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Review

# Diffusion of molecules and macromolecules in thylakoid membranes ☆

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ABSTRACT

The survival and fitness of photosynthetic organisms is critically dependent on the flexible response of the photosynthetic machinery, harbored in thylakoid membranes, to environmental changes. A central element of this flexibility is the lateral diffusion of membrane components along the membrane plane. As demonstrated, almost all functions of photosynthetic energy conversion are dependent on lateral diffusion. The mobility of both small molecules (plastoquinone, xanthophylls) as well as large protein supercomplexes is very sensitive to changes in structural boundary conditions. Knowledge about the design principles that govern the mobility of photosynthetic membrane components is essential to understand the dynamic response of the photosynthetic machinery. This review summarizes our knowledge about the factors that control diffusion in thylakoid membranes and bridges structural membrane alterations to changes in mobility and function. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.



## 1. Introduction

Life on earth requires constant energetic fueling of cell metabolism that is ensured by photosynthetic conversion of solar radiation into metabolic energy forms. Along the trajectory of photosynthetic evolution, the invention of oxygenic photosynthesis by ancient cyanobacteria is considered a big bang of evolution because it made available the almost infinite reservoir of water as an electron donor [1]. Geological records show a rise in the atmospheric oxygen level about 1.8 billion years ago indicating that oxygenic photosynthesis must have occurred way before this time [2,3]. Indeed, it was predicted that oxygenic photosynthesis was already operative more than three billion years ago [4]. During these eons, photosynthetic energy conversion was optimized and tuned to tackle the multiple challenges dictated by the dynamic environment where terrestrial and aquatic photosynthetic organisms lived and still live in. An understanding of photosynthetic energy conversion must appreciate that natural photosynthesis was shaped by evolution to flexibly react to environmental alterations that occur on different time scales. The dynamic response of the photosynthetic machinery depends on diffusion processes. Therefore, knowledge about diffusion of molecules and macromolecules is essential to understanding the plasticity of photosynthetic membranes in a challenging nature.

The evolution of the photosynthetic machinery leads to the highly specialized thylakoid membrane system that separates two aqueous phases, the stroma (plants and green algae) or cytoplasm (cyanobacteria) and the thylakoid lumen. Four highly conserved protein complexes are responsible for energy conversion. They are all membrane integral multi-subunit complexes embedded in the thylakoid membrane bilayer. Three of the four complexes, photosystem II (PSII), cytochrome $b_6f$ complex (cyt *bf*), and photosystem I (PSI) constitute the light-driven electron transport chain from water to ferredoxin. Reduced ferredoxin is a universal reducing equivalent used for many metabolic reactions but mainly for assimilation of $CO_2$ via NADPH + $H^+$. The electron transport is strictly coupled to proton translocation from the stroma side of the thylakoid membrane to the lumen that acidifies the thylakoid lumen. The resulting proton motive force is used for ATP formation catalyzed by the fourth protein complex, the ATPase. The photosynthetic machinery is complemented by light harvesting antenna complexes (LHC) that are connected to the photosystems and increase their apparent absorption cross section for harvesting sunlight. In contrast, to the conserved electron transport complexes and the ATPase, LHC complexes show a high degree of variability [5] ranging from hydrophobic membrane embedded LHCs (plants, green and red algae) to extrinsic hydrophilic phycobilisomes (red algae and cyanobacteria). Finally, in addition to this core set of protein complexes, low-abundance small proteins exist in thylakoid membranes. They have come more and more into focus since they are often involved in regulation, repair, turnover, and biogenesis processes.

This review focuses on diffusion-dependent processes in thylakoid membranes of plants and cyanobacteria because of the high knowledge base that exists for these organisms. Care must be taken to extrapolate data from these organisms to other photosynthetic organisms (diatoms, red and green algae) because their thylakoid membrane architecture can deviate. As will be seen below, almost all functional aspects of photosynthetic energy transformation are dependent on the migration of metabolites and protein complexes. This includes diffusion of the small electron carriers, plastoquinone (PQ) and plastocyanin (PC), which wire

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electron transport between the large protein complexes as well as the mobility of protein supercomplexes necessary for adaptation and repair processes. The efficiency of these multiple diffusion processes is highly dependent on the exact structural boundary conditions in thylakoid membranes that in turn are dynamic and controlled by the environment. It will be demonstrated that small structural changes cause switches between efficient and inefficient diffusion. In this context, this section has strong connections to other sections in this special issue dealing with supramolecular protein organization (chapters 12, 13), the dynamic changes of thylakoid membranes (chapters 16, 17), and its modulation by posttranslational protein modifications (chapter 18). Our knowledge about the plasticity of diffusion processes in thylakoid membrane is fragmentary. This is partially caused by technical limitations and partially because the importance to understand the interdependency between membrane structure and mobility and its functional consequences is often underestimated. However, recent reviews on this emergent topic exist [6,7] and the interested reader should refer to these reviews.

