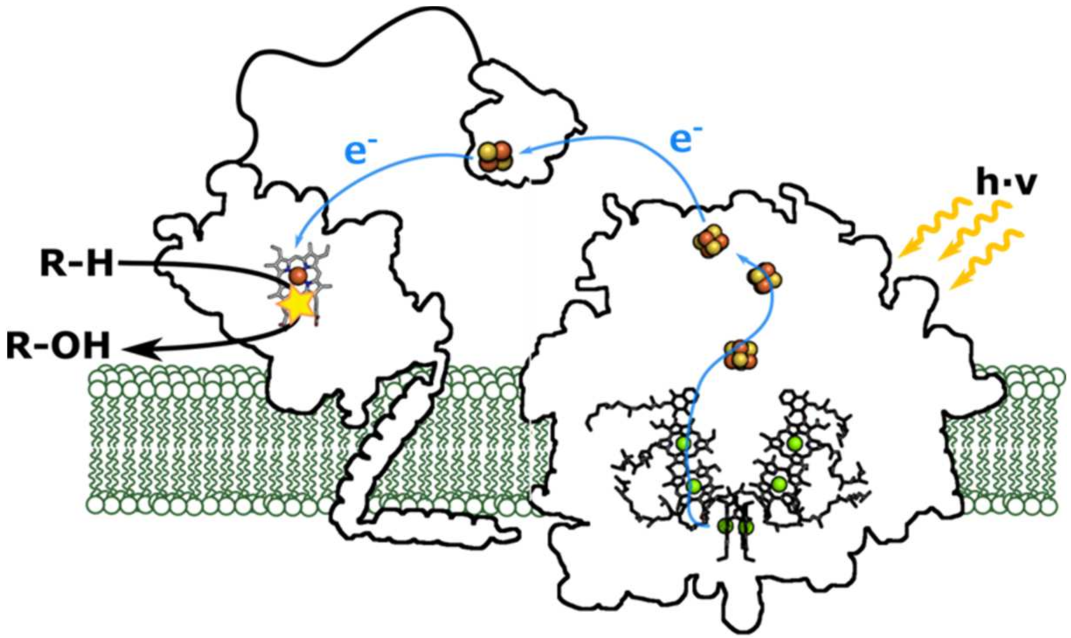
UNIVERSITY OF COPENHAGEN

Faculty of Science

Metabolic engineering for high-value compounds and efficient secretion

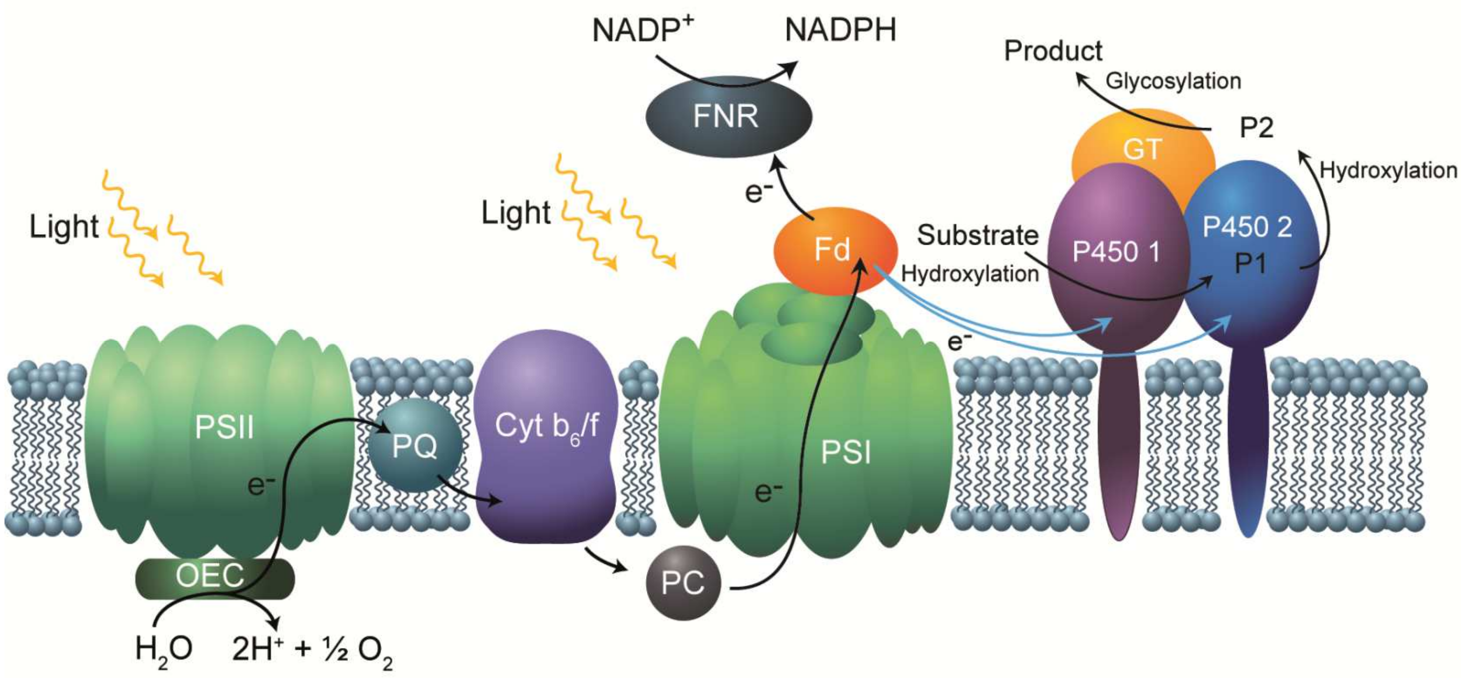
Poul Erik Jensen



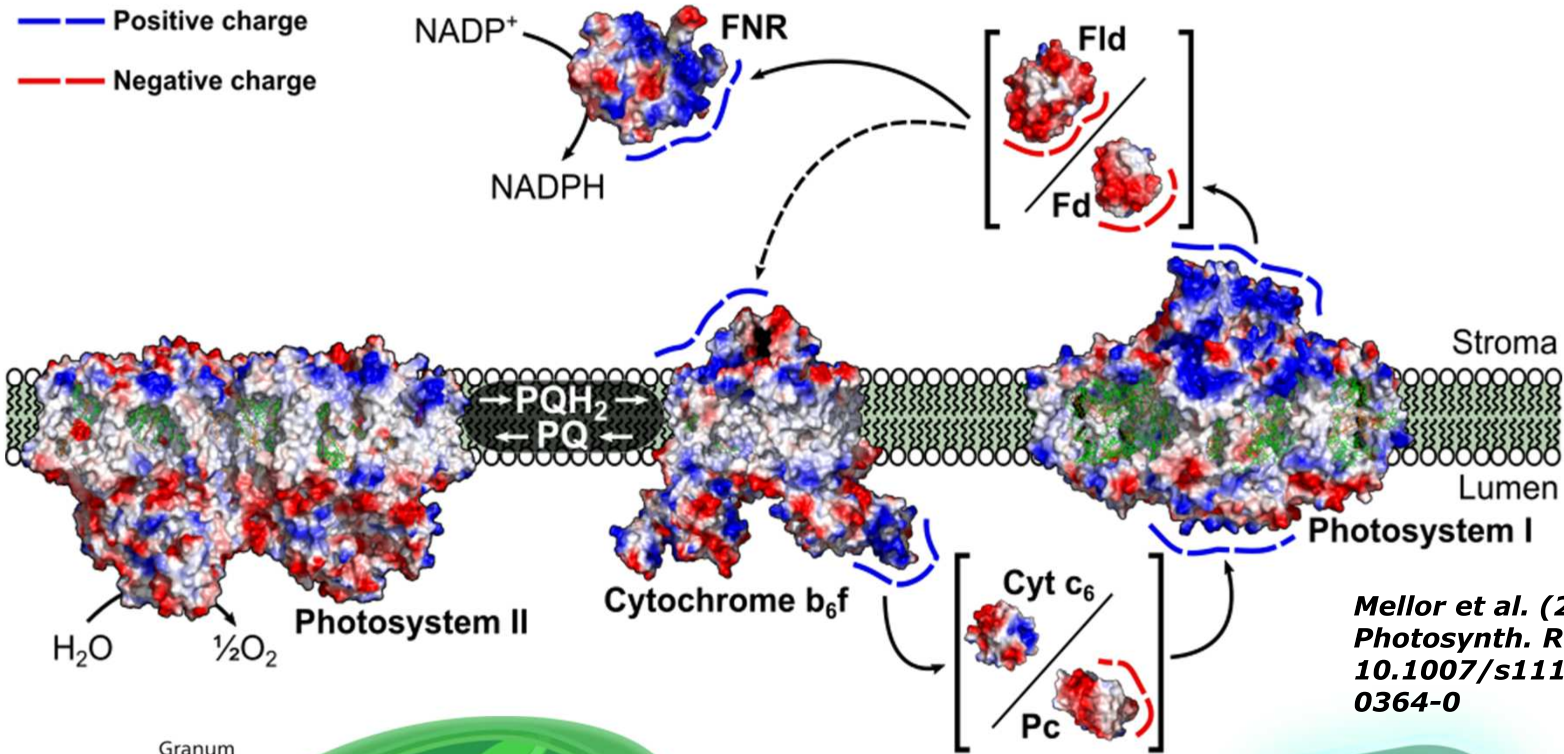
Metabolic engineering for high-value compounds and efficient secretion

