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REVIEW

Study of Electron Transfer in Photosystem I Using High-Frequency EPR Spectroscopy. In Memory of Professor Klaus Möbius (1936-2024)

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Abstract—Klaus Möbius, Professor at the Free University of Berlin, was an outstanding physical chemist and biophysicist. He was a pioneer in the development of high-field/high-frequency EPR spectroscopy methods and their application in the study of photosynthesis. Among the most essential are the applications in studying the charge transfer kinetics and properties of the ion-radical pairs in photosynthetic reaction centers (RC). Under his leadership and with his direct participation a unique setup allowing registration of the kinetics of the electron transfer between the (bacterio)chlorophyll dimer and quinone in the bacterial photosynthetic RC and plant photosystem I (PSI) was created. This setup also allowed precise determining of the distance between separated charges based on measuring the frequencies of the Electron Spin Echo Envelope Modulation (ESEEM). This setup made it possible to prove that electron transfer in PSI occurs mainly along the *A* branch of redox cofactors. The kinetics of backward electron transfer reaction (reoxidation of the phyllosemiquinone anion A_1^- and reduction of the photooxidized chlorophyll dimer P_{700}^+) in PSI were measured under the same conditions. The essential data on the bioprotective effect of the disaccharide trehalose on the kinetics of forward and backward electron transfer in PSI complexes were obtained. A significant slowdown in the kinetics of electron transfer due to the restriction of protein conformational mobility, as well as long-term maintaining of functional activity of PSI dried in a vitreous trehalose matrix at room temperature (i.e., subjected to a reversible anhydrobiosis) was demonstrated. These results obtained in collaboration with Prof. Möbius and Prof. Venturoli (Bologna) allowed elucidating the role of hydrogen bond network and the conformational mobility of the protein subunits in facilitating electron transfer in the photosynthetic RC.

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INTRODUCTION

Professor Klaus Möbius (1936-2024) was one of the pioneers in the development of high-frequency EPR spectroscopy methods, including multiresonance methods such as electron-nuclear double resonance (ENDOR), electron-electron double resonance (ELDOR), triple resonance (TRIPLE), and their applications in photosynthesis studies. We were fortunate to interact with this remarkable scientist. For one of the co-authors of this article (V. V. P.), communications with Klaus Möbius were (alas!) only episodic, occurring during his several visits to Russia. However, during these relatively short meetings and conversations, his deep interest not only for scientific (including general scientific) issues, but also for the personality of his interlocutor was always felt. Both his explanations of some ideas on EPR spectroscopy and his benevolent advice on life evoke grateful memories. Communication with Klaus Möbius for another co-author (A. Y. S.)

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was much closer and more regular (Fig. 1); we would like to devote a separate section to the memories of this communication.

PERSONAL MEMORIES OF ONE OF THE CO-AUTHORS (A. Y. S.) ON MEETING KLAUS MÖBIUS

Klaus and I first met at the Gordon Conference on the biophysics of photosynthesis in the small town of Plymouth, New Hampshire (USA) in 1997. A year later, he invited me to visit Berlin with my wife, and we stayed in the hospitable home of Klaus and his wife Uta in Dahlem. From that moment until 2024, we often saw each other in Berlin, Moscow, Bologna, Mülheim and Kazan. Based on common scientific interests and mutual sympathy, we developed good scientific and close personal relations.

Klaus was a friendly person with deep knowledge in various fields, the breadth of his interests was simply amazing. Klaus was exceptionally modest and democratic; he never demonstrated his superiority to any interlocutors. I was amazed that during one of our first visits to Berlin, he considered it necessary to show me many places connected with the Holocaust of the Jews under the Nazi Regime, in particular, the platform from which trains used to depart for the extermination camps in 1943.

In 2003, together with the laboratories of S. Ciurli (Bologna, Italy), R. Hasanov (Baku, Azerbaijan) and A. N. Tikhonov (Moscow), we received a joint INTAS grant, within the framework of which, during a visit to Bologna, I introduced Klaus to my long-time colleague Professor Giovanni Venturoli. This acquaintance developed into a long and successful collaboration and friendship between Möbius and Venturoli. As a result of the joint work of the laboratories of Venturoli, Möbius, his student Anton Savitsky and Professor Wolfgang Lubitz from the Max Planck Institute in Mülheim (Germany), most important results were obtained on the effect of the disaccharide trehalose (a bioprotector) on the kinetics of electron transfer in bacterial photosynthetic reaction centers (RC). Sometime later, similar work was performed with the participation of our laboratory on photosystem I (PSI) complexes from cyanobacteria. It was shown that drying in a glassy trehalose matrix at room temperature leads to a slowdown in the kinetics of electron transfer in both bacterial RC and PSI due to the limitation of the conformational mobility of the protein and long-term preservation of its functional activity in a state of reversible anhydrobiosis.

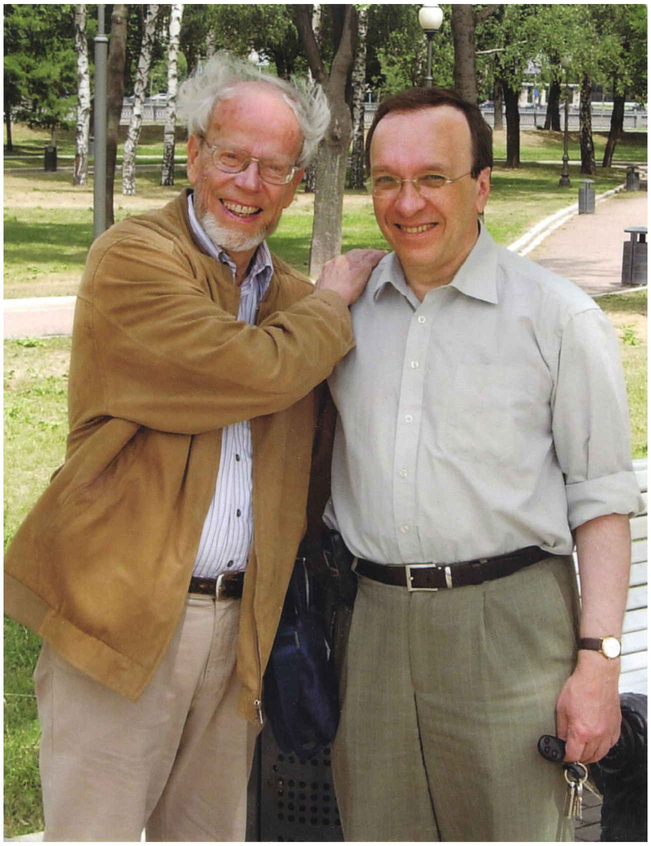
Fig. 1. K. Möbius and A. Semenov, Moscow, 2007. Photo by W. Lubitz.

