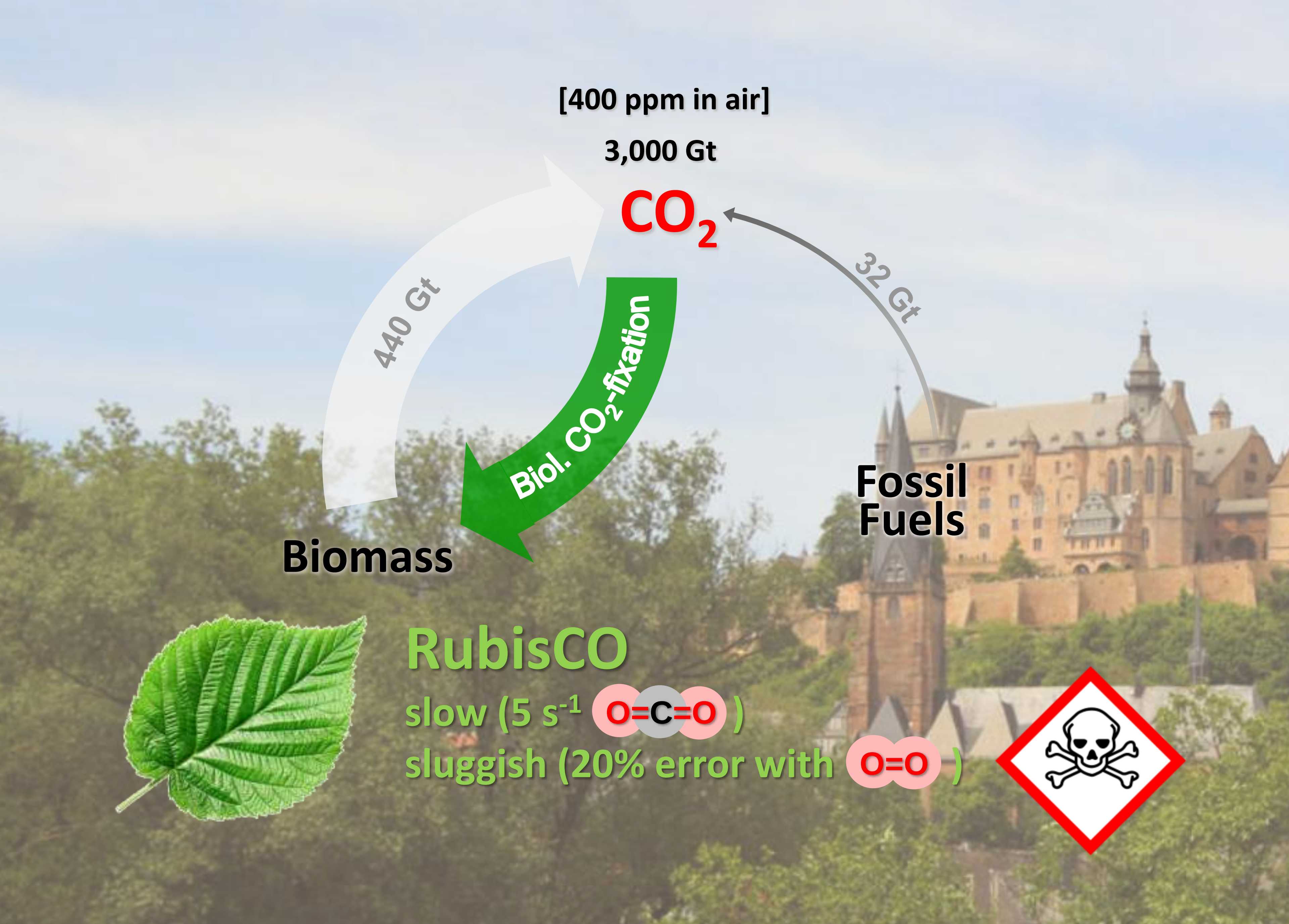
CETCH me if you can

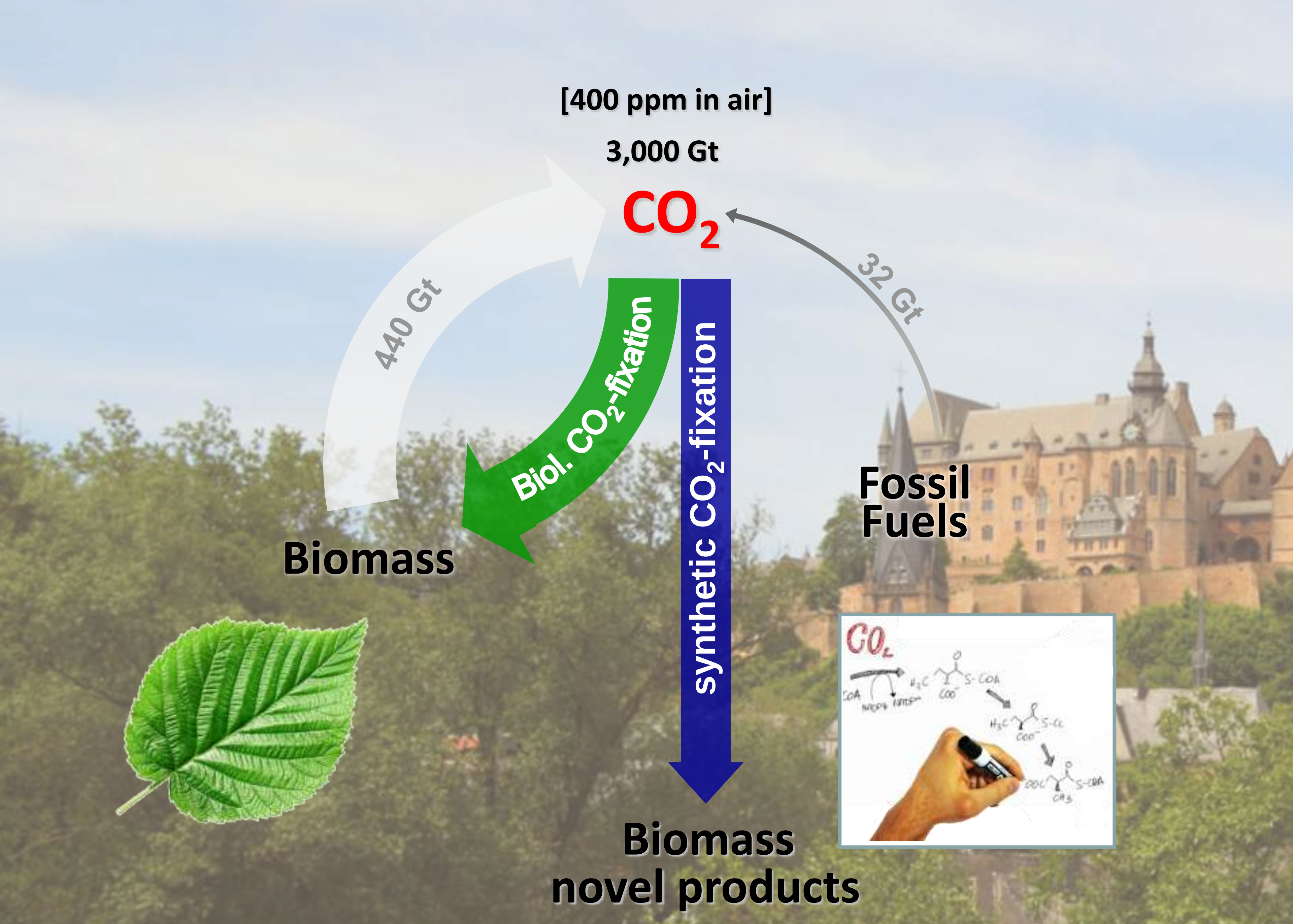
Designing new solutions for carbon capture & conversion with synthetic biology

Tobias J. Erb
Max Planck Institute for Terrestrial Microbiology, Marburg
Center for Synthetic Microbiology (synmikro)

@erblabs



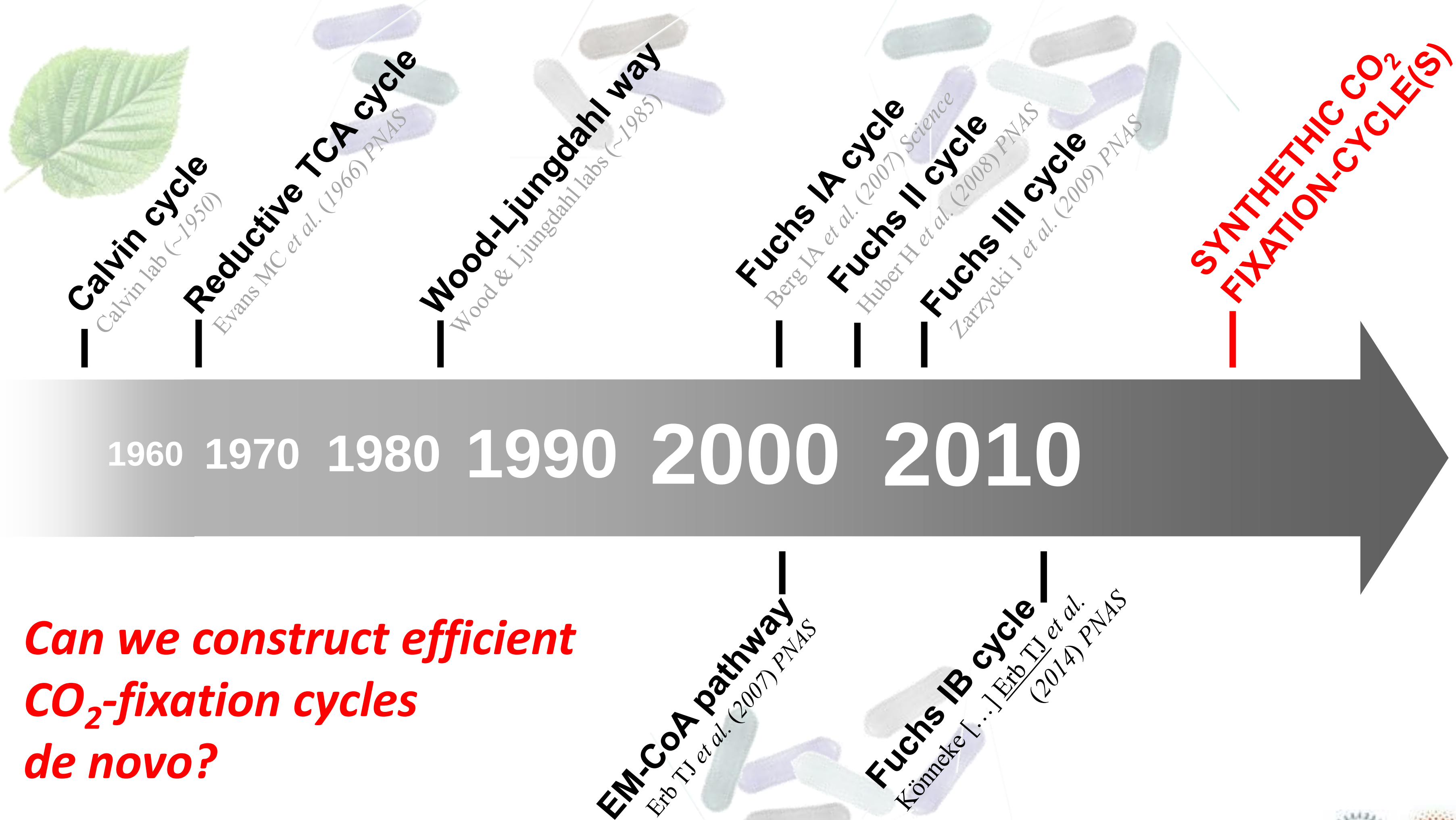
Erb *et al.* (2016) **Curr Op Chem Biol**



Erb *et al*. (2016) **Curr Op Chem Biol**

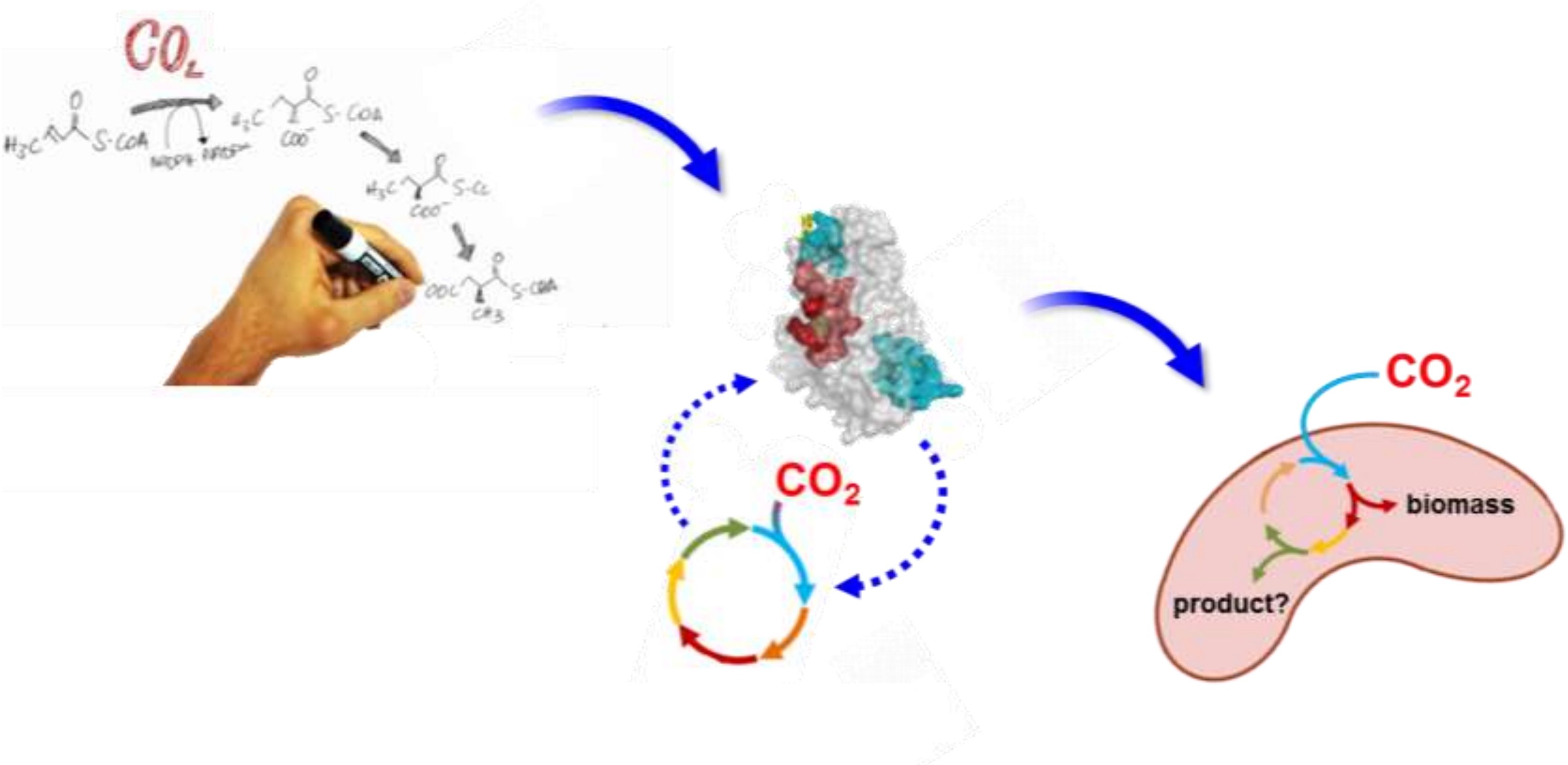
Alternative CO_2-fixation pathways

Nature evolved several CO_2-fixation pathways & enzymes



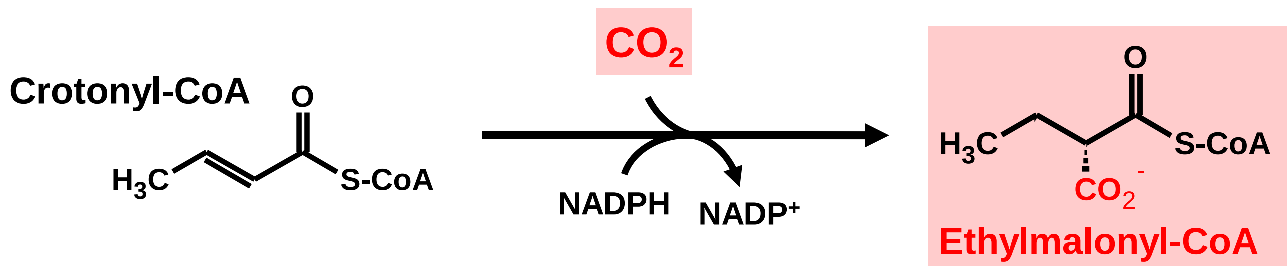
Can we construct efficient CO_2-fixation cycles de novo?

