Engineering yeast for production of advanced biofuels

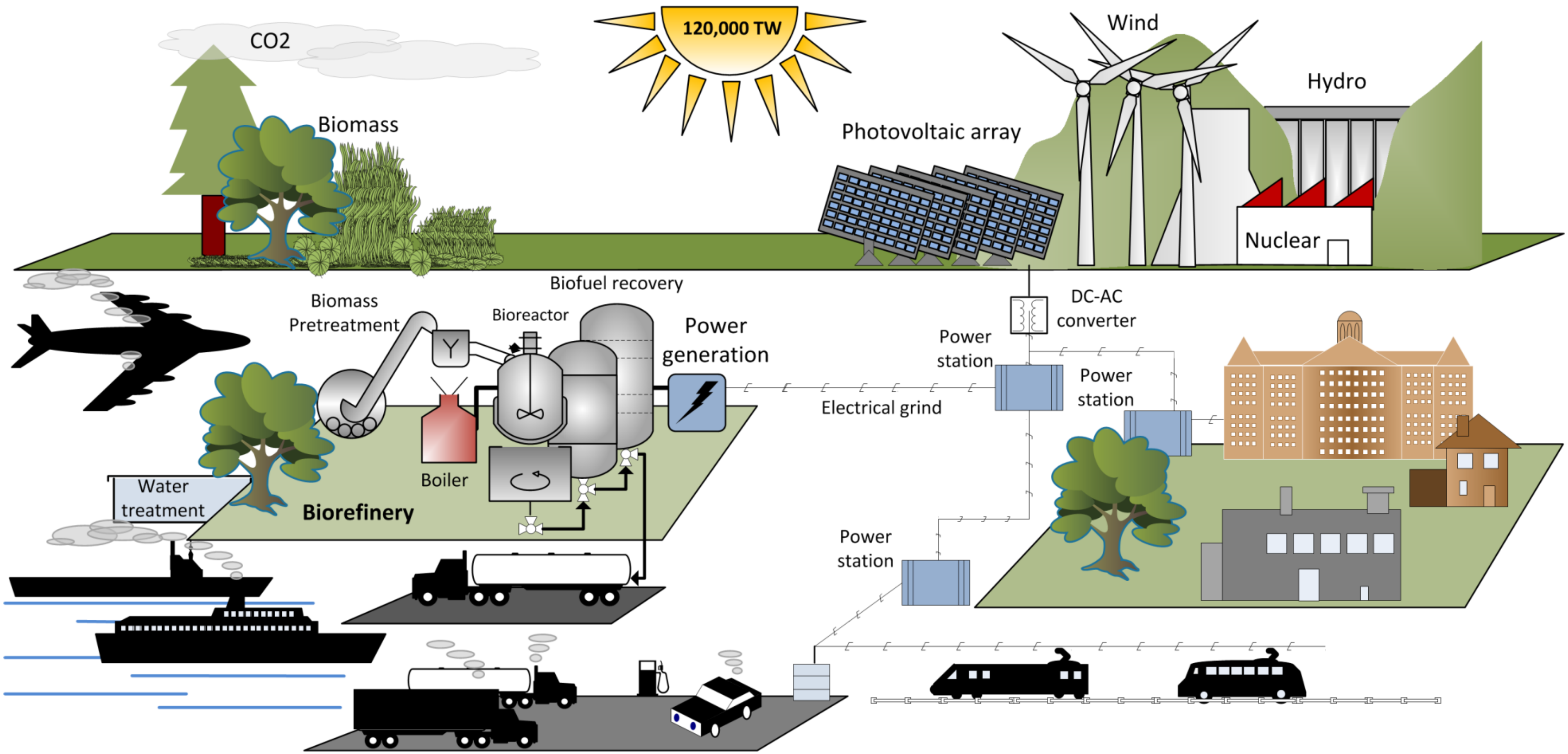
Jens Nielsen

Department of Biology and Biological Engineering, Chalmers University of Technology, Sweden

Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark

Sustainability

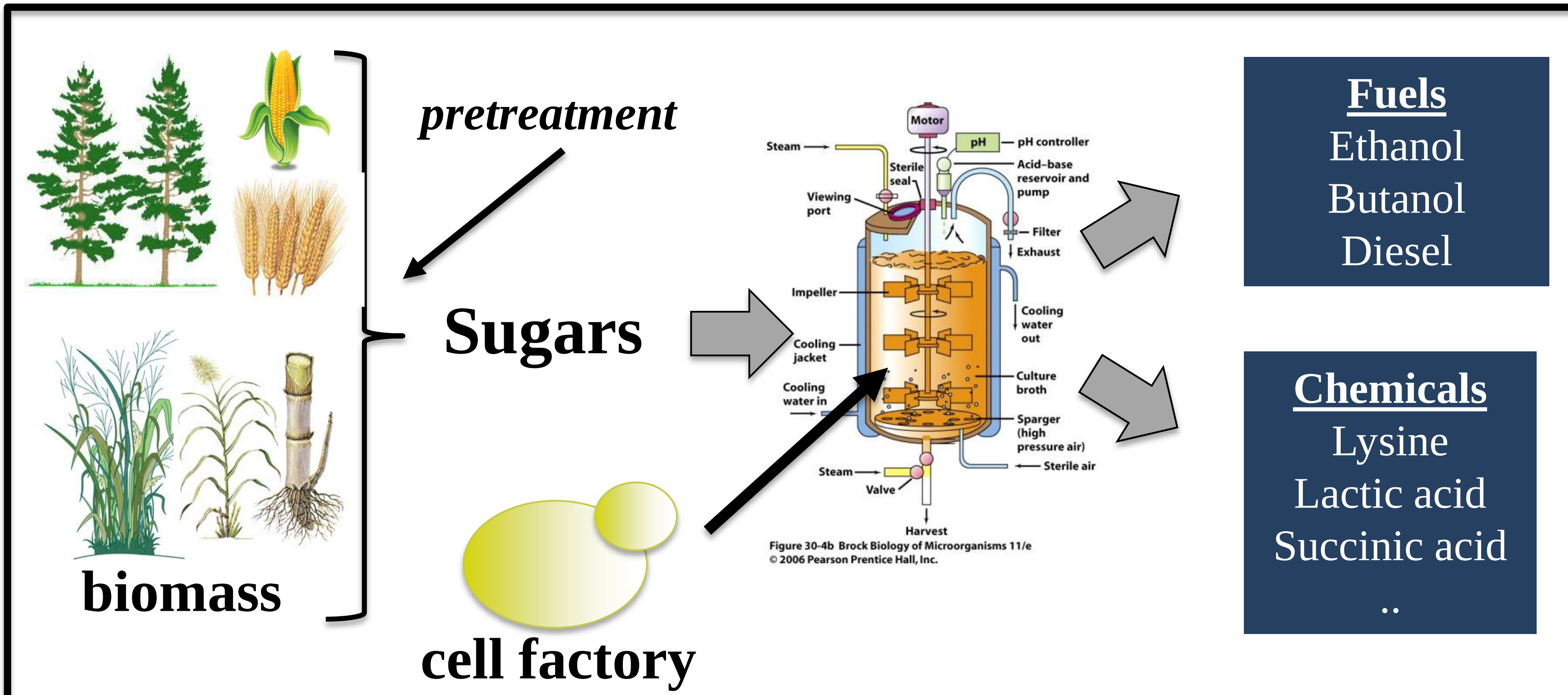
Metabolic engineering is an enabling science that can contribute to establish a sustainable society



The Biorefinery Concept

Metabolic Engineering of Cell Factories enables development of novel cell factories

Engineered cell factories can be used in biorefineries for sustainable production of fuels and chemicals



FEMS Yeast Res. (2012) **12**:228-248

The Value Chain

sys²bio www.sysbio.se

Raw Material → **Pre-treatment** → **Fermen-tation** → **Purifi-cation** → **Formu-lation** → **Chemicals & Fuels**

The research intensive part
Desire to develop **plug and play solutions**, i.e. common cell factory platforms for many products

Typical costs for developing a cell factory is **USD50 million**

There is a trend towards partnerships – sharing **competences**, **risks**, and **capital investments**