Klaus was a great connoisseur of music, painting, and literature; he was interested in the history of science. In his house he organized a science museum, where he collected unique examples of scientific instruments and working models of some technical devices. Klaus was interested in the discoveries of his famous ancestor, the mathematician August Möbius (known to everyone for discovering the "Möbius strip"). He knew a lot about the life of August Möbius's teacher, the great mathematician Carl Gauss. Once Klaus surprised me by telling that Gauss had specifically learned Russian in order to read Nikolai Lobachevsky's articles, which had been available only in Russian at the time. His interest in the Möbius strip made Klaus, together with his long-time colleague Martin Plateau and Anton Savitsky, write and publish a book about Möbius strip-like structures in mathematics, astronomy, physics, chemistry, biology, music, painting and architecture near the end of his life [1].

Klaus witnessed the important historical events in Germany: the entry of Soviet troops into Berlin in 1945, the Soviet blockade of West Berlin in 1948-1949, the construction of the Berlin Wall and the closing of the border between East and West Berlin in 1961, the youth movement of the 1960s and the destruction of the Berlin Wall in 1989. His stories about these historical events, as well as the history of German science at the Kaiser Wilhelm Institutes in Dahlem, were extremely interesting.

Klaus lived a long and eventful life, till the very end he fully retained his interest in science, art, politics, and was a convinced supporter of the development of environmentally friendly energy. His stories and discussions with him are sorely missed by many of his colleagues and friends in different countries of the world.

A BRIEF SCIENTIFIC BIOGRAPHY OF KLAUS MÖBIUS

In this brief introduction we will review the main scientific achievements of K. Möbius, in which his fundamental research and methodological developments are closely intertwined, forming an inherent unity in his scientific journey.

Klaus Möbius's scientific career was linked to EPR spectroscopy from the very beginning. His supervisor at the Free University of Berlin, where Klaus Möbius studied (and later worked throughout his life), Richard Honerjäger, was a specialist in microwave electromagnetic radiation [2] – its generation, propagation, and its various applications, from plasma physics to microwave spectroscopy of molecules. In the second half of the 1950s, the scientist's attention was drawn to the new microwave method of electron paramagnetic resonance and its prospects in chemistry [3]. K. Möbius's thesis, completed in 1960-1961, was devoted to anisotropy of g-tensor and hyperfine interaction constants in crystals of organic molecules. Although the era of serial EPR spectrometers had already arrived, K. Möbius had to assemble an EPR spectrometer from available materials himself to complete his research. This first experience became the basis for K. Möbius's further scientific studies always combining fundamental scientific research with the development of new technology for magnetic resonance studies, which ensured his scientific achievements. The main focus of K. Möbius's instrument development was the high-frequency EPR spectrometry. The spectrometer created by K. Möbius and his colleagues at the Free University of Berlin is one of the best in the world, exceeding commercial instruments of well-known brand owners in its capabilities. His work in this area brought Klaus together with the famous Russian scientist Yakov Sergeevich Lebedev, a pioneer in high-frequency EPR spectroscopy. K. Möbius highly valued this researcher and believed that his contribution to world EPR spectroscopy was very significant, despite all the restrictions on the international exchange of scientific knowledge in the USSR in the 1970s and 1980s [4].

Nevertheless, the use of a non-self-made EPR spectrometer (manufactured by the famous German company AEG) which was the most modern at the time ensured efficient start of scientific work of K. Möbius [5]. Fritz Schneider, who was working at the AEG Research Institute (AEG Forschungsinstitut), provided to K. Möbius this fortunate opportunity [6]. It was a joint article with Schneider in the German journal *Zeitschrift für Naturforschung* [7] – a review introducing chemists to a new research method including the introduction to the theory of hyperfine structure (HFS) of EPR spectra and its application to the analysis of organic radical structure – that apparently became the first published work of K. Möbius. His subsequent works in those years, including collaborations with colleagues, among whom was Martin Plato, were also devoted mainly to EPR spectroscopy of organic radicals (for example, Möbius and Plato [8]). Later, K. Möbius turned to using ENDOR as a promising method for studying organic radicals in solutions [9]. This was facilitated by an internship in the USA with August (Gus) Mackie, who had first applied this method several years earlier together with James Hyde. The method had been previously proposed by J. Feher [10] for studying free radicals in solutions [11, 12]. The internship resulted in a data on changes in spin density in aromatic compounds and the experience of creating ENDOR observation equipment, which turned out to be very useful for his subsequent scientific work [13].

Since the late 1970s, K. Möbius also began to use the ENDOR, ELDOR, and TRIPLE methods [14-16], including their applications with strong magnetic fields. It should be noted that, despite the fact that high-field EPR methods promised a breakthrough in free radical research, by that time only a few research groups around the world had attempted to master these techniques and create the necessary equipment. K. Möbius's group was actually one of the first three teams of researchers and developers in the field of high-field EPR spectrometry, along with the groups of Harold Box et al. [17] in the USA and Ya. S. Lebedev in Moscow [18, 19]; only later J. Schmidt's group in Leiden [20] and the laboratories of Thomas Priesner [21] and Jack Fried in the USA [22] joined these studies.

The "instrument-building" stage of K. Möbius's activity coincided remarkably with the emergence of his interest in photosynthesis, a phenomenon that looks like tailor-made for the application of magnetic resonance methods. In his autobiographical notes, K. Möbius recalled how this interest was sparked in him by Arnold Hoff [6], with whom he published his first work on the of bacteriochlorophyll cation radical (Hoff and Möbius [23]). From this moment, K. Möbius began studying electron transfer reactions in photosynthesis, first on bacterial RCs [24], and then on photosystems I and II (PSI and PSII) pigment–protein complexes from oxygenic organisms (see the reviews [4, 25] and references therein). The development of another resonance method, electron-spin-echo-envelope-modulation (ESEEM), specifically in the high-frequency W-band [26], proved invaluable for these studies, with this approach remaining one of the most modern methods of EPR spectroscopy to this day. In subsequent years, the use of this particular technique allowed K. Möbius to obtain the most interesting results related to photosynthesis.

