Photosystem II Subunit S (PsbS): a nano regulator of plant photosynthesis

Marulanda Valencia, W.; Pandit, A.

Review Article

Photosystem II Subunit S (PsbS): A Nano Regulator of Plant Photosynthesis

Willem Marulanda Valencia and Anjali Pandit *

Leiden Inst. of Chemistry, *Gorlaeus Laboratory, Einsteinweg 55, 2300 RA Leiden, The Netherlands*

Correspondence to Anjali Pandit: *a.pandit@lic.leidenuniv.nl (A. Pandit)*
https://doi.org/10.1016/j.jmb.2023.168407
Edited by Volha Chukhutsina

Abstract

Light is required for photosynthesis, but plants are often exposed to excess light, which can lead to photodamage and eventually cell death. To prevent this, they evolved photoprotective feedback mechanisms that regulate photosynthesis and trigger processes that dissipate light energy as heat, called non-photochemical quenching (NPQ). In excess light conditions, the light reaction and activity of Photosystem II (PSII) generates acidification of the thylakoid lumen, which is sensed by special pH-sensitive proteins called Photosystem II Subunit S (PsbS), actuating a photoprotective "switch" in the light-harvesting antenna. Despite its central role in regulating photosynthetic energy conversion, the molecular mechanism of PsbS as well as its interaction with partner proteins are not well understood. This review summarizes the current knowledge on the molecular structure and mechanistic aspects of the light-stress sensor PsbS and addresses open questions and challenges in the field regarding a full understanding of its functional mechanism and role in NPQ.

Introduction

During photosynthesis, sunlight energy is converted to chemical energy: a global process that provides the primary source of energy for most life on Earth. In higher plants this conversion takes place via the light-harvesting complexes (LHC) and photosystem II and I (PSII and PSI) in the thylakoid membrane. Light excites chlorophyll molecules in the LHCs and this excited state is transferred across the LHCs to the PSII reaction center where the excitation is transferred to a chlorophyll dimer (P680).[1] Excitation of P680 results in a charge-separated state and electron transfer, reducing plastoquinone to plastoquinol, and further transportation of electrons across the electron transport chain to form NADPH. $P680^+$ oxidizes two water molecules, with help of the manganese cluster Mn_4CaO_5, into $4H^+$ and O_2. This process, along with proton transport via cytochrome b6f, creates a pH gradient up to two units' difference[2] between the stroma and the lumen of the thylakoid membrane. The pH gradient provides the proton-motive force to generate ATP via F1Fo ATP synthase.[3] The efficiency of photosynthesis is tightly coupled with plant fitness under fluctuating environmental conditions. In excess light, the balance between light excitation and substrate availability is disturbed. Overexcitation takes place which can lead to formation of chlorophyll triplets, to saturation of the reaction center, and creation of reactive oxygen species (ROS) which are highly damaging to the plant.[4] To protect PSII and the plant against ROS, the excess of excitation energy needs to be dissipated before reaching PSII. The process in which the excess excitation energy is dissipated as heat is called non-photochemical quenching (NPQ),[5–7] which is triggered upon acidification of the thylakoid lumen, caused by the imbalance between

light excitation and substrate supply. Different mechanisms exist to accomplish this dissipation of energy and they are distinguished on the time scale they occur[4,8,9]: qE induces rapid de-excitation of chlorophylls (Chls) in the light-harvesting antennas and occurs within seconds; qT is associated with membrane state transitions that take seconds to minutes. It is worth noting that in plants exposed to saturating light, qT does not seem to contribute to NPQ[10]; qZ is associated with the conversion of violaxanthin into zeaxanthin and takes minutes to hours to revert to a non-dissipative state; qI is associated with photo inhibition and reduction of the photosynthetic capacity, taking place within minutes to hours. qE is the most prominent form in higher plants and is ΔpH dependent.[11] The LHC superfamily includes specific light-harvesting complex proteins that do not have a primary role in light harvesting, but play a crucial role in the qE component of photoprotection, safeguarding photosynthesis.[12] In plants and green algae, those are the LHC stress-related (LHCSR) proteins and Photosystem II subunit S (PsbS). LHCSR is active for photoprotection in green algae and diatoms, whereas PsbS is essential for qE in higher plants, and in moss both proteins are active for light energy dissipation.[12,13] Both LHCSRs and PsbS are pH-sensitive proteins that contain protonable residues at the lumen side that respond to a change in luminal pH. LHCSR is a pigment-binding protein with three transmembrane helices, resembling other LHC antenna proteins, and contains a pH-sensitive C-terminal domain. At low pH, protonation of C-terminal residues in LHCSR switches the protein into a quenched state so that it functions as a pH-responsive, photoprotective switch.[14] PsbS, in contrast, is a four-transmembrane helix protein that does not bind pigments at specific binding sites.[15] Two lumen-faced protonable glutamate residues are essential for the function of PsbS in establishing NPQ.[16,17] Current models for PsbS function assume that PsbS is a light-stress sensor that responds to thylakoid lumen acidification in excess light by actuating a photoprotective switch in the photosynthetic antenna.[18] Different from the energy dissipation process in green algae, where pH-sensing and chlorophyll (Chl) de-excitation is combined in LHCSR, or moss, where both LHCSR and PsbS are active,[19] in higher plants the two processes of pH sensing and excitation quenching are decoupled. PSII and PsbS together can be considered as a photosensory network, in which over-excitation of PSII results in a low luminal pH that activates the light-stress sensor PsbS. Activated PsbS subsequently gives the feedback signal for photoprotection to the LHC-PSII network, establishing quencher states in the LHCs for dissipation of excess light energy.

The Light-stress Sensor PsbS: Identification of Active Sites

In the early work by Li et al., PsbS was identified as a protein that was essential for regulation of photosynthetic light harvesting, by analyzing isolated mutants of *Arabidopsis thaliana* that cannot dissipate excess absorbed light energy.[21] Their study found that the gene encoding for PsbS was cconsidered a pigment-binding protein, like other members of the LHC family, because the protein isolated from plants contained chlorophyll and xanthophyll.[22] However, attempts to reconstitute recombinant PsbS with pigments failed[23] and for native PsbS, the authors of the same study concluded that despite the homology with chlorophyll*a*/*b*/xanthophyll-binding proteins of the LHC family, PsbS protein does not show tight binding chlorophylls or carotenoids, unlike other LHC members. Bergantino et al. found that PsbS exists in monomeric and dimeric form, and monomerization of PsbS dimers was found to be reversible and pH-inducible.[24] The monomer form was most present at high-light/low pH conditions whereas the dimer was most present at moderate light condition in neutral/alkaline pH. Site-directed mutagenesis studies of Li et al. were able to identify two essential amino acid residues for qE, two lumen- exposed glutamates (Glu122 and Glu226 in *Arabidopsis*, equivalent to Glu69 and Glu173 in spinach) of PsbS, for qE activation.[16,17] Mutations of either of the active Glu into Gln reduced qE by 60–70%, while mutating both Glu essentially abolished all fast qE. The similar effect of mutating either of the two Glu was ascribed to the pseudo-symmetry of the PsbS: according to its sequence, the protein displays a pseudo-two-fold symmetry with the two halves having high sequence identity. Mutation of either of Glu108 and Glu212 in *Arabidopsis* (equivalent to Glu55 and Glu159 in spinach), two other Glu residues at the luminal side, also strongly affected qE.[17] The early studies formed the basis of current hypotheses for PsbS function, in which light-inducible activation of PsbS via protonation of specific residues in the luminal loops promotes monomerization and subsequently its action establishing qE via interaction with LHC proteins or PSII. Further indication that PsbS acts as a regulator, but is not the site of qE, was given by the fact that qE could be activated in intact chloroplasts lacking PbsS when ΔpH was enhanced by using the agents diaminodurene (DAD) or phenazine methosulfate (PMS) as mediators of cyclic electron flow.[25]

