485 phases for each of three angles leading to a total of 15 images per axial slice. The 3D image was  
486 acquired via sectioning with a 2D slice separation of 125 nm. The final super-resolution image was  
487 reconstructed with the SoftWoRx OMX 6.0 software (GE Healthcare). Grana diameter was measured  
488 as the full-width half-maximum of a line profile across the granal-midpoint in images that had been  
489 thresholded and 16-bit converted with the SIMcheck plugin for ImageJ.

490

### 491 **Electron Microscopy**

492 Spinach leaves were fixed by infiltrating with 3% glutaldehyde/0.1M sodium cacodylate buffer  
493 overnight, washed in buffer and post fixed 2% osmium tetroxide, washed briefly in water and

494 dehydrated through a graded series of ethanol treatments, cleared in epoxypropane (EPP) and  
495 infiltrated in a 50/50 araldite resin:EPP mixture overnight on a rotor. This mixture was replaced twice,  
496 over 8 hours, with fresh araldite resin mixture before being embedded and curing in a 60°C oven for  
497 48-72 hours. Ultrathin sections, approximately 85 nm thick, were cut on a Leica UC 6 ultramicrotome  
498 onto 200 mesh copper grids and stained for 30 mins with saturated aqueous Uranyl Acetate followed  
499 by Reynold's Lead Citrate for 5 mins. Sections were examined using a FEI Tecnai Transmission Electron  
500 Microscope at an accelerating voltage of 80kV. Electron micrographs were recorded using a Gatan  
501 Orius 1000 digital camera and Digital Micrograph software.

502

### 503 **Native-PAGE, SDS-PAGE and Phosphoprotein Staining**

504 Thylakoid membranes were prepared according to Albertsson et al., (1994), from spinach or  
505 Arabidopsis leaves either dark adapted or adapted to low-intensity white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ),  
506 high-intensity white light ( $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), PSI ( $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 730 nm) or  
507 PSII ( $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 660 nm) light conditions for 1 hour with the addition that 10 mM NaF  
508 was included in all buffers. Thylakoids ( $5 \mu\text{g}$  of chlorophyll) were solubilized in Laemmli buffer and  
509 separated by SDS-PAGE on a 12% gel (Benson et al., 2015). Diamond Pro-Q Phospho staining (Life  
510 technologies) was performed as previously described by Mekala et al. (2015). For Blue Native-PAGE,  
511 thylakoids were solubilized in 2% (w/v) digitonin for 10 minutes at room temperature.

512

### 513 **Low-temperature fluorescence spectroscopy.**

514  $1 \mu\text{M}$  of chlorophyll from thylakoids prepared as above was suspended in the fluorescence buffer  
515 (60% glycerol, 300 mM sucrose, 5 mM  $\text{MgCl}_2$ , 20 mM HEPES pH 7.8) and measured in 1 cm polymethyl  
516 methacrylate cuvettes in a Opistat liquid nitrogen cooled bath cryostat (Oxford Instruments).

517 Fluorescence emission measurements were performed as previously described (Benson et al., 2015)  
518 using a FluoroLog FL3-22 spectrofluorimeter (Jobin Yvon).

519

#### 520 **Accession numbers**

521 The sequence data from this article can be found in The Arabidopsis Information Resource or  
522 GenBank/EMBL database under the following accession numbers: STN7 (At1g68830), STN8  
523 (At5g01920), TAP38/PPH1 (At4t27800), PBCP (At2g30170), CURT1A (At4g01150), CURT1B  
524 (At2g46820), CURT1C (At1g52220), CURT1D (At4g38100), PSAL (At4g12800), LHCA4 (At3g47470),  
525 LHCB1 (At1g29930), LHCB2 (At2g05100), D1 (Atcg00020), D2 (Atcg00270), CP43 (Atcg00280).

526

#### 527 **Supplemental Data**

528 **Supplemental Table 1.** Results of analysis of variance (ANOVA) and Tukey's multiple comparisons  
529 test for F685/F735 low temperature fluorescence emission ratio data shown Fig 1C.

530 **Supplemental Table 2.** Results of analysis of variance (ANOVA) and Tukey's multiple comparisons  
531 test for the FWHM of grana fluorescence data shown in Fig 2F.

532 **Supplemental Table 3.** Results of analysis of variance (ANOVA) and Tukey's multiple comparisons  
533 test for the average number of grana per chloroplast data shown in Fig 2G.

534 **Supplemental Table 4.** Results of analysis of variance (ANOVA) and Tukey's multiple comparisons  
535 test for grana diameter thin-section EM data shown in Fig 3D.

536 **Supplemental Table 5.** Results of analysis of variance (ANOVA) and Dunn's multiple comparisons  
537 test for average number of membrane layers per grana data shown in Fig 3E.

538 **Supplemental Table 6.** Results of analysis of variance (ANOVA) and Tukey's multiple comparisons  
539 test for average grana area data shown in Fig 3F.

540 **Supplemental Table 7.** Results of analysis of variance (ANOVA) and Tukey's multiple comparisons  
541 test for FWHM of grana fluorescence data shown in Fig 5B-F, Fig 6D and Fig7D,E.