## 2. Diffusion theory

In 1906, Albert Einstein published a theory on diffusion that describes how far a particle will move from a given position if it performs random Brownian type motion [8]. For two-dimensional (2D) systems like biomembranes, the Einstein equation reads:

$$\langle r\rangle^2 = 4 \cdot D \cdot t \tag{1}$$

where $\langle r\rangle^2$ is the mean squared displacement of the particle, $t$ is the time, and $D$ a constant (diffusion coefficient). It follows that in this ideal case of infinite diluted membranes, the plot $\langle r\rangle^2$ versus time gives a straight line where slope is $4D$. From Eq. (1), it follows that for quantitative predictions of lateral diffusion processes, knowledge of the diffusion coefficient $D$ is necessary. The hydrodynamic Saffman–Delbrück theory [9] calculates $D$ for membrane proteins as a function of the membrane thickness ($h$), membrane viscosity ($\mu m$), viscosity of the aqueous fluid around the membrane ($\mu w$), and the radius ($R$) of the diffusing cylinder (approximating that the protein has a cylindrical shape):

$$D = \frac{k_B \cdot T}{4 \cdot \pi \cdot \mu m \cdot h} \cdot \left[ ln\left(\frac{\mu m \cdot h}{\mu w \cdot R}\right) - 0.5772 \right]. \tag{2}$$

An important conclusion is that Eq. (2) predicts a weak logarithmic dependence of $D$ on the radius of the protein. Recent experimental evidence indicates a stronger dependency of $D$ on the radius of the protein, i.e. instead of $D$ being proportional to $\ln(1/R)$, it is proportional to $1/R$ [10,11]. However, these theories (i.e. Einstein's diffusion theory) are valid only for very diluted membranes in contrast to most biomembranes. In particular, bioenergetic membranes [12] are the opposite to the diluted Singer Nicolson membrane model suggesting that a few proteins are dispersed in a sea of lipids (see the Macromolecular crowding section below). Since diffusion in crowded membranes can be very different to diffusion in diluted membranes, knowledge of the impact of crowding on mobility is central to understanding diffusion-dependent processes in photosynthetic membranes.

A diffusion theory that includes the protein packing density (obstacle concentration) in membranes is percolation theory [13,14]. Percolation theory predicts that $D$ is no longer a constant but can become distance dependent if obstacles hinder diffusion. The higher the obstacle concentration (c) the stronger the decline in $D$ as a function of diffusion distances. At a critical obstacle concentration (protein density) called percolation threshold (cP), the obstacles form enclosed diffusion domains that prevent any long-range diffusion of a small tracer (e.g. plastoquinone). This can be regarded as a phase transition because of an abrupt change of a fundamental physicochemical membrane property, i.e. a switch from long-range to short range diffusion. In the following, the enclosed diffusion areas will be named microdomains. cP is dependent not only on obstacle density but also on obstacle shape, interaction, self-diffusion, and more [13,14], i.e. on many structural and physicochemical parameters that make it difficult to predict. However, as a rule of thumb, cP occurs at relative obstacle densities (area occupations) around 0.6 to 0.7. As will be seen below, these are interesting values because they are close to protein densities in thylakoid membranes. For an understanding of the multiple diffusion-dependent processes in photosynthetic membranes, percolation theory is a helpful concept because it guides us to ask the right questions about critical parameters that determine mobility of photosynthetic components. In particular, it asks for the exact molecular architecture of membranes because small changes in structural parameters like protein density can have a huge impact on long-range diffusion [14].

## 3. Structural boundary conditions for diffusion in thylakoid membranes

There is a good knowledge base about structural boundary conditions realized in photosynthetic thylakoid membranes. Since these are addressed in other contributions of this special issue, only aspects related to diffusion processes are summarized in the following and the reader is referred to chapters 12, 13, 16, and 17 of this special issue for more detailed information.

### 3.1. Overall thylakoid membrane architecture

In oxygenic photosynthetic organisms, the energy converting apparatus is localized in the thylakoid membrane system. In plants and green algae, the thylakoid membrane partially folds to characteristic grana stacks (Fig. 1) that are interconnected by unstacked stroma lamellae [15–17]. The cylindrical grana stacks are missing in free-living aquatic cyanobacteria. It was hypothesized that membrane stacking to grana was invented after photosynthetic organism colonized terrestrial habitats and reflect an adaptation to life on land [18]. In grana-containing organisms, the photosynthetic protein complexes are not homogenously distributed between stacked and unstacked thylakoid regions [17,19–21] (see also chapter 12 of this special issue). PSII with LHCII complexes are concentrated in grana thylakoids whereas PSI with LHCI and the ATPase are found preferentially in unstacked regions. The existence of grana thylakoid membranes sets special structural constraints to molecular traffic between stacked and unstacked regions. For example, for the lateral movement of protein complexes between stacked and unstacked regions, the relation between the width of the stromal gap in grana stacks and the stromal protrusion of proteins is critical. Recent cryo-electron microscopic (EM) image analysis [22,23] shows that the stromal gap in grana is only about 3.5 nm (Fig. 1). This value gives a structural basis for the exclusion of PSI and the ATPase from stacked grana because their stromal protrusions are larger [24,25]. Critical for protein diffusion is also the width of the thylakoid lumen on the opposite membrane side (Fig. 1). This width was determined to be variable between 4.5 and ~10 nm [22,23]. The implication of the luminal width on proteins localized in this tiny compartment will be addressed in Section 4.3.