Outline

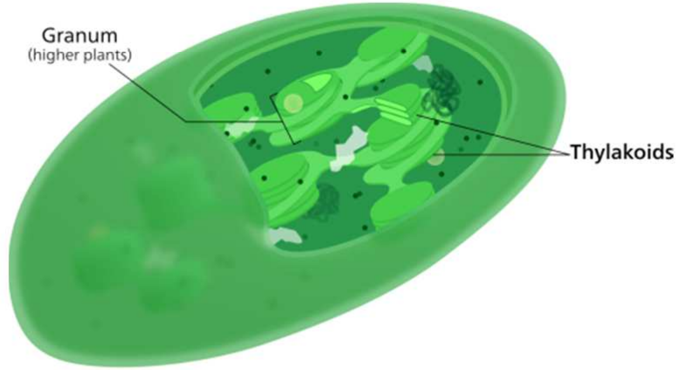
1. Light-driven cytochrome P450s for biosynthesis of high-value compounds in chloroplasts and cyanobacteria
2. Recombinant protein secretion using the green algae Chlamydomonas



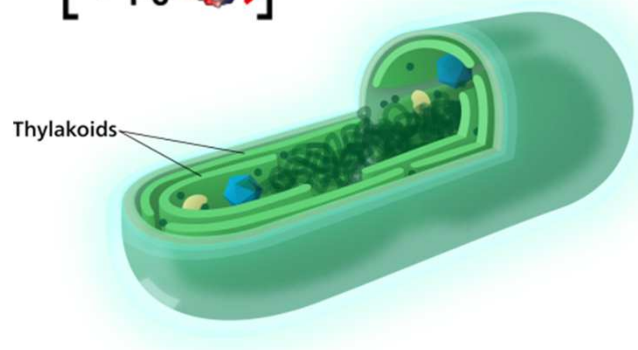
Can photosynthetic electrons be redirected to other pathways?