Isobutanol

bp

DUPONT The miracles of science™

Farnesane

TOTAL

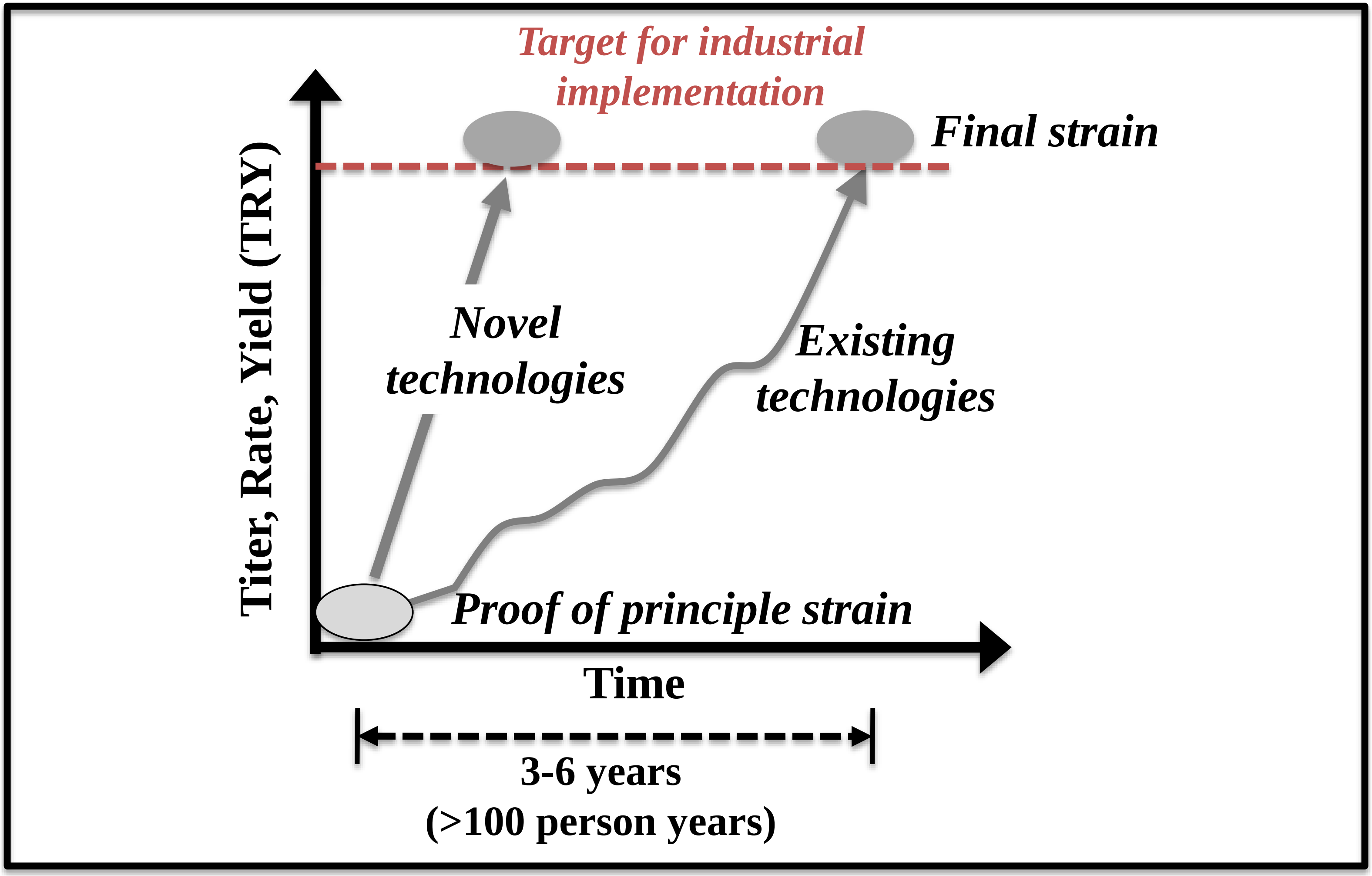
AMYRIS

1,4 Butanediol

genomatica sustainable chemicals

BASF The Chemical Company

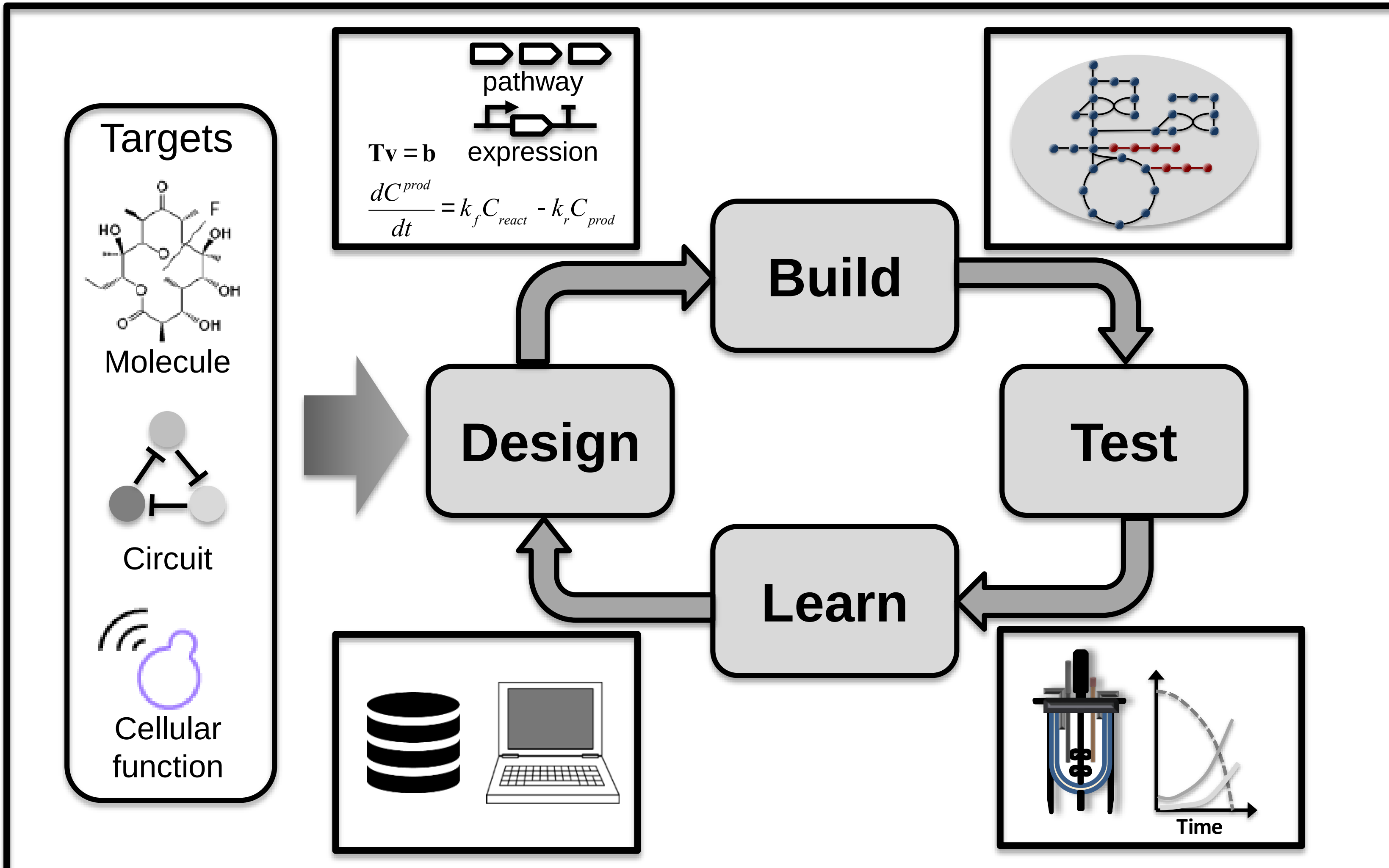
Cell Factory Development



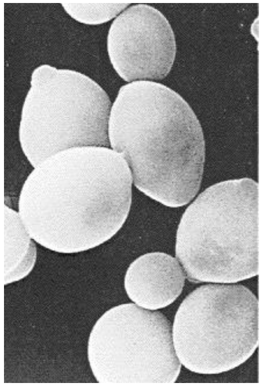
New **enabling technologies** are needed to substantially reduce the **time for developing efficient cell factories** for industrial production

Design-Build-Test Cycle

Metabolic engineering involves the design-build-test cycle



Yeast as a Cell Factory



Some of many advantages:

- Extremely well-characterized
- Many online databases with information on genome, as well as different omics data
- Genetically tractable
- GRAS (Generally Regarded as Safe)
- **Robust industrial organism**