### 3.2. Macromolecular crowding

The density of the membrane integral protein complexes in photosynthetic membranes is one of the highest for biomembranes [12,26]. In the light of the percolation theory, this is a critical quantity because if the density is below or above the percolation threshold, it determines whether long-distance diffusion is possible or not. Estimates of the actual number for the relative protein area for grana and whole thylakoids were derived from EM, atomic force microscopy and biochemical data [27–29]. These different approaches give a remarkable constant value of 0.7 to 0.8 for grana thylakoid membranes and about 0.69 (corresponding to a lipid/protein ratio of 0.34 [w/w], [27]) for whole thylakoid

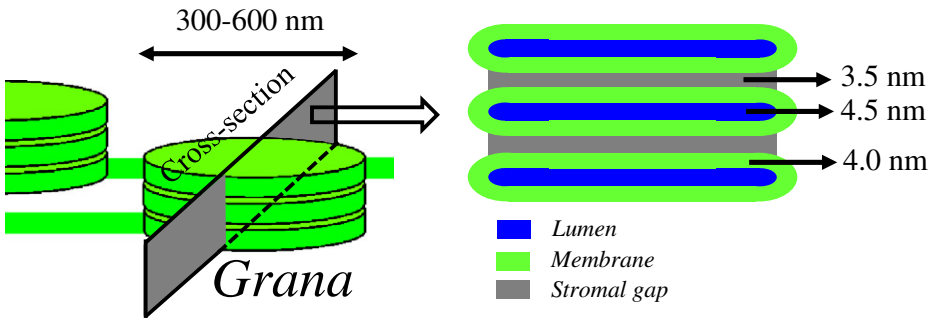


**Fig. 1.** Structural quantities of grana thylakoid membranes. The cartoon to the right shows part of the thylakoid membrane system. The cross-section on the right is in scale for vertical dimensions but off scale laterally.

membranes (see also Fig. 3 for visualization). It is assumed that the protein density in stroma lamellae is lower but concrete numbers are rare. According to [26], the protein density in stroma lamellae membranes is about 1/3 lower than in grana membranes indicating that the difference between stacked and unstacked thylakoid membranes is not high. However, this is the only report about protein packing densities in stroma lamellae, which are based on rough estimates of the molecular area of the protein complexes.

A physiological advantage of macromolecular crowding in photosynthetic membranes is that it enables a high concentration of protein bound pigments and therefore leads to high probability for absorbing sunlight that is a dilute energy source on molecular scales [5]. The chlorophyll concentration in thylakoid membranes was estimated to about 0.3 M [27]. Furthermore, we recently found that high protein densities in grana membranes are required for efficient intermolecular exciton energy transfer between LHCII complexes and PSII [30]. However, macromolecular crowding can significantly impede lateral diffusion (see below).

### 3.3. Supercomplexes

On the molecular scale, a central feature of photosynthetic membranes (reviewed in [15,21]) as well as respiratory membranes (reviewed in [31,32]) is that protein complexes assemble to higher non-covalently linked supercomplexes. For photosynthetic membranes of higher plants, almost all main protein complexes form supercomplexes: LHCII-trimers, dimeric PSII with variable amount of bound LHCII-trimers, dimeric cyt *bf* complexes, and PSI with four LHCI. It is unknown why supercomplexes are formed. For the PSII–LHCII supercomplexes formation could facilitate exciton energy transfer that requires a well-defined and exact orientation between light absorbing pigments [33].

However, concerning small hydrophobic molecules in thylakoid membranes, supercomplexes can facilitate diffusion. As illustrated in Fig. 2, the efficiency of obstacles for hindering tracer diffusion is higher if the obstacles are smaller (assuming the same obstacle area occupation). This is understandable because diffusion between smaller obstacles means more collisions leading to longer diffusion path length and consequently longer diffusion time (Fig. 2). In contrast in membranes with larger supercomplexes, the tracer experiences less collisions leading to a shortening of the diffusion time. These more intuitive considerations are strongly supported by Monte Carlo computer simulations for biomembranes in general [14] and for PQ diffusion in grana membranes in particular (Fig. 2, right, [28]). Thus, in addition to a role in the intrinsic functionality of protein complexes, the formation of supercomplexes is significant for the mobility of small molecules that have to diffuse through crowded thylakoid membranes.

### 3.4. Supramolecular organization

Here, the supramolecular level is defined as spatial arrangements of many proteins (supercomplexes) in the thylakoid membrane. It is in-between the molecular nm-scale and the whole membrane μm-scale. A long known feature of supramolecular organization in plant grana membranes [34] is that PSII can rearrange into highly ordered semicrystalline arrays [21]. The functional consequences of crystalline protein array formation are unknown as well as the physicochemical forces that drive their formation. Very recently, computer calculations that simulate the supramolecular protein phase behavior showed that native grana membranes are often in a state of co-existence of disordered and crystalline arrays [35]. The simulations predict that the coexistence of supramolecular states exists at protein packing densities above 0.7, i.e. as found for grana thylakoids. Changes in the LHCII/PSII ratio, protein density, or protein interaction energies can shift the equilibrium towards