Mellor et al. (2017) Photosynth. Res.: DOI: 10.1007/s11120-017-0364-0



chloroplast



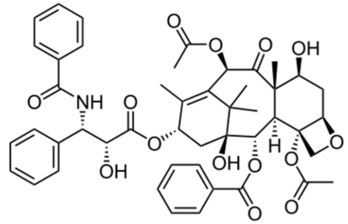
cyanobacterium

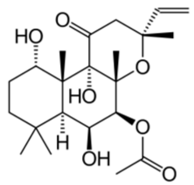
Plant–derived specialized metabolites

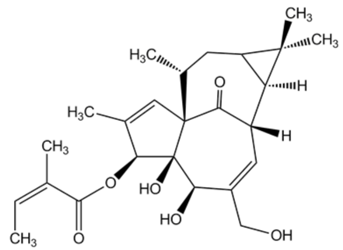
Taxol

Forskolin

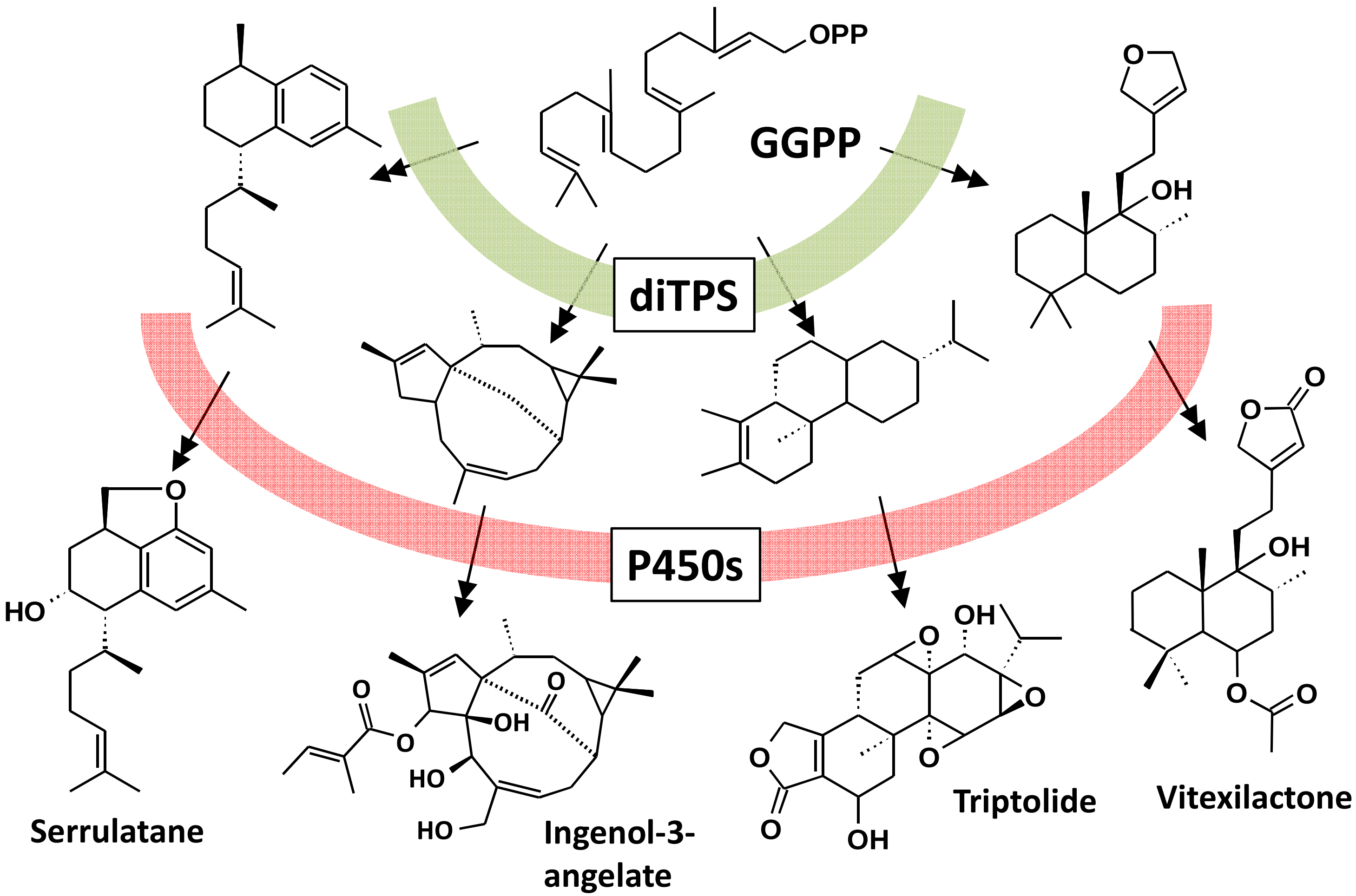
Ingenol-3-
angelate





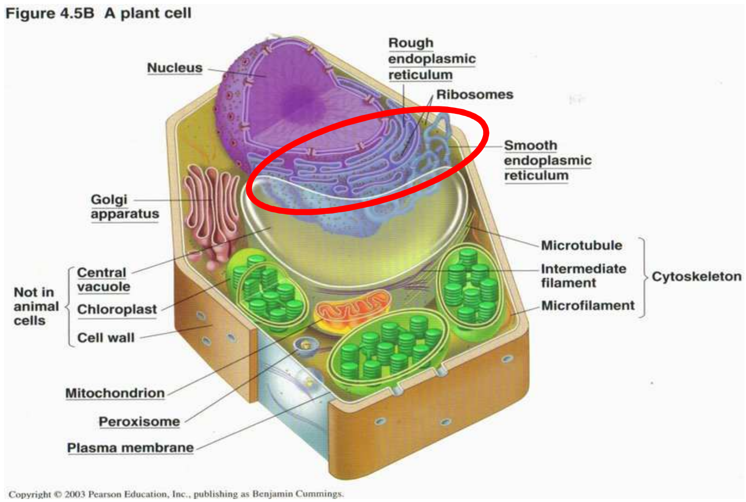


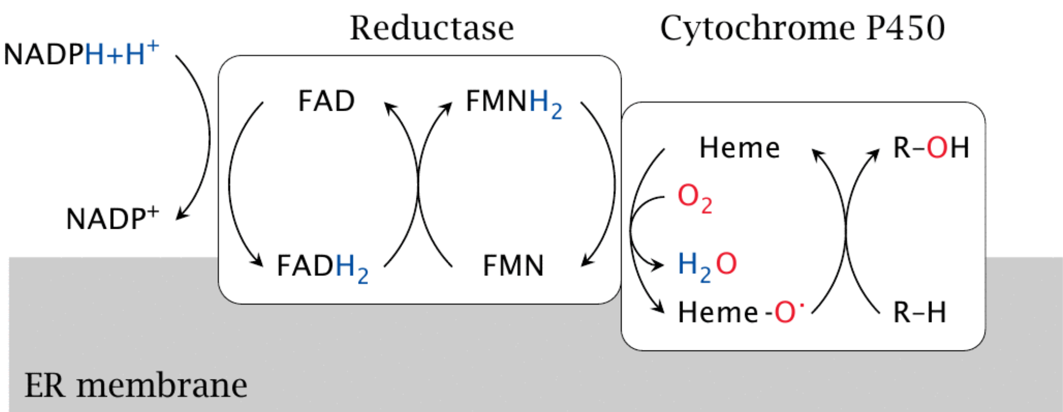
Diterpenoids – two classes of enzymes

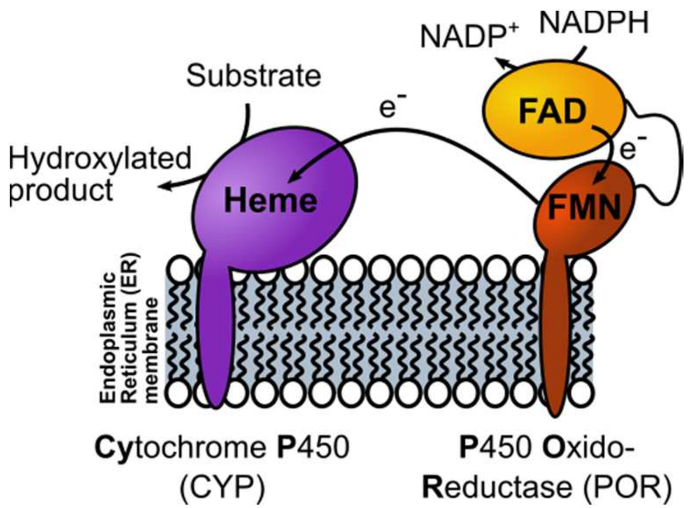


Cytochrome P450s are key for functionalization

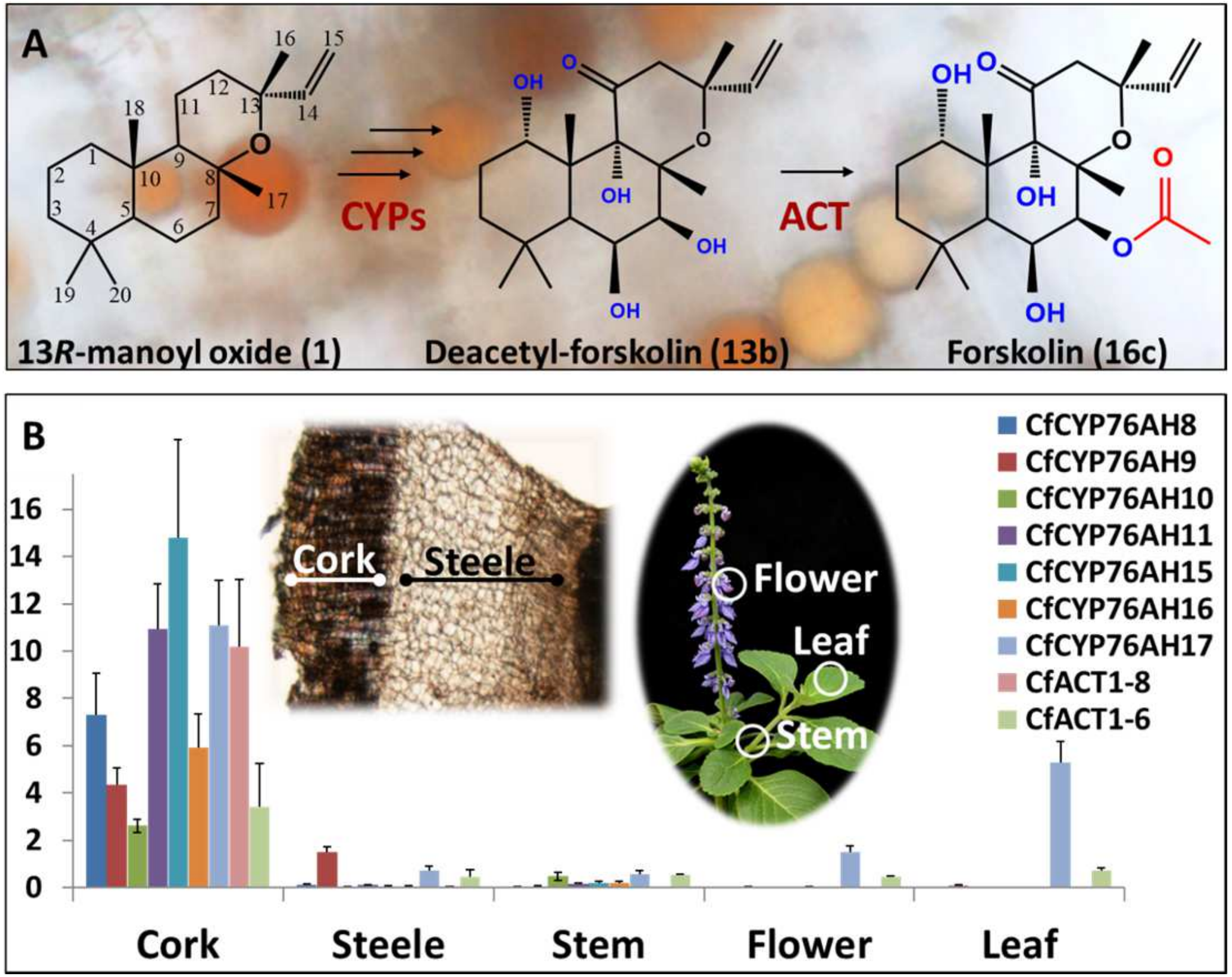
- **Heme-containing monooxygenases**
- **Stereo and regio-specific**
- **Membrane bound**
- **Require dedicated reductase (POR) and NADPH, i.e. electrons**





