Project Closing Report

Conformational changes of photosystem II reaction center complex: uncovering, mechanism(s) and functional significance

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The present report will focus on our most important findings on the structural dynymics of Photosystem II (PSII) (Part I) – also highlighted in the Summary section; in Part II, further studies which are related to the structure, function and structural/functional plasticity of PSII and thylakoid membranes will be briefly outlined; in Part III, we list and shortly describe the contents of our methodological or review papers.

Part I. Structural dynamics of Photosystem II – related publications: *Magyar et al. 2018 Scientific Reports; Sipka et al. 2019 Physiologia Plantarum; Sipka et al. 2021 Plant Cell*

I/1. Background. Structure and basic functions of Photosystem II

Photosystem II (PSII) is a multi-subunit pigment–protein complex embedded in the thylakoid membrane of plants, algae, and cyanobacteria. PSII is often called the 'engine of life' because of its pivotal roles in the evolution of life and in feeding virtually all life on Earth (Barber, 2004 Photosynth Res 80, 137). It uses light energy to catalyze the electron transfer from water to plastoquinone and supplies the reducing equivalents necessary to fix carbon dioxide. PSII is probably the most-studied light-induced enzyme, not only for its relevance to biochemistry (it is the O_2-producing enzyme) but also because it serves as source of inspiration for artificial photocatalyzers for water oxidation and the production of H_2. The structure and function of PSII and its reaction center (RC) complex are well known (Umena et al. 2011 Nature 473, 55; Cardona et al. 2012 BBA 1817, 26). Each PSII core complex (PSII CC) contains the reaction center incorporated in the D1/D2 proteins, the α and β subunits of cyt b_{559} and two integral antenna proteins, CP43 and CP47, which carry 13 and 16 Chl-*a* molecules (Chl), respectively.

The RC D1/D2 proteins are placed roughly symmetrically with regard to the transmembrane region, which is analogous to the arrangement of the L/M subunits in bacterial RC (bRC). Four chlorophylls (Chls) (P_{D1} and P_{D2}, traditionally denoted as P_{680}, two "accessory" Chls Chl_{D1} and Chl_{D2}), two pheophytins ($Pheo_{D1}$ and $Pheo_{D2}$), two plastoquinones (Q_A and Q_B) are arranged in two pseudosymmetrical branches. As pointed out by Romero et al. (2017 Nature 543, 355) - the traditional assignment of P_{680} does not describe the early events of charge separation; for such cases, instead of P_{680}* or P_{680}^+, respectively, RC* and P_{D1}^+ should be used. Here, we use the simplified approach, and thus the term P_{680}, denoting the primary electron donor, irrespective of its molecular identity.

The absorption of a photon or transfer of an exciton to the primary electron donor P_{680} forms an electronically excited state. As in the simpler purple bacterial RC (Michel and Deisenhofer, 1988), the subsequent charge separation in PSII is known to proceed asymmetrically, only along the D1 branch with the formation of $P_{680}^+Pheo^-$. This primary charge separation is stabilized by a re-oxidation of $Pheo^-$ by Q_A, forming $P_{680}^+Q_A^-$; the primary electron donor is re-reduced by electron donation from the nearby redox active tyrosine (Tyr161 of D1), forming a neutral tyrosyl ($Y_Z^{\cdot}$) radical, which is then able to oxidize the Mn_4CaO_5 cluster, leading to the $S_2^{(+)}$ state, which denotes the state of the oxygen-evolving complex (OEC) after a single-turnover saturating flash

(STSF) excitation. When Q_A is pre-reduced, only the primary radical pair $P_{680}^{+}Pheo^{-}$ can be formed. This pair then decays by charge recombination within a few nanoseconds.

I/2. Monitoring the functional activity of PSII by Chl-*a* fluorescence induction kinetics – controversies

Functional activity of PSII – both in vivo and in vitro - is routinely characterized by measuring the Chl-*a* fluorescence induction kinetics, i.e the rise of fluorescence intensity upon the dark-to-light transition (Papageorgiou and Govindjee, 2004 Springer). To characterize this transition, either the yield or the intensity of the fluorescence emission is recorded as a function of time of illumination. In both cases, the fluorescence levels rise from the minimum to the maximum, from F_o to F_m, or from O to P, respectively. The fast fluorescence transients usually contain intermediary states, J and I; these are absent when the electron transfer from Q_A to Q_B is inhibited, and then the O-to-P rise assumes a sigmoidal shape (**Fig. 1**). In general, the variable Chl-*a* fluorescence ($F_v = F_m - F_o$ or the O-J-I-P) transients carry information on the light reactions of the entire photosynthetic machinery (Kalaji et al. 2017 Photosyn Res 132, 13). Thus, Chl-*a* fluorescence induction is arguably one of the most commonly used techniques in plant biology.

