PHD THESIS

Bacteriochlorophyll fluorescence as indicator of physiological state of photosynthetic bacteria

Gábor Sipka

Supervisor: Péter Maróti, PhD, DSc

Doctoral School of Physics
University of Szeged

Faculty of Medicine and Faculty of Science and Informatics
Department of Medical Physics and Informatics

Szeged
2017

Introduction

Photosynthesis is a biological process whereby the energy of the Sun is captured and stored by series of events that convert the free energy of light into different forms of free energy needed to feed cellular processes. (Blankenship 2014). The photosynthesis provides the foundation for essentially all life and has altered the Earth itself over geologic time profoundly. It provides all of our foods and most of our energy resources. Since essentially all energy used on Earth can be traced back to the photosynthetic transformation of solar energy into chemical energy, it is not surprising that the study of photosynthesis is at the center of scientific interest (Govindjee et al. 2005; Eaton-Rye et al. 2012; Niederman 2017).

In photosynthetic bacteria, the energy conversion processes are considerably simpler than in green plants. While there are two photochemical reactions in green plants, there is only one in the bacteria. In contrast to the linear electron transport chain of green plants, the electron transport in bacteria is cyclic, in which the free energy of the charge pair produced in the reaction center (RC) is utilized by a cyclic pathway of electron building up a proton gradient across the photosynthetic membrane. The reaction center and the cytochrome bc_1 complex (via the Q-cycle) constitute a proton-pump mechanism that translocates protons from the cytoplasmic side to the periplasmic side of the membrane.

In the modern photosynthesis research, the non-sulfur type of purple bacteria plays a significant role, because the three-dimensional determination of the reaction center at atomic level (Deisenhofer et al. 1984) has made it possible to identify the structure and function of a photosynthetic energy conversion system. Although the details of the transformation of energy may vary in different species, there are structural and functional similarities. The bacterial reaction center has a very high photochemical quantum yield (~ 100%) since nearly all of the absorbed photons create charge pairs (Wraight and Clayton 1974). The highest free-energy loss relates to the reduction of the primary quinone (Q_A), which also means that physiological conditions make this process irreversible.

The photosynthetic bacteria protect and operate their energy conversion system with remarkable efficiency and rate. An important part of this process is the light-dependent production and protection of tripled states of bacteriochlorophylls (BChl) essential for the survival of photosynthetic organisms. The energy of the BChl tripled state can be transmitted easily to triplet molecular oxygen ($^3O_2$) that generates harmful singlet excited oxygen ($^1O_2^*$, strong oxidant). To avoid this reaction, several pathways are operating in all of which carotenoid (Car) pigments play prominent role.

In addition to high light intensity, photosynthetic bacteria are exposed to numerous stress effects including heavy metal ions. The organisms can maintain their functions even under harmful conditions. How do they do it and what can be learned from these experiences? What makes the intact photosynthetic bacterium and its reaction center robust and yet flexible enough to function efficiently under different stress conditions? These are the fundamental questions I set in the frontline of the dissertation.

Aims

The photosynthetic purple bacteria are an excellent model for studying light-induced structural changes, electron- and proton transfer, electrostatic processes and protection against damaging effects such as high light intensity and heavy metal contamination. Therefore, my studies were performed with intact photosynthetic purple bacteria and isolated reaction center protein.

1. By modifying some of the key amino acids in bacterial RC, the role of the protein matrix in electron transfer can be explored. Therefore, I expected answers to the following questions:
   1.1. What energetic changes are caused on the RC donor side by point mutations of the dimer close to the P_A and P_B 2 -acetyl and the 9-keto groups that result in modification of hydrogen bonds?
   1.2. What changes of the thermodynamic properties of Q_A can be expected from amino acid mutations on the acceptor side near the iron-ligand?
   1.3. How does the triple mutant (LH^{L131} - LH^{M160} - FH^{M197}) modify the quantum yield and anisotropy of the BChl prompt fluorescence? The answer would serve better understanding of the transfer of the electron excitation energy between the BChl dimer and BChl monomer in the RC protein.

To answer the above questions, (quasi) steady-state (fluorescence anisotropy) and kinetic (fluorescence induction, delayed fluorescence) spectroscopic measurements were carried with selective excitation of BChl. These methods have proven to be suitable and sensitive to characterize the kinetic and thermodynamic properties of bacterial photosynthesis.

2. Heavy metal ions such as mercury(II) (Hg^{2+}) ion are predominantly harmful to the wildlife. As the photosynthetic bacteria and its reaction center protein are highly sensitive to heavy metal ions and are simple organisms/protein with well studied structure and functions, they are ideal objects for these investigations. What structural and functional changes are caused by the Hg^{2+} ions and what are the specific binding sites (if they are) in the RC?
   2.1. Are there any energetic changes if the Hg^{2+} ion binds to the RC donor side? Does the mercury(II) ion modify the docking of the reduced cyt c_2 to the RC and/or the electron transfer from cyt c_2^{2+} to P^+?
   2.2. Can we detect any effects of binding Hg^{2+} ion to the RC acceptor side on the energetics of the quinone-acceptor system, the interquinone electron transfer or proton uptake?
3. In addition to heavy metal ion contamination, strong light intensity belongs also to common environmental (stress) effects. The details of the protection mechanisms are put in the center of our interest.