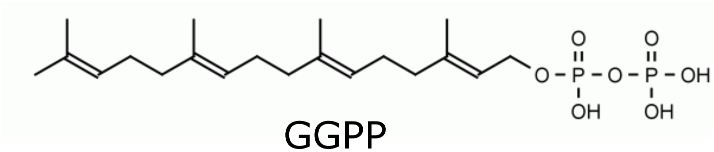


Biosynthesis of forskolin in the root cork cells of *C. forskohlii*



Irini Pateraki et al., eLIFE: 10.7554/eLife.23001

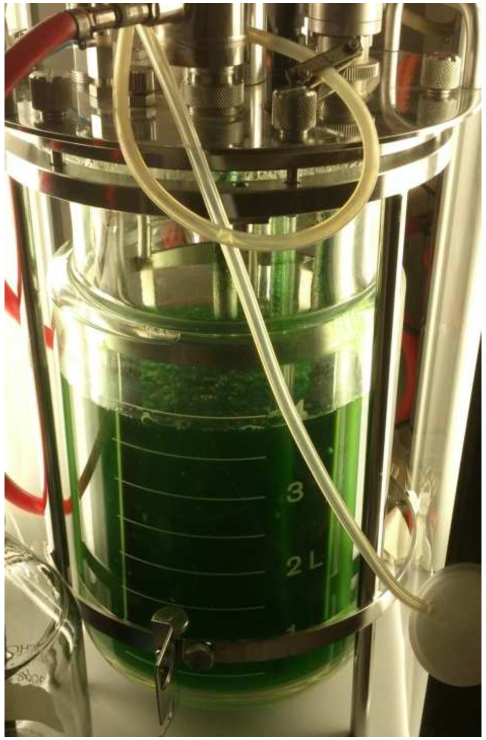
Light-driven synthesis of forskolin

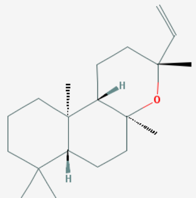


GGPP
(geranylgeranyl pyrophosphate)

Photosynthetic fixed CO_2

diterpene synthases





manoyl oxide

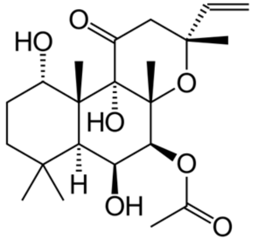
Photosynthetic electron transport

e^-

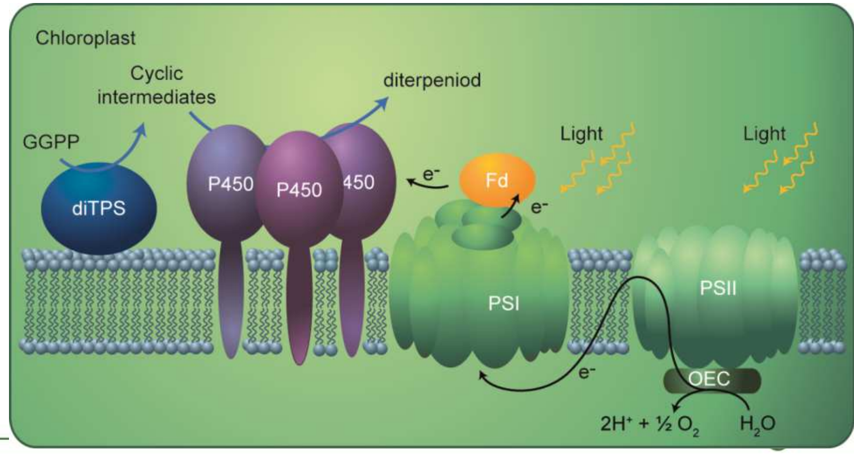
Fd

e^-

cytochrome P450s



Forskolin



BUT…

Low yields!

N. tabacum

1-2 mg/g
leaf DW

Synechocystis sp. PCC 6803

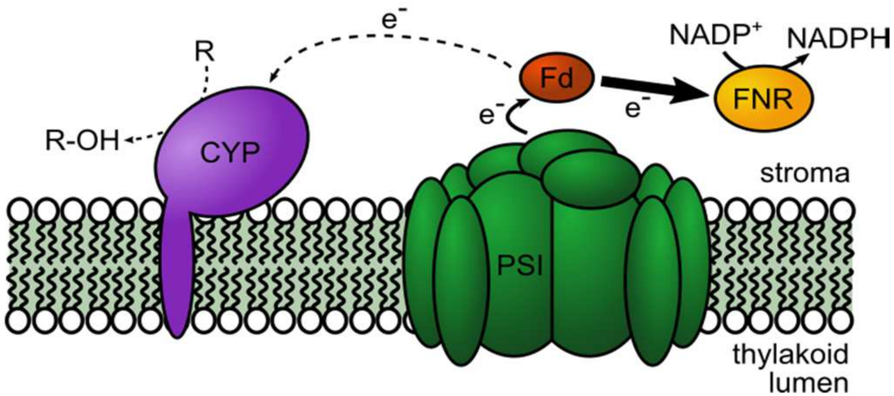
low μg/L

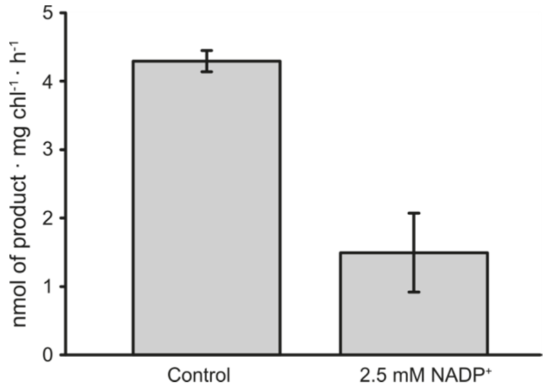
Gnanasekaran, T. *et al. J. Exp. Bot.* **67,** 2495–506 (2016)

Wlodarczyk, A. *et al. Metab. Eng.* **33,** 1–11 (2016)

Photosynthetic electrons have many fates

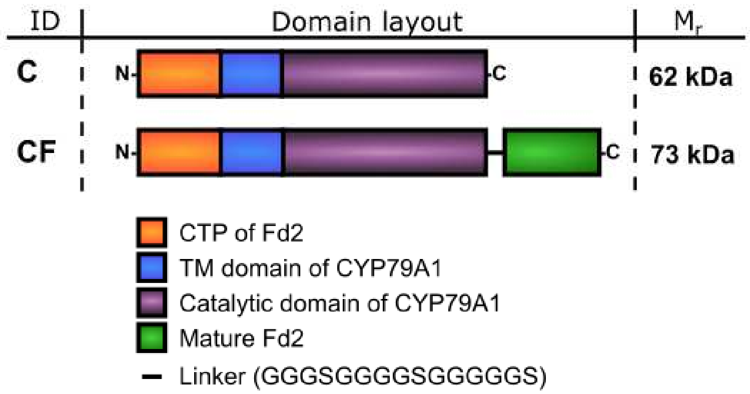
- **Competition from native sinks severely limits P450 activity**





Nielsen AZ, Ziersen B, Jensen K, Lassen LM, Olsen CE, Møller BL, Jensen PE (2013) ACS Synth. Biol. 6: 308-315

Overcoming competition from native electron sinks: Ferredoxin-CYP79A1 fusion



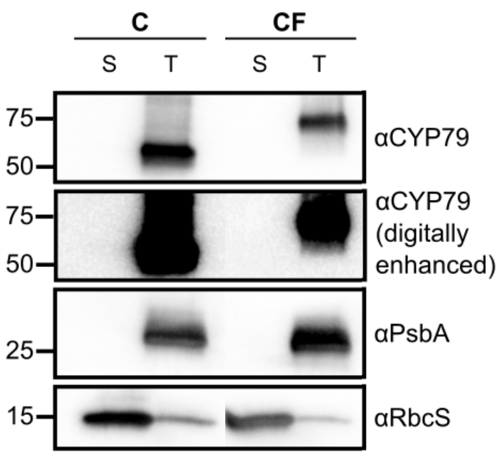
Transient expression in tobacco plants

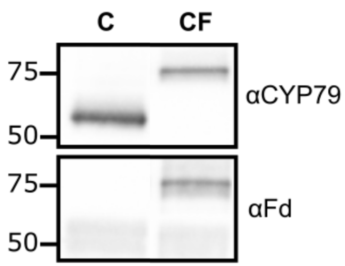
Chloroplasts
Thylakoids
Protein
Metabolites

