ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

Kick-off session: "Biotechnology for a sustainable bioeconomy"

MicroalgaE as Renewable Innovative green cell facTories

MERIT

Olaf Kruse, Alison Smith, Rene Wijffels, Josue Heinrich, Andrew Spicer

presenting author: Lutz Wobbe (Kruse group)

MERIT

MicroalgaE as Renewable Innovative green cell facTories

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

Frankfurt am Main, 14.06.2018

ERA CoBioTech

Prof. Olaf Kruse (coordinator)

Universität Bielefeld

- Bielefeld University / Center for Biotechnology (CeBiTec) / Algae Biotechnology & Bioenergy Group / Germany
- Total project budget: 495 k€
- Project start: 1st Month

CeBiTec
Center for Biotechnology

ALGAE BIOTECHNOLOGY AND BIOENERGY GROUP

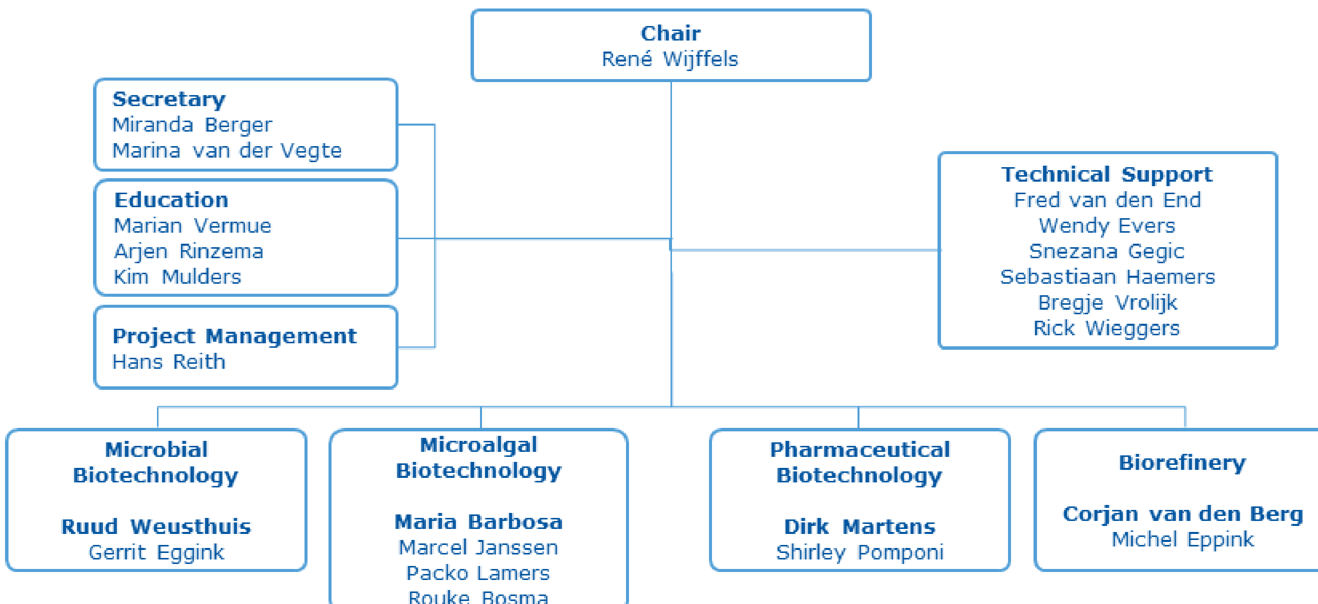
Prof. Alison Smith

- University of Cambridge / Department of Plant Sciences / United Kingdom
- Total project budget: 428 k€
- Project start: 1st Month

DEPARTMENT OF
Plant Sciences

Prof. René Wijffels

- Wageningen University / Bioprocess Engineering / Netherlands
- Total project budget: 250 k€
- Project start: 1st Month



In **bold** the theme group leaders

Prof. Josue Heinrich

- Universidad Nacional del Litoral (Santa Fe) / Faculty of Biochemistry and Biological Sciences / Argentina

- Total project budget: 100 k€

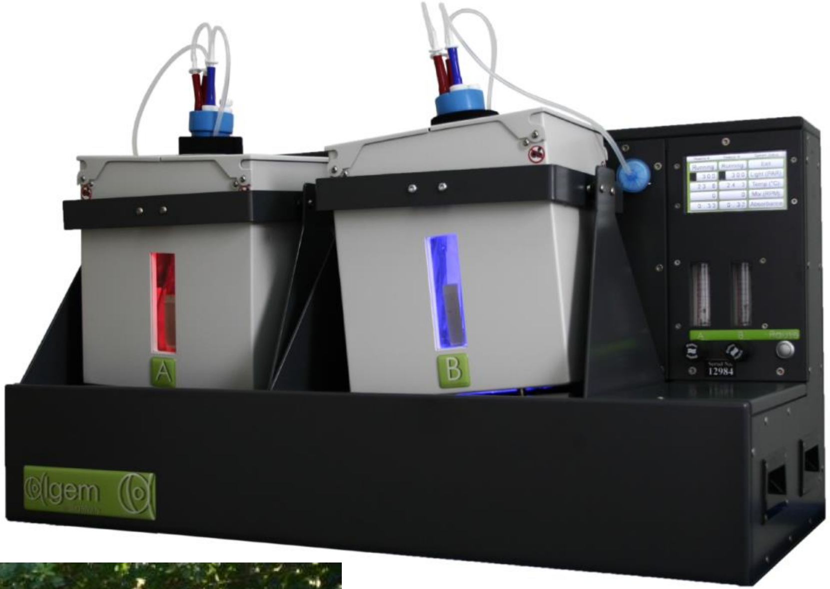
- Project start: 1st Month

GRUPO DE INNOVACIÓN EN INGENIERÍA DE BIOPROCESOS

Dr. Andrew Spicer

Algenuity
cultivating algal biotech

- Spicer Consulting Limited / Algenuity / United Kingdom
- Total project budget: 384 k€
- Project start: 1st Month



algem

algae environment modelling
labscale photobioreactor

End user committee

Swiss biotech company which develops and commercializes ingredients for use in food, nutrition and personal healthcare, including terpenoids (evenootkatone®)

✓ Other companies to be identified during the project

End-user committee members:

- participate at annual MERIT meetings to discuss results in order to ensure that the project goals and milestones are reached
- give advice regarding actions that need to be taken to realize commercial opportunities at the end of the project

Introduction

- Project objectives

✓ To establish a synthetic biology platform for the two microalgal organisms *Chlamydomonas reinhardtii* and *Phaeodactylum tricornutum*, enabling "green combinatorial diterpene chemistry"

✓ To generate versatile phototrophic chassis for the efficient conversion of carbon dioxide into diterpenoids

✓ To design a diterpenoid production process with integrated product recovery at laboratory scale

✓ To scale-up this process and to perform techno-economic modelling and sustainability analysis

Introduction

- Project topic area / topics covered

✓ Sustainable production and conversion of different types of feedstocks and bioresources into added value products

✓ Development of new products, value-added products and supply services

✓ Sustainable industrial processes

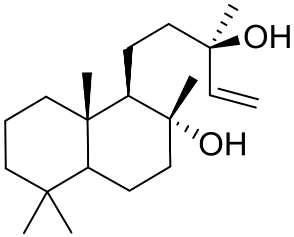
- Scientific approach

Example high-value diterpenoids from plants to be synthesized in MERIT

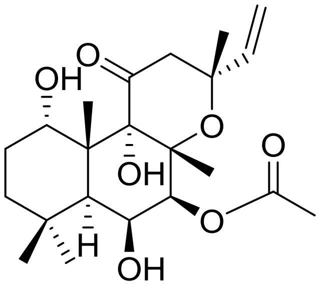
Salvia sclarea
(Clary sage;
Muskatellersalbei)

Coleus forskohlii
(Plectranthus barbatus,
Indian coleus

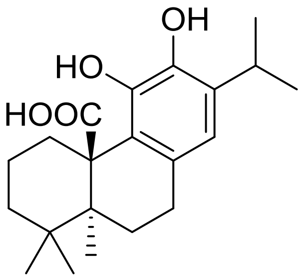
Rosmarinus officinalis
Rosemary



(+)-sclareol



forskolin



carnosic acid

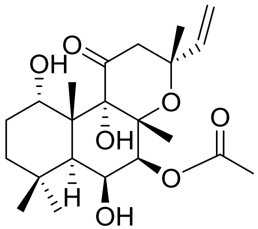
Introduction

- Scientific approach

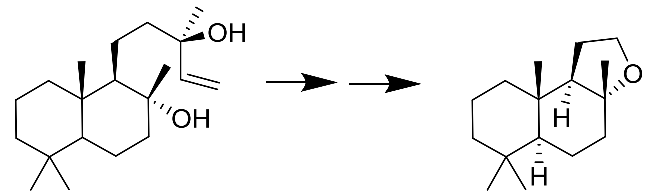
Stoll, M.; Hinder, M.,
Helv. Chim Acra 1950,33, 1251-1261.

