Organization and Flexibility of Cyanobacterial Thylakoid Membranes Examined by Neutron Scattering

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Running title: Dynamic changes in cyanobacterial cells examined by neutron scattering

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Background: In cyanobacteria, light harvesting and photosynthesis occur in the thylakoid membranes.

Results: The distances between thylakoid membranes are correlated with the size of the phycobilisome antenna and change reversibly and rapidly upon illumination.

Conclusion: Thylakoid membranes have a structural plasticity tied to the regulation of photosynthesis.

Significance: Characterizing the structural changes in photosynthetic membranes is crucial for understanding light harvesting and photosynthetic productivity.

SUMMARY

Cyanobacteria are prokaryotes that can use photosynthesis to convert sunlight into cellular fuel. Knowledge of the organization of the membrane systems in cyanobacteria is critical to understanding the metabolic processes in these organisms. We examined the wild type strain of Synechocystis sp. PCC 6803 and a series of mutants with altered light harvesting phycobilisome antenna systems for changes in thylakoid membrane architecture under different conditions. Using small-angle neutron scattering (SANS), it was possible to resolve correlation distances of subcellular structures in live cells on the nanometer scale and capture dynamic light-induced changes to these distances. Measurements made from samples with varied scattering contrasts confirmed that these distances could be attributed to the thylakoid lamellar system. We found that the changes to the thylakoid system were reversible between light- and dark-adapted states, demonstrating a robust structural flexibility in the architecture of cyanobacterial cells. Chemical disruption of photosynthetic electron transfer diminished these changes, confirming the involvement of the photosynthetic apparatus. We have correlated these findings with electron microscopy data in order to understand the origin of the changes in the membranes and found that light induces an expansion in the center-to-center distances between the thylakoid membrane layers. These combined data lend a dynamic dimension to the intracellular organization in cyanobacterial cells.

In photosynthetic organisms like plants, algae, and cyanobacteria, the light reactions occur in the highly specialized thylakoid membranes that house the components of the photosynthetic electron transfer chain. While plant thylakoids are arranged in stacked grana and unstacked stroma membranes, and algal
thylakoids can be appressed or separated depending on the strain (1), cyanobacterial thylakoids typically do not stack or appress and are more uniformly sheet-like (2). Cyanobacterial strains display differences in intracellular organization, in particular having various arrangements of the thylakoid membranes within the cell interior (2). The organization of the thylakoid membrane system in cyanobacterial cells has been explored by different techniques, including recent work using electron tomography to show the three-dimensional arrangement of the membrane system (3,4). While these studies have begun to show the complexity and spatial detail of these membrane systems, such work provides static views of what are undoubtedly dynamic structures. To date there is little information exploring the rearrangements of the thylakoid membrane system in response to environmental stimuli such as light or temperature in live cyanobacterial cells. In order to thoroughly understand the form and function of cyanobacterial thylakoid membranes, studies are needed that incorporate high resolution measurements of membrane structure with information of how the organization of these membranes change in response to environmental inputs.

Probing the structural responses of the thylakoid membrane systems of photosynthetic organisms to light is of particular interest. In *Synechocystis* 6803, growth in low light (0.5 µmol photons m⁻² s⁻¹) with glucose as a carbon source resulted in inflated thylakoid lumens measuring up to 300 nm (5). Kirchhoff and colleagues used cryoelectron microscopy to examine vitreous sections and found that the grana lumen in *Arabidopsis thaliana* expands in the light, thereby increasing the lumenal volume and presumably facilitating protein diffusion (6). Earlier studies of the thylakoid membranes in the marine algae *Ulva* and *Porphyra* demonstrated a reproducible and reversible light-induced flattening of the thylakoid lumenal space as measured by electron microscopy of chemically fixed samples and microdensitometry (7). Recently, Nagy and coworkers used small-angle neutron scattering (SANS) to examine the *Synechocystis* 6803 PAL mutant and found a light-induced increase in repeat distances that they attributed to periodic spacing between adjacent thylakoid membrane pairs (8). These data demonstrate the need for additional studies to understand the effect of light on the organization of thylakoids in detail, and to clarify whether illumination results in expansion or contraction of the membrane system.