According to the mainstream model, which has been widely accepted in the past more than half a century, "to reach F_m it is necessary, and sufficient, to have Q_A completely reduced in all the active PSII centers" (Duysens and Sweers 1963 Univ Tokyo Press; Stirbet and Govindjee 2012 Photosynth Res 113, 15). Correspondingly, in this model, F_o and F_m belong to the open ($PSII_O$) and closed ($PSII_C$) states of the reaction center - states which, respectively, are ready and incapable of utilizing the absorbed light for stable charge separation. In this model, the F_v/F_m parameter is equated with the quantum efficiency of PSII. The multiphasic (O–J–I–P) rise is explained by whole-chain electron-transfer reactions affecting the reduction state of Q_A, and the sigmoidal rise is ascribed to an energetic coupling (connectivity) between PSII units (Stirbet 2013 Photosynth Res 116, 189).

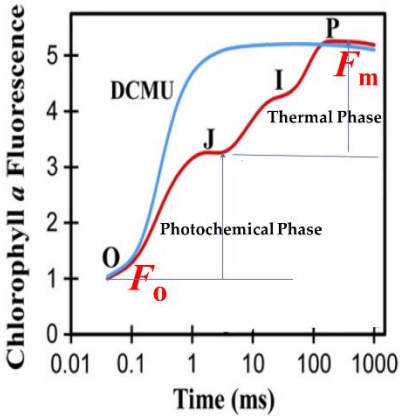

Figure 1. *Fast Chl-a fluorescence induction (O-J-I-P) kinetics in the absence and presence of the Q_A-to-Q_B electron transfer inhibitor, DCMU. The figure also displays the usual terminology, the photochemical and thermal phases, and marks the F_o(=O) and F_m(=P) levels.*

While Chl-*a* fluorescence measurements have provided a wealth of information on the mechanisms of photosynthetic light-energy conversion (Papageorgiou and Govindjee 2004), the mainstream model has never been free of controversies (Joliot and Joliot 1979 BBA 546, 93; Vredenberg 2011 Biosystems 103, 138; Schansker et al. 2014 Photosynth Res 120, 43). Early measurements indicated that only the O-J transition could be assigned unequivocally to the photochemical activity of PSII; the additional rise (J-I-P) was termed as thermal phase (Delosme 1967 BBA 143, 108). This phase, i.e. the rise consecutive to the O-J step, was proposed to be caused by the action of secondary quenchers and/or by a partial re-oxidation of Q_A via the activity of Photosystem I (PSI) (Stirbet and Govindjee 2012 Photosynth Res 113, 15). However, this explanation did not resolve the controversial features of the O-J-I-P rise - as shown by the experiments of Schansker et al. (2011 BBA 1807, 1032), among others, by pointing out that the J step, peaking around 1 ms, lagged behind the P level even at very high light intensities (15,000 μmol photons m^{-2} s^{-1}), when each RC was excited once every ~40 μs. Also, in contrast to the

expectations, F_m could not be generated by a STSF, despite it fully reduced Q_A; to resolve this controversy, a hypothetical quencher molecule (called Q_2, was invoked, which was proposed to be reduced after Q_1(=Q_A) (Joliot and Joliot 1979 BBA 546, 93). However, as pointed out by Magyar et al. (2018 Sci Rep 8, 1), all potential candidates of Q_2 could be excluded. Further, contradicting the Q_A model, in some cyanobacterial mutants (Vavilin et al. 1999 Biochem 38, 14690) and wild-type green algal cells (Treves et al. 2016 New Phytol 210 1229) no or very poor correlation was found between the measured F_v/F_m parameter and the oxygen evolution activity of PSII.

The observation that in the presence of DCMU the fluorescence induction adopts a strong sigmoidal rise, instead of the expected exponential rise, was explained by assuming connectivity of the RCs, i.e. excitation energy exchange between PSII supercomplexes (reviewed by Stirbet 2013), despite some alternative explanations (Vredenberg 2011 Biosystems 103, 138). However, the fact that the rise remained sigmoidal in isolated PSII core complexes irrevocably proved that connectivity of PSII units is not required to explain the sigmoidal rise (Magyar et al. 2018 Sci Rep 8, 1).

