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FRET-based nanocommunication in organic structures of plant origin

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Abstract

The intention of this article is to put researchers' attention on the molecules of plant origin, like carotenoids and chlorophylls, which can serve very well for the purposes of nanocommunication. Carotenoids are additionally able to harvest energy from their environment absorbing sunlight. They can further pass signals to chlorophyll molecules via Foerster resonance energy transfer. Carotenoids and chlorophylls can thus perform functions of nanotransmitters and nanoreceivers, respectively, and their communication can be fuelled by the locally acquired energy.

We performed a laboratory experiment using a membrane protein supercomplex termed as photosystem II that plays a crucial role in the process of photosynthesis on Earth. Photosystem II (PSII) is a well-organized and self-driven structure containing photosynthetic pigments such as carotenoids and chlorophylls. We measured the absorption and excitation spectra of the PSII complex and then calculated the efficiency of the signal transfer between the carotenoid, beta-carotene, and the chlorophyll-a. We discussed the obtained results comparing them with other carotenoids.

Section 1

Introduction

Foerster resonance energy transfer (FRET) is a phenomenon, recently gaining much attention when discussing the possibilities of communication in nano scale. FRET seems to be a very promising nanocommunication mechanism, especially thanks to its low propagation delay and high possible throughput. A still open issue is how the FRET-based communication could be fuelled. Having an external laser exciting donors (FRET nanotransmitters) does not look like a feasible option for many applications. One of the solutions would be using energy of local chemical reactions with some bioluminescent molecules, which could be a solution suitable for medical applications inside human body [1]. In this paper, we are going to consider another possibility which is harvesting the sunlight energy. Some molecules, e.g. from the family of carotenoids, are able to effectively acquire energy from sunlight and then pass it, via FRET, to other molecules, e.g. chlorophylls. Such a signal transfer can be observed in so called photosystem II, which is a complex containing both carotenoids and chlorophylls in plants, but also in algae and cyanobacteria.

The contribution of this paper is as follows. We first propose the molecules of plant origin, carotenoids and chlorophylls, for the purpose of FRET-based nanocommunication. We then present the results of our laboratory experiment on the system PSII containing carotenoids and chlorophylls, showing the efficiency of the FRET signal transfer between these

molecules. Finally, we discuss other molecules and scenarios that could help to further increase the efficiency of the FRET signal transfer.

The rest of the paper contains a general description of FRET, especially in the context of carotenoids and chlorophyll molecules (Section 2), a report on the performed experiment (Section 3) and the discussion of the results together with comparison to other similar molecules (Section 4).

Section 2

Foerster resonance energy transfer

Foerster resonance energy transfer is a mechanism where an excited molecule, called a donor, non-radiatively passes its energy to another molecule, called acceptor. Both molecules must be located close to each other, usually up to 10 nm, and they must be spectrally matched, i.e. the donor emission spectrum should, at least partially, overlap the acceptor absorption spectrum. The donor molecule may be excited in many ways, e.g. accepting a photon, using energy of a chemical reaction or via another FRET process. After excitation, the donor keeps its energy usually few nanoseconds; this delay time is exponentially distributed with the average value depending on the molecule type. Then, the energy transfer between the donor and the acceptor is realized without any radiation and nearly immediately (in picoseconds).
The FRET efficiency is highly dependent on the separation between the donor and acceptor molecules, it was shown to be equal to [2]:

$$E = \frac{{R_0}^6}{r^6 + {R_0}^6} \tag{1}$$

The parameter r is the separation between the donor and acceptor molecules and R_0 is the so called Foerster distance, which depends on the spectral match between the donor and acceptor. The value of R_0 can be derived experimentally or calculated having known the donor quantum yield, donor emission spectrum and acceptor absorption spectrum; depending on the molecule type, R_0 varies from 2 to 9 nm.

From the communication viewpoint, the energy transfer between a donor and an acceptor can be treated as a way to send information bits over a channel consisting of a physical space between these two molecules. The donor plays the role of a nanotransmitter and the acceptor is the nanoreceiver. In such a scenario, it has been already proposed to use ON-OFF modulation [3]. Keeping a common assumption of a kind of synchronization between the transmitter and receiver sides, a bit ‘1’ is sent exciting the donor and expecting the FRET occurs, while a bit ‘0’ is sent keeping the donor in the ground state (low energy level) so that no energy reaches the acceptor (no FRET).