Obtaining structural information from photosynthetic organisms under changing conditions requires non-destructive techniques capable of probing the membrane systems of live cells. Studies using light microscopy approaches such as fluorescence recovery after photobleaching (FRAP) and hyperspectral confocal fluorescence microscopy (HCFM) have led to intriguing findings regarding the mobility of photosynthetic components and the organization of photosynthetic pigments within cyanobacterial cells (9,10). SANS can also be used to characterize periodically organized biological membrane systems on smaller length scales by observing diffraction from regularly spaced structural features separated by ~1-200 nm (11). Both the accessible length scales and the ability to examine individual components of biological systems using contrast matching has led to the application of SANS in studies of cyanobacteria, eukaryotic algae, plant thylakoids (8,12), and mammalian mitochondria (13).

In order to examine thylakoid membrane organization in cyanobacterial cells under different conditions, we used a correlative approach incorporating both transmission electron microscopy and SANS. These techniques both provide complementary data at the length scales relevant to the study of biological structures, including membranes. We compared the thylakoid membrane organization in wild type *Synechocystis* 6803 cells to that of mutants in which the phycobilisomes have been truncated by varying degrees. These mutants are CB (containing one phycocyanin hexamer per rod), CK (lacking phycocyanin rods but still possessing the allophycocyanin core), and PAL (lacking functionally assembled phycobilisomes) (14-16). Our results showed that the distances between thylakoid membrane layers is correlated with the size of the phycobilisome light harvesting antenna complexes and that these distances change reversibly and rapidly upon illumination, with strain-specific differences.
Furthermore, our data indicated that the photosynthetic membranes in *Synechocystis* 6803 have a structural plasticity that is tied to the function of the photosynthetic electron transfer chain.

**EXPERIMENTAL PROCEDURES**

**Electron microscopy**--Cells were prepared for electron microscopy as previously described in (3). Cultures of WT, CB, CK, and PAL cells were grown for 5 days in liquid BG11 (17) with appropriate antibiotics, harvested by centrifugation, resuspended in a small volume, and loaded into planchettes for high pressure freezing (Bal-Tec). Thin sections (~80 nm) were cut from resin-embedded material and imaged using a LEO 912AB electron microscope equipped with a ProScan digital camera. Measurements were taken using Soft Imaging Systems’ iTEM software and are given as the results of 20-30 measurements per cell type.

**Small angle neutron scattering**--SANS measurements were performed using the Bio-SANS instrument (18) at the High Flux Isotope Reactor, Oak Ridge National Laboratory. Cyanobacterial suspensions were in BG11 media prepared with H$_2$O (H-media), D$_2$O (D-media), or a mixture of the two. Cells grown in standard H-BG11 were harvested by centrifugation, washed twice in 10 ml of fresh media (with D$_2$O depending on the experiment), resuspended in 5 ml of media, and equilibrated overnight at 30°C under ~25 μmol photons m$^{-2}$s$^{-1}$ white fluorescent light with 150 rpm shaking. All cultures were then adjusted to the same optical density (measured at λ = 730 nm). SANS measurements were carried out in 1 mm path cylindrical cells at 30°C. The sample enclosure was equipped with a lighting apparatus that illuminated each sample position with ~ 20 μmol photons m$^{-2}$s$^{-1}$ cool white LED light. A dark enclosure was also installed to allow for dark adaptation of individual samples. Three different configurations of the Bio-SANS instrument were employed to collect data over the scattering vector range of 0.003 < q < 0.7 Å$^{-1}$ with sample-to-detector distances of 1.1, 6.8 and 15.3 m and a neutron wavelength of 6 ± 0.15 Å.

**Quasi-elastic neutron scattering**--QENS experiments were performed on the backscattering spectrometer (BASIS) at the Spallation Neutron Source, Oak Ridge National Laboratory (19). The samples were prepared as described above and placed in rectangular aluminum cells with colorless sapphire windows to allow for illumination with white light (~280 μmol photons m$^{-2}$s$^{-1}$). The raw data were converted from time-of-flight to energy transfer at selected q values using standard BASIS data reduction software, and data analysis was performed using the DAVE software package (20). Data fitting was carried out using the jump diffusion model, $G(q) = \frac{\eta Dq^2}{(1 + Dq^2\tau)}$, where $\eta$ is the reduced Planck’s constant, D is the translational diffusion coefficient, and $\tau$ is the residence time between diffusion jumps.

