Supplementary Information

Photosystem II antenna phosphorylation-dependent protein diffusion determined by fluorescence correlation spectroscopy

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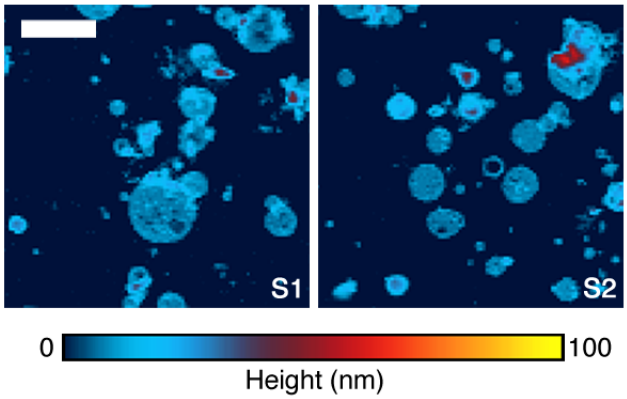

Figure S1 | The thickness of the isolated membranes determined by AFM. Representative AFM images of the isolated membranes were shown. S1, state 1; S2, state 2. Color scale, membrane height in nanometer scale. Scale bar, 1 μm. The average thickness of the membranes isolated from cells in state 1 and state 2 was 14.8 ± 0.6 and 14.5 ± 1.2 nm, respectively, which was calculated from the membrane areas of at least 50 μm^2.

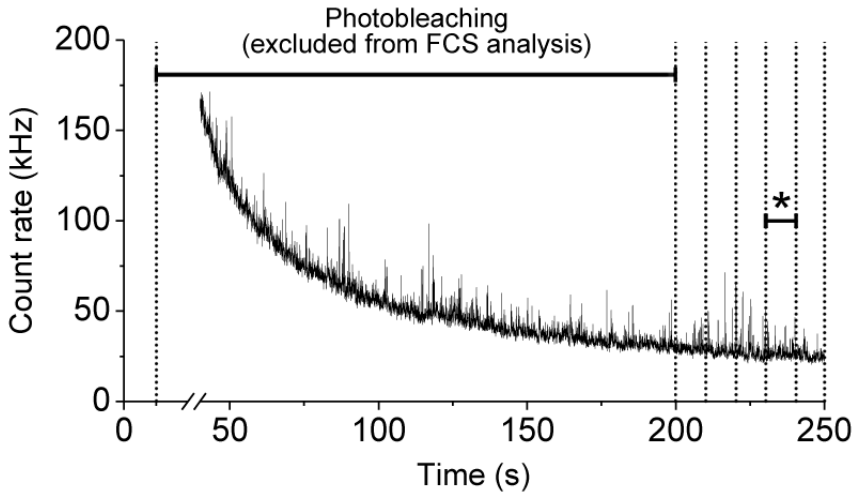

Figure S2 | Chl fluorescence fluctuations observed during FCS measurements. Chl fluorescence intensity was reduced due to the laser-induced photobleaching and subsequently became the stationary state. This photobleaching process is completely different from the one using in fluorescence recovery after photobleaching technique because of using very weak laser illumination in only the diffraction-limited detection volume that results in the slow reduction of fluorescence intensity. Thus, the effect of photobleaching on protein diffusion in other parts of the membrane should be minimal. Such continuous reduction is due to the immobilized or very slow proteins existed in thylakoid membranes. The FCS measurements were performed after Chl fluorescence reduced enough to observe the fluorescence fluctuations, as indicated dotted lines. This method is particularly effective to distinguish fast mobile proteins in the immobilized fractions with high fluorescence intensity[27,28]. Asterisk indicates the representative 10-s measurement, shown in **Fig. 2b**.