Adenylate cyclase activator

- *Treatment of glaucoma or hypertension*



forskolin



sclareol

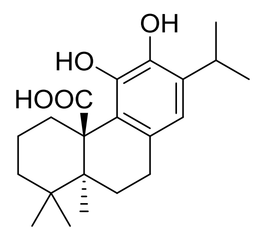
fragrance compound

(-)-Ambrox

higher value fragrance compound

The use of plants as a natural diterpenoid source is difficult, because of

- low diterpenoid contents
- cultivation and harvesting issues
- extraction processes not being transferable to an industrial scale



carnosic acid

- *Together with carnosol the main component of the food additive E392, rosemary extract with antioxidative activities*
- *Antimicrobial activity*

Chemical synthesis?

- Stereospecific synthesis is often uneconomical due to a low efficiency

Transfer biosynthetic pathways into microbial hosts (e.g. *E.coli* or *S. cerevisiae*)

Introduction

Communications
Angewandte Chemie International Edition

Biosynthesis

International Edition: DOI: 10.1002/anie.201510650
German Edition: DOI: 10.1002/ange.201510650

Expanding the Landscape of Diterpene Structural Diversity through Stereochemically Controlled Combinatorial Biosynthesis

*Johan Andersen-Ranberg, Kenneth Thermann Kongstad, Morten Thrane Nielsen, Niels Bjerg Jensen, Irini Pateraki, Søren Spanner Bach, Britta Hamberger, Philipp Zerbe, Dan Staerk, Jörg Bohlmann, Birger Lindberg Møller, and Björn Hamberger**

eLIFE
elifesciences.org

RESEARCH ARTICLE

Total biosynthesis of the cyclic AMP booster forskolin from *Coleus forskohlii*

Irini Pateraki[1,2*†], Johan Andersen-Ranberg[1,2†‡], Niels Bjerg Jensen[3], Sileshi Gizachew Wubshet[4§], Allison Maree Heskes[1,2], Victor Forman[1], Björn Hallström[5], Britta Hamberger[1,2¶], Mohammed Saddik Motawia[1,2], Carl Erik Olsen[1,2], Dan Staerk[4], Jørgen Hansen[3], Birger Lindberg Møller[1,2], Björn Hamberger[1,2¶]

[1]Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark; [2]Center for Synthetic Biology "bioSYNergy", Copenhagen, Denmark; [3]Evolva A/S, Copenhagen, Denmark; [4]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [5]Science for Life Laboratory, KTH - Royal Institute of Technology, Stockholm, Sweden

nature COMMUNICATIONS

ARTICLE

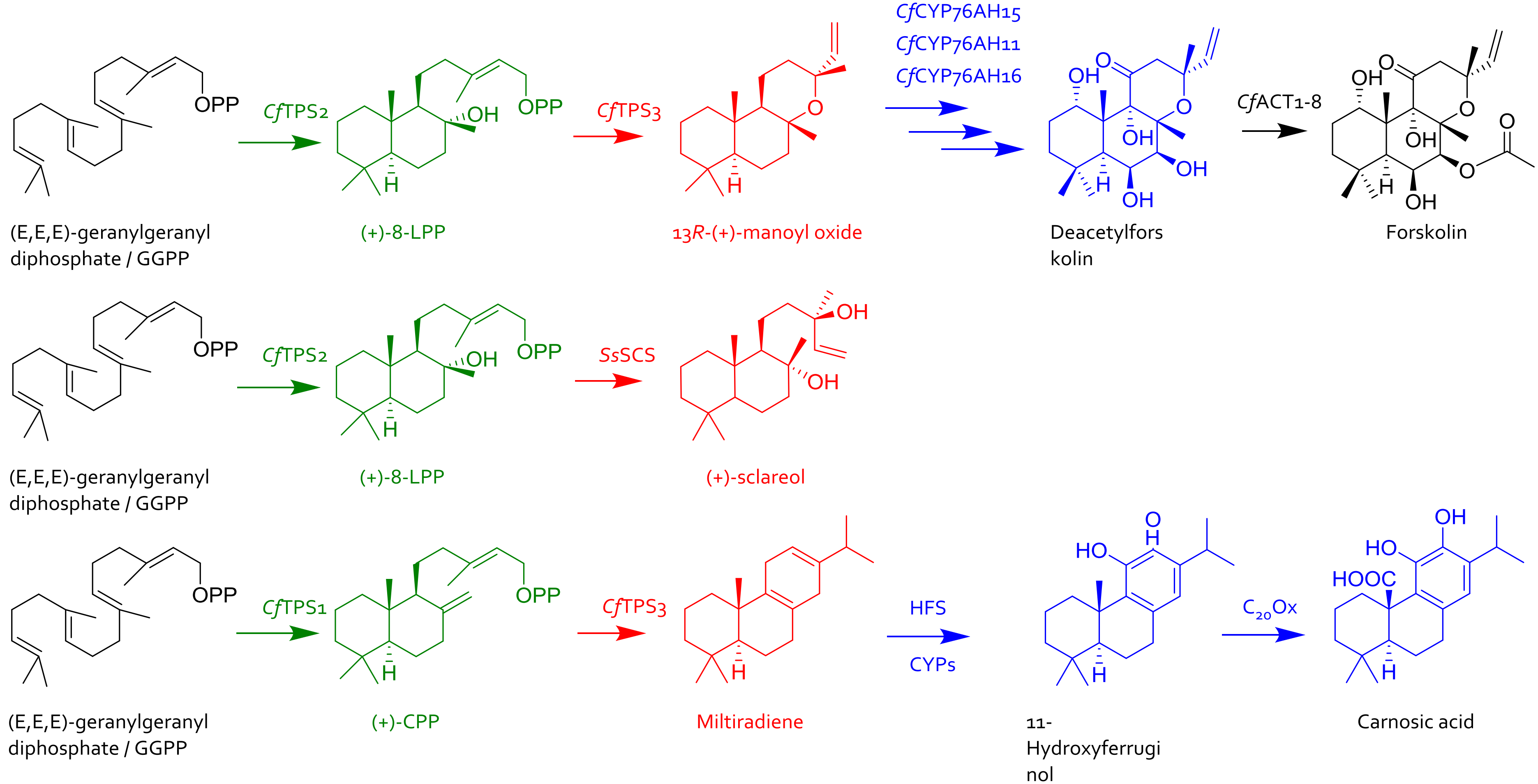
Received 7 Jan 2016 | Accepted 11 Aug 2016 | Published 5 Oct 2016 DOI: 10.1038/ncomms12942 OPEN

Elucidation of the biosynthesis of carnosic acid and its reconstitution in yeast

Ulschan Scheler[1], Wolfgang Brandt[2], Andrea Porzel[2], Kathleen Rothe[1], David Manzano[3,4], Dragana Božić[5,†], Dimitra Papaefthimiou[5], Gerd Ulrich Balcke[1], Anja Henning[1], Swanhild Lohse[1], Sylvestre Marillonnet[1], Angelos K. Kanellis[5], Albert Ferrer[3,4] & Alain Tissier[1]

Pathways for the synthesis of forskolin, sclareol and carnosic acid have been characterized