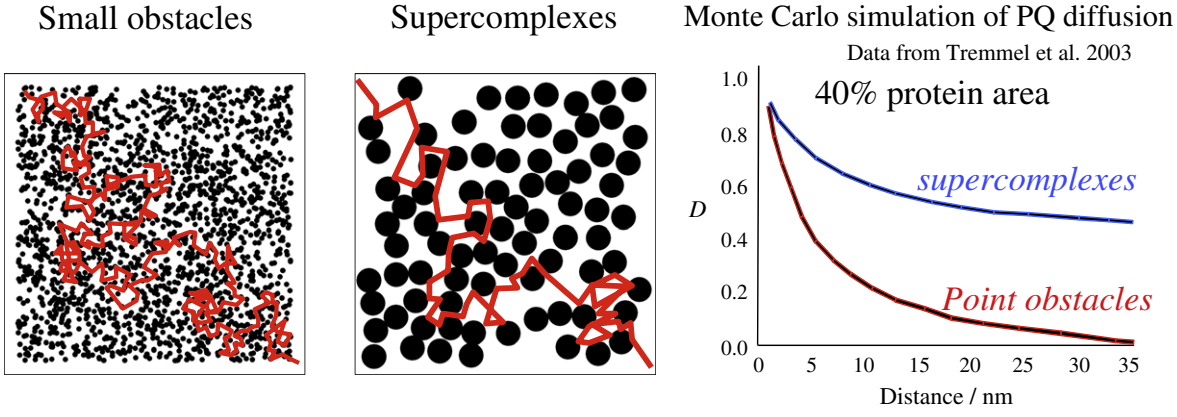


**Fig. 2.** Dependence of lateral tracer diffusion on obstacle size. Small obstacles (left) are more efficient in hindering the diffusion of tracers (red lines) than larger obstacles like supercomplexes (middle). This was demonstrated by Monte Carlo computer simulations (right) showing a much stronger distance-dependent decline in *D* for smaller obstacles.

arrays. With respect to diffusion processes, highly ordered protein arrays can have distinct impacts on the mobility of small and large molecules as discussed in Section 5.1.

## 4. Diffusion of small electron carriers and metabolites

### 4.1. Plastoquinone

The ~750 Da sized plastoquinone shuttles electrons between PSII and cyt *bf* complexes by diffusion through the hydrophobic lipid space of the thylakoid membranes. Although PQ is the smallest electron transport component, it has multiple central roles [36]. Besides electron shuttling between PSII and cyt *bf* complexes, the redox state of the PQ pool controls the activities of protein kinases that regulate energy conversion. In addition, the PQ redox status acts as a sensor for differential gene expression and determines the vulnerability of PSII to photodamage. Since the PQ redox status is directly dependent on the efficiency of electron shuttling, it is also a function of its diffusion efficiency in the thylakoid lipid bilayer. Impaired diffusion would increase the reduction level. Therefore, knowledge of PQ diffusion is important to understand electron flux and its regulation.

The diffusion space in grana membranes 'seen' by PQ is visualized in Fig. 3 (upper panel). As mentioned in Section 3.2, this high protein density in grana membranes is close to the percolation threshold and thus microdomain formation is likely. Direct structural evidence for PQ microdomains is missing since no techniques are available to visualize the local arrangement of PSII, LHCII, and cyt *bf* complexes in the same membrane. However, there is functional data showing that PQ cannot equilibrate freely, providing indirect evidence for the existence of microdomains [37,38]. In support for the microdomain concept, PQ diffusion coefficient in thylakoid membranes shows a drastic retardation compared to free diffusion in pure lipid liposomes (Table 1). Finally, PQ microdomains were also predicted by Monte Carlo computer simulations [28,39]. Thus, different approaches reveal that PQ diffusion is highly restricted in grana thylakoid membranes of plants (Fig. 3). For grana-free thylakoid membranes of cyanobacteria, lipid diffusion was determined by measuring the mobility of fluorescence lipophilic dyes (Table 1). The derived diffusion coefficient is on the higher end compared to grana-containing thylakoids but is still much lower than for PQ diffusion in protein-free liposomes. This indicates that lipid diffusion in thylakoid membranes is retarded in cyanobacteria as well but so far no evidence exists for microdomain formations. It has to be taken into account that thylakoid membranes in cyanobacteria are significantly different compared to plants and green algae. First, their light-harvesting phycobilisome antenna system sits on top of the membrane in contrast to the membrane embedded LHCs. Second, in cyanobacteria, all the proteins involved in respiration share the same membrane as the photosynthetic components. However, the degree of protein crowding is similar as in higher plants [40]. Therefore, it cannot be excluded that PQ-microdomains exist in thylakoid membranes of cyanobacteria as well. In the study of [41], it was demonstrated that increasing the desaturation level of fatty acids in cyanobacterial thylakoid membranes speeds up lipid mobility more than 6-times (Table 1). This shows that the microviscosity in the hydrophobic core of the membrane is critical for the mobility of lipid-like membrane components.

