Graduate School NTU-BOKU

PROPOSAL
SURFACE PLASMON SPECTROSCOPY MEETS PHOTOSYNTHESIS

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Motivation and Aim

We would like to exploit the structure and function of polymer-embedded protein assemblies for light sensing and actuation. In this interdisciplinary proposal, we investigate nature-derived concepts of energy conversion with the help of optical detection/imaging methods – to learn from evolutionary-optimized concepts and structures. The efficiency of nature in light-energy conversion hosts many aspects worthwhile of being understood and eventually translated into synthetic approaches. The concept of light-harvesting has been so successful in nature that among all the various photosynthesizing organism on Earth, be it bacteria, algae, or plants, every single one possesses light-harvesting components. The light-harvesting complex (LHCII) of green plants, funneling its collected light energy into photosystem II of the photosynthetic apparatus (Fig. 1) contains an apoprotein that organizes its pigments (chlorophylls, carotenoids) at a particularly high density. This complex is highly self-organizing, i.e. it can be reconstituted in the reaction tube starting out from its recombinant apoprotein. Therefore it seems worthwhile to try and use this light-harvesting complex to improve the performance of artificial solar energy-converting constructs such as photovoltaic devices.

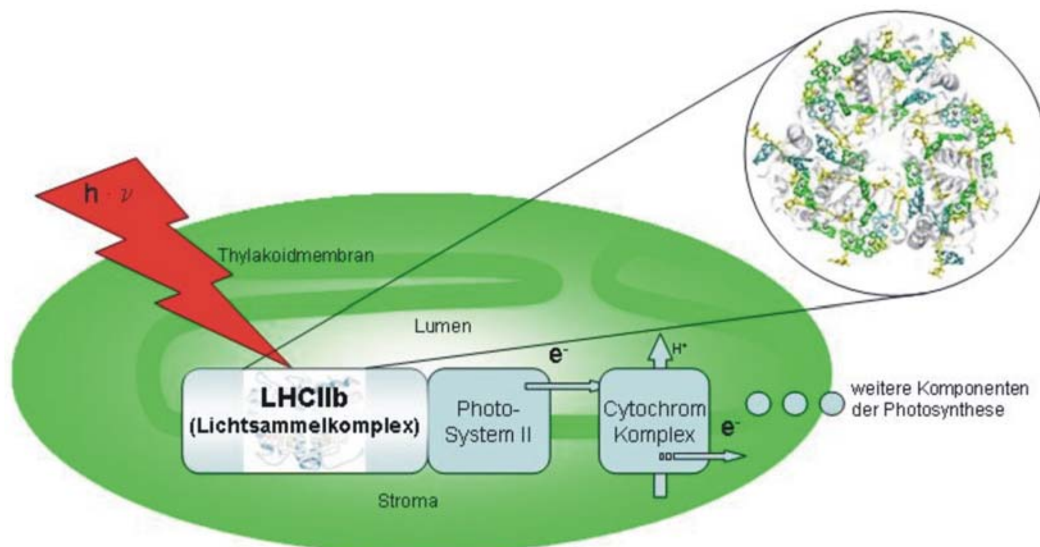


Fig 1: Schematic presentation of the processes, involved in photosynthesis in higher plants. The LHCIIb complex is important for organizing the light-absorbing pigments.

In this project, we would like to focus on the *in vitro* synthesis of the light harvesting complex of higher plants (LHCII), in a surface-bound synthetic architecture of di-block copolymers, mimicking the structure and chemical nature of cellular glyco- and phospholipids-composed thylakoid membranes. The *in vitro* synthesis strategy has already been successful with the synthesis of the LHCII protein> Here, we aim to translate this process into a functional structure embedded in an artificial membrane (Fig. 2), which we would like to explore in detail. By application of the commercial surface plasmon spectrometer, BIAcore™, we would like to analyze the presence, quantity and orientation of in vitro synthesized LHCIIb making use of its recognition by specific proteins, recently identified in the lab of Harald Paulsen. The surface bound membrane-like matrix will be in contact with a cell-free extract from wheat germs, processing transcription and translation of the LHCIIb. For this purpose, an L1 chip will be used (mere Gold) from BIcore ™ and polymersomes will be immobilized, as published from Nallani et al. 2011, BioInterphases, *in press*. The resulting surface structure is ready for pigment reconstitution. The peak molar absorption coefficient of the porphyrine-ring based chlorophylls exceeds 10^5 M^{-1} cm^{-1}, which is among the highest for small-molecule organic compounds. The high refractive index of such molecules results in a strong signal-to-noise ratio of the SPR– so even little amounts of chlorophyll can be detected. This is an indispensable prerequisite in our experiment as the water-solubility of the porphyrins is generally very low. LHCII apoprotein. Pigments have been shown to bind spontaneously to the folding apoprotein. As an alternative route towards the same structure we propose to bind recombinant, fully pigmented LHCII to a solid surface first, via a histidine$_6$ tag or the sulfhydryl of a cysteine side group engineered into the apoprotein, to a gold surface or an affinity surface for histidine$_6$ tags, respectively. Subsequently the membrane-mimicking membrane will be assembled using LHCII to tether it to the surface.