The Eq. (1) shows that, especially for the distances larger than R_0, FRET may be very inefficient. The energy, instead of being passed to the acceptor, is emitted in form of a photon (in case of fluorescent molecules) or dissipated as heat. For the communication purposes, such energy is lost and a transmission error occurs. The errors may happen only when transmitting bits ‘1’, as there is no energy transfer when sending a ‘0’. Thus, the corresponding bit error rate (BER) is given as:

$$\mathrm{BER} = 0.5 \cdot (1 - E) \tag{2}$$

When the donor and acceptors molecules are separated by about Foerster distance, the related BER is clearly not acceptable for the communication (usually the required BER about be at least 10^{-3} or better). This is a reason why the MIMO-FRET technique was proposed, where multiple donors and multiple acceptors are used at the same time, similarly as in MIMO wireless systems, in order to enhance the reliability of the signal transfer. It was already shown that having *n* donors and *m* acceptors, the related BER is much smaller than in the case with single molecules and can be expressed as [4]:

$$\mathrm{BER}_{\mathrm{n,m}} = 0.5 \cdot \left(\frac{r^6}{r^6 + m \cdot {R_0}^6} \right)^n \tag{3}$$

Having 5-6 molecules both at transmitter and receiver sides enables BER below 10^{-3} for the communication distances equal to the Foerster distance [4-5].

The FRET phenomenon is a natural mechanism of the energy transfer between the molecules occurring in plants as well. Chlorophyll molecules, performing the photosynthesis reaction, get the energy partially directly from sunlight (mostly at 400-460 and 640-680 nm), but partially via FRET from carotenoids. Carotenoids also use the sunlight as a source of their energy, but they absorb a different part of the visible spectrum: 460-500 nm. Both types of molecules, especially carotenoids are quire efficient in sunlight energy harvesting as might be judged from their molar extinction coefficients ε. In the case of beta-carotene it reaches as much as 13.4 x 10^4 l·mol^{-1}·cm^{-1} (at 454 nm in acetone) [6], whereas for chlorophyll-a, ε = 71.43 x 10^3 l·mol^{-1}·cm^{-1} (665 nm, 100% methanol) [7]. For synthetic dyes, such as for example Alexa Fluor 488 (roughly comparable to beta-carotene spectral range), ε = 73 x 10^3 l·mol^{-1}·cm^{-1} (ddH_2O or PBS) [8].

Except of their communication capabilities, carotenoids and chlorophylls might be attractive for the future development of nanotechnology also from other reasons. First, they could be considered as parts of nanostructures for intelligent fabrics and materials. When applied on surfaces exposed to sunlight, they could be a source of energy for other nanomachines. Moreover, the process of energy harvesting might be controlled by numerous filters. The first option is an optical thin-film resonant cavity filter based on a Fabry-Perot or Mach-Zehnder interferometer [9]. Other options are nanoparticles or nanowires filtering the energy gathered by light harvesting complexes (LHC) [10-15]. All these filters can be used for the modulation of the transferred signals or control over the functions of nanomachines or other hybrid nanostructures [16].

Carotenoids and chlorophylls are present together in all the plants, in algae and cyanobacteria. In the next section, we report the laboratory experiment focused on photosystem II (PSII), a 10 x 11 x 21 nm molecular structure containing both types of molecules of our interest (Fig. 1).