At first sight, a restricted PQ diffusion by microdomains seems disadvantageous. However, PQ microdomains give PSII a high control over the whole electron transport chain [38]. Since the capacity of electron transport through PSII is significantly higher than for the rate-limiting cyt *bf* complex [42], a down-regulation of PSII would have minor impact on the whole electron flux. In terms of the control theory [43] this means that PSII has a low control coefficient in contrast to the cyt *bf* complex that has a high coefficient [38]. However, this is only valid for a free equilibrating PQ pool in which the 'overcapacity' of PSII can become effective. Microdomains break the free redox equilibration and functionally connect only a small number of PSII and cyt *bf* complexes. In the microdomain scenario, a down-regulation of PSII has direct consequences for the overall rate through the cyt *bf* complex and thus the

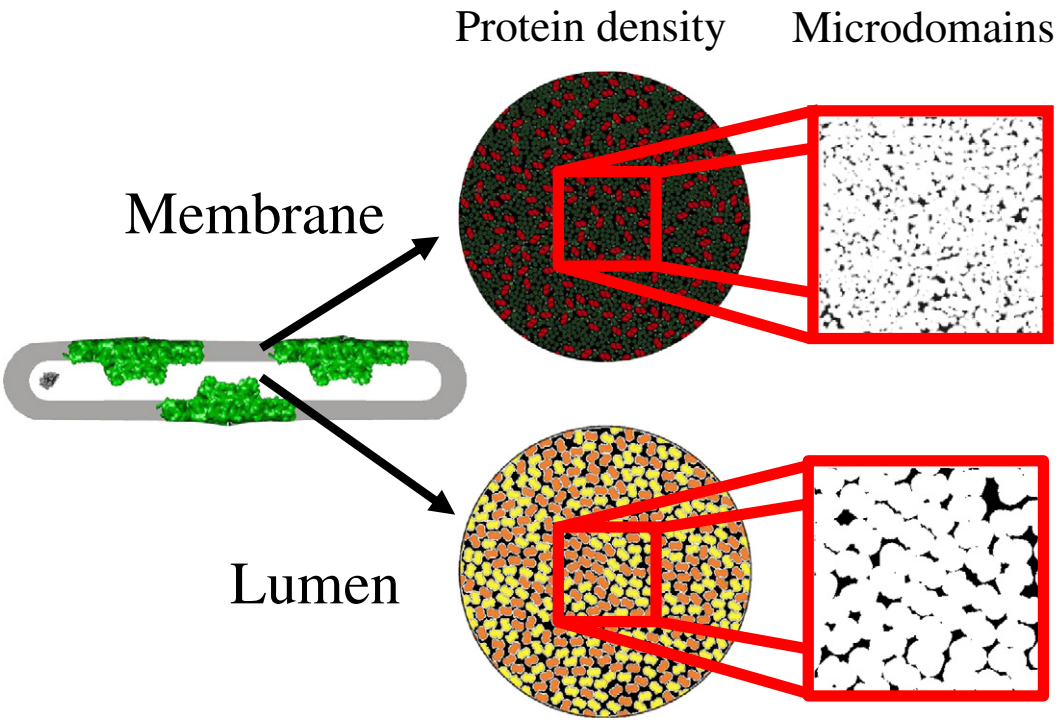


**Fig. 3.** Macromolecular crowding and diffusion microdomains. Both the grana thylakoid membrane bilayer (top) as well as the aqueous grana thylakoid lumen (bottom) are heavily packed by protein complexes. In the bilayer, LHCII and PSII cause crowding whereas in the lumen the water splitting apparatus contributes to crowding. From this, it follows that both diffusion spaces form microdomains for PQ/xanthophylls (upper right) or PC (bottom right). Note that these models are for dark-adapted plants and considerable changes can occur in illuminated plants. The different colors (yellow, orange) for the lumen model indicate PSII protrusion coming from two different membranes (upper and lower membranes in the cartoon on the left).

**Table 1**
Lipid and protein diffusion coefficients in thylakoid membranes [80–84].

| | Method | Diffusion coefficient $\mu m^2 s^{-1}$ | Reference |
|---|---|---|---|
| **Lipids** | | | |
| Plants: | | | |
| PQ thylakoids | Pyrene quenching | 0.01-0.3 | [84]Blackwell et al. 1994 |
| PQ liposomes | | 10-30 | [85, 86]Blackwell et al. 1987 Blackwell and Whitmarsh 1990 |
| Cyanobacteria: | | | |
| Lipids (WT) | FRAP on BODIPY | 0.28 +/-0.05 | [41]Sarcina et al. 2003 |
| Lipids (desA+) | | 1.8 +/-0.3 | [41]Sarcina et al. 2003 |
| **Proteins** | | | |
| Plants: | | | |
| PSII grana | Fluorescence rise after restacking | 0.0006 (20°C) | [87]Rubin et al. 1981 |
| PSII grana | FRAP | <0.0001 | [58]Kirchhoff et al. 2008 |
| Cyanobacteria: | | | |
| PSII | FRAP | 0.00002 | [88]Sarcina andMullineaux 2004 |
| Simulation | | | |
| PSII grana | Monte Carlo | <0.000001 | [29]Kirchhoff et al. 2004 |
| Plants: | | | |
| LHCII (PSII) grana | FRAP | 0.0046 | [58]Kirchhoff et al. 2008 |
| LHCII unstacked | SPT | 0.0084 | [83]Consoli et al. 2005 |
| LHCII-P unstacked | SPT | 0.027 | [83]Consoli et al. 2005 |
| Cyanobacteria: | | | |
| IsiA | FRAP | 0.003 | [88]Sacrina & Mullineaux 2004 |
| Simulation | | | |
| LHCII grana | Monte Carlo | 0.000013 | [39]Drepper et al. 1993 |