All the scientific activities of K. Möbius had also another aspect: he never closed himself off within his laboratory or even the university, and many of his works were carried out within the framework of international cooperation. Together with his outstanding scientific achievements, this humanistic result of his work was recognized by many international and national prizes and honorary titles, of which Klaus Möbius especially valued the Order of Merit of the Federal Republic of Germany (2006), awarded for the development of broad international cooperation; the Max Planck Prize of the Alexander von Humboldt Foundation (jointly with Chaim Levanon; 1992); the E. K. Zavoisky Prize (jointly with James Norris and Ya. S. Lebedev; 1994) and the V. V. Voevodsky Prize of the Siberian Branch of the Russian Academy of Sciences (2006); Silver (1996) and Gold (2001) Medals of the International EPR Society; and the AMPERE (Atomes et Molécules Par Études Radio-Électriques) Prize, 1998. He was elected a member of a number of academies and scientific societies, including the International Society of Magnetic Resonance (ISMAR) (2009); International EPR/ESR Society (IES) (2011) and the Istituto Veneto di Scienze, Lettere ed Arti in Venice (2002).

COLLABORATIONS WITH KLAUS MÖBIUS AND HIS COLLEAGUES

Our laboratory was fortunate to collaborate with Klaus Möbius for the last 15 years of his life.

Asymmetry of electron transfer in PSI. Our first joint work was the study of the asymmetry of electron transfer along the branches of redox cofactors in PSI complexes [27]. All photosynthetic RCs are known to have two practically symmetrical branches of redox cofactors between the dimer of chlorophyll (Chl) (or bacteriochlorophyll) and quinone molecules, but electron transfer in PSII complexes and in bacterial RCs occurs only along one of these branches. At the same time, electron transfer in PSI occurs along both branches (*A* and *B*), but with different efficiency and predominantly along branch *A*.

Conservative Met amino acid residues (M688 in the cofactor branch *A* and M668 in the branch *B*) are axial ligands of the third Chl molecule pair (called Chl_{3A} and Chl_{3B} in the literature), which correspond to the primary electron acceptors A_{0A} and A_{0B} in the symmetric *A* and *B* branches of redox cofactors in PSI (Fig. 2). Substitution of Met molecules by Leu in any of the redox cofactor branches can lead to a change in the degree of asymmetry of electron transfer along *A* and *B* branches from the primary acceptors A_{0A} and A_{0B} to the secondary quinone acceptors A_{1A} and A_{1B}. In PSI from cyanobacterium *Synechocystis* sp. PCC 6803 the degree of asymmetry of electron transfer along the branches of redox cofactors was investigated on complexes isolated from the wild type and from mutant strains with symmetrical point substitutions of Met to Leu in *A* (M688LPsaA) and *B* (M668LPsaB) branches of cofactors.

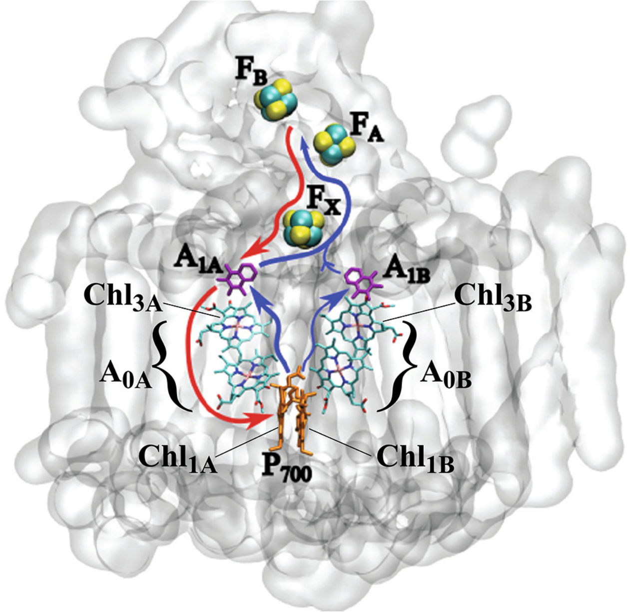

Fig. 2. Electron transfer between redox cofactors in PSI. The arrangement of redox cofactors within the protein complex and the pathways of forward (blue arrows) and backward (red arrows) electron transfer are shown. P_{700} – the primary electron donor, a dimer of Chl molecules; A_{1A}, A_{1B} – phylloquinone electron acceptors in *A* and *B* branches of cofactors; F_X, F_A, F_B – 4Fe4S clusters. Electron transfer occurs predominantly along branch *A*.

The results obtained using femtosecond laser spectroscopy [28], time-resolved EPR spectroscopy in microwave X (~9 GHz) [29] and Q (~35 GHz) ranges [30] showed that in PSI from the M688LPsaA mutant, the efficiency of electron transfer from the primary PSI donor, P_{700}, to the terminal F_A/F_B acceptors is reduced, while an increase in the formation of the triplet state P_{700}^* was assumed. In our joint work, it was shown that the decay kinetics of the laser-induced EPR signals of the oxidized primary PSI donor $P_{700}^{\bullet+}$ from wild type *Synechocystis* sp. and from the M668LPsaB mutant are very similar, while in the case of the M688LPsaA mutant, the decay kinetics is significantly slowed down [27]. For an illustration of this, see Fig. 3, which demonstrates the kinetics of $P_{700}^{\bullet+}$ decay (which is formed as a result of excitation of an isolated PSI complex by a nanosecond laser pulse).

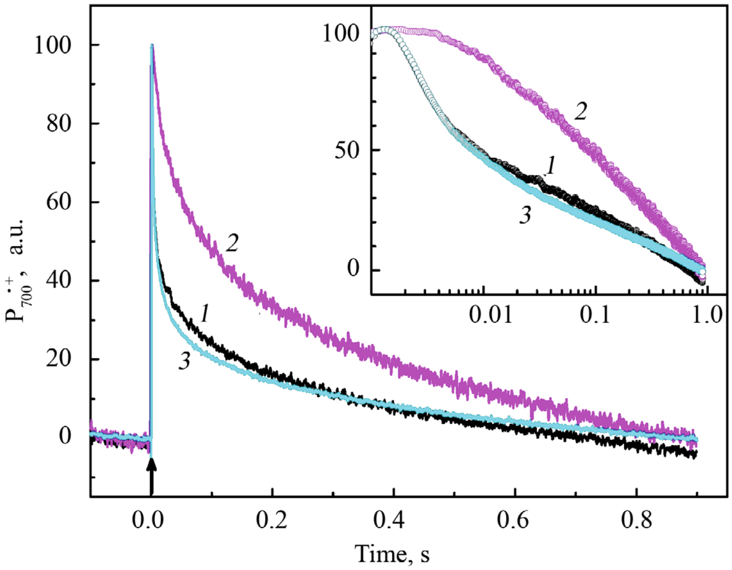

Fig. 3. Light-dependent changes in the magnitude of the $P_{700}^{\bullet+}$ EPR signal in PSI from the wild type (*1*), M688NPsaA (*2*) and M668NPsaB (*3*) mutants after illumination with a nanosecond laser pulse in linear and semi-logarithmic (insert) coordinate systems (based on the data from Savitsky et al. [27]).