Erb TJ (2011) **Appl Env Microbiol**

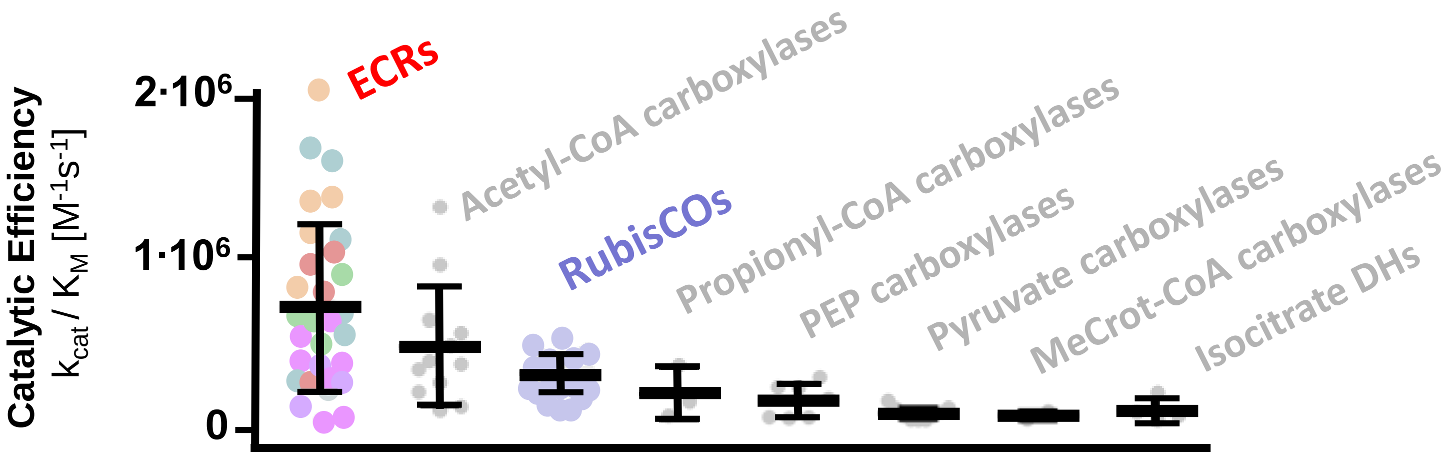


Finding & engineering an efficient CO_2-fixation reaction for carbon capture and conversion

The efficiency of CO_2-fixing enzymes

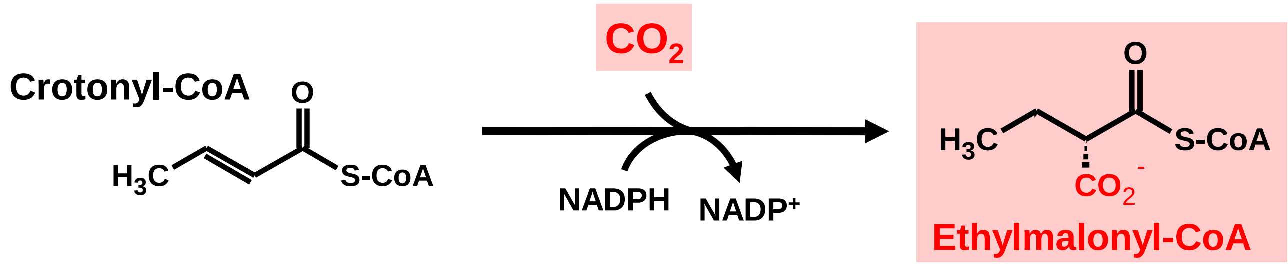


Enoyl-CoA Carboxylases/reductases (ECRs) are up to 4 x more efficient than RubisCO



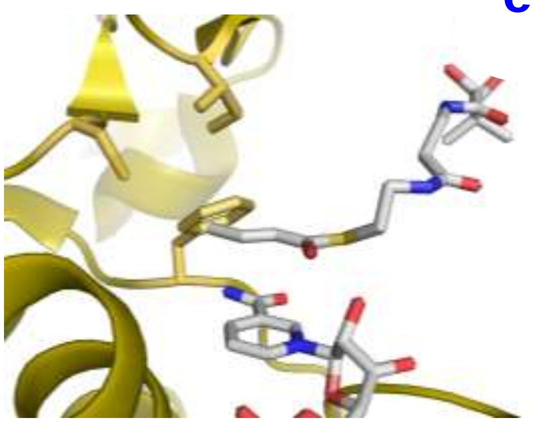
*Erb *et al.* (2007 & 2009) **PNAS**; Rosenthal *et al.* (2014, 2015 & 2017) **Nat Chem Biol**

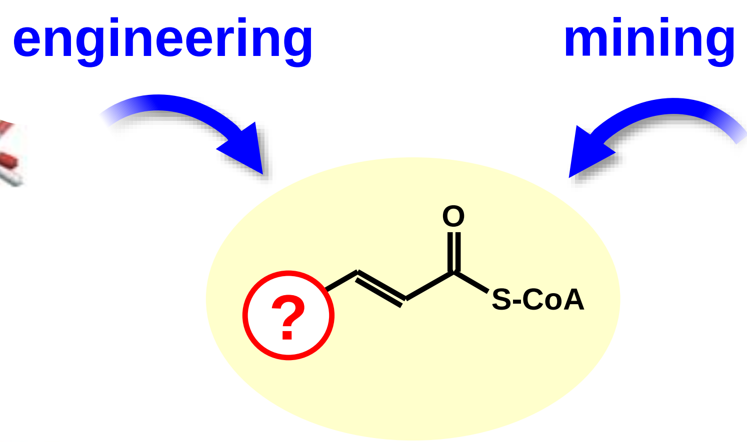
Expanding the (bio)synthetic space of ECRs



*Erb *et al.* (2007 & 2009) **PNAS**; Rosenthal *et al.* (2014, 2015 & 2017) **Nat Chem Biol**

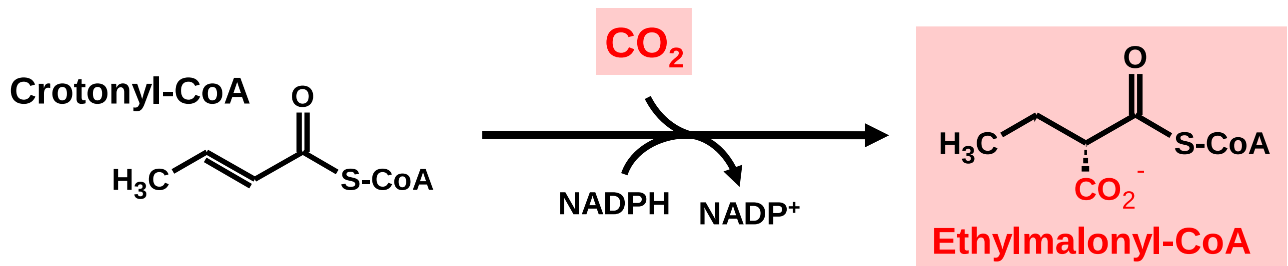
Can we design or discover new CO_2-fixation reactions?





Peter D *et al.* (2015) **Angew Chem Int Ed**

Expanding the (bio)synthetic space of ECRs

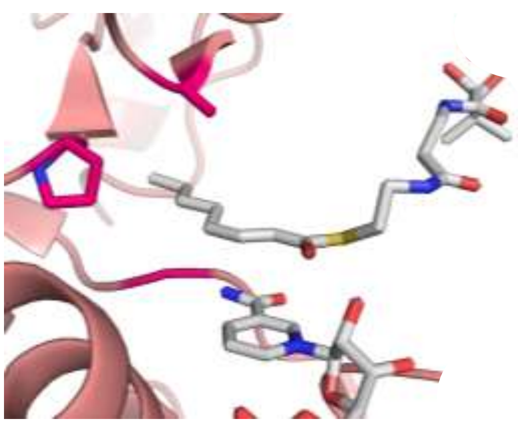


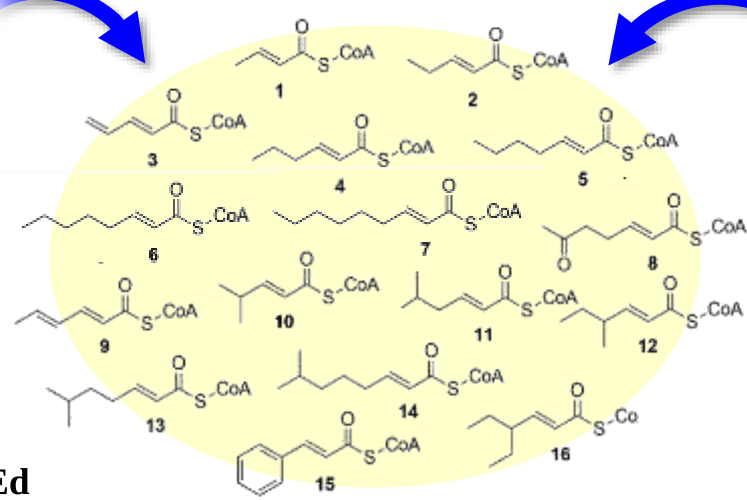
*Erb *et al.* (2007 & 2009) **PNAS**; Rosenthal *et al.* (2014, 2015 & 2017) **Nat Chem Biol**

Can we design or discover new CO_2-fixation reactions?