**RESULTS**

We have used transmission electron microscopy (TEM) to examine the thylakoid membrane systems in *Synechocystis* 6803 wild type (WT) and the phycobilisome mutant strains CB, CK, and PAL (10). Thin section electron micrographs of high pressure frozen cells showed that the distances between thylakoid membranes were greatest in WT cells with intact
phycobilisomes and smallest in the PAL mutant in which phycobilisomes were absent (Figure 1). In all strains, we measured the thickness of the membrane pairs that enclose the lumenal space and form a single thylakoid, and the interthylakoidal distances between the membrane pairs. The thickness of the thylakoid membrane pair was consistently ~150 Å in all strains, but the distances between membrane pairs varied considerably. We report the distance between membrane pairs in two ways: as the membrane-to-membrane boundary distance across the width of the interthylakoidal space, and as the center-to-center repeat distance between the thylakoid layers. The membrane-to-membrane distance is relevant for discussing the space available to the phycobilisome antenna in the cytoplasmic region, while the center-to-center repeat distance is the measurable quantity directly obtained in SANS.

Wild type cells (Fig. 1A) showed the characteristic intracellular arrangement of Synechocystis 6803: layers of ~3-6 concentric membrane pairs generally conforming to the curvature of the cell envelope, with a central cytoplasmic region largely devoid of thylakoid membranes, but in which other cellular components were found. We measured the interthylakoidal distances between membrane pairs as averaging 400 ± 80 Å in WT cells, consistent with reported measurements of 300-500 Å (21,22). The center-to-center thylakoid membrane distance was 550 Å for WT cells (Fig. 1B and Table 1). In terms of overall cell morphology and thylakoid membrane organization, the CB strain (which contains only one phycocyanin hexamer per rod) appears very similar compared to WT (Fig. 1C). Measurements of interthylakoidal distances averaged 330 ± 60 Å, and center-to-center membrane distance was 480 Å for CB (Fig. 1D and Table 1). In the CK strain, which possesses only the APC core, distances between thylakoid membrane layers averaged 320 ± 20 Å (Fig. 1E), with center-to-center distances of 470 Å (Fig. 1F and Table 1). In the PAL mutant (Fig. 1G), thylakoid membranes consistently formed straight bands with little curvature (10). The interthylakoidal distances in PAL averaged 190 ± 30 Å, with center-to-center membrane distances of 340 Å (Fig. 1H and Table 1).

Periodic cellular structures were indicated in the SANS profiles obtained from samples suspended in 100% D-media by the appearance of diffraction peaks above power-law and incoherent scattering backgrounds as shown for WT in Fig. 2A. A series of samples equilibrated in 0%, 20%, 40%, 60%, 80%, and 100% D-media were measured to determine the scattering contrast match points(s) of the diffracting structures and to identify their general molecular composition (viz., protein, lipid, etc.). Fig. 2B shows that the contrast for the structure diffracting at the lowest- was “matched” (i.e., disappeared) at ~17% D-media, a value known to correspond to lipid structures. The scattering profile of WT in 20% D-media (Fig. 2A) demonstrated that all diffraction peaks were largely matched at this media scattering length density (SLD) and pointed to the ordered thylakoid membranes observed by TEM as the source of all of the diffraction measured from these samples.

Including the thylakoid repeat features, up to five distinct peaks, depending upon the strain, could be observed with small strain-to-strain variations in both the q positions and relative intensities (Table 1). A putative assignment of all the diffraction features observed from the WT sample is presented in the following discussion. Furthermore, to ensure that the observed peaks were originating from physiological phenomenon in live cyanobacterial cells, we performed plating experiments comparing cells exposed to neutrons for two hours with cells treated identically but not exposed to neutrons. We found that exposing cells to neutrons for this amount of time did not decrease cell viability, and therefore concluded that our results showed the responses of live cyanobacterial cells to our experimental conditions.