This result showed that at least under the experimental conditions, electron transfer occurs asymmetrically in favor of branch *A*.

Registration of the decay kinetics of the $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ signals. Another important result that was obtained by measuring the kinetics of the EPR signal in the W-band of microwave frequencies is the demonstration of the possibility of recording the decay of the signals of the cation radical $P_{700}^{\bullet+}$ and the anion radical $A_1^{\bullet-}$ in one experiment. Following the illumination of PSI photosynthetic complex by a laser flash, P_{700} transitions to the excited state P_{700}* and subsequent electron transfer from P_{700}* to A_1 occurs. As a result, an ion-radical pair $P_{700}^{\bullet+}A_1^{\bullet-}$ is formed, the lifetime of which is limited both by the recombination of charges within the pair and by the further forward electron transfer to the distal PSI acceptors, iron-sulfur clusters F_X, F_A, and F_B. Since EPR signals of the paramagnetic centers $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ are characterized by different g-factor values [27, 31], this makes it possible to observe them in different (non-overlapping) ranges of magnetic field strength using high-frequency EPR spectroscopy methods (Fig. 4) and, consequently, to simultaneously record the kinetics of EPR signal decay for both of these radicals after the excitation by light pulse. As demonstrated on Fig. 5, the kinetics of EPR signal decay for $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ differ significantly, since the disappearance of the $P_{700}^{\bullet+}$ signal is due only to the backward electron transfer (i.e., recombination, $A_1^{\bullet-} \rightarrow P_{700}^{\bullet+}$), while the $A_1^{\bullet-}$ signal can be associated with both the recombination of charges in the ion radical pair $P_{700}^{\bullet+}A_1^{\bullet-}$ and with the forward electron transfer from $A_1^{\bullet-}$ to the subsequent acceptors (4Fe4S clusters). Thus, the obtained data

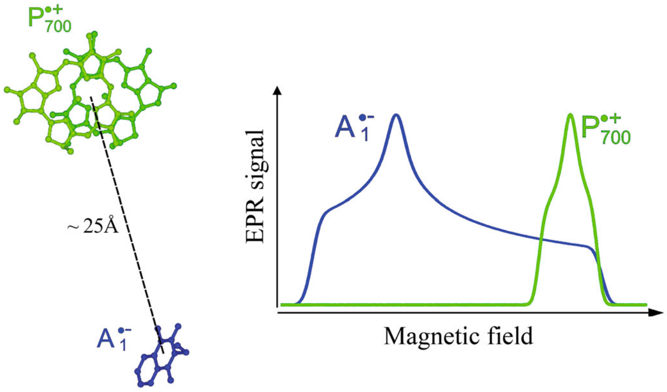

Fig. 4. Calculated EPR spectra of $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ radicals in the W-band (95 GHz). The relative positions of these cofactors in PSI structure are shown. (The spectra were calculated in the article by Savitsky et al. [27] based on the values of the g-tensor components obtained in the work by Zech et al. [31]).

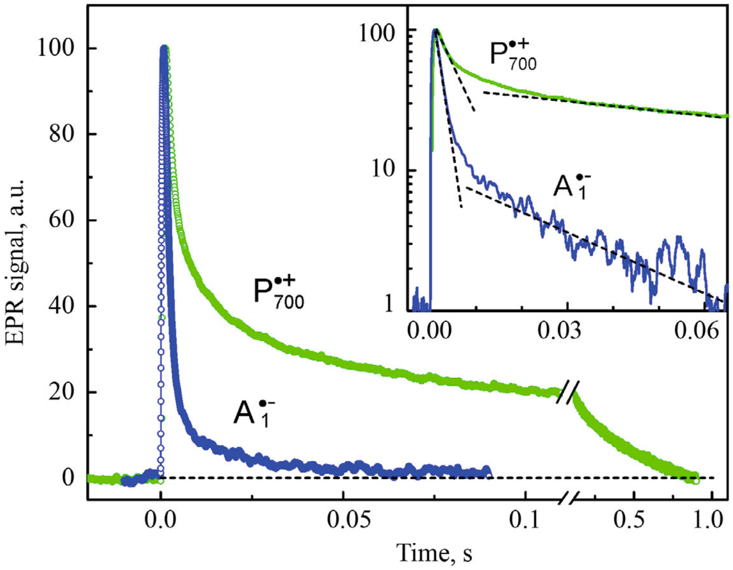

Fig. 5. Kinetics of signal decay in the W-band, obtained in the EPR absorption maxima of $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ radicals for PSI complexes from M668NPsaB strain cells (data obtained in the work by Savitsky et al. [27] were used).

demonstrated that, under the experimental conditions, at least in some PSI complexes, direct electron transfer occurs from the reduced semiquinone anion $A_1^{\bullet-}$ to the iron-sulfur clusters.

Measuring the distance between the spin density centers of $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$. To determine the distance between the components of the ion-radical pair, the pulse EPR method (ESEEM) was used, which allows to obtain signals sensitive to the interaction of magnetic moments of two unpaired electrons ($P_{700}^{\bullet+}$ and $A_1^{\bullet-}$). These interactions depend on the distance between the interacting unpaired electrons (or, more precisely, on the spin density distribution in the radicals). The accuracy of the method is such that it allows distinguishing distances that differ by 0.3 Å. Experiments have shown that in PSI from the M668NPsaB mutant, smaller distances are observed between the spin density centers in $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ radicals than in PSI from M688NPsaA. This difference reflects the different charge distribution in the radical pairs formed in PSI of the two mutant strains.

