Supporting Information

Liu et al. 10.1073/pnas.1120960109

SI Materials and Methods

Strains and Growth Conditions. For GFP-fusion strains, BG-11 medium was supplemented with 50 $\mu g \cdot mL^{-1}$ apramycin. Growth of the cells was monitored by turbidity at 750 nm with an SLM Aminco DW-2000 UV/Vis spectrophotometer (SLM Aminco). The *Escherichia coli* strains used in this work were DH5α and BW25113. *E. coli* was grown aerobically at 30–37 °C in Luria-Bertani (LB) medium. Medium supplements were used where appropriate at the following final concentrations: ampicillin 100 $\mu g \cdot mL^{-1}$, chloramphenicol 10 $\mu g \cdot mL^{-1}$, apramycin 50 $\mu g \cdot mL^{-1}$ and arabinose 100 μM.

Respiratory Electron Transport. O_2 uptake of cell cultures was measured in the dark at room temperature in a Clarke-type oxygen electrode (OxyLab 2; Hansatech). One milliliter of cell suspension with Chlorophyll (Chl) concentration of 10 μM was placed into the electrode chamber, aerated, and sealed from the atmosphere.

P_{700}^+ Rereduction Measurements. Cells grown at late log phase in fresh BG-11 with a Chl concentration of 10 μM were incubated in the absence or presence of 20 μM DCMU (a PSII inhibitor), in a glass cuvettete with dimensions of 1 × 1 cm. Cells were dark adapted for about 5 min and incubated in the presence of DCMU for 1 min before the measurement (except for cells preadapted for 30 min in ML in the presence of DCMU, where no additional DCMU was added). Eight saturating flashes from a xenon lamp (6 μs half peak width, 20 J per flash, filtered with a Schott BG-39 filter; 30 Hz) were applied perpendicular to the measuring beam to both sides of the cuvettete to illuminate the cells evenly. The kinetics of P_{700}^+ rereduction were recorded by a photomultiplier tube screened by narrow band-pass (695–707 nm interference notch) and RG 660 long-pass filters. For each measurement, 25 transients, with 2-s dark adaptation before each train of flashes, were averaged.

Membrane Preparation, Protein Analysis, and Immunoblotting. *Synechococcus* membrane fractions and soluble fractions for SDS-polyacrylamide gel electrophoresis (SDS/PAGE) analysis were prepared by glass bead (212–300 μm in diameter) breakage at 4 °C followed by centrifugation (1). For Blue-Native polyacrylamide gel electrophoresis (BN-PAGE) isolated membranes were prepared according to the previous method (2). Chl *a* content was determined by extraction into methanol and absorption measurement at 666 and 750 nm (3). Protein concentration was determined using a NanoVue spectrophotometer (GE Healthcare). Similar amounts of protein samples with 6 μg Chl were loaded on the gels for total protein and membrane fraction samples, and 80 μg of proteins were loaded for the soluble fraction samples. Membrane protein samples were either separated on 15% (vol/vol) denaturing SDS/PAGE gels containing 6 M urea (4) or on 5–12.5% (vol/vol) linear gradient native BN-PAGE gels (5, 6) as described previously (7) with modifications (8, 9). For the serial dilution Western blots, the samples were loaded on the gel with 1 μg Chl for 100%, 0.50 μg Chl for 50%, 0.25 μg Chl for 25%, and 0.1 μg Chl for 10%. Gels were either stained with Coomassie Blue R-250 or electroblotted onto nitrocellulose membrane (0.2-μm pore size; Bio-Rad Laboratories). Immunoblotting analyses were performed with primary rabbit polyclonal anti-GFP antibody diluted 1:6,000, and horseradish peroxidase-conjugated goat antirabbit IgG secondary antibody (GE Healthcare) diluted 1:10,000. Signals were visualized using a chemiluminescence kit (SuperSignal West Pico; Pierce).