3.1. There is a need to develop a sensitive method for the measurement of the excited triplet states of Cars and/or BChls. The assay should be based on the measurement of fast kinetics of BChl fluorescence.

3.2. The goal is to examine the widest range of photosynthetic bacteria and to establish relationship between conjugation length and triplet lifetime of different carotenoids. The following bacteria were included in our experiments: *Rvx. gelatinosus* (anaerobic and semianaerobic), *Rsp. rubrum*, *Thio. roseopersicina*, *Rba. sphaeroides* 2.4.1 and carotenoid and cytochrome modified mutants: *Rba. sphaeroides* Ga, R-26 and *cycA*, respectively.

3.3. Since the photoprotection function of ^{3}Car in intact cells is not limited to RC, it is straightforward to work out a model of ^{3}Car photoprotection function in which the carotenoids are not localized to the RC but distributed in the BChl antenna system.

3.4. What is the sequence of light-induced charge separation (photochemical quenching) and ^{3}Car production (triplet quenching)? Does the carotenoid triplet formation follow the charge separation or they are simultaneous and competitive processes?

3.5. To develop our home-built instrument to determine the lifetime of ^{3}Car with different number of conjugated double bonds in several bacterial strains.

3.6. It has been observed that the ^{3}Car lifetime measured in the light is considerably shorter than in the dark. What could be the reason of that?

Materials and Methods

Bacterial strains, conditions and carotenoid biosynthesis

Cells of purple non-sulfur photosynthetic bacterium *Rhodobacter* (*Rba.*) *sphaeroides* strain 2.4.1 (Maróti and Wraight 1988), *Rhodospirillum* (*Rsp.*) rubrum, *Rubrivivax* (*Rvx.*) *gelatinosus* (Vermeglio et al. 2012) and carotenoidless *Rba*. *sphaeroides* R-26 were cultivated anaerobically in Siström minimal medium in 1 liter screw top flasks under continuous illumination of about 13 W m^{-2} provided by tungsten lamps (40 W) after incubation in the dark for 5-7 h. The cytochrome c_2 deficient mutant of *Rba*. *sphaeroides* cycA I (kindly provided by Prof. Dr. T. Donohue, University of Wisconsin, USA) was cultivated in the dark on a shaker (1 Hz) in the presence of antibiotic kanamycin and spectinomycin in concentrations of 50 μg/mL. The phototrophic purple sulfur bacterium *Thiocapsa* (*Thio*.) *roseopersicina* (obtained from Dr. Cs. Bagyinka, Biological Research Center Hungarian Academy of Sciences, Institute of Biophysics, Hungary) was grown anaerobically in a modified Pfennig's medium (Bagyinka et al. 1981).

Fluorescence measurements and conditions

The kinetics and yield of the BChl fluorescence generated by a rectangular excitation profile from a high power laser diode (2 W, Roithner LaserTechnik) were recorded by a homebuilt spectrofluorometer (Maróti 2008). The emission wavelength and bandwidth of the laser diodes

were 804 ± 0.5 nm which assured close to perfect excitation, because of its coincidence with the 800 nm absorption band of the LH2 antenna complex of *Rba. sphaeroides*. The measurements were carried out with bacterial cell cultures in a 3x3 mm prismatic quartz cuvette in a temperature-controlled sample holder. The maximum intensity of excitation was 2.1 einstein/m^2/s that was attenuated by calibrated neutral density filters. Due to the reciprocity of the intensity of light excitation and the photochemical rise time, the photochemical rate was used as an internal calibration to measure the intensity of light excitation. The excitation induced by a flash of the laser diode had the form of a step function with a rise time less than 100 ns. For determination of the yield and kinetics of the BChl fluorescence, a fluorescent dye IR-806 (Sigma) was used as reference. The BChl fluorescence was detected by a large area (diameter 10 mm) and high gain Si-avalanche photodiode as detector (APD; model 394-70-72-581; Advanced Photonix, Inc., USA) that was protected from the scattered laser light by an IR cutoff filter (Schott RG-850). The timing of the experiments was controlled by a Digital Delay-Pulse Generator (Berkeley Nucleonics Corporation (BNC) 555) via custom-designed LabVIEW software.

Light-induced absorption change

The kinetics of absorption changes of the whole cells induced by laser diode (Roithner LaserTechnik LD808-2-TO3), using a wavelength of 804 nm and maximum power 2 W, and a rectangular excitation profile of variable duration was detected by a home-constructed spectrophotometer. For the measuring light, a 130-W tungsten lamp was used. A monochromator (Jobin-Yvon H-20 with a concave holographic grating) was used to disperse the measuring light and to protect the detector from the scattered laser light. The monochromatic transmitted measuring light was detected by photomultiplier (R928 Hamamatsu) which was connected to a differential amplifier and to a digital oscilloscope (Tektronix TDS 3032). Samples were placed in a quartz cuvette (3 × 3 mm cross section). The light-induced energization of the membrane was monitored by electrochromic band shift of the carotenoids at wavelengths between 510 nm and 600 nm. The kinetic traces of absorption changes of RC induced by saturating Xe flash or by continuous illumination were detected by a home-constructed spectrophotometer (Maróti and Wraight 1988). The charge recombination was followed at wavelengths 430 nm or 860 nm, the first electron transfer at 402 nm and the second electron transfer at 450 nm.