These controversies prompted us to reinvestigate the origin of F_v and the assignment of F_v/F_m parameter to the quantum efficiency of PSII; and, in general, the correlation between the functioning of PSII and the structural and functional parameters during the dark-to-light transition of this photosystem.

I/3. Discovery of rate-limiting steps, $PSII_L$; F_v/F_m vs structural dynamics of PSII

We studied STSF-induced Chl-*a* fluorescence transients in dark-adapted isolated plant thylakoid membranes and cyanobacterial PSII CCs in the presence of DCMU. We have shown, in accordance with Joliot and Joliot (1979 546, 93), that upon exciting PSII by a STSF (generating the charge separated closed state, $PSII_C$) the fluorescence yield rises from F_o to F_1 ($F_1 < F_m$); and F_m could only be reached by a train of STSFs. Surprisingly, however, this could be achieved only when considerably long waiting times ($\Delta\tau$) were allowed between the consecutive flashes (F_1-to-F_2, F_2-to-F_3, etc.) (**Fig. 2**). This led to the discovery of rate-limiting steps in PSII photoreactions, i.e. reactions associated with multiple light-induced events following the single photochemical event of stable charge separation (Magyar et al. 2018 Sci Rep 8, 1). Waiting times were found in all samples tested, from PSII CC to whole cells and in preparations lacking the oxygen-evolving complex and were shown to exhibit strong temperature dependences.

A priori, it could not be ruled out that the rate limitation originates from gating of the formation of the $P_{680}^+Pheo^-$ radical pair, i.e. the charge separation that is allowed in $PSII_C$. To answer this question, we performed nanosecond transient absorption spectroscopy measurements, detecting at 819 nm the photooxidation and re-reduction kinetics of the primary electron donor P_{680} in DCMU-treated PSII CC. We used double laser-flash excitations with variable $\Delta\tau$ between the flashes. The experiments showed a rapid (~4 ns) recombination of $P_{680}^+Pheo^-$ upon the second flash for all $\Delta\tau$ values tested between 5 μs and 100 ms (Sipka et al. 2019, Physiol Plant 166, 22). These data have shown that the rate-limiting steps in PSII for fluorescence does not arise from a gating of the primary photochemistry, and provided irrevocable evidence that the F_o-F_1 rise and the F_1-F_m increments are generated by different physical mechanisms. The presently available data strongly suggest the role of stationary and transient electric fields and associated dielectric relaxation processes (Sipka et al. 2021 Plant Cell).

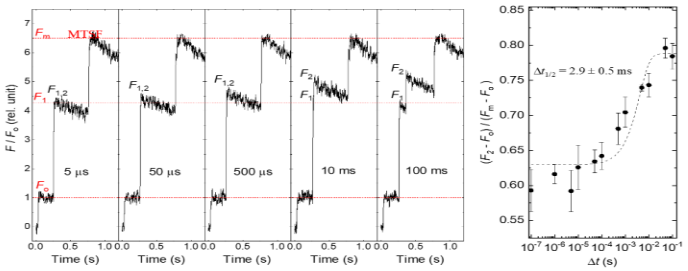

Figure 2. *Kinetic traces of Chl-a fluorescence induced by double-STSFs and multiple-turnover saturating flashes (MTSFs) in DCMU-treated PSII CC at 296 K with different waiting times between the first and second STSFs; and the dependence of the F_1-to-F_2 increment on the waiting time.*

In our most recent work (Sipka et al. 2021 Plant Cell), we showed that, in addition to the open ($PSII_O$) and closed ($PSII_C$) states, this photosystem can also be found in charge-separated (closed) light-adapted state ($PSII_L$). We studied the formation of this state and established its basic features. Main elements of our recent results are schematically illustrated in **Fig. 3** and are outlined in the following paragraphs.

