Abstract:

Improved photosystem coupling and a novel spectral-tuning approach in light-driven formate production

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Abstract:

Electrocatalytic- and solar-driven fuel synthesis from the greenhouse gas CO_2 is a desirable approach to simultaneously produce sustainable energy carriers, and combat increasing atmospheric CO_2 levels. Formate is a stable intermediate in the reduction of CO_2 and is utilised in a wide range of downstream applications. Recent approaches focussed on using an all-protein, light-triggered, catalytic circuit based on photosystem I, cytochrome c and formate dehydrogenase, which would convert CO_2 into formate. However, various challenges remained. We aim to *(i)* determine and optimise the structural basis for efficient electron transfer from cytochrome *c* to a genetically engineered photosystem I-formate dehydrogenase fusion complex using cryo-electron microscopy and state-of-the-art genetic tools, and *(ii)* explore the potential of using different spectral regions of light to drive CO_2 reduction via photosystem I, especially in near-infrared light. The prospective PhD student should be interested in molecular biology, biochemistry and structural biology.

Extended description version of the project:

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1. Overall goal of the project

Various strategies have been proposed to reduce CO_2. Here we focus on a purely enzymatic approach using the well-studied biocatalysts formate dehydrogenase (FDH), the mediator cytochrome (Cyt) c_6 and the power engine photosystem I (PSI). An efficient coupling of these proteins would allow the light-driven reduction of CO_2 and the formation of formate which can be used as liquid energy carrier in fuel cells, as a hydrogen storage material, or feedstock for the synthesis of fine chemicals. In particular, we aim to

(i) determine and optimise the structural basis for efficient electron transfer from Cyt c_6 to a genetically engineered PSI-FDH fusion complex, and

(ii) explore the potential of using different spectral regions of light to drive CO_2 reduction via PSI.

Despite various attempts, the actual binding site of Cyt c_6 to PSI has not been identified and binding affinities were not optimised. Here, this challenge is addressed by combining modified PSI and heterologous expressed Cyt c_6 variants with varied (higher) binding affinities to PSI. The structure of the complex will be then determined by cryo-electron microscopy (cryo-EM). In parallel, the direct coupling of FDH to PSI will be attempted by genetically fusing the FDH to the PsaD subunit of PSI. This offers the opportunity to express the hybrid complex heterologous in *Escherichia coli* and combine it with a PsaD-less PSI isolated from its native organism. In the second part of the project, we will explore the possibility of using a different region of the solar spectrum to drive CO_2 reduction and increase the efficiency of the process. For this approach, we will use PSI from cyanobacteria that incorporate the far-red chlorophylls *d* and *f*, which allow them to use near-infrared light.

2. State of the art

In recent studies we have structurally investigated the binding behaviour of the electron donor Cyt *c* to trimeric PSI using small-angle X-ray scattering (SAXS) and cryo-EM. Based on the SAXS data, the formation of a PSI-Cyt *c* complex was proven [1]. However, in a cross-linked cryo-EM Cyt c_6-PSI structure, only unspecific binding sites on the protein could be identified [1, see also 2], which is in contradiction to the previously suggested models and does not explain its functionality [1]. For efficient electron transfer binding of Cyt c_6 needs to occur near the photochemically active P700. To validate our model, we identified various amino acid residues that could play a critical role in the interaction and used site-directed mutagenesis to generate Cyt c_6 variants. They were expressed in *E. coli*, purified by affinity chromatography and analysed in respect to their binding affinity to PSI. In this way, we could generate a mutant with an 11-fold higher binding affinity than the wild-type Cyt c_6 and therefore consider it as a prime candidate for our proposed structural studies.
Various strategies have been tested to couple enzymatic reactions to PSI. A promising approach involves the direct coupling of FDH to PsaD of PSI. The possibility of fusing a hydrogenase to PSI via PsaD has been shown for the cyanobacterium *Synechocystis* [3]. In their study, the authors were able to produce H_2 *in vivo* by introducing *psaD-hoxYH* into a *psaD*-less *Synechocystis* mutant. We will use a different strategy that allows the assembly of the complex *in vitro* by combining PsaD-less PSI with heterologous expressed *psaD*-FDH. The deletion of *psaD* in *Synechocystis* has been achieved as has the expression of *psaD* in *E. coli,* and its assembly to a functional PSI *in vitro* [3,4]. For biotechnological applications, it is desirable to increase the incident photon-to-electron conversion efficiency (IPCE); so is being able to fine-tune two enzymatic reactions by using different light. Recent studies have shown that the ICPE values in the green gap could be doubled by using light harvesting antenna of cyanobacteria [5]. However, little is known about the efficiency of photosynthesis using light beyond 700 nm. In fact, near-infrared light was considered to be energetically too weak to drive oxygenic photosynthesis. We have shown that this is not the case.