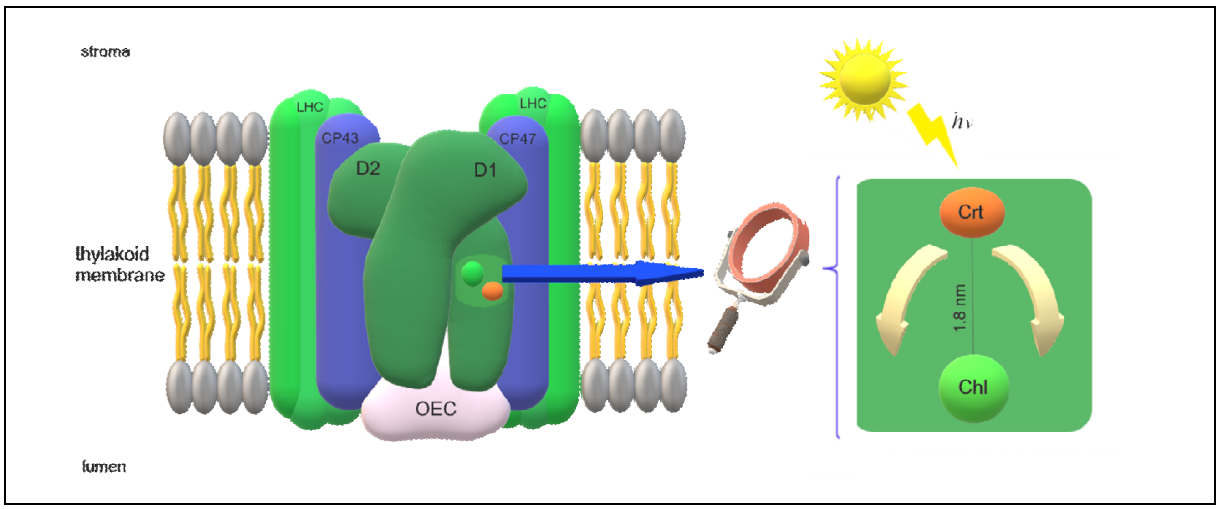

Fig. 1. The structure of the photosystem II (PSII). Used abbreviations: LHC – light harvesting complex; CP43, CP47 – intrinsic proteins of PSII reaction center; D1, D2 – protein subunits of the PSII complex; OEC – oxygen evolving complex of PSII; Crt – carotenoids molecule; Chl – chlorophyll molecule; lumen – the space inside the thylakoid membrane; stroma – the space outside the thylakoids.

Section 3

The experiment with the PSII system

The main goal of the experimental part of this research was to measure the FRET efficiency between the carotenoids, in this case the beta-carotene, and chlorophylls, in this case the chlorophyll-a, both located in the PSII system. The FRET can be observed from the beta-carotene to the chlorophyll-a, as the beta-carotene emission spectrum and the chlorophyll-a absorption spectrum overlap for the wavelengths about 500 nm (the beta-carotene absorption and emission spectra are strongly overlapped, as the respective Stoke shift is very small [17-18]). The fluorescence of beta-carotene molecules is very low [17-18], so the FRET efficiency cannot be measured calculating the decrease of the donors (beta-carotene) lifetime in the presence of the acceptors (chlorophyll-a), like in [4-5]. Instead, it was decided to use another technique described in [19-20]. This approach is based on comparing two spectra of the PSII system. The first one is the PSII absorption spectrum. The second one is the so-called PSII excitation spectrum, which is recorded by measuring the PSII emission at 684 nm for a wide range of input laser excitation (from 350 to 600 nm). Thus, the excitation spectrum shows how much energy was absorbed **and passed** through the PSII system. Both these spectra can be normalized to each other, as we know that their values at 600 nm must be equal. At 600 nm only chlorophyll-a absorbs energy, so 100% absorbed energy is later emitted.

3.1. Isolation of photosystem II

Photosystem II particles were isolated from spinach (*Spinaciaoleracea* L.) according to the method described in [21] with minor modifications. Thylakoids (photosynthetic membranes) were isolated from fresh leaves purchased from a local market. After removing stems from the spinach, leaves were homogenised by the use of blender in wash medium (pH 7.8) containing 400 mM sucrose, 50 mM TRIS-HCl, 10 mM NaCl and 5 mM $MgCl_2$. The ground spinach leaves were filtered and remaining suspension was centrifuged at 4200x g for 15 minutes in 6 ^{0}C. After the supernatant was removed, the pellet was re-suspended with

buffer (pH=6.5) containing 20 mM HEPES, 5mM $MgCl_2$ and 15 mM NaCl. This step was triplicated. Then thylakoids enriched in PSII particles were suspended in buffer containing 400 mM sucrose, 20 mM HEPES, 5mM $MgCl_2$ and 15 mM NaCl to the total concentration of chlorophyll of 1mg/ml. Thylakoids enriched in photosystem II particles were solubilized on ice in the presence of 15 % Triton X-100 for 25 minutes in darkness. After that, they were centrifuged (62000 x g, 10 minutes, 4 ^{0}C) 4-5 times to obtain transparent supernatant. The final chlorophyll concentration in the sample was adjusted to the total concentration of chlorophyll of 1.5 mg/ml. Only fresh samples (not frozen) were used for further experiments.

3.2. Measurement of chlorophyll concentration

The concentration of the chlorophyll in thylakoids enriched in PSII was spectrophotometrically determined as described in [22]. Thirty microliters of sample were suspended in 2.7 ml of 80% of acetone and 270 µl of distilled water and suspended solution was centrifuged at 2200 x g for 5 minutes. Then the solution was gently pipetted and the absorbance was measured at 645 nm and 663 nm.

