Construction of a synthetic light-driven enzymatic supra-metabolon

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Photosystem I (PSI) from plants, algae and cyanobacteria utilizes energy harvested from sunlight to mediate light-driven electron transport across the thylakoid membrane and produce reducing equivalents for the metabolic reactions of the photosynthetic organism. Cytochrome P450s constitute a very large and highly versatile superfamily of membrane-bound enzymes that catalyse a wide variety of different reactions, the most frequent being highly stereo- and regiospecific monooxygenations. In this project we are aiming at coupling PSI directly to a cytochrome P450 (P450) to develop a system in which the enzymatic reaction of some P450s is driven directly by the energy of solar light. As photosynthetic host organism we use the cyanobacterium *Synechococcus* sp. PCC 7002, which can be transformed, is fast growing and is tolerant to high light intensities.

In nature, PSI transports electrons from plastocyanin or cytochrome c_6 on the lumenal side to ferredoxin (Fd) or flavodoxin (Fld) on the stromal side of the thylakoid membrane. The electrons supplied by PSI may via the soluble electron carriers Fd or, during iron deficiency in some algae and cyanobacteria, Fld be donated to ferredoxin $NADP^+$ oxidoreductase (FNR), which reduces $NADP^+$ to NADPH. *In vivo*, the electrons required for the reactions of the P450s are taken from NADPH through the NADPH-cytochrome P450 oxidoreductase (CPR) enzyme. Our recent results from *in vitro* experiments indicate that the delivery of electrons to the P450 may not have to involve the production and oxidation of NADPH, but that direct electron transfer from PSI via Fld or Fd to a P450 adjacent to the PSI may be achievable.

A supra-metabolon containing PSI and a cytochrome P450 provides the opportunity for direct utilization of solar energy for production of complex chemical products. In this synthetic enzymatic supercomplex, the energy of the photons harvested by PSI will be utilized to mediate electron transport to the cytochrome P450 enzyme. To facilitate highly efficient electron transfer, the fusion protein complex has been designed to keep the interacting proteins in close proximity to each other. Thus we are aiming at linking the P450 directly to one of the small subunits of PSI containing one transmembrane domain. The P450 is fused to a PSI transmembrane domain so it can integrate directly into the PSI complex. Ultimately, the aim is to generate a system in which the fusion protein is stably expressed in the thylakoid membrane of *Synechococcus* and utilized to carry out the desired enzymatic reactions *in vivo*.