The Low-pH Structure of PsbS

In 2015, the low-pH crystal structure of PsbS was resolved,[15] paving the way for recent structural and

in-silico studies to understand the relationship between PsbS structure and function. PsbS is a 22-kDa transmembrane protein that consists of four transmembrane helices (TM1-TM4) and two amphipathic helical stretches (H1 and H2) at the luminal side of the thylakoid membrane (see Figure 1). In comparison with structures of other members of the LHC superfamily, PsbS has a remarkable different architecture and contains a fourth TM helix. Compared to the structure of LHCII, the two crossing TM helices of PsbS (TM1 and TM3, equivalent to helix A and B in LHCII) are shorter and TM2 (equivalent to helix C in LHCII) is positioned close to the central supercoil whereas for LHCII this site contains a gap that is filled with pigments. Next to H1 there is a 3_{10} short helix domain, recently named H3.[26] Despite the pseudo-symmetry of the two halves of PsbS, the crystal structure of PsbS shows that the two luminal loops of PsbS that contain the essential glutamates, Glu69 and Glu173 respectively, assume quite different conformations: Glu69 is part of the short 3_{10} helix fragment called H3 whereas Glu173 is located inside the amphipathic α-helix domain H2. The crystal structure presents a PsbS dimer that is stabilized by an intensive network of inter-monomer hydrogen bonds, in addition to hydrophobic interactions involving TM2 and TM3, one salt bridge and one arginine-arginine pair. Four hydrogen bonds are formed at the luminal side between Glu173 and the backbone amide nitrogen of Ile74 and Tyr75 (Figure 2A, B). Furthermore, Glu55 and Glu159 anchor H2 and H1 to respectively TM1 and TM3, stabilizing the stability of the luminal helices and the tertiary structure of PsbS. Although the PsbS crystals contained some Chls, the PsbS dimer structure is too tight to fit any pigment molecules and potential Chl-binding sites that are conserved among the LHC superfamily cannot bind Chl in PsbS due to steric hindrance imposed by TM2 and TM4. The structure reveals that PsbS is not a canonical pigment-binding protein and the authors suggested that free Chls may be trapped at the PsbS dimer interfaces. The outer side of the dimer contains a hydrophobic interface formed by a cluster of phenylalanine (Phe) residues, which potentially could have a role in establishing interactions with other protein partners.[27]

PsbS is a pH-dependent Molecular Switch

The pH-response molecular mechanism of PsbS has been investigated by molecular dynamics (MD) and coarse-grained (CG) simulations of spinach PsbS in lipid bilayer at constant-pH sampling, and by a combined FTIR, 2D-IR and NMR spectroscopy study on *Physcomitrella patens* PsbS.[26,28,29] The lumen-exposed 3_{10}-helix fragment H3, located next to Glu69 in the low-pH PsbS structure, forms an equilibrium between 3_{10}-helix and a loop/turn conformation that shifts towards the unfolded state at neutral-pH conditions[26,28,29] and the amphipathic H2 helix containing Glu173 moves from the hydrophobic membrane phase to the aqueous phase at neutral pH (Figure 2B, C).[28,29] Notably, in an E176Q mutant (numbering according to the *patens* sequence, mutation equivalent to E173Q of PsbS from spinach), both the pH conformational changes of H2 and H3 were suppressed, and IR spectra of this mutant resembled the low-pH structure of PsbS at both low and neutral pH conditions.

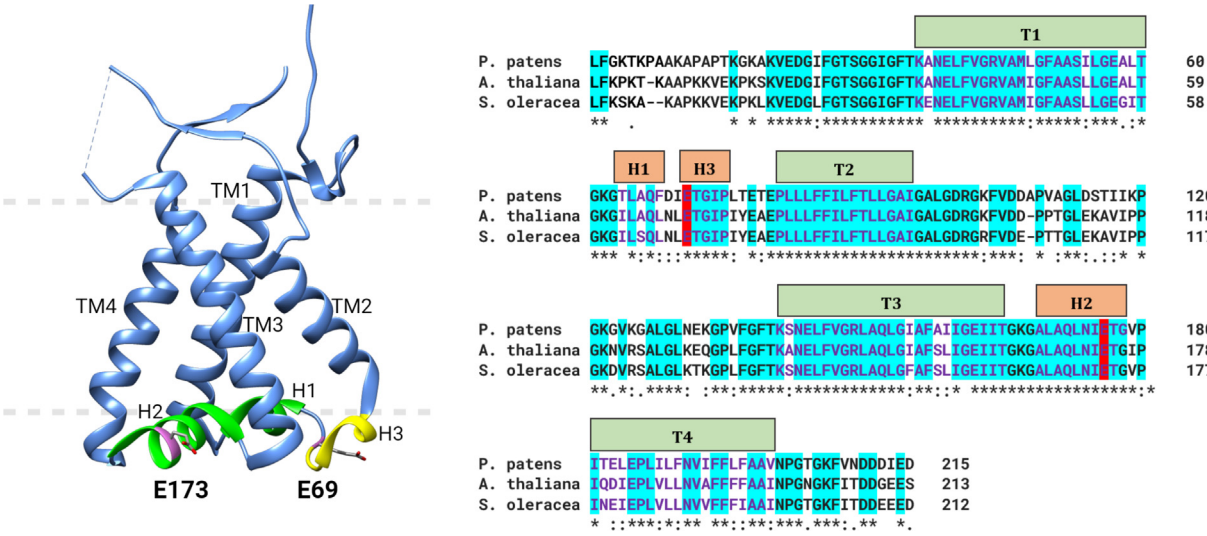

Figure 1. Crystal structure of PsbS[15] (PDB-ID 4RI2) with the amphipathic helices H1 and H2 in green and H3 in yellow and the two active Glu highlighted. The stromal loops are not resolved. The right side presents a protein sequence alignment (Clustal Omega, EMBL-EBI) of PsbS from Physcomitrella patens, Arabidopsis thaliana and Spinacia oleracea. The active Glu are indicated in red. The PsbS structure was created using UCSF Chimera.[20]

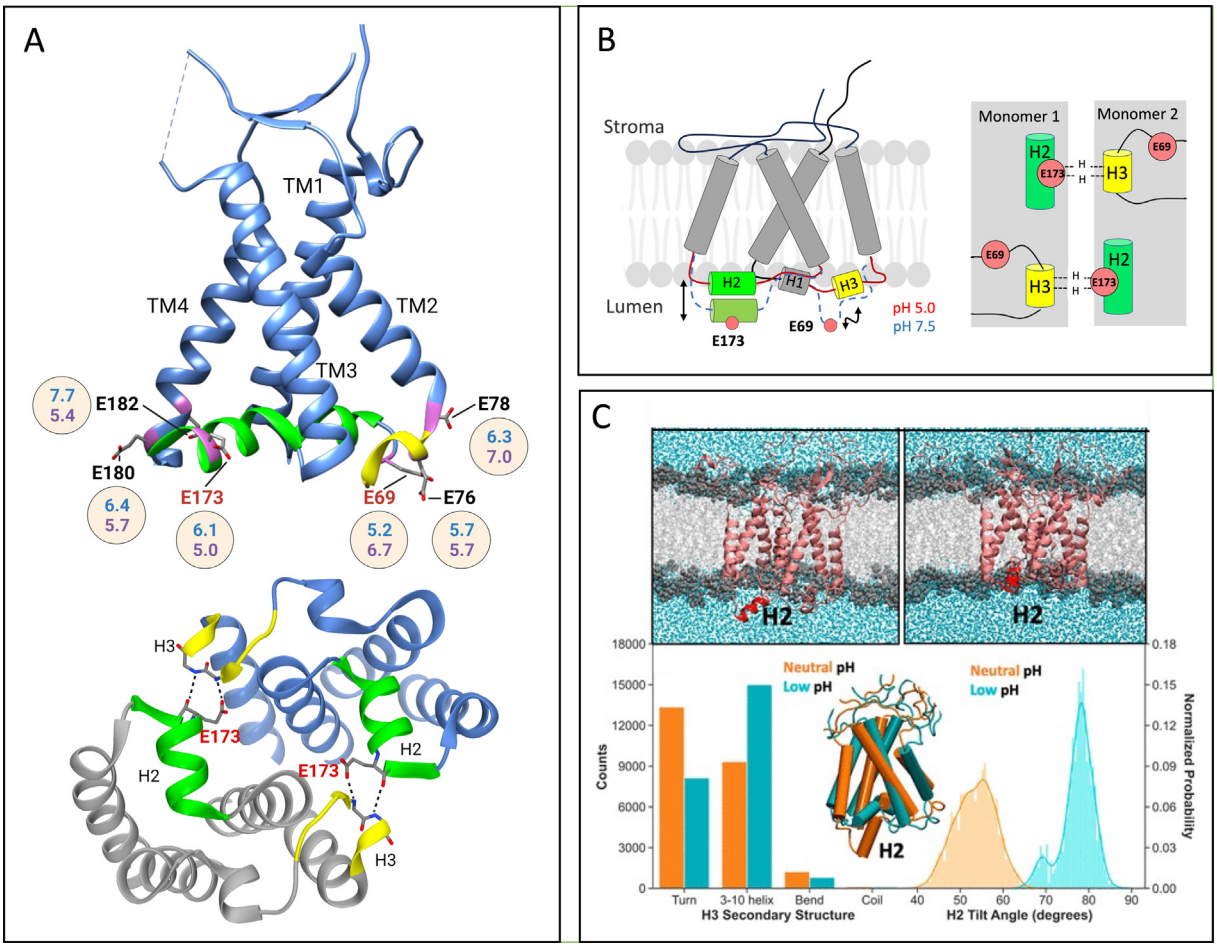

Figure 2. Molecular pH-response mechanism of PsbS. A: Upper structure: Crystal structure of PsbS[15] (PDB-ID 4R12) with amphipatic helix H3 in yellow and H1 and H2 in green. Lumen-exposed Glu with elevated pKa values are highlighted. Numbers are the pKa values predicted from MD simulations on PsbS monomer (Liguori et al.)[26] in blue and predicted from CG simulations on PsbS dimer (Chiarello et al.)[29] in purple. Glu 55 and Glu 159 are not shown. Lower structure: PsbS dimer facing the lumen side. H-bonds formed by Tyr75 and Ile74 to Glu173 are indicated. B: schematic picture of the pH response of PsbS (left) and H-bonds that stabilize the PsbS dimer at the lumen side (right), figures adapted from 28. C: CG simulations of PsbS dimer indicating the molecular response to changes in pH. PsbS structures were created using UCSF Chimera.[20] pH. PsbS structures were created using UCSF Chimera.[20] (C) has been adapted from 29 with permission according to Creative Commons Attribution 4.0 International Public License https://creativecommons.org/licenses/by/4.0/.