Color code: green — plants, red — cyanobacteria, blue — computer simulations. FRAP — fluorescence recovery after photobleaching, SPT — single particle tracking, desA+ is a mutant defective in fatty acid desaturation, LHCII-P — phosphorylated LHCII.

whole electron transport chain. Why is a high PSII control coefficient important? A possible answer is because PSII is the main target of regulation. Microdomains enable that down-regulation of PSII can down-regulate the whole electron transport chain and in this way minimize production of toxic reactive oxygen species (ROS) around PSI. Furthermore, in case that photoprotective mechanisms are not sufficient to avoid damage by ROS, then PQ microdomains would shift the redox pressure towards PSII and lower the pressure to PSI. Since PSII is better equipped to deal with damage than PSI (PSII repair cycle), this would be physiologically advantageous.

However, the microdomain concept awaits direct approval. A method that promises to test its existence is EM tomography that has the potential to visualize local protein organization in intact thylakoid membranes. A further aspect of microdomains is that they may not always be existent. For example, in membranes with the semicrystalline protein organization, it is unlikely that microdomains are present.

### 4.2. Xanthophylls

A related problem to PQ diffusion in thylakoid membranes is the mobility of xanthophylls because they share the same protein crowded lipid diffusion space. For example, the conversion of zeaxanthin to violaxanthin by the xanthophyll cycle is an essential element for activation of photoprotective high energy quenching qE [44–46]. The de-epoxidation of violaxanthin to zeaxanthin is catalyzed by the enzyme violaxanthin-deepoxidase (VDE) localized in the thylakoid lumen [47]. During qE induction, violaxanthin unbinds from LHCII, migrates to VDE where it is converted to zeaxanthin, and then moves back to LHCII to activate energy dissipation [47]. There are estimates that the VDE concentration is low in thylakoid membranes [47] and that its mobility in the lumen is highly restricted (see Section 4.3). Therefore, long-range diffusion of xanthophylls from LHCII to VDE and back is expected. However, since there is good evidence for restricted PQ diffusion (see Section 4.1), the diffusion-dependent conversion of xanthophylls is expected to be restricted as well. Consequently the zeaxanthin-dependent activation of qE could be diffusion-limited [48]. Indeed, strong evidence for the diffusion-limitation of qE induction is the observation that it is slow if dark-adapted plants are illuminated the first time but it is fast if they are illuminated again [49]. The interpretation of this observation is that during the first light-cycle zeaxanthin has to be formed (diffusion-limited), but in the second cycle qE is fast because zeaxanthin is already present. Diffusion-limitation of qE induction is further supported by qE induction analysis in the *npq2* mutant that lacks the enzyme zeaxanthin-epoxidase [50]. In the *npq2* mutant, the xanthophyll pool is highly de-epoxidized [50]. In contrast to wildtype plants, dark-adapted *npq2* mutants show a fast qE induction most likely because zeaxanthin is already present in the dark. This gives strong support to the idea that diffusion of xanthophylls kinetically restricts the activation of qE and gives interesting possibilities for designing plants with manipulated energy dissipation kinetics.

### 4.3. Plastocyanin and other luminal proteins

Besides PQ, the second small electron carrier in the electron transport chain is the copper-containing 10.5 kDa protein plastocyanin [51]. PC electronically wires the cyt *bf* complex with PSI and diffuses in the lumen of the thylakoids. The size of PC is about 3 × 3 × 4 nm [52]. Recently, it was determined by cryo EM that the luminal width in dark adapted or low light illuminated plants is about 4.5 nm (see Section 3.1 and Fig. 1), putting it in a similar size range as that of PC. Modeling the available diffusion space for PC (Fig. 3, bottom) reveals that similarly to PQ in the lipid bilayer diffusion microdomains also exist for PC in the aqueous lumen (Fig. 3). Interestingly, the mobility of both mobile electron transport carriers is highly restricted to small diffusion domains. As discussed for PQ in Section 4.1, this could be beneficial for keeping the redox pressure at PSI low. We could show that the existence of PC microdomains depends on the light conditions [23]. In light adapted leaves, the lumen expands to about 9.5 nm (e.g. by more than 100%), which has the consequences that microdomains disappear. Thus, PC mobility in light is highly facilitated which makes physiological sense because under those conditions electron transport has to work with high efficiency. In conclusion, the dynamic swelling of the lumen in higher plants controlled by the environment can be a strategy to control the mobility of PC and in that way electron transport. This can be different in other photosynthetic organism in which the antenna system is in the lumen as for example in cryptophytes [53].

Since under some conditions the mobility of both electron transport carriers in grana-containing organisms is restricted, the sub-localization of the cyt *bf* complexes in thylakoid membranes becomes an important question. For sharing the burden of long-range diffusion from PSII to PSI between PQ and PC, a cyt *bf* complex localization in the grana margins or at the periphery of grana core could be beneficial. However, the exact localization of this central electron transport complex in thylakoid membranes of higher plants is under debate. It was either suggested that it is present in stacked grana or that it is excluded from these regions (reviewed in [21]). Also, a preferential localization in grana margins was found [54,55]. Thus, further studies are required to identify the localization of cyt *bf* complexes and the dynamics of its localization.