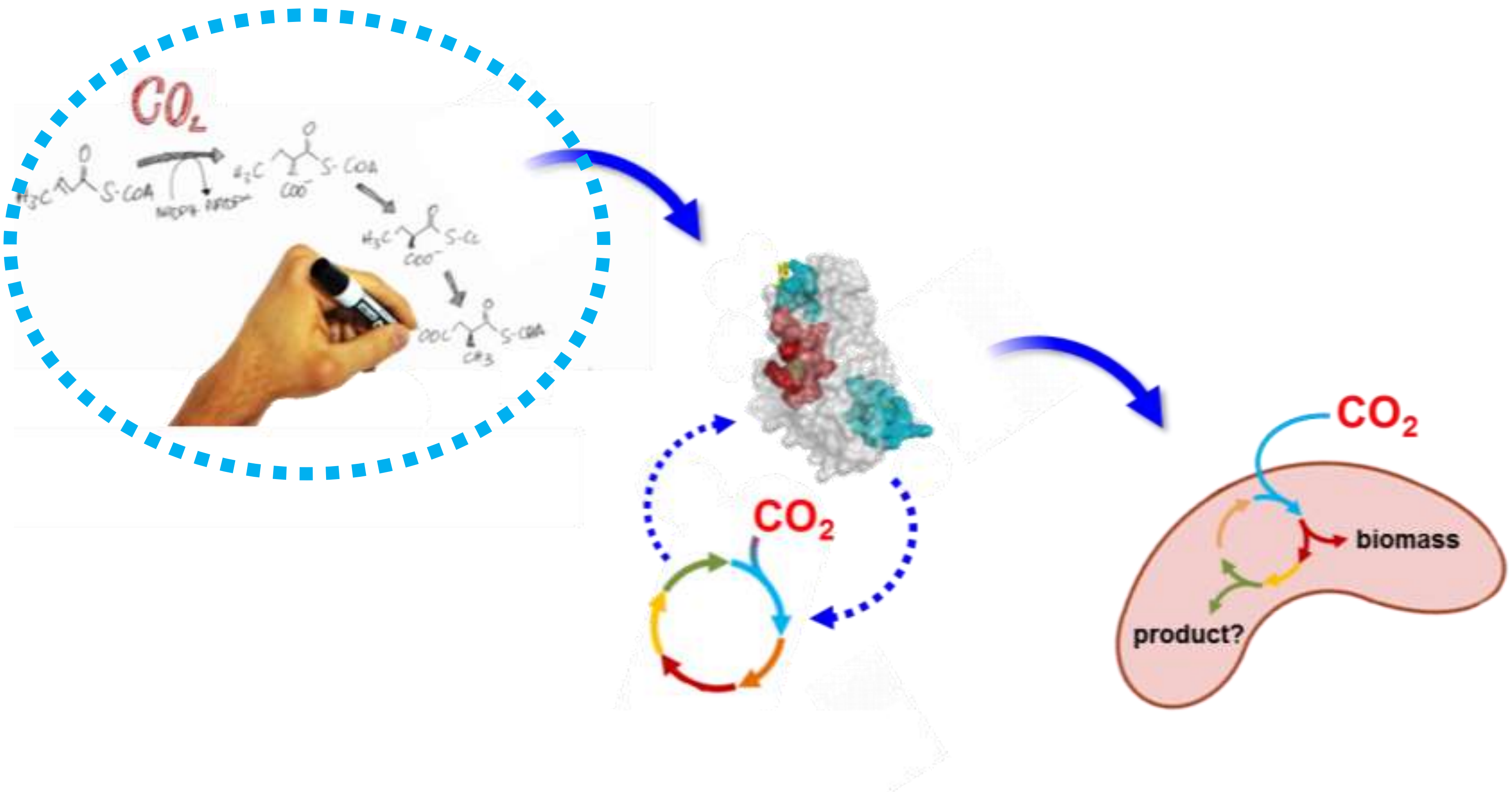
engineering

mining





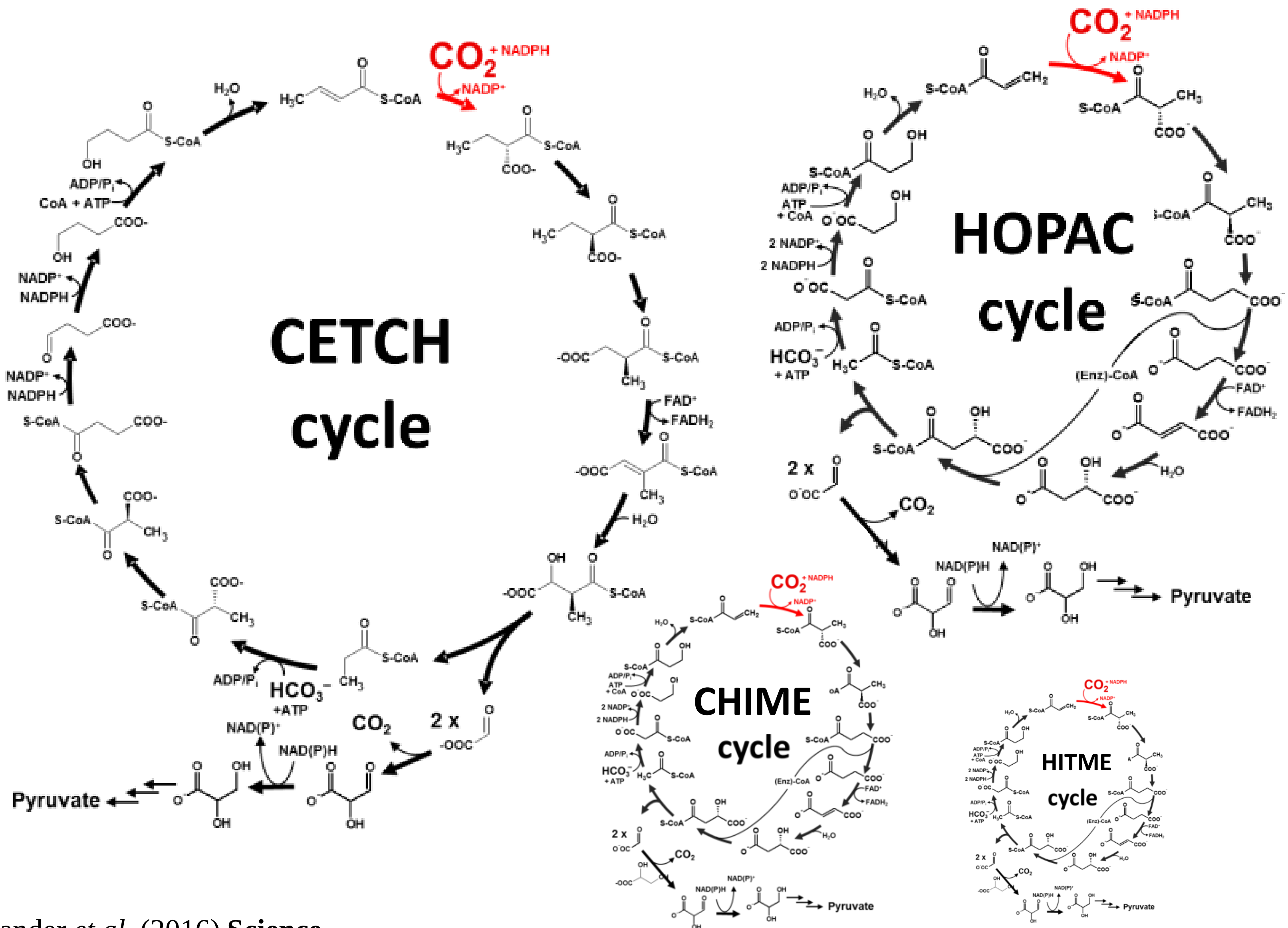
Peter D *et al.* (2015) **Angew Chem Int Ed**



How to **design** synthetic biological networks for carbon capture and conversion?

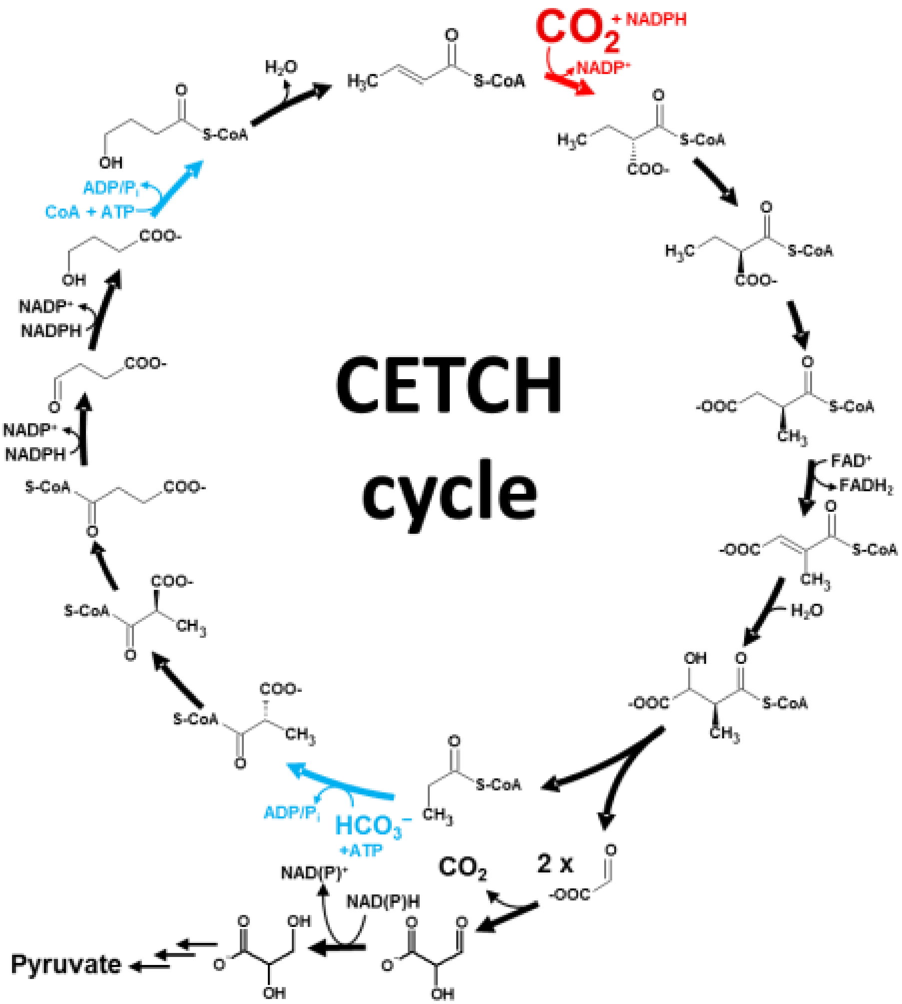
Design of synthetic CO_2-fixation pathways

(centered on an ECR reaction)



Schwander *et al.* (2016) **Science**

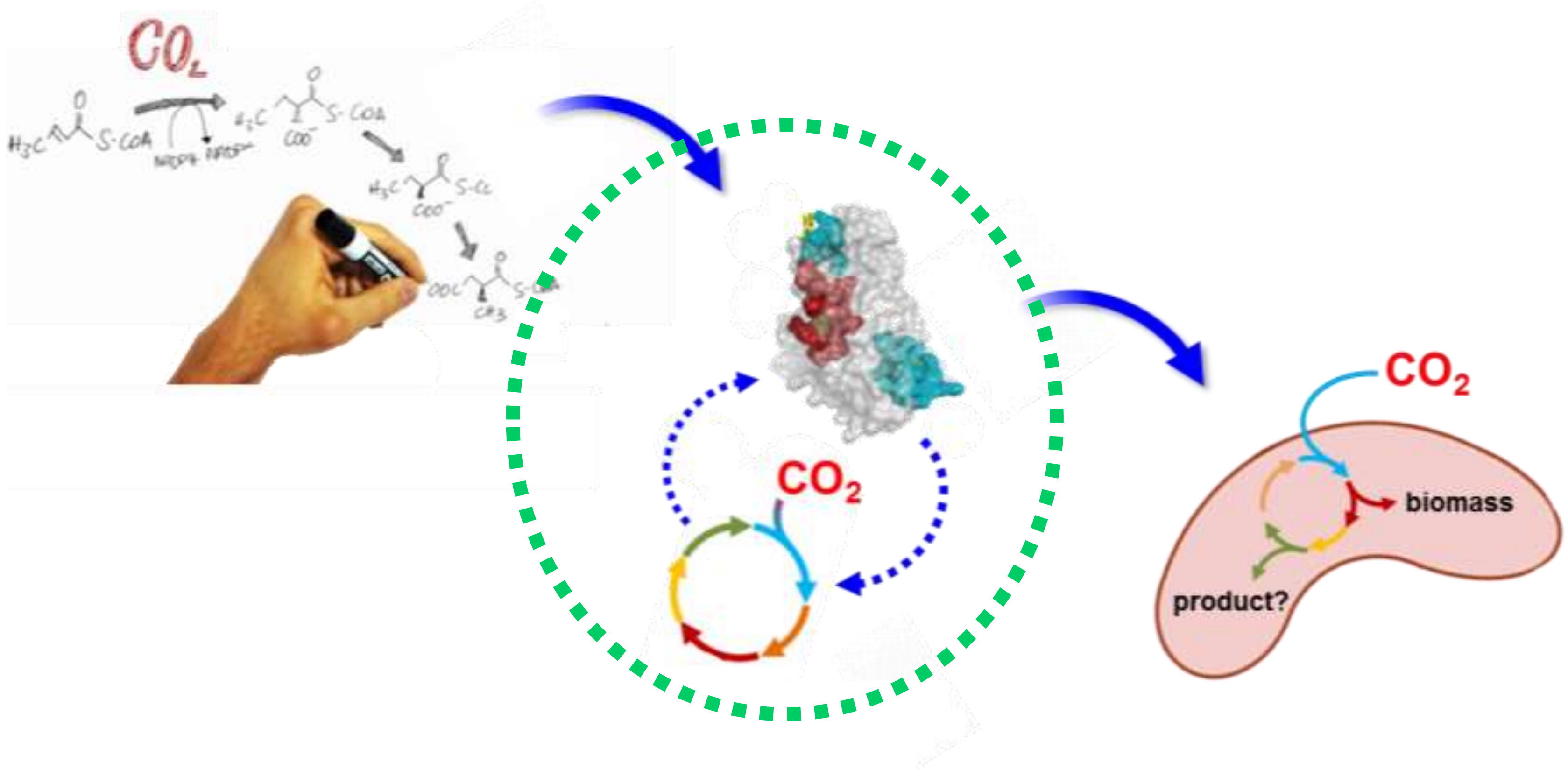
Evaluation of synthetic CO_2-fixation cycles



Evaluation criteria:

- ☑ **Kinetically favored**
  fast & efficient enzyme reactions
- ☑ **Thermodynamically favored**
  energy per CO_2 molecule fixed
- ☑ **Thermodynamically feasible**
  all equilibrium constants ≥ 1

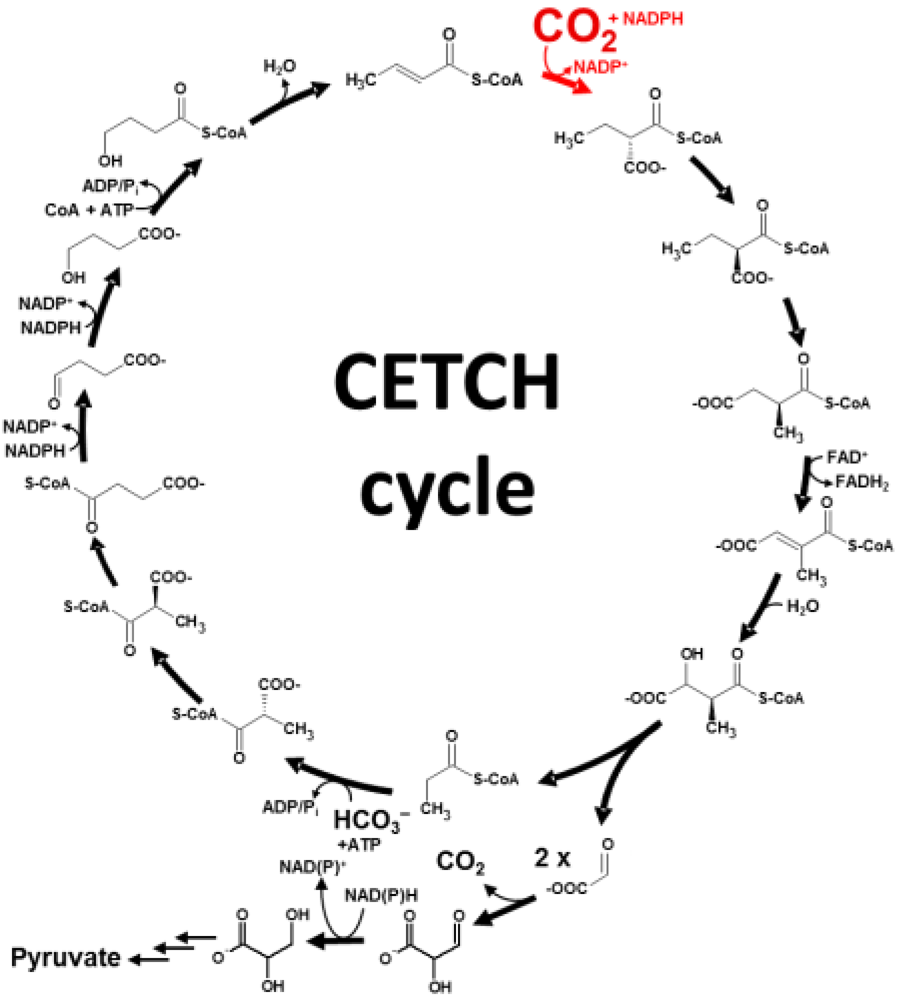
Schwander *et al.* (2016) **Science**



How to **realize** synthetic biological networks for carbon capture and conversion?