Enzyme assays

Immunoblots

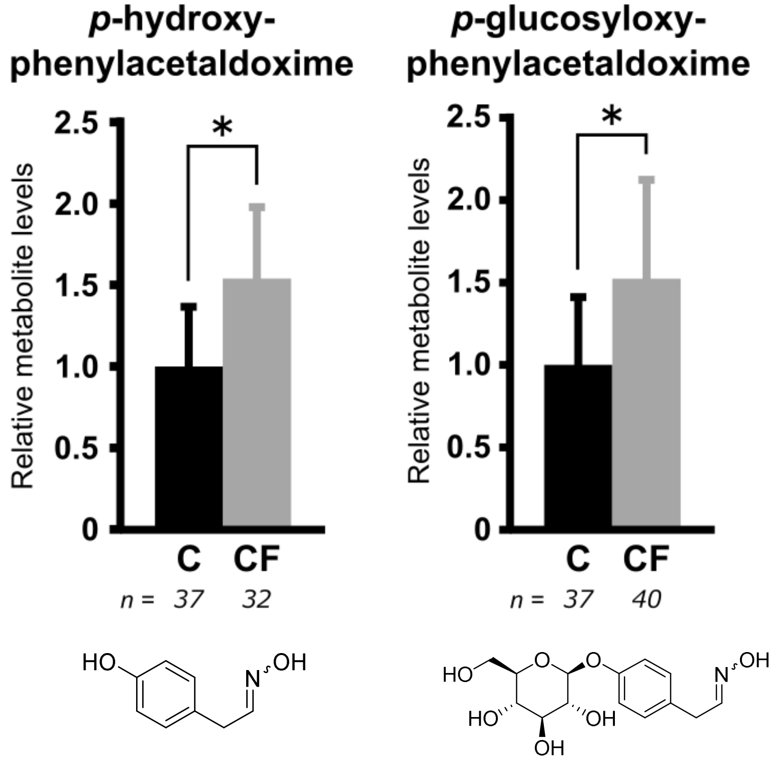
LC-MS/MS quantification

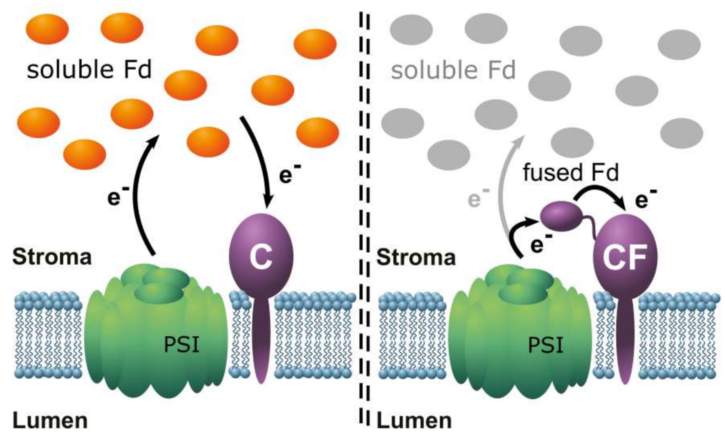




Mellor et al., (2016). ACS Chemical Biology, DOI: 10.1021/acschembio.6b00190.

Ferredoxin fusion shows better *in vivo* activity



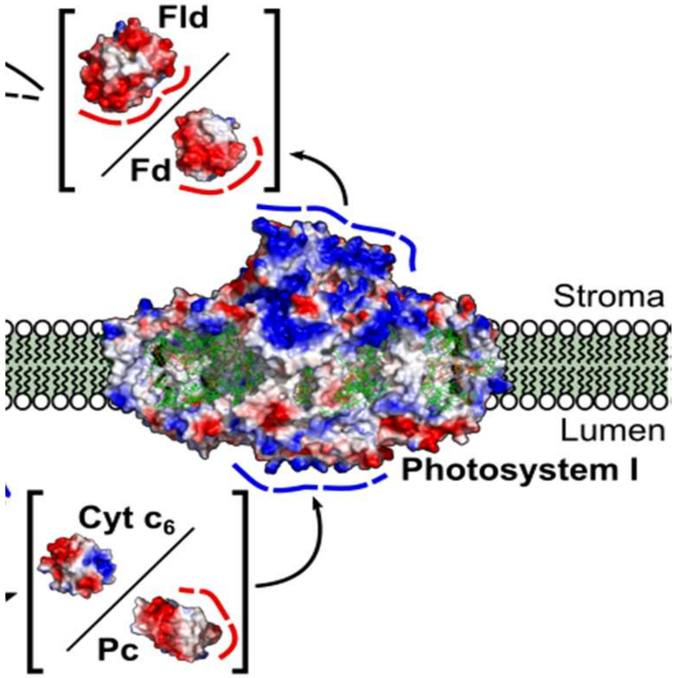


LC-MS/MS analysis of total leaf extracts
Metabolite levels were normalised to protein abundance

→ **Specific activity of fusion is 50% higher than unfused P450**

Mellor et al., (2016). ACS Chemical Biology, DOI: 10.1021/acschembio.6b00190.

Conclusions-1

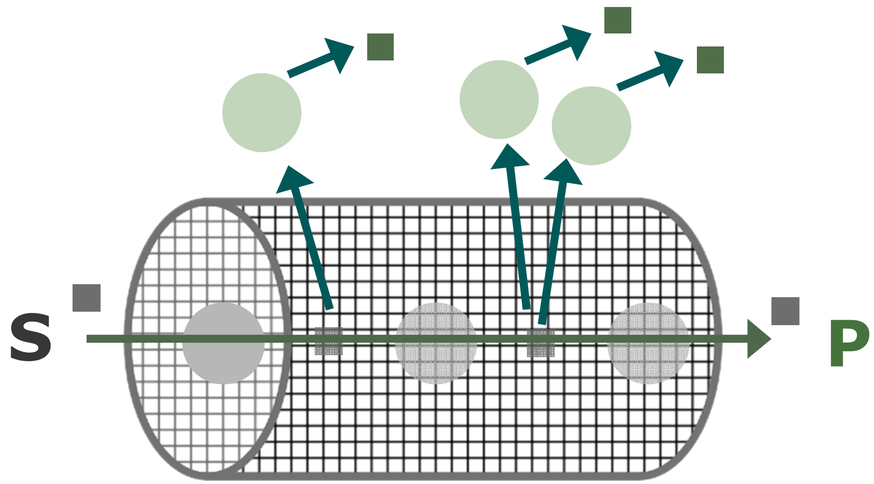


- **Competition for electrons**
- **Fusion between P450s and ferredoxin improves electron transfer and reduces electron loss to competing enzymes**
- **Stability of the fusion protein is compromised**