The binding of both the protein and the pigments will be detected by surface plasmon resonance. The immobilized LHCII can be shown to be functional by demonstrating intra-molecular energy transfer from chlorophyll *b* to chlorophyll *a*. This will be achieved by surface-plasmon-enhanced fluorescence (SPFS).

Detection of small molecules by surface plasmons has been explored extensively in the past. However, the use of molecules with special light absorbing features, correlating to the evanescent field - is still not employed as a tool to probe label-free detection – we would like to take advantage of the highly absorbing porphyrin structures of pigments in order to enhance the signal in label-free SPR technology for the characterization of matrix-integration of the synthetically generated LHCIIb/pigment complex.

Task overview

I. We would like to analyze the in vitro synthesized LHCII complex in its orientation and structural integrity. For this purpose, **a wheat germ based cell-free expression system will be established** in the lab of Eva Sinner at the BOKU University to enable detailed studies in synthesis and integration of the LHCII complex. In collaboration with Prof. Harald Paulsen, Univ. Mainz. Close contact and exchange of protocol and materials will be part of the project, to enable fast and efficient LHCIIb synthesis.

II. The functionality of LHCII will be assessed by SPFS as detailed above, taking advantage of the fact that only in correctly folded and assembled LHCII intra-complex energy transfer takes place.

II. We like to **address the orientation** of the synthesized LHCIIb complex and **control its integrity** by removal of the pigments *via* a recently identified protein from the lab of Prof.

Harald Paulsen, specifically interacting with the pigments, bound to the complex. By analysis of this interaction using the BIAcore system in the lab of Prof. Susana Geifman-Shochat, we will learn about the orientation, quantification and stoichiometry of the LHCIIb complex, employing specific immuno-based detection assays.I. The orientation of LHCII within the membrane-like structure is unambiguous if LHCII is first immobilized on the solid surface and then used as a tether of the polymer matrix (second route above). If, however, the protein is inserted co-translationally, it can in principle adopt a right-side up or an upside down orientation. This will be assessed by SPR using antibodies specifically binding to tags engineered into the N- or C-terminal domain of the apoprotein (Fig. 2).
This will be important in order to understand finally the boundary condition of the LHCIIb complex in light detection – continuing the work of Dr. Shaohua Ding, which successfully synthesized LHCIIb in a cell-free setting. With the method of surface plasmon enhanced fluorescence spectroscopy (SPFS), we
IV. Once the immobilization of recombinant LHCII in a membrane-mimicking polymer scaffold is established, we will use it to improve the light utilization by a sensitizer dye bound to a semiconductor surface in an electrochemical solar cell (Fig. 3). This will analyse the orientation of the LHCIIb complex (fig. 2), followed by electrochemical characterization on a semiconductor surfacebe done in collaboration with SONY Deutschland, active in the field of Dye-sensitized solar cells.

