ERA CoBioTech

BIOTECH RESEARCH AND INNOVATION HACK 2021

Final seminar of the cofunded projects of ERA CoBioTech

Project name: **Microbial conversion of C1 to value-added products by integrated systems and synthetic biology**

Project acronym: **C1Pro**

Name: **Trygve Brautaset, PL**

C1Pro

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

29.09.2021

Project partners

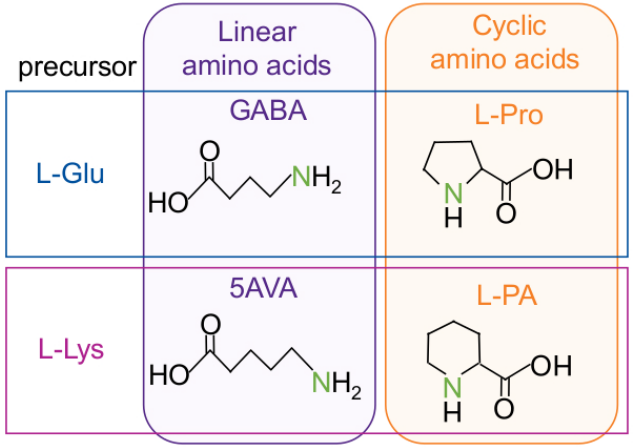
- **Partners**
  - Partner 1: Trygve Brautaset, NTNU, Norway
  - Partner 2: Volker Wendisch, University of Bielefeld, Germany
  - Partner 3: Stephanie Heux, INSA Toulouse, France
  - Partner 4: Oskar Zelder, BASF, Germany
  - Partner 5: Ingemar Nærdal, SINTEF, Norway
  - Partner 6: Gregor Kosec, Acies Bio, Slovenia
- Total project budget: **1.767.000 Euro**
- Project start: **01.03.2018**

Project objective

C1Pro project aims to establish a sustainable platform for **methanol-based production of four value-added products:**

1) gamma-aminobutyric acid (**GABA**)
2) 5-aminovaleric acid (**5AVA**)
3) L-proline (**L-Pro**)
4) L-pipecolic acid (**L-PA**)

with proven industrial applications!



Scientific approach and project topic area

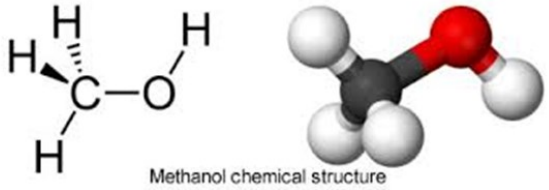
Gram-positive, methylotrophic and thermophilic bacterium ***Bacillus methanolicus*** was chosen as model organism in this project for several reasons:

- It utilizes **methanol** as raw material for growth and energy
- It is **thermophilic** and grows at elevated temperatures (50 – 55 °C)
- It naturally overproduces **L-glutamate**, and its classical mutants have demonstrated a high potential to overproduce **L-lysine**.

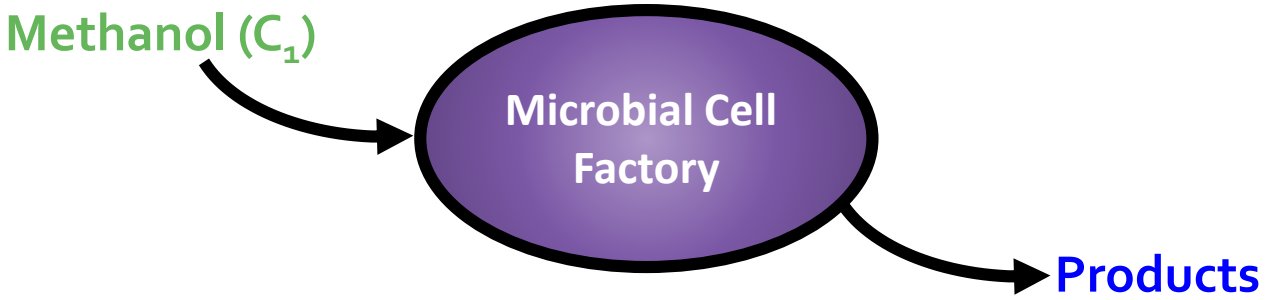
Introduction

Methanol as feedstock for industrial biotechnology:

- Non-food
- Price and availability
- Completely consumed



Methanol chemical structure

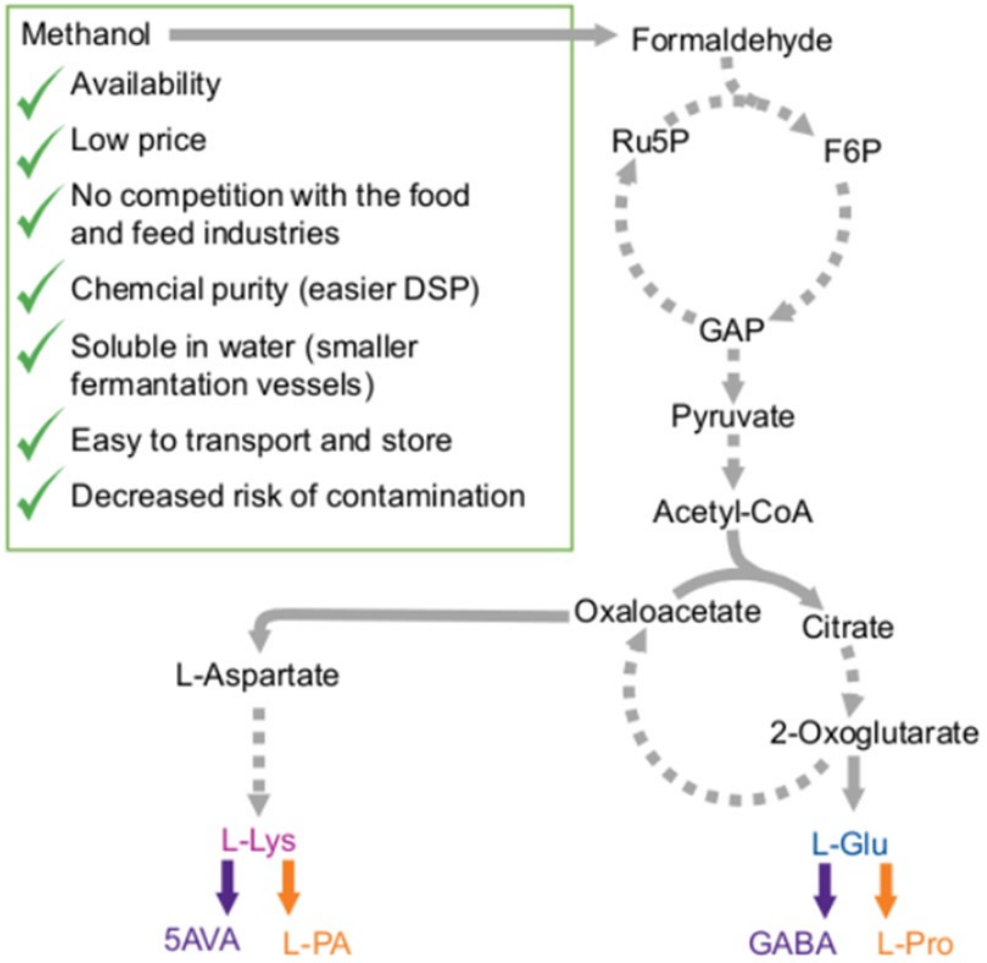


Methylotrophic bacteria can utilize **methanol** as the sole carbon and energy source!

Introduction

- **GABA**, **5AVA**, **L-Pro** and **L-PA** were chosen as they share
  - biosynthesis pathways
  - functional characteristics
  - industrial applications

- The products serve as building blocks of polymers or precursors of pharmaceuticals



Scientific approach and project topic area

Systems and **synthetic biology** approaches are key to the proposed strain and process development, which is facilitated by common biosynthesis pathways

- Novel genetic tools used to simplify regulated gene expression via CRISPR interference and riboswitches for **synthetic regulatory circuits**
- Pathway design guided by the **genome-scale metabolic model**, iteratively fine-tuned based on experimental test results
- Strain performance in **methanol-based fermentations** characterized in-depth by **multi omics approach**

Technical overview

C1Pro consist of 7 interlinked Work packages (WPs):

- *WP1: Establishment of technological platform for **GABA** production and downstream processing*
- *WP2: Development of **5AVA** production strains and application of **synthetic regulatory circuits***
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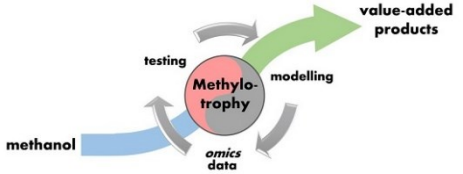
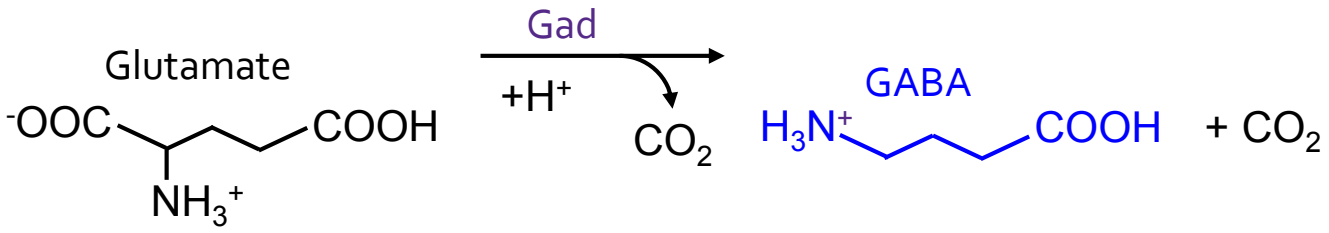