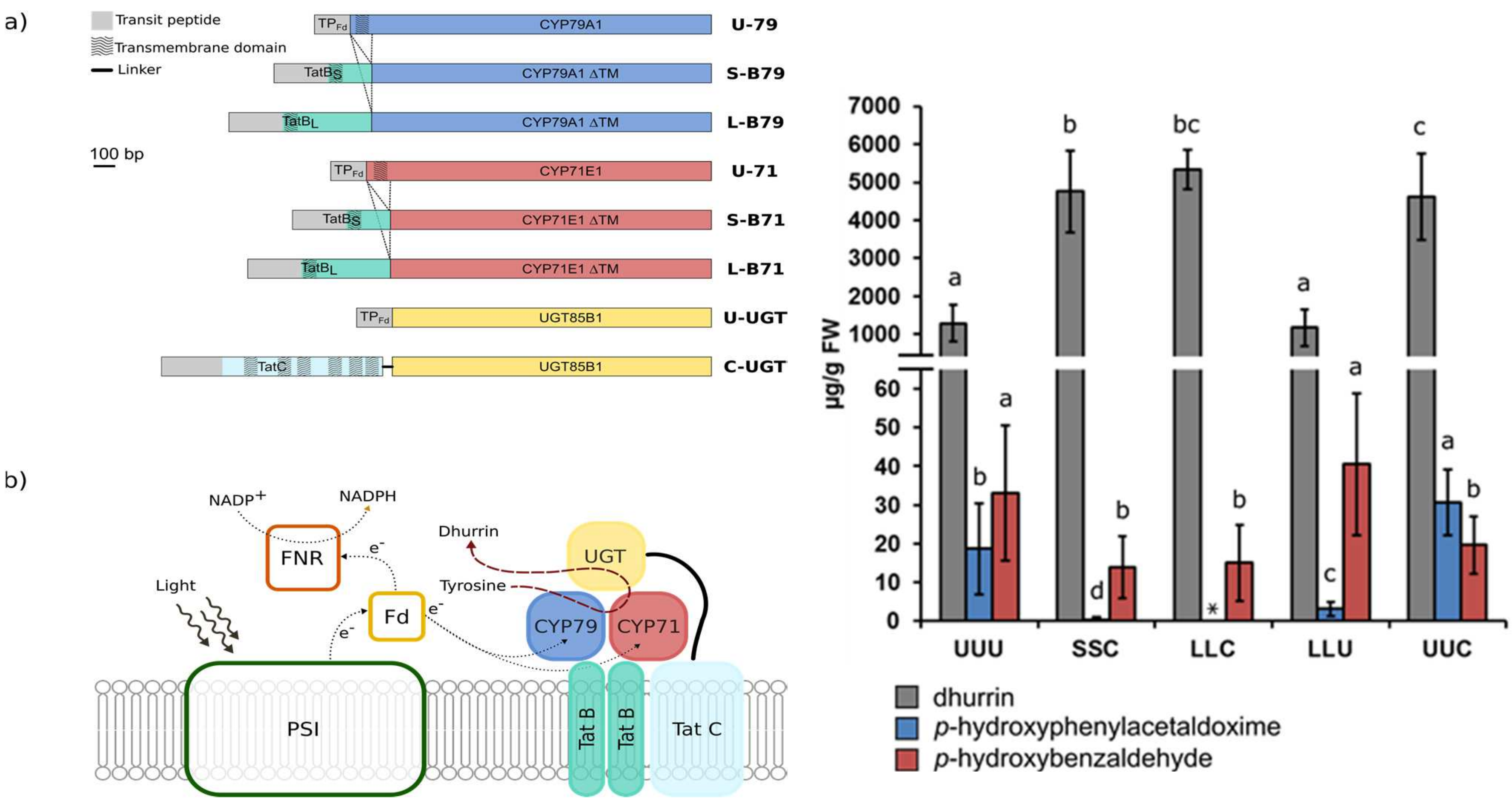
Scaffolding -
Need for spatial Organization of the pathway!

Why?

- **Facilitates substrate flow between interacting enzymes**
- **Limits cross-talk between signaling pathways**
- **Increases yields of sequential metabolic reactions**



Tat-mediated scaffolding lead to 5-fold increase in product formation

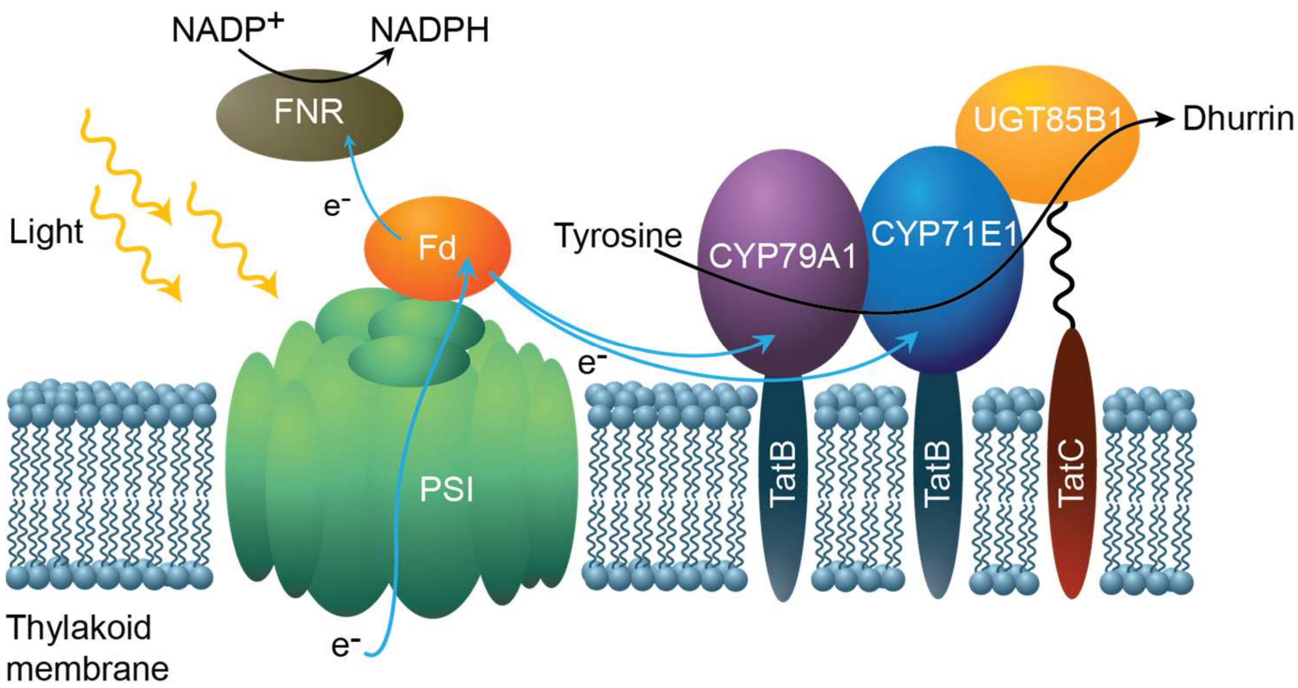


- clear reduction in the amount of both intermediates in both SSC and LLC combinations
- targeting of the UGT to the membrane is key to increase the channeling of the nitrile towards dhurrin

Maria Perestrello Jesus et al., (2017). Metabolic Engineering 44: 108–116

Conclusions-2

- P450s are functional after exchange of the membrane anchors.
- Targeting the soluble glycosyl transferase (UGT) to the membrane is key to maximize product formation.
- Synergy between TatB fused P450s and the TatC fused UGT: efficient substrate channelling and less intermediates and side-products.