Realizing synthetic CO_2-fixation cycles:

Building the CETCH cycle version 1.0



Finding the parts:

- ☑ Searching enzyme databases
- ☑ Testing enzyme homologs
- ☑ (Re)-engineering enzymes

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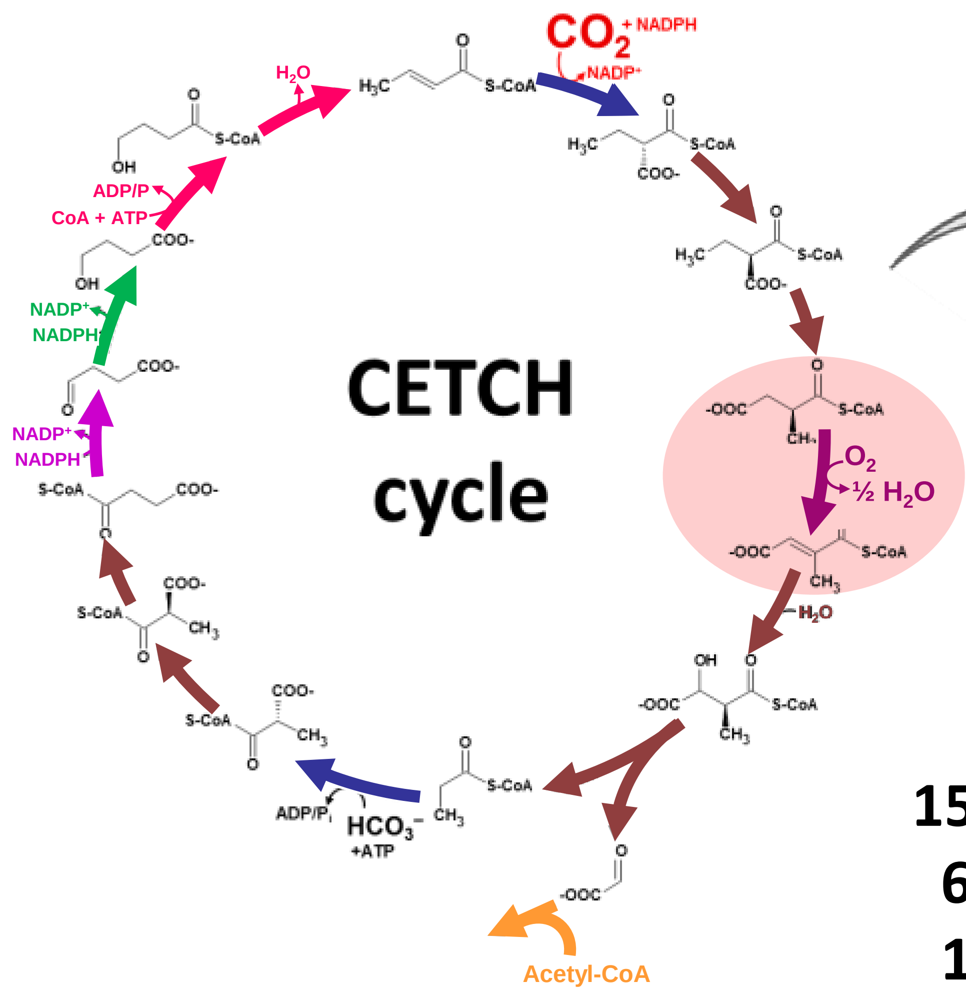
Realizing synthetic CO_2-fixation cycles:

Building the CETCH cycle version 1.0



Methylobacterium

Rhodobacter

Clostridium

E. coli

Nitrosopumilus

Human liver

15 different enzymes

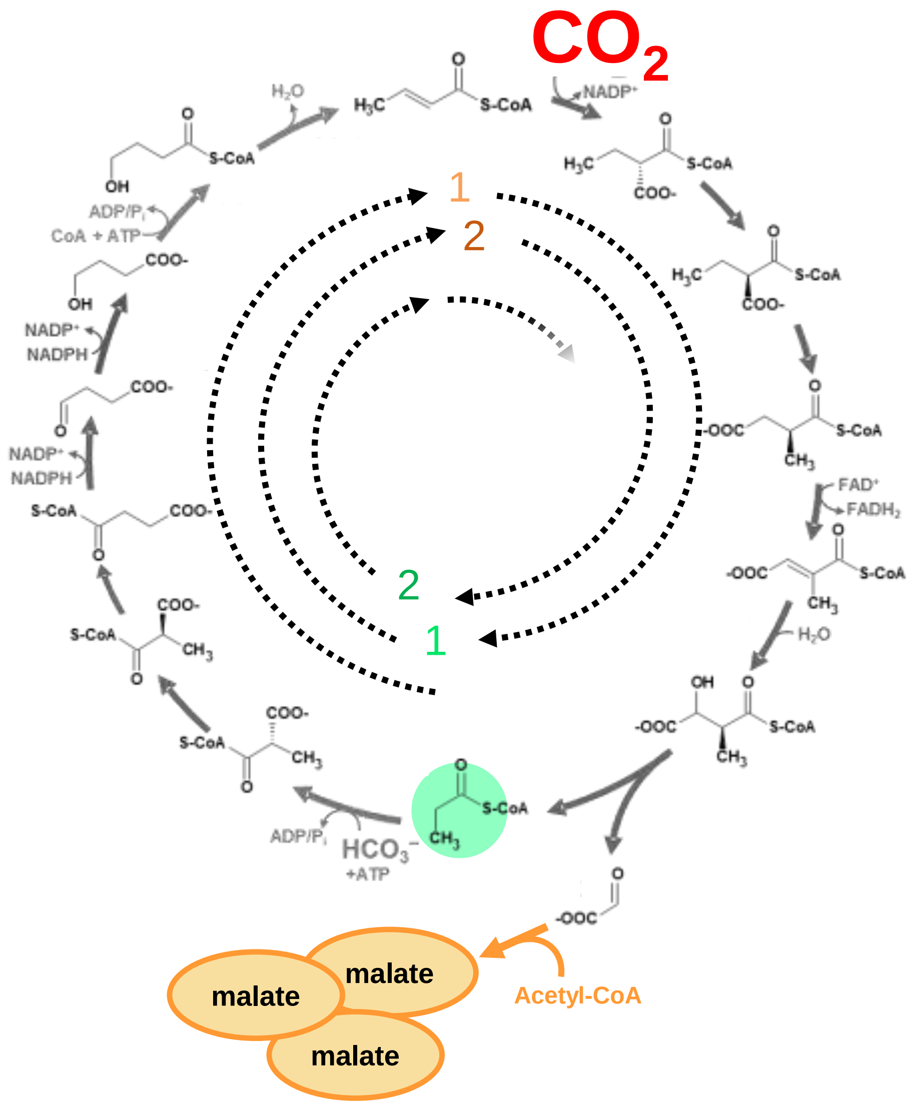
6 different organisms

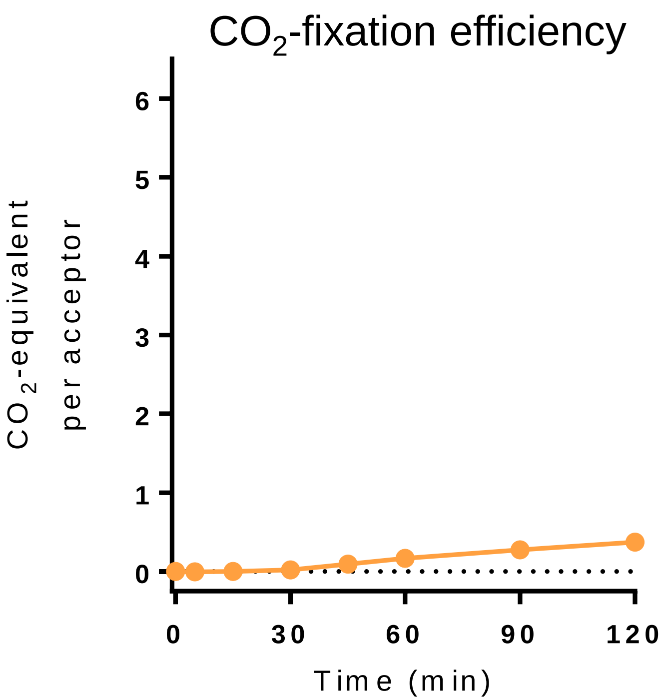
1 engineered enzyme

Schwander *et al.* (2016) **Science**

Realizing synthetic CO_2-fixation cycles:

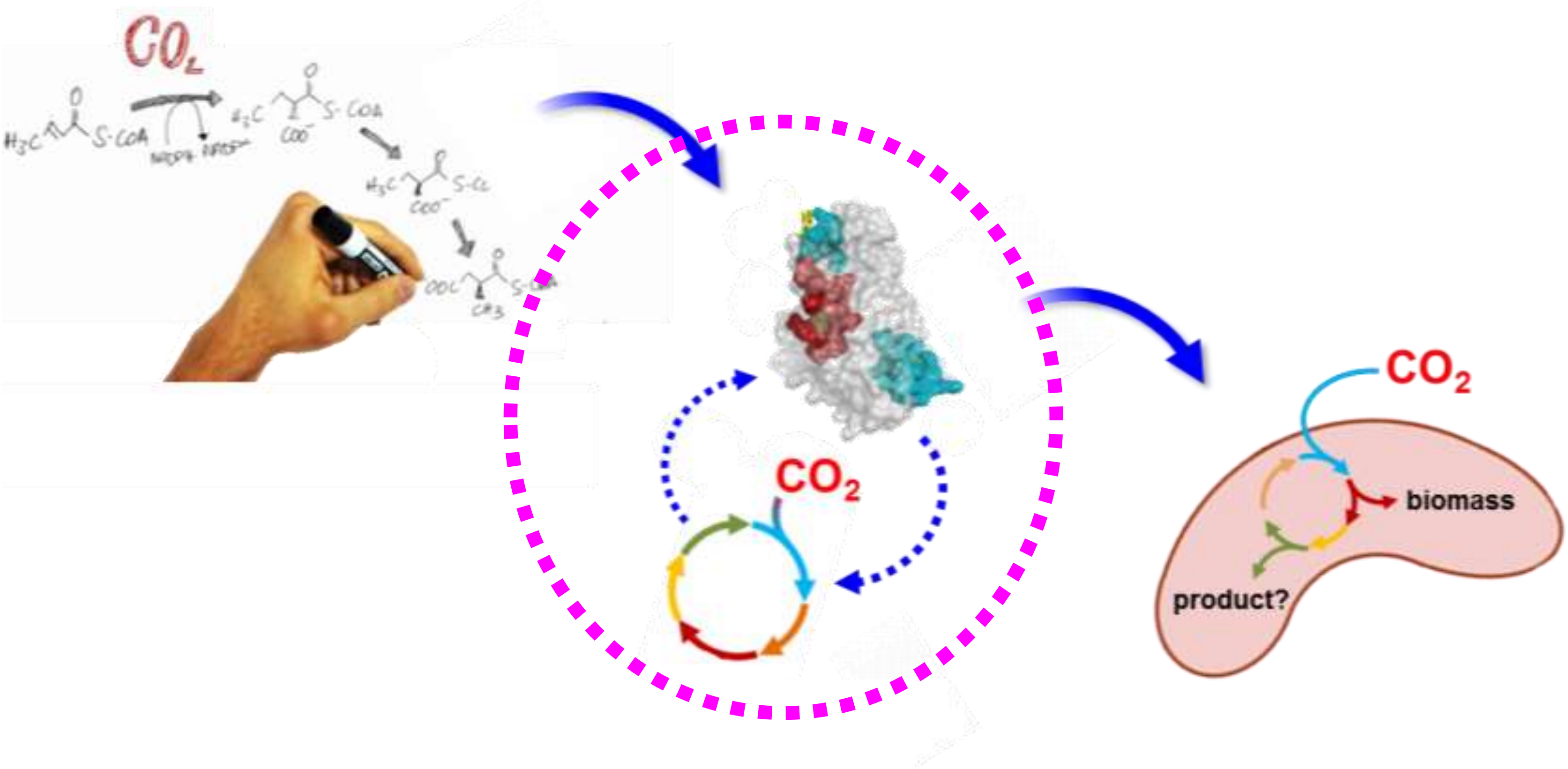
Building the CETCH cycle version 1.0





0.2 CO_2 molecules acceptor^{-1} and h^{-1}

Schwander *et al.* (2016) **Science**

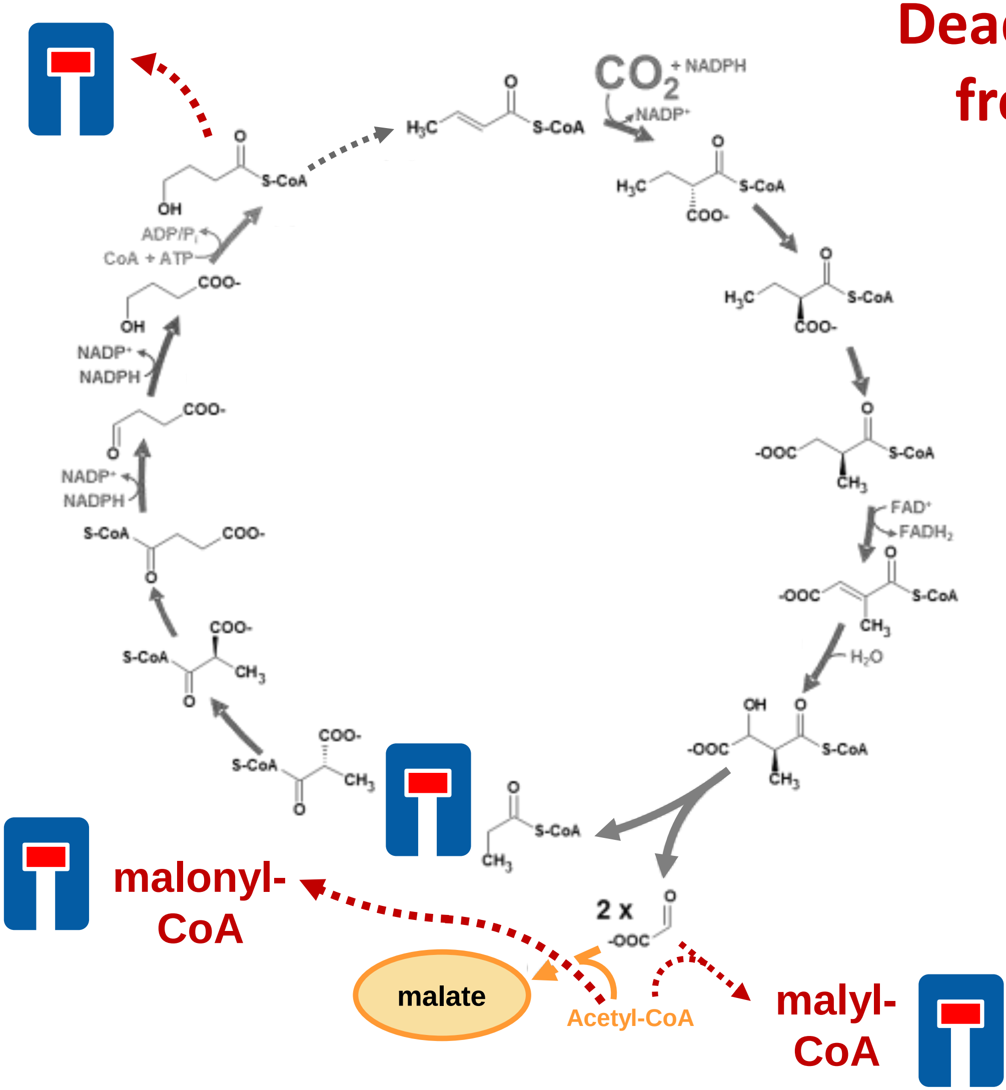


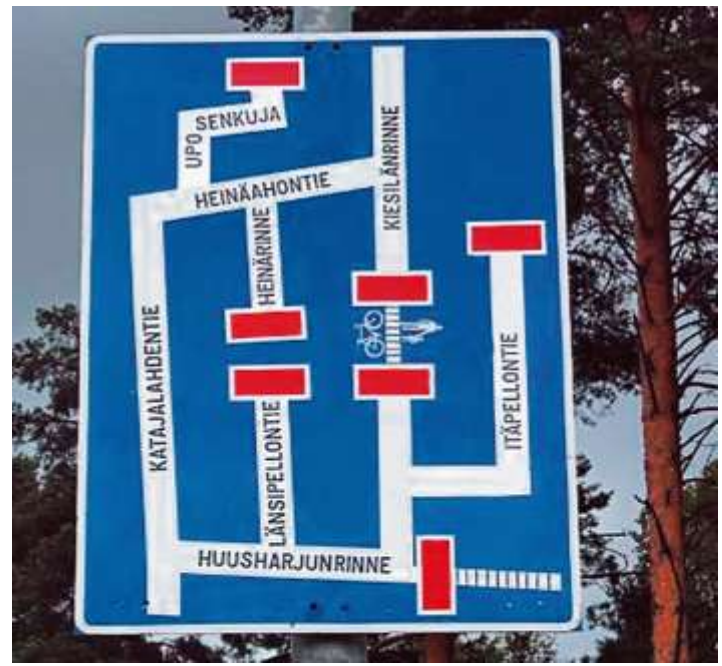
How to **optimize** synthetic biological networks for carbon capture and conversion?