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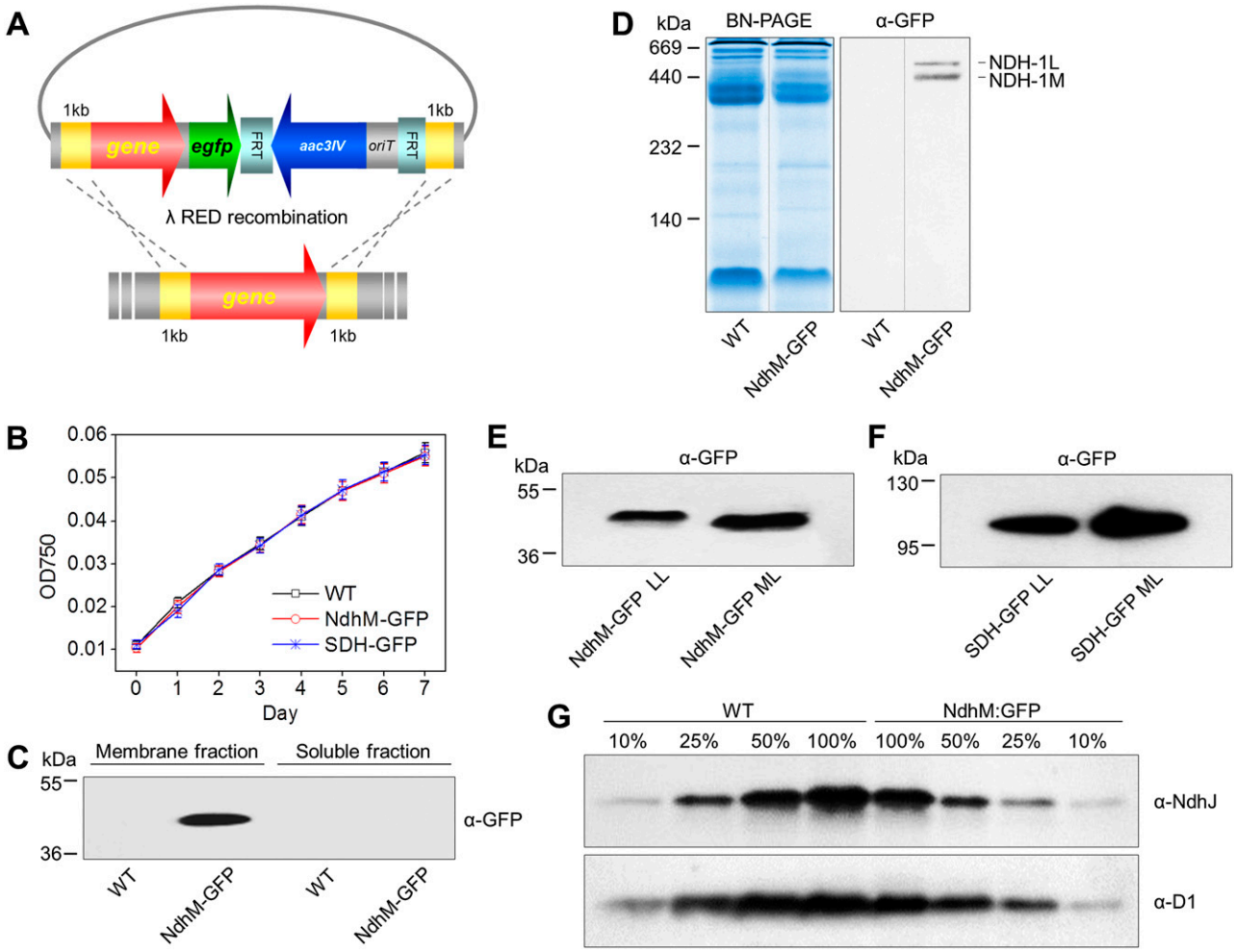