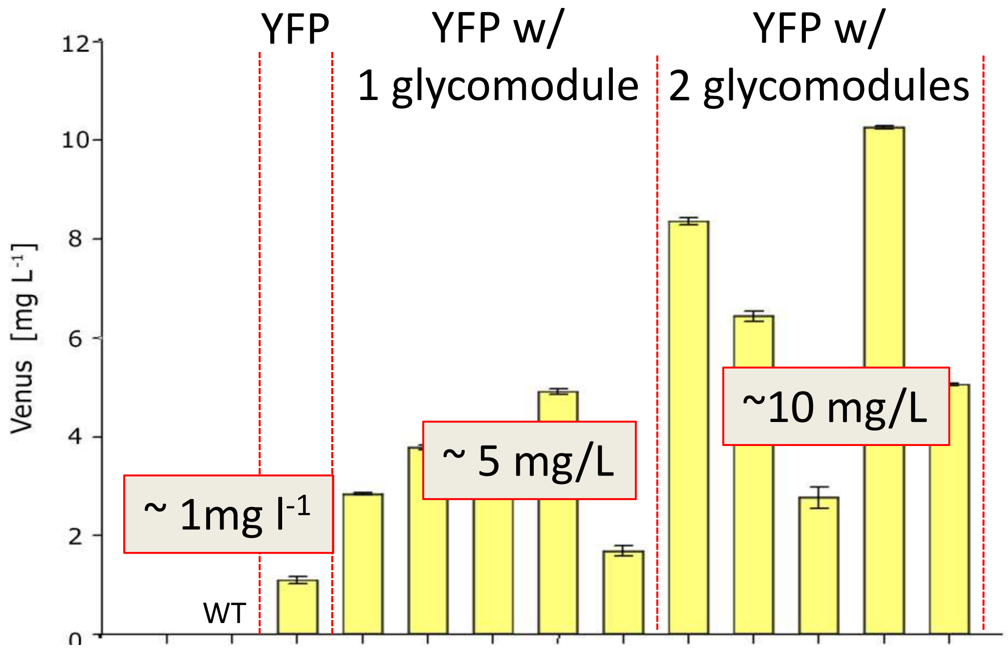


Sakuragi group

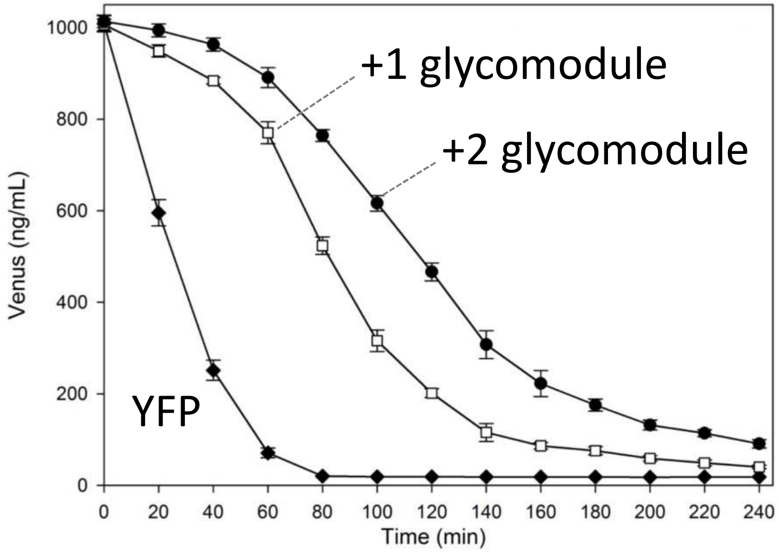
Recombinant protein secretion using the green microalgae *Chlamydomonas redinhardtii*

Benefits: Glycosylation

Increased yields



Increased stability

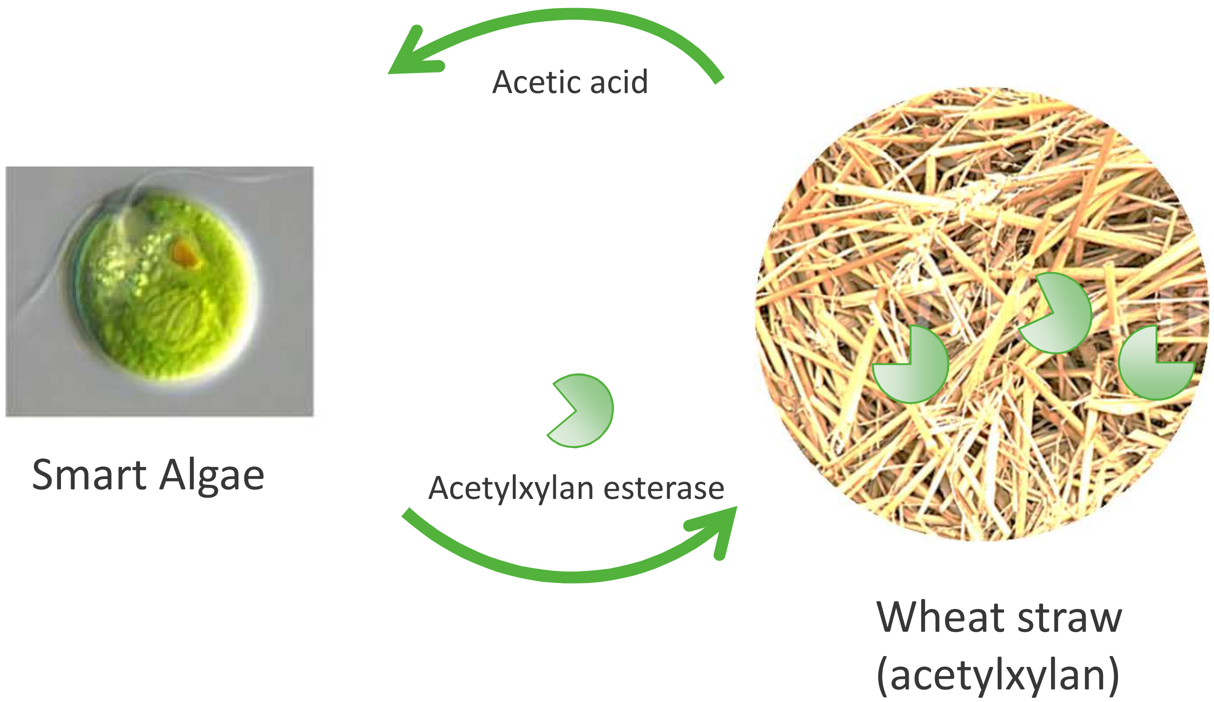


Ramos-Martinez EM, Fimognari L, Sakuragi Y (2017) Plant Biotech J

Smart Algae: use of microalgae and recombinant protein secretion for upgrading lignocellulosic biomass

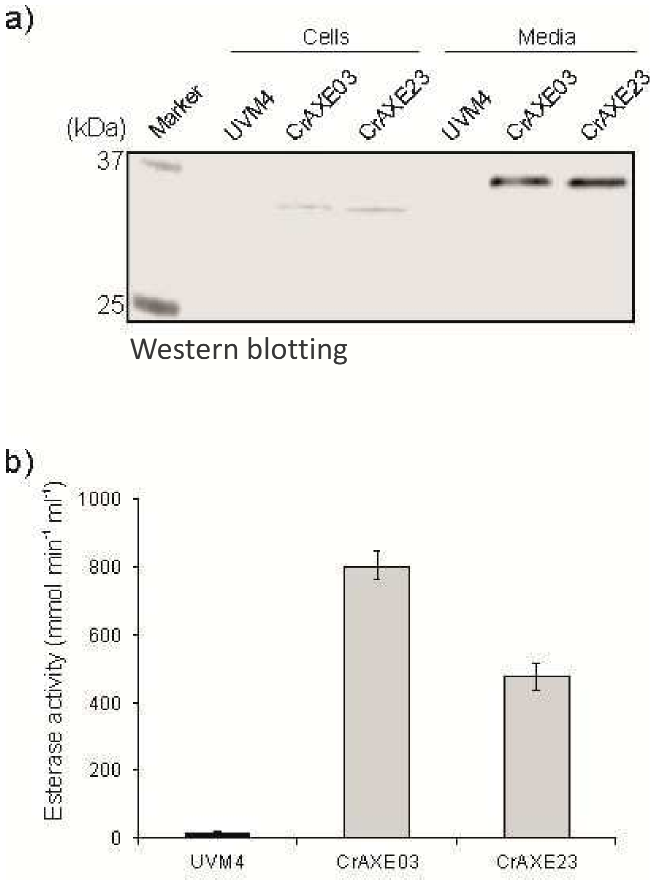
A problem: **acetic acid** released from lignocellulosic biomass inhibits yeast fermentation in 2G biofuel production.

Solution: removal of acetylesters by transgenic *Chlamydomonas reinhardtii* secreting a fungal acetylxylan esterase (smart algae)

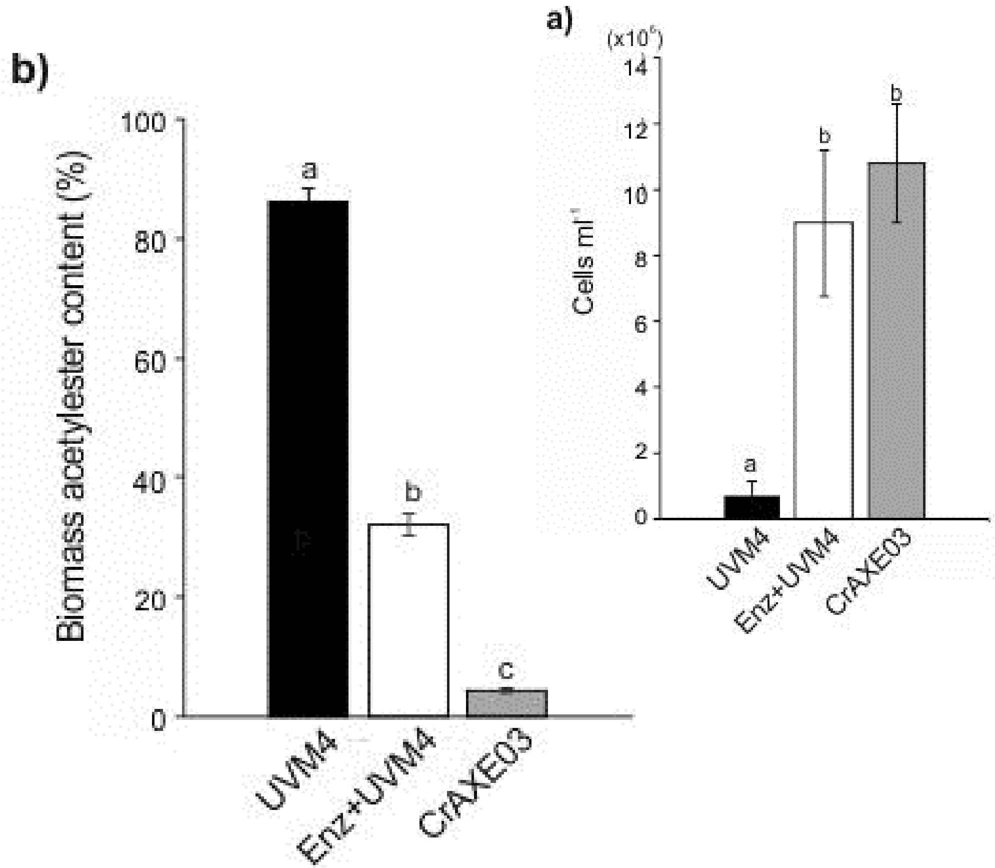


Transgenic lines CrAXE03 and CrAXE23 reduced acetylester contents in wheat biomass