Introduction



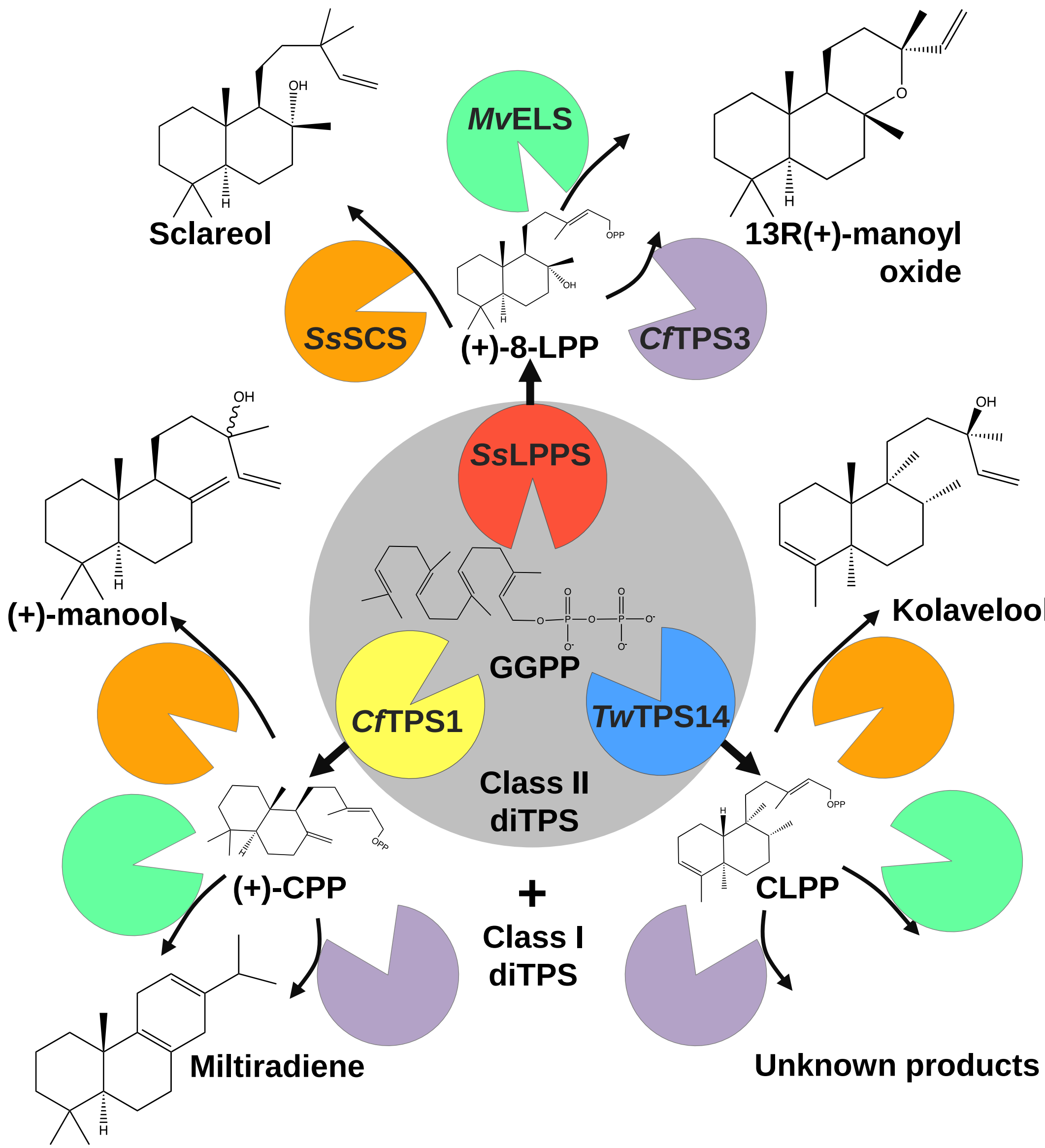
Common precursor + ClassII diTPS + ClassI diTPS + CYPs (+ other)

Diterpenoid biosynthetic pathways can be dissected into distinct modules

Introduction

ClassI and Class II diTPS can be combined in a LEGO®-like fashion to even produce "new-to-nature" diterpenoids

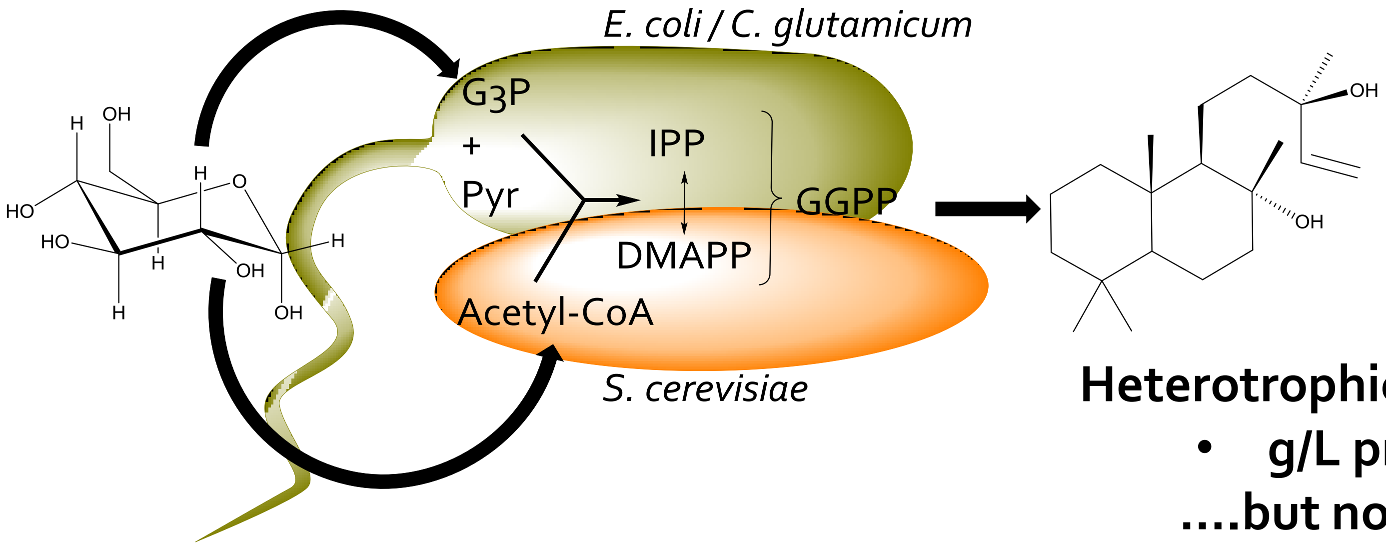
Which options exist in regard to the microbial host?



Andersen-Ranberg J, Kongstad KT, Nielsen MT, Jensen NB, Pateraki I, Bach SS, Hamberger B, Zerbe P, Staerk D, Bohlmann J, Moller BL, Hamberger B (2016) Expanding the Landscape of Diterpene Structural Diversity through Stereochemically Controlled Combinatorial Biosynthesis. Angew Chem Int Ed Engl **55:** 2142-2146

Zerbe P, Bohlmann J (2015) Plant diterpene synthases: exploring modularity and metabolic diversity for bioengineering. Trends Biotechnol **33:** 419-428