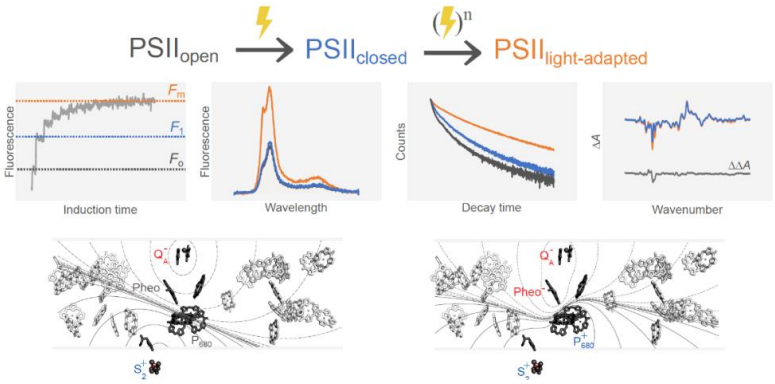

Figure 3. *Illustration of the observed effects of the $PSII_O$-$PSII_C$-$PSII_L$ transitions (black-blue-red lines, respectively) in PSII CC, as reflected in STSF-induced Chl-a fluorescence induction, the 80 K fluorescence emission, the fluorescence lifetimes and the rapid-scan FTIR difference spectra. The figure also shows the configurations of the stationary and transient electric fields, due to the stable and transient charge separations.*

Literature data, including our own studies and references therein (Schansker et al. 2011 Photosynth Res 120, 43; Schansker et al. 2014 BBA 1807, 1032; Magyar et al. 2018 Sci Rep 8, 1), and numerous data on bRCs (not listed here) indicated the involvement of light-induced conformational changes in the RCs. To obtain information on the nature of these temperature-dependent putative conformational changes, we performed light-induced rapid-scan FTIR experiments which is a widely used technique to study the mechanism of photo-induced reactions in proteins. Our FTIR experiments provided direct evidence for changes in the amide I region during the light adaptation process, correlated with the F_1-to-F_m fluorescence increment in the presence of DCMU. Earlier, light-induced conformational changes were identified in PSII CC of *T. vulcanus*; these occurred around the Q_B pocket and the OEC, identified by using femtosecond X-FEL crystallography (Suga et al. 2017 Nature 543, 131). However, in the presence of DCMU these reactions are blocked; consequently, our data show that the structural dynamics of PSII is not confined to the OEC and the Q_B pocket.

The formation of $PSII_L$, via light-induced conformational changes, has been shown to facilitate the stabilization of the charge-separated state. $PSII_L$ is characterized by distinct features in the energy landscape of trapping/detrapping of excitations in the core-antenna reaction-center complex.

After ruling out the gating of the primary charge separation causing the waiting time, we assumed that the fluorescence increments in F_v arise from specific light-induced reorganizations which affect the yield of Chl-*a* fluorescence via an interplay between the RC and the core-antenna complexes. The emission is known to originate – after energy transfer from the RC – from the two antenna complexes, CP47 and CP43, of the CC (Shibata et al. 2013 JACS 135, 6903). Indeed, low

temperature (80 K) fluorescence emission spectroscopy revealed significant changes in the excitation energy distribution in the core antenna during the transition from F_1-to-F_m, whereas only minor intensity and spectral distribution changes were discerned upon the F_o-to-F_1 transition. Hence, low temperature fluorescence spectroscopy appears to be a suitable diagnostic tool to identify the $PSII_O$-$PSII_C$-$PSII_L$ transitions.

The $PSII_C$-to-$PSII_L$ transition, i.e. the light adaptation process, was further investigated by time-resolved fluorescence spectroscopy at 278 K. We observed that the forward reactions were slowed down in the F_m ($PSII_L$) state compared to the F_1 ($PSII_C$) state - shifting the equilibrium to the antenna exciton states and increasing the fluorescence lifetime and yield. We proposed a model with key roles of radical pair states, different oxidation-reduction states of RC cofactors as well as protein structural dynamics.