CrAXE03 and CrAXE23 secrete acetylxylan esterase in media

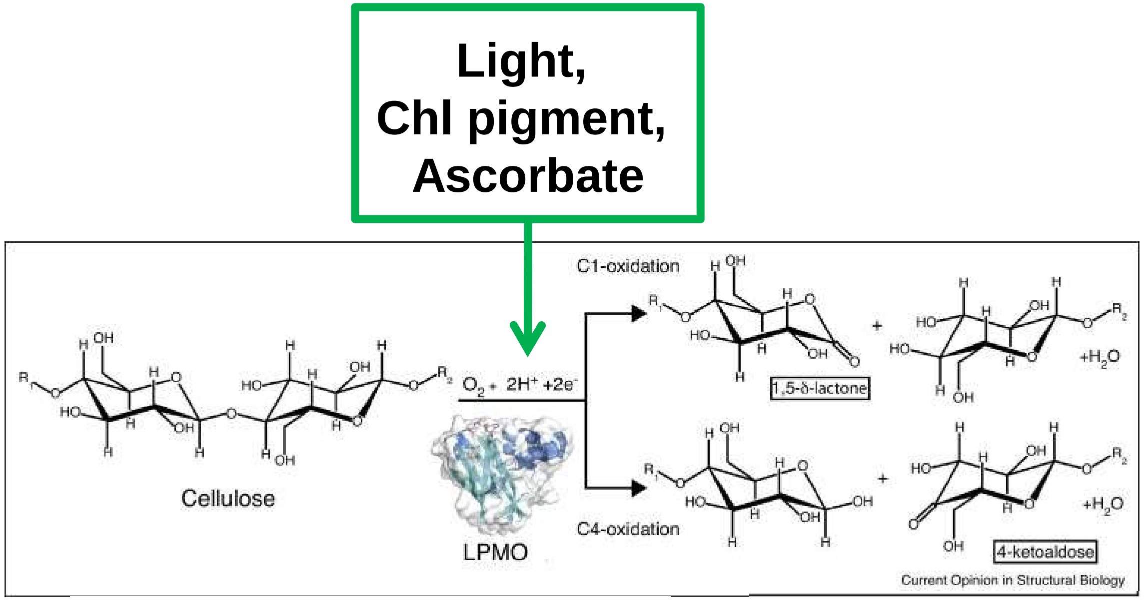


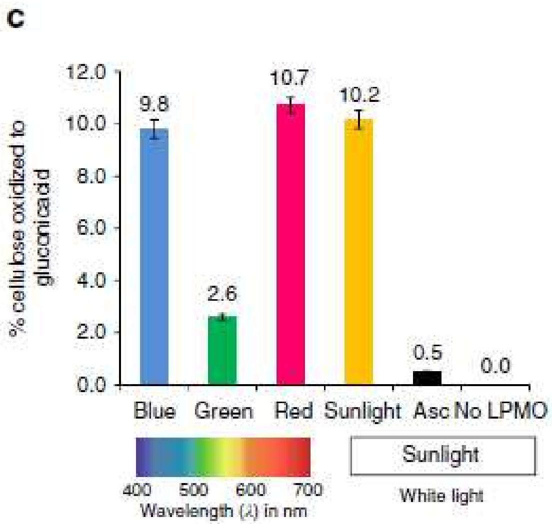
CrAXE03 removed acetylesters from wheat biomass and increase algal biomass

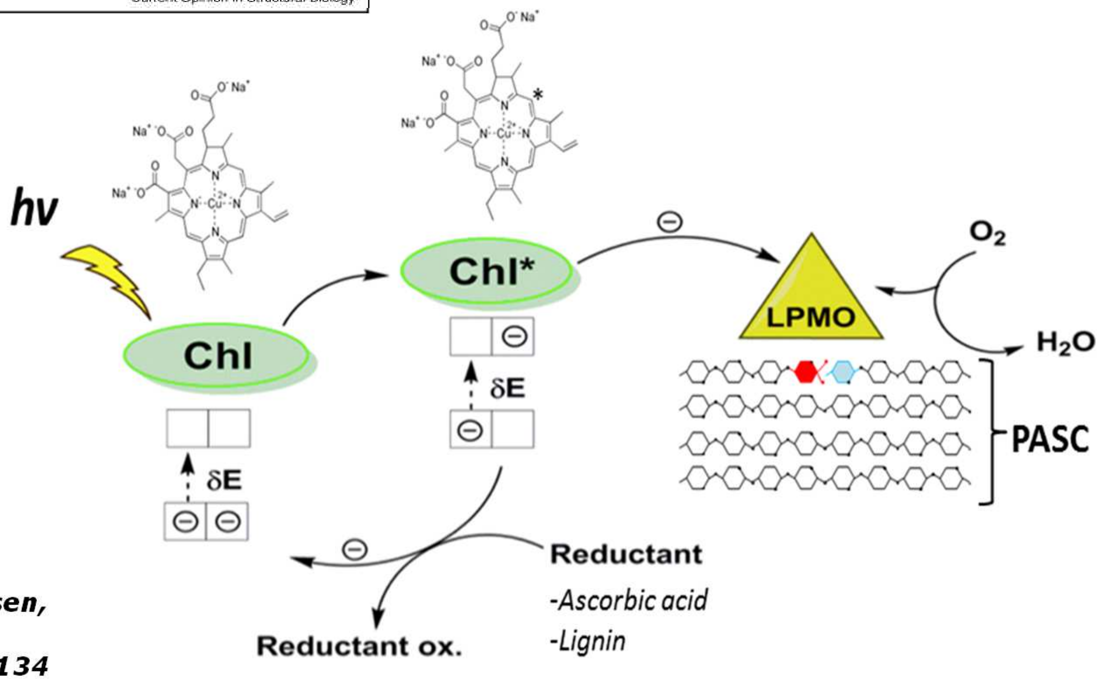


Ramos-Martinez EM, Fimognari L, Rasmussen MR, Sakuragi Y. (2017) PCT application

Light-driven LPMOs – Cellulose oxidation by light







D. Cannella, K.B. Möllers, N-U. Frigaard, P.E. Jensen, Bjerrum, K.S. Johansen and C. Felby (2016) Nature Comm. 7: 11134 doi:10.1038/ncomms11134

Acknowledgments

Photosynthesis group

- Kamil Bakowski
- Lawrence Sutardja
- Dainius Jakubauskas
- Dr. Lars Scharff
- Dr. Annemarie Matthes
- Laura Maria Furelos Brey
- Dr. Julie Zedler
- Dr. David Russo
- (Dr. Agnieszka Zygadlo Nielsen)
- (Maria Perestrello Jesus)

Others

- Dr. Yumiko Sakuragi
- Dr. Mathias Pribil
- Dr. Meike Burow
- Prof. Birger Lindberg Møller
- Dr. Mohammed Saddik Motawia
- Prof. Ralph Bock, MPI Golm
- Dr. Guy Thomas Hanke, QMU London
- Prof. Colin Robinson, U.Kent
- Prof. Claus Felby

ITN: Photo.comm
Funded by the EU