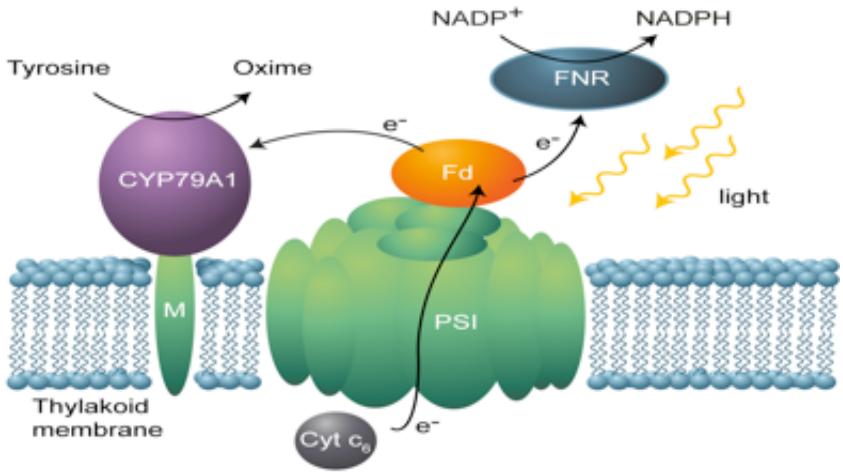
Driving metabolic pathways on with sunlight

December 1 2015, by Konstantinos Vavitsas



Credit: Lassen et al.

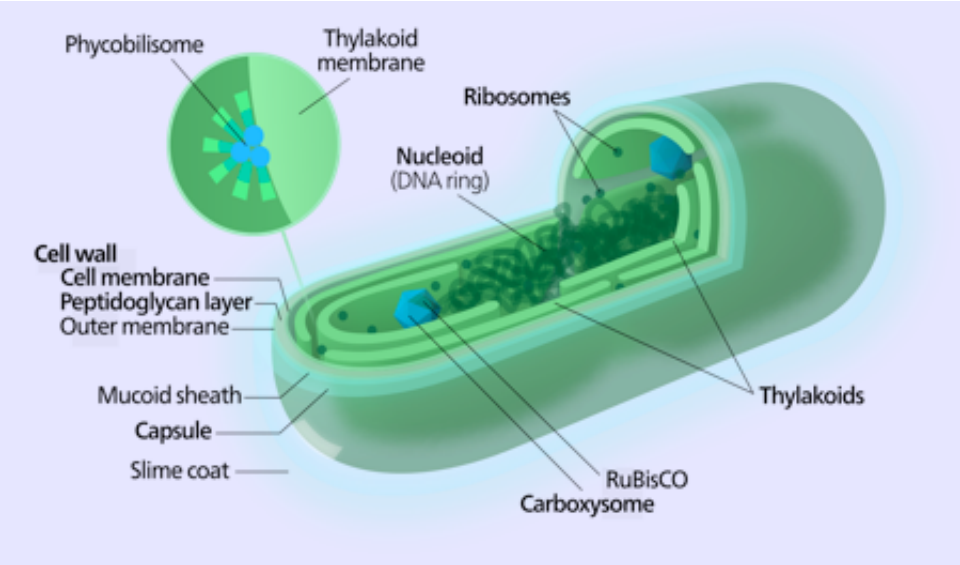
Most life forms are directly or indirectly dependent on photosynthesis. The question is, can we exploit sunlight more broadly than in carbohydrate production, making it effectively a synthetic biology part? As an answer to this, a research group from University of Copenhagen published an article on using photosynthetic electrons to drive cytochrome P450 enzymes in cyanobacteria.

Cytochrome P450s are enzymes that oxygenate organic compounds stereo-specifically and are involved in numerous metabolic routes. Their reaction mechanism requires electrons, usually obtained from redox cofactors such as NADPH. This availability of reducing power is often the bottleneck in heterologous expression plant pathways in microbes: in

the aremisinin (1) and taxol (2) production in yeast and E. coli respectively, the biosynthetic steps catalyzed by P450s required laborious optimization. There might be however another way to overcome this hurdle, the answer could lie in photosynthesis.

The subject of the latest research article from my research group is around harnessing sunlight and redirecting it towards desired metabolic compounds. The paper, titled "Metabolic engineering of light-driven cytochrome P450 dependent pathways into Synechocystis sp. PCC 6803" (3), was recently published in Metabolic Engineering. In this work, my colleagues engineer Synechocystis sp. PCC6803, a popular cyanobacterium model, to produce dhurrin (a cyanogenic glucoside from Sorghum bicolor).