The small-angle scattering observed for all strains equilibrated in 100% D-media and incubated in light is shown in Fig. 3A. The scattering profiles from all strains showed significant differences in the observed diffraction at the lowest q (Peak 1). Based on the length scale of both this periodicity and the periodic thylakoid arrangements from TEM, we attributed this diffraction feature to the thylakoid membrane center-to-center period (Table 1).
The systematic shortening of this period with antenna truncation can be seen in the shift to higher $q$ values for this feature.

To determine whether changes to the SANS profiles would occur if cells were incubated in the dark, cells were dark adapted for 1 hour, and data were collected in an identical manner as for cells in the light. SANS data from cells in the dark showed changes in the $q$ positions and relative intensities, but the overall pattern of the profiles remained fairly consistent for each strain (Fig. 3B). All strains have a first peak at 0.010 Å$^{-1}$ to 0.017 Å$^{-1}$ and a third peak centered at ~0.042 Å$^{-1}$. However, Peaks 2, 4, and 5 are only present in a subset of the strains (Table 1). Peak 2 is clearly observed in WT in the dark at approximately 0.022 Å$^{-1}$. In the light this peak is strongly reduced but still discernible as a shoulder (Fig. 3A), and the curve shape suggests yet another peak at ~0.016 Å$^{-1}$ in WT in light. Peak 2 is weakly present in CB but not in the other mutant strains. There is a fourth peak in all strains except WT in dark from 0.045-0.071 Å$^{-1}$. A fifth peak is present in WT and CK (Table 1).

The trend in changes between light and dark was not consistent among the strains (Table 1). The values that corresponded to the largest scale (Peak 1, Table 1) showed a small dark-induced expansion in the CB mutant, but CK and PAL showed a contraction in this distance in the dark with changes of 37.4 Å for CK and 25.8 Å for PAL. No change in position of Peak 1 was discernible for WT, but its intensity was clearly reduced in response to illumination. Peaks 3, 4, and 5, observed at larger $q$, which corresponds to smaller distances, mostly showed expansion in light. This effect was clearest for Peak 3 with ~8% expansion in the wild type and 4% to 5% expansion in the mutants. All four strains showed a reduction in scattering intensity for the peaks at smaller $q < 0.025$ Å$^{-1}$ (distances > 250 Å) when exposed to light. In contrast, scattering intensity for the higher $q$ peaks clearly increased for WT. Similarly, in all 3 mutants the intensity of Peak 4 at $q \sim 0.045$ Å$^{-1}$ increased when cells were exposed to light.

Time-series measurements were made to characterize the timescale of the transition between the dark- and light-adapted states. Samples of WT, CK and PAL strains were adapted to darkness for one hour before a series of five-minute measurements during illumination began. SANS from the WT strain (Fig. 4A) showed that the dark-to-light changes occurred rapidly, with the majority happening within the first ten minutes of illumination. In contrast, however, structural changes in the PAL mutant only began to appear after 30 minutes of illumination (Fig. 4B), with change occurring up to 2 hours. Reversed light-to-dark time-series measurements were conducted for the CK strain. The dark adapted structures were largely recovered within 20 minutes (Figs. 4C-D).

To determine if these changes depended upon functional photosynthetic electron transport, we incubated dark-adapted cells with 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), an inhibitor of electron transport from photosystem II. DCMU treatment did not perturb the dark-adapted structure. However, the SANS measured from these samples after 30 minutes of illumination (Fig. 5) showed little change from the corresponding dark state and demonstrated the dependence of the light-induced changes on an intact electron transport chain.