Steady-state absorption spectroscopy

The steady-state near infrared absorption spectra of the cells during the growth were recorded at room temperature by a single beam spectrophotometer (Thermo Spectronic Helios). The baselines were corrected for light scattering, and the spectra were decomposed into Gaussian components by least square Marquardt procedure.

Delayed fluorescence

The decay of the yield of delayed fluorescence of the BChl dimer after flash excitation was measured by a home-made kinetic fluorometer described earlier (Turzó et al. 2000; Filus et al. 2004; Maróti and Wraight 2008; Asztalos and Maróti 2009). The free energy drop from P^* to $P^+Q_A^-$, $\Delta G_{P^*Q_A}$, was calculated by comparison of the delayed and prompt fluorescence yields, according to Arata and Parson (Arata and Parson 1981):

$$\Delta G_{P^*Q_A} = k_B T \cdot \ln\left(\frac{\int F_{DL}(t)dt}{\int F_{PF}(t)dt} \cdot \frac{k_{DL}}{k_{fl}} \cdot \frac{\eta_{fl}}{\eta_{ph}}\right) \quad (1)$$

$\int F_{DL}(t)dt$ and $\int F_{PF}(t)dt$ are the integrated intensities of delayed and prompt fluorescence, measured in the same sample but at very different excitation intensities (both in the linear region) to give similar emission intensities. Highly purified RC with negligible fluorescence from impurities is needed. The Boltzmann factor is k_{BT} (25 meV at room temperature), kfl is the radiative rate constant of prompt fluorescence ($8 \cdot 10^7$ s^{-1}, (Arata and Parson 1981; McPherson et al. 1990)), k_{DL} is the rate of decay of the delayed fluorescence, ηph is the quantum yield of photochemical trapping (≈1.0, (Wraight and Clayton 1974)), and η_{fl} is the quantum yield of the prompt fluorescence ($4 \cdot 10^{-4}$, (Woodbury et al. 1985)).

Circular dichroism

The circular dichroism (CD) spectra in the spectral range of 550–900 nm were recorded with a JASCO 815 spectropolarimeter. RC samples were solubilized in TL buffer (10 mM Tris and 0.03% LDAO at pH 8). The measurements were performed in a standard UV quartz cell of 1-cm optical path length at room temperature.

Electrochemical redox titration.

The redox midpoint potential of P/P^+ of the RC was measured by a homebuilt electrochemical cell with three-electrode-arrangement (Mäntele 1993) equipped in a steady-state spectrophotometer. The working electrode was a fine gold mesh with 55% optical transparency, the counter electrode was a Pt wire ring and Ag/AgCl served as the reference electrode. The electric potentials of the working and reference electrodes were controlled by a PGSTAT10 potentiostat/galvanostat at ambient temperature. The typical medium contained the following: 1 mM 4,4' Bipiridyl, 10 mM Tris, 0.03% LDAO and 150 μM potassium ferricyanide. The relative amount of oxidized P at each potential was determined from the magnitude of the absorption maximum at 865 nm and 835 nm compared to the absorption maximum at 800 nm. The optical path and the volume of the cell were 50 μm and 200 μL, respectively..

Thesis points

1. **Multiple modifications of the hydrogen bonding pattern to P and near Q_A resulted in systematic changes of the free energy gap between the metastable charge separated state $P^+Q_A^-$ and the excited bacteriochlorophyll dimer P^* [1].**

The free energy level of charge separation is significantly altered by the mutation of amino acids at key sites both on the donor and acceptor sides of the RC. For demonstration, amino acid mutations were specifically targeted at different sites of the RC protein and the kinetic properties of the delayed fluorescence of the dimer of different mutants were measured.

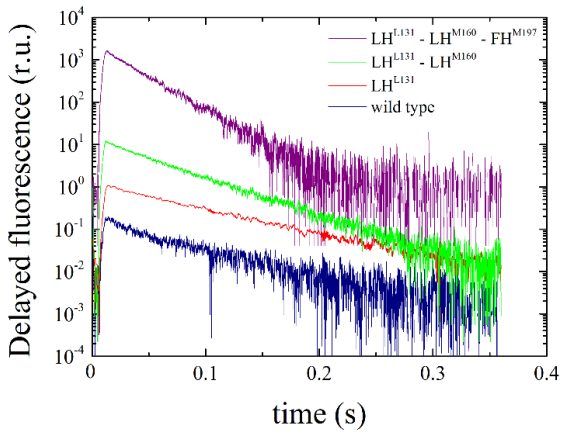

Figure 1. Kinetic traces of the DL of donor side mutants with increasing number of H-bonds in the vicinity of the dimer. The DL increases in single (LH^{L131}), double (LH^{L131}–LH^{M160}) and triple (LH^{L131}–LH^{M160}–FH^{M197}) mutants relative to that of wild type (WT) RC. The introduction of histidine residue at the donor side reflects addition of one H-bond to the network around the dimer. Conditions: 2 µM RC, 100 mM NaCl, 0,03% LDAO, 120 µM terbutryn, pH 8 and wavelength of fluorescence 915 ± 10 nm.