3.3. Spectroscopic measurements

Steady-state absorption spectra were recorded with a Cary50 Bio UV-VIS spectrophotometer (Varian) in a standard quartz cell in the range of 300-850 nm. Steady-state fluorescence (excitation and emission) spectra were measured on a CaryEclipse spectrofluorometer (Varian). Emission spectra were recorded within a range of 650-800 nm upon excitation at 475 nm, 485 nm, or 500 nm (carotenoid band), and 438 nm (chlorophyll band), respectively. Excitation spectra were collected in the range of 350-600 nm, while emission from chlorophyll was measured at 684 nm. In each case the number of recorded spectra was set to twenty, but for the purpose of data processing the first four recorded spectra were rejected (warming up of the equipment).

3.4. Spectra normalization and FRET calculation

As the PSII absorption, excitation and emission spectra were recorded 20 times, in the cases the first four records were rejected (they might not be reliable due to the measurement equipment warming up). The final spectra were calculated as the averages of the remaining 16 records, although the differences between the records were negligible. The PSII absorption and emission spectra are presented in Fig. 2. One can clearly notice the absorption maxima related to the presence of both beta-carotene and chlorophyll-a molecules. In Fig. 3, the absorption and excitation spectra are compared and normalized assuming they are equal for wavelength of 600 nm (as explained in Section 3).

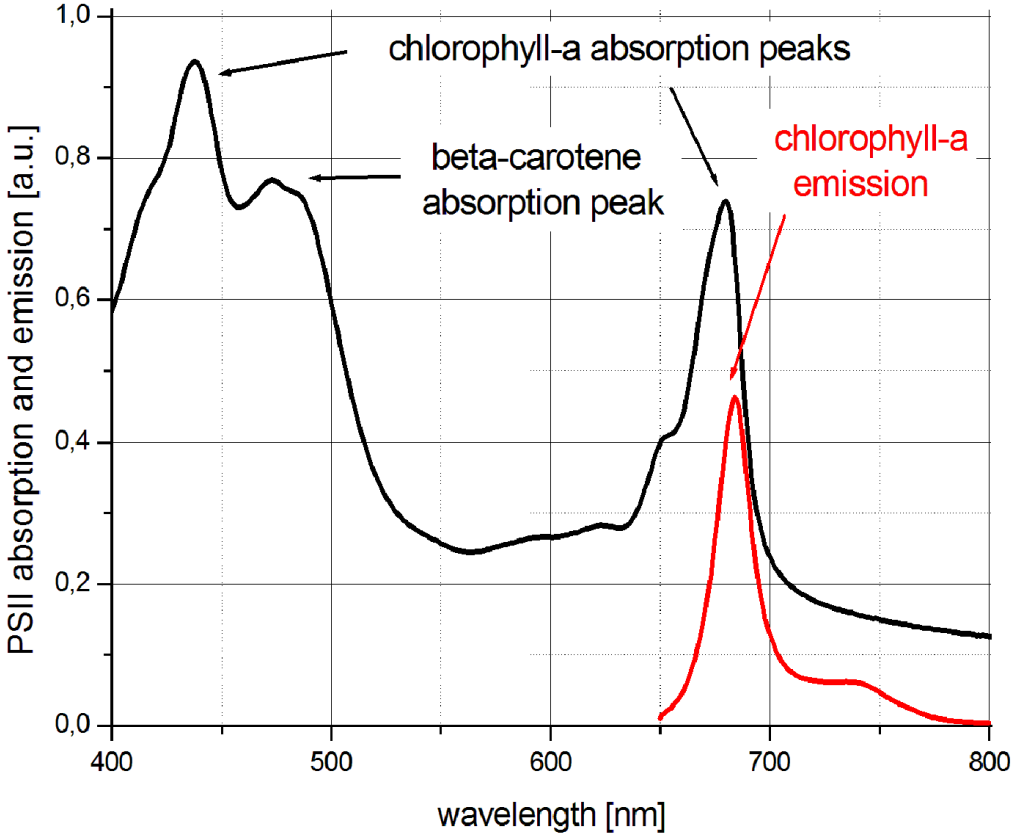

Fig. 2. The PSII absorption and emission spectra.

