Excitation Energy Transfer and Trapping in Cyanobacterial Photosynthesis

Ph.D. Dissertation

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Background

Cyanobacteria, also known as blue-green algae, are among the most ancient organisms on Earth, dating back approximately 3.5 billion years. These photosynthetic prokaryotes have played a pivotal role in shaping Earth's atmosphere by contributing to the evolution of oxygenic photosynthesis. Although cyanobacteria share a similar ecological niche with algae, they differ in their simpler, prokaryotic cellular structure, lacking membrane-bound organelles such as nuclei and chloroplasts.

Cyanobacteria exhibit a remarkable diversity in form, ranging from unicellular types like *Synechocystis* and *Synechococcus* to filamentous species such as *Anabaena* and *Nostoc*, which are capable of forming complex multicellular colonies. This morphological diversity allows cyanobacteria to thrive in a wide array of ecological environments, from freshwater bodies to extreme habitats like thermal springs and Arctic ice.

Their simple yet versatile structure, along with well-characterized genetics, makes cyanobacteria valuable models for studying photosynthesis and other biological processes. They also hold significant biotechnological potential, particularly in sustainable applications like biofuel production and environmental remediation. Their ability to perform oxygenic photosynthesis, fix nitrogen, and synthesize a variety of compounds further enhances their relevance in addressing global challenges.

The photosynthetic machinery in cyanobacteria is housed within thylakoid membranes, where light energy is converted into chemical

energy. These membranes contain key components such as photosystems I and II (PSI, PSII), cytochrome b_6f, and ATP synthase. Unlike the organized grana stacks found in plant chloroplasts, cyanobacterial thylakoids are more uniformly distributed. Nevertheless, they efficiently support photosynthesis and respiration, crucial for the survival and energy production of these organisms.

An essential aspect of cyanobacterial photosynthesis is the role of phycobilisomes (PBS), large protein complexes attached to the thylakoid membranes. PBS efficiently capture sunlight within a broad spectral range of 550–650 nm, extending the absorption capacity of chlorophyll and creating an energetic funnel towards the photosystems. Rapid and efficient energy transfer within PBS is vital for effective light harvesting, making it crucial to understand the dynamics of excitation energy transfer (EET) within PBS and to identify potential bottlenecks. The architectural differences in PBS among various cyanobacterial species, such as the pentacylindrical PBS of *Anabaena* and the tricyndrical core of *Synechocystis*, may significantly influence these energy transfer dynamics, although this has not been thoroughly studied.

PBS are generally believed to transfer energy directly to PSII due to their structural proximity. However, there is also the possibility of an indirect energy transfer route to PSI via PSII, known as the "spillover" route. The direct coupling of PBS to PSI and the associated energy transfer rates remains areas of ongoing research, with a need for more extensive studies.

Aims

1. Investigate the structural and functional aspects of energy transfer in *Anabaena* and *Synechocystis* by analysing the picosecond fluorescence kinetics of isolated PBS and intact cells recorded with high signal-to-noise ratio, using TCSPC, and with high time resolution, using a streak camera setup.

2. Develop a minimal functional compartment model of intact cells that includes the spectrally and kinetically resolvable groups of pigments (PC, APC, Chls) and the kinetics of energy transfer between them. Determine the kinetics of energy transfer by simultaneously fitting to the experimental fluorescence kinetics obtained from isolated PBS, PSI and intact cells under different excitation conditions.

3. Investigate the direct energy transfer from PBS to PSI in PSII-deficient *Synechocystis* cells by analysing the time-resolved fluorescence spectroscopy data obtained using streak camera and TCPSC at room temperature and 77 K.

4. Construct and refine a detailed kinetic model of energy transfer dynamics in *Synechocystis* ΔPSII and determine the rate constants of energy transfer at RT and 77 K by simultaneous fitting to the experimental fluorescence decay kinetics. At 77 K, the challenge lies in accurately modeling the fluorescence kinetics due to the significant emission from various pools of red chlorophylls within PSI.

Materials and Methods

Cell Cultures and Sample Preparation: Cyanobacterial strains (*Synechocystis* sp. PCC6803; WT, PSII-deficient mutant ΔPSII, and *Anabaena variabilis* sp. PCC 7120) were grown photoautotrophically. Phycobilisomes and Photosystem I were isolated from thylakoid membranes using gradient ultracentrifugation.

Spectroscopic Analysis:

- Room-temperature steady-state absorption spectra
- Steady-state fluorescence spectra measured at RT and 77 K
- Time-resolved fluorescence spectroscopy: time-correlated single photon counting and synchroscan streak camera.