Fig. S1. Construction and characterization of NDH-1:eGFP and SDH:eGFP strains of *Synechococcus*. (*A*) Strategy of GFP fusion using REDIRECT protocol. (*B*) Comparison of the growth of WT *Synechococcus*, NDH-1:eGFP, and SDH:eGFP strains. Growth was monitored by turbidity of cell suspensions at 750 nm. Results are a mean ± SD of three independent cultures. (*C*) Immunoblot with anti-GFP antibody of membrane fractions and soluble fractions of the NDH-1:eGFP strain based on SDS/PAGE (15%). The band at around 40 kDa is consistent with the fusion of NdhM and eGFP. (*D*) BN-PAGE and immunoblots using anti-GFP antibody show that NDH-1:eGFP is incorporated into complete NDH-1 complexes. Western blot bands were assigned on the basis of their molecular weights according to (2, 8). Gel lanes were grouped from the same gel. (*E* and *F*) SDS/PAGE immunoblots showing that eGFP is fused to NdhM and SdhA respectively, under complexes under both ML and LL conditions. (*G*) Relative amounts of NDH-1 complexes in wild-type and NDH-1:eGFP strain measured by SDS/PAGE immunoblots with anti-NdhJ. Antibody to the D1 protein of Photosystem II is used as a reference. Samples are loaded on the basis of chlorophyll concentration (1 μg for 100%) and diluted to 50, 25, and 10% of initial concentration. Ratios of NDH-1:D1 staining at increasing dilution are 0.58, 0.57, 0.50, 0.48 in wild-type and 0.58, 0.57, 0.49, 0.48 in the NDH-1:eGFP strain.

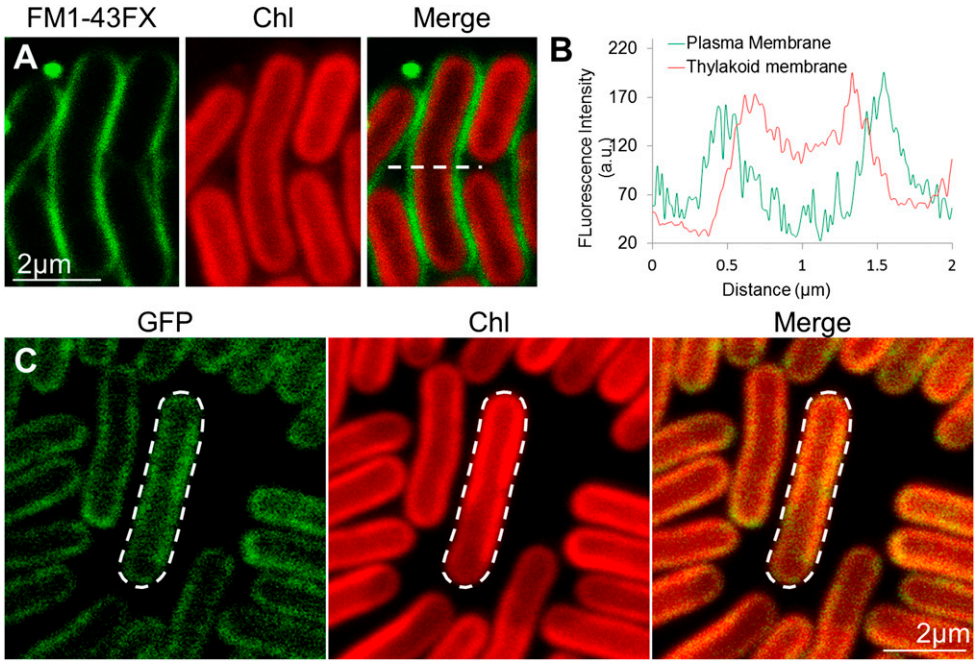

Fig. S2. *Synechococcus* cytoplasmic and thylakoid membranes can be distinguished in confocal images. (*A*) FM1-43FX, a green fluorescent membrane marker, was used to stain the cytoplasmic membrane (1). (*B*) Cross-section analysis of green (FM1-43FX) and red (chlorophyll) fluorescence along the white line in *A*, indicating a separation of 190 ± 19 nm (SD, *n* = 50) between the cytoplasmic and thylakoid membranes (*C*). Outlines of the cytoplasmic membranes (broken lines) based on the cross-section data, superimposed on images of NDH-1:eGFP and chlorophyll fluorescence.