Hence, deletion of Glu173 locks PsbS in the low-pH conformational state. The lack of response by H3 was explained by the following: without rearrangement of H2, H-bonding to H3 in the opposite monomer within PsbS dimer persists at neutral pH conditions and prevents unfolding of H3. Although glutamates in aqueous solution have pKa values around pH 4.0,[30] the bulk of protonable residues in PsbS are protonated at pH 5.0[28] and simulations predict that many lumen-exposed Glu have elevated pKa values around 6.0 and some even above 7.0.[26,29] The H3 fragment containing Glu76 is connected to TM2 which contains Glu78 at the luminal edge, suggesting that the conformational pH switch of H3 may involve protonation of Glu69, Glu76 and Glu78. The H2 fragment contains Glu173 and is connected to TM4 that contains the residues Glu180 and Glu182 at the lumen edge. Protonation of Glu173 neutralizes the charge of the H2 fragment, by which this fragment can enter the hydrophobic membrane phase. A structural rearrangement of H2 occurs with increasement of its tilt angle with the membrane normal that stabilizes embedment of H2 in the membrane environment.[28,29] The elevated pKa values of the lumen exposed Glu renders those residues sensitive to protonation within the physiological pH range between 5.5–7.5 (see Figure 2A). Intriguingly, the pH-response mechanism of PsbS suggest that PsbS dimers are destabilized at neutral pH due to unfolding of H3 and rearrangement of H2, which disrupts the four hydrogen bonds at the luminal side. Nonetheless, CG simulations

predict that both at neutral and low pH conditions, the PsbS dimer is the most stable form[29] and PsbS in detergent micelles is capable of forming dimers at both pH conditions.[31] Those findings and the low-pH crystal structure of the PsbS dimer seem in contradiction with the earlier results of Bergantino et al.[24] and with proposed models that assume monomerization of PsbS at low pH.[16,32] However, *in-vitro* and *in-silico* studies were performed on isolated PsbS proteins that can only interact with each other, in contrast to native thylakoid membranes where PsbS may interact with various interaction partners. An alignment of LHC proteins onto PsbS monomers at low pH shows that docking interactions could be formed between the H3 domain of PsbS facing the C-terminal luminal amphipathic helix of the most abundant LHC antenna complexes, Light-Harvesting Complex II (LHCII), that resembles H2 in PsbS.[26] It is thus conceivable that in the native thylakoid membrane, switching of PsbS H3 into a 3_{10} helix at low pH may establish docking interactions between monomeric PsbS and the C-terminus of LHCII.[26,28,29] A very recent cryo-electron microscopy (cryo-EM) study uncovered a pH-induced conformational change in LHCII. At low pH conditions, an 3_{10} helix fragment at the luminal side (helix E) transforms into a normal α-helix and a random-coil fragment at the C terminus folds into a short α-helix.[33] Moreover, the amphipathic helices D and E at the luminal side rearrange and make an inward movement, resembling the rearrangement of H2 of PsbS. Similar structural motifs of luminal helices containing protonable residues exist for the minor antenna protein CP29.[34]

Taken together, the pH response mechanism of PsbS can be described as follows: At neutral pH, the luminal loops of PsbS reside in the aqueous phase. Protein-protein interactions may occur at the stromal side via weak H-bonding and electrostatic interactions of the stromal loop segments and through hydrophobic interactions in the membrane phase. Upon protonation at low pH, the amphipathic short helices H2 and H3 at the luminal side undergo conformational changes and structural rearrangements. Those changes establish a network of four H bonds that stabilize the PsbS dimer *in vitro*, and *in vivo* may serve to enable H-bonding at the lumen side with LHC partner proteins. It would be highly interesting to explore if to which extent the pH-responsive motifs of PsbS are shared among LHC complexes and serve to establish flexible protein–protein interactions that are controlled by pH.

Signaling Light Stress: PsbS Interactions in the Thylakoid Membrane

Various efforts have been made to find the location of PsbS in the thylakoid membrane.[35–37] The PSII super complexes consist of the PSII core surrounded by the LHC minor antennas (CP29, CP26 and CP24) and can bind the peripheral antennas LHCII (forming PSII-LHCII). PsbS was not found to be an intrinsic part of the PSII–LHCII super complex although the structure of $C_2S_2M_2$-PSII-LHCII super complex contains a cleft that could potentially fit a PsbS protein.[38] It was noted however, that PsbS also was able to modulate NPQ in membranes of lincomycin-treated plants that were depleted of photosystems, suggesting that the specific binding side is not essential for its activity.[39] PsbS was shown to control the assembly and dissociation of a super complex consisting of the monomeric minor antennae CP29 and CP24 and the trimeric complex LHCII.[40] Dissociation in high light (HL) of this complex, reducing the size of the antenna system associated with PSII, was found to be indispensable for the onset of NPQ. The results suggest that PsbS serves to reorganize protein domains in thylakoid grana membranes, in line with observations that PsbS reduces the number of crystalline domains in thylakoid membranes and increases membrane fluidity[41,42]. Both the monomeric, minor antennae close to the PSII core and the accessory trimeric antennae LHCII have been indicated as quencher sites for light energy dissipation in higher plants[43] that may be activated by PsbS. Based on the results of[40] an NPQ model was proposed in which the dissociated super complex in HL enables association with protonated PsbS, inducing a conformational change by which CP29 forms a quencher state.[44] However, a study on mutant plants lacking LHCII stated that PsbS-dependent NPQ mainly occurs in LHCII, while there is an additional quenching site in the PSII core.[45] To localize PsbS interaction partners, two immunolabeling studies have been performed. Correa-Galvis et al. used chemical crosslinking and immunolabelling to localize PsbS binding partners.[37] Based on their results, a model was proposed in which in dark-adapted leaves, PsbS is associated with PSII-LHCII and interacts with the PSII core proteins, whereas in HL conditions, enhanced interaction of PsbS with LHCII occurs, accompanied by the detachment and/or aggregation of LHCII proteins. Sacharz et al. explored the thylakoid location of PsbS using magnetic-bead linked antibodies and a pull-down assay.[36] In this work, in the presence of ΔpH alone, PsbS was found mainly associated with LHCIIs whereas the combination of ΔpH and zeaxanthin (the NPQ state) increased the proportion of PsbS bound to the minor antennas, and no significant interaction was observed with the PSII core. An additional role for PsbS in protection of PSII was recently proposed based on characterization of a monomeric PSII complex with PsbS and PSb27 bound (PSII/PsbS/PSb27) that was isolated from thylakoid stromal lamellar membranes.[46] This complex was suggested to be an intermediate in the assembly of PSII in which binding of PsbS and/or

Psb27 serve to protect PSII and restrict its activity while in transit, via a bicarbonate-mediated switch.