The long-wavelength chlorophylls *d* and *f* can drive the process even above 700 nm by introducing the pigments into key positions in the photosystems [6]. These photosystems open up interesting opportunities to use a region of the solar spectrum which is largely unused by standard photosystems to drive enzymatic reactions such as CO_2 reduction.

3. Specific aims and how they may be reached:

The project consists of three work packages which will allow the student to learn and apply various biophysical and molecular biology methods in the three associated labs at the FU, HU and Charité.

WP1: Cryo-EM structure analysis of Cyt c_6-PSI

The recent Cryo-EM structure of a PSI-Cyt *c* complex by [1] revealed various binding sites, but not the predicted functional site near P700. To overcome this limitation, we aim to achieve a higher occupancy of Cyt c_6 at each reaction centre by using monomeric PSI (mPSI) with either cross-linked Cyt c_6 or with heterologous expressed Cyt c_6 variants with higher binding affinities to PSI. To monomerise PSI, biochemical and genetics methods will be used, which have been shown to have a minimal effect on the functionality of PSI, Cyanobacterial mutants, we will be generated by using molecular biology techniques that are well established in the Nürnberg lab [7]. PSI complexes will be then isolated using either sucrose density gradient ultracentrifugation [6] or chromatography approaches established in the Zouni and Nürnberg lab [1]. Oligomerisation states of PSI will be determined by blue-native PAGE and the sample quality evaluated by Dynamic Light Scattering measurements. Various Cyt c_6 variants are already available and their heterologous expression in *E. coli* has been optimised based on the previous published protocol by [1] to obtain high protein yields. These proteins will be used for pull-down assays in which one protein is bound to an affinity column (here: Cyt c_6) and the potential interaction partner (mPSI) is run through the column. The complexes will be then eluted and used for sample vitrification and subsequent cryo-EM studies in the Scheerer lab [8]. Similar experiments have recently helped to resolve the structure of the Cyt b_6f complex with a regulator [9]. In addition, we will use various cross-linking agents to connect Cyt c_6 and PSI [4]. The formation of the complexes can be verified using time-resolved optical spectroscopy following the transient absorbance changes at 820 nm and by oxygen reduction measurements.

WP2: Efficient coupling of FDH to PSI

We aim to simplify the reconstitution process by fusing FDH to PsaD and using a *psaD*-less PSI that can be directly isolated from the cyanobacterium. For this we will first generate a *psaD* knock-out strain using standard molecular cloning techniques published previously [7]. In parallel, various *psaD*-FDH constructs will be generated and their expression in *E. coli* optimised using the protocol by [4]. Here we will use *psaD* from *Synechocystis* and the FDH from *Rhodobacter capsulatus*. To allow efficient electron transfer between FeS clusters, their distance should be around 14 Å [3]. To place the FeS cluster of FdsB in close proximity of the 4Fe4S cluster F_B of PsaC, the PsaD clamp will be removed by fusing it to Q106 similar to the strategy by [3]. A structural model for the design will be developed with M.A. Mroginski (TU Berlin). The FDH-PsaD complex will be isolated in collaboration with S. Leimkühler (Potsdam) and analysed by native PAGE, SDS-PAGE and Western Blots. The PSI-(PsaD-FDH) complex will be reconstituted and its functionality tested by GC-MS.

WP3: Spectral tuning of enzymatic reactions using red-shifted PSI

Here we will explore the use of PSI variants that absorb in the near-infrared by using the far-red chlorophylls *d* and *f*. The Nürnberg lab has optimised the growth for the two cyanobacteria, *Acaryochloris marina* (containing chl *d*) and *Chroococcidiopsis thermalis* (containing chl *f*) in large-scale photobioreactors and successfully isolated photosystems [6]. After biophysical and biochemical characterisation, we will test, in collaboration with F. Lisdat (TH Wildau), whether these photosystems with enhanced spectral range can generate higher photocurrents.

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