542 **Supplemental Table 8.** Grana diameters determined from the FWHM of grana fluorescence in SIM  
543 images of Arabidopsis and Spinach.

544

545

## 546 **ACKNOWLEDGMENTS**

547 We wish to thank Professor Dario Leister (Munich) and Dr Mathias Pribil (Copenhagen) for  
548 providing seeds of the *psaL*, *curt1abcd*, *oeCURT1A* and *tap38* lines, Professor Michel Goldschmidt-  
549 Clermont (Geneva) for providing seeds of the *stn8* and *pbcp* mutant seeds, Professor Lutz Eichacker  
550 (Stavenger) for providing seeds of *stn7* and Professor Stefan Jansson (Umeå) for seeds of *lhca4*.  
551 Chris Hill (University of Sheffield) is acknowledged for assistance with the EM. M.P.J. acknowledges  
552 funding from the Leverhulme Trust grant RPG-2016-161 and Grantham Centre for Sustainable  
553 Futures. C.N.H. and M.P.J. also gratefully acknowledge financial support from the Biotechnology  
554 and Biological Sciences Research Council (BBSRC UK), award number BB/M000265/1. The SIM  
555 imaging was performed at the University of Sheffield Wolfson Light Microscopy Facility and was  
556 partly funded by MRC Grant MR/K015753/1.

557

## 558 **Figure legends**

559 **Figure 1. Low-intensity white light induces State II, high-intensity white light induces State I.**

560 Biochemical and spectroscopic characterisation of thylakoids isolated from spinach leaves  
561 previously treated for 1 hour with PSI light (730 nm, 20  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), PSII light (660 nm, 20  
562  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), darkness, low-intensity white light (150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) or high-

563 intensity white light ( $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). A, Spectra of white, far-red and red lights used for  
564 the treatments. B, Low-temperature (77 K) fluorescence emission spectra (435 nm excitation) of  
565 thylakoids from spinach leaves treated with PSI light, PSII light, dark (D), low light (LL) or high light  
566 (HL). Mean of 5 spectra in each condition. C, Mean ratio of the 77K fluorescence emission at 685  
567 nm relative to 735 nm in each sample  $\pm$  standard deviation (N=5, statistical analysis in  
568 Supplemental Table 1). D, Phospho-protein staining of total thylakoid proteins separated by SDS-  
569 PAGE in each sample. E, BN-PAGE analysis of protein complexes in digitonin-solubilized thylakoids  
570 from each sample.

571 **Figure 2. Low-intensity white light reduces grana size, high-intensity white light increases grana**  
572 **size.** 3D-SIM analysis of chloroplast ultrastructure in spinach induced by 1 hour of PSI light (730 nm,  
573  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), PSII light (660 nm,  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), darkness, low-intensity white  
574 light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) or high-intensity white light ( $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). A,  
575 Representative 3D-SIM images of each sample, bars 1  $\mu\text{m}$ . B, Representative 3D-SIM z-projection of  
576 each sample, bars 1  $\mu\text{m}$ . C, Individual 125 nm SIM z-slices of each sample, bars 10  $\mu\text{m}$ . D, Mean  
577 grana diameter (FWHM of fluorescence signal) in each sample  $\pm$  standard deviation  
578 (N=100,99,49,40,50, statistical analysis in Supplemental Table 2). E, Mean number of grana per  
579 chloroplast in each sample  $\pm$  standard deviation (N=12,8,21,18,14, statistical analysis in  
580 Supplemental Table 3).

581 **Figure 3. Low-intensity white light reduces grana stacking, high-intensity white light increases**  
582 **grana stacking.** Thin-section transmission EM analysis of chloroplast ultrastructure in spinach  
583 induced by 1 hour of darkness, low-intensity white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) or high-intensity  
584 white light ( $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). A, Representative EM images of chloroplasts from each  
585 sample, bars 1  $\mu\text{m}$ . B, Mean grana diameter from EM images in each sample  $\pm$  standard deviation

586 (N=627,897,958, statistical analysis in Supplemental Table 4). C, Mean number of layers per grana  
587 stack in each sample  $\pm$  standard deviation (N=75,99,74, statistical analysis in Supplemental Table 5).  
588 D, Mean area of grana membrane as a percentage of the total area of thylakoids in EM images in  
589 each sample  $\pm$  standard deviation (N=5, statistical analysis in Supplemental Table 6).