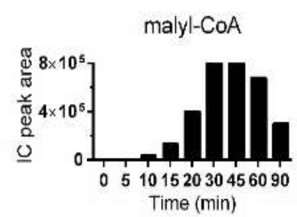
Optimizing synthetic CO_2-fixation cycles:

The CETCH cycle (v2.0) is limited in CO_2-fixation

Dead end-metabolites accumulate from unwanted side reactions



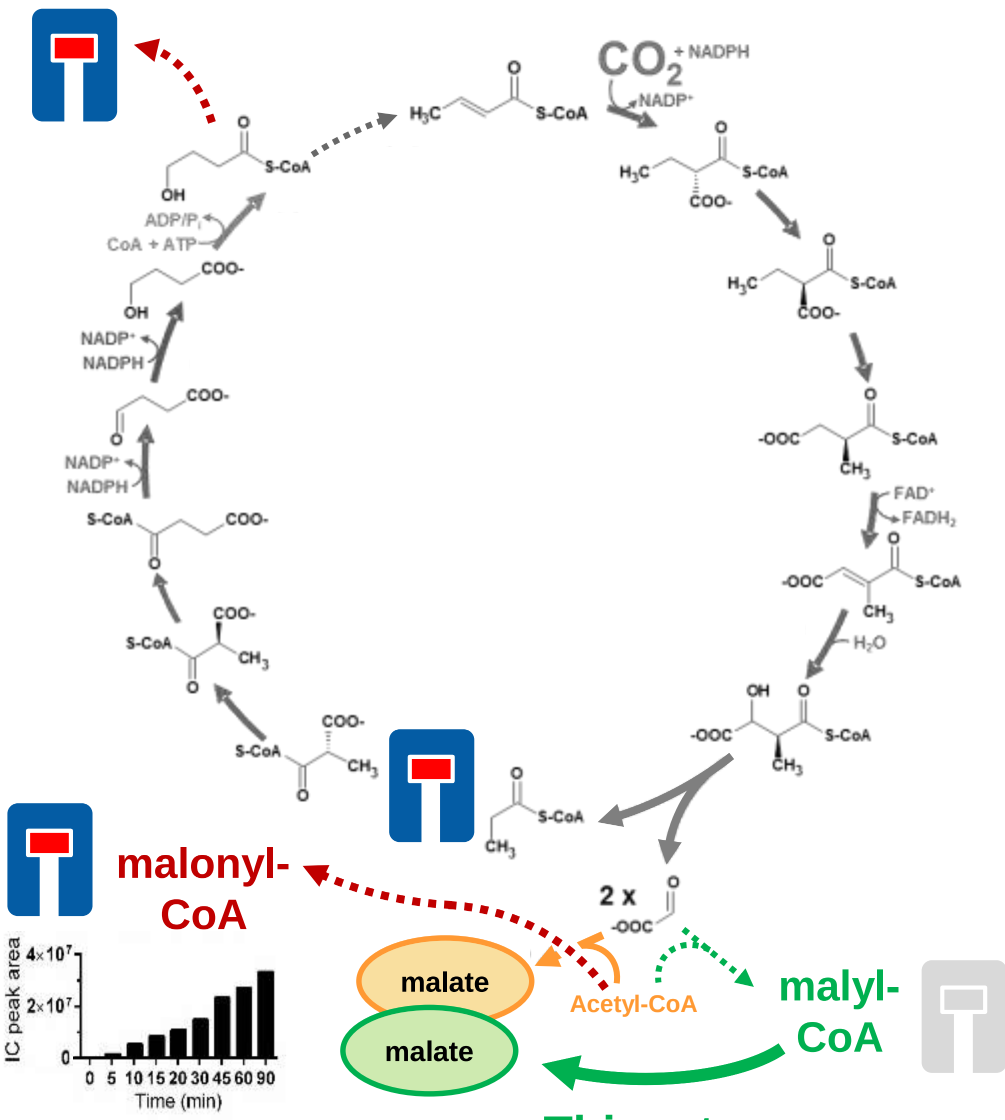


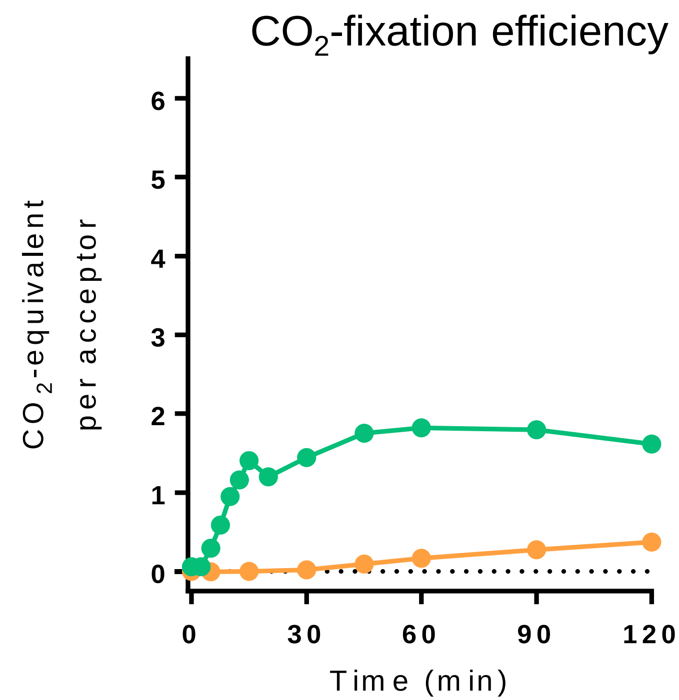


Schwander *et al.* (2016) **Science**

Optimizing synthetic CO_2-fixation cycles:

Overcoming side reactions in the CETCH cycle (v3.0)



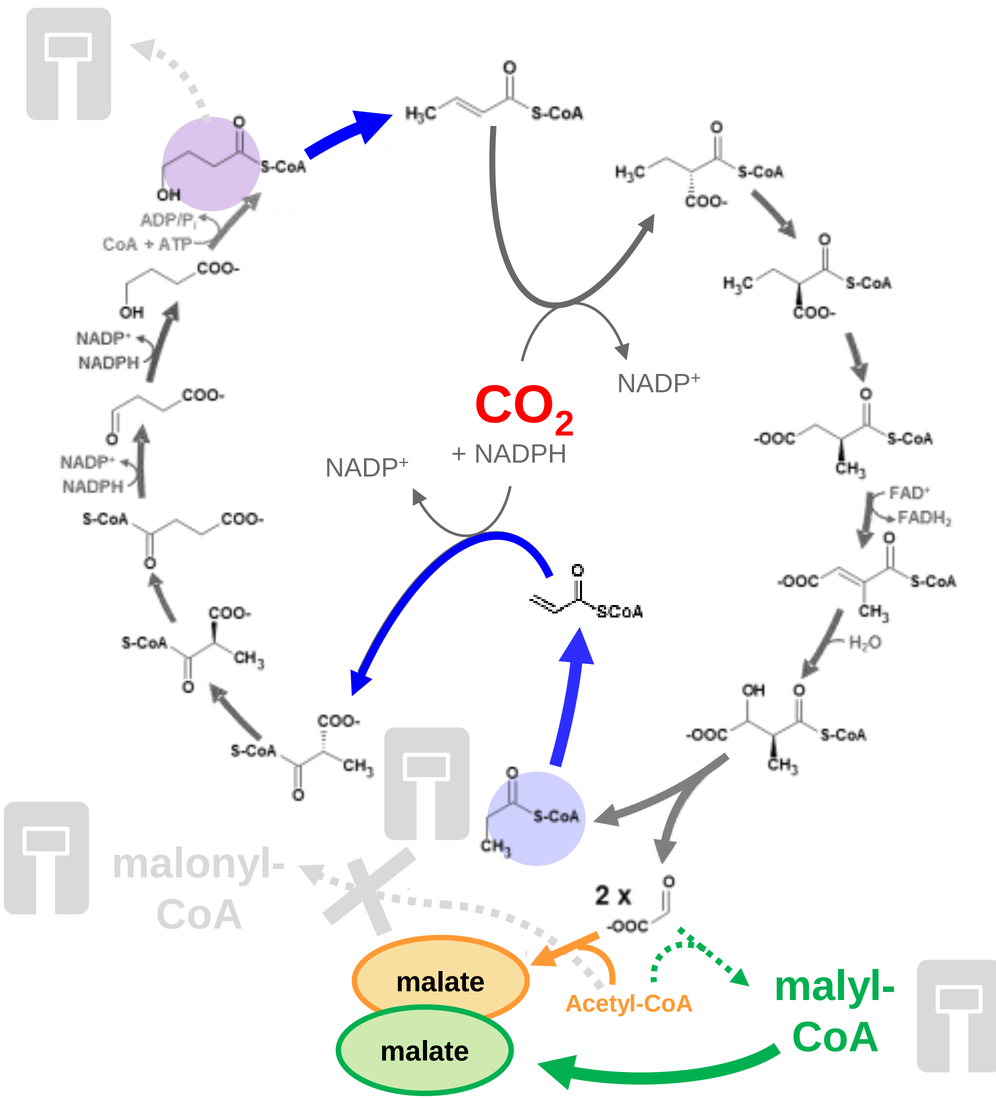


Proof reading & recycling

Schwander *et al.* (2016) **Science**

Optimizing synthetic CO_2-fixation cycles:

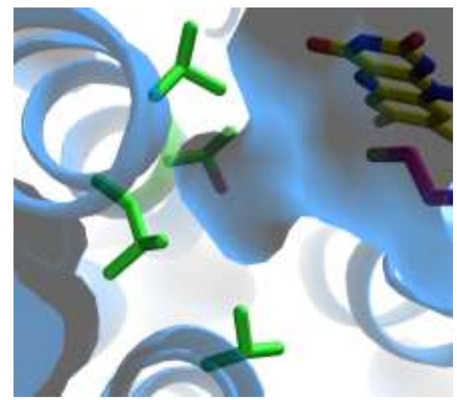
Overcoming side reactions in the CETCH cycle (v4.0)



Schwander *et al.* (2016) **Science**

Discovery of a propionyl-CoA oxidase

ACX4 - wt: $2.7 \cdot 10^6$ M s^{-1} (with propionyl-CoA)
$0.7 \cdot 10^6$ M s^{-1} (with 4-OH-butyryl-CoA)



***Engineering* to create a *specific* propionyl-CoA oxidase**

T134L: $2.2 \cdot 10^5$ M s^{-1} (with propionyl-CoA)
$1.2 \cdot 10^3$ M s^{-1} (with 4-OH-butyryl-CoA)