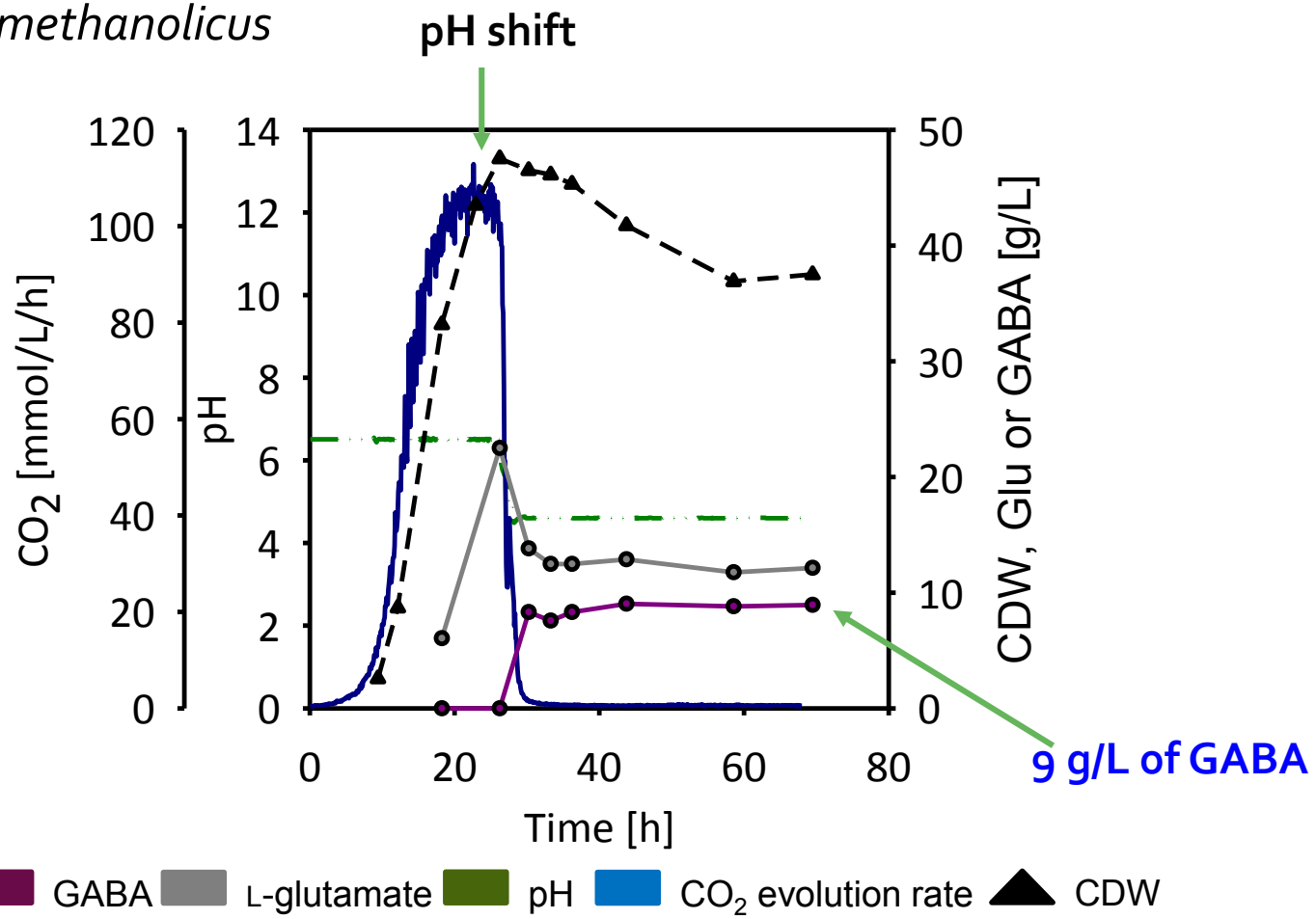
Production of GABA from methanol



- Expression of several hererologous **glutamate decarboxylase** (*gad*) genes in *B. methanolicus*
- Use of *gadST* gene from *Sulfobacillus thermosulfidooxidans* led to the best results

Technical overview

Preliminary results: Methanol controlled fed batch fermentation of genetically engineered *B. methanolicus*



Irla et al., 2017

Strategies to improve GABA bioprocess and its downstream processing

- Increase of precursor (L-glutamate) availability through classical mutagenesis and media optimization
- GABA fermentation optimization
- Downstream processing
  - ✓ Demonstration of GABA extraction from fermentation broth
  - ✓ Conversion to 2-pyrrolidone