It was of interest to investigate if the light-induced structural rearrangements observed by SANS that are attributed to a swelling of the thylakoid lumen resulted in changes in the intracellular dynamics of the cyanobacteria. Quasi-elastic neutron scattering is ideally suited to investigate dynamics on a pico- to nanosecond time frame. Measurements on the SNS backscattering spectrometer, BASIS, were carried out in order to assess the effect of light on the microscopic dynamics of the cyanobacteria and its intracellular water. Both the faster water dynamics and the slower cellular dynamics could be measured simultaneously due to the combination of a high energy resolution and a wide dynamic range of the BASIS. It was possible to analyze the translational dynamics of the intracellular water up to $q = 0.7$ Å$^{-1}$ and the internal dynamic motions of cellular components up to $q = 0.9$ Å$^{-1}$. Fig. 6 shows the $q$-dependence of the quasielastic signal broadening (half-width at half-maximum) for WT *Synechocystis* 6803 cells measured with and without light. Data for *Escherichia coli* are presented for comparison. The broader $q$-dependent component originates...
from the intracellular water, whereas the narrower \( q \)-independent component originates from the cellular components of the bacteria. Unlike the measured dynamics of water, which is clearly translational, the internal dynamics of the bacteria is of localized character resulting in \( q \)-independent broadening of the scattering signal. The \( q \)-dependence of the water data shown in Fig. 6 was fitted with the jump diffusion model described above. The results of the fitting are shown in Table 2. Within the accuracy of the experiment, the fit values obtained are essentially the same for cells in the light- and dark-adapted states and also similar to the translational diffusion coefficient bulk water \( (29.8 \times 10^{-10} \text{ m}^2 \text{s}^{-1}) \) (23). Thus, the space accessible to the intracellular water in cyanobacteria is not sufficiently restricted to affect its translational dynamics, despite the profound structural changes in the membranes observed in the SANS experiment. Similarly, the measured internal dynamics of the cyanobacteria are not affected by light either; the relaxation times calculated from the \( q \)-independent broadening component as \( \tau = \frac{\hbar}{\Gamma(q)} \) are \( (157 \pm 21) \times 10^{-12} \text{ s} \) and \( (153 \pm 21) \times 10^{-12} \text{ s} \) with and without light, respectively. In the case of \( E. \text{coli} \), the diffusion coefficient of water was similar to the values obtained with the cyanobacteria (Table 2) but the dynamics cellular components exhibited a significantly slower relaxation time, \( (175 \pm 15) \times 10^{-12} \text{ s} \), as is evident in Fig. 6. This possibly reflects the notable differences in the cellular architectures of the two bacterial species.

**DISCUSSION**

Cyanobacteria such as *Synechocystis* 6803 have a highly organized thylakoid membrane architecture, and it has been proposed that rearrangements in the membrane system, and the spacing between membrane layers, play a role in the cellular regulation of photosynthesis (10). Ideally, investigation of membrane architecture in cyanobacteria requires real-time *in situ* studies that can follow the time course of reorganization in response to external stimuli such as light or other environmental conditions. However, techniques that combine the required spatial resolution with the capability to resolve changes over time are rare. TEM is the method of choice for determining cell physiology at the nano- to meso-scale and has been used extensively to determine thylakoid architecture (4); however, it can rely heavily on fixation of the specimen and does not provide a dynamic picture. Solution scattering techniques, on the other hand, can in principle provide this missing link to the understanding of photosynthesis, but have hardly been used in this field of research until recently (24).

The length scales of interest in this context range from the thickness of individual lipid bilayers to the spacing between thylakoid membrane layers (a few nanometers to just under 100 nanometers), which is covered by small-angle scattering (SAS) techniques using x-rays (SAXS) or neutrons (SANS). However, well-defined peaks are not commonly observed in the small-angle range of scattering data obtained from complex biological systems. In order to observe diffraction-like peaks in biological SAS, well-defined discrete repeat distances must be present in the system, and there must be good contrast between the scattering length densities of the ordered cellular component and the surrounding medium. Both of these prerequisites are fulfilled in cyanobacteria that are equilibrated in D\(_2\)O media: the membrane system has been shown to exhibit well defined spacings, and the D\(_2\)O-rich cytoplasm and lumen have strong contrast relative to hydrogen-rich lipid membranes. For a system that is as complex as entire cells, however, the convoluted scattering contributions from a mixture of structural features generally pose great challenges to the interpretation of SAS data. Fortunately, this situation can be greatly improved for SANS by contrast enhancement through selective replacement of hydrogen atoms with its heavier isotope deuterium (\( ^2\text{H} \) or D). In cyanobacterial cells, well-organized arrangements of phycobilisomes have been reported (21) and a number of other structural features could in principle produce peaks. By using contrast variation, we were able to conclude that the observed peaks are indeed due to membrane repeat distances (Figure 2). We attributed these distances to the thylakoid membrane system due to the repeating nature of the concentric thylakoid membrane layers, and considered it unlikely that the membrane
components of the cell envelope are able to contribute to the observed peaks. Recent work by Nagy et al. has successfully applied SANS to photosynthetic systems (8,12). Their pioneering study, however, has left many open questions. In the current study, we have developed a more comprehensive picture through correlation of SANS data with TEM images of the same cyanobacterial strains, observation and interpretation of additional diffraction peaks, and application of scattering contrast variation.