Yeast is already a widely used for:

- Baker's yeast, wine & beer
- Bioethanol
- Isobutanol & farnesane
- Succinate
- Isoprenoids (fragrances)
- Resveratrol
- Insulin precursors & vaccines

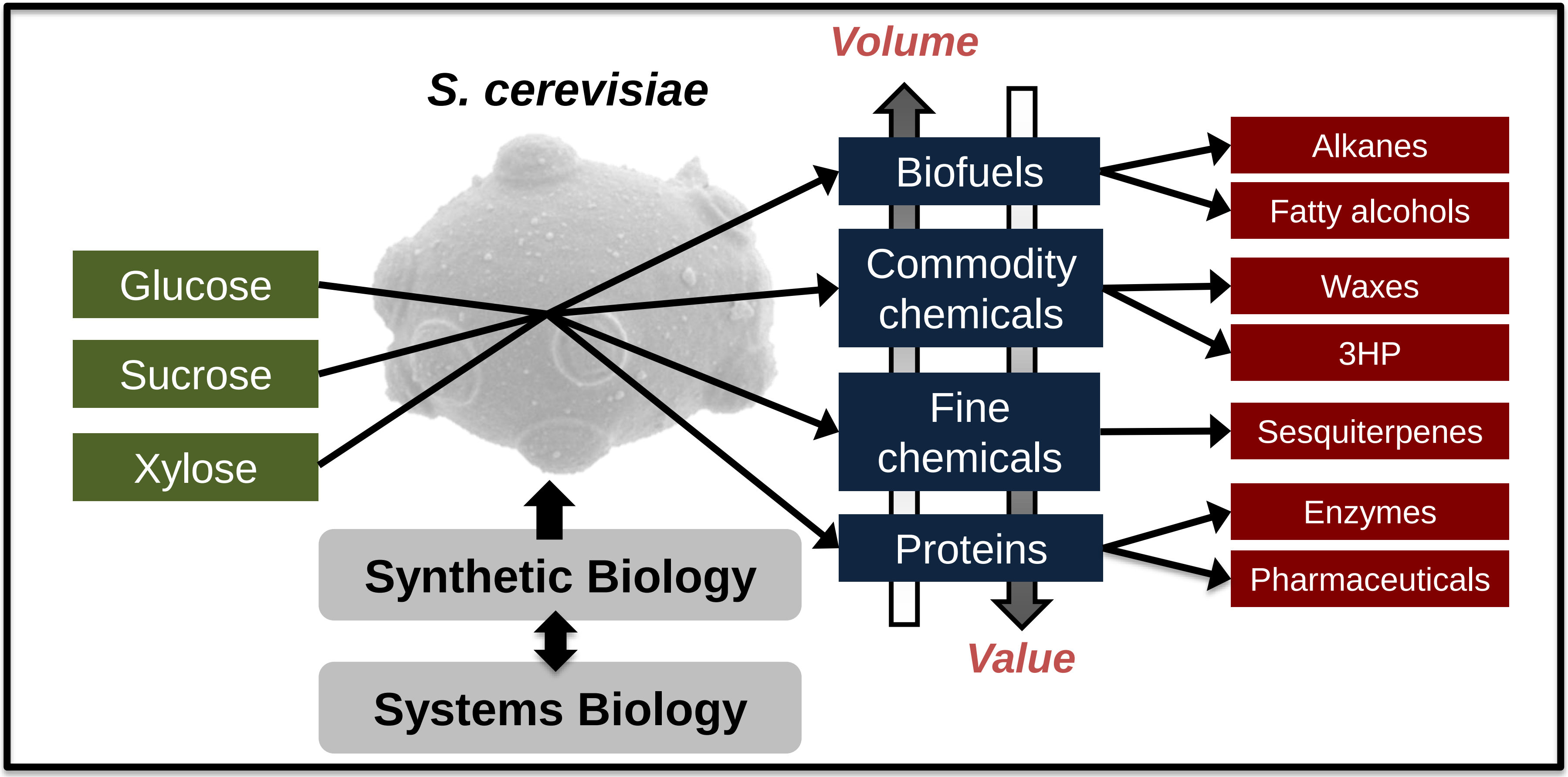
Ongoing developments of novel cell factories:

- **Fuels** (alkanes, monoterpenes)
- **Commodity chemicals** (malate, 3-OH propionic acid)
- **Fine chemicals** (isoprenoids**)**
- **Food ingredients** (PUFAs)
- **Protein drugs**

FEMS Yeast Research (2008) **8**:122-131
FEMS Yeast Research (2012) **12**:228-248