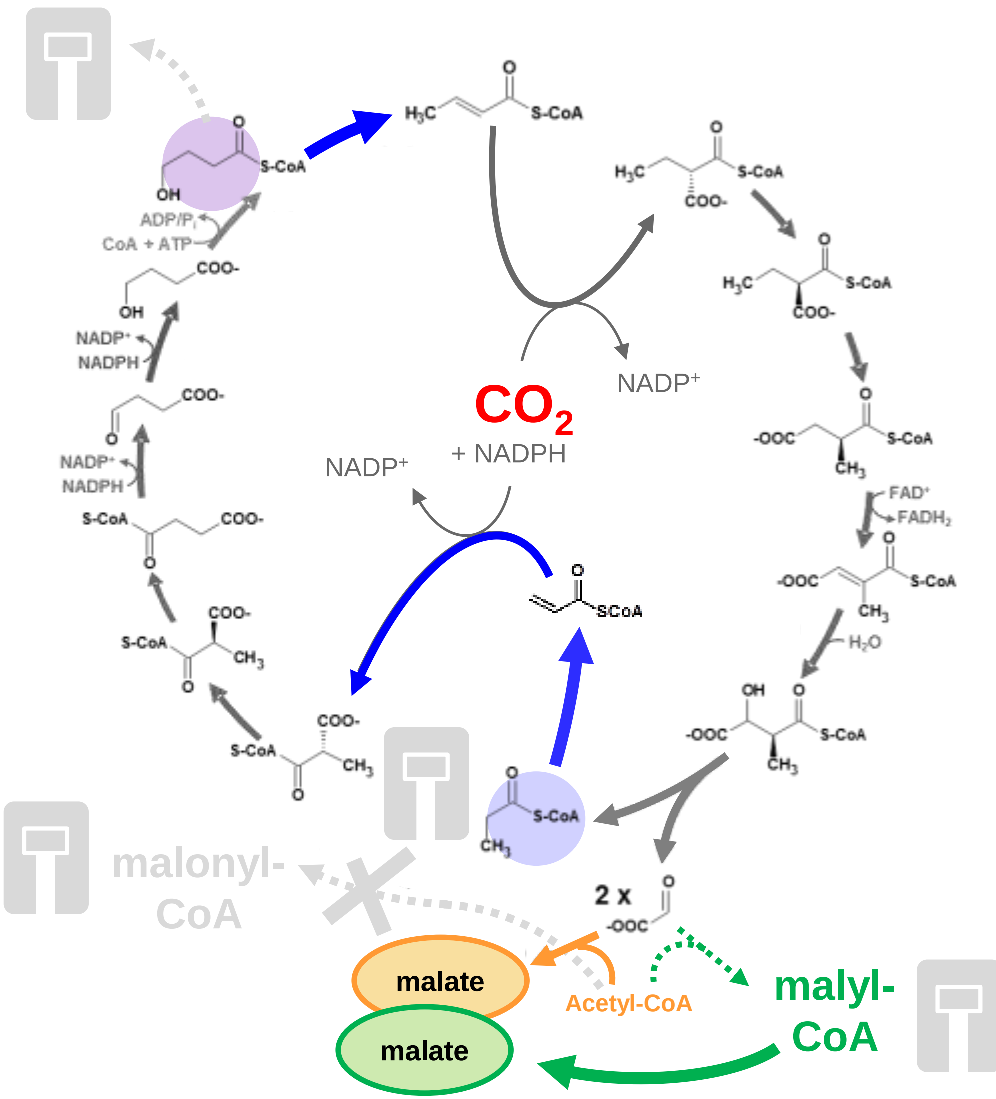
Proof reading & recycling

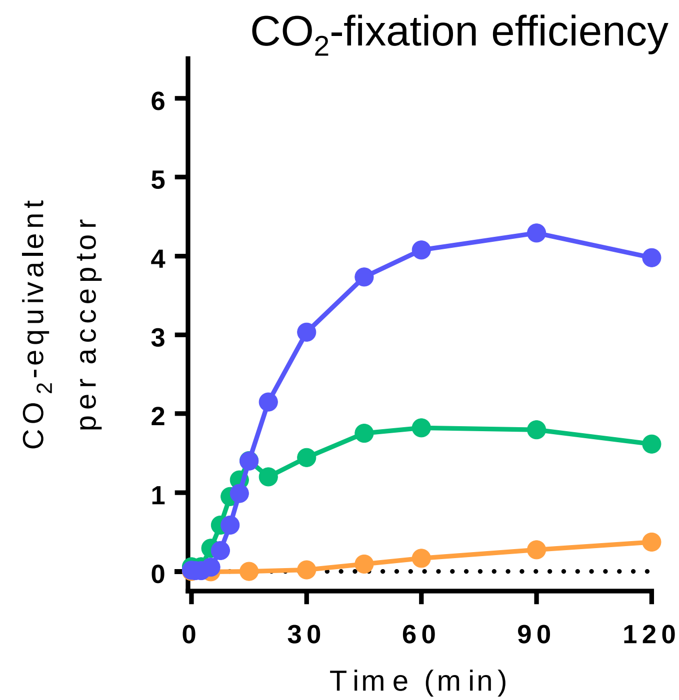
Pathway redesign

Enzyme engineering

Optimizing synthetic CO_2-fixation cycles:

Overcoming side reactions in the CETCH cycle (v4.0)





Proof reading & recycling

Pathway redesign

Enzyme engineering

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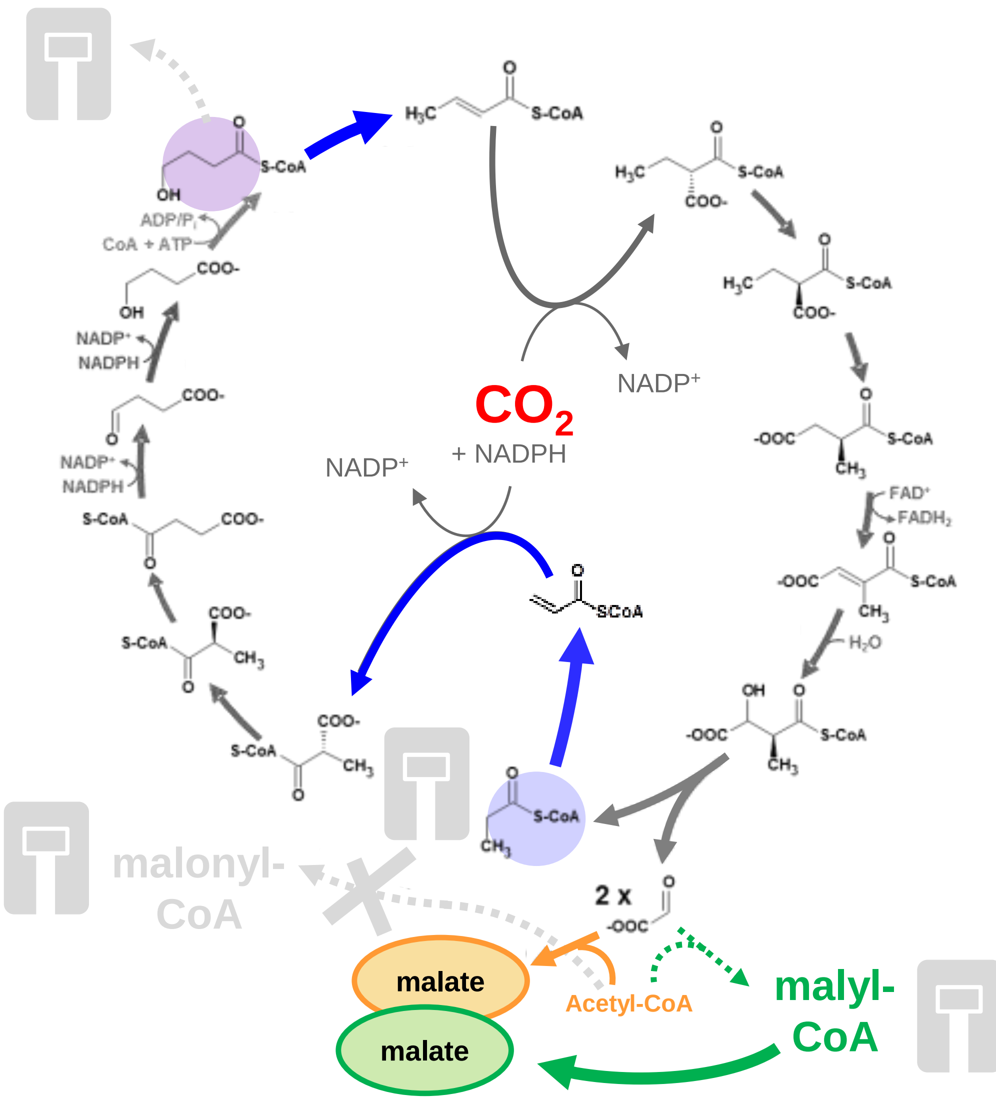
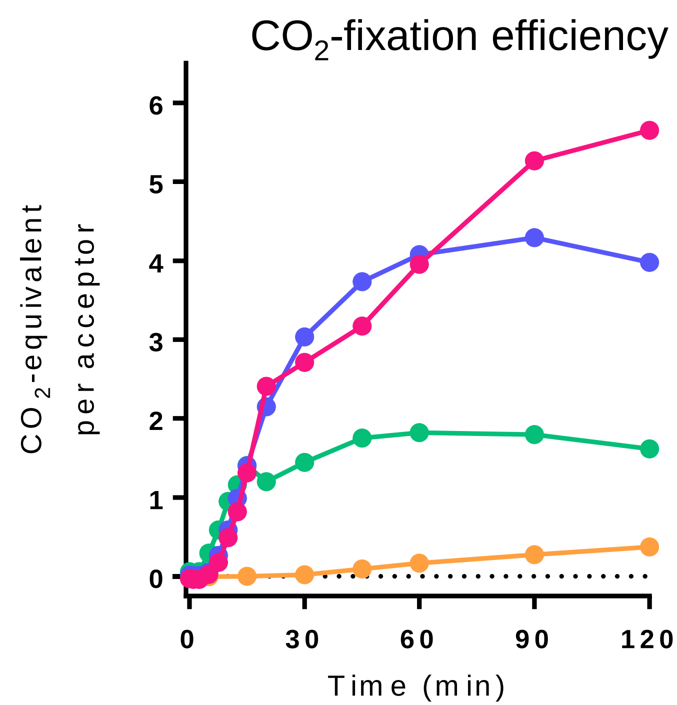
Optimizing synthetic CO_2-fixation cycles:

Overcoming side reactions in the CETCH cycle (v5.4)





3.6 CO_2 molecules acceptor^{-1} and h^{-1}

Schwander *et al.* (2016) **Science**

You might have

great **individual**

players…

…but they

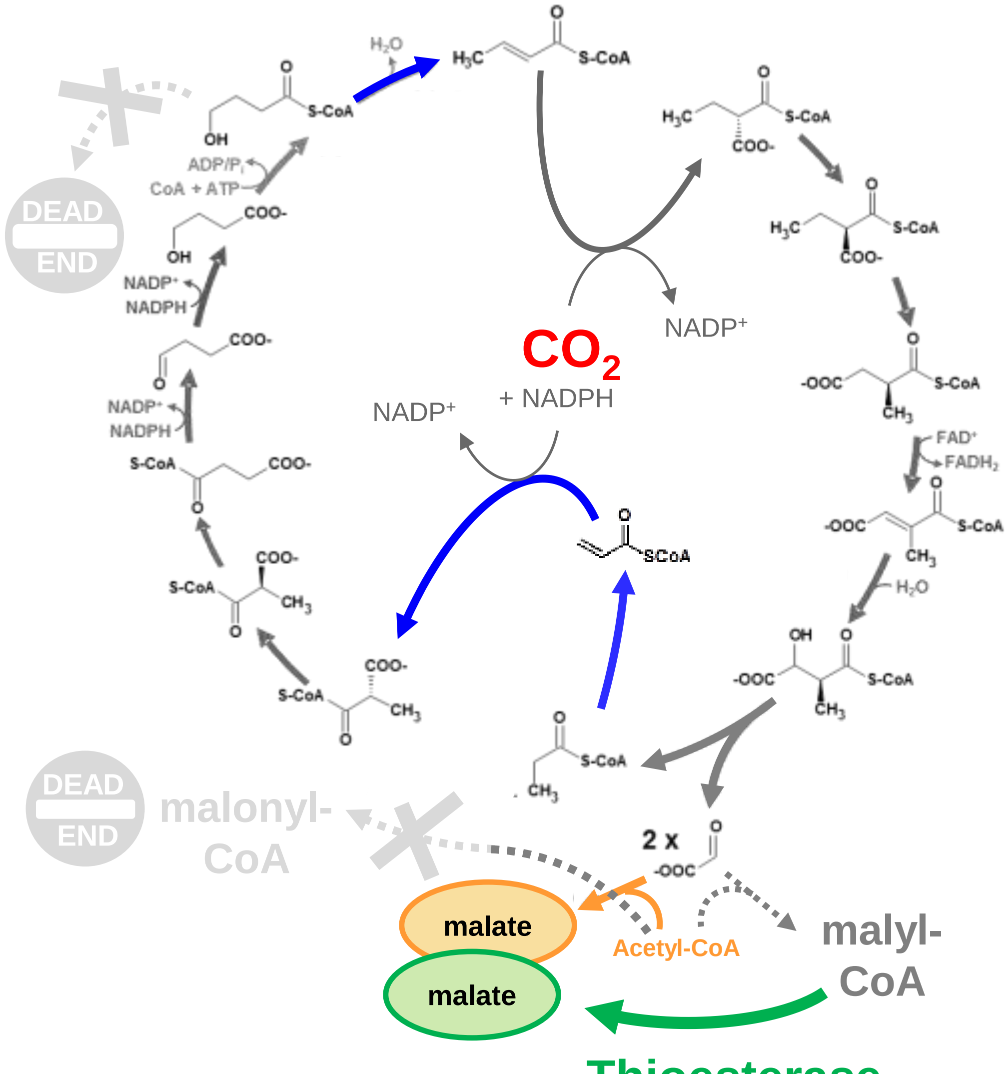
need

to play

together

in a **team!**

The CETCH cycle version 5.4



Schwander *et al.* (2016) **Science**

17 different enzymes

9 different organisms

3 engineered enzymes

5 nmol min^{-1} mg^{-1} prot.

A starting point for several research directions…

Next steps: Further optimization

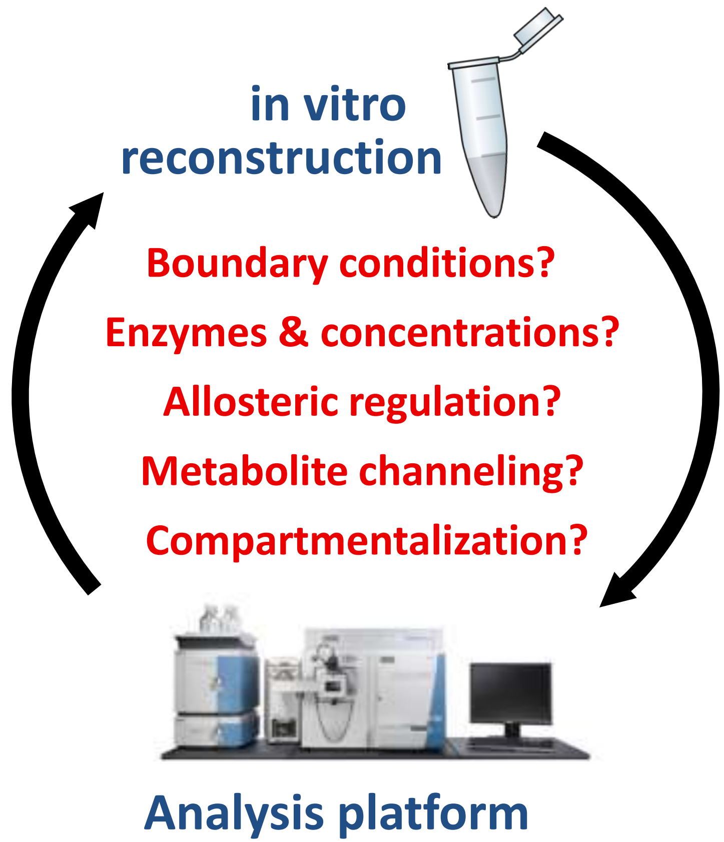
Entering the ‚Design – Build – Test – Analyze‘ cycle