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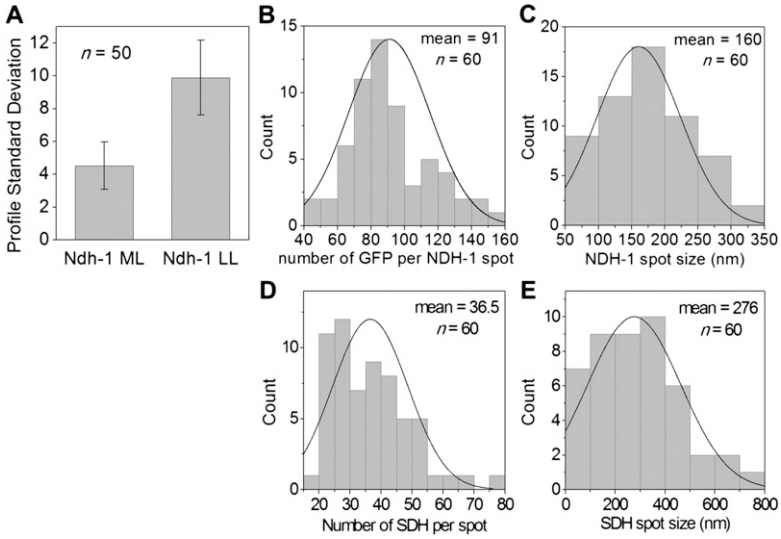

Fig. S3. Analysis of NDH-1:eGFP and SDH:eGFP distributions. (*A*) SDs of NDH-1:eGFP fluorescence profiles at ML and LL ($n = 50$, $P = 10^{-7}$). (*B*) There are around 91 ± 30 (SD, $n = 60$) NDH-1:eGFP complexes per NDH-1 patch. (*C*) The diameter of the NDH-1 spots (corrected for the microscope point spread function) varies from 60 to 350 nm, with a mean of 160 ± 28 nm (SD, $n = 60$). (*D*) There are around 37 ± 12 (SD) SDH:eGFP complexes per SDH patch ($n = 60$). (*E*) The mean diameter of the SDH patches (corrected for the microscope point spread function) is 276 ± 188 nm (SD, $n = 60$).

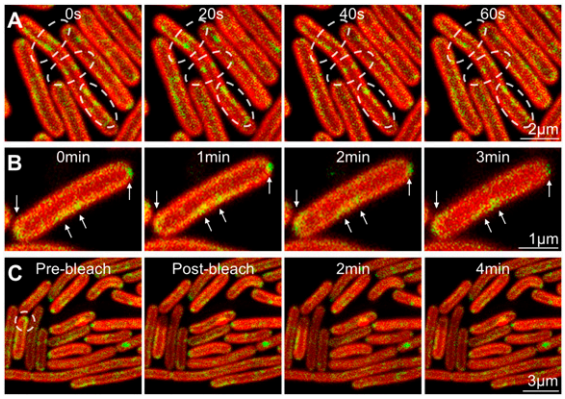

Fig. S4. Immobility of the NDH-1:eGFP patches in vivo. (*A*) Successive confocal images recorded over 60 s. (*B*) Successive confocal images recorded over 3 min. (*C*) FRAP analysis of the NDH-1 patches. No recovery was observed, indicating that diffusion of NDH-1 complexes between neighboring spots is not detectable on this timescale.

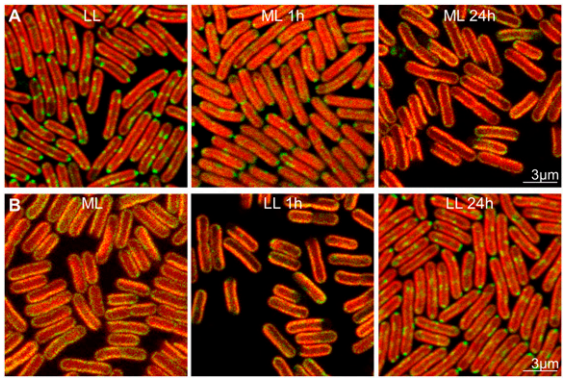

Fig. S5. Redistribution of NDH-1 complexes in response to light changes. (*A*) Fluorescence image under LL, after transferring to ML for 1 h and after 24 h. (*B*) Fluorescence image of ML-adapted cells, after transferring to LL for 1 h and after 24 h.