590 **Figure 4. Dynamic thylakoid stacking changes occur with a half-time of <10 minutes.** Kinetics of  
591 white light-induced changes in thylakoid stacking in spinach determined by 3D-SIM analysis. A,  
592 Change in mean grana diameter (FWHM of the fluorescence signal)  $\pm$  standard deviation (N=100  
593 each time point) and B, Change in mean number of grana per chloroplast  $\pm$  standard deviation  
594 (N=18,15,11,14), versus time in 1 hour dark-adapted (D) spinach illuminated with low-intensity  
595 white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). C, Change in mean grana diameter (FWHM of the  
596 fluorescence signal)  $\pm$  standard deviation (N=100 each time point) and D, Change in mean number  
597 of grana per chloroplast  $\pm$  standard deviation (N=10,17,10,11), versus time in 1 hour low white light  
598 treated spinach ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) illuminated with high-intensity white light ( $1000 \mu\text{mol}$   
599  $\text{photons m}^{-2} \text{s}^{-1}$ ). E, Change in mean grana diameter (FWHM of the fluorescence signal)  $\pm$  standard  
600 deviation (N=100 each time point) and F, Change in mean number of grana per chloroplast  $\pm$   
601 standard deviation (N=12,11,8,9), versus time in 1 hour high white light treated spinach ( $1000 \mu\text{mol}$   
602  $\text{photons m}^{-2} \text{s}^{-1}$ ) illuminated with low-intensity white light ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). G, Change in  
603 mean grana diameter (FWHM of the fluorescence signal)  $\pm$  standard deviation (N=100 each time  
604 point) with sequential 20 minute treatments of low- (LL,  $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and high- (HL,  
605  $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) intensity white light. H, Change in the ratio of 77K fluorescence emission  
606 at 685 nm relative to 735 nm (435 nm excitation) with sequential 30 minute treatments of low-  
607 ( $150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and high- ( $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) intensity white light, with  
608 fluorescence ratios obtained during 1 hour treatment with PSII light (660 nm,  $20 \mu\text{mol photons m}^{-2}$

609  $s^{-1}$ ) or PSI light (730 nm, 20  $\mu\text{mol photons m}^{-2} s^{-1}$ ) shown for comparison as dashed lines.

610 **Figure 5. Dynamic thylakoid stacking depends on LHCII phosphorylation.** 3D-SIM analysis of  
611 chloroplast ultrastructure in wild-type *Arabidopsis* and mutants lacking the LHCII kinase (*stn7*),  
612 LHCII phosphatase (*tap38*), PSII kinase (*stn8*) or PSII phosphatase (*pbc1*) induced by 1 hour of  
613 darkness, low-intensity white light (LL, 150  $\mu\text{mol photons m}^{-2} s^{-1}$ ) or high-intensity white light (HL,  
614 1000  $\mu\text{mol photons m}^{-2} s^{-1}$ ). A, Representative 3D-SIM z-projection of each sample, bars 1  $\mu\text{m}$ . B-F,  
615 Mean grana diameter (FWHM of fluorescence signal) in each sample  $\pm$  interquartile range (box) and  
616 range (whiskers) (N=100 each light intensity, statistical analysis in Supplemental Table 7). G,  
617 Phospho-protein staining of total thylakoid proteins separated by SDS-PAGE from LL- and HL-  
618 treated wild-type, *tap38* and *stn7* leaves. H-J, Heat maps showing changes in grana diameter ( $\mu\text{m}$ )  
619 between *Arabidopsis* mutants (read left compared to bottom) in Dark, Low light and High light  
620 conditions as labelled.

621 **Figure 6. CURT1A oligomerization is unaffected in the *stn7* and *tap38* mutants of *Arabidopsis*.**

622 Analysis of CURT1A oligomerization in wild-type *Arabidopsis* and mutants lacking the LHCII kinase  
623 (*stn7*) or LHCII phosphatase (*tap38*), and 3D-SIM analysis of chloroplast ultrastructure in wild-type  
624 *Arabidopsis* and mutants lacking the CURT1 proteins (*curt1abcd*) or overexpressing CURT1A  
625 (*oeCURT1A*) induced by 1 hour of darkness, low-intensity white light (LL, 150  $\mu\text{mol photons m}^{-2} s^{-1}$ )  
626 or high-intensity white light (HL, 1000  $\mu\text{mol photons m}^{-2} s^{-1}$ ). A, BN-PAGE analysis of protein  
627 complexes in digitonin-solubilized thylakoids from wild-type, *stn7* and *tap38* plants. B, Immunoblot  
628 of CURT1A protein distribution in BN-PAGE gel shown in (A). C, 3D-SIM images of chloroplasts in  
629 *curt1abcd* and *OEcurt1a* *Arabidopsis* in dark, low light and high light; bars represent 1  $\mu\text{m}$ . D, Mean  
630 grana diameter (FWHM of the fluorescence signal) in *OEcurt1a* *Arabidopsis* in dark, low light and  
631 high light  $\pm$  interquartile range (box) and range (whiskers) (N=100 each light intensity, statistical

632 analysis in Supplemental Table 7).

633

634 **Figure 7. Dynamic thylakoid stacking is independent of phospho-LHCII interaction with PSI.**

635 Analysis of wild-type Arabidopsis and mutants lacking the PSAL (*psaL*) or LHCI (*lhca4*) phospho-LHCII  
636 binding sites on PSI induced by 1 hour of darkness (D), low-intensity white light (LL, 150  $\mu\text{mol}$   
637 photons  $\text{m}^{-2} \text{s}^{-1}$ ) or high-intensity white light (HL, 1000  $\mu\text{mol}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ). A, Low-temperature  
638 (77 K) fluorescence emission spectra (435 nm excitation) of thylakoids from LL- or HL-treated wild-  
639 type, *lhca4*, and *psaL* Arabidopsis. B, BN-PAGE analysis of digitonin-solubilized thylakoids from LL-  
640 or HL-treated wild-type, *lhca4*, and *psaL* Arabidopsis. C, 3D-SIM images of chloroplasts from Dark-,  
641 LL- or HL-treated wild-type, *lhca4*, and *psaL* Arabidopsis; bars 1  $\mu\text{m}$ . D,E, Mean grana diameter  
642 (FWHM of the fluorescence signal)  $\pm$  interquartile range (box) and range (whiskers) in Dark-, LL- or  
643 HL-treated *lhca4* (D), and *psaL* (E) Arabidopsis; wild-type data found in Fig. 5A and B (N=100 each  
644 light intensity, statistical analysis in Supplemental Table 7).