PsbS interactions within the dimer and with different partner proteins in the thylakoid membrane have been explored *in silico* in work of Daskalakis et al.[47–49] For the PsbS dimer, their simulations predicted enhanced flexibility and destabilization at the lumen side at low pH.[48] Their result differs from the more recent CG study of PsbS dimer by Chiarello et al., obtained by simulations at constant-pH sampling, where protein–protein interactions are weakened at neutral pH.[29] The differential results could be due to differences in the simulation approaches. A challenging factor in simulations of large membrane systems at relatively long timescales is the treatment of pH and identifying the pKa of protonable residues.[50] This complicates the use of sophisticated constant-pH sampling techniques as applied by Liguori et al.[26] and by Chiarello et al.[29] In the simulations of Daskalakis et al., pKa values were defined based on experimental findings or using the computational PROPKA method.[48,51] Although caution is needed concerning the assignment of pKa values that can be influenced by multiple factors[50] the approach allows study of larger membrane systems with higher complexity. In a simulation performed on LHCII and PsbS in thylakoid lipid bilayers, a more compact shape of LHCII at low pH was accompanied by an accumulation of the lipid digalactosyldiacylglycerol (DGDG) at the lower leaflet and luminal side of LHCII trimers.[49] In accordance with *in vivo* observations,[52] the model indicates a thinning of the membrane at low pH. The accumulation of DGDG occurred to lower the cost of a hydrophobic mismatch induced either by thylakoid membrane thinning, or by the change of the LHCII scaffold and rendered LHCII trimers almost immobile. LHCII mobility was only restored in the presence of PsbS that inhibited DGDG binding, suggesting a role for PsbS to ensure LHC mobility at low pH conditions. The relevance of membrane thinning for energy dissipative processes was further demonstrated in membrane models consisting of LHCII proteoliposomes prepared from lipids with different acyl chain lengths, showing a correlation between LHCII excitation quenching and hydrophobic mismatch.[53] All-atom simulations on CP29 and PsbS in fused lipid micelles predicted that PsbS-CP29 interactions occurred effectively at low pH only in the presence of Zea[47]. Protein-protein interactions occurred at the hydrophobic interaction interface and between amino acids close to the lumen side, the latter electrostatic of nature.[47] Moreover, in complexation with PsbS, Glu128 in CP29 had a pKa shift from 4.46 to 5.24, which would make this residue pH-sensitive within the physiological pH range. Based on the simulations, a model was proposed where PsbS dimers bind close to CP29 in the PSII super complex. Herein, PsbS could also interact with CP47 and CP24 and could slightly reorganize the super complex. This slight reorganization could then facilitate quenching by disrupting energy transfer pathways and altering the environment of quenching sites. Such a reorganization of the membrane would be possible with the increase of protein mobility in the presence of PsbS, as observed *in vivo* and *in silico*.[54,55]

Interplay Between PsbS and LHCII May Form a Photo Protective Switch

A key question regarding the role of PsbS in qE is how a pH response of PsbS enables the photoprotective switch that leads to dissipation of excitations in the LHC antennas. This question has been addressed by studying interaction of PsbS with the most well-studied antenna protein, the major antenna complex LHCII. LHCII has a structural response to pH[33] and to binding of Zea,[56] two key elements in NPQ and qE, but those two factors do not influence its photo physics[57]. Individual LHCII complexes can switch between fluorescent and dissipative states[58,59] and are known to display aggregation-dependent quenching *in vivo*[60] and in aggregates or proteoliposomes *in vitro*.[61–64] To interrogate the effect of PsbS on the quenching properties of LHCII, several studies have investigated PsbS-LHCII interactions in model membranes through their co-insertion in proteoliposomes.[65–69] Because clustering of LHCII in proteoliposomes is known to induce aggregation-related fluorescence quenching,[61–63] fluorescence results were typically compared to those of LHCII-only proteoliposomes as control. PsbS-induced quenching was observed already at neutral-pH conditions using recombinant PsbS and liposomes prepared from thylakoid lipids[65,66,68] and the effect was enhanced at low pH,[66,68] whereas proteoliposomes containing co-reconstituted native PsbS and LHCII only displayed PsbS-dependent quenching at low pH.[69] No significant effect of PsbS at either pH conditions was observed in a study that used asolectin proteoliposomes.[67] LHCII aggregation is known give rise to far-red fluorescence emission that has been attributed to Chl-Chl charge-transfer states[70,71] and also is observed *in vivo* during qE.[72] Far-red emission of LHCII was reproduced in proteoliposomes and enhanced upon co-reconstitution with PsbS.[68,69] The addition of zeaxanthin (Zea) was reported to have a strong enhancing effect on the quenching of LHCII-PsbS proteoliposomes according to Wilk et al.,[65] but no effect was observed by Pawlak et al..[68] The latter study applied time-resolved fluorescence spectroscopy with high temporal resolution and report that co-insertion of PsbS in LHCII proteoliposomes produces weakly fluorescent red states that are quenched within ~50 picoseconds.

The proteoliposome studies suggest that direct PsbS-LHCII interactions can be established in thylakoid membranes, promoting energy

dissipative states, of which the effectiveness may depend on pH, protein densities, lipid composition and Zea. An overall comparison of the studies is complicated by the fact that they were carried out using varying protein to lipid ratios, using different reconstitution procedures, and applying different spectroscopic techniques for fluorescence detection. Furthermore, the role of PsbS dimerization has not been clarified and in the various studies, PsbS may have been membrane inserted in its dimeric or monomeric form, or a combination of both.

Possible Roles of PsbS in Photo Protection

From the various research studies on PsbS interactions in thylakoid membranes the following possible scenarios can be drawn for PsbS activity (see also Figure 3):

(i) Protonation of PsbS at low pH/HL conditions enables its binding to the PSII super complex near CP29, destabilizing the super complex. In this scenario, the binding of PsbS disrupts energy transfer pathways towards PSII and could produce dissipative states of CP29.[40,43,44,47] As a counterargument, it has been demonstrated that the efficiency of energy capture by the reaction center exceeds the efficiency of trapping by non-photochemical quenchers.[73]

(ii) Protonated PsbS serves to ensure the mobility of LHCII and its detachment of super complexes, enabling self-aggregation of LHCII and formation of energy dissipative states.[37,43,45]

(iii) Proteoliposome studies suggest that protonated PsbS directly interacts with LHCII and stabilizes its quenched conformational state, via one-to-one interactions or via enhanced LHCII aggregation.[65,68,69]

In all scenarios, the effects of hydrophobic mismatch and electrostatic interactions at the luminal sites are key factors that can drastically affect the lateral distribution of LHCII complexes. Together with PsbS and Zea those factors seem to control thylakoid mobility and provide the driving force for tuning protein–protein interactions in the membrane.

Role of PsbS in the Induction and Relaxation of NPQ

The two central components that regulate qE in higher plants are PsbS and Zea. Zea is accumulated in high light through the xanthophyll cycle, associated with qZ. The xanthophyll cycle is activated by a ΔpH, in which violaxanthin is converted to zeaxanthin in high-light conditions via de-epoxidation enzymes, and reversibly converted back to violaxanthin in moderate light.[74] This process typically takes place on a minutes time scale. PsbS is essential for fast induction of NPQ on a seconds time scale and determines the amplitude of qE, while Zea has a role in the kinetics of qE induction and recovery.[32] Plants adapted to high light containing elevated levels of Zea have accelerated qE induction and slower recovery from qE. Therefore Zea is considered to acts as a light-stress memory in plants that allows rapid reactivation of

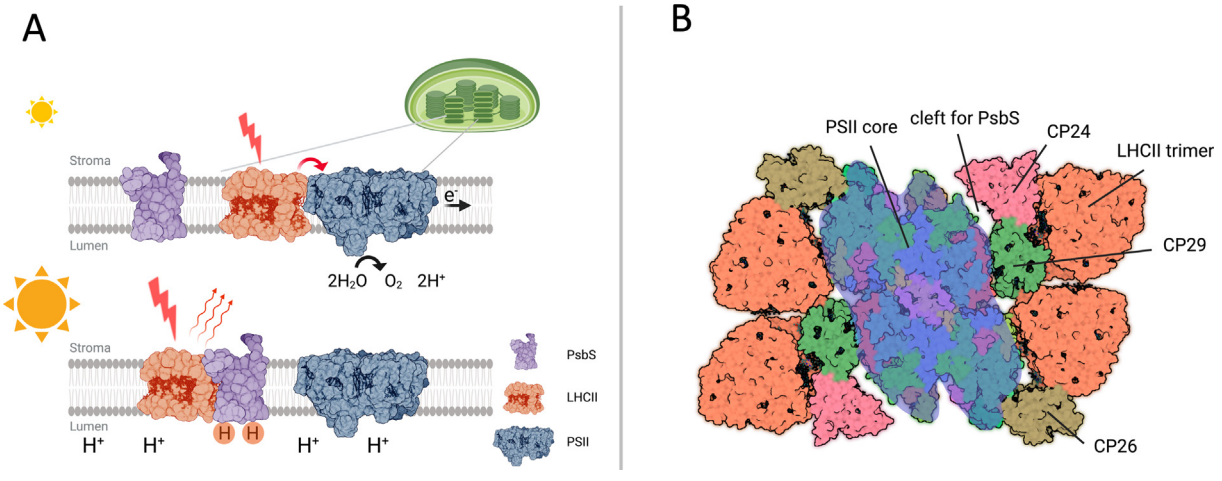

Figure 3. A: Proposed model of PsbS in interaction with LHCII. Under moderate light conditions, photosynthesis is active: excitations absorbed by LHCII are transferred to PSII, resulting in water oxidation and electron transfer processes. Under HL conditions, protonated PsbS interacts with LHCII, inducing a quenched state in which excitation energy is dissipated as heat. B: PSII super complex (top view) with LHCII in red, CP24 in pink, CP26 in brown and CP29 in green surround the PSII core. The structure indicates a cleft for potential binding of PsbS, supporting models in which associated PsbS promotes protein reorganization and energy dissipation via interaction with CP29. Pictures were created with BioRender (https://BioRender.com).