Data Analysis: Workflow for Developing a Functional Kinetic Model:

- Creating the Model: Visualize experimental data, perform SVD to identify components, define initial models and parameters.
- Global Analysis: Fit multiple decay curves simultaneously to separate spectral (DAES) and kinetic properties (lifetimes).
- Solving the Model: Iteratively adjust parameters using mathematical decomposition and non-linear least squares fitting to optimize and simulate fluorescence kinetics, aligning the model with experimental data.
- Evaluating and Fine-tuning the Model: Assess model accuracy through chi-squared analysis, residual examination, and ensuring physical plausibility.

Results

This thesis investigates the EET processes in cyanobacterial light-harvesting systems, focusing on the two model species *Anabaena variabilis* PCC 7120 and *Synechocystis* sp. PCC 6803. Using time-resolved fluorescence spectroscopy and advanced data analysis techniques, the research elucidates the pathways and kinetics of EET in intact cells and isolated complexes, with particular emphasis on the interactions between PBS and photosystems. The study begins with a detailed examination of EET in *Anabaena*, revealing the complex EET dynamics within the PBS and from PBS to both PSI and PSII. A comprehensive model is developed, describing the stepwise energy migration from the peripheral rods to the core of PBS, and subsequently to the photosystems. The research highlights the rate-limiting step of rod-to-core transfer and demonstrates efficient and balanced energy distribution to both PSI and PSII. The thesis then focuses on PSII-deficient Synechocystis mutants, providing insights into direct energy transfer from PBS to PSI. Through room temperature and 77K studies, the research reveals the robustness of PBS-PSI energy coupling and the role of PSI red chlorophylls. The work demonstrates that even in the absence of PSII, PBS effectively channel excitation energy to PSI, with transfer rates comparable to those observed in wild-type cells.

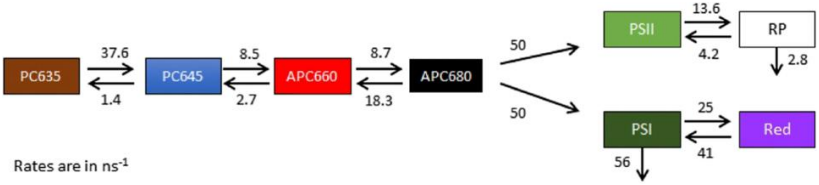
The following list summarizes the main novel scientific results of this work:

1. The kinetics of energy equilibration in the PBSs of Anabaena PC 7120 at room temperature entails major time scales of about 30 ps and 120

ps that reflect the migration excitations through the PBS rods and to the core, whereas equilibration with the terminal emitters of APC is significantly faster. The EET kinetics is highly similar to other cyanobacterial PBSs, notably Synechocystis. This similarity is notable given the differences in composition and architecture between the two PBS systems.

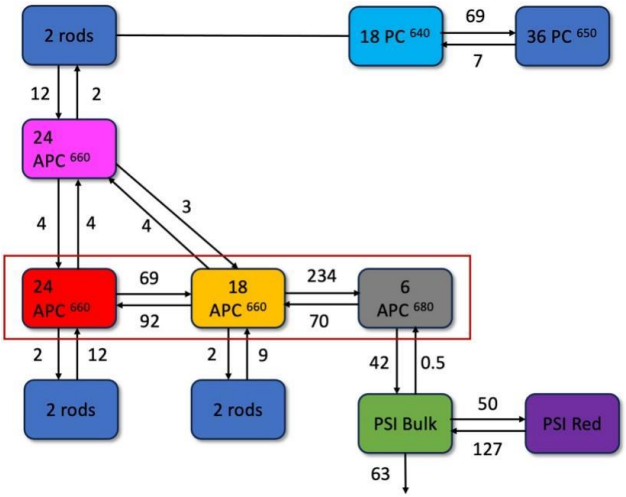
2. In intact cells of both *Anabaena* and *Synechocystis*, PBS efficiently transfer energy to the photosystems where it is photochemically utilized. The cascade of EET in the PBS (PC–APC–APC_{680}) can be identified in vivo with similar transfer lifetimes as in isolated PBSs. The effective trapping time of PBS excitations is about 200 ps, which includes both EET and charge separation.