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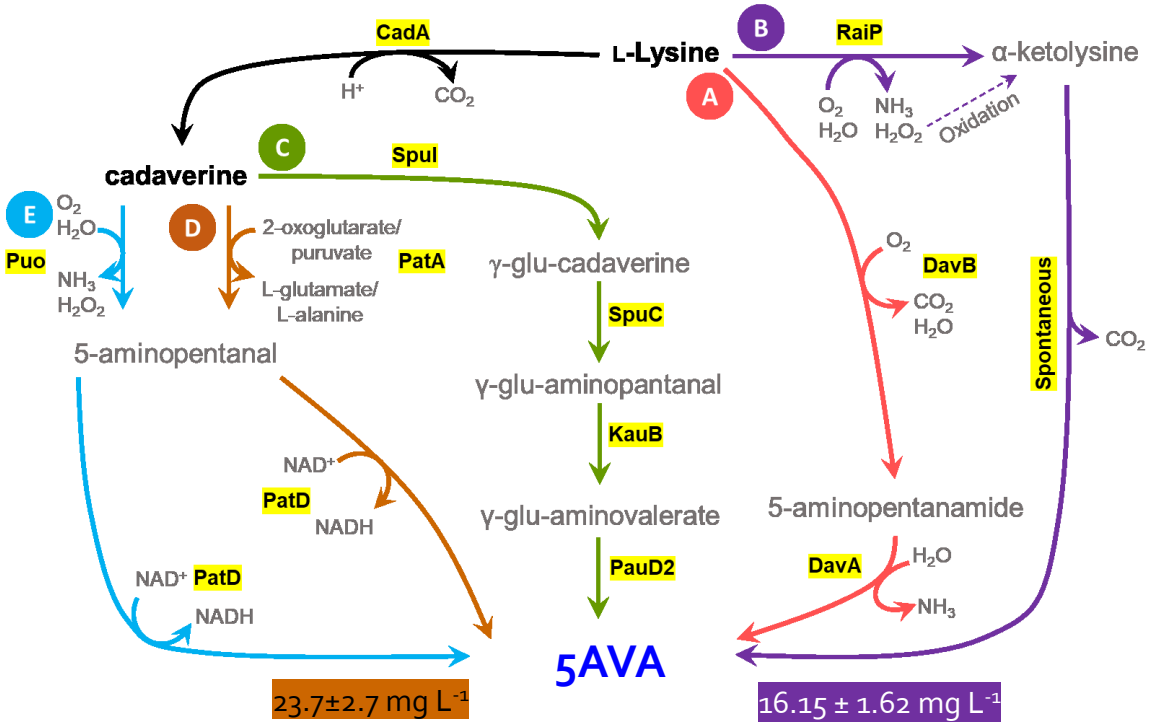
Possible routes for 5AVA biosynthesis



wo pathways with lysine as precursor
'sine

A- DavAB: employing lysine 2-monooxygenase and 5-aminovaleramidase

B- RaiP: employing lysine α-oxidase in presence of hydrogen peroxide

hree pathways with **cadaverine** as an ıtermediate

C- SpuI: cadeverine to γ-glutamine-cadaverine by glutamine synthetase

D- PatAD: cadaverine to 5-aminiopentanal through activity of putrescine aminase

E- Puo-PatD: cadaverine to 5-aminiopentanal through activity of putrescine oxidase

- The pathways relying on **RaiP** and **PatA** activities were functional in shake flask cultures of *B. methanolicus*

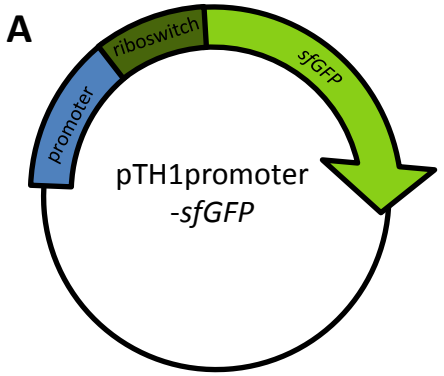
Brito et al., 2021

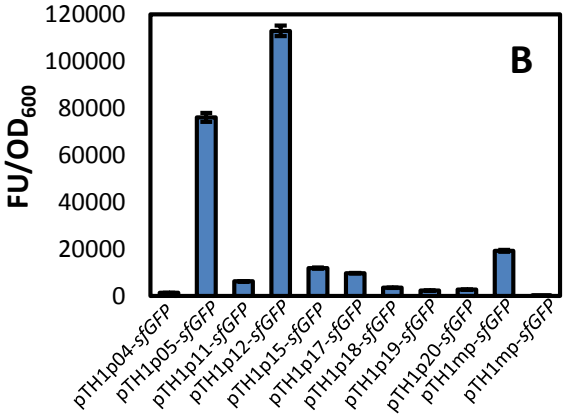
Tools for creation of synthetic regulatory circuits

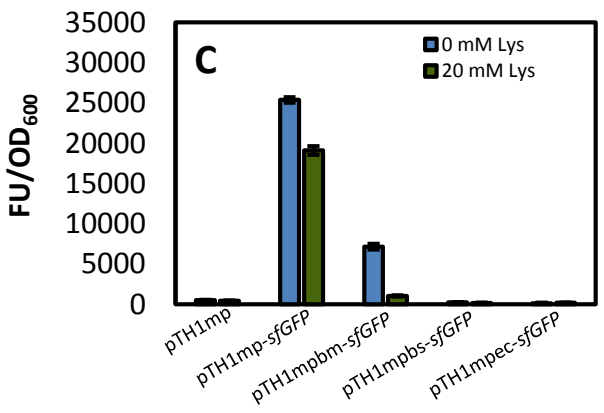
System for testing of promoters and other regulatory elements was developed (A).