According to the structural data, the distance between the centers of P_{700} and A_{1B} quinone ring should be 25.2 Å, and the distance between P_{700} and A_{1A} should be 25.0 Å [32]. Since the spin density in $P_{700}^{\bullet+}$cation radical dimer is significantly shifted towards Chl_{1B} [33, 34], taking into consideration the preferential electron transfer along the *A* branch, the observed distance between the spin density centers of $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ should be 25.8 Å (Fig. 6). Experiments using pulse ESEEM showed that the distance between $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$, measured in PSI from the wild type and the mutant strain M668LPsaB, is 26.0 ± 0.3 Å, i.e. close to the distance corresponding to electron transfer along the *A* branch. A similar distance was measured for PSI complexes containing plastoquinone-9 and 2,3-dichloro-1,4-naphthoquinone in the A_1 binding site [35], as well as for PSI dried in a trehalose matrix at 150 and 220 K [36].

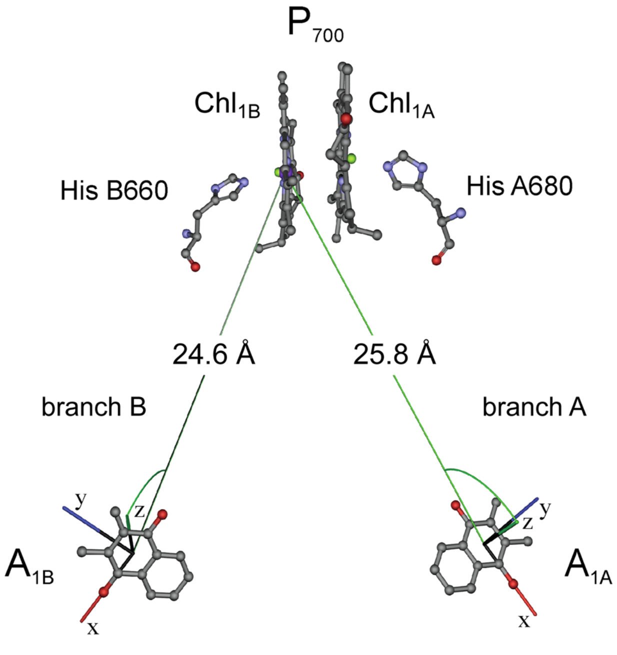

Fig. 6. The relative positions of the primary PSI electron donor (P_{700}, a dimer of Chl_{1A} and Chl_{1B} molecules) and phylloquinone molecules A_{1A} and A_{1B} based on the structure of PSI [32]. The distances between the Chl P_{700} dimer and A_{1A} (25.8 Å) in branch *A* and P_{700} and A_{1B} (24.6 Å) in branch *B* correspond to the distances between the spin density center in the P_{700} radical (see [33, 34]) and the geometric centers of the quinone rings of phylloquinones A_{1A} and A_{1B}.

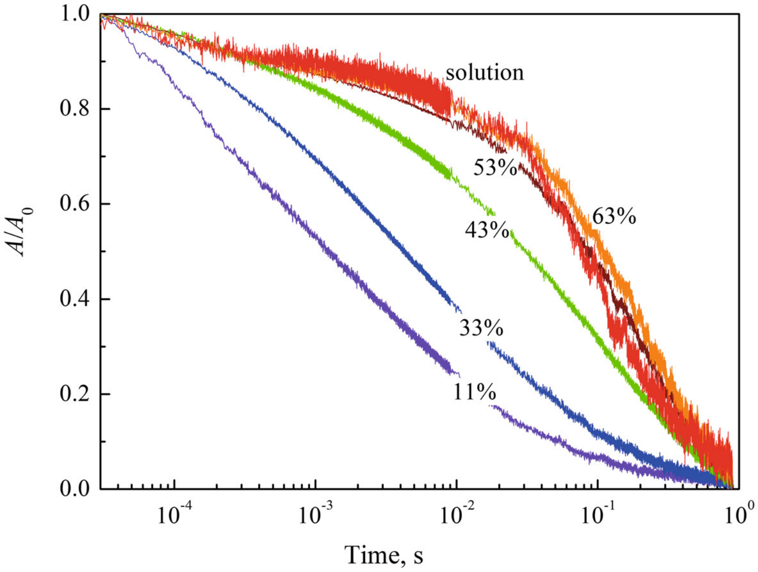

Fig. 7. Kinetics of reduction of the laser-flash photooxidized $P_{700}^{\bullet+}$ in PSI, recorded at a wavelength of 820 nm, at different degrees of relative humidity of the trehalose matrix (shown as a percentage near the kinetic curves) (data obtained in the work by Malferrari et al. [37]).

Thus, the data obtained by using pulse ESEEM demonstrate that the distances between the spin density centers $P_{700}^{\bullet+}$ and $A_1^{\bullet-}$ in various PSI complexes from the *Synechocystis* sp. PCC 6803 cyanobacterium correspond to preferential electron transfer along the *A* branch of PSI redox cofactors. It should be emphasized that such structural and functional data cannot be obtained by such methods as X-ray structural analysis or even by cryoelectron microscopy, which is the most popular approach today.

The effect of dehydration in trehalose matrix on electron transfer in PSI. The kinetics of electron transfer in pigment–protein complexes depends on many factors, including the distance between redox cofactors, the free energy of reactions and the reorganization energy, which in turn depends on the conformational mobility of proteins. It has been previously shown that successive dehydration of bacterial RCs in a trehalose matrix, as well as a decrease in temperature, leads to a slowdown in the forward reactions between the primary and secondary ubiquinone acceptors Q_A and Q_B and an increase in the contribution of backward electron transfer reactions to the observed kinetics of reduction of the laser flash-oxidized primary donor, the bacteriochlorophyll dimer (see reviews [4, 25] and references therein). In a joint study with the laboratories of K. Möbius, G. Venturoli, and W. Lubitz, we investigated the effect of successive decreases in the relative humidity of a glassy trehalose matrix at room temperature on the kinetics of reduction of the photooxidized Chl dimer P_{700} in PSI complexes from cyanobacteria. W-band EPR spectroscopy showed structural homogeneity of the protein-trehalose matrix and the absence of changes in the distances and mutual orientation of the redox cofactors upon drying [37]. At high humidity, the kinetics of $P_{700}^{\bullet+}$ reduction was mainly due to the backward electron transfer from the terminal iron-sulfur F_A/F_B clusters. With successive drying, the kinetics of $P_{700}^{\bullet+}$ reduction accelerated and became less monoexponential and more heterogeneous (Fig. 7).