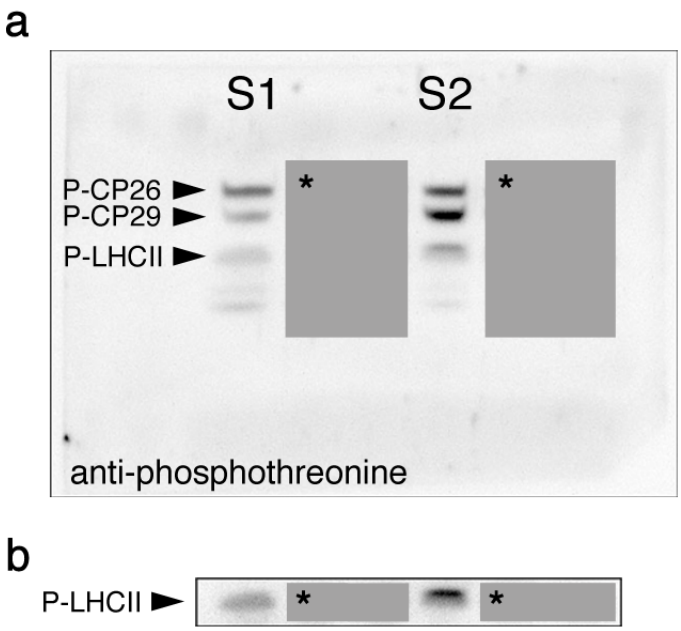

Figure S3 | The full length run of the blot for Fig. 3. (**a**) The samples for S1 and S2 were run on the same electrophoresis and blot. Please note that the parts of the blot are redacted and covered in gray squares with asterisks as these are the parts of unpublished data, which are not related to this study. (**b**) Because it is important to compare the phosphorylation levels between S1 and S2, not between the proteins, we cropped the bands for the phosphorylated LHCII and performed the linear contrast adjustment by using ImageJ software. The parts of unpublished data were then covered in gray squares with asterisks.