SANS data from the four cyanobacterial strains investigated at both light and dark conditions show qualitative agreement (Fig. 3). All profiles can be described by sets of correlation peaks above a power-law baseline (exponent ~ -2.5). The largest repeat distance observed in our studies was found in WT cells at 627 Å (Peak 1). This peak has been predicted previously (8), but this is the first time that it was observed experimentally. The repeat distance agrees well with the expected sum of ~150 Å for thylakoid membrane thickness and an interthylakoidal spacing of ~450 Å (21) or 300-500 Å (22) as has been reported from TEM data, as well as our own measured interthylakoidal distance of ~400 Å (Fig. 1). The differences seen here among the TEM data are likely due to the inherent biological variability of the membrane spacing when measured within individual cells, and sample preparation and variations in growth conditions may also contribute.

Studies have shown that modulation of phycobilisome antenna size results in changes to the interthylakoidal distances (21). The largest SANS repeat distance (position of Peak 1) systematically decreases with successive reduction of the light harvesting antenna in the order WT, CB, CK, PAL, giving further strong evidence that the size of the phycobilisome is closely related to membrane spacing as reported previously (10,21). In particular, removal of the phycocyanin rods in CK leads to a reduction of SANS-derived membrane spacing by 170 Å (in light) or 207 Å (in the dark), and the latter number agrees perfectly with the result reported for the phycocyanin-less strain PMB11 by Olive et al. (21). In the mutant strain PÆE, which lacks assembled phycobilisomes, a small interthylakoidal distance of 40 Å was measured (21,22). However, our TEM data did not show as severe a decrease in the interthylakoidal distance in the PAL mutant that lacks phycobilisomes, where the interthylakoidal distances averaged 190 Å. The origin of the differences found in the interthylakoidal distances measured by TEM between these two strains lacking phycobilisomes, PAL and PÆE, is unknown, but may be due in part to fixation methods (high pressure freezing versus chemical fixation). It is interesting that our SANS data showed a repeat distance of 383 Å (light) or 357 Å (dark) for the PAL strain (Peak 1), and this value is similar to our center-to-center membrane distance from PAL TEM images (340 Å) (Table 1).

For a system with appressed membranes, a repeat distance of about 190 Å would be expected, based on the thickness of a thylakoid pair (~150 Å) and an interthylakoidal distance of ~40 Å, as seen in the PÆE strain and as has been measured for appressed thylakoids in other systems (1). This value was observed by Nagy et al (8) and also shows in our SANS data for the PAL mutant as Peak 3. However, a value very close to this is also present in WT, CB, and CK, and this repeat distance varies relatively little among the different strains. These data suggest that all the strains may contain some regions of nearly appressed thylakoids with interthylakoidal distances of 20 Å to 40 Å. We did not see evidence for this in our TEM data; however, SANS gives data averaged over a large population. Moreover, if such appressed membranes form extended stacks, then the diffraction signal would be strongly amplified and even a small concentration of such regions could be visible in the diffraction pattern. In all strains this repeat distance increases in response to illumination. This may be interpreted as an increase in interthylakoidal spacing from 20 to 30 Å in the dark to 30 to 40 Å in the light. However, an alternative interpretation can be given in that the thickness of the lumen also increases in response to light.

SANS data from WT in the dark exhibited a repeat distance of 300 Å (Peak 2 at 0.022 Å⁻¹). This corresponds to an interthylakoidal spacing too narrow to accommodate phycobilisomes. In light this peak
is attenuated and accompanied by an additional repeat distance of 393 Å (at ~0.016 Å⁻¹). The latter value suggests that such thylakoids are separated by a single layer of phycobilisomes. This peak is also weakly present in the CB mutant.