Both the amplitude (area) and the rate constant of the delayed fluorescence were increased for all selected mutants (LH^{L131}; HL^{M202}; LH^{L131} - LH^{M160}; LH^{L131} - HL^{M202}; LH^{L131} - LH^{M160} - FH^{M197}; IT^{M265}; EH^{M234}; EL^{M234}; EA^{M234}; ER^{M234}) compared to the wild type RC. The increase of the number of hydrogen bonds increases the intensity of the DL and the increase is stepwise: the triple mutant shows about twice as large increase as the double mutant relative to that of the wild type.

The quinone-side mutations modify the flash-induced uptake of H^+ ions by an acidic cluster in anticooperative interaction near the Q_B. The similarity of the pH-dependence of the free energy gap between P^* and $P^+Q_A^-$ of the IT^{M265} mutant and wild type is consistent with and suggestive that the extended hydrogen bond network between Q_A and the acidic cluster is not modified. The EX^{M234} (where X = H, L, A and R) mutants, however, have major impact on the iron ligand and the H-bond network and do not show any stabilization (rather slight destabilization) upon lowering the pH.

2. **A significant drop of the yield of BChl fluorescence related to the monomeric BChl was experienced in the triple mutant (LH^{L131} - LH^{M160} - FH^{M197}) of the RC from *Rhodobacter sphaeroides*, but the major (prompt) fluorescence properties of the dimeric BChl – the spectrum, the orientation and strength of the transition dipoles, the magnitude of the Stokes-shift – were hardly modified by the mutation [2].**

As the mutations affected the energetics of the dimer, and the primary charge pair $P^+Q_A^-$ was destabilized, the interaction between the BChl monomer (B) and the BChl dimer (P) should also change.

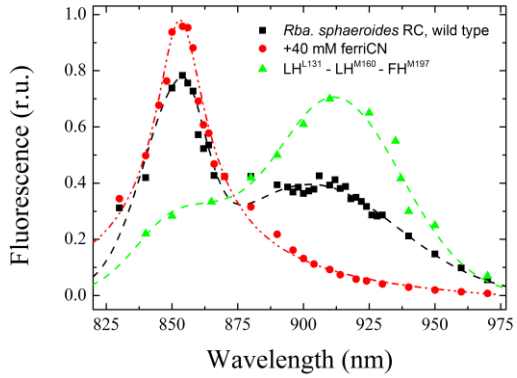

Figure 2. Spectral changes of fluorescence induction evoked by 808 nm laser diode excitation in wild-type and triple mutated (L131LH–M160LH–M197FH) reaction centers of Rba. sphaeroides. Fluorescence spectra of WT (black dots) and mutant (green dots) RCs in reduced and (chemically) oxidized states of the dimer (red dots).

Using diode laser emitting at 808 nm, both B and the upper exciton state (P^+) of the dimer in wild-type RC can be directly excited resulting in two well-defined fluorescence bands at 850 and 910 nm corresponding to radiative transitions from $^1B^*$ and from $^1P_-$ (the lower exciton state of the dimer) to the ground state, respectively. The kinetics of fluorescence induction observed at these wavelengths show remarkable changes. The opposite shapes of fluorescence kinetics with identical fall and rise times at 910 and 850 nm, respectively, serve as clear cut evidence for the electronic excitation energy transfer from $1B^*$ to the dimer.

The anisotropy of the fluorescence excited at 865 nm (P_-) was very close to the limiting value (0.4) across the whole spectral range. The excitation of both B and P_- at 808 nm resulted in wavelength-dependent depolarization of the fluorescence from 0.35 to 0.24 in the wild type and from 0.30 to 0.24 in the reaction center of triple mutant. The additivity law of the anisotropies of the fluorescence species accounts for the wavelength dependence of the anisotropy. The measured fluorescence yields and anisotropies are interpreted in terms of very fast energy transfer from $^1B^*$ to $^1P_-$ (either directly or indirectly by internal conversion from $^1P_+$) and to the oxidized dimer.

3. The Hg^{2+} ion bound to the RC from Rhodobacter sphaeroides changes the pattern of hydrogen bond around the dimer but has no effect on cytochrome docking and inhibits the electron transfer and proton uptake on the RC acceptor side [3].

The steady-state absorption spectrum of the dimer band (at 865 nm) shows a clear blue shift by ~ 30 nm upon mercury(II) treatment up to $[Hg^{2+}]/[RC]$ = 100 without loss of the amplitude. While the positions and the amplitudes of the absorption bands of the monomeric BChl (at 800 nm) and the BPheo (at 760 nm) remain unchanged, the shift of the dimer band is reversible and a clear isosbestic point is observable. This indicates that there is an interconversion of two components that absorb at 865 nm and 835 nm.