NPQ during recurrent light stress periods.[75] To quantify the contribution of qE in NPQ, and to separate the input of different qE components, mathematical feedback models have been developed that are able to reproduce the NPQ dynamics under high-light (HL) and dark cycles.[76–80] According to the models, PsbS and Zea together determine both the capacity for qE and rate of NPQ formation and relaxation.[76–79] Without PsbS, the generated quenching response was observed to be "out of phase" with the HL/dark cycles,[78] hinting that PsbS is necessary for plant response to both HL and dark conditions. According to the model in 78 interactions between protonated PsbS and LHCII not only produce quenched states, but may also actively enable recovery from those. Further, a study using overexpressed PsbS indicated that plant PsbS levels have a regulatory role in cyclic electron flow.[81] Under high light, plants with overexpressed PsbS were more protected from singlet oxygen at the level of PSII, whereas lack of cyclic electron flow in those plants rendered them susceptible to damage at PSI.

PsbS is a Key Parameter for Improvement of Photosynthesis

The mathematical models serve as a framework to identify parameters for manipulation to improve photosynthesis. PsbS is considered one of the key factors in plant photoprotection. Manipulation of PsbS levels in plants has been proposed as a means to improve plant resilience to high or fluctuating light conditions and optimize photosynthetic efficiency.[11,32] In particular accelerating the recovery from photoprotection would allow plants to respond more accurately to rapid changing light conditions, reducing solar energy losses without compromising the essential photo protection.[82] Indeed, promising results were obtained with engineered tobacco and soy plants with elevated levels of PsbS and the xanthophyl (de) epoxidation enzymes violaxanthin de-epoxidase (VDE) and zeaxanthin epoxides (ZE) that would faster respond to fluctuating light conditions in the field. The tobacco plants had increased biomass and more-efficient photosynthetic electron transport and carbon fixation,[83] and the soybean plants had improved photosynthetic efficiency and seed yield.[84] Moreover, elevated PsbS levels in rice plants increased canopy radiation use efficiency and grain yield in fluctuating light[85] and overexpression of PsbS in tobacco plants increased their water-use efficiency.[86] A potential two-fold advantage of manipulating NPQ is foreseen: Overexpression of genes central to the regulation on NPQ, including those of PsbS, can increase phototolerance and increase the kinetics of NPQ, reducing the lag time between changes in light intensity and response. Secondly, manipulation of NPQ could lead to indirect changes in canopy structural properties.[87] (Figure 4).

PsbS in Green Algae

PsbS genes are present both in plants and green algae.[88] PsbS homologs have been found in several lineages of green algae,[89] and the green alga *Chlamydomonas reinhardtii* (*Cr*) contains two genes for PsbS: PSBS1 and PSBS2. PsbS proteins were long believed to be absent in green algae as the protein was not detected and the genes not expressed. However, investigations of the kinetics of gene expression of PsbS in *Cr* during HL stress uncovered that PsbS accumulates very rapidly, but only transiently.[90,91] PsbS accumulation in *Cr* is controlled by CO_2 availability and the protein is detected for several hours after exposure to HL at low CO_2 while degrading after $\sim$1 hour at high CO_2 levels.[91] PsbS could not compensate for LHCSR in an LHCSR-deficient *Cr* mutant[91] indicating that in green algae, LHCSR is essential for qE and NPQ. PsbS however impacts the NPQ capacity and cell viability under HL conditions. *Cr* cells with overexpressed LHCSR and PsbS could be grown under HL conditions where wildtype cells do not survive.[92] The mutant functional characteristics indicated detachment of LHCII complexes in the NPQ state and a decreased excitation pressure on PSII with the excess energy directed toward PSI. Lower amounts of (transiently expressed) PsbS in specific *Cr* mutants negatively affected NPQ capacity and modified electron transport and antenna organization, resulting in lower cell vitality.[93] Higher amounts of PsbS did not affect NPQ capacity but provided higher tolerance against HL stress. Notably, constitutively expressed PsbS was degraded during HL acclimation. This led to the hypothesis that transiently expressed PsbS in HL is required for membrane and protein reorganization, adjustment of electron transport characteristics and activation of NPQ, whereas its degradation is essential in the fully HL acclimated state.[93]

Outstanding Questions and Future Perspectives

In recent years, tremendous progress has been made in resolving the high-resolution structure of PsbS and unveiling its pH response mechanism. Yet, many open questions remain regarding its function and method of action. Key questions are listed below.

- **Structure of PsbS at neutral pH?** The neutral-pH high-resolution structure of PsbS has not been resolved by any structural method.
- **Function of the PsbS monomer/dimer state?** *In-vivo* studies suggest that PsbS dimers monomerize at HL/low pH conditions, whereas the PsbS crystal structure and structural pH-response mechanism

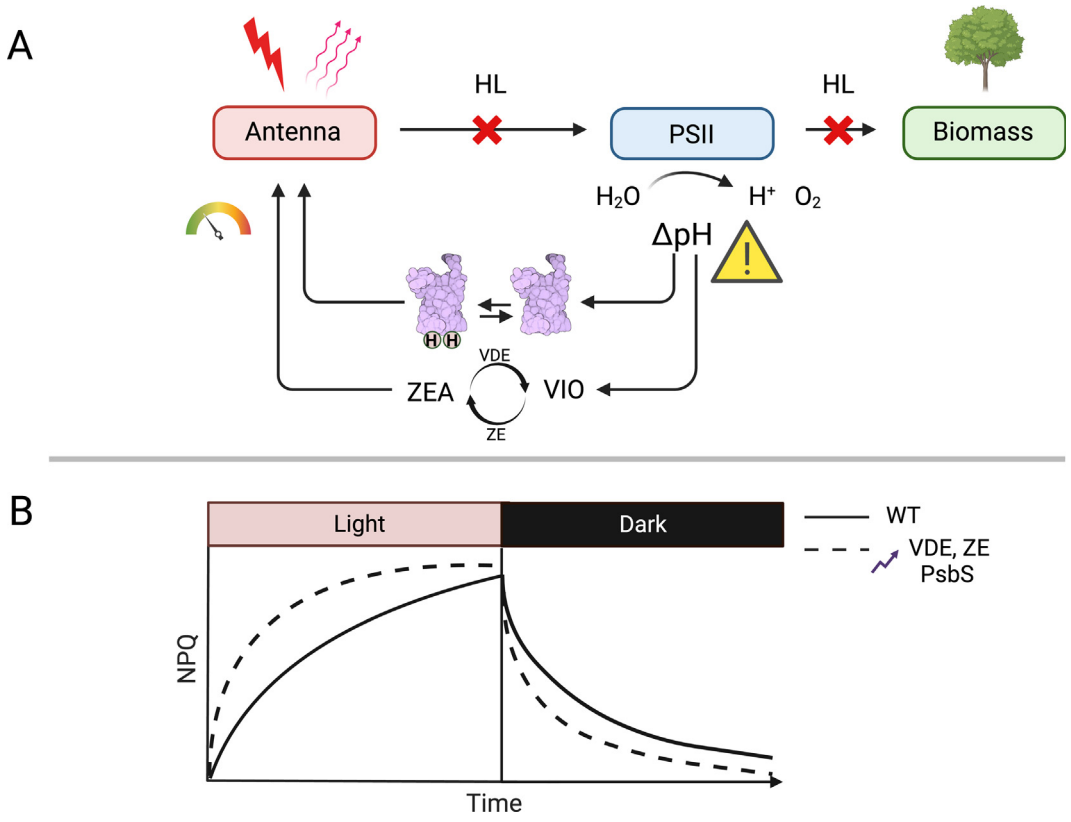

Figure 4. Scheme of the fast photo protective feedback response to HL. A: Acidification of the thylakoid lumen (ΔpH) in HL activates VDE enzymes in the xanthophyll cycle that convert violaxanthin (VIO) to Zea (time scale of minutes) and protonates PsbS (time scale of seconds). Protonated PsbS and Zea interact with the light-harvesting antenna, dimming its activity. Energy from sunlight is dissipated as heat and photosynthesis stops. In dark conditions, the reverse process occurs. B: NPQ response during a light/dark cycle. Overexpression of PsbS and the xanthophyll-cycle enzymes VDE and ZE accelerates the induction and relaxation of NPQ. Pictures were created with BioRender (https://BioRender.com).

suggest that the dimeric form is stabilized at low pH. The function of the PsbS monomer and dimer states is unclear.