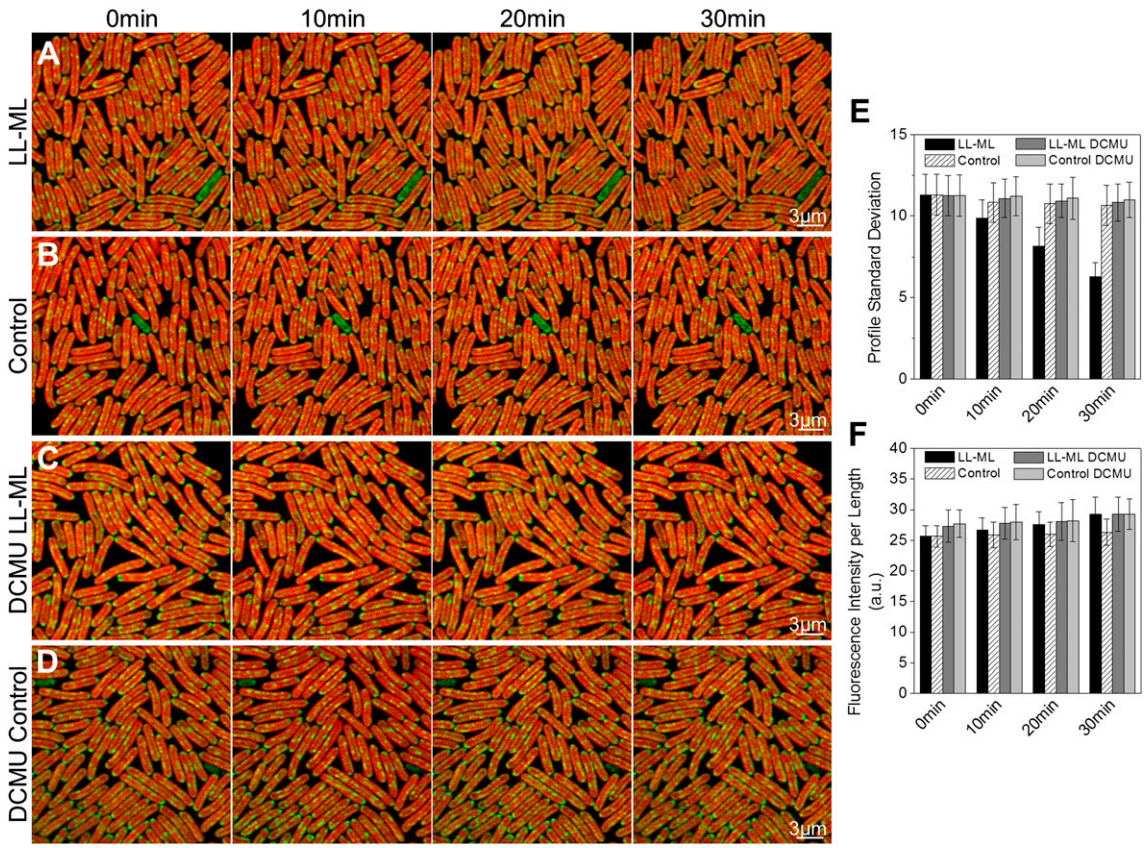

Fig. S6. Tracking of NDH-1 complexes within a single set of cells during illumination with continuous white-light at 500 μmol·m^{-2}·s^{-1} on the microscope stage in 30 min. (*A*) Confocal images of the same set of NDH-1:eGFP cells. (*B*) Control for *A* showing that the NDH-1:eGFP patches do not disperse or move when cells are kept in the dark between recording of images. (*C*) The same set of NDH-1:eGFP cells in the presence of DCMU. (*D*) Control for *C* showing that the NDH-1:eGFP patches do not disperse or move when cells in the presence of DCMU are kept in the dark between recording of images. (*E*) Fluorescence profile variances and (*F*) normalized fluorescence intensities for the low-light grown NDH-1:eGFP cells with/without DCMU exposed to intense light (LL-ML) and control (in the dark) for 10, 20, and 30 min (n = 50). Data analysis is shown in Fig. 1 in the main text.