The lumen hosts about eighty different proteins involved in regulation, protein degradation, and maturation processes [56]. Since most of these proteins are larger than PC, it is expected that the dynamic swelling of the lumen controls their mobility. For example, it is assumed that the active form of VDE is a dimer that has a molecular mass of about 80 kDa [57], i.e. its diameter would be doubled compared to PC. If PC mobility is confined to microdomains, it is expected that VDE mobility is confined as well. The light triggered swelling of the lumen could have a significant impact on VDE mobility and therefore on the light-regulation of qE. In the future, interesting discoveries are expected because the dynamic control of the mobility and sublocalization of luminal

proteins by shrinkage/swelling will have an impact on the associated functions.

## 5. Diffusion of protein complexes

Percolation theory predicts that the mobility of molecules in membranes is highly dependent on their size [14]. Therefore, the quantitative size difference between small metabolites (PQ, xanthophylls) and larger protein supercomplexes can have a qualitative impact on their mobility. What is apparent for PQ must not be realized for PSII and vice versa. This is the rationale why both molecule size classes are addressed separately in this review. The following sub-sections review protein complexes where diffusion data exists. It shows that our knowledge is very fragmentary.

### 5.1. Photosystem II

Experimentally derived diffusion coefficients for both plants and cyanobacteria PSII are both very low (Table 1). For example, a diffusion coefficient of 0.0006 $\mu m^2\ s^{-1}$ translates into diffusion time of ~4 s for 100 nm, ~17 s for 200 nm, and ~38 s for 300 nm (Eq. (1), assuming Brownian behavior). It has to be mentioned that this $D_{PSII}$ was determined by very indirect measurements (Table 1). More recent determinations of $D_{PSII}$ measured with isolated grana membranes by the fluorescence recovery after photobleaching technique (FRAP) reveal a very low $D_{PSII}$ of $<0.0001\ \mu m^2\ s^{-1}$ [58] indicating that PSII is almost immobile. This number is in accordance with $D_{PSII}$ determined for cyanobacterial thylakoid membranes (Table 1). It is interesting to compare these values with respiratory membranes in mitochondria. For a fraction (~50%) of the mitochondrial ATPase complex a diffusion coefficient ($D_{ATPase}$) of 0.0005 $\mu m^2\ s^{-1}$ was deduced from FRAP experiments [59]. In the same study a more than 10-times higher $D$ was derived for ~50% of the cytochrome c oxidase (COX) proteins (0.007 $\mu m^2\ s^{-1}$). The similarities between $D_{PSII}$ in plants and cyanobacteria and $D_{ATPase}$ in respiratory membranes indicate that the main reason for retardation in lateral protein mobility is crowding because all these membranes have a similar protein packing density [12]. However, other factors could be involved because $D_{COX}$ and $D_{LHCII}$ are much higher than $D_{PSII}$ and $D_{ATPase}$. This will be addressed in the next section.

Computer simulations predict an even more retarded long-range diffusion of PSII (Table 1). From the simulations, it follows that a PSII complex in the center of 500 nm diameter grana would need 260 min(!) to reach the grana periphery. The computer simulation reveals that this extreme retardation in mobility is caused by crowding [28] and is in sharp contrast to the requirement of a brisk PSII traffic between stacked and unstacked regions for the PSII repair cycle. These considerations raise the question what mobilizes damaged PSII from an almost immobile protein network in stacked grana to reach its repair machinery in distant stroma lamellae [60]. There are several possibilities that can mobilize photoinhibited PSII including (i) disassembly of the dimeric PSII–LHCII holocomplex [61], (ii) increase of repulsive forces [62] by phosphorylation of PSII subunits [61,63,64] or (iii) lateral [65] or vertical [66] destacking of grana stacks or luminal swelling [60]. Future research is required to unravel how these structural alterations alone or in combination help to mobilize PSII from grana [60].

An intriguing observation that comes up from Table 1 is that the diffusion coefficient derived from computer simulations is always much slower than experimental data (see also LHCII). This indicates that the simulations miss a critical component, which is realized in native thylakoid membranes. At this point, it is only possible to speculate about this missing component(s). A potential candidate that can explain the difference between simulation and experiments is protein order. Mathematical analysis of electron microscopic images from thylakoid membranes reveals that even at first sight disordered protein arrangements posses a certain degree of order [28]. Under some conditions, membranes can switch to even highly ordered semicrystalline states (Section 3.4). There are two possibilities how semicrystalline protein arrays can facilitate lateral diffusion. First, crystalline arrays can form high-speed diffusion channels that would be relevant for diffusion of small molecules like PQ or xanthophylls. The existence of these channels was proposed from EM tomographic data [22]. The size of the 1 to 2 nm lipid channel would accommodate lipid like molecules but not protein complexes. Second, due to the more efficient protein packing in the crystal, the density in these arrays is higher than in disordered membranes. Packing proteins tighter together in arrays will make more diffusion space in the remaining disordered membrane regions. Therefore, the lower packing density in disordered regions can have a significant impact on protein mobility. In particular, if the density falls below the percolation threshold. Again, a small change in a structural parameter (protein packing) can have a huge impact on functionality.