- **Structural basis for PsbS interactions?** The LHC proteins share motifs of lumen-faced amphipathic helices and large stromal loops that could form recognition motifs. Identification of specific docking sites may reveal how the pH response of PsbS is transduced into dissipative states.
- **Role of PsbS in controlling qE?** Different scenarios have been proposed how PsbS could contribute to qE quenching, but a comprehensive picture is lacking. Plants lacking PsbS display qE quenching at enhanced ΔpH,[25] suggesting a cooperative function. The role and functional mechanism of PsbS in green algae is largely unknown.
- **Interplay between PsbS and Zea?** Both are key players in the process of qE, but none of them acts as a direct quencher. Enhancement of qE in PsbS-overexpressed plants has not been demonstrated, to the best of our knowledge, in the absence of Zea. The role of Zea and its interplay with PsbS in controlling qE is still elusive.

Current advancements in biophysical techniques and computational approaches could accelerate research on PsbS to shed light on its molecular mechanism of action. The application of 2D-IR spectroscopy has proven successful to observe the pH response of PsbS experimentally.[28] In combination with 2D-IR spectral simulations[94] 2D-IR data could provide input to build experimental-based structural models of PsbS at varying pH conditions. NMR spectroscopy can detect changes in protonation states, as has been observed for PsbS.[28] Although more challenging for membrane proteins, pH titration experiments can determine pKa values of individual protonable residues via shifts of the carboxyl NMR chemical shift signals. For instance, the role of a protonated Glu in the membrane transporter EmrE was assessed applying MAS NMR spectroscopy.[95,96] The recently published cryo-EM structures of LHCII in detergent and lipid nanodiscs demonstrate the possibility to access LHC structures under non-crystallized conditions[33] and may open new ways to assess the neutral-pH structure of PsbS. Recent developments

in hardware and algorithms for classical and quantum mechanical approaches now enables researchers to simulate photosynthetic processes from atom to mesoscale and predict how pigment-protein complexes respond to changes in the microenvironment.[97,98] Advanced computational models combining quantum mechanical, classical and CG approaches may resolve how PsbS interaction with other LHC complexes affects their pigment-protein scaffolds or how the interplay of PsbS and Zea controls energy dissipative states. In our lab, NMR dynamic spectral-editing approaches have been applied to intact thylakoid membranes for assessing the influence of Zea, temperature and stacking conditions on protein dynamics and membrane fluidity.[56,99,100] Such methods may be suitable to assess the influence of PsbS on protein and lipid mobility. Understanding the complex roles of PsbS, Zea and membrane transitions in qE induction and kinetics would provide further means for targeting parameters for manipulation of plant photo protection. Research in these areas continue to advance and will contribute to a deeper understanding of the functional mechanism of PsbS and its significance in NPQ. Eventually, this knowledge may be implemented in further engineering approaches to enhance plant resilience and photosynthetic efficiency for food security in a global changing climate.

CRediT authorship contribution statement

Willem Marulanda Valencia: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Anjali Pandit:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

W. Marulanda Valencia is financially supported through the NWO Groot grant “Nanoregulators of photosynthesis” of the Netherlands Organization of Scientific research (NWO), file nr. OCENW. GROOT.2019.086 to A. Pandit.

Received 3 October 2023;
Accepted 13 December 2023;
Available online 16 December 2023

Keywords:
non-photochemical quenching;
membrane protein;
photo protection;
pH sensor;
thylakoid membrane

References

1. Johnson, M.P., (2016). Photosynthesis. *Essays Biochem.* **60**, 255–273.
2. Kramer, D.M., Sacksteder, C.A., Cruz, J.A., (1999). How acidic is the lumen?. *Photosynth. Res.* **60**, 151–163.
3. Blankenship, R.E., (2002). Chemiosmotic coupling and ATP synthesis. Molecular mechanisms of photosynthesis. Wiley-Blackwell, pp. 157–170.
4. Derks, A., Schaven, K., Bruce, D., (2015). Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. *BBA* **1847**, 468–485.
5. Horton, P., Ruban, A.V., Walters, R.G., (1996). Regulation of light harvesting in green plants. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.* **47**, 655–684.
6. Demmig-Adams, B., Cohu, C.M., Muller, O., Adams 3rd., W.W., (2012). Modulation of photosynthetic energy conversion efficiency in nature: from seconds to seasons. *Photosynth. Res.* **113**, 75–88.
7. Ruban, A.V., Murchie, E.H., (2012). Assessing the photoprotective effectiveness of non-photochemical chlorophyll fluorescence quenching: a new approach. *BBA* **1817**, 977–982.
8. Patricia, M., Li, X.-P., Niyogi, K.K., (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiol.* **125**, 1558–1566.
9. Maxwell, K., Johnson, G.N., (2000). Chlorophyll fluorescence–a practical guide. *J. Exp. Bot.* **51**, 659–668.
10. Nilkens, M., Kress, E., Lambrev, P., Miloslavina, Y., Müller, M., Holzwarth, A.R., et al., (2010). Identification of a slowly inducible zeaxanthin-dependent component of non-photochemical quenching of chlorophyll fluorescence generated under steady-state conditions in Arabidopsis. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1797**, 466–475.
11. Murchie, E.H., Ruban, A.V., (2020). Dynamic non-photochemical quenching in plants: from molecular mechanism to productivity. *Plant J.* **101**, 885–896.
12. Levin, G., Schuster, G., (2023). LHC-like proteins: The guardians of photosynthesis. *Int. J. Mol. Sci.* **24**, 2503
13. Pinnola, A., (2019). The rise and fall of Light-Harvesting Complex Stress-Related proteins as photoprotection agents during evolution. *J. Exp. Bot.* **70**, 5527–5535.
14. Liguori, N., Roy, L.M., Opacic, M., Durand, G., Croce, R., (2013). Regulation of light harvesting in the green alga Chlamydomonas reinhardtii: The C-terminus of LHCSR is the knob of a dimmer switch. *J. Am. Chem. Soc.* **135**, 18339–18342.
15. Fan, M., Li, M., Liu, Z., Cao, P., Pan, X., Zhang, H., et al., (2015). Crystal structures of the PsbS protein essential for photoprotection in plants. *Nature Struct. Mol. Biol.* **22**, 729
16. Li, X.-P., Gilmore, A., Caffarri, S., Bassi, R., Golan, T., Kramer, D., et al., (2004). Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein*. *J. Biol. Chem.* **279**, 22866–22874.