The repeat distances obtained from Peaks 4 and 5 are too small to encompass the width of a thylakoid membrane plus interthylakoidal spacing. Instead, these peaks correspond to distances that are similar to the width of a single thylakoid membrane. Indeed, the repeat distance of the pair of lipid bilayers that enclose the width of the lumen is expected to be approximately 100 to 110 Å, and Peak 5 matches this distance. However, the reason for the absence of Peak 5 in CB and PAL is unknown at this time. Peak 4, with values slightly larger than Peak 5, might suggest a swollen thylakoid membrane in which the lumen width is increased by approximately 30 Å. Peak 4 is absent in WT in the dark but rises in response to light. Thus, SANS data suggest a swelling of the lumen in response to light exposure in WT. It is at present unknown what the significance of lumen swelling in response to light is, but this is consistent with the findings of Kirchhoff et al. (6).

In summary, SANS data of WT and mutant Synechocystis 6803 cells revealed at least five distinct peaks that could be correlated with TEM images of these same strains. These findings are summarized in Fig. 7, which shows a schematic drawing of our interpretation of these data: Peak 1 is the center-to-center thylakoid membrane repeat distance that may accommodate phycobilisomes, while Peak 2 is a center-to-center repeat distance that is too small for phycobilisomes. Peak 3 originates from a population of appressed membranes in all strains, and Peaks 4 and 5 originate from single thylakoid membrane layers with variable luminal widths. While raising further questions, these descriptions are the most comprehensive analysis of SANS/TEM data sets to date and may serve as a basis for future such work in other photosynthetic membrane systems.

Our findings show that cyanobacterial cells undergo reversible structural changes in response to light, and that these changes are significantly different among the phycobilisome antenna mutants. These intracellular rearrangements within the thylakoid system were reproducible between biological and technical replicates in Synechocystis cells in both the wild type and mutant strains, suggesting that these responses are robust. Future measurements under different light qualities and intensities will probe these intracellular reorganizations further. In Synechocystis 6803, it is known that many components, including photoreceptors, can play a role in the response of cells to light, and probing the membrane changes in mutant strains lacking such components will be particularly informative. The ability to directly observe membrane rearrangement in response to light exposure and its dependence on antenna mutations points towards a rich arsenal of interdependent membrane regulation processes that allow cyanobacteria a rapid and efficient adaptation to changes in light intensity.
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Dynamic changes in cyanobacterial cells examined by neutron scattering

FIGURE LEGENDS

Figure 1. Transmission electron micrographs of WT and phycobilisome antenna mutants. Whole cell images and enlargements showing thylakoid membrane spacing are shown for WT (A-B), CB (C-D), CK (E-F), and PAL (G-H). White bars depict the center-to-center thylakoid membrane spacing. Labeled are T, thylakoid membranes; P, polyphosphate bodies. Bar = 500 nm in (A), (C), (E), (G) and 50 nm in (B), (D), (F), (H).

Figure 2. Contrast variation for WT in light and dark. SANS intensity was measured for samples in both light and dark conditions for a range of ratios of D_2O to H_2O. (A) Scattering profiles for samples in dark and equilibrated in 100% D_2O (solid) and 20% D_2O (open). In (B), the square root of scattering intensity for the diffraction peak at lowest q is plotted as a function of D_2O concentration for samples in light (open) and dark (solid). The intensities from samples in 20% D_2O were excluded from the linear regression fits (solid lines). The contrast match point for the major peak is 17.9% in the light and 16.5% in the dark, within the range of a peak that is primarily composed of lipids.

Figure 3. SANS data from cyanobacterial cells under light and dark conditions. Scattering intensities from WT (red), CB (blue), CK (green), and PAL (gold) in light (A) and dark (B) over the entire experiment q range are shown. Peaks are labeled 1-5. In (A) intensities for WT and PAL are vertically offset by -12 cm\(^{-1}\) and 6 cm\(^{-1}\), respectively. In (B) intensities for WT, CB and PAL are vertically offset by -12 cm\(^{-1}\), -3 cm\(^{-1}\) and 6 cm\(^{-1}\), respectively.

Figure 4. Time course of dynamic changes in cyanobacterial strains during dark–light transition. (A) WT during the transition from dark to light. (B) PAL during the transition from dark to light, showing that peaks shift slightly in the Q range but do not appear or disappear. (C) CK during light to dark and (D) dark to light transition.