645 **Figure 8. Model of dynamic thylakoid stacking in plants.** A model depicting chloroplast  
646 ultrastructure in response to environmental light intensity. In low-intensity white light and PSII  
647 light, STN7 is activated leading to maximal LHCII phosphorylation; the effect is to decrease the  
648 grana diameter and increase the number of grana stacks per chloroplast compared to darkness and  
649 high-intensity white light. In darkness, PSI light and high-intensity white light, STN7 activity is low  
650 allowing TAP38 to dephosphorylate LHCII, increasing the grana diameter and decreasing the  
651 number of grana stacks per chloroplast compared to low-intensity light.

652

653

654 **Supplemental Table 1.** Analysis of variance (ANOVA) and Tukey's multiple comparisons test for  
655 data shown Fig 1C. P values for the difference in mean ratio of fluorescence at 685 nm over 735 nm  
656 emission at 77 K (435 nm excitation) for Spinach in dark (D), low light (LL), high light (HL), far-red  
657 light (FR), and red light (R)..

658

659

| Tukey's multiple comparisons test | Adjusted P Value |
|-----------------------------------|------------------|
| D vs. LL                          | 0.0069           |
| D vs. HL                          | 0.9097           |
| D vs. FR                          | 0.2434           |
| D vs. R                           | <0.0001          |
| LL vs. HL                         | 0.0440           |
| LL vs. FR                         | <0.0001          |
| LL vs. R                          | 0.0231           |
| HL vs. FR                         | 0.0499           |
| HL vs. R                          | <0.0001          |
| FR vs. R                          | <0.0001          |

660

661

662

663

664

665

666

667

668

669

670 **Supplemental Table 2.** Analysis of variance (ANOVA) and Tukey's multiple comparisons test for  
671 data shown in Fig 2F. P values for the difference in mean grana FWHM (from SIM) for Spinach in  
672 dark (D), low light (LL), high light (HL), PSI light (FR), and PSII light (R).

673

| Tukey's multiple comparisons test | Adjusted P Value |
|-----------------------------------|------------------|
| D vs. LL                          | <0.0001          |
| D vs. HL                          | <0.0001          |
| D vs. FR                          | 0.9781           |
| D vs. R                           | <0.0001          |
| LL vs. HL                         | <0.0001          |
| LL vs. FR                         | <0.0001          |
| LL vs. R                          | 0.8654           |
| HL vs. FR                         | <0.0001          |
| HL vs. R                          | <0.0001          |

674

|          |         |
|----------|---------|
| FR vs. R | <0.0001 |
|----------|---------|

675

676

677

678

679

680

681

682

**Supplemental Table 3.** Analysis of variance (ANOVA) and Tukey's multiple comparisons test for data shown in Fig 2G. P values for the difference in mean number of grana per chloroplast (from SIM) for Spinach in dark (D), low light (LL), high light (HL), PSI light (FR), and PSII (R).

683

684

685

686

687

688

689

690

691

692

693

694

695

696

697

698

699

| Tukey's multiple comparisons test | Adjusted P Value |
|-----------------------------------|------------------|
| D vs. LL                          | 0.0072           |
| D vs. HL                          | 0.9925           |
| D vs. FR                          | 0.8674           |
| D vs. R                           | 0.0067           |
| LL vs. HL                         | 0.0595           |
| LL vs. FR                         | 0.2446           |
| LL vs. R                          | 0.9303           |
| HL vs. FR                         | 0.9858           |
| HL vs. R                          | 0.0331           |
| FR vs. R                          | 0.1187           |

**Supplemental Table 4.** Analysis of variance (ANOVA) and Tukey's multiple comparisons test for data shown in Fig 3D. P values for the difference in mean grana diameter (from thin-section EM) for Spinach in dark (D), low light (LL), and high light (HL).

| Tukey's multiple comparisons test | Adjusted P Value |
|-----------------------------------|------------------|
| D vs. LL                          | <0.0001          |
| D vs. HL                          | 0.0350           |
| LL vs. HL                         | <0.0001          |

700

701 **Supplemental Table 5.** Analysis of variance (ANOVA) and Dunn's multiple comparisons test for  
702 data shown in Fig 3E. P values for the difference in mean number of layers per grana stack (from  
703 thin-section EM) for Spinach in dark (D), low light (LL), and high light (HL).

704

705

| Dunn's multiple comparisons test | Adjusted P Value |
|----------------------------------|------------------|
| D vs. LL                         | 0.0208           |
| D vs. HL                         | 0.0105           |
| LL vs. HL                        | <0.0001          |

706

707

708

709 **Supplemental Table 6.** Analysis of variance (ANOVA) and Tukey's multiple comparisons test for  
710 data shown in Fig 3F. P values for the difference in percentage of grana area in comparison to total  
711 thylakoid area (from thin-section EM) for Spinach in dark (D), low light (LL), and high light (HL).