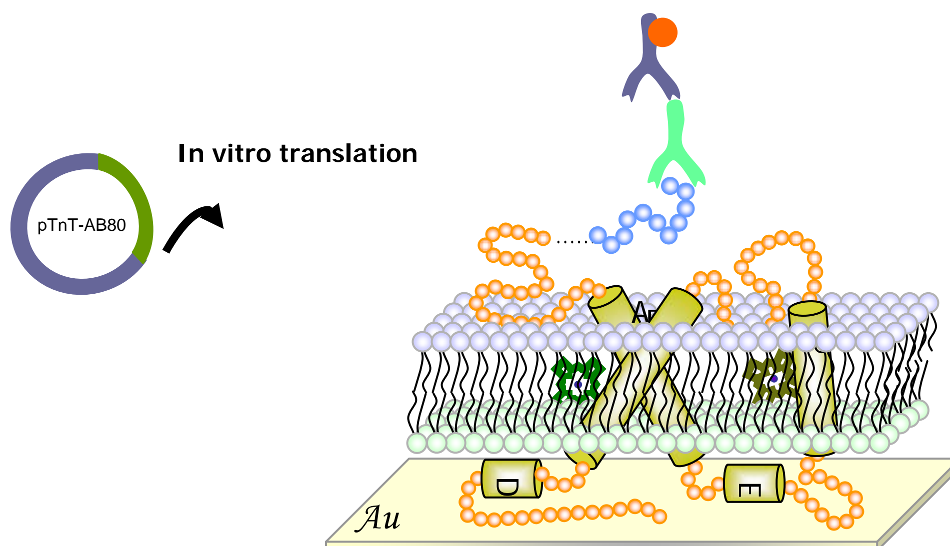
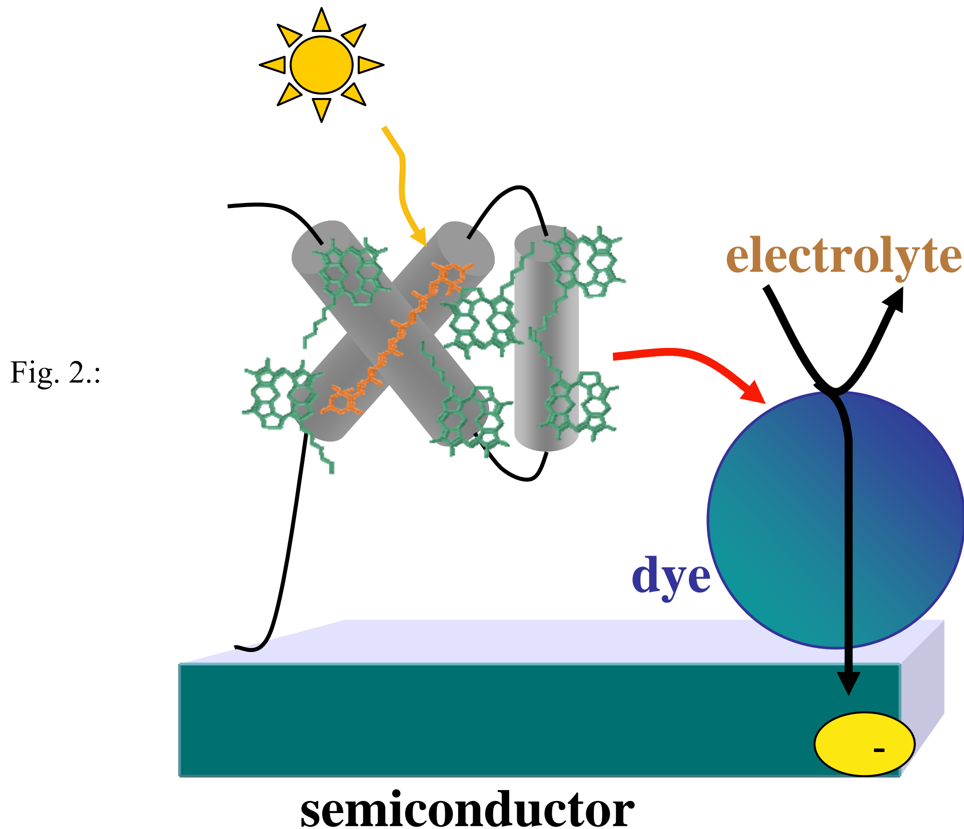


Figure 2: schematice view on a membrane architecture, hosting cell-free synthesized LHCIIb for antibody detection employing side specific antibody binding.

III. Ultimately, we would like to address the functionality of the surface-bound complex – pigment reconstitution which will be monitored as a function of refractive index change – and by means of protein binding. Being able to implement the complex into a corresponding architecture, we will try to understand the composition as well as the function of LHCIIb.

Fig. 2.:



Organiszation of a LHCIIb complex on a “synthetic” surface, enabling electron transfer upon light absorption.

Consortium and Mutual Benefits for the IGS

In this interdisciplinary project, we would like to point out the unique combination between Nano-science and Biochemistry/Botany. We would like to address fundamental principles in photosynthesis, addressed in the research of Prof. Harald Paulsen with a new combination of methods and architectures/materials, developed in this project. Prof. Geifman-Shochat has a long standing expertise in BIAcore –based analytics of protein composition and functional analysis with a strong background in plant physiology. Prof. Sinner has a similar history in membrane proteins in higher plants – developing new methods for synthesis and analysis. With this consortium, we address fundamental questions in the program of IGS – acquiring an excellent environment for the respective PhD students to develop an independent profile in protein synthesis and characterization, employing the relevant and promising example of energy harvesting and conversion of plants.