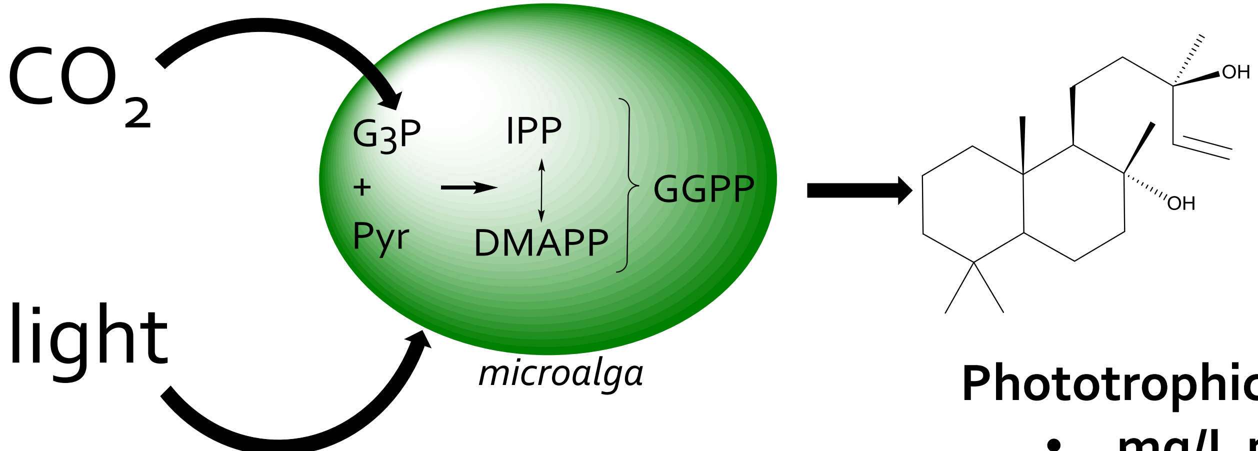
Introduction



Heterotrophic microorganisms

- **g/L productivities**

....but not sustainable



Phototrophic microorganisms

- **mg/L productivities**

....but sustainable

Introduction

Chlamydomonas reinhardtii | *Phaeodactylum tricornutum*

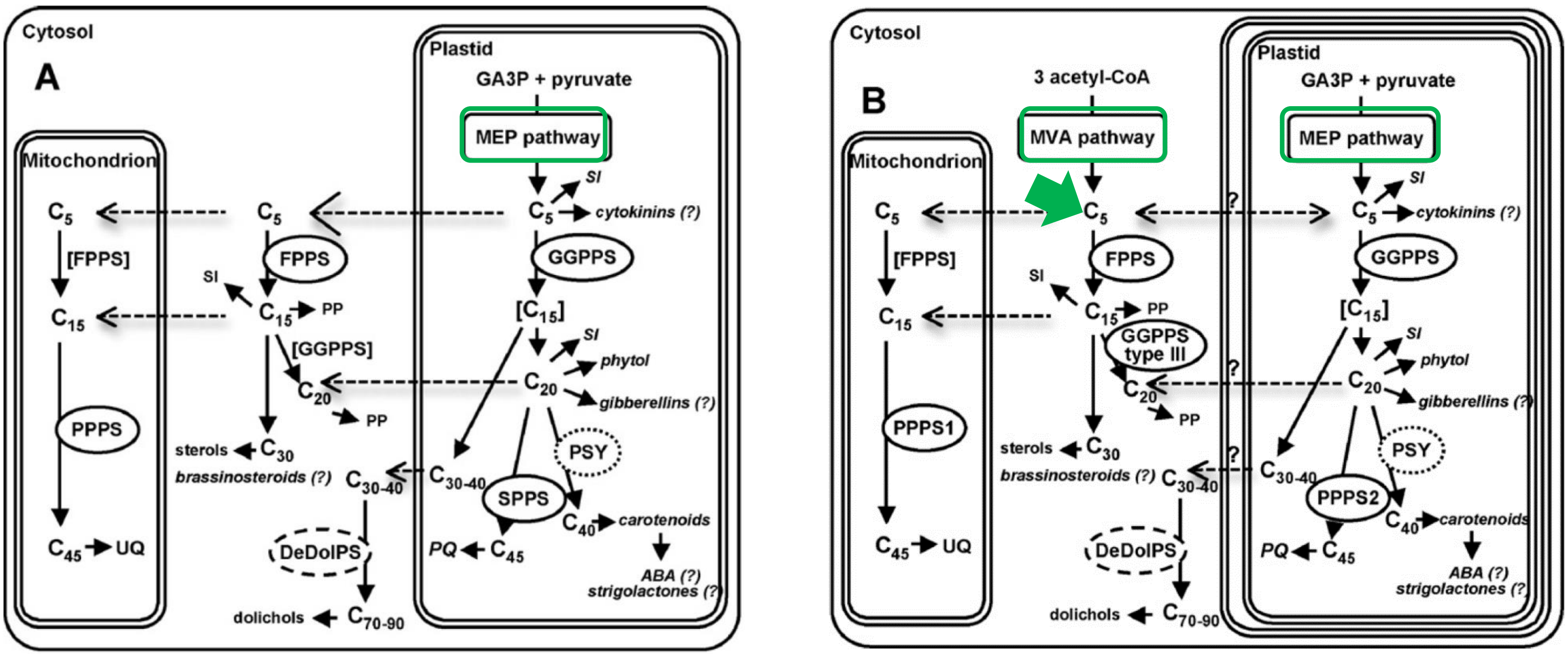
....but molecular toolbox still needs to be expanded to enable the application of synthetic biology principles to microalgae

- ✓ eukaryotic photosynthetic microorganisms / microalgae
- ✓ genomes sequenced and annotated
- ✓ a molecular toolbox exists for both organisms:
  - constitutive/inducible promoters; regulatory elements
  - Reporters (luciferase, fluorescent proteins etc.)
  - RNAi techniques
  - Genome modification via CRISPR approaches / TALEN (Phae)

Photograph taken from: Scaife Mark A, Smith Alison G (2016) Towards developing algal synthetic biology. Biochemical Society Transactions **44:** 716-722

Introduction

Chlamydomonas reinhardtii *Phaeodactylum tricornutum*

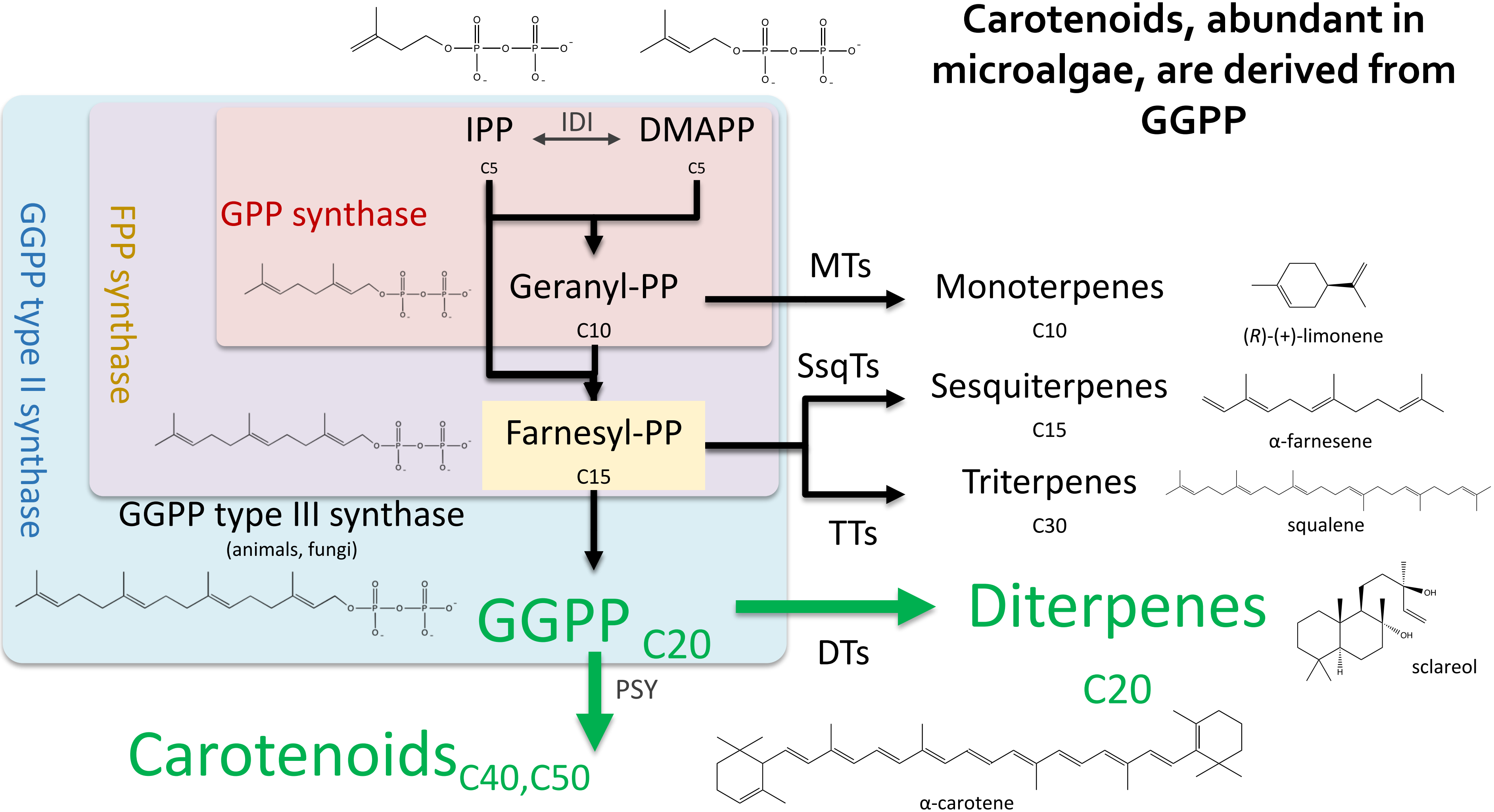


Lohr M, Schwender J, Polle JE (2012) Isoprenoid biosynthesis in eukaryotic phototrophs: a spotlight on algae. Plant Sci **185-186:** 9-22

Cytosolic and plastidic diterpenoid production feasible in *Phaeodactylum*?