3. A detailed kinetic model of the EET in *Anabaena* PC 7120 is developed and parametrized by simultaneous fitting to the experimental kinetics of intact cells and isolated complexes. The model describes the energy flow from the PBS to both photosystems. The rate-limiting step in EET is rod-core equilibration with an effective rate constant of 8.5 ns^{-1}. In contrast, the transfer of excitations from the terminal emitters in APC to both photosystems is significantly faster, with an estimated rate constant of 50 ns^{-1}.



Estimated kinetic model of the Megacomplex 1 consisting of PBS-PSII-PSI from simultaneous target analysis of all in vitro and in vivo datasets, rates in ns−1. Fluorescence decay rates (0.71 ns−1) have been omitted for clarity. Key: PC635 (maroon), PC645 (blue), APC660 (red), APC680 (black), PSII Chl a (green), PSI Chl a (dark green) and PSI "Red Chl" (purple)

4. PBSs can transfer energy to PSI directly, as evidenced by measurements on the PSII-deficient mutant of *Synechocystis*. A kinetc model of EET in the ΔPSII mutant based on simultaneous analysis of the fluorescence kinetics of intact cells and isolated complexes resolves a microscopic PBS-PSI transfer rate constant of 42 ns^{-1} – much faster than the energy equilibration within the PBS and comparable to the transfer of excitations from the PBS to PSII. This is evidence for the existence of specific interactions between the APC core and PSI.



Functional compartmental model of ΔPSII cells. This diagram illustrates a minimal kinetic model of the PBS-PSI complex at RT, featuring microscopic rate constants in ns^{-1}. The model includes a detailed view of a rod consisting of three lumped hexamers in the upper right corner. The rods are composed of PC640 (cyan) and PC650 (blue). The magenta compartment labelled APC660 represents the top cylinder. The red rectangle highlights the two basal cylinders, which contain APC660 (orange) and APC680 (black) across four discs, as well as APC660 (red) in four additional discs. The PSI complex includes a bulk Chl *a* compartment (green) and a red Chl *a* compartment (purple), which is connected to APC680.

5. A detailed investigation of the EET in the ΔPSII mutant of *Synechocystis* at 77 K revealed that 40% of the PBSs in the cells efficiently transfer energy to PSI. The study also resolved the dynamics of equilibration within the APC core. Different spectral forms of the terminal APC emitters were identified, emitting at 675 and 680 nm, as well as slow equilibration dynamics (on a time scale of 800 ps) between the PBS core cylinders.

List of publications

(MTMT: 10070022); Cumulative impact factor: 43

Publications related to the thesis:

1. Akhtar P, Biswas A, Petrova N, Zakar T, Van Stokkum IH, Lambrev PH. Time-resolved fluorescence study of excitation energy transfer in the cyanobacterium Anabaena PCC 7120. Photosynthesis research. 2020 May;144:247-59. (IF_{2020} :3.573)
2. Biswas A, Huang X, Lambrev PH, Van Stokkum IH. Modelling excitation energy transfer and trapping in the filamentous cyanobacterium Anabaena variabilis PCC 7120. Photosynthesis research. 2020 May;144:261-72. (IF_{2020} :3.573)
3. Van Stokkum IH, Akhtar P, Biswas A, Lambrev PH. Energy transfer from phycobilisomes to photosystem I at 77 K. Frontiers in Plant Science. 2023 Nov 22;14:1293813. (IF_{2023} :4.1)
4. Biswas A, Akhtar P, Lambrev PH, van Stokkum IH. Energy transfer from phycobilisomes to photosystem I at room temperature. Frontiers in Plant Science. 2024 Jan 8;14:1300532. (IF_{2024} : 4.1)

Other Publications:

1. Akhtar P, Biswas A, Kovács L, Nelson N, Lambrev PH. Excitation energy transfer kinetics of trimeric, monomeric and subunit-depleted Photosystem I from Synechocystis PCC 6803. Biochemical Journal. 2021 Apr 16;478(7):1333-46. (IF_{2021} :3.76)