712

713

| Tukey's multiple comparisons test | Adjusted P Value |
|-----------------------------------|------------------|
| D vs. LL                          | 0.3944           |
| D vs. HL                          | 0.0054           |
| LL vs. HL                         | 0.0005           |

714

715

716

717

718 **Supplemental Table 7.** Analysis of variance (ANOVA) and Tukey's multiple comparisons test for  
719 data shown in Fig 5B-F, Fig 6D and Fig7D,E. P values for the difference in the grana FWHM of  
720 Arabidopsis (various mutants) in dark, low light and high light.

721

|              | Dark to low light | Dark to high light | Low light to high Light |
|--------------|-------------------|--------------------|-------------------------|
| Wild type    | <0.0001           | 0.0115             | <0.0001                 |
| <i>stn7</i>  | 0.8395            | 0.7672             | 0.3528                  |
| <i>tap38</i> | 0.0015            | 0.0027             | <0.0001                 |

722  
723  
724  
725  
  
726  
727  
728  
729  
730  
731  
732  
733  
734  
735  
736

|                 |         |         |         |
|-----------------|---------|---------|---------|
| <i>stn8</i>     | <0.0001 | 0.0213  | <0.0001 |
| <i>pbcp</i>     | 0.0002  | <0.0001 | <0.0001 |
| <i>oeCURT1a</i> | 0.0003  | 0.1291  | 0.1131  |
| <i>psaL</i>     | <0.0001 | 0.5501  | <0.0001 |
| <i>lhca4</i>    | <0.0001 | 0.8546  | <0.0001 |

737 **Supplemental Table 8.** Grana diameters determined from the FWHM of grana fluorescence in SIM  
738 images of Arabidopsis and Spinach.

739  
740  
741  
742

|                  | Dark        | Low light   | High light  | PSI Light   | PSII light  |
|------------------|-------------|-------------|-------------|-------------|-------------|
| Spinach          | 0.46 ± 0.09 | 0.38 ± 0.06 | 0.58 ± 0.12 | 0.49 ± 0.06 | 0.36 ± 0.07 |
| Wild type        | 0.41 ± 0.07 | 0.35 ± 0.07 | 0.47 ± 0.09 |             |             |
| <i>stn7</i>      | 0.49 ± 0.11 | 0.50 ± 0.12 | 0.44 ± 0.09 |             |             |
| <i>tap38</i>     | 0.37 ± 0.06 | 0.35 ± 0.06 | 0.4 ± 0.07  |             |             |
| <i>stn8</i>      | 0.49 ± 0.09 | 0.37 ± 0.07 | 0.45 ± 0.1  |             |             |
| <i>pbcp</i>      | 0.37 ± 0.07 | 0.33 ± 0.06 | 0.43 ± 0.07 |             |             |
| <i>curt1abcd</i> | 1.35 ± 0.38 | N/A         | N/A         |             |             |
| oeCURT1a         | 0.3 ± 0.05  | 0.28 ± 0.04 | 0.29 ± 0.04 |             |             |
| <i>lhca4</i>     | 0.48 ± 0.09 | 0.4 ± 0.08  | 0.47 ± 0.11 |             |             |
| <i>psaL</i>      | 0.49 ± 0.11 | 0.42 ± 0.1  | 0.5 ± 0.1   |             |             |

743 **LITERATURE CITED**

744

745 **Albertsson P-Å** (2001) A quantitative model of the domain structure of the photosynthetic  
746 membrane. *Trends Plant Sci.* **6**: 349–354

747 **Albertsson P-Å, Andreasson E, Stefansson H, Wollenberger L** (1994) Fractionation of the thylakoid  
748 membrane. *Methods Enzymol.* **228**: 469–482

749 **Allen JF** (2003) State transitions: a question of balance. *Science* **299**: 1530– 1532

750 **Allen JF, Nield J, Krauß N.** (2019). Molecular Recognition: How Photosynthesis Anchors the Mobile  
751 Antenna. *Trends in Plant Science.* **24**: 388-392

752 **Andersson B, Anderson JM** (1980) Lateral heterogeneity in the distribution of chlorophyll–protein  
753 complexes of the thylakoid membranes of spinach chloroplasts. *Biochim Biophys Acta* **593**:  
754 427–440

755 **Anderson JM** (1986) Photoregulation of the composition, function and structure of thylakoid  
756 membranes. *Annual Review of Plant Physiology* **37**: 93–136

757 **Anderson JM, Chow WS, Goodchild DJ** (1988) Thylakoid membrane organisation in sun/ shade  
758 acclimation. *Aust J Plant Phys* 15: 11-26

759 **Anderson JM, Horton P, Kim EH, Chow WS** (2012) Towards elucidation of dynamic structural  
760 changes of plant thylakoid architecture. *Philosophical Transactions of the Royal Society B:*  
761 *Biological Sciences* **367**: 3515–3524

762 **Armbruster U, Labs M, Pribil M, Viola S, Xu W, Scharfenberg M, Hertle AP, Rojahn U, Jensen PE,**  
763 **Rappaport F, Joliot, P, Dörmann, I, Wanner G, Leister D (2013)** Arabidopsis CURVATURE  
764 THYLAKOID1 proteins modify thylakoid architecture by inducing membrane curvature. *Plant*  
765 *Cell* **25**: 2661–2678

766 **Austin JR, Staehelin LA** (2011). Three-dimensional architecture of grana and stroma thylakoids of  
767 higher plants as determined by electron tomography. *Plant Physiology* **155**: 1601-1611.