Yeast as a Platform Organism

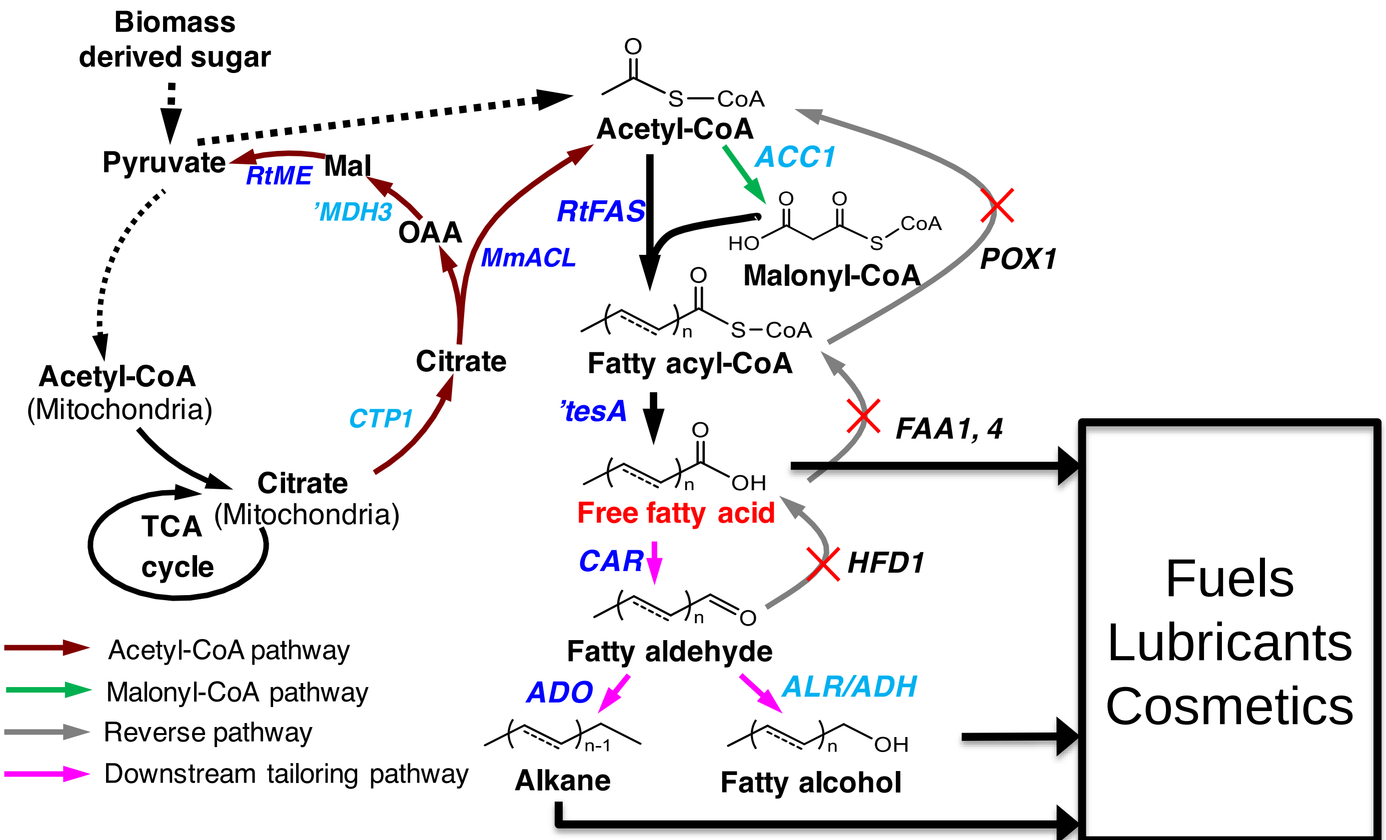
Our objective is to establish an extensive technology base for wider use of yeast as platform cell factory and demonstrate its use for production of a range of different products

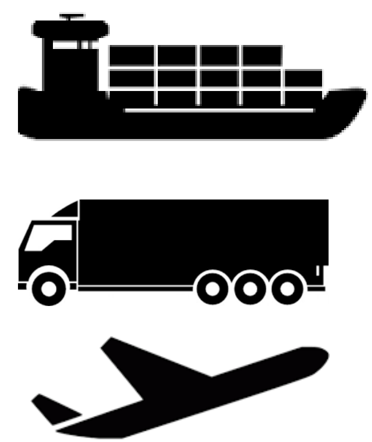


Fatty Acid Production Platform

Through a multiple engineering strategy we improved the production of free fatty acids >100 fold; enabling >10 g/L titer in fed-batch fermentations