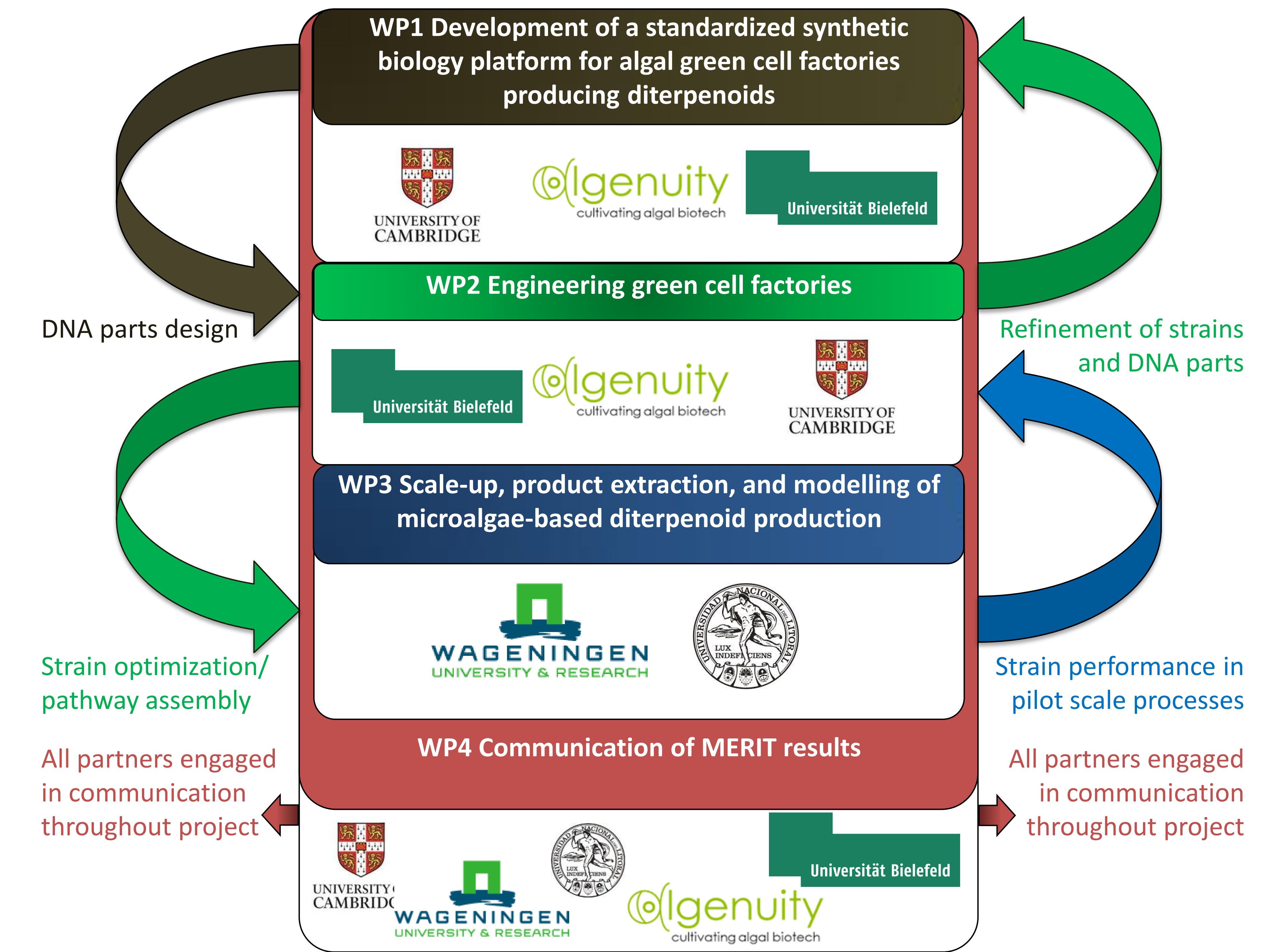
Introduction

Carotenoids, abundant in microalgae, are derived from GGPP



Concepts taken from Lohr M., Schwender J., Polle J.E.W., 2012. Isoprenoid biosynthesis in eukaryotic phototrophs: A spotlight on algae. Plant Sci. 185–186, 9–22.

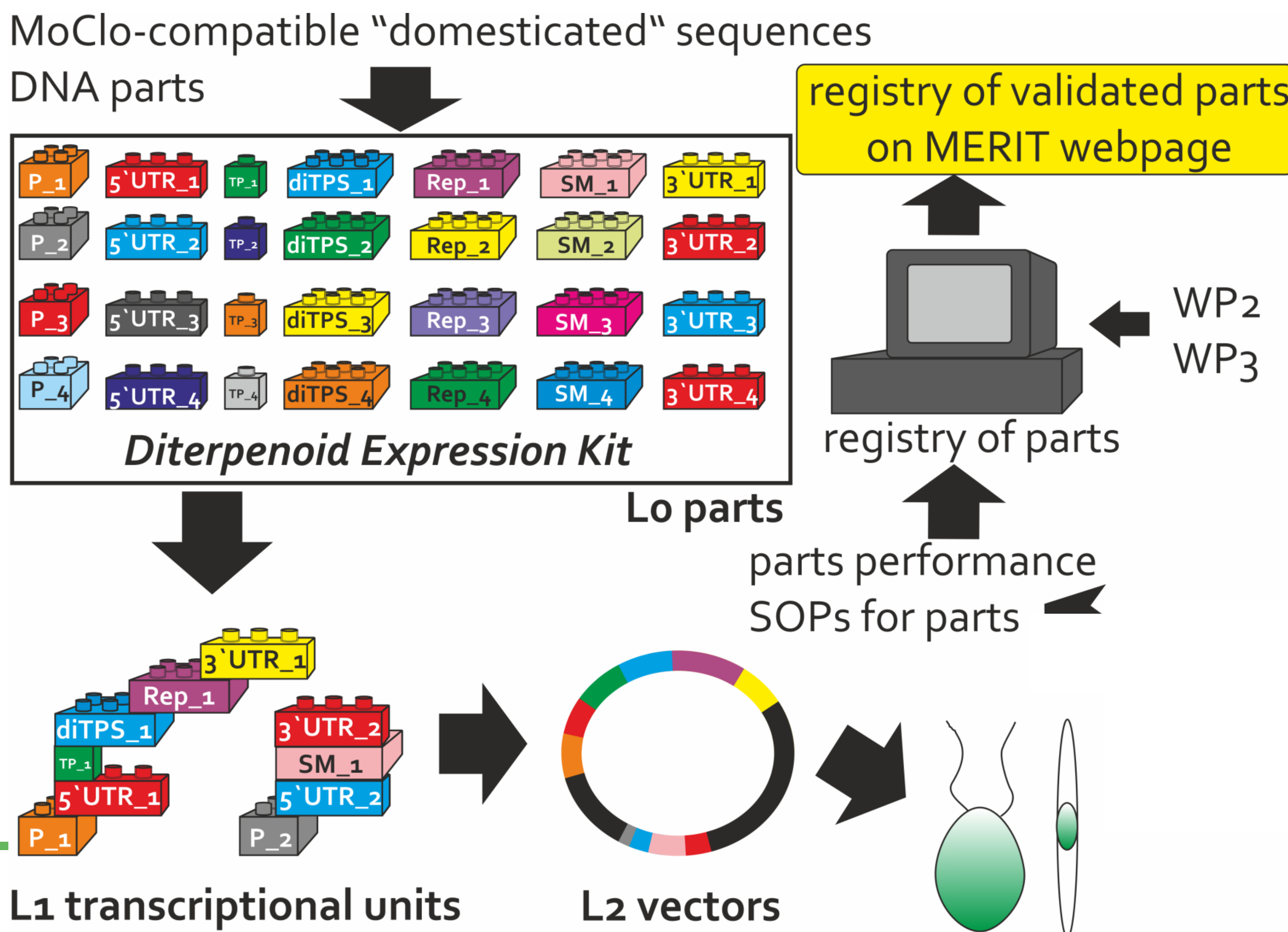
Work to be conducted in the MERIT project


WP1 Development of a standardized synthetic biology platform for algal green cell factories producing diterpenoids
UNIVERSITY OF CAMBRIDGE
algenuity cultivating algal biotech
Universität Bielefeld
WP2 Engineering green cell factories
Universität Bielefeld
algenuity cultivating algal biotech
UNIVERSITY OF CAMBRIDGE
WP3 Scale-up, product extraction, and modelling of microalgae-based diterpenoid production
WAGENINGEN UNIVERSITY & RESEARCH
UNIVERSIDAD NACIONAL DEL LITORAL
WP4 Communication of MERIT results
Universität Bielefeld
WAGENINGEN UNIVERSITY & RESEARCH
algenuity cultivating algal biotech
DNA parts design
Strain optimization/ pathway assembly
All partners engaged in communication throughout project
Refinement of strains and DNA parts
Strain performance in pilot scale processes
All partners engaged in communication throughout project

WP1 :Development of a standardized synthetic biology platform for algal green cell factories producing diterpenoids

Project plan

WP1 (partners UCAM (WP leader), UniBi, Algenuity)



WP2 :Engineering green cell factories for the conversion of carbon dioxide and organic carbon derived from waste material into high value and novel diterpenoids