768 **Barber J** (1982) Influence of surface-charges on thylakoid structure and function. *Ann Rev Plant*  
769 *Phys Plant Mol Biol* **33**: 261–295

770 **Bell AJ, Frankel LK, Bricker TM.** (2015). High Yield Non-detergent Isolation of Photosystem I-Light-  
771 harvesting Chlorophyll II Membranes from Spinach Thylakoids IMPLICATIONS FOR THE  
772 ORGANIZATION OF THE PS I ANTENNAE IN HIGHER PLANTS. *J Biol Chem* **290**: 18429-18437

773 **Bellafiore S, Barneche F, Peltier G, Rochaix JD** (2005) State transitions and light adaptation require  
774 chloroplast thylakoid protein kinase STN7. *Nature* **433**: 892–895

775 **Benson SL, Maheswaran P, Ware MA, Hunter CN, Horton P, Jansson S, Ruban AV, Johnson MP**  
776 (2015). An intact light harvesting complex I antenna system is required for complete state  
777 transitions in *Arabidopsis*. *Nature Plants* **12**: 15176

778 **Bressan M, Bassi R, Dall'Osto L** (2018) Loss of LHCI system affects LHCII redistribution between  
779 thylakoid domains upon state transitions. *Photosynt Res* **135**: 251–261

780 **Chuartzman SG, Nevo R, Shimoni E, Charuvi D, Kiss V, Ohad I, Brumfeld V, Reich Z** (2008)  
781 Thylakoid membrane remodeling during state transitions in *Arabidopsis*. *Plant Cell* **20**: 1029–  
782 1039

783 **Chow WS, Miller C, Anderson JM** (1991) Surface charges, the heterogeneous lateral distribution of  
784 the two photosystems, and thylakoid stacking. *Biochim Biophys Acta* **1057**: 69–77

785 **Chow WS, Thorne SW, Duniec JT, Sculley MJ, Boardman NK** (1982) The stacking of chloroplast  
786 thylakoids: evidence for segregation of charged groups into nonstacked regions. *Arch Biochem*  
787 *Biophys* **216**: 247–254

788 **Cox RP, Andersson B** (1981) Lateral and transverse organisation of cytochromes in the chloroplast  
789 thylakoid membrane. *Biochem Biophys Res Comm* **103**: 1336-1342

790 **Damkjær JT, Kereïche S, Johnson MP, Kovacs L, Kiss AZ, Boekema EJ, Ruban AV, Horton P Jansson**  
791 **S** (2009) The photosystem II light-harvesting protein Lhcb3 affects the macrostructure of  
792 photosystem II and the rate of state transitions in *Arabidopsis*. *Plant Cell* **21**: 3245-3256

793 **Daum B, Nicastro D, Austin J, McIntosh JR, Kühlbrandt W** (2010) Arrangement of photosystem II  
794 and ATP synthase in chloroplast membranes of spinach and pea. *Plant Cell* **22**: 1299–1312

795 **Day DA, Ryrie IJ, Fuad N** (1984) Investigations of the role of the main light-harvesting chlorophyll–  
796 protein complex in thylakoid membranes. Reconstitution of depleted membranes from  
797 intermittent-light-grown plants with the isolated complex. *J Cell Biol* **98**: 163–172

798 **Engel BD, Schaffer M, Cuellar LK, Villa E, Plitzko JM, Baumeister W** (2015) Native architecture of  
799 the *Chlamydomonas* chloroplast revealed by in situ cryo-electron tomography. *ELife* **4**: e04889.

800 **Fernyhough P, Foyer CH, Horton P** (1984) Increase in the level of thylakoid protein phosphorylation  
801 in maize mesophyll chloroplasts by decrease in the transthylakoid pH gradient. *FEBS Lett* **176**:  
802 133-138

803 **Fristedt R, Willig A, Granath P, Crèvecoeur M, Rochaix JD, Vener AV** (2009) Phosphorylation of  
804 photosystem II controls functional macroscopic folding of photosynthetic membranes in  
805 *Arabidopsis*. *Plant Cell* **21**: 3950–3964

806 **Goral TK, Johnson MP, Brain AP, Kirchhoff H, Ruban AV, Mullineaux CW** (2010). Visualizing the  
807 mobility and distribution of chlorophyll proteins in higher plant thylakoid membranes: effects  
808 of photoinhibition and protein phosphorylation. *Plant Journal* **62**: 948-959

809 **Grieco M, Suorsa M, Jajoo A, Tikkanen M, Aro EM** (2015). Light-harvesting II antenna trimers  
810 connect energetically the entire photosynthetic machinery—including both photosystems II  
811 and I. *Biochim Biophys Acta* **1847**: 607-619