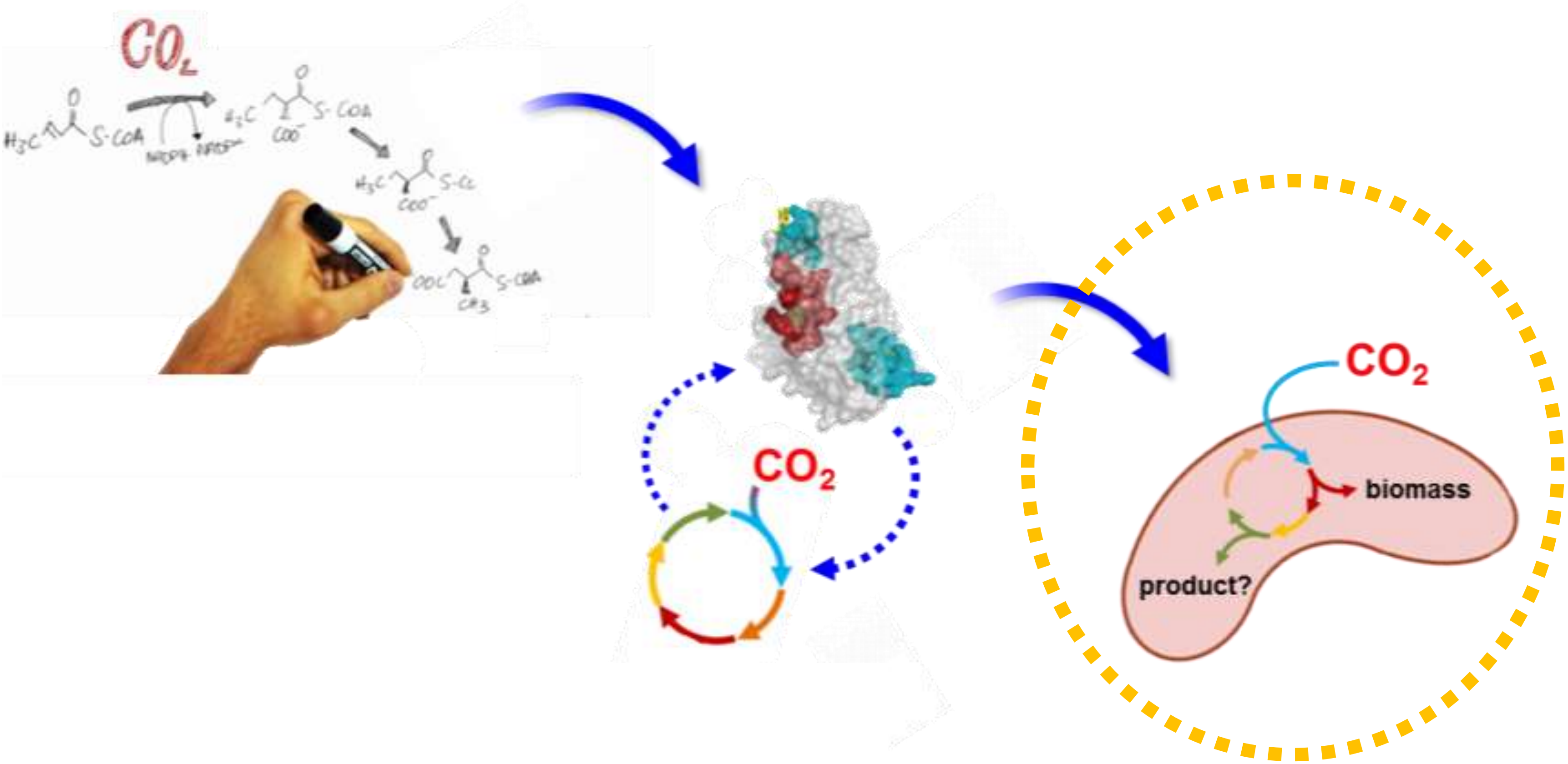
17 different enzymes

9 different organisms

3 «designer-enzymes»

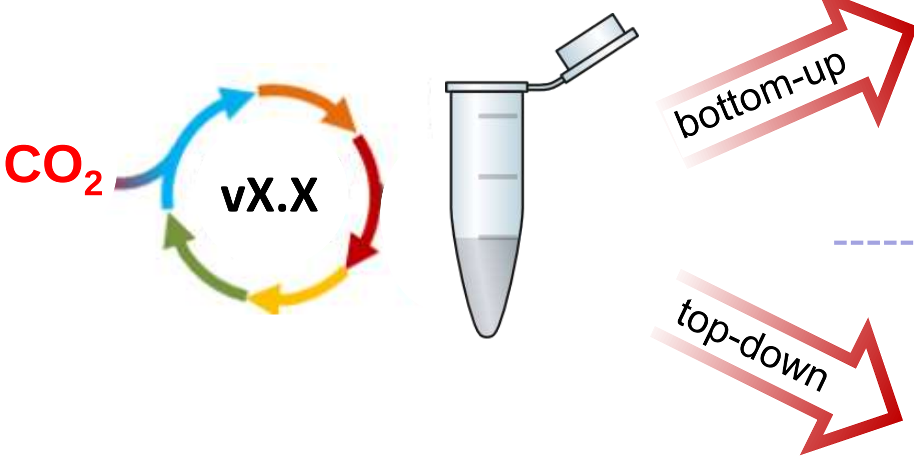
5 nmol min^{-1} and mg^{-1} protein





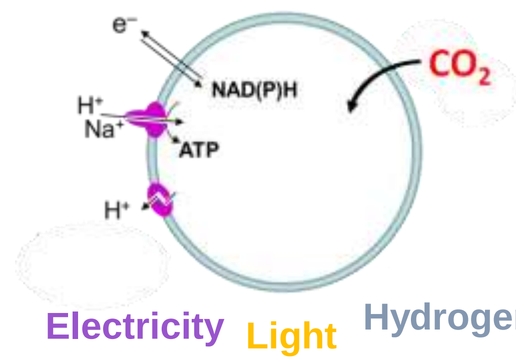
How to **transplant** synthetic metabolic networks for carbon capture and conversion?

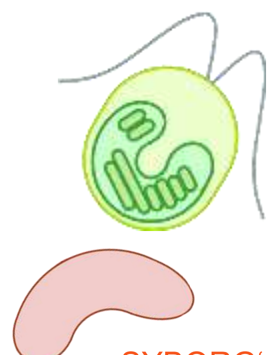
Next steps: Transplanting the CETCH cycle



Artificial Cells & Chloroplasts

maxsynbio
MAX PLANCK RESEARCH NETWORK IN SYNTHETIC BIOLOGY





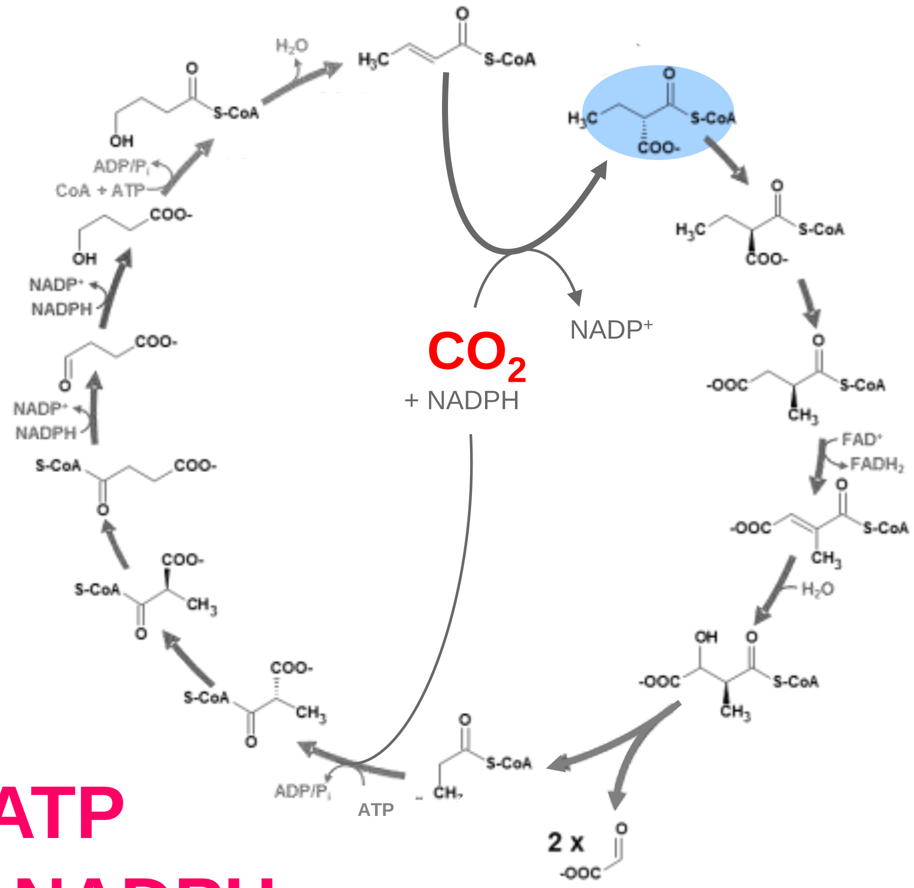
‚SYBORG'

erc

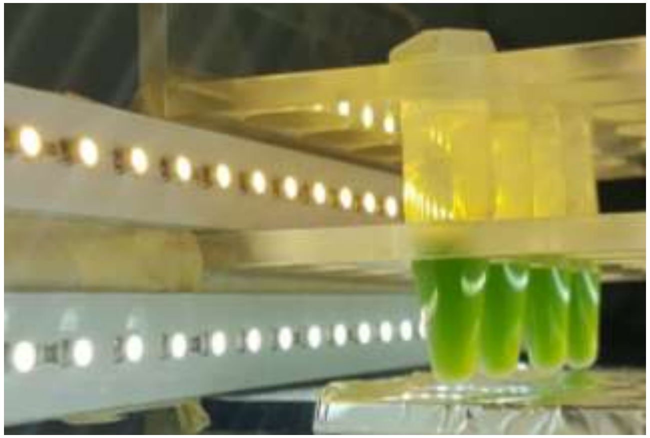
in vivo transplantation

Powering the CETCH cycle by light

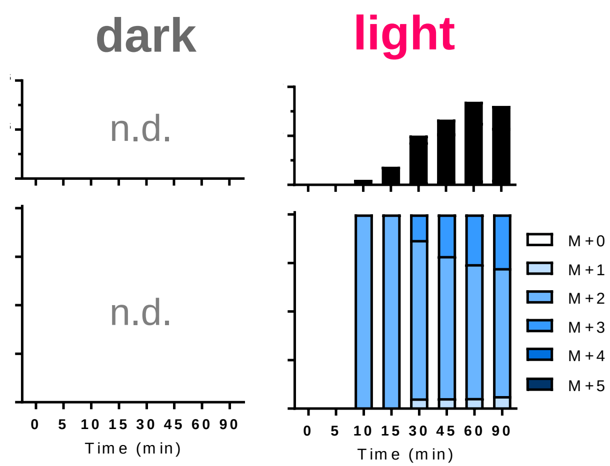
Coordinating the CETCH cycle with the photosynthetic machinery



ATP
NADPH



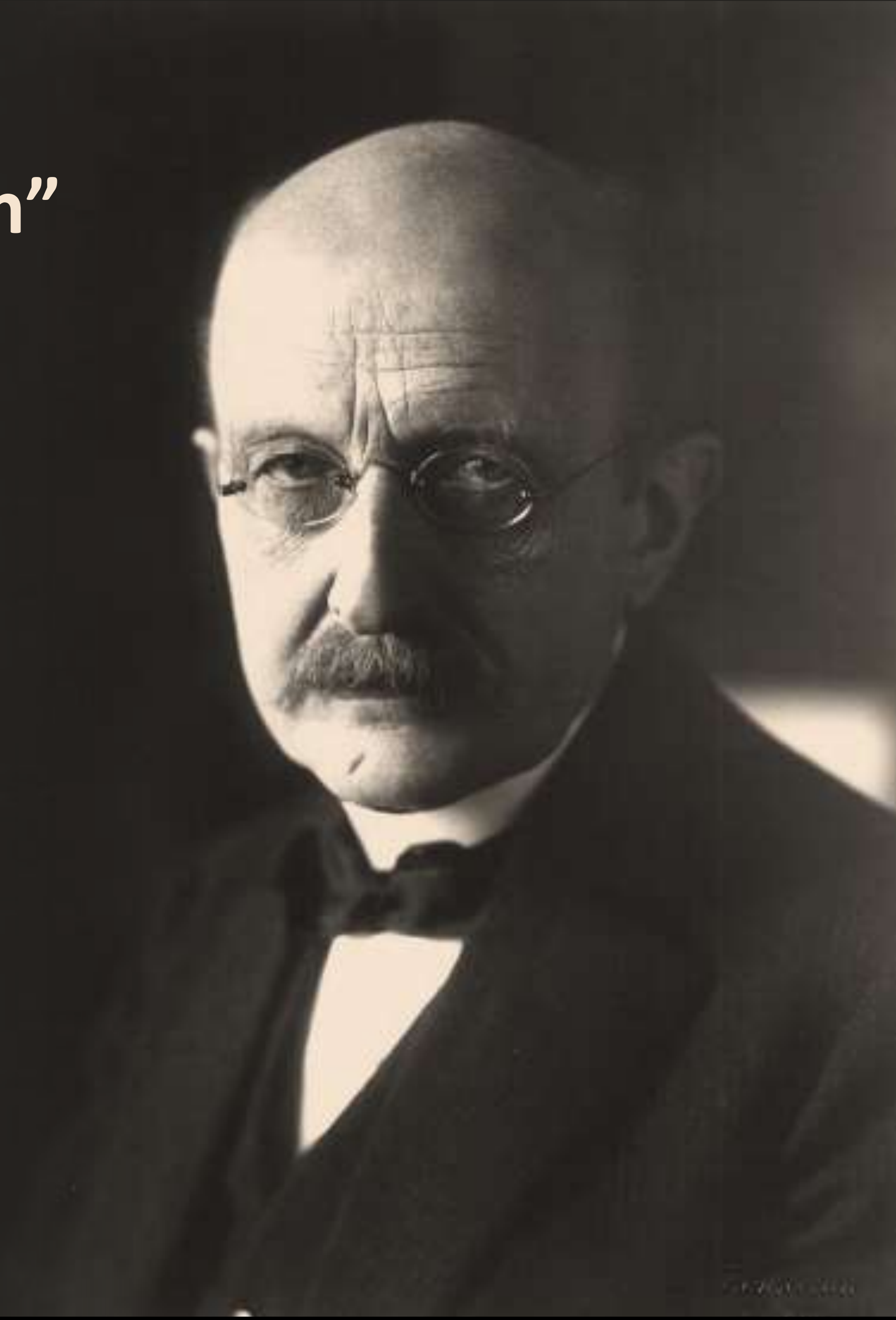
Thylakoid powered CETCH



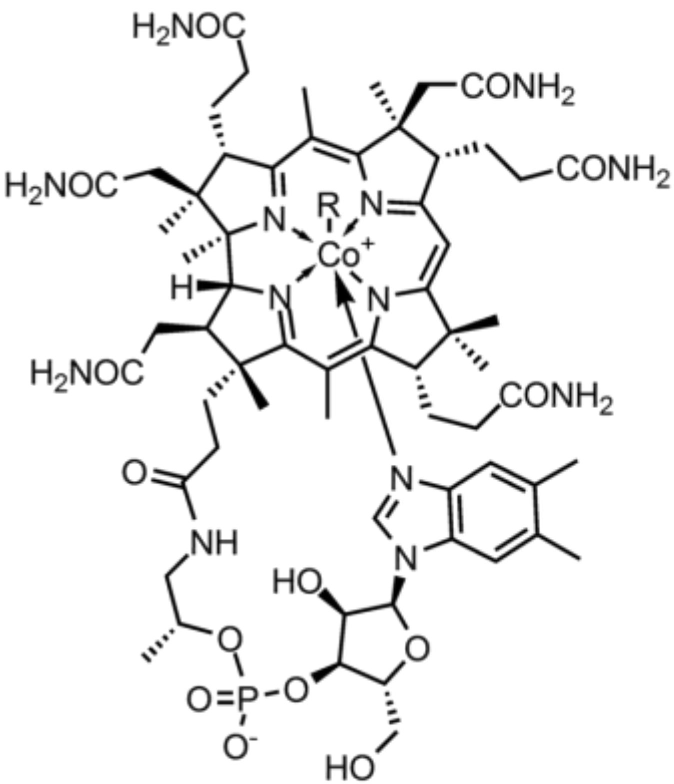
“Dem Anwenden muss
das Erkennen vorausgehen”

“Insight must
preceed application.”