Figure 5. Inhibition of electron transport prevents light/dark induced changes. Strains were pre-incubated in the dark in the presence of DCMU and data were collected in the dark and upon exposure to light for (A) WT, (B) CB, (C) CK and (D) PAL strains. Untreated light (solid red) and dark (solid black) scattering profiles are reproduced from Figure 2 for comparison with the DCMU treated light (open red) and DCMU treated dark (open black) profiles. In (A) intensities were vertically offset by 0.4 cm\(^{-1}\) for treated and untreated samples exposed to light. In (B) intensities were vertically offset by 0.7 cm\(^{-1}\) for the untreated and by 0.3 cm\(^{-1}\) for the treated samples exposed to light. In (C-D) intensities for treated and untreated samples exposed to light were vertically offset by 0.3 cm\(^{-1}\).

Figure 6. Quasi-elastic linewidths and fits to jump-diffusion model used to determine average water diffusion coefficients. The half-width at half-maximum values of the q-dependent and q-independent Lorentzian components of the quasi-elastic energy spectra are shown for WT *Synechocystis* in dark (solid circles), WT *Synechocystis* illuminated with white light (open circles) and BL-21 *E. coli* (solid squares), respectively. Fits of the WT dark, WT illuminated, and *E. coli* dark q-dependent linewidths to the jump-diffusion model are shown as solid, dashed, and dotted lines, respectively.

Figure 7. Schematic drawing of thylakoid membrane organization in cyanobacterial cells from SANS data and TEM analysis. Different membrane and phycobilisome arrangements are numbered 1-5 to correlate with SANS peaks: 1, center-to-center repeat distance between thylakoid membrane pairs, shown with WT phycobilisomes; 2, repeat distance between thylakoid membrane pairs insufficient for phycobilisomes; 3, repeat distance originating from closely appressed thylakoid membrane pairs; 4 and 5, repeat distance originating from a single thylakoid membrane layer with lumen of varying size.
## TABLES

Table 1. Distances and peak positions in WT, CB, CK, and PAL cells under light (L) and dark (D) conditions. All measurements are in Å.

|       | TEM<sup>a</sup> | SANS          |
|-------|----------------|---------------|
|       | Peak 1 | Peak 2 | Peak 3 | Peak 4 | Peak 5  |
| 6803 L | 550    | 627.3  | 295.7  | 181.1  | 138.9  | 113.7  |
| 6803 D | 480    | 627.1  | 299.5  | 167.8  | -      | 114.0  |
| CB L   | 470    | 524.1  | 311.2  | 181.3  | 130.3  | -      |
| CB D   |        | 529.7  | 308.7  | 175.0  | 128.5  | -      |
| CK L   | 470    | 457.3  | -      | 197.8  | 144.1  | 112.1  |
| CK D   |        | 419.9  | -      | 188.4  | 142.9  | 106.0  |
| PAL L  | 340    | 382.7  | -      | 192.4  | 129.0  | -      |
| PAL D  |        | 356.9  | -      | 184.8  | 122.6  | -      |

<sup>a</sup>TEM data report the center-to-center thylakoid membrane distances.

Table 2. Translational diffusion coefficients (D) and mean residence times (τ) obtained from the jump diffusion model of the QENS data. Error is reported as standard deviation.

| Organism                              | D<sup>b</sup> (10<sup>-10</sup> m<sup>2</sup>/s) | τ<sup>b</sup> (10<sup>-12</sup> s) |
|---------------------------------------|---------------------------------|---------------------------------|
| *Synechocystis* 6803 (dark)           | 28.8 ± 9.7                      | 4.7 ± 3.3                       |
| *Synechocystis* 6803 (light)          | 27.7 ± 9.7                      | 5.2 ± 3.5                       |
| *Escherichia coli* (dark)             | 32.1 ± 6.8                      | 6.7 ± 1.8                       |
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Figure 1
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Figure 2
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Figure 4
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Figure 5
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Figure 6
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Figure 7
Organization and Flexibility of Cyanobacterial Thylakoid Membranes Examined by Neutron Scattering
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