812 **Herbstova M, Tietz S, Kinzel C, Turkina MV, Kirchhoff H** (2012). Architectural switch in plant  
813 photosynthetic membranes induced by light stress. *Proc Nat Acad Sci USA* **109**: 20130–20135

814 **Hind G, Nakatani HY, Izawa S (1974)** Light-Dependent Redistribution of Ions in Suspensions of  
815 Chloroplast Thylakoid Membranes. Proc Nat Acad Sci USA **71**: 1484-1488

816 **Iwai M, Roth MS, Niyogi KK (2018)** Subdiffraction-resolution live-cell imaging for visualizing  
817 thylakoid membranes. Plant J **96**: 233-243

818 **Johnson MP (2018)** Metabolic regulation of photosynthetic membrane structure tunes electron  
819 transfer function. Biochem J **475**: 1225–1233

820 **Johnson, MP, Vasilev, C, Olsen, JD, Hunter, CN (2014)** Nanodomains of cytochrome b6f and  
821 photosystem II complexes in spinach grana thylakoid membranes. Plant Cell **26**: 3051 -3061

822 **Joliot P, Joliot A (2002)** Cyclic electron transfer in plant leaf. Proc Natl Acad Sci USA **99**: 10209–  
823 10214

824 **Khatoun M, Inagawa K, Pospíšil P, Yamashita A, Yoshioka M, Lundin B, Horie J, Morita N, Jajoo A,**  
825 **Yamamoto Y, Yamamoto Y (2009)** Quality control of photosystem II: thylakoid unstacking is  
826 necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under  
827 light stress in spinach thylakoids. J Biol Chem **284**: 25343–25352

828 **Kiss A, Crouchman S, Ruban AV, Horton P (2008)** The PsbS protein controls the organisation of the  
829 photosystem II antenna in higher plant thylakoid membranes. J Biol Chem **283**: 3972–3978

830 **Kou J, Takahashi S, Oguchi R, Fan DY, Badger MR, Chow WS (2013)** Estimation of the steady-state  
831 cyclic electron flux around PSI in spinach leaf discs in white light, CO<sub>2</sub>-enriched air and other  
832 varied conditions. Funct Plant Biol **40**: 1018-1028.

833 **Kouril R, Zygadlo A, Arteni AA, de Wit CD, Dekker JP, Jensen P-E, Scheller HV, Boekema EJ (2005)**  
834 Structural characterization of a complex of photosystem I and light-harvesting complex II of  
835 *Arabidopsis thaliana*. Biochem **44**: 10935–10940

836 **Kowalewska L, Mazur R, Suski S, Garstka M, Mostowska A** (2016) Three-Dimensional  
837 Visualization of the Tubular-Lamellar Transformation of the Internal Plastid Membrane  
838 Network during Runner Bean Chloroplast Biogenesis. *Plant Cell* **28**: 875-891

839 **Kyle DJ, Staehelin LA, Arntzen CJ** (1983) Lateral mobility of the light- harvesting complex in  
840 chloroplast membranes controls excitation energy distribution in higher plants. *Arch Biochem*  
841 *Biophys* **222**: 527–541

842 **Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV** (2000) The PSI-H subunit of photosystem I is  
843 essential for state transitions in plant photosynthesis. *Nature* **408**: 613-615.

844 **Lunde C, Jensen PE, Rosgaard L, Haldrup A, Gilpin MJ, Scheller HV** (2003) Plants Impaired in State  
845 Transitions Can to a Large Degree Compensate for their Defect. *Plant Cell Physiol* **44**: 44-54.

846 **MacGregor-Chatwin C, Sener M, Barnett SF, Hitchcock A, Barnhart-Dailey MC, Maghlaoui K,**  
847 **Barber J, Timlin JA, Schulten K, Hunter CN** (2017) Lateral Segregation of Photosystem I in  
848 Cyanobacterial Thylakoids. *Plant Cell* **29**: 1119–1136

849 **Mekala NR, Suorsa M, Rantala M, Aro EM, Tikkanen M** (2015) Plants actively avoid state-  
850 transitions upon changes in light intensity: role of light- harvesting complex II protein  
851 dephosphorylation in high light. *Plant Physiol* **168**: 721–734

852 **Mustárdy L, Garab G** (2003) Granum revisited. A three-dimensional model—where things fall into  
853 place. *Trends Plant Sci* **8**: 117–122

854 **Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T** (2004) Cyclic  
855 electron flow around photosystem I is essential for photosynthesis. *Nature* **429**: 579– 82

856 **Pan X, Ma J, Su X, Cao P, Chang W, Liu Z, Zhang X, Li M.** (2018). Structure of the maize  
857 photosystem I supercomplex with light-harvesting complexes I and II. *Science*, **360**: 1109-1113.

858 **Paolillo DJ Jr.** 1970. The three-dimensional arrangement of intergranal lamellae in chloroplasts. *J*  
859 *Cell Sci* **6**: 243–255

860 **Pribil M, Labs M, Leister D** (2014) Structure and dynamics of thylakoids in land plants. *J Exp Bot* **65**:  
861 1955-1972

862 **Pribil M, Pesaresi P, Hertle A, Barbato R, Leister, D** (2010) Role of plastid protein phosphatase  
863 TAP38 in LHCII dephosphorylation and thylakoid electron flow. *PLoS biology*, **8**(1), e1000288.