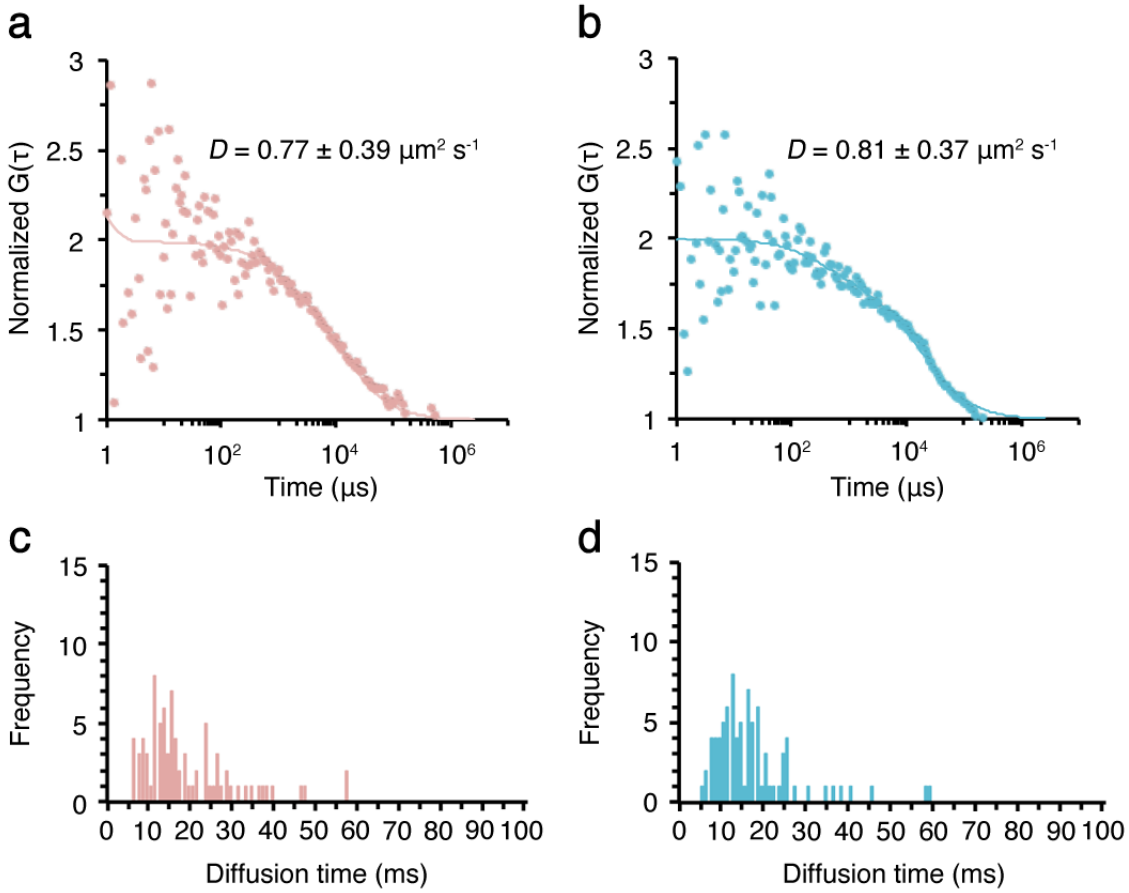

Figure S4 | FCS measurements using the stroma lamellae isolated from the *stt7* mutant treated with DCMU or CCCP. Before thylakoid membrane isolation, the *stt7* mutant was incubated with 10 μM DCMU or 5 μM CCCP for 20 min in Tris-acetate-phosphate liquid media under low light (~20 μmol photons m^{-2} s^{-1}) at 23°C, which were exactly the same treatments done with wild type. FCS measurements were similarly done as in **Fig. 2**. (**a, b**) The FAFs (dots) obtained from Chl fluorescence fluctuations were used to calculate the diffusion coefficients (*D*) of the CBPs in the membrane isolated from the *stt7* mutant treated with (**a**) DCMU or (**b**) CCCP. (**c, d**) The diffusion time of the CBPs in the membrane treated with (**c**) DCMU or (**d**) CCCP was also measured by FCS. Distribution of mobile CBPs with certain diffusion time is shown. Frequency was calculated during a total of ~5 min measurements observed in each membrane.

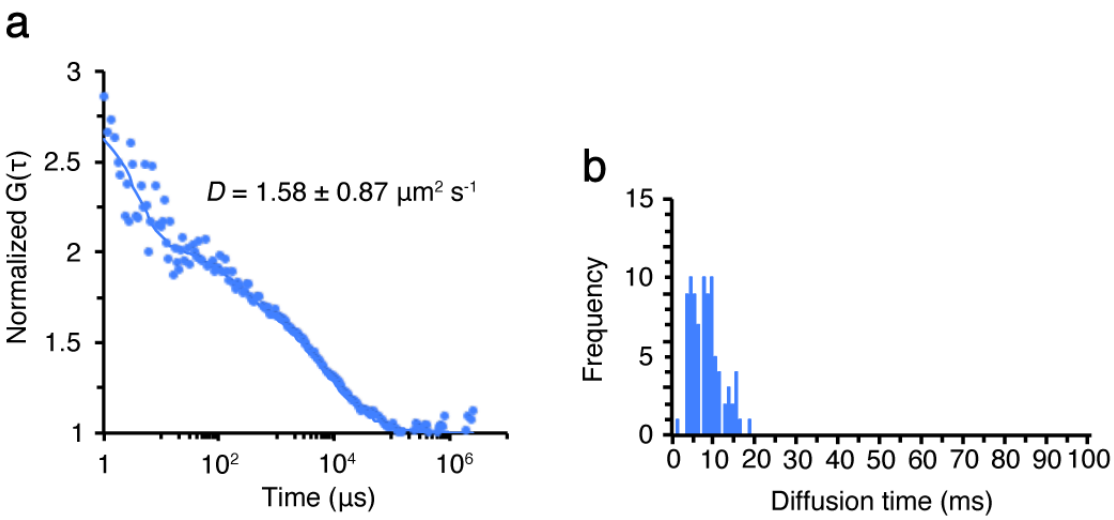

Figure S5 | FCS measurements using the stroma lamellae isolated from wild type cells in state 2 induced by anaerobic incubation in the dark. Before thylakoid membrane isolation, wild-type cell was anaerobically incubated in the dark, a well-known alternative method to induce state 2 condition in *C. reinhardtii*[32]. FCS measurements were similarly done as in **Fig. 2**. (**a, b**) The calculations of (**a**) the diffusion coefficients (*D*) and (**b**) diffusion time were done similarly.