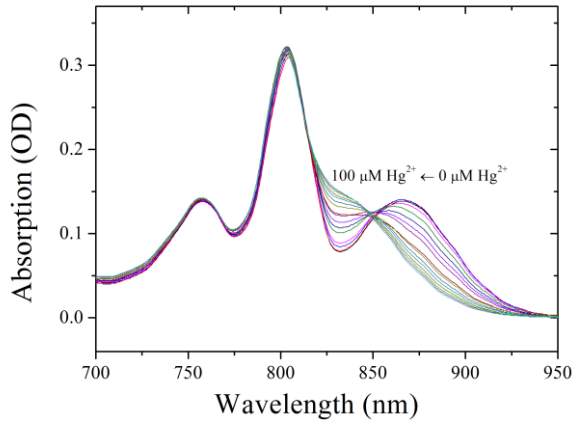

Figure 3. Blue-shift of the steady-state absorption band of the RC dimer due to increase of Hg^{2+} concentration. Conditions: 1 µM RC, TL buffer and increase of $[HgCl_2]$ up to 100 $[Hg^{2+}]/[RC]$

The electrochemical redox titration of P/P^+ follows single (Nernst) component of midpoint redox potential E_m ~500 mV in the untreated RC and E_m ~545 mV in the presence of 100 $[Hg^{2+}]/[RC]$. The electrostatic influence of bound Hg^{2+} ion in the vicinity of P causes an increase of E_m by 47±12 mV. Taking into account the relevant energetic changes, the +47 meV increase in the P/P^+ state obtained from electrochemical measurements accounts entirely for the +49 meV increase of the P/P* gap causing the observed 30 nm blue shift of the dimer absorption band of the mercury(II) treated RC. This donor side (electrostatic) effect does not modify the docking of the reduced cyt *c* to the RC nor the electron transfer from cyt c^{2+} to P^+.

The proton gate at the cytoplasmic side had the highest affinity for Hg^{2+} binding (K~0.2 $(\mu M)^{-1}$) and blocked the proton uptake. Reduced affinity (K~0.05 $(\mu M)^{-1}$) was measured for the Hg^{2+} binding site close to the secondary quinone that resulted in inhibition of the interquinone electron transfer.

By measuring the free energy level of P/P^* upon substitution of the native UQ by AQ (anthraquinone) at the Q_A site, we found that the free energy level of Q_A/Q_A^- was also insensitive to Hg^{2+}. The independent energetic measurements clearly demonstrate that the mercury(II) ions exercise much larger energetic influence to the dimer side than to the primary acceptor side of the RC.

4. **Triplet carotenoid quenching of the BChl fluorescence yield is observed during and not exclusively after the photochemical rise indicating that the charge separation in the RC and the carotenoid triplet formation are not consecutive but parallel and competitive processes [4,5].**

The formation and lifetime of light-induced triplet carotenoid (^{3}Car) were observed by measuring the yield and kinetics of bacteriochlorophyll fluorescence. The ^{3}Car quenching is observed during and not exclusively after the photochemical rise indicating that the charge separation in the reaction center and the carotenoid triplet formation are competing processes.

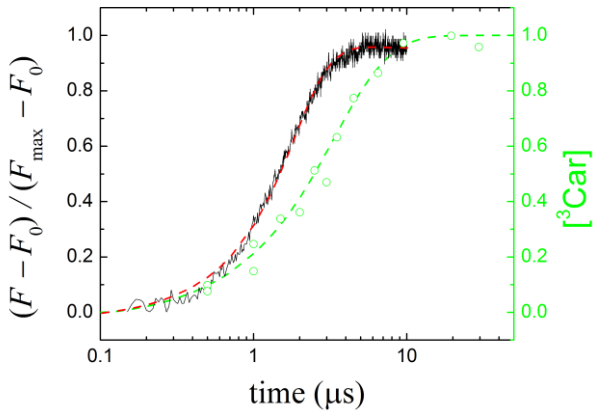

Figure 5. Simultaneous measurement of the rise of the normalized variable BChl fluorescence (induction) and carotenoid triplet (^{3}Car) formation (triplet quenching) in whole cells of *Rba. sphaeroides*. Both kinetics saturate but the ^{3}Car suffers some delay relative to the fluorescence induction. The two kinetics are normalized to the same value.

Based on the measurement of the BChl fluorescence, I developed 1) a sensitive method to detect the excited triplet states of carotenoid and bacteriochlorophyll and 2) a model to describe the generation and relaxation of light-induced carotenoid triplets in the antenna system of the pigments around the RC. The model is based on the observation that the photo-protective function of ^{3}Car is not limited to the RC only but is distributed to the whole pigment bed.

5. The observed rate of relaxation of ^{3}Car depends on the length of the conjugated double bonds and is the weighted average of those of the carotenoids with various numbers of conjugated double bonds in the bacterial strain [5].

The generation and relaxation of flash-induced carotenoid triplets (^{3}Car) were studied by observation of the quenching of bacteriochlorophyll (BChl) fluorescence in different strains of photosynthetic bacteria including *Rvx. gelatinosus* (anaerobic and semianaerobic), *Rsp. rubrum*, *Thio. roseopersicina*, *Rba. sphaeroides* 2.4.1 and carotenoid and cytochrome deficient mutants *Rba. sphaeroides* Ga, R-26 and *cycA*, respectively.