Excellent platform strain for production of fatty alcohols and hydrocarbons

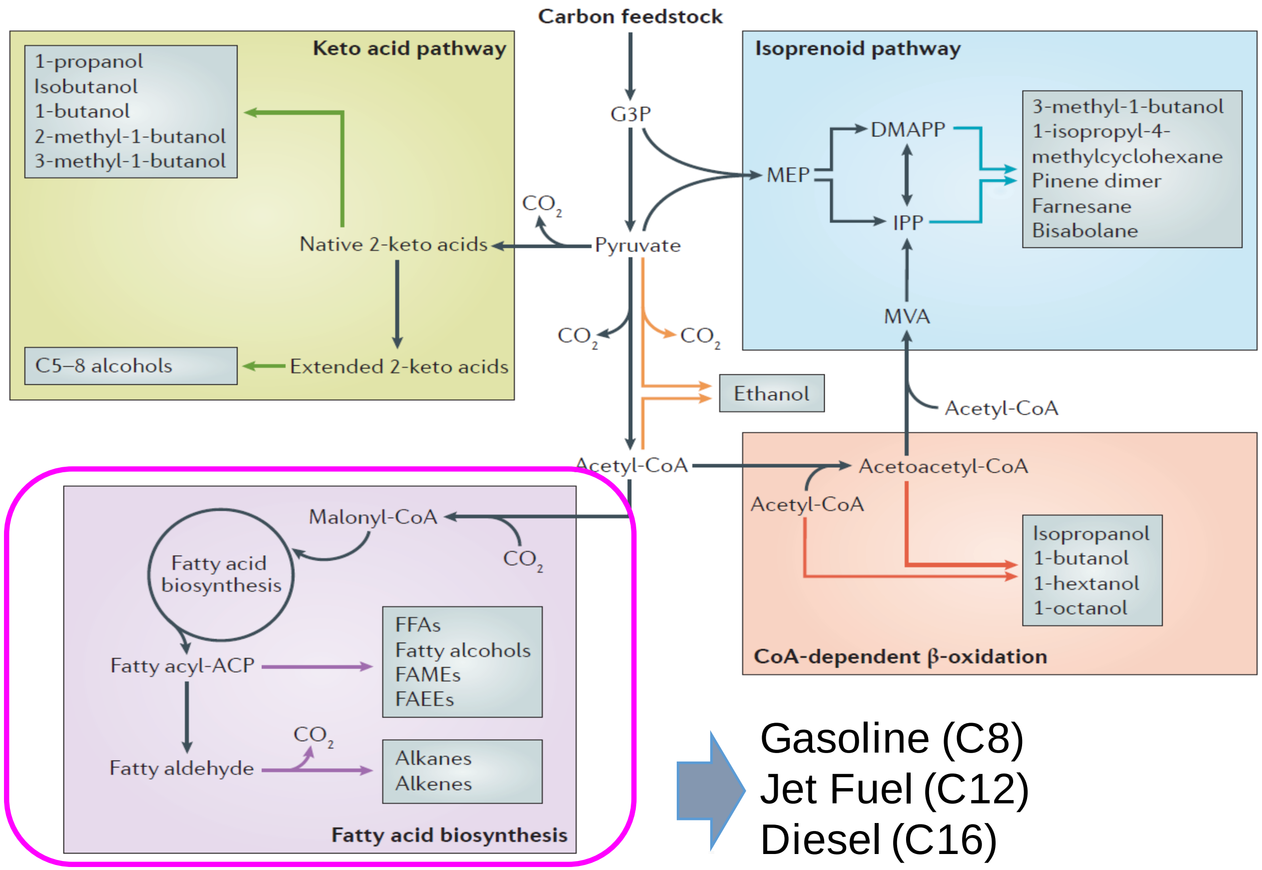




Nature Com. (2016) **7**:11709; *JACS* (2016) **138**:115368-15377

Production of drop-in fuels

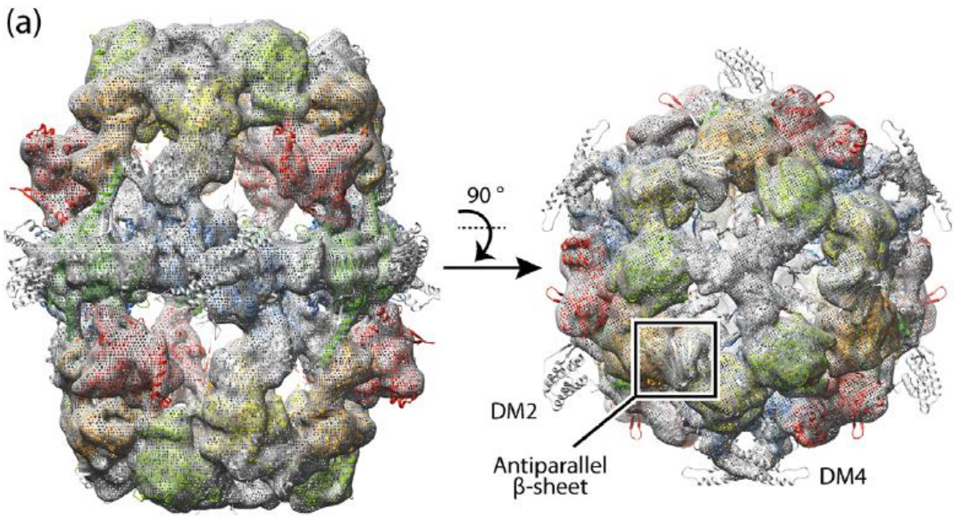
Sugar 17.0 MJ/kg → Ethanol 26.6 MJ/kg → 1-Octanol 40.7 MJ/kg → Octane 47.9 MJ/kg



Gasoline (C8)
Jet Fuel (C12)
Diesel (C16)