The $PSII_C$–$PSII_L$ transition has been shown to be responsible for a large part of F_v, which thus appears to reflect predominantly the structural dynamics of PSII, rather than the reduction of Q_A. Clearly the F_v/F_m parameter cannot be equated with the quantum efficiency of PSII: F_m cannot be reached by a STSF, despite closing all PSII, i.e. despite reducing all Q_A (Sipka et al. 2021 Plant Cell). It must be pointed out that the F_v/F_m values of $PSII_C$, i.e. measured after the first STSF inducing stable charge separation in all active centers under physiologically relevant conditions, were typically not higher than 0.5 and were much lower at cryogenic temperatures. In all cases, additional excitations, trains of STSFs (at 80 K more than 500 STSFs) or intense and long flashes (MTSFs) are required to reach F_m. Even after MTSFs the maximum F_v/F_m values are typically found between 0.75 and 0.85. By contrast, the quantum efficiency of the stable charge separation in PSII is close to unity at all temperatures (Romero et al. 2017 Nature 543, 355). Further, closing the RC by chemically pre-reducing Q_A did not prevent the light-induced Chl-*a* fluorescence transitions and the STSF-induced increments (Sipka et al. 2021 Plant Cell). Hence, the F_v/F_m parameter, while it evidently carries useful information about the functional activity of PSII, must not be equated with the quantum efficiency of PSII. Hence, it is mandatory to re-interpret the F_v/F_m parameter – while it evidently will remain a useful parameter of PSII. It is noteworthy that this parameter, as well as the parameters of the fast O-J-I-P parameters, are sensitive to variations in the physico-chemical environment of PSII complexes; in particular, to the lipid polymorphism of thylakoid membranes (Ughy et al. 2019 Physiol Plant 166, 278, cf. also Dlouhý et al. 2020 Sci Rep 10, 11959– publications in Part II). Earlier we have also revealed that the F_v/F_m parameter and its thermal susceptibility is closely correlated with the macrodomain organization of PSII-LHCII supercomplexes (Kotakis et al. 2018 Photosynthetica 56, 254).

Our data suggest key roles of strong local stationary and transient electric fields (around Q_A^- and the rapidly recombining primary radical pair, respectively), and dielectric relaxation processes associated with these local electric fields generated in PSII.

Part II. Structural/functional plasticity of PSII and of the thylakoid membranes

II/1. Effects of the physico-chemical envronments and stresses – related publications: *Zsiros et al. 2019 Photosynthesis Research; Zsiros, Nagy et al. 2020 Frontiers in Plant Science; Zsiros, Ünnep et al. 2020 Frontiers in Plant Science; Ünnep et al. 2020 Open Biology* – described in this order.

Selenium (Se) is a natural trace element, which shifts its action in a relatively narrow concentration range from nutritional role to toxicity. Although it has been well established that in plants

chloroplasts are among the primary targets, the mechanism of toxicity on photosynthesis is not well understood. Here, we compared selenate and red-allotrope elemental selenium nanoparticles (red nanoSe) in in vitro tobacco cultures to investigate their effects on the structure and functions of the photosynthetic machinery – using mainly electron microscopy (EM), small-angle neutron scattering (SANS), circular dichroism (CD) spectroscopy and chlorophyll a fluorescence transient measurements. We revealed changes in the ultrastructure and significantly diminished PSII activity, and an enhanced capability of non-photochemical quenching (NPQ) in the presence of 10 mg/L selenate. In contrast, red nanoSe, even at 100 mg/L and selenate at 1 mg/L, exerted only marginal effects on the thylakoid membrane ultrastructure and the photosynthetic functions.

Trace metal contaminations in natural waters, wetlands, and wastewaters pose serious threats to aquatic ecosystems—mainly via targeting microalgae. We investigated the effects of toxic amounts of chromium and cadmium ions on the structure and function of the photosynthetic machinery of Chlorella variabilis cells – using biochemical tools, as well as absorbannce (P700) and chlorophyll fluorescence transient measurements, CD spectroscopy, EM and SANS. Our data revealed that despite their similar toxicity, the response of the photosynthetic machinery of C. variabilis to these two trace metal ions substantially differ from each other.