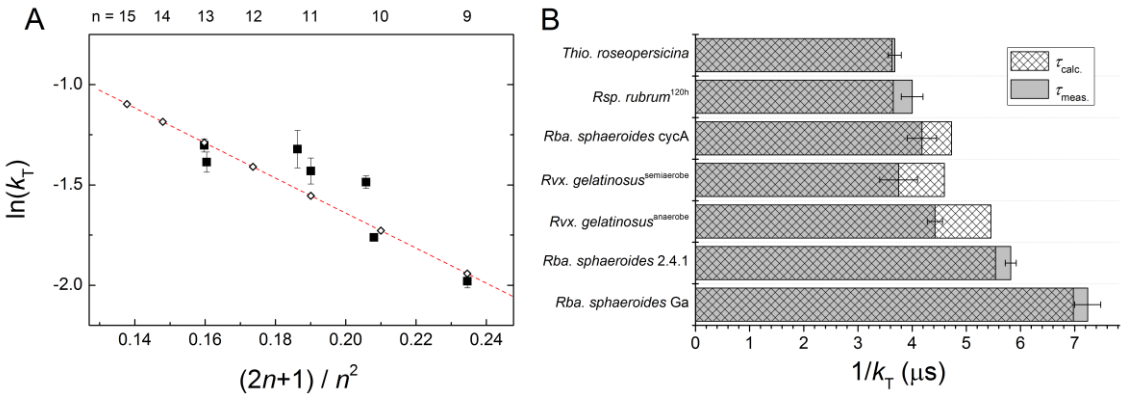

Figure 6. Triplet relaxation rate constants (k_T) of carotenoids of different conjugation lengths (n) in intact cells of photosynthetic bacteria. Panel A: triplet rate constants for carotenoids determined from set of linear equations constructed from carotenoid components and from measured triplet lifetimes of the different strains (open squares). Triplet rate constants of the bacteria calculated from the weighted average of the corresponding carotenoids hosted by the different strains (black squares). Panel B: Comparison of measured and calculated carotenoid triplet relaxation rate constants in different strains.

The rate of triplet relaxation was dependent on the length of the conjugated double bonds of the carotenoid: the longer was the chain, the faster was the decay. In appropriate representation, a linear dependence can be obtained.

The observed rate of relaxation of ^{3}Car in intact cells containing carotenoids with different numbers of conjugated double bonds is the weighted average of the rates of the constituting carotenoids in the bacterial strain.

Publication used in the thesis:

[1] Onidas D., **Sipka G.**, Asztalos E., Maróti P. (2013) Mutational control of bioenergetics of bacterial reaction center probed by delayed fluorescence, Biochim Biophys Acta - Bioenergetics, 1827 1191-1199. IF=4,932; Biophysics D1

[2] **Sipka G.**, Maróti P. (2016) Induction and anisotropy of fluorescence of reaction center from photosynthetic bacterium *Rhodobacter sphaeroides*, Photosynth. Res., 127 61-68. IF=3,864, Plant Science Q1

[3] **Sipka G.**, Kis M., Maróti P. (2017) Emergence of mercury(II)-induced inhibition of photochemistry in the reaction center of photosynthetic bacteria, Photosynth. Res., IF(2016)= 3,864, Plant Science Q1, *submitted*

[4] **Sipka G.**, Kis M., Smart J., Maróti P. (2017) Fluorescence induction of photosynthetic bacteria, Photosynthetica. IF(2016)=1,507, Plant Science Q2, *in press*

[5] **Sipka G.,** Maróti P. (2017) Photoprotection in intact cells of photosynthetic bacteria: quenching of bacteriochlorophyll fluorescence by carotenoid triplets, Photosynth. Res., IF(2016)= 3,864, Plant Science Q1, *in press*

Other publications

1. Kis M., **Sipka G.**, Maróti P. (2017) Stoichiometry and kinetics of mercury uptake by photosynthetic bacteria, Photosynthesis Research 132:(2) pp. 197-209. Plant Science Q1
2. Kis M., **Sipka G.**, Ayaydin F., Maróti P., (2017) The biophysics of a critical phenomenon: colonization and sedimentation of the photosynthetic bacteria *Rubrivivax* gelatinosus. European Biophysics Journal. doi:10.1007/s00249-017-1236-4
3. Asztalos E., **Sipka G.**, Maróti P. (2015) Fluorescence relaxation in intact cells of photosynthetic bacteria: donor and acceptor side limitations of reopening of the reaction center, Photosynthesis Research, 124 31-44. IF=4,122; Plant Science Q1
4. Kis M., **Sipka G.**, Asztalos E., Rázga Zs., Maróti P. (2015) Purple non-sulfur photosynthetic bacteria monitor environmental stresses, Journal of Photochemistry and Photobiology. B, Biology, 151 110-117. IF=3,035; Biophysics Q2
5. Kis M., Asztalos E., **Sipka G.**, Maróti P. (2014) Assembly of photosynthetic apparatus in Rhodobacter sphaeroides as revealed by functional assessments at different growth phases and in synchronized and greening cells, Photosynthesis Research, 122 261-273. IF=3,502; Plant Science Q1
6. Maróti P., Asztalos E., **Sipka G.** (2013) Fluorescence Assays for Photosynthetic Capacity of Bacteria, Biophysical Journal, 104 545a-545a. IF=3,632; Biophysics Q1
7. Asztalos E., **Sipka G.**, Kis M., Trotta M., Maróti P. (2012) The reaction center is the sensitive target of the mercury(II) ion in intact cells of photosynthetic bacteria, Photosynthesis Research, 112 129-140. IF=4,122; Plant Science Q1

Training schools

PicoQuant's Microscopy Course - 9th European Short Course on "Time-resolved Microscopy and Correlation Spectroscopy" 2017. 02. 28. – 03.02.