864 **Pribil M, Sandoval-Ibáñez O, Xu W, Sharma A, Liu Q, Galgenmüller C, Schneider T, Wessels M,**  
865 **Matsubara S, Jansson S, Wanner G, Leister D** (2018) Fine-Tuning of Photosynthesis Requires  
866 CURVATURE THYLAKOID1-Mediated Thylakoid Plasticity. *Plant Physiol* **176**: 2351-2364.

867 **Pietrzykowska M, Suorsa M, Semchonok DA, Tikkanen M, Boekema EJ, Aro EM, Jansson S** (2014)  
868 The light-harvesting chlorophyll a/b binding proteins Lhcb1 and Lhcb2 play complementary  
869 roles during state transitions in *Arabidopsis*. *Plant Cell* **26**: 3646–3660

870 **Puthiyaveetil S, Tsabari O, Lowry T, Lenhart S, Lewis RR, Reich Z, Kirchhoff H.** (2014).  
871 Compartmentalization of the protein repair machinery in photosynthetic membranes. *Proc Nat*  
872 *Acad Sci USA* **111**: 15839-15844

873 **Puthiyaveetil S, van Oort B, Kirchhoff H** (2017) Surface charge dynamics in photosynthetic  
874 membranes and the structural consequences. *Nature Plants* **3**: 17020

875 **Rintamäki E, Martinsuo P, Pursiheimo S, Aro EM** (2000) Cooperative regulation of light-harvesting  
876 complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in  
877 chloroplasts. *Proc Natl Acad Sci USA* **97**: 11644–11649

878 **Rozak PR, Seiser RM, Wacholtz WF, Wise RR** (2002) Rapid, reversible alterations in spinach  
879 thylakoid appression upon changes in light intensity. *Plant Cell Environ* **25**: 421–429

880 **Samol I, Shapiguzov A, Ingelsson B, Fucile G, Crèvecoeur M, Vener AV, Rochaix JD, Goldschmidt-**  
881 **Clermont M** (2012) Identification of a photosystem II phosphatase involved in light acclimation  
882 in *Arabidopsis*. *Plant Cell* **24**: 2596–2609

883 **Sathish Yadav KN, Semchonok DA, Nosek L, Kouřil R, Fucile G, Boekema E, Eichacker L (2017)**  
884 Supercomplexes of plant photosystem I with cytochrome b6f, light-harvesting complex II and  
885 NDH. *Biochim Biophys Acta* **1858**: 12–20

886 **Schumann T, Paul S, Melzer M, Dörmann P, Jahns P. (2017).** Plant growth under natural light  
887 conditions provides highly flexible short-term acclimation properties toward high light stress.  
888 *Front Plant Science*, **8**: 681.

889 **Shimoni E, Rav-Hon O, Ohad I, Brumfeld V, Reich Z (2005)** Three- dimensional organization of  
890 higher-plant chloroplast thylakoid membranes revealed by electron tomography. *Plant Cell* **17**:  
891 2580–2586

892 **Staehelein LA. (1976).** Reversible particle movements associated with unstacking and restacking of  
893 chloroplast membranes *in vitro*. *J Cell Biology*, **71**: 136-158.

894 **Theis J, Schroda M (2016)** Revisiting the photosystem II repair cycle. *Plant Signalling and Behavior*.  
895 **11**: e1218587

896 **Tikkanen M, Nurmi M, Suorsa M, Danielsson R, Mamedov F, Styring S, Aro EM (2008)**  
897 Phosphorylation-dependent regulation of excitation energy distribution between the two  
898 photosystems in higher plants. *Biochim Biophys Acta* **1777**: 425–432

899 **Tikkanen M, Grieco M, Kangasjärvi S, Aro EM (2010)** Thylakoid protein phosphorylation in higher  
900 plant chloroplasts optimizes electron transfer under fluctuating light. *Plant Physiol* **152**: 723–  
901 735

902 **Trissl HW, Wilhelm C (1993)** Why do thylakoid membranes from higher plants form grana  
903 stacks? *Trends Biochem Sci* **18**: 415–419

904 **Vallon O, Bulte L, Dainese P, Olive J, Bassi R, Wollman FA (1991)** Lateral redistribution of  
905 cytochrome b6/f complexes along thylakoid membranes upon state transitions. *Proc Nat Acad*  
906 *Sci USA* **88**: 8262-8266.

907 **Wientjes E, van Amerongen H, Croce R** (2013) LHCII is an antenna of both photosystems after long-  
908 term acclimation. *Biochim Biophys Acta* **1827**: 420-426.

909 **Wood WHJ, MacGregor-Chatwin C, Barnett S, Mayneord G, Huang X, Hobbs J, Hunter CN, Johnson**  
910 **MP** (2018) Dynamic thylakoid stacking regulates the balance between linear and cyclic  
911 photosynthetic electron transfer. *Nature Plants* **4**: 116–127

912

913

914

915

916

917

918

919

920

921

922