The thylakoid membranes of vascular plants are differentiated into stacked granum and unstacked stroma regions. The formation of grana is triggered by the macrodomain formation of photosystem II and light-harvesting complex II (PSII-LHCII) and thus their lateral segregation from the photosystem I—light-harvesting complex I (PSI-LHCI) supercomplexes and the ATP-synthase; which is then stabilized by stacking interactions of the adjacent PSII-LHCII enriched regions of the thylakoid membranes. The self-assembly and dynamics of this highly organized membrane system and the nature of forces acting between the PSII-LHCII macrodomains are not well understood. By using CD spectroscopy, SANS and transmission EM, we investigated the effects of Hofmeister salts on the organization of pigment-protein complexes and on the ultrastructure of thylakoid membranes. We found that the kosmotropic agent (NH4)2SO4 and the Hofmeister neutral NaCl, up to 2 M concentrations, hardly affected the macro-organization of the protein complexes and the membrane ultrastructure. In contrast, chaotropic salts, NaClO4, and NaSCN destroyed the mesoscopic structures, the multilamellar organization of the thylakoid membranes and the chiral macrodomains of the protein complexes but without noticeably affecting the short-range, pigment-pigment excitonic interactions, i.e. the molecular organization of protein complexes. Comparison of the concentration- and time-dependences of SANS, TEM and CD parameters revealed the main steps of the disassembly of grana in the presence of chaotropes. It begins with a rapid diminishment of the long-range periodic order of the grana membranes, apparently due to an increased stacking disorder of the thylakoid membranes; this step is followed by a somewhat slower disorganization of the TEM ultrastructure, due to the gradual loss of stacked membrane pairs; and last, the stepwise decrease and disappearance of the long-range chiral order of the protein complexes occurs. These data are interpreted in terms of a theory, from the BRC (András Dér), according to which Hofmeister salts primarily affect the hydrophylic-hydrophobic interactions of proteins, and the stroma-exposed regions of the intrinsic membrane proteins of LHCII and PSII and point to the role of protein-water interface in the interactions.

NPQ is an important photoprotective mechanism in plants and algae. Although the process is extensively studied, little is known about its relationship with ultrastructural changes of the thylakoid membranes. In order to better understand this relationship, we studied the effects of illumination on the organization of thylakoid membranes in Monstera deliciosa leaves – using chlorophyll fluorescence transient and time-resolved SANS measurements. We have shown that NPQ-inducing illumination causes a strong decrease in the periodic order of granum thylakoid membranes. Development of NPQ and light-induced ultrastructural changes, as well as the relaxation processes, follow similar kinetic patterns. Surprisingly, whereas whenh NPQ is suppressed by the PSII electron transport (Q_A-to-Q_B) inhibitor (diuron), it impedes only the relaxation of the structural changes and not its formation, suggesting that structural changes do not cause but enable NPQ.

II/2. Effects of the lipid polymorphism of thylakoid membranes and PSII functions – related publications: Ughy et al. 2019 Physiologia Plantarum; Dlouhy et al. 2020 Scientific Reports.

Our earlier experiments, using 31P-NMR and time-resolved merocyanine fluorescence spectroscopy, have shown that isolated intact, fully functional plant thylakoid membranes, in addition to the bilayer phase, contain three non-bilayer (or non-lamellar) lipid phases (Garab et al. 2017 Scientific Reports 7: 13343). It has also been shown that the lipid polymorphism of thylakoid membranes can be characterized by remarkable plasticity, i.e. by significant variations in ^{31}P-NMR signatures – which, however, could not be assigned to changes in the overall membrane organization and the magnitude of Fv/Fm also showed only marginal variations. In this work, we investigated in more detail the temporal stability of the different lipid phases by recording ^{31}P-NMR spectra on isolated thylakoid membranes and observed – using fast chlorophyll fluorescence (OJIP) and electrochromic (ΔA515) absorbance transients that substantial gradual enhancement of the isotropic lipid phases and diminishment of the bilayer phase were associated with the gradually increasing membrane permeability. These observations suggest that non-bilayer lipids and non-lamellar lipid phases play significant roles in the structural dynamics and functional plasticity of thylakoid membranes, also affecting PSII functions (as already suggested in our earlier work – Kotakis et al. 2018 Photosynthetica 56: 254–264, and references therein).

The role of non-bilayer lipids and non-lamellar lipid phases in biological membranes is an enigmatic problem of membrane biology. Non-bilayer lipids are present in large amounts in all membranes; in energy-converting membranes they constitute about half of their total lipid content—yet their functional state is a bilayer. In vitro experiments revealed that the functioning of the water-soluble violaxanthin de-epoxidase (VDE) enzyme of plant thylakoids (which de-epoxidizes the violaxanthin of LHCII into antera- and zeaxanthin) requires the presence of a non-bilayer lipid phase. Here we reveal reversible pH- and temperature-dependent changes of the lipid-phase behaviour, particularly the flexibility of isotropic non-lamellar phases, of isolated spinach thylakoids. These reorganizations are accompanied by changes in the permeability and thermodynamic parameters of the membranes and appear to control the activity of VDE and the photoprotective mechanism of NPQ of chlorophyll-a fluorescence. These data demonstrate, for the first time in native membranes, the dominant role of (a) non-bilayer lipid phase(s) in the modulation of the activity of a water-soluble enzyme.