EBSA Biophysics Course on: “Membranes and Lipid-protein interactions” Montpellier, Franciaország, 2014. 09. 08-12.

LAMELIS Lasers in Medicine and Life Sciences Szeged, Magyarország, 2014. 07. 14-25.

COST Action on “Molecular machineries for ion translocation across biomembranes” Lisszabon, Portugália, 2013. 03. 15-19.

Lectures of conferences

P. Maróti, **G. Sipka**, P. Sebban, C.A. Wraight, Bioenergetics of bacterial reaction center protein, Szent-Györgyi Albert Konferencia, Molecular Biology & Genetics section, 2012. Szeged, Hungary.

Péter Maróti, Asztalos Emese, **Gábor Sipka**, Delphine Onidas, Light-induced proton uptake in reaction center of photosynthetic bacteria: Role of the bacteriopheophytine and donor side mutation. COST Action on “Molecular machineries for ion translocation across biomembranes” (CM0902). 2012. Bari, Italy.

Sipka Gábor, Maróti Péter. Bacterial reaction center as a model protein for the detection of heavy metal ion contamination. COST Action on “Molecular machineries for ion translocation across biomembranes” Lisszabon, Portugália, 2013. 03. 15-19.

Sipka Gábor, Maróti Péter. Bacterial reaction center as a model protein for the detection of heavy metal ion contamination. EBSA Biophysics Course on: “Membranes and Lipid-protein interactions” Montpellier, Franciaország, 2014. 09. 08-12.

Magyar Melinda, **Sipka Gábor**, Kovács László, Ughy Bettina, Qingjun Zhu, Guangye Han, Vladimír Špunda, Petar H. Lambrev, Jian-Ren Shen és Garab Győző. Fényindukált konformáció-változások igazolása a második fotokémiai rendszer reakciócentrumában klorofill fluoreszcencia tranziensek mérésével. MBFT XXVI. Kongresszusa. Konferencia helye, ideje: Szeged, Magyarország, 2017. 07. 22-25.

Sipka Gábor, Kis Mariann, Maróti Péter. A fotoszintetizáló baktériumok fluoreszcenciájának kinetikai összetevői. MBFT XXVI. Kongresszusa. Konferencia helye, ideje: Szeged, Magyarország, 2017. 07. 22-25.

Steinbach Gábor, **Sipka Gábor**, Garab Győző. Differenciálpolarizációs lézerpásztázó mikroszkóp (DP-LSM) biológiai alkalmazása anizotróp szerkezetek feltárásában. MBFT XXVI. Kongresszusa. Konferencia helye, ideje: Szeged, Magyarország, 2017. 07. 22-25.

Conference posters

Maróti P, **Sipka G**, Asztalos E, Kis M- A higanyszennyezés támadási pontjai és mechanizmusai fotoszintetizáló baktériumokban. (Multiple effects of mercury (II) ion on bacterial reaction center: Biophysical study) A Magyar Anatómus Társaság, a Magyar Biofizikai Társaság, a Magyar Élettani Társaság, és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság Kongresszusa. Debrecen, 2012. 06. 10-13

Gábor Sipka, Péter Maróti - Bacterial reaction center as a model protein for the detection of heavy metal ion contamination. COST Action CM0902 Training School - Molecular machinery in membranes, Lisbon, 2013. 03. 15-18.

Sipka Gábor, Maróti Péter - Interactions of protonatable groups and light-induced charges in photosynthetic reaction center. MBFT XXIV. Kongresszusa. Veszprém, 2013. 08. 27-30.

Kis Mariann, Asztalos Emese, **Sipka Gábor**, Rázga Zsolt, Maróti Péter: Környezeti stressz hatásai fotoszintetizáló baktériumokra, Magyar Biofizikai Társaság XXV. Kongresszusa, 2015 aug. 26-28. Budapest

Gábor Steinbach, **Gábor Sipka**, András Barta, István Pomozi, Győző Garab. 16th ELMI congress: Transform your Laser Scanning Microscope to Differential-Polarization Laser Scanning Microscope. Konferencia helye, ideje: Debrecen, Magyarország, 2016.05.24-27.

Magyar Melinda, **Sipka Gábor**, Kovács László, Ughy Bettina, Qingjun Zhu, Guangye Han, Vladimír Špunda, Petar H. Lambrev, Jian-Ren Shen és Garab Győző. Light-induced conformational changes in photosystem II reaction centers – as revealed by chlorophyll fluorescence transients. Sfphi2017. Meeting of the French Photosynthesis Society. Paris (France) 2017. 06. 1-2.

Kis Mariann, **Sipka Gábor**, Maróti Péter. Rubrivivax gelatinosus sejtek higanyfelvételének kinetikája és sztöchiometriája. MBFT XXVI. Kongresszusa. Konferencia helye, ideje: Szeged, Magyarország, 2017. 07. 22-25.

Sipka Gábor, Maróti Péter. Erős fény elleni védelem intakt fotoszintetizáló baktériumokban: hogyan oltják ki a bakterioklorofill fluoreszcenciát a karotenoid triplettek? MBFT XXVI. Kongresszusa. Konferencia helye, ideje: Szeged, Magyarország, 2017. 07. 22-25.