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WP2_preliminary work

MERIT

Collaboration on diterpenoid production in *Chlamydomonas*

Birger Lindberg Møller

Olaf Kruse

Sortirios Kampranis

Kyle J. Lauersen

Irini Pateraki

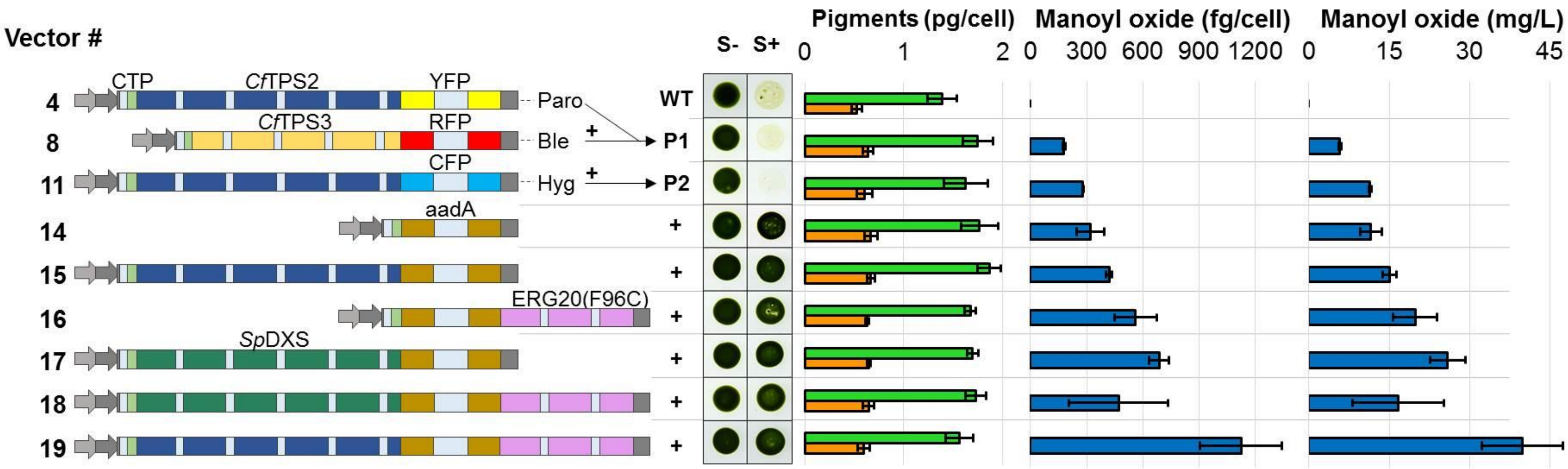
UNIVERSITY OF COPENHAGEN

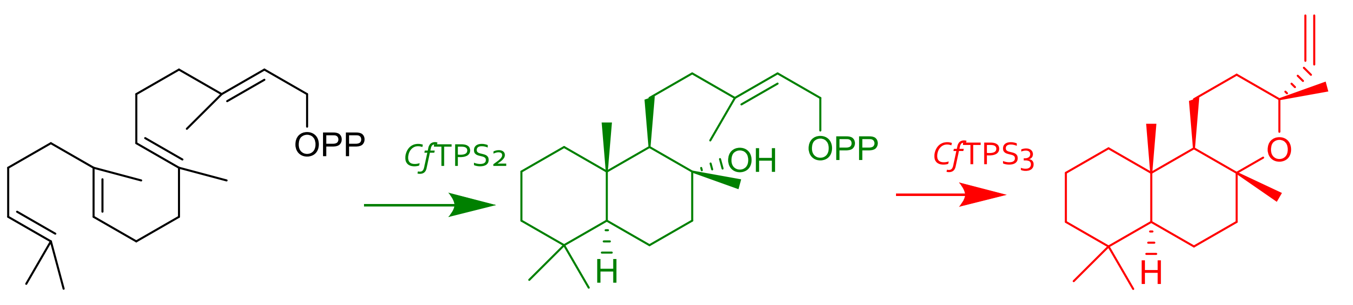
ALGAE BIOTECHNOLOGY AND BIOENERGY GROUP

Universität Bielefeld

CeBiTec
Center for Biotechnology

WP2_preliminary work





(E,E,E)-geranylgeranyl diphosphate / GGPP

(+)-8-LPP

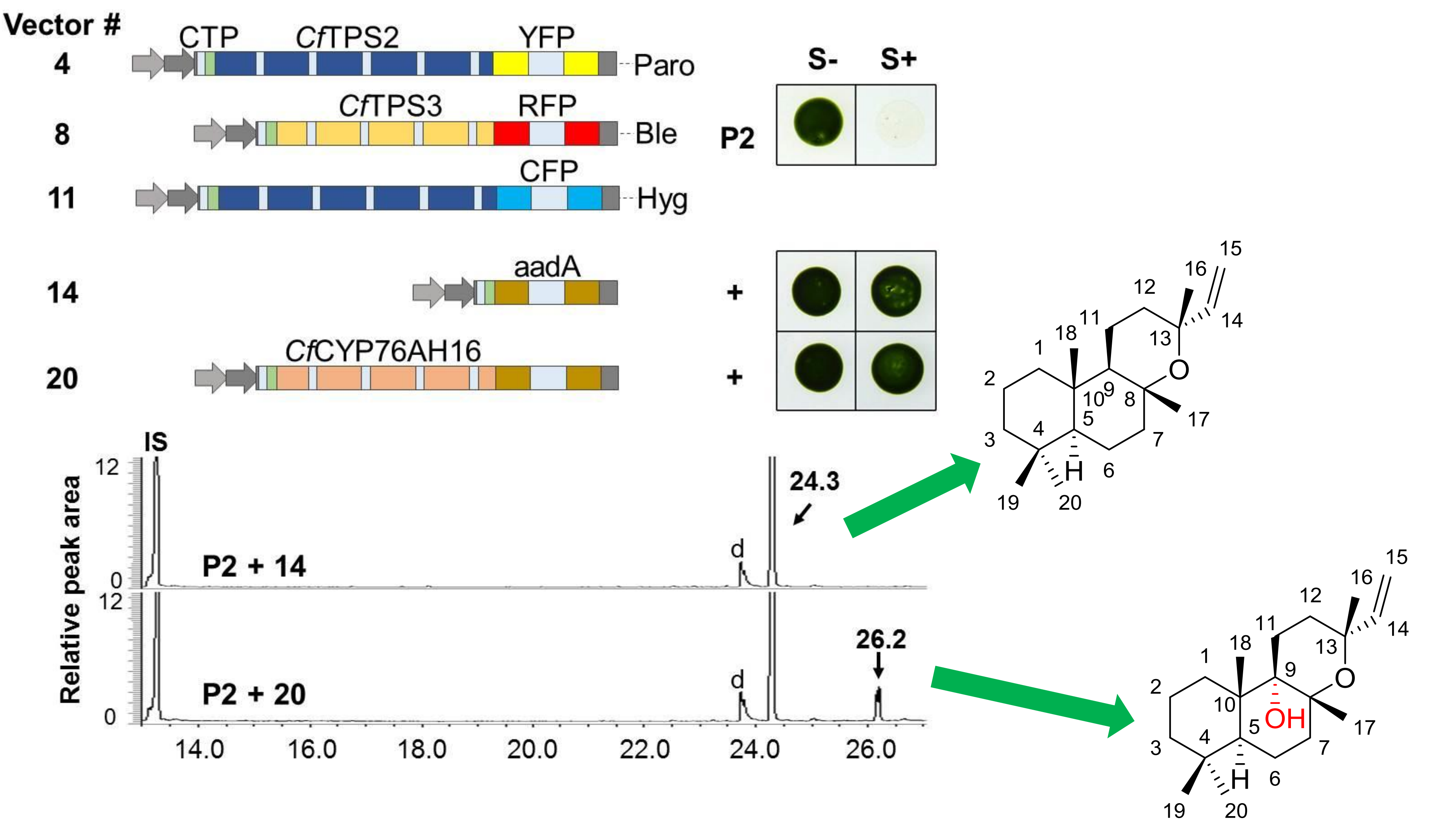
13*R*-(+)-manoyl oxide

ERG20(F96C):
GGPP synthase

SpDXS:
Catalyzes first step of MEP pathway

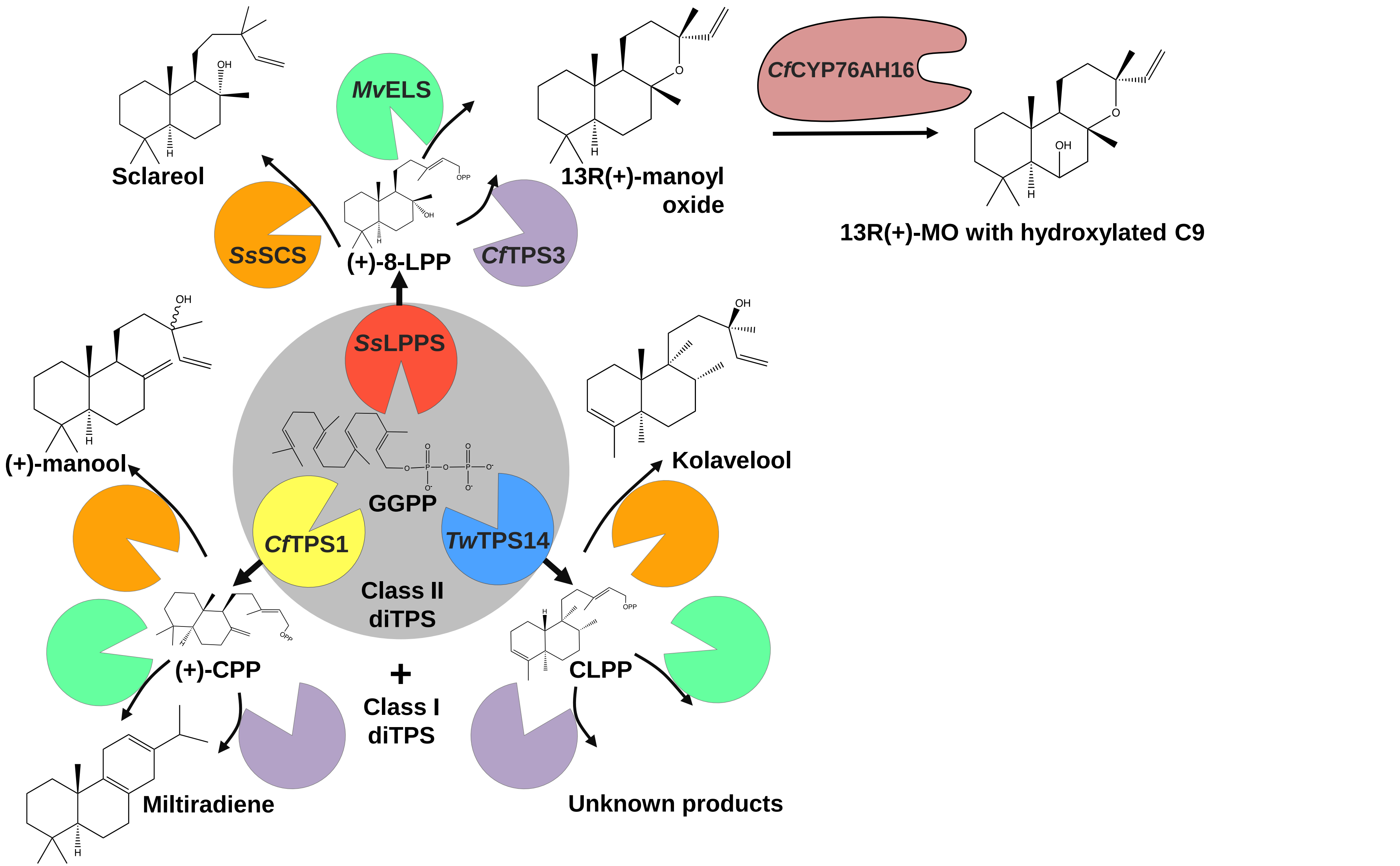
Kyle J. Lauersen, Julian Wichmann, Thomas Baier, Sotirios Kampranis, Irini Pateraki, Birger Møller, Olaf Kruse (2018) Phototrophic production of heterologous diterpenoids and a hydroxyfunctionalized derivative from *Chlamydomonas reinhardtii (submitted to Metabolic Engineering)*