Production of short chain FAs

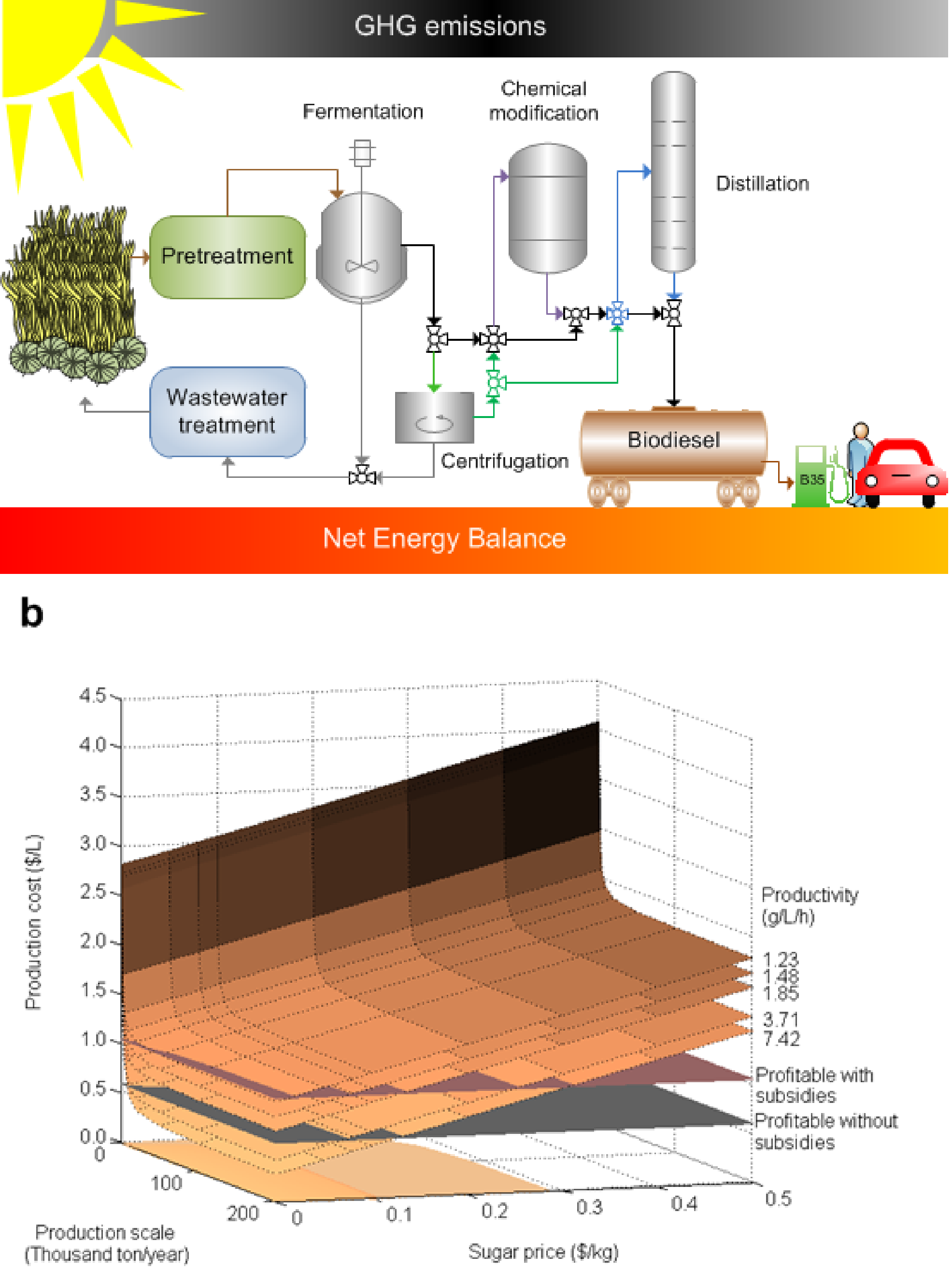
Fatty acid biosynthesis involves a multi-functional enzyme that operates in an iterative fashion where two-carbons are added in each cycle, eventually resulting in release of fatty acids with a chain length of 16 carbon atoms (C16)



We inserted a new enzyme activity, a thioesterase, in the catalytic core of this multi-functional enzyme and this allowed us to produce C8 and C10 fatty acids that can be converted further to alcohols and alkanes

These molecules can be used in gasoline – octane has an energy content of 47.9 MJ/kg compared with 17.0 MJ/kg for ethanol

Technology licensed to the French energy giant **Total** and the Swedish start-up **Biopetrolia AB**