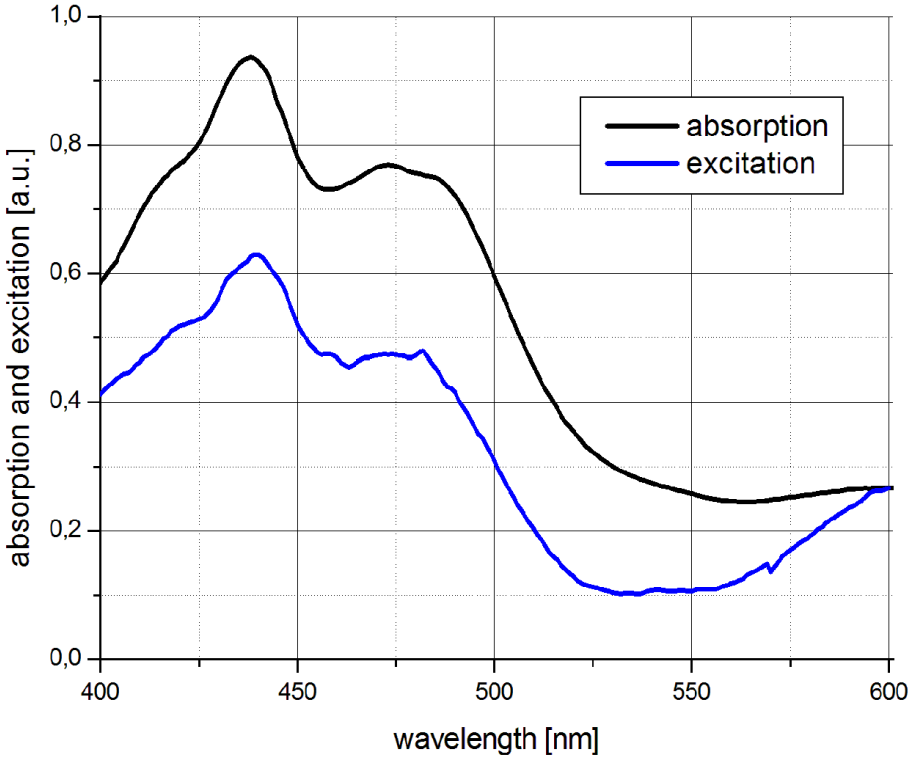

Fig. 3. The PSII absorption and excitation spectra together, normalized assuming their equality for 600 nm.

Finally, the FRET efficiency was calculated. Among the wavelengths of the beta-carotene absorption spectrum (about 480-500nm), five wavelengths were chosen: 480, 485, 490, 495 and 500 nm. For these wavelengths, the ratio of excitation to absorption was calculated which, according to the approach from [19-20] is equal to the FRET efficiency between the beta-carotene and the chlorophyll-a. The calculated FRET efficiencies are given in Table 1.

TABLE 1
MEASURED FRET EFFICIENCIES

Absorbed light wavelength	**FRET efficiency**
480 nm	62.6%
485 nm	60.6%
490 nm	57.7%
495 nm	54.3%
500 nm	52.1%

The resulting FRET efficiencies, being in the range 52–63% can be compared with theoretical values. The Foerster distance for beta-carotene – chlorophyll-a pair is assessed for 1.8–2.5 nm [23]. In the PSII system, the physical distance between these molecules is about 1.8 nm (see Fig. 1), so indeed, comparing with Eq. (1), we should expect the FRET efficiency about 50% or a little higher.

Section 4

Discussion with comparison to other carotenoids

The results presented in this paper are just a preliminary work on the application of carotenoids and chlorophylls in nanocommunication. However, even at this early stage of research, it can be seen that the signal transfer via FRET between carotenoids and chlorophylls can be quite effective. The experimentally measured FRET efficiencies between beta-carotene and chlorophyll-a molecules varied from 52% to 63%. Other authors assess that the FRET efficiency can be even higher when other carotenoids are used instead of beta-carotene. A short summary of FRET efficiency values reported in literature for carotenoids and chlorophylls is given in Table 2. Both the experimental results presented in this paper and the reported literature values prove a great potential of photosynthetic structures for future nanocommunication networks.

TABLE 2
FRET EFFICIENCIES FOR OTHER CAROTENOID AND CHLOROPHYLL MOLECULES

pairs of molecules	**FRET efficiency [%]**	**reference**
peridinin $\rightarrow$ chlorophyll-a	$88 \pm 2\%$	[24]
lutein + neoxanthin $\rightarrow$ chlorophyll-a	76%	[25]
violaxanthin + isofucoxanthin $\rightarrow$ chlorophyll-a	90	[26]
neurosporene $\rightarrow$ bacteriochlorophyll-a	92	[27]
spheroidene $\rightarrow$ bacteriochlorophyll-a	$56 \pm 3\%$	[28]

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