Part III. Methodological and review papers – related publications: *Akhtar et al. 2019 The Journal of Physical Chemistry B; Akhtar et al. 2019 Chemical Physics; Nagy and Garab 2020 Photosynthesis Research; Wilhelm et al. Journal of Plant Physiology* – descibed in the following paragraphs, in this order.

Anisotropic circular dichroism (ACD) spectroscopy of macroscopically aligned molecules reveals additional information about their excited states that is lost in the CD of randomly oriented solutions. ACD spectra of LHCII, the main peripheral antenna of PSII of plants in oriented lipid bilayers were recorded between 180 and 750 nm. ACD spectra show a drastically enhanced magnitude and level of detail compared to the isotropic CD spectra, resolving a greater number of bands and weak optical transitions. We propose ACD spectroscopy to be a valuable tool linking the three-dimensional structure and the photophysical properties of pigment−protein complexes.

The quenching of chlorophyll-a fluorescence was investigated in plant LHCII embedded in reconstituted membranes containing thylakoid lipids and a lipid label. The proteoliposomes were further separated by density and the protein and lipid contents of the fractions were quantified spectrophotometrically, allowing tighter control over the lipid/protein (L/P) ratios in a wide range of values. Using time-resolved fluorescence, we found a strong correlation between the fluorescence quenching and the L/P ratio. It is hypothesized that plants can exploit the intrinsic quenching propensity of LHCII-only membrane domains to safely store extra antenna units.

The photosynthetic performance of crop plants under a variety of environmental factors and stress conditions, at the fundamental level, depends largely on the organization and structural flexibility of thylakoid membranes. These highly organized membranes accommodate virtually all protein complexes and additional compounds carrying out the light reactions of photosynthesis. Most regulatory mechanisms fine-tuning the photosynthetic functions affect the organization of thylakoid membranes at different levels of the structural complexity. In order to monitor these reorganizations, non-invasive techniques are of special value. On the mesoscopic scale, SANS has been shown to deliver statistically and spatially averaged information on the periodic organization of the thylakoid membranes in vivo and/or, in isolated thylakoids, under physiologically relevant conditions, without fixation or staining. SANS investigations have revealed rapid reversible reorganizations on the timescale of several seconds and minutes. In this paper, we give a short introduction into the basics of SANS technique, advantages and limitations, and briefly overview recent advances and potential applications of this technique in the physiology and biotechnology of crop plants. We also discuss future perspectives of neutron crystallography and different neutron scattering techniques, which are anticipated to become more accessible and of more use in photosynthesis research at new facilities with higher fluxes and innovative instrumentation.

Since the publication of the fluid-mosaic membrane theory by Singer and Nicolson in 1972 generations of scientists have adopted this fascinating concept for all biological membranes. Assuming the membrane as a fluid implies that the components embedded in the lipid bilayer can freely diffuse like swimmers in a water body. During the detailed biochemical analysis of the thylakoid protein components of chloroplasts from higher plants and algae, in the ‘80 s and ‘90 s it became clear that photosynthetic membranes are not homogeneous either in the vertical or the

lateral directions. The lateral heterogeneity became obvious by the differentiation of grana and stroma thylakoids, but also the margins have been identified with a highly specific protein pattern. Further refinement of the fluid mosaic model was needed to take into account the presence of non-bilayer lipids, which are the most abundant lipids in all energy-converting membranes, and the polymorphism of lipid phases, which has also been documented in thylakoid membranes. These observations lead to the question, how mobile the components are in the lipid phase and how this ordering is made and maintained and how these features might be correlated with the non-bilayer propensity of the membrane lipids. Assuming instead of free diffusion, a "controlled neighborhood" replaced the model of fluidity by the model of a "mixed crystal structure". In this review we describe why basic photosynthetic regulation mechanisms depend on arrays of crystal-like lipidprotein macro-assemblies. The mechanisms which define the ordering in macrodomains are still not completely clear, but some recent experiments give an idea how this fascinating order is produced, adopted and maintained. We use the operation of the xanthophyll cycle as a rather well understood model challenging and complementing the standard Singer-Nicolson model via assigning special roles to non-bilayer lipids and non-lamellar lipid phases in the structure and function of thylakoid membranes.