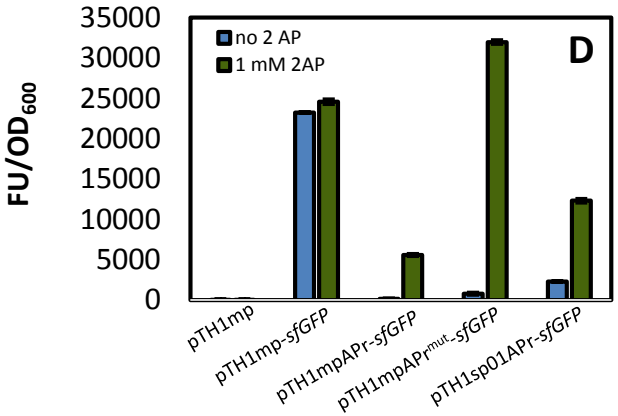
A wide range of promoters was tested and established for futher use (B).

Lysine and 2-aminopurine riboswitches were succesfully applied for control of gene expression (C and D).





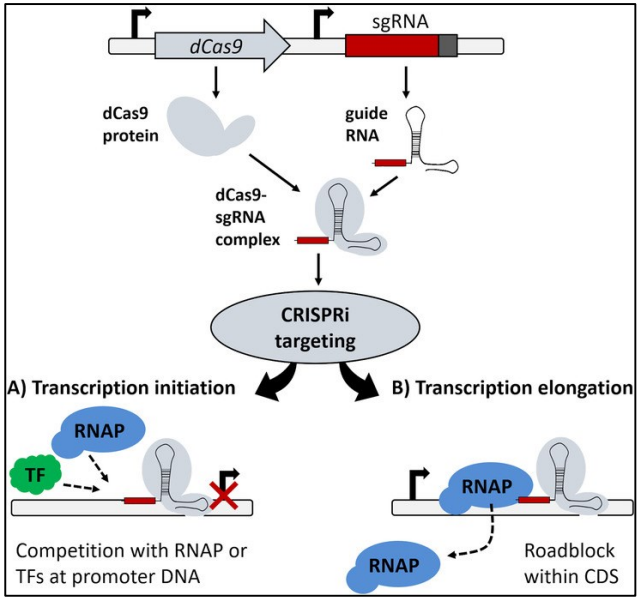


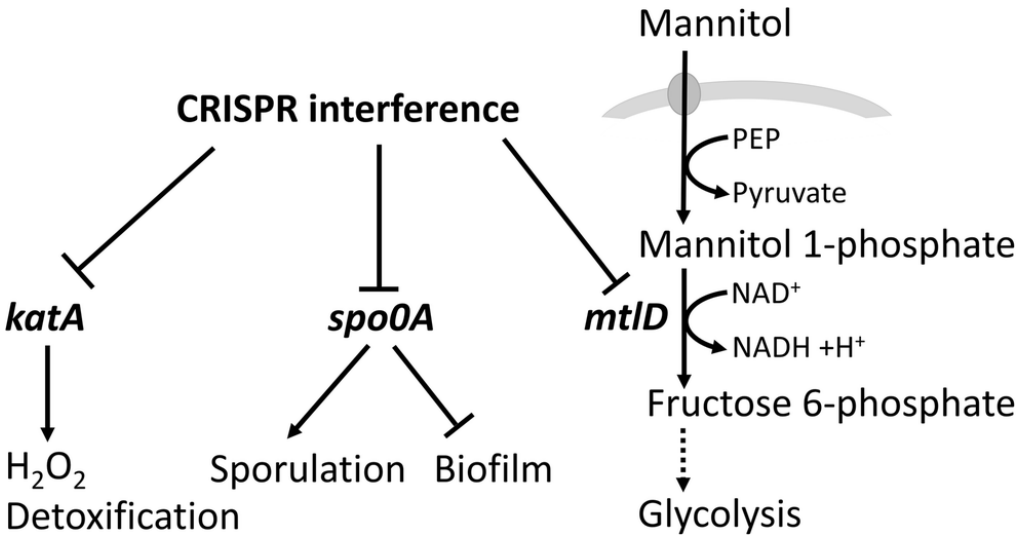


Irla et al., 2021

Tools for creation of synthetic regulatory circuits

Establishment and application of CRISPR interference in *B. methanolicus*





Control of sporulation and biofilm formation, characterization of MtlD for mannitol catabolism, and of catalase in hydrogen peroxide dismutation