The kinetics analysis using the Maximum Entropy Method (MEM), demonstrated in Fig. 8, showed that dehydration is accompanied by a decrease in the contributions of the slow components (τ ≈ 300 ms and ≈ 60 ms) due to charge recombination between $[F_A/F_B]^-$ and $P_{700}^{\bullet+}$, in parallel with an increase in the contribution of the fastest component (τ ≈ 150 μs) due to the backward electron transfer from phylloquinone A_1^- and the intermediate components in the 1-10 ms time range (see the diagram in Fig. 8a). The intermediate components can most likely be attributed to the backward electron transfer from the F_X^- cluster, captured in one of several conformational states.

In our joint work with the laboratories of John Golbeck and Klaus Möbius, it was shown that in PSI dried in a trehalose matrix at room temperature, a significant slowdown in direct electron transfer from $A_1^{\bullet-}$ to the F_X cluster occurs. Meanwhile, the preferential electron transfer along the *A* branch of redox cofactors slows down from 220 ns in solution to 13-14 μs in a trehalose matrix. A similar result was obtained both using EPR spectroscopy in the W-band and by recording the electrochromic carotenoid shift [38].

PSI dehydration in the trehalose matrix changes the kinetics of forward and backward electron transfer

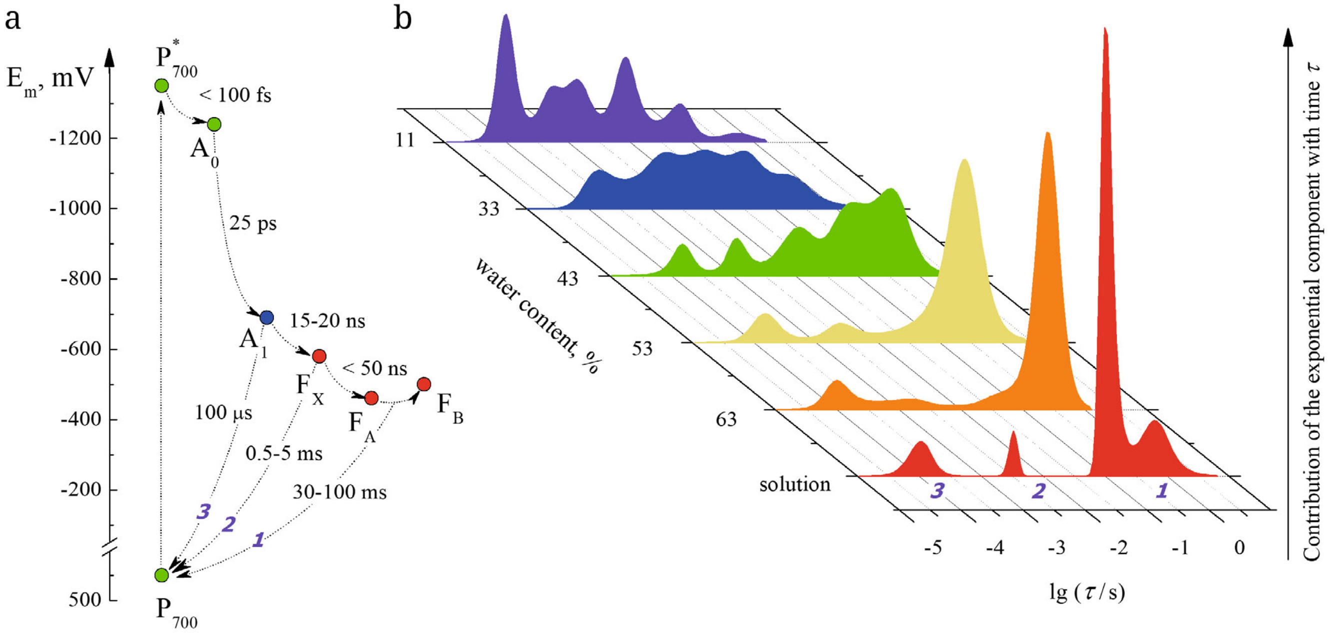

Fig. 8. Energy diagram of electron transfer in PSI (a) and recombination distribution functions of the $P_{700}^{\bullet+}$ reduction over various hydrations levels (11%, 33%, 43%, 53%, 63%, and in solution) for PSI in a trehalose glass (b). Italic numbers near the peaks on the lifetime (τ) distribution graph indicate the corresponding charge recombination pathways in PS I (data obtained in the work by Malferrari et al. [37] were used).

reactions, similar to what occurs when temperature decreases in a water–glycerol mixture below the phase transition temperature [4, 25, 39]. This is apparently due to the fact that in both cases there is a decrease in the conformational mobility of the PSI pigment–protein complex. It is assumed that the effect of drying in a trehalose matrix is due to changes in the hydrogen bond network of the protein complex. Meanwhile, a layer of water molecules surrounded by trehalose molecules is preserved around the protein globule. Under such conditions, the protein complex transits into a state of reversible anhydrobiosis and can be preserved for many months at room temperature.

CONCLUSION

As a result of joint works with Professor Klaus Möbius, important results were obtained that clarify the molecular mechanisms of PSI functioning.

1. Using high-frequency EPR spectroscopy in the W-band for studying PSI from wild type cyanobacteria and mutant strains with point substitutions of axial ligands to the primary chlorophyll electron acceptor A_0 in the symmetric *A* and *B* branches of redox cofactors, it was shown that at low temperatures, electron transfer occurs along the *A* branch.

2. It was demonstrated that the decay of the $A_1^{\bullet-}$ anion radical signal at liquid nitrogen temperature is caused not only by backward electron transfer to $P_{700}^{\bullet+}$, but also by forward transfer to the subsequent acceptors, 4Fe4S clusters. It was shown that the distance between the spin density centers of the $P_{700}^{\bullet+}$ cation radical and the $A_1^{\bullet-}$ anion radical in native PSI complexes containing a full set of redox cofactors and in PSI complexes lacking the terminal 4Fe4S F_A and F_B clusters corresponds to electron transfer along branch *A*.

3. It was found that successive drying of PSI in a glassy trehalose matrix at room temperature results in slowing down the forward reactions kinetics and the increase in contribution of backward electron transfer reactions. Meanwhile, the distance and mutual arrangement of the redox cofactors P_{700} and A_1 remain unchanged.

4. When dried in a trehalose matrix, PSI enters a state of reversible anhydrobiosis and can be preserved at room temperature for many months. Subsequent rehydration leads to a complete restoration of electron transfer in PSI.