17. Li, X.-P., Phippard, A., Pasari, J., Niyogi, K.K., (2002). Structure–function analysis of photosystem II subunit S (PsbS) *in vivo*. *Funct. Plant Biol.* **29**, 1131–1139.
18. Niyogi, K.K., Truong, T.B., (2013). Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Curr. Opin. Plant Biol.* **16**, 307–314.
19. Gerotto, C., Alboresi, A., Giacometti, G.M., Bassi, R., Morosinotto, T., (2012). Coexistence of plant and algal energy dissipation mechanisms in the moss Physcomitrella patens. *New Phytol.* **196**, 763–773.
20. Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G. S., Greenblatt, D.M., Meng, E.C., et al., (2004). UCSF Chimera–a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605–1612.
21. Li, X.-P., Björkman, O., Shih, C., Grossman, A.R., Rosenquist, M., Jansson, S., et al., (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* **403**, 391–395.
22. Funk, C., Adamska, I., Green, B.R., Andersson, B., Renger, G., (1995). The nuclear-encoded chlorophyll-binding photosystem II-S protein is stable in the absence of pigments. *J. Biol. Chem.* **270**, 30141–30147.
23. Dominici, P., Caffarri, S., Armenante, F., Ceoldo, S., Crimi, M., Bassi, R., (2002). Biochemical properties of the PsbS subunit of photosystem II either purified from chloroplast or recombinant. *J. Biol. Chem.* **277**, 22750–22758.
24. Bergantino, E., Segalla, A., Brunetta, A., Teardo, E., Rigoni, F., Giacometti, G.M., et al., (2003). Light- and pH-dependent structural changes in the PsbS subunit of photosystem II. *PNAS* **100**, 15265–15270.
25. Johnson, M.P., Ruban, A.V., (2011). Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced DeltapH. *J. Biol. Chem.* **286**, 19973–19981.
26. Liguori, N., Campos, S.R.R., Baptista, A.M., Croce, R., (2019). Molecular anatomy of plant photoprotective switches: The sensitivity of PsbS to the environment, residue by residue. *J. Phys. Chem. Lett.* **10**, 1737–1742.
27. Beraldo, C., Guyon-Debast, A., Alboresi, A., Nogué, F., Morosinotto, T., (2023). Functional analysis of PsbS transmembrane domains through base editing in Physcomitrium patens. *Plant J.* **113**, 1049–1061.
28. Krishnan-Schmieden, M., Konold, P.E., Kennis, J.T.M., Pandit, A., (2021). The molecular pH-response mechanism of the plant light-stress sensor PsbS. *Nature Commun.* **12**, 2291
29. Chiariello, M.G., Grünewald, F., Zarmiento-Garcia, R., Marrink, S.J., (2023). pH-dependent conformational switch impacts stability of the PsbS dimer. *J. Phys. Chem. Lett.* **14**, 905–911.
30. Forsyth, W.R., Antosiewicz, J.M., Robertson, A.D., (2002). Empirical relationships between protein structure and carboxyl pKa values in proteins. *Proteins Struct. Funct. Bioinf.* **48**, 388–403.
31. Krishnan, M., Moolenaar, G.F., Gupta, K., Goosen, N., Pandit, A., (2017). Large-scale in vitro production, refolding and dimerization of PsbS in different microenvironments. *Sci. Rep.* **7**, 15200.
32. Murchie, E.H., Niyogi, K.K., (2011). Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiol.* **155**, 86
33. Ruan, M., Li, H., Zhang, Y., Zhao, R., Zhang, J., Wang, Y., et al., (2023). Cryo-EM structures of LHCII in photo-active and photo-protecting states reveal allosteric regulation of light harvesting and excess energy dissipation. *Nature Plants* **9**, 1547–1557.
34. Pan, X., Li, M., Wan, T., Wang, L., Jia, C., Hou, Z., et al., (2011). Structural insights into energy regulation of light-harvesting complex CP29 from spinach. *Nature Struct. Mol. Biol.* **18**, 309–315.
35. Nield, J., Funk, C., Barber, J., (2000). Supermolecular structure of photosystem II and location of the PsbS protein. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1337–1344.
36. Sacharz, J., Giovagnetti, V., Ungerer, P., Mastroianni, G., Ruban, A.V., (2017). The xanthophyll cycle affects reversible interactions between PsbS and light-harvesting complex II to control non-photochemical quenching. *Nature Plants* **3**, 16225
37. Correa-Galvis, V., Poschmann, G., Melzer, M., Stühler, K., Jahns, P., (2016). PsbS interactions involved in the activation of energy dissipation in Arabidopsis. *Nature Plants* **2**, 15225
38. Su, X., Ma, J., Wei, X., Cao, P., Zhu, D., Chang, W., et al., (2017). Structure and assembly mechanism of plant C(2)S (2)M(2)-type PSII-LHCII supercomplex. *Science* **357**, 815–820.
39. Ware, M.A., Giovagnetti, V., Belgio, E., Ruban, A.V., (2015). PsbS protein modulates non-photochemical chlorophyll fluorescence quenching in membranes depleted of photosystems. *J. Photochem. Photobiol. B Biol.* **152**, 301–307.
40. Betterle, N., Ballottari, M., Zorzan, S., de Bianchi, S., Cazzaniga, S., Dall'osto, L., et al., (2009). Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction. *J. Biol. Chem.* **284**, 15255–15266.
41. Kereïche, S., Kiss, A.Z., Kouřil, R., Boekema, E.J., Horton, P., (2010). The PsbS protein controls the macro-organisation of photosystem II complexes in the grana membranes of higher plant chloroplasts. *FEBS Lett.* **584**, 759–764.
42. Kiss, A., Ruban, A., Horton, P., (2008). The PsbS protein controls the organization of the photosystem II antenna in higher plant thylakoid membranes. *J. Biol. Chem.* **283**, 3972–3978.
43. Dall'Osto, L., Cazzaniga, S., Bressan, M., Paleček, D., Židek, K., Niyogi, K.K., et al., (2017). Two mechanisms for dissipation of excess light in monomeric and trimeric light-harvesting complexes. *Nature Plants* **3**, 17033
44. Guardini, Z., Bressan, M., Caferri, R., Bassi, R., Dall'Osto, L., (2020). Identification of a pigment cluster catalysing fast photoprotective quenching response in CP29. *Nature Plants* **6**, 303–313.
45. Nicol, L., Nawrocki, W.J., Croce, R., (2019). Disentangling the sites of non-photochemical quenching in vascular plants. *Nature Plants* **5**, 1177–1183.
46. Fantuzzi, A., Haniewicz, P., Farci, D., Loi, M.C., Park, K., Büchel, C., et al., (2023). Bicarbonate activation of the monomeric photosystem II-PsbS/Psb27 complex. *Plant Physiol.* **192**, 2656–2671.
47. Daskalakis, V., (2018). Protein–protein interactions within photosystem II under photoprotection: the synergy between CP29 minor antenna, subunit S (PsbS) and

zeaxanthin at all-atom resolution. *PCCP* **20**, 11843–11855.
48. Daskalakis, V., Papadatos, S., (2017). The photosystem II subunit S under stress. *Biophys. J.* **113**, 2364–2372.
49. Daskalakis, V., Papadatos, S., Kleinekathöfer, U., (2019). Fine tuning of the photosystem II major antenna mobility within the thylakoid membrane of higher plants. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1861**, 183059
50. Navakoudis, E., Stergiannakos, T., Daskalakis, V., (2023). A perspective on the major light-harvesting complex dynamics under the effect of pH, salts, and the photoprotective PsbS protein. *Photosynth. Res.* **156**, 163–177.
51. Olsson, M.H.M., Søndergaard, C.R., Rostkowski, M., Jensen, J.H., (2011). PROPKA3: consistent treatment of internal and surface residues in empirical pKa predictions. *J. Chem. Theory Comput.* **7**, 525–537.
52. Johnson, M.P., Brain, A.P., Ruban, A.V., (2011). Changes in thylakoid membrane thickness associated with the reorganization of photosystem II light harvesting complexes during photoprotective energy dissipation. *Plant Signal. Behav.* **6**, 1386–1390.
53. Li, D.-H., Wilson, S., Mastroianni, G., Ruban, A.V., (2023). Altered lipid acyl chain length controls energy dissipation in light-harvesting complex II proteoliposomes by hydrophobic mismatch. *J. Photochem. Photobiol. B Biol.* **246**, 112758
54. Daskalakis, V., Papadatos, S., Kleinekathöfer, U., (2019). Fine tuning of the photosystem II major antenna mobility within the thylakoid membrane of higher plants. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1861**
55. Goral, T.K., Johnson, M.P., Duffy, C.D., Brain, A.P., Ruban, A.V., Mullineaux, C.W., (2011). Light-harvesting antenna composition controls the macrostructure and dynamics of thylakoid membranes in Arabidopsis. *Plant J.* **69**, 289–301.
56. Azadi-Chegeni, F., Thallmair, S., Ward, M.E., Perin, G., Marrink, S.J., Baldus, M., et al., (2022). Protein dynamics and lipid affinity of monomeric, zeaxanthin-binding LHCII in thylakoid membranes. *Biophys. J.* **121**, 396–409.
57. Son, M., Pinnola, A., Schlau-Cohen, G.S., (2020). Zeaxanthin independence of photophysics in light-harvesting complex II in a membrane environment. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1861**, 148115
58. Schlau-Cohen, G.S., Yang, H.-Y., Krüger, T.P.J., Xu, P., Gwizdala, M., van Grondelle, R., et al., (2015). Single-molecule identification of quenched and unquenched states of LHCII. *J. Phys. Chem. Lett.* **6**, 860–867.
59. Krüger, T.P., Novoderezhkin, V.I., Ilioaia, C., van Grondelle, R., (2010). Fluorescence spectral dynamics of single LHCII trimers. *Biophys. J.* **98**, 3093–3101.
60. Shukla, M.K., Watanabe, A., Wilson, S., Giovagnetti, V., Moustafa, E.I., Minagawa, J., et al., (2020). A novel method produces native light-harvesting complex II aggregates from the photosynthetic membrane revealing their role in nonphotochemical quenching. *J. Biol. Chem.* **295**, 17816–17826.
61. Tutkus, M., Chmeliov, J., Trinkunas, G., Akhtar, P., Lambrev, P.H., Valkunas, L., (2021). Aggregation-related quenching of LHCII fluorescence in liposomes revealed by single-molecule spectroscopy. *J. Photochem. Photobiol. B Biol.* **218**, 112174
62. Natali, A., Gruber, J.M., Dietzel, L., Stuart, M.C., van Grondelle, R., Croce, R., (2016). Light-harvesting Complexes (LHCs) cluster spontaneously in membrane environment leading to shortening of their excited state lifetimes. *J. Biol. Chem.* **291**, 16730–16739.
63. Crisafi, E., Pandit, A., (2017). Disentangling protein and lipid interactions that control a molecular switch in photosynthetic light harvesting. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1859**, 40–47.
64. Kirchhoff, H., Hinz, H.-J., Rösgen, J., (2003). Aggregation and fluorescence quenching of chlorophyll a of the light-harvesting complex II from spinach in vitro. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1606**, 105–116.
65. Wilk, L., Grunwald, M., Liao, P.N., Walla, P.J., Kühlbrandt, W., (2013). Direct interaction of the major light-harvesting complex II and PsbS in nonphotochemical quenching. *PNAS* **110**, 5452–5456.
66. Liu, C., Gao, Z., Liu, K., Sun, R., Cui, C., Holzwarth, A.R., et al., (2016). Simultaneous refolding of denatured PsbS and reconstitution with LHCII into liposomes of thylakoid lipids. *Photosynth. Res.* **127**, 109–116.
67. Emanuela, C., Maithili, K., Anjali, P., (2018). Time-resolved fluorescence analysis of LHCII in the presence of PsbS at neutral and low pH. *bioRxiv*, 456046.
68. Pawlak, K., Paul, S., Liu, C., Reus, M., Yang, C., Holzwarth, A.R., (2020). On the PsbS-induced quenching in the plant major light-harvesting complex LHCII studied in proteoliposomes. *Photosynth. Res.* **144**, 195–208.
69. Nicol, L., Croce, R., (2021). The PsbS protein and low pH are necessary and sufficient to induce quenching in the light-harvesting complex of plants LHCII. *Sci. Rep.* **11**, 7415.
70. Chmeliov, J., Gelzinis, A., Songaila, E., Augulis, R., Duffy, C.D.P., Ruban, A.V., et al., (2016). The nature of self-regulation in photosynthetic light-harvesting antenna. *Nature Plants* **2**, 16045
71. Miloslavina, Y., Wehner, A., Lambrev, P.H., Wientjes, E., Reus, M., Garab, G., et al., (2008). Far-red fluorescence: A direct spectroscopic marker for LHCII oligomer formation in non-photochemical quenching. *FEBS Lett.* **582**, 3625–3631.
72. Miloslavina, Y., de Bianchi, S., Dall'Osto, L., Bassi, R., Holzwarth, A.R., (2011). Quenching in Arabidopsis thaliana mutants lacking monomeric antenna proteins of photosystem II. *J. Biol. Chem.* **286**, 36830–36840.
73. Belgio, E., Kapitonova, E., Chmeliov, J., Duffy, C.D.P., Ungerer, P., Valkunas, L., et al., (2014). Economic photoprotection in photosystem II that retains a complete light-harvesting system with slow energy traps. *Nature Commun.* **5**, 4433
74. Demmig-Adams, B., Adams, W.W., (1993). The xanthophyll cycle. In: Young, A.J., Britton, G. (Eds.), *Carotenoids in Photosynthesis*. Springer, Netherlands, Dordrecht, pp. 206–251.
75. Kress, E., Jahns, P., (2017). The dynamics of energy dissipation and xanthophyll conversion in arabidopsis indicate an indirect photoprotective role of zeaxanthin in slowly inducible and relaxing components of non-photochemical quenching of excitation energy. *Front. Plant Sci.* **8**