– Max Planck



Building to understand



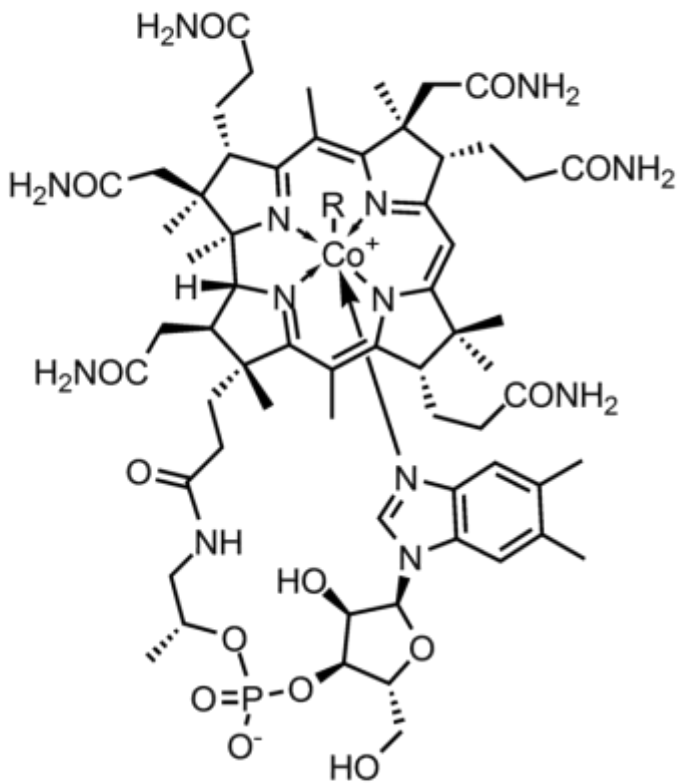
Vitamin B_{12}
synthetic chemistry
1961 - 1972

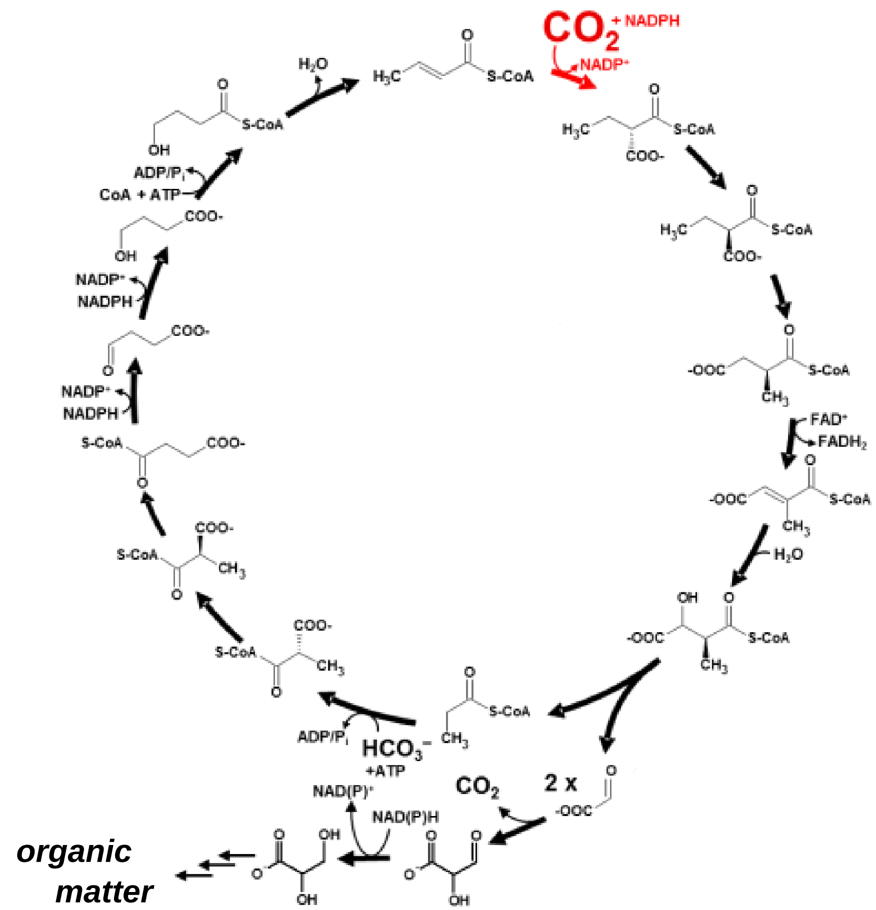
Re-synthesis of the most complex natural product

Milestone for synthetic chemistry

General rules for organic chemistry

Building to understand

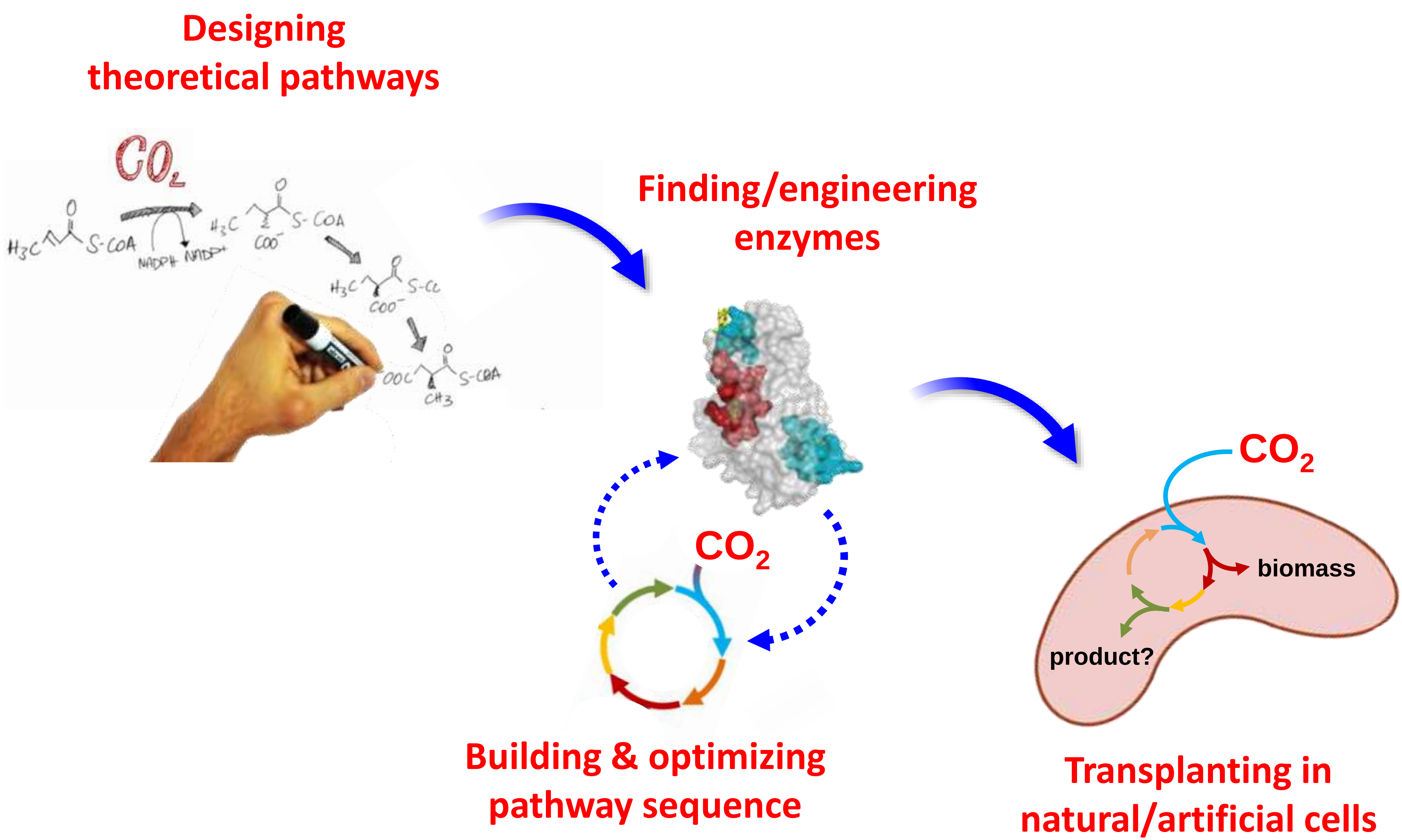




Vitamin B_{12}
synthetic chemistry
1961 - 1972

artificial CO_2 fixation
synthetic biochemistry
2013 - ?

The concept of synthetic metabolism



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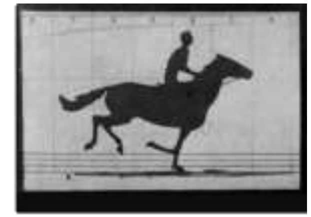
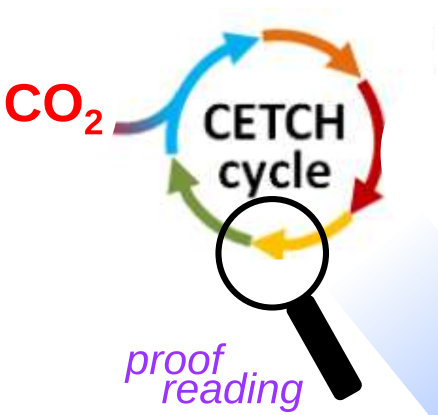
Conclusions and perspectives

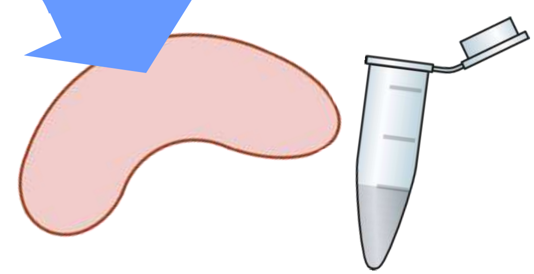
1. **Reductive carboxylation** a **new principle** to study fundamental questions in CO_2-fixation opening new options for **synthetic CO_2-fixation**



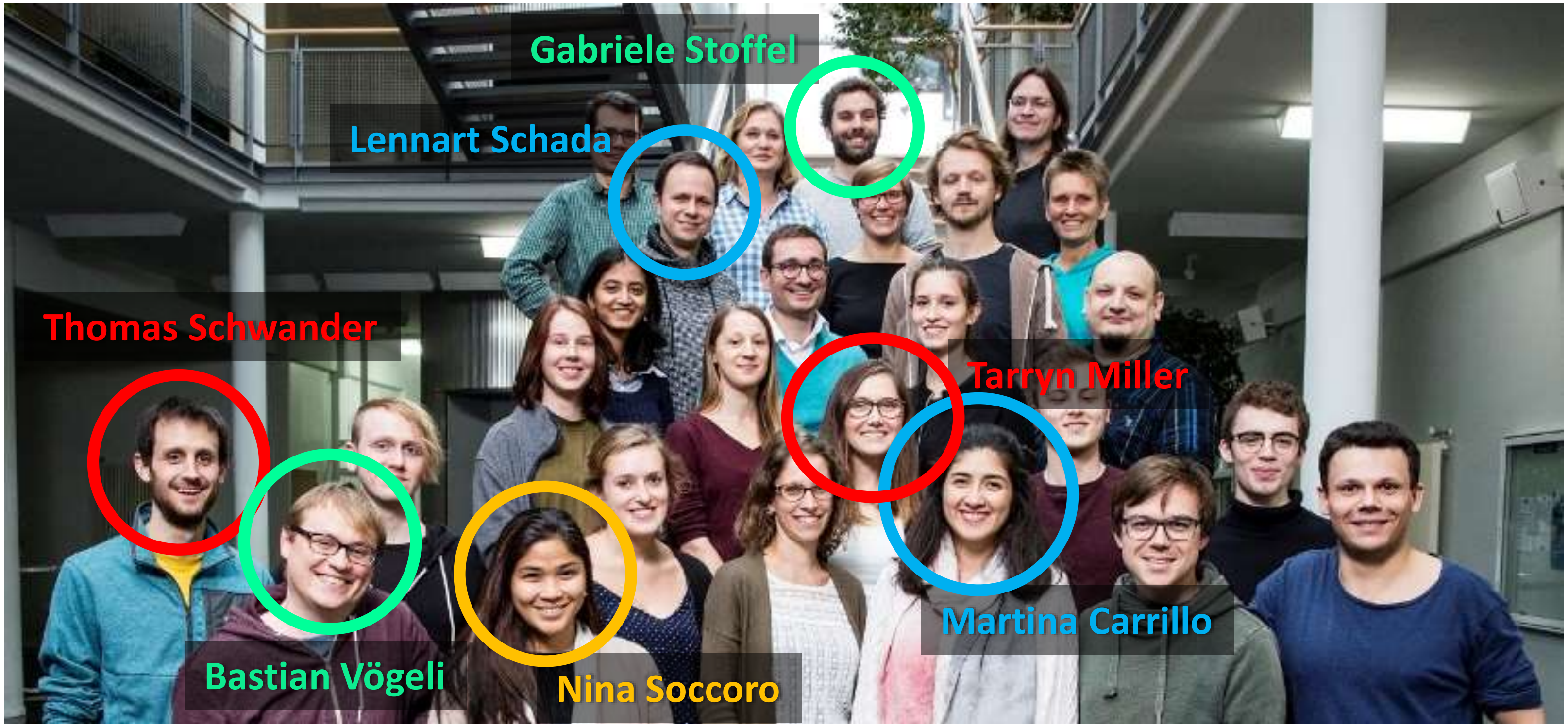


2. Synthetic CO_2-fixation cycles can be **realized with chemical logic** and further **optimized following biological design principles**

3. Established **in vitro** and **in vivo platforms** for the implementation of synthetic CO_2-fixation



Gracias Merci Tado Danke Grazie Kiitos Thanks



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U.S. DEPARTMENT OF ENERGY

SLAC NATIONAL ACCELERATOR LABORATORY

Future AGRICULTURE

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ETH - Reserach Grant

ETH - Fellowship

LOEWE

JGI

FNSNF FONDS NATIONAL SUISSE SCHWEIZERISCHER NATIONALFONDS

GEBERT RÜF STIFTUNG WISSENSCHAFT.BEWEGEN

DFG Deutsche Forschungsgemeinschaft