Abbreviations. A_1, phylloquinone, the electron acceptor in photosystem I; Chl, chlorophyll; ESEEM, Electron Spin Echo Envelope Modulation; PSI (PSII), photosystem I (II); P_{700}, primary electron donor in photosystem I; RC, photosynthetic reaction center.

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Contributions. V. V. Ptushenko – writing sections of the manuscript, discussion, editing; A. Y. Semenov – concept of the work, writing sections of the manuscript, discussion, editing.

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Ethics approval and consent to participate. This work does not contain any studies involving human and animal subjects.

Conflict of interest. The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Möbius, K., Plato, M., and Savitsky, A. (2022) The Möbius Strip Topology: History, Science, and Applications in Nanotechnology, Materials, and the Arts, Jenny Stanford Publishing, https://doi.org/10.1201/9781003256298.
2. Honerjäger, R. (1951) Microwave spectroscopy [in German], *Naturwissenschaften*, **38**, 34-39, https://doi.org/10.1007/BF00716170.
3. Honerjäger, R. (1958) Paramagnetic electron resonance [in German], *Fortsch. Chem. Forsch.*, **3**, 722-737, https://doi.org/10.1007/BFb0051770.
4. Möbius, K., Savitsky, A., Malferrari, M., Francia, F., Mamedov, M. D., Semenov, A. Y., Lubitz, W., and Venturoli, G. (2020) Soft dynamic confinement of membrane proteins by dehydrated trehalose matrices: high-field EPR and fast-laser studies, *Appl. Magn. Reson.*, **51**, 773-850, https://doi.org/10.1007/s00723-020-01240-y.
5. Möbius, K. (1965) Investigation of simple π-electron systems using electron spin resonance and polarography: Part I. Experiment and theory [in German], *Zeitschr. Naturforsch. A*, **20**, 1093-1102.
6. Möbius, K. (2022) Autobiographical sketches (2021), *Appl. Magn. Reson.*, **53**, 467-489, https://doi.org/10.1007/s00723-021-01410-6.
7. Schneider, F., and Moebius, K. (1963) Report: the evaluation of electron resonance spectra of organic radicals [in German], *Zeitschr. Naturforsch. B*, **18**, 1111-1119.
8. Möbius, K., and Plato, M. (1967) EPR-investigation of dissolved free-radical ions of non-alternating hydrocarbons [in German], *Zeitschr. Naturforsch. A*, **22**, 929-939, https://doi.org/10.1515/zna-1967-0612.
9. Möbius, K., and Dinse, K.-P. (1972) ENDOR of organic radicals in solution, *Chimia*, **26**, 461, https://doi.org/10.2533/chimia.1972.461.
10. Feher, G. (1956) Observation of nuclear magnetic resonances via the electron spin resonance line, *Phys. Rev.*, **103**, 834, https://doi.org/10.1103/PhysRev.103.834.
11. Hyde, J. S., and Maki, A. H. (1964) ENDOR of a free radical in solution, *J. Chem. Phys.*, **40**, 3117-3118, https://doi.org/10.1063/1.1724957.
12. Hyde, J. S. (1965) ENDOR of free radicals in solution, *J. Chem. Phys.*, **43**, 1806-1818, https://doi.org/10.1063/1.1697013.
13. Möbius, K., Van Willigen, H., and Maki, A. H. (1971) ENDOR study of pentaphenylcyclopentadienyl radicals in solution. Lifting of orbital degeneracy by methyl substitution, *Mol. Phys.*, **20**, 289-304, https://doi.org/10.1080/00268977100100271.
14. Möbius, K., and Biehl, R. (1979) Electron-nuclear-nuclear TRIPLE resonance of radicals in solutions, in *Multiple Electron Resonance Spectroscopy* (Dorio, M. M., and Freed, J. H., eds) Springer, pp. 475-507, https://doi.org/10.1007/978-1-4684-3441-5_14.
15. Biehl, R., Moebius, K., O'Connor, S. E., Walter, R. I., and Zimmermann, H. (1979) Evaluation and assignment of proton and nitrogen hyperfine coupling constants in the free-radical 1-picryl-2, 2-diphenylhydrazyl. An NMR, electron-nuclear double resonance, and electron-nuclear-nuclear triple resonance study, *J. Phys. Chem.*, **83**, 3449-3456, https://doi.org/10.1021/j100489a027.
16. Haindl, E., and Möbius, K. (1985) A 94 GHz EPR spectrometer with Fabry-Perot resonator, *Zeitschr. Naturforsch. A*, **40**, 169-172, https://doi.org/10.1515/zna-1985-0211.
17. Box, H. C., Budzinski, E. E., and Potter, W. (1971) Effects of ionizing radiation on potassium hydrogen malonate, *J. Chem. Phys.*, **55**, 315-319, https://doi.org/10.1063/1.1675523.
18. Grinberg, O. Ya., Dubinskii, A. A., Shuvalov, V. F., Oranskii, L. G., Kurochkin, V. I., and Lebedev, Ya. S. (1976) Submillimeter EPR spectroscopy of free radicals, *Dokl. Akad. Nauk SSSR*, **230**, 884-887.
19. Galkin, A. A., Grinberg, O. Y., Dubinskii, A. A., Kabdin, N. N., Krymov, V. N., Kurochkin, V. I., Lebedev, Y. S., Oranskii, L. G., and Shuvalov, V. F. (1977) EPR spectrometer in 2-mm range for chemical-research, *Instr. Exp. Tech.*, **20**, 1229-1233.
20. Weber, R. T., Disselhorst, J., Prevo, L. J., Schmidt, J., and Wenckebach, W. T. H. (1989) Electron spin-echo spectroscopy at 95 GHz, *J. Magn. Reson.*, **81**, 129-144, https://doi.org/10.1016/0022-2364(89)90272-2.
21. Prisner, T. F., Un, S., and Griffin, R. G. (1992) Pulsed ESR at 140 GHz, *Isr. J. Chem.*, **32**, 357-363, https://doi.org/10.1002/ijch.199200042.
22. Lynch, W. B., Earle, K. A., and Freed, J. H. (1988) 1-mm wave ESR spectrometer, *Rev. Sci. Instrum.*, **59**, 1345-1351, https://doi.org/10.1063/1.1139720.
23. Hoff, A. J., and Möbius, K. (1978) Nitrogen electron nuclear double resonance and proton triple resonance experiments on the bacteriochlorophyll cation in solution, *Proc. Natl. Acad. Sci. USA*, **75**, 2296-2300, https://doi.org/10.1073/pnas.75.5.2296.
24. Lendzian, F., Lubitz, W., Scheer, H., Hoff, A. J., Plato, M., Tränkle, E., and Möbius, K. (1988) ESR, ENDOR and TRIPLE resonance studies of the primary donor radical cation P960$^{+\bullet}$ in the photosynthetic bacterium *Rhodopseudomonas viridis*, *Chem. Phys. Lett.*, **148**, 377-385, https://doi.org/10.1016/0009-2614(88)87191-4.