76. Zaks, J., Amarnath, K., Kramer, D.M., Niyogi, K.K., Fleming, G.R., (2012). A kinetic model of rapidly reversible nonphotochemical quenching. *PNAS* **109**, 15757–15762.
77. Sylak-Glassman, E.J., Malnoë, A., De Re, E., Brooks, M. D., Fischer, A.L., Niyogi, K.K., et al., (2014). Distinct roles of the photosystem II protein PsbS and zeaxanthin in the regulation of light harvesting in plants revealed by fluorescence lifetime snapshots. *PNAS* **111**, 17498
78. Steen, C.J., Morris, J.M., Short, A.H., Niyogi, K.K., Fleming, G.R., (2020). Complex roles of PsbS and xanthophylls in the regulation of nonphotochemical quenching in arabidopsis thaliana under fluctuating light. *J. Phys. Chem. B* **124**, 10311–10325.
79. Matuszyńska, A., Heidari, S., Jahns, P., Ebenhöh, O., (2016). A mathematical model of non-photochemical quenching to study short-term light memory in plants. *BBA* **1857**, 1860–1869.
80. Bennett, D.I.G., Amarnath, K., Park, S., Steen, C.J., Morris, J.M., Fleming, G.R., (2019). Models and mechanisms of the rapidly reversible regulation of photosynthetic light harvesting. *Open Biol.* **9**, 190043
81. Roach, T., Krieger-Liszkay, A., (2012). The role of the PsbS protein in the protection of photosystems I and II against high light in Arabidopsis thaliana. *BBA* **1817**, 2158–2165.
82. Long, S.P., Taylor, S.H., Burgess, S.J., Carmo-Silva, E., Lawson, T., De Souza, A.P., et al., (2022). Into the shadows and back into sunlight: photosynthesis in fluctuating light. *Annu. Rev. Plant Biol.* **73**, 617–648.
83. Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K., et al., (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* **354**, 857.
84. De Souza, A.P., Burgess, S.J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., et al., (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. *Science* **377**, 851–854.
85. Hubbart, S., Smillie, I.R.A., Heatley, M., Swarup, R., Foo, C.C., Zhao, L., et al., (2018). Enhanced thylakoid photoprotection can increase yield and canopy radiation use efficiency in rice. *Commun. Biol.* **1**, 22.
86. Glowacka, K., Kromdijk, J., Kucera, K., Xie, J., Cavanagh, A.P., Leonelli, L., et al., (2018). Photosystem II Subunit S overexpression increases the efficiency of water use in a field-grown crop. *Nature Commun.* **9**, 868
87. Foo, C.C., Burgess, A.J., Retkute, R., Tree-Intong, P., Ruban, A.V., Murchie, E.H., (2020). Photoprotective energy dissipation is greater in the lower, not the upper, regions of a rice canopy: a 3D analysis. *J. Exp. Bot.* **71**, 7382–7392.
88. Büchel, C., (2015). Evolution and function of light harvesting proteins. *J. Plant Physiol.* **172**, 62–75.
89. Bonente, G., Passarini, F., Cazzaniga, S., Mancone, C., Buia, M.C., Tripodi, M., et al., (2008). The occurrence of the psbS gene product in Chlamydomonas reinhardtii and in other photosynthetic organisms and its correlation with energy quenching. *Photochem. Photobiol.* **84**, 1359–1370.
90. Tibiletti, T., Auroy, P., Peltier, G., Caffarri, S., (2016). Chlamydomonas reinhardtii PsbS protein is functional and accumulates rapidly and transiently under high light. *Plant Physiol.* **171**, 2717–2730.
91. Correa-Galvis, V., Redekop, P., Guan, K., Griess, A., Truong, T.B., Wakao, S., et al., (2016). Photosystem II subunit PsbS is involved in the induction of LHCSR protein-dependent energy dissipation in Chlamydomonas reinhardtii. *J. Biol. Chem.* **291**, 17478–17487.
92. Wilson, S., Kim, E., Ishii, A., Ruban, A.V., Minagawa, J., (2023). Overexpression of LHCSR and PsbS enhance light tolerance in Chlamydomonas reinhardtii. *J. Photochem. Photobiol. B Biol.* **244**, 112718
93. Redekop, P., Rothhausen, N., Rothhausen, N., Melzer, M., Mosebach, L., Dülger, E., et al., (2020). PsbS contributes to photoprotection in Chlamydomonas reinhardtii independently of energy dissipation. *Biochimica et Biophysica Acta (BBA) – Bioenergetics* **1861**, 148183
94. van Hengel, C.D.N., van Adrichem, K.E., Jansen, T.L.C., (2023). Simulation of two-dimensional infrared Raman spectroscopy with application to proteins. *J. Chem. Phys.* **158**
95. Gayen, A., Leninger, M., Traaseth, N.J., (2016). Protonation of a glutamate residue modulates the dynamics of the drug transporter EmrE. *Nature Chem. Biol.* **12**, 141–145.
96. Li, J., Sae Her, A., Traaseth, N.J., (2021). Asymmetric protonation of glutamate residues drives a preferred transport pathway in EmrE. *PNAS* **118**, e2110790118
97. Cupellini, L., Liguori, N., (2023). Special issue on quantum and classical computational methods in photosynthesis: from the atom to the mesoscale. *Photosynth. Res.* **156**, 1–2.
98. Liguori, N., Croce, R., Marrink, S.J., Thallmair, S., (2020). Molecular dynamics simulations in photosynthesis. *Photosynth. Res.* **144**, 273–295.
99. Nami, F., Tian, L., Huber, M., Croce, R., Pandit, A., (2021). Lipid and protein dynamics of stacked and cation-depletion induced unstacked thylakoid membranes. *BBA Adv.* **1**, 100015
100. Pandit, A., (2022). Structural dynamics of light harvesting proteins, photosynthetic membranes, and cells observed by spectral editing solid-state NMR. *J. Chem. Phys.* **157**, 025101