WP2_preliminary work



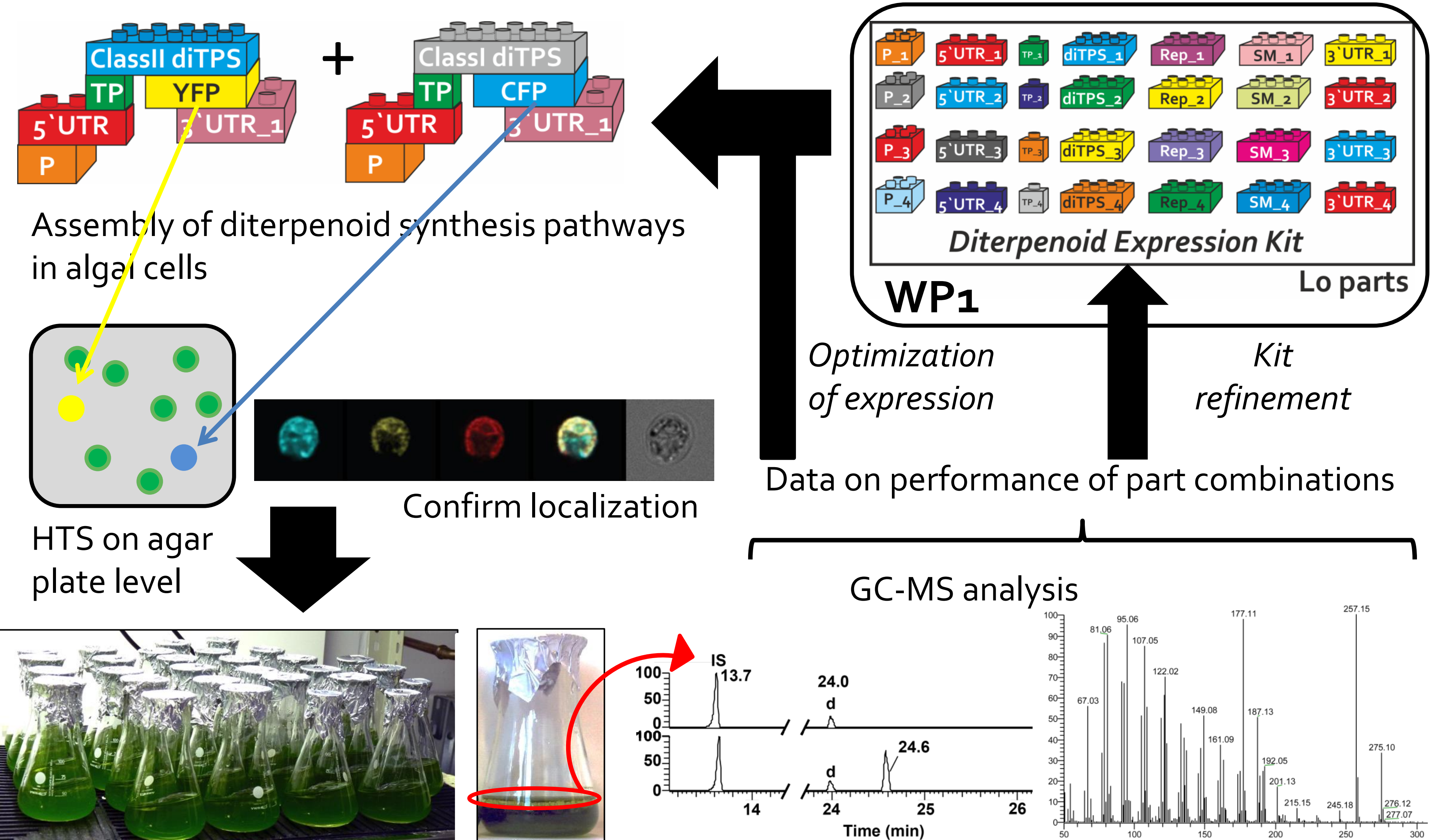
Kyle J. Lauersen, Julian Wichmann, Thomas Baier, Sotirios Kampranis, Irini Pateraki, Birger Møller, Olaf Kruse (2018) Phototrophic production of heterologous diterpenoids and a hydroxyfunctionalized derivative from *Chlamydomonas reinhardtii (submitted to Metabolic Engineering)*

Project plan



Project plan

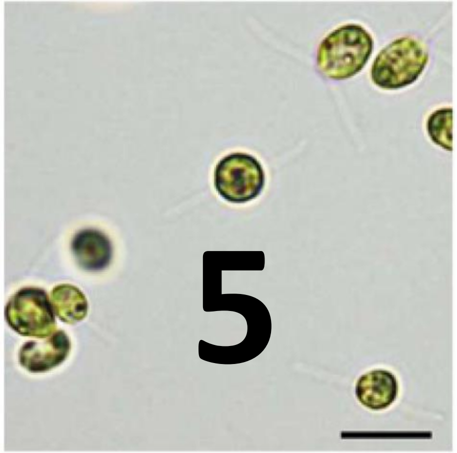
WP2 (partners UniBi (WP leader), UCAM, Algenuity)



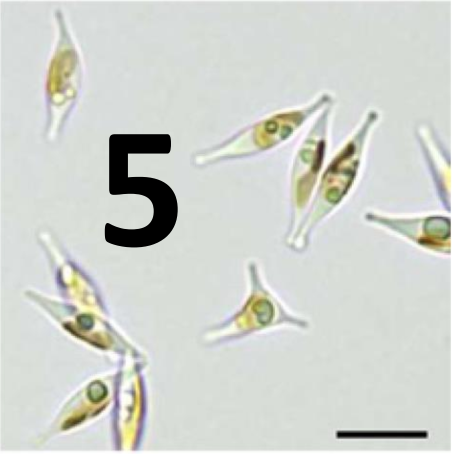
WP2 (partners UniBi (WP leader), UCAM, Algenuity)

Five best-performing strains per organism for cultivation and product extraction in WP3