Schultenkämper et al., 2019; Schultenkämper et al., 2020

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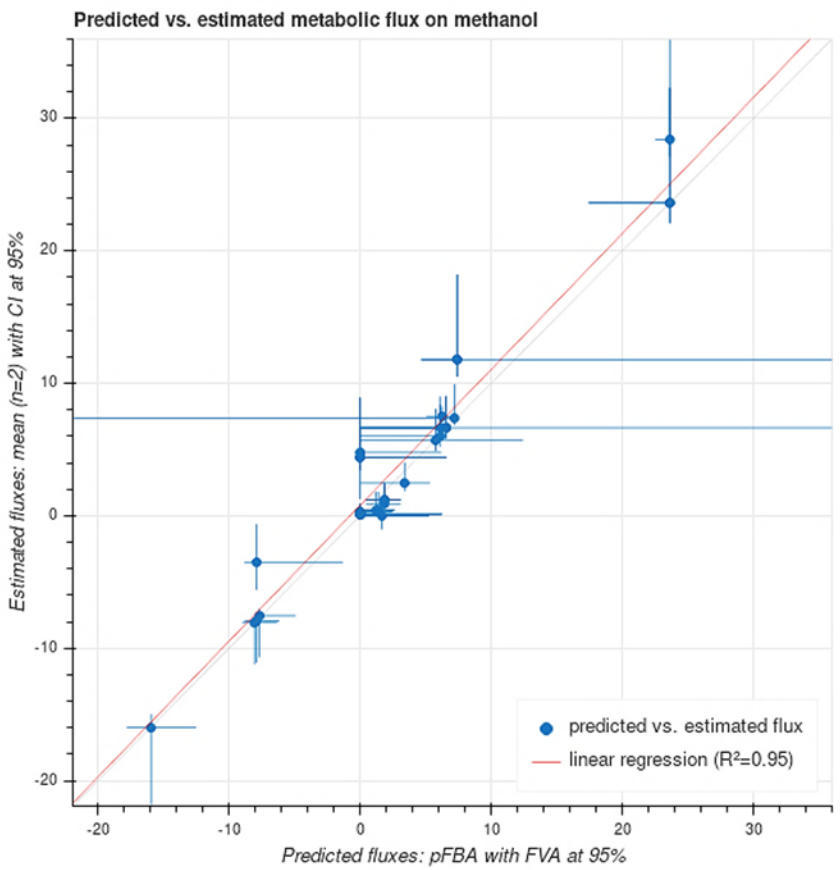
Genome scale model

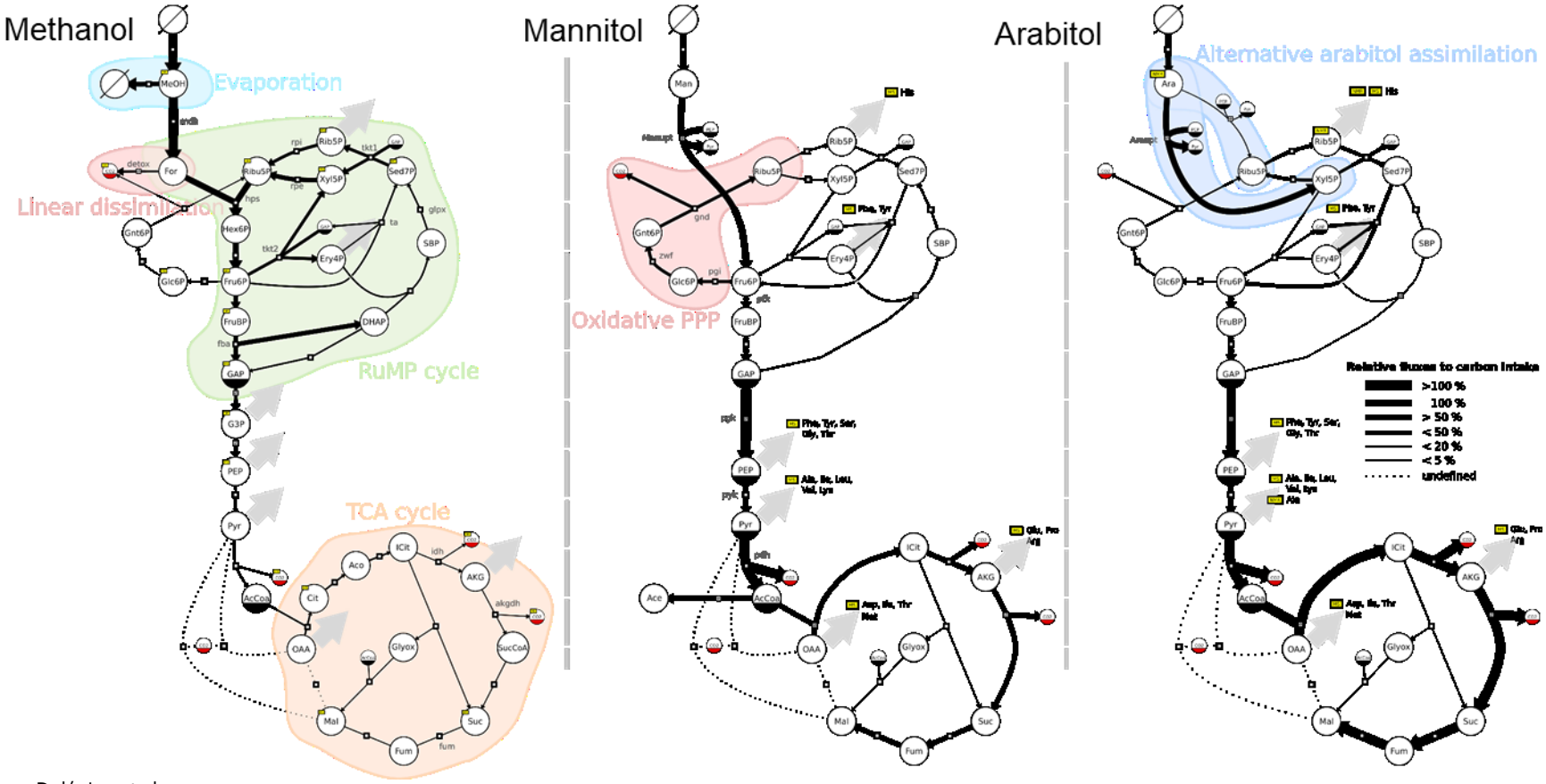
- Full **genome sequence** of *B. methanolicus* MGA3 strain
- **Annotation** software (Inparanoid)
- Comparison other available GSMs