References

Arata H, Parson WW (1981) Delayed fluorescence from *Rhodopseudomonas sphaeroides* reaction centers - Enthalpy and free-energy changes accompanying electron-transfer from P870 to quinones. Biochim Biophys Acta 638 (2):201-209. http://dx.doi.org/10.1016/0005-2728(81)90228-0

Asztalos E, Maróti P (2009) Export or recombination of charges in reaction centers in intact cells of photosynthetic bacteria. Biochim Biophys Acta 1787 (12):1444-1450. http://dx.doi.org/10.1016/j.bbabio.2009.06.007

Bagyinka C, Kovacs KL, Rak E (1981) Localization of hydrogenase in the photosynthetic membrane of *Thiocapsa roseopersicina*. Acta Biochim Biophys 16 (3-4):235-235

Blankenship RE (2014) Molecular Mechanisms of Photosynthesis. 2nd edn. Wiley-Blackwell,

Deisenhofer J, Epp O, Miki K, Huber R, Michel H (1984) X-ray structure-analysis of a membrane-protein complex - electron-density map at 3Å resolution and a model of the chromophores of the photosynthetic reaction center from *Rhodopseudomonas viridis*. J Mol Biol 180 (2):385-398. http://dx.doi.org/10.1016/S0022-2836(84)80011-X

Eaton-Rye JJ, Tripathy BC, Sharkey TD (eds) (2012) Photosynthesis - Plastid biology, energy conversion and carbon assimilation. Advances in Photosynthesis and Respiration, vol 34. Springer, Netherlands. http://dx.doi.org/10.1007/978-94-007-1579-0

Filus Z, Laczkó G, Wraight CA, Maróti P (2004) Delayed fluorescence from the photosynthetic reaction center measured by electronic gating of the photomultiplier. Biopolymers 74 (1-2):92-95. http://dx.doi.org/10.1002/bip.20051

Govindjee, Beatty JT, Gest H, Allen JP (eds) (2005) Discoveries in Photosynthesis. Advances in Photosynthesis and Respiration, vol 20. Springer, Netherlands. http://dx.doi.org/10.1007/1-4020-3324-9

Mäntele W (1993) Reaction-induced infrared difference spectroscopy for the study of protein function and reaction-mechanisms. Trends Biochem Sci 18 (6):197-202. http://dx.doi.org/Doi 10.1016/0968-0004(93)90186-Q

Maróti P (2008) Kinetics and yields of bacteriochlorophyll fluorescence: redox and conformation changes in reaction center of *Rhodobacter sphaeroides*. Eur Biophys J 37 (7):1175-1184. http://dx.doi.org/10.1007/s00249-008-0300-5

Maróti P, Wraight CA (1988) Flash-induced H^+ binding by bacterial photosynthetic reaction centers - comparison of spectrophotometric and conductimetric methods. Biochim Biophys Acta 934 (3):314-328. http://dx.doi.org/10.1016/0005-2728(88)90091-6

Maróti P, Wraight CA (2008) The redox midpoint potential of the primary quinone of reaction centers in chromatophores of *Rhodobacter sphaeroides* is pH independent. European Biophysics Journal : EBJ 37 (7):1207-1217. http://dx.doi.org/10.1007/s00249-008-0301-4

McPherson PH, Nagarajan V, Parson WW, Okamura MY, Feher G (1990) pH dependence of the free energy gap between DQ_A and $D^+Q_A^-$ determined from delayed fluorescence in reaction centers from *Rhodobacter sphaeroides* R-26. Biochim Biophys Acta 1019 (1):91-94. http://dx.doi.org/10.1016/0005-2728(90)90128-Q

Niederman RA (2017) Photosynthesis in the Purple Bacteria. In: Hallenbeck PC (ed) Modern Topics in the Phototrophic Prokaryotes: Metabolism, Bioenergetics, and Omics. Springer International Publishing, Cham, pp 193-224. http://dx.doi.org/10.1007/978-3-319-51365-2_6

Turzó K, Laczkó G, Filus Z, Maróti P (2000) Quinone-dependent delayed fluorescence from the reaction center of photosynthetic bacteria. Biophys J 79 (1):14-25. http://dx.doi.org/10.1016/S0006-3495(00)76270-9

Vermeglio A, Nagashima S, Alric J, Arnoux P, Nagashima KV (2012) Photo-induced electron transfer in intact cells of Rubrivivax gelatinosus mutants deleted in the RC-bound tetraheme cytochrome: insight into evolution of photosynthetic electron transport. Biochim Biophys Acta 1817 (5):689-696. http://dx.doi.org/10.1016/j.bbabio.2012.01.011

Woodbury NW, Becker M, Middendorf D, Parson WW (1985) Picosecond kinetics of the initial photochemical electron-transfer reaction in bacterial photosynthetic reaction centers. Biochem 24 (26):7516-7521. http://dx.doi.org/10.1021/Bi00347a002

Wraight CA, Clayton RK (1974) The absolute quantum efficiency of bacteriochlorophyll photooxidation in reaction centres of Rhodopseudomonas spheroides. Biochim Biophys Acta 333 (2):246-260. http://dx.doi.org/10.1016/0005-2728(74)90009-7