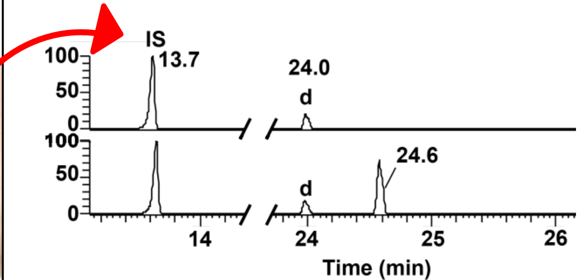
Chlamydomonas reinhardtii

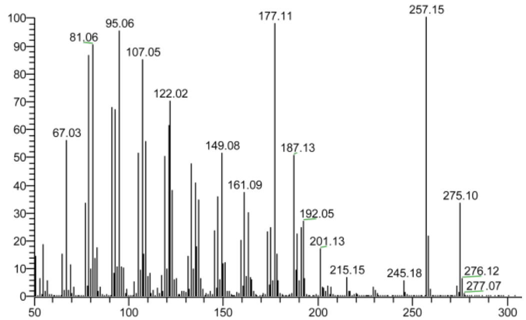


Phaeodactylum tricornutum



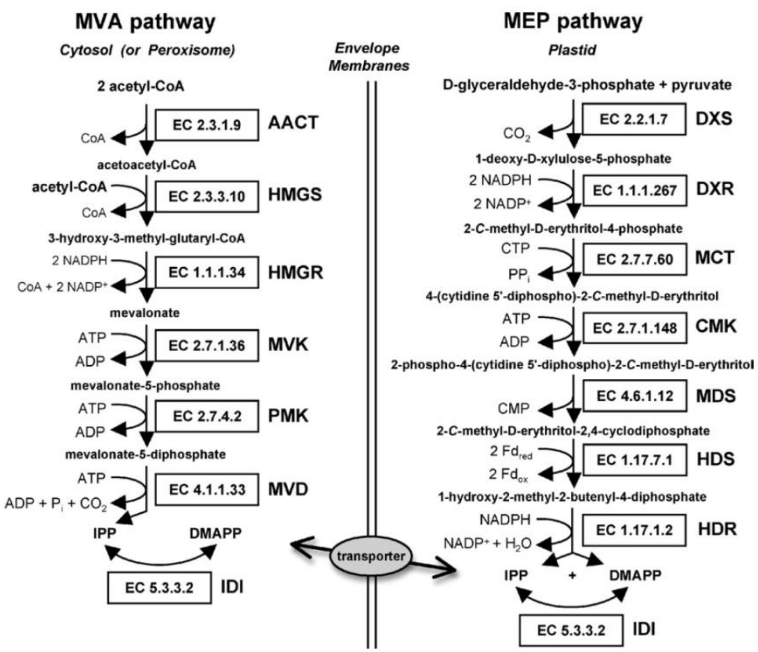
GC-MS analysis



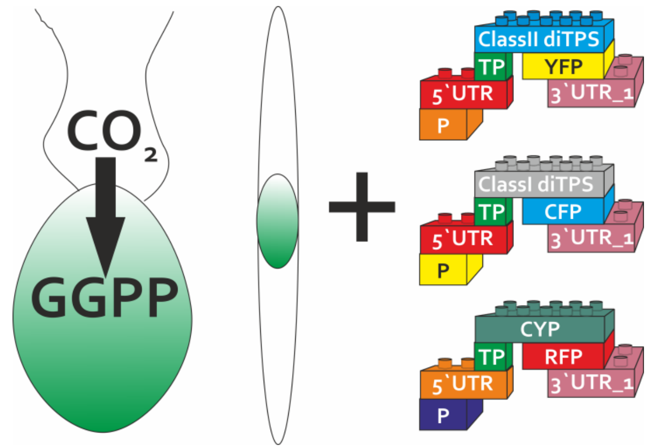


Project plan

WP2 (partners UniBi (WP leader), UCAM, Algenuity)



MEP/MVA pathway engineering for enhanced flux of fixed carbon into diterpenoid synthesis pathways



most efficient MVA/MEP engineering strategy

Most productive diterpenoid producer from WP3

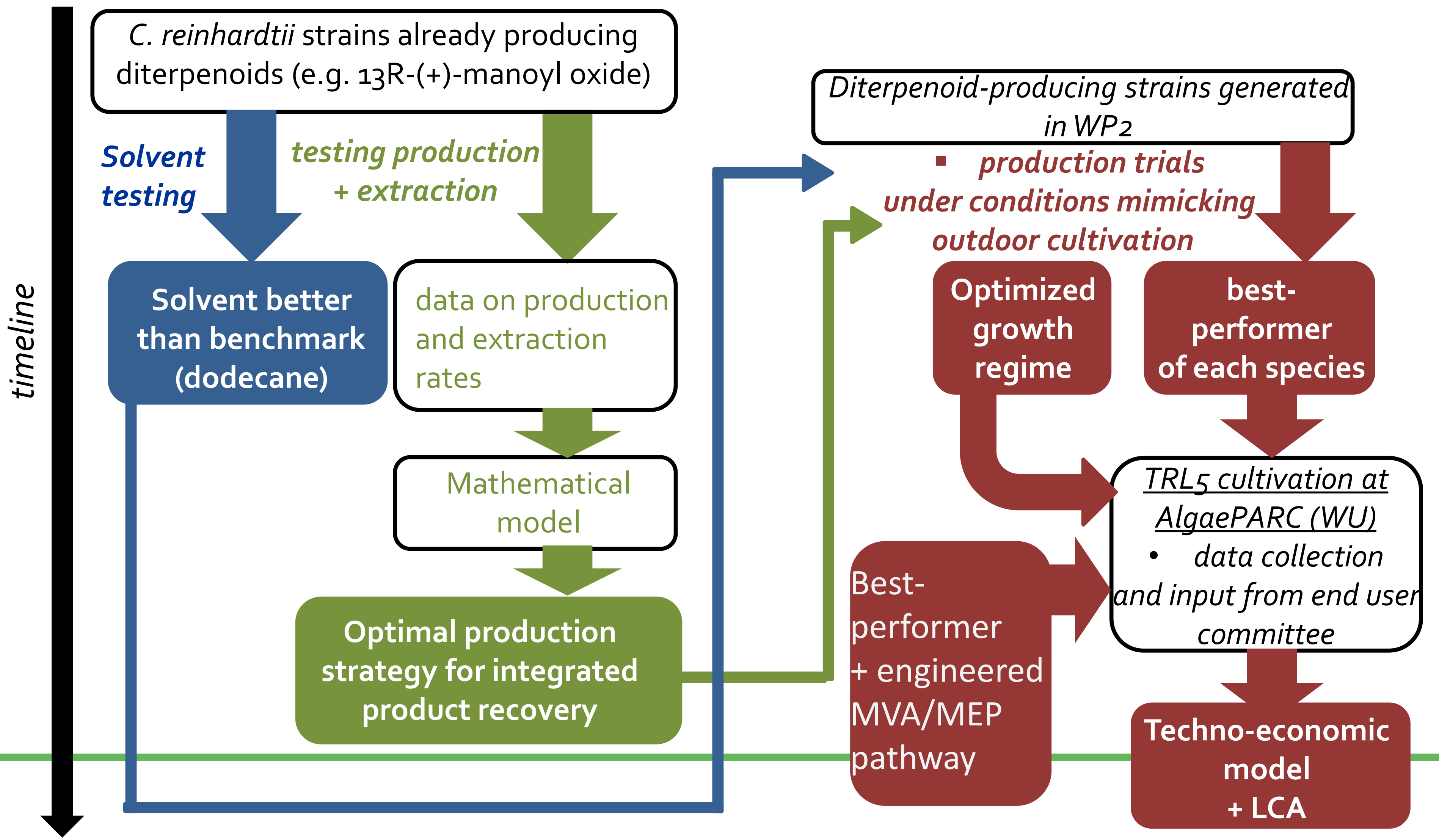
One efficient diterpenpoid-producing strain per species grown at TRL5 in WP3

Lohr M, Schwender J, Polle JE (2012) Isoprenoid biosynthesis in eukaryotic phototrophs: a spotlight on algae. Plant Sci **185-186:** 9-22

WP3: Scale-up, product extraction, and modelling of microalgae-based diterpenoid production

Project plan

WP3 (partners WU (WP leader), UNDL, Algenuity)



WP4: Communication of MERIT results

WP4 (partners UniBi (WP leader), UNDL, Algenuity, WU, UCAM)

Aim: To increase the stakeholder`s awareness for the great innovation potential of microalgae as green cell factories, which should foster the development of a sustainable European bio-based economy by including microalgae as production hosts.

Objective: To communicate with stakeholders via various communication channels

Deliverables:

- ✓ Creation of a MERIT webpage
- ✓ Articles in business press
- ✓ MERIT webpage with non-expert- and "ask a scientist"-sections
- ✓ Youtube clip presenting the MERIT idea and project
- ✓ International MERIT summer school
- ✓ Teutolab briefing meetings with High school teachers

Milestones:

- ✓ At least two briefing meetings with industrial stakeholders from the pharmaceutical, nutritional or chemical sector
- ✓ At least two local media reports about MERIT
- ✓ Public interest documented by webpage visits and use of the discussion platform

Expected outcomes

- ***Major outcomes to be achieved***

✓ synthetic biology platform for *C. reinhardtii* and *P. tricornutum* + diterpenoid production hosts

✓ diterpenoid production in an industrially-relevant environment (TRL5)

✓ LCA + techno-economic model based on TRL5-production, enabling extrapolation to industrial-scale production (TRL8) for envisaged TRL6-8 processes (beyond the scope)

Expected outcomes

- ***Economic exploitation of results during/after the project***

<u>Actions to be taken:</u>

- ✓ Form an end user committee for product commercialization and market evaluation during the course of the project
- ✓ To identify and evaluate the market potential for diterpene products during the project
- ✓ To identify and prepare suitable conditions for commercialization in long-term after the project
- ✓ Follow-up activities beyond MERIT: include proof of concept trials at demonstration scale (TRL6-8, see comment above), thus providing a basis for the later industrial production and commercialization of at least one product.

Contact details of the coordinator

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Mobile: 0049 15122312184
Fax: 0049 52110612290
http://uni-bielefeld.de/

Thanks for your attention!!!!