25. Gorka, M., Cherepanov, D. A., Semenov, A. Y., and Golbeck, J. H. (2020) Control of electron transfer by protein dynamics in photosynthetic reaction centers, *Crit. Rev. Biochem. Mol. Biol.*, **55**, 425-468, https://doi.org/10.1080/10409238.2020.1810623.
26. Prisner, T. F., Rohrer, M., and Möbius, K. (1994) Pulsed 95 GHz high-field EPR heterodyne spectrometer with high spectral and time resolution, *Appl. Magn. Reson.*, **7**, 167-183, https://doi.org/10.1007/BF03162610.
27. Savitsky, A., Gopta, O., Mamedov, M., Golbeck, J. H., Tikhonov, A., Möbius, K., and Semenov, A. (2010) Alteration of the axial met ligand to electron acceptor A_0 in photosystem I: effect on the generation of $P_{700}^{\bullet+}$ $A_1^{\bullet-}$ radical pairs as studied by W-band transient EPR, *Appl. Magn. Reson.*, **37**, 85-102, https://doi.org/10.1007/s00723-009-0052-0.
28. Dashdorj, N., Xu, W., Cohen, R. O., Golbeck, J. H., and Savikhin, S. (2005) Asymmetric electron transfer in cyanobacterial photosystem I: charge separation and secondary electron transfer dynamics of mutations near the primary electron acceptor A_0, *Biophys. J.*, **88**, 1238-1249, https://doi.org/10.1529/biophysj.104.050963.
29. Cohen, R. O., Shen, G., Golbeck, J. H., Xu, W., Chitnis, P. R., Valieva, A. I., van der Est, A., Pushkar, Y., and Stehlik, D. (2004) Evidence for asymmetric electron transfer in cyanobacterial photosystem I: analysis of a methionine-to-leucine mutation of the ligand to the primary electron acceptor A_0, *Biochemistry*, **43**, 4741-4754, https://doi.org/10.1021/bi035633f.
30. Salikhov, K. M., Pushkar, Y. N., Golbeck, J. H., and Stehlik, D. (2003) Interpretation of multifrequency transient EPR spectra of the P_{700}^{+} A_0 Q_K^{-} state in photosystem I complexes with a sequential correlated radical pair model: wild type versus A_0 mutants, *Appl. Magn. Reson.*, **24**, 467-482, https://doi.org/10.1007/BF03166949.
31. Zech, S. G., Hofbauer, W., Kamlowski, A., Fromme, P., Stehlik, D., Lubitz, W., and Bittl, R. (2000) A structural model for the charge separated state in photosystem I from the orientation of the magnetic interaction tensors, *J. Phys. Chem. B*, **104**, 9728-9739, https://doi.org/10.1021/jp002125w.
32. Jordan, P., Fromme, P., Witt, H. T., Klukas, O., Saenger, W., and Krauß, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution, *Nature*, **411**, 909-917, https://doi.org/10.1038/35082000.
33. Plato, M., Krauß, N., Fromme, P., and Lubitz, W. (2003) Molecular orbital study of the primary electron donor P_{700} of photosystem I based on a recent X-ray single crystal structure analysis, *Chem. Phys.*, **294**, 483-499, https://doi.org/10.1016/S0301-0104(03)00378-1.
34. Lubitz, W. (2006) EPR studies of the primary electron donor P700 in photosystem, in *Photosystem I. Advances in Photosynthesis and Respiration* (Golbeck, J. H., ed) Vol. 24, Springer, Dordrecht, https://doi.org/10.1007/978-1-4020-4256-0_17.
35. Mula, S., Savitsky, A., Möbius, K., Lubitz, W., Golbeck, J. H., Mamedov, M. D., Semenov, A. Y., and van der Est, A. (2012) Incorporation of a high potential quinone reveals that electron transfer in photosystem I becomes highly asymmetric at low temperature, *Photochem. Photobiol. Sci.*, **11**, 946-956, https://doi.org/10.1039/c2pp05340c.
36. Sukhanov, A. A., Mamedov, M. D., Möbius, K., Semenov, A. Y., and Salikhov, K. M. (2018) The decrease of the ESEEM frequency of P_{700}^{+} A_1^{-} ion-radical pair in photosystem i embedded in trehalose glassy matrix at room temperature can be explained by acceleration of spin-lattice relaxation, *Appl. Magn. Reson.*, **49**, 1011-1025, https://doi.org/10.1007/s00723-018-1017-y.
37. Malferrari, M., Savitsky, A., Mamedov, M. D., Milanovsky, G. E., Lubitz, W., Möbius, K., Semenov, A. Y., and Venturoli, G. (2016) Trehalose matrix effects on charge-recombination kinetics in photosystem I of oxygenic photosynthesis at different dehydration levels, *Biochim. Biophys. Acta*, **1857**, 1440-1454, https://doi.org/10.1016/j.bbabio.2016.05.001.
38. Shelaev, I., Gorka, M., Savitsky, A., Kurashov, V., Mamedov, M., Gostev, F., Möbius, K., Nadtochenko, V., Golbeck, J., and Semenov, A. (2017) Effect of dehydrated trehalose matrix on the kinetics of forward electron transfer reactions in photosystem I, *Zeitschr. Phys. Chem.*, **231**, 325-345, https://doi.org/10.1515/zpch-2016-0860.
39. Milanovsky, G., Gopta, O., Petrova, A., Mamedov, M., Gorka, M., Cherepanov, D., Golbeck, J. H., and Semenov, A. (2019) Multiple pathways of charge recombination revealed by the temperature dependence of electron transfer kinetics in cyanobacterial photosystem I, *Biochim Biophys Acta Bioenerg.*, **1860**, 601-610, https://doi.org/10.1016/j.bbabio.2019.06.008.