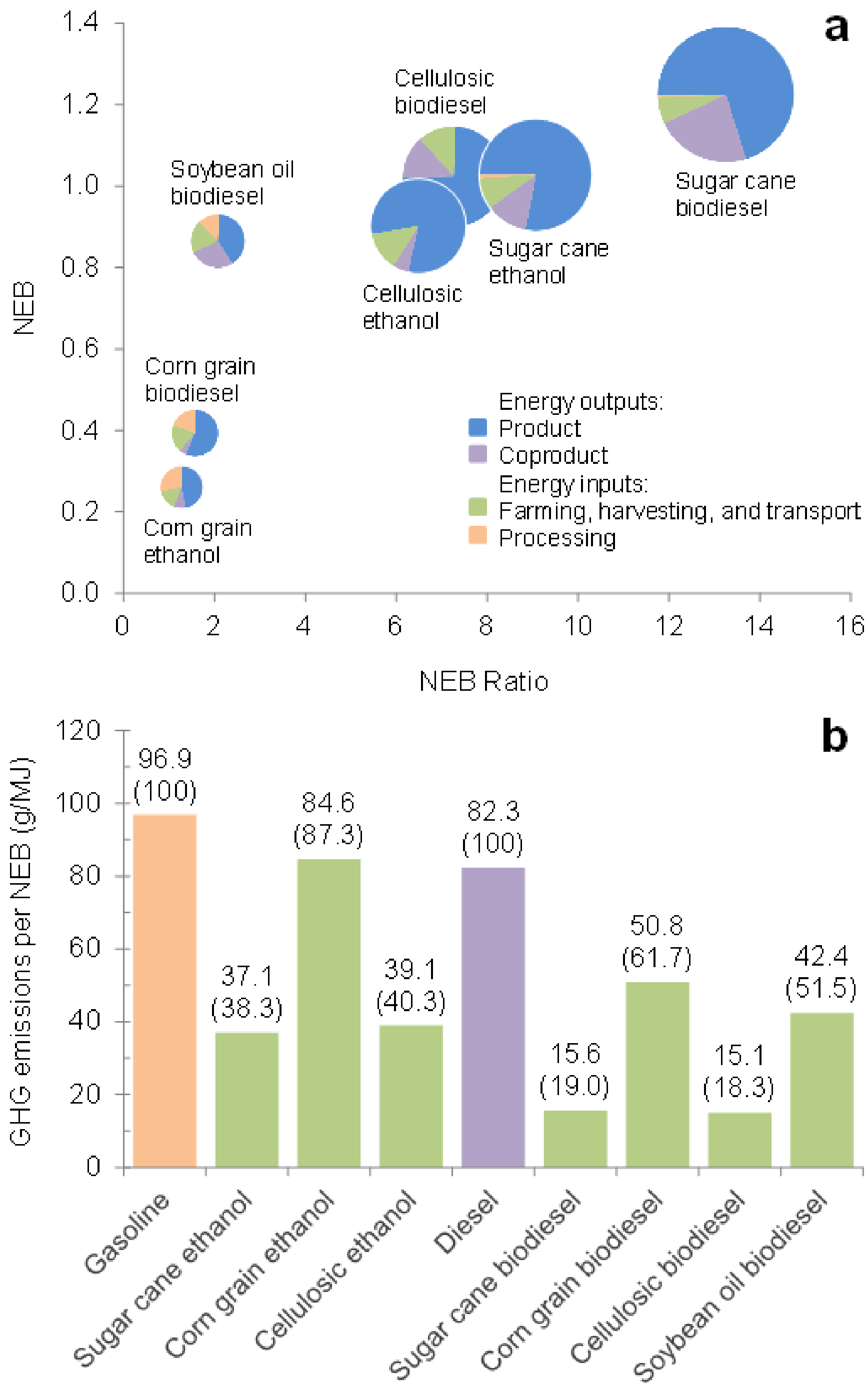
Total Energy Balance Calculations

At yields of about 90% of the theoretical yield (as for current ethanol production) hydrocarbon production can be profitable with a sugar price of about :

- 0.10 USD/Kg without subsidy
- 0.28 USD/Kg with subsidy

It will require subsidy or mandates to get the biofuels industry to develop

Significant reduction in GHG emission with production of advanced biofuels



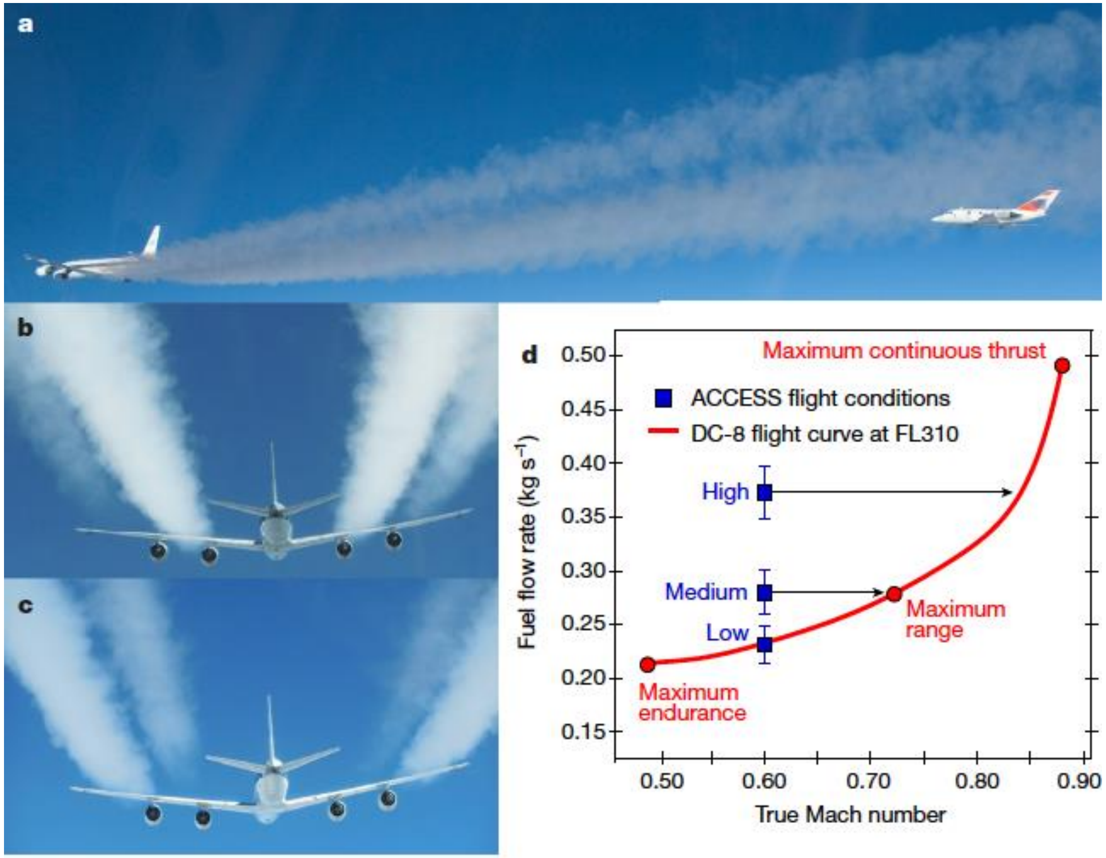
Combining genome-scale modeling for calculating theoretical yields with techno-economic modeling we performed analysis of economic feasibility of producing farnesane, FAEEs and alkanes

Production of these products results in significant reduction in Green House Gas (GHG) emission

Nature Biotechnol. (2013) **9:**789-793

Clean Technology

Use of biofuels in jet may significantly reduce particle emission and hereby global warming



LETTER

doi:10.1038/nature21420

Biofuel blending reduces particle emissions from aircraft engines at cruise conditions

Richard H. Moore[1], Kenneth L. Thornhill[1,2], Bernadett Weinzierl[3,4], Daniel Sauer[3,5], Eugenio D'Ascoli[3,5], Jin Kim[3], Michael Lichtenstern[3], Monika Scheibe[3], Brian Beaton[1], Andreas J. Beyersdorf[1,6], John Barrick[1,2], Dan Bulzan[7], Chelsea A. Corr[1,8], Ewan Crosbie[1,9], Tina Jurkat[3], Robert Martin[1], Dean Riddick[1], Michael Shook[1,2], Gregory Slover[1], Christiane Voigt[3,10], Robert White[1], Edward Winstead[1,2], Richard Yasky[1], Luke D. Ziemba[1], Anthony Brown[11], Hans Schlager[3] & Bruce E. Anderson[1]

Acknowledgement

Docent Verena Siewers
Dr. Yongjin Zhou
Dr, Yun Chen
Dr. Zhiwei Zhu
Klaas Buijs