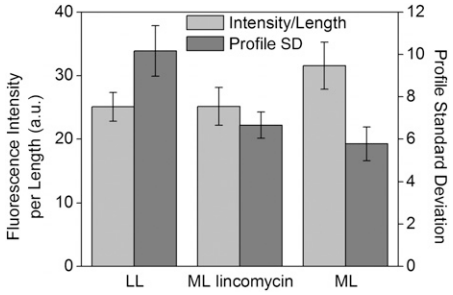

Fig. S7. Redistribution of the NDH-1 complexes does not depend on de novo protein biosynthesis. Lincomycin was added in the culture medium (final concentration, 400 μg·ml^{-1}) (1, 2) to inhibit protein synthesis. After switching from LL to ML, the total GFP fluorescence increases in untreated cells but not in lincomycin-treated cells (light gray bar). The SD of the NDH-1 distribution profile (gray) decreases (two-tailed Student t test, $P = 10^{-11}$) from 10.16 ± 1.19 in LL (SD, n = 30) to 6.65 ± 0.62 in ML (SD, n = 30) with the presence of lincomycin, indicating that protein biosynthesis is not required for the NDH-1 redistribution.

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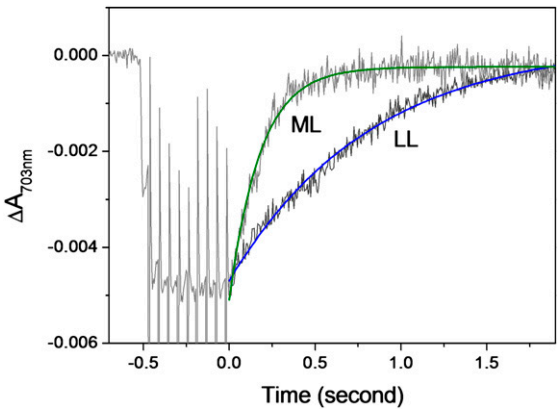

Fig. S8. Kinetics of the P_{700}^+ rereduction in the cells under ML and LL. P_{700} rereduction kinetics were determined at room temperature by analysis of flash-induced absorbance changes at 703 nm as indicated in *Materials and Methods*. Cells grown at late log phase in fresh BG-11 were incubated in the presence of DCMU before the measurement. Eight saturating flashes from a xenon lamp were applied, and the kinetics of P_{700}^+ rereduction were fitted with a single exponential decay.

Table S1. Primers used in this study (sequences 5′–3′)

Primer	Sequence
Respiratory genes	
Fsynpcc7942_1982	TCCGGCAACAGTAGCTTGAG
Rsynpcc7942_1982	CCATCCGCAAGGTGATTGAC
Fsynpcc7942_0641	ACCTCAGCTCTGGGTGATAG
Rsynpcc7942_0641	CGGCATTACTCGGCTTTACG
GFP fusion	
Fsynpcc7942_1982GFP	GTGCTGAACTACAGCATGGGCGTGCCGCAAGCCGTTGTCCTGCCGGGCCCGGAGCTGCC
Rsynpcc7942_1982GFP	GTTGCCCTGCGAGGTGAGTCCGTAGACTCAACGCCTGCAATTCCGGGGATCCGTCGACC
Fsynpcc7942_0641GFP	GAGTTCAAGACGTTAGTTCCCAGCCAGCGAAACTATCAACTGCCGGGCCCGGAGCTGCC
Rsynpcc7942_0641GFP	GGCGGGGGACTAAAGCCCCTTATTGCTTAAATGGAGGCGATTCCGGGGATCCGTCGACC
Segregation	
Fsynpcc7942_1982seg	GTACAATCTGCGTCGTATCG
Rsynpcc7942_1982seg	AGCCAAGGCCAAGGTGATTC
Fsynpcc7942_0641seg	GCTCTGCACCGTGAAGAATC
Rsynpcc7942_0641seg	TGTTAGCGACGGAAGGACTC
Sequencing	
Seq1synpcc7942_1982	TTTGGCTGGAAGGGCAGAAG
Seq2synpcc7942_1982	GTACAATCTGCGTCGTATCG
Seq1synpcc7942_0641	GCCGGTGATTCATAGCAAAG
Seq2synpcc7942_0641	TGTTAGCGACGGAAGGACTC