Sorghum utilizes dhurrin as a defense compound: when the leaf tissues are mechanically disrupted (chewed), dhurrin degrades and hydrogen cyanide releases, poisoning the unfortunate herbivores. Its biosynthesis commences from tyrosine, and involves two distinct cytochrome P450s and a glycosyltransferase. Although dhurrin has no commercial use, its biosynthesis has evolved into a model pathway for understanding plant P450 functionality (I refer the curious reader to the work of Professor Birger Møller 's research and the University of Copenhagen Center for Synthetic Biology).

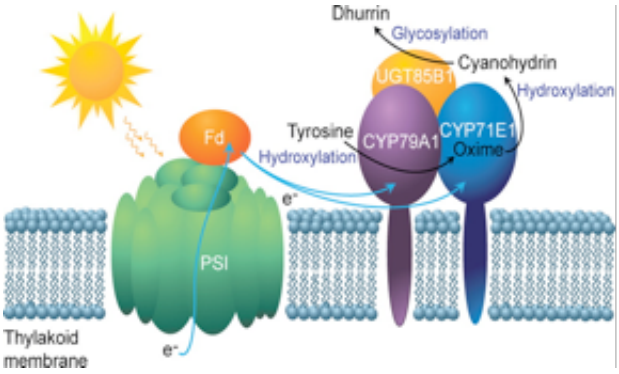


Cyanobacterium-inline Credit: Kelvinsong CC BY-SA 3.0 via Wikimedia Commons

More interestingly, the dhurrin P450s were used to demonstrate that it is possible for P450s to obtain electrons directly from the photosynthetic apparatus. Eukaryotic P450s are normally located in the endoplasmic reticulum and rely on a NADPH-dependent dedicated oxidoreductase as a redox partner. However, in vitro and in vivo experiments have shown that if the P450s localize in the proximity of the photosystem I, they can retain activity by gaining electrons from photoreduced ferredoxin, thus bypassing the specialized reductase requirement (4,5). Since this concept worked quite well in plant chloroplasts, our group saw no reason that this principle is not transferable to cyanobacteria.

Coming back to our recent paper, the authors introduced the three enzyme sequences of the dhurrin pathway into a self-replicating vector as an operon, their expression controlled by a strong inducible promoter. Under theophyline induction, cyanobacteria produce dhurrin and excrete most of it to the growth medium. The productivity was also tested in 8

Liter bioreactors, where dhurrin accumulation reached 3.2 mg dhurrin L-1OD-1 after 7 days of cultivation.



Schematic representation of the dhurrin pathway, localized in the thylacoid membranes, using ferredoxin as electron donor.

The effect of dhurrin production on cell fitness was also tested. When the whole pathway was expressed, there was a small delay in cell growth. But the strains expressing only the two first enzymes (the p450s without the glycosyltransferase) displayed a severe poisoning phenotype. Electron microscopy revealed rearrangements of the thylacoid structures and the lack of glycogen granules. These side-effects were not observed in the full pathway-expressing strain, suggesting that some intermediate may have toxic effect and that the glycosilation is crucial for the detoxification and secretion of this potential poison.

Hijacking electrons from photosynthesis is a promising bioengineering alternative, especially in the cases where reducing power and co-factor availability are limiting. This study shows that Synechocystis is receptive to this practice, and paves the way for further metabolic engineering work, aiming to produce more and commercially interesting compounds. Cyanobacteria are prominent vessels for synthetic biology approaches,

recently receiving attention from NASA as polymer construction hosts (see the hangout with Dr. F. Zhang). Even though it might be some time before seeing photosynthetic organisms doing large scale production in space (or in Mars, according to our 2015 iGEM SpaceMoss team), the principle remains the same: light, CO2 and water—feedstock plentifully available in a resource-limited planet—are captured by photosynthetic microbial cell factories to produce any fuel, nutrient, or pharmaceutical.

More information: Paddon CJ, Westfall PJ, Pitera DJ, Benjamin K, Fisher K, McPhee D, et al. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature*. 2013 Apr 25;496(7446):528–32.

Ajikumar PK, Xiao W-H, Tyo KEJ, Wang Y, Simeon F, Leonard E, et al. Isoprenoid Pathway Optimization for Taxol Precursor Overproduction in Escherichia coli. *Science*. 2010 Oct 1;330(6000):70–4.

Wlodarczyk A, Gnanasekaran T, Nielsen AZ, Zulu NN, Mellor SB, Luckner M, et al. Metabolic engineering of light-driven cytochrome P450 dependent pathways into Synechocystis sp. PCC 6803. *Metab Eng*. 2016 Jan;33:1–11.

Jensen K, Jensen PE, Møller BL. *Light*-Driven Cytochrome P450 Hydroxylations. ACS Chem Biol. 2011 Jun 17;6(6):533–9.

Nielsen AZ, Ziersen B, Jensen K, Lassen LM, Olsen CE, Møller BL, et al. Redirecting Photosynthetic Reducing Power toward Bioactive Natural Product Synthesis. *ACS Synth Biol*. 2013 Jun 21;2(6):308–15.