2. Akhtar P, Biswas A, Balog-Vig F, Domonkos I, Kovács L, Lambrev PH. Trimeric photosystem I facilitates energy transfer from phycobilisomes in Synechocystis sp. PCC 6803. Plant Physiology. 2022 Jun 1;189(2):827-38. (IF_{2022} :8.0)
3. Arshad R, Saccon F, Bag P, Biswas A, Calvaruso C, Bhatti AF, Grebe S, Mascoli V, Mahbub M, Muzzopappa F, Polyzois A. A kaleidoscope of photosynthetic antenna proteins and their emerging roles. Plant physiology. 2022 Jul 1;189(3):1204-19. (IF_{2022} :8.0)
4. Böde K, Javornik U, Dlouhý O, Zsíros O, Biswas A, Domonkos I, Šket P, Karlický V, Ughy B, Lambrev PH, Špunda V. Role of isotropic lipid phase in the fusion of photosystem II membranes. Photosynthesis Research. 2024 Apr 25:1-4. (IF_{2024} :2.9)
5. Nápoles-Duarte JM, Biswas A, Parker MI, Palomares-Baez JP, Chávez-Rojo MA, Rodríguez-Valdez LM. Stmol: A component for building interactive molecular visualizations within streamlit web-applications. Frontiers in molecular biosciences. 2022 Sep 23;9:990846. (IF_{2022} :5.0)
6. Nantasenamat C, Biswas A, Nápoles-Duarte JM, Parker MI, Dunbrack Jr RL. Building bioinformatics web applications with Streamlit. InCheminformatics, QSAR and Machine Learning Applications for Novel Drug Development 2023 Jan 1 (pp. 679-699). Academic Press.

Összefoglalás

Dolgozatom első részében az *Anabaena variabilis* cianobaktériumban zajló gerjesztési energia transzfer (EET) dinamikáját vizsgáltam időfelbontott fluoreszcencia spektroszkópia segítségével. A cél a fikobiliszómák (PBS) szerkezeti és funkcionális tulajdonságainak, valamint a PBS és az I. (PSI) és II. fotokémiai rendszer (PSII) közötti kölcsönhatások feltárása volt. Pikoszekundumos fluoreszcencia élettartam méréseket végeztem különböző gerjesztési hullámhosszokon, izolált rendszerekben és intakt sejtekben. Az eredmények az energiaátviteli folyamatok részletes elemzését tették lehetővé, amely során két kulcsfontosságú élettartam komponenst azonosítottam az izolált PBS-ben: egy 30-35 ps-os komponenst, amely a pálcikákon belüli energiaátvitelhez kapcsolódik, és egy 110-130 ps-os komponenst, amely a pálcikák és az allofikocianint (APC) tartalmazó mag közötti energiaátvitelhez kötődik. Az izolált tetramer PSI komplexekben a fluoreszcencia lecsengése biexponenciális mintázatot mutatott, 10 és 40 ps-os komponensekkel, jelezve az antenna-klorofillok és a "vörös" állapotok közötti kiegyenlítődés és a gerjesztési csapdázás dinamikáját. Intakt filamentumokban a PBS által csapdázott energia szinte teljes egészében a fotokémiai rendszerekbe jutott, körülbelül 180-190 ps alatt. A dolgozat második részében egy kinetikai modellt mutatok be, amely az *Anabaena* energiatranszferének és csapdázásának kinetikáit írja le. A modell alapján a PBS-PSII-PSI megakomplexben a végső emitter egyenletesen osztja szét a gerjesztési energiát a PSI és PSII között, körülbelül 50 ns^{-1} sebességgel. PSII hiányos *Synechocystis* mutánsokban végzett vizsgálatokkal kimutattam, hogy a PSI-PBS megakomplex rugalmasan alkalmazkodik a PSII hiányához, biztosítva az energiaátvitel hatékonyságát, amelynek sebessége eléri a 42 ns^{-1} -t szobahőmérsékleten. Ezen eredmények bizonyítják az APC mag és a PSI közötti specifikus kölcsönhatások jelenlétét, és rávilágítanak a PBS és a PSI közötti közvetlen kapcsolódás fontosságára még alacsony hőmérsékleten is.

Co-author declaration

I, Dr. Petar H. Lambrev, in the capacity of supervisor of the Ph.D. candidate Avratanu Biswas and a co-author of the publications listed below, declare that:

I am familiar with the dissertation work of Avratanu Biswas.

The candidate had a significant contribution in obtaining the results presented in the dissertation and the listed articles.

These results have not been used previously for obtaining any other Ph.D. degree and will not be used in the future.

Publications

1. Biswas A, Akhtar P, Lambrev PH, van Stokkum IH. Energy transfer from phycobilisomes to photosystem I at room temperature. Frontiers in Plant Science. 2024, 14:1300532.
2. Akhtar P, Biswas A, Petrova N, Zakar T, Van Stokkum IH, Lambrev PH. Time-resolved fluorescence study of excitation energy transfer in the cyanobacterium Anabaena PCC 7120. Photosynthesis research. 2020, 144:247-59.

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Petar H. Lambrev