	1st draft	After curation
GENES	723	610
REACTIONS	2132	1019
METABOLITES	1638	791



Delépine et al., 2020

Comparison between different carbon sources



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Data Management

- In C1Pro different types of data are generated: experimental meta-data, physiologic and fermentation data, product/substrate/metabolite analytic data from HPLC/GC/MS/NMR experiments, transcriptomics data from RNAseq experiments and fluxomics data from ^{13}C-labeling experiments

- Long term storage of the processed data intended for dissemination together with metadata and scripts in interlinked form in FAIRDOMHub

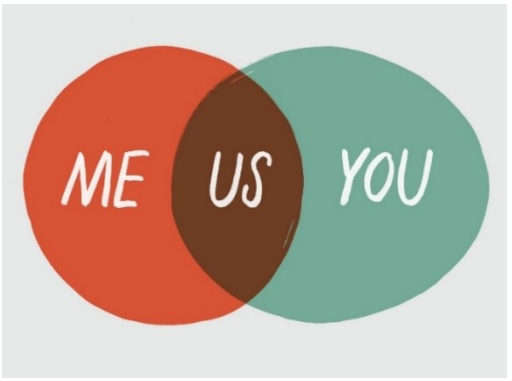
- Data-sharing within the project is organized via a project-own cloud maintained at UNIBI and via SEEK

Communication strategy

- Dissemination activities
  - Publication in the scientific literature
  - Presentation at national and international scientific meetings
  - Patents pending
  - Organization of conferences (9th International CeBiTec Research Conference Bielefeld)
- Communication
  - Active webpage
  - Meetings with school pupils and trainees (e. g. TeutoLab-Academy Systems Biology)

Responsible Research & innovation approach

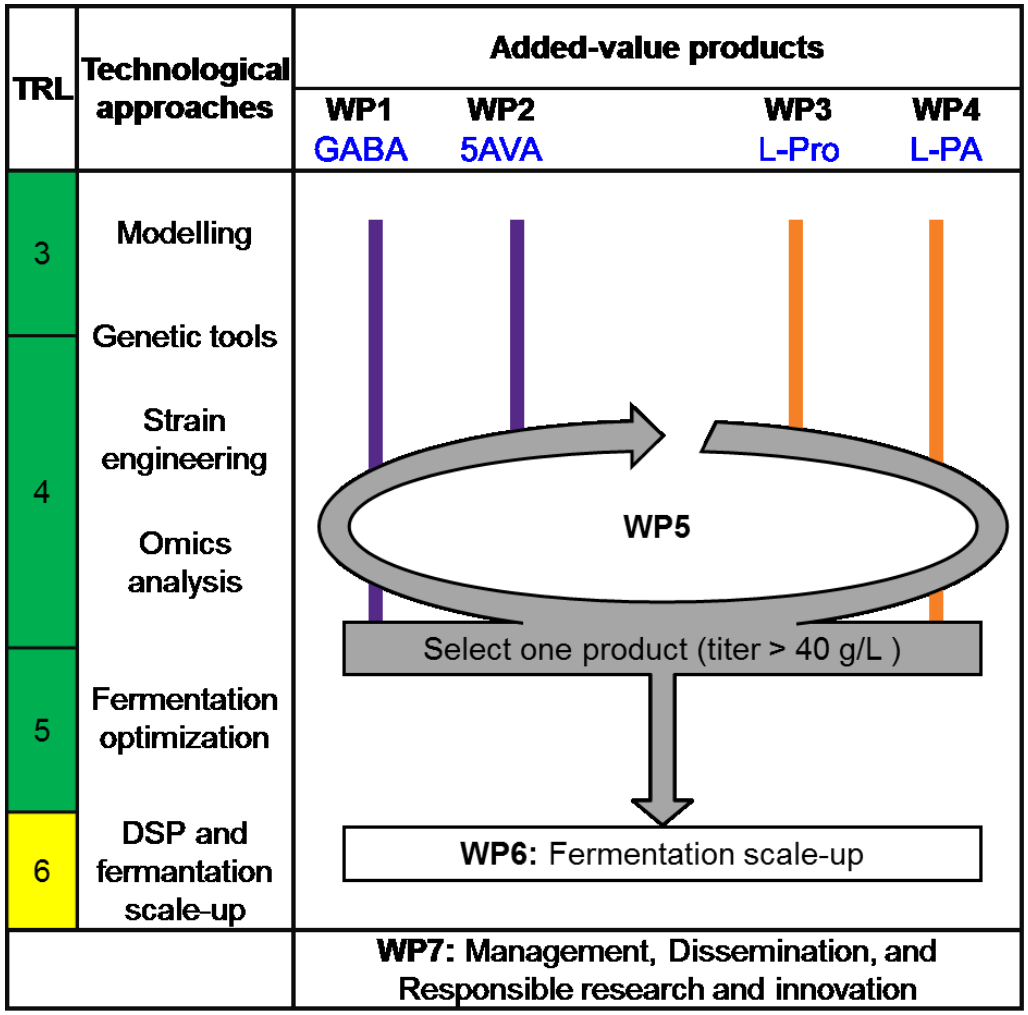
- **Scientists** should increasingly reflect on their visions and presumptions, including **positive and negative impacts** of their work on **society**.
- Science, technology and innovation shape the future. **But what kind of future do we want?** This question goes beyond science.
- An effective process of learning about making research & innovation responsible to the needs of society should emerge through processes of anticipation, reflection, and inclusion