### 5.2. Light-harvesting complexes

Measured diffusion coefficients for LHCII in plants and IsiA in cyanobacteria are more than ~100 faster than for PSII (Table 1). For LHCII, this can be due to the fact that trimeric LHCII (75 kDa) is much smaller than the PSII holocomplex (1.4 MDa, [67]). The situation for IsiA is more complex because its organization can range between monomeric (~100 kDa) and an 18mer giving a total size of 1.9 MDa [68]. The similar diffusion coefficient of IsiA compared to LHCII suggests that the FRAP experiments probed mainly smaller oligomerization states of IsiA. Besides the size difference, a further structural difference between LHCII/IsiA on one side and PSII on the other side is a luminal protrusion. PSII has a 4.5 nm protrusion [69] that sticks into the lumen that is missing in IsiA and LHCII. As suggested in [6], this protrusion could be relevant for the stronger retardation in PSII mobility that is in accordance with the macromolecular crowding in this tiny compartment (Fig. 3).

## 6. Dynamic control of diffusion processes

As summarized above, the structural boundary conditions in photosynthetic membranes are key for the mobility of membrane components. It is essential to appreciate the dynamics of the thylakoid architecture because its changes can form a switch that significantly alters mobility. This was demonstrated for light-induced swelling of the thylakoid lumen and the mobility of lumen-hosted proteins (see Section 4.3). Other examples of architectural changes of the thylakoid system are the supramolecular change from disordered to semicrystalline or partial destacking of grana thylakoids. In addition, the thylakoid membrane composition is also dependent on environmental factors. A well-established example is that in low-light adapted plants the abundance of LHCII increases where as the abundance of PSII and cyt *bf* complexes decreases [71,72]. With respect to diffusion processes, this change in composition implies a change in the protein size distribution. In low-light grown plants, much smaller LHCII and less larger supercomplexes exist. According to the percolation theory, the consequence would be that diffusion of small molecules is challenged (see Fig. 2). In light of a challenged diffusion in low-light plants, the observation is interesting that under these conditions the abundance of semicrystalline structures is higher in these thylakoid membranes [72,73] that could improve for example PQ diffusion. As discussed in Section 5.1, semicrystalline array formation can facilitate PQ/xanthophyll diffusion.

Almost no data exists about changes in crowding. By comparing diffusion in grana-containing C3 plants with agranal bundle sheath chloroplasts in C4 plants and isolated stroma lamellae, we demonstrated that changes in protein crowding have huge impact on protein diffusion [74]. This is in agreement with percolation theory that predicts that thylakoid membranes are close to cP. Therefore, knowledge about density fluctuations and alterations under different growth conditions can be very valuable to understanding mobility switches.

Since architectural changes can control diffusion of membrane components, it is worthwhile to understand the factors that trigger these

changes. For the swelling of the lumen, the proton motive force (pmf) is the driving force [23]. Most likely, the pmf causes ion in/effluxes to the thylakoid lumen that causes osmotic swelling. In this respect, ion channels can play a central role. Ion channels in thylakoid membranes were detected [75,76] or were predicted [77,78] to be voltage gated. Thus, it is likely that control of thylakoid ion channels controls diffusion of lumen-hosted proteins by swelling/shrinkage. Further research in this area is necessary.

A second important modulator of structural membrane features is posttranslational protein modifications (see chapter 17 in this special issue). In plants, essentially two kinases catalyze protein phosphorylation. The stn7 kinase mainly phosphorylates LHCII [70] whereas the targets for the stn8 kinase are PSII subunits [70]. Both computer simulation [39] as well as experimental data [65,79] show that LHCII or PSII phosphorylation speeds up their molecular mobility. This can be due to an increase in lateral electrostatic repulsion triggered by the negative chargers from the phosphate groups that are added to the proteins [62]. Another possibility is that protein phosphorylation modifies stacking of grana thylakoids that loosen steric restrictions/frictions [60]. In any case, both the pmf as well as protein phosphorylation are signal transducers involved in the sensing of environmental and metabolic changes and convert them into structural changes that in turn control diffusion processes.

## 7. Conclusions

An interesting feature of photosynthetic membranes is that several structural quantities are in critical regions that in the context of lateral diffusion make the system sensitive to small changes. This is obvious for the protein packing density in grana thylakoids that is near the percolation threshold, cP. In addition the sizes of protein supercomplexes could be tuned to allow to support or retard long-range diffusion processes by assembly/disassembly. Also the composition, density and interaction energies between grana hosted proteins are placed in a sensitive region that leads to the co-existence of disordered and semicrystalline arrangements. Small changes in the stoichiometric and physicochemical membrane properties have significant impact on the equilibrium between ordered and disordered states that in turn determines mobility of small and larger membrane components. Finally, the luminal width has a similar size as the proteins that must diffuse in this tiny aqueous space that can restrict or alleviate their mobility by minor shrinkage/swelling. Due to the long timeframe of photosynthetic evolution, it is unlikely that these structural features are pure coincidence. More likely is that these features form a selection advantage. The advantage is that small changes can switch the system between confined and long-range diffusion. Since the structural features that control lateral diffusion are under environmental control, it enables the system to tune diffusion efficiencies to the needs that are dictated by the environment and/or metabolism. The high sensitivity to small structural changes is advantageous for this tuning.

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