Responsible Research & innovation approach

- C1Pro is in well accordance with several sustainability goals and the bioeconomy
- C1Pro has two active and engaged industry partners; communication and mutual understanding
- C1Pro results well communicated in peer review articles, book chapters, conferences, workshops, newsletters and social media
- Norwegian C1Pro partners (SINTEF, NTNU) active collaboration with RRI competence hub in Centre for Digital Life Norway; including starting a new PhD course in Transdisciplinarity in Biotechnology
- C1Pro PL presented on RRI on ERA CoBioTech workshop 22.06.2020

Technology transfer and exploitation of results



Summary (1)

Goals

- Establishment of a sustainable platform for methanol-based production of four value-added products GABA, 5AVA, L-Pro, L-PA

Achievements

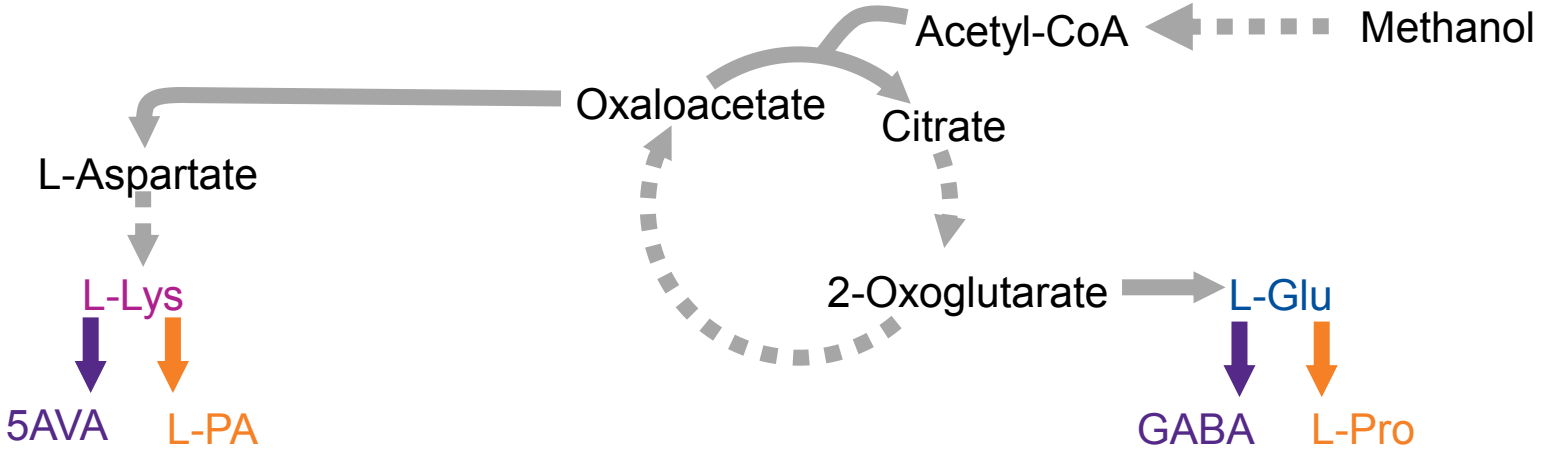
- Proof-of-concept for production of all targeted compounds and strain characterization
- Improvement of precursor supply (L-glutamate) through high-throughput screening of mutants
- Transfer of knowledge about 5AVA production to create 5AVA-production *Corynebacterium glutamicum* strains
- Development of gene silencing tools and tools to control gene expression
- Downstream process for GABA purification

Plans for future

- Work is still on-going! Final analysis of recently conducted experiments
- Some results still not published (up-coming papers)
- A new project (McM4SB) involving 3 partners of C1Pro has recently started
  - Expanding beyond methanol as C-source and amino acids as products

Project outcomes

- Four different chemicals produced from methanol by genetically engineered *B. methanolicus* strains
- Several joint publications; patents pending
- Sharing of competence, technology and knowledge among project partners
- Industrial interest in methanol as feedstock (BASF SE, Acies Bio)
- PhD education and postdoc training



General Evaluation

Benefits of creating scientific consortium within ERA CoBiotech

- Initiation of international collaboration,
- Creation of scientific networks for researchers, including young scientists (PhDs, postdocs),
- Collaboration academia-research institutes-industry,
- Joint publications,
- Trandisiplinarity due to partners with different scientific expertise
- Student exchange,
- New projects (MCM4SB) and new proposals ideas.

General Evaluation

Positive feedback to ERA CoBioTech

- Efficiently sized projects,
  - Good communication between partners
  - Limited administration needs,
  - Preexisting structure for data management and other managment needs,
  - Support in comminication with public
- Biohacks/networking arenas.

Contact details

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https://www.ntnu.edu/c1pro/