CETCH me if you can

Designing new solutions for carbon capture & conversion with synthetic biology

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CO₂

[400 ppm in air]
3,000 Gt

Biomass

32 Gt

Fossil Fuels

CO₂

440 Gt

Biom. CO₂-fixation

RubisCO
slow (5 s⁻¹ O=C=O)

sluggish (20% error with O=O)

CO₂

[400 ppm in air]

3,000 Gt

440 Gt

Biomass

Biomass

novel products

[400 ppm in air]

32 Gt

Fossil Fuels

Can we construct efficient CO₂-fixation cycles de novo?

Alternative CO₂-fixation pathways

Nature evolved several CO₂-fixation pathways & enzymes

- Calvin cycle (Calvin lab (~1950))
- Reductive TCA cycle (Evans et al. (1966) PNAS)
- Wood-Ljungdahl way (Wood & Ljungdahl labs (~1985))
- Fuchs IA cycle (Berg IA et al. (2007) Science)
- Fuchs II cycle (Huber H et al. (2006) PNAS)
- Fuchs III cycle (Zarraga J et al. (2009) PNAS)
- EM-CoA pathway (Erb TJ et al. (2007) PNAS)
- Fuchs IB cycle (Könnecke L et al. (2014) PNAS)

SYNTHEtic CO₂ fixation-cycle(s)

Can we construct efficient CO₂-fixation cycles de novo?
Finding & engineering an efficient CO₂-fixation reaction for carbon capture and conversion
Enoyl-CoA Carboxylases/reductases (ECRs) are up to 4 x more efficient than RubisCO

Expanding the (bio)synthetic space of ECRs

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

\[
\text{Crotonyl-CoA} \quad \stackrel{\text{CO}_2}{\longrightarrow} \quad \text{Ethylmalonyl-CoA}
\]

\[
\text{NADPH} \quad \text{NADP}^+ \quad \text{H}_3\text{C} - \quad \text{S-CoA} \quad \text{O} \quad \text{CO}_2 \quad \text{S-CoA}
\]


Can we design or discover new CO\textsubscript{2}\text{-fixation reactions?}

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 $\geq 1$

Schwander et al. (2016) Science
How to realize synthetic biological networks for carbon capture and conversion?
Realizing synthetic CO₂-fixation cycles: Building the CETCH cycle version 1.0

Finding the parts:

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

Schwander et al. (2016) Science
Realizing synthetic CO$_2$-fixation cycles:
Building the CETCH cycle version 1.0

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

CO$_2$-fixation efficiency

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 CO₂-fixation cycles: Overcoming side reactions in the CETCH cycle (v3.0)

Schwander et al. (2016) Science
Optimizing synthetic CO$_2$-fixation cycles: Overcoming side reactions in the CETCH cycle (v4.0)

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)

Schwander et al. (2016) Science
Optimizing synthetic CO$_2$-fixation cycles: Overcoming side reactions in the CETCH cycle (v4.0)

Proof reading & recycling
Pathway redesign
Enzyme engineering

Schwander et al. (2016) Science
Optimizing synthetic CO$_2$-fixation cycles: Overcoming side reactions in the CETCH cycle (v5.4)

Schwander et al. (2016) Science

3.6 CO$_2$ molecules acceptor$^{-1}$ and h$^{-1}$
You might have great individual players...

...but they need to play together in a team!
The CETCH cycle version 5.4

17 different enzymes
9 different organisms
3 engineered enzymes
5 nmol min\(^{-1}\) mg\(^{-1}\) prot.

A starting point for several research directions...

Schwander et al. (2016) Science
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

CO₂ → vX.X → CO₂

bottom-up

top-down

Electricity → Light → Hydrogen

'SYBORG'

in vivo transplantation
Powering the CETCH cycle by light

Coordinating the CETCH cycle with the photosynthetic machinery

ATP

NADPH

\( \text{CO}_2 \) + \( \text{NADPH} \)

Thylakoid powered CETCH

dark

n.d.

light

n.d.
“Dem Anwenden muss das Erkennen vorausgehen”

“Insight must proceed application.”

– Max Planck
Building to understand

Re-synthesis of the most complex natural product

Milestone for synthetic chemistry

General rules for organic chemistry

Vitamin $\text{B}_{12}$

synthetic chemistry

1961 - 1972
Building to understand

Vitamin $\text{B}_{12}$
synthetic chemistry
1961 - 1972

artificial $\text{CO}_2$ fixation
synthetic biochemistry
2013 - ?
The concept of synthetic metabolism

Designing theoretical pathways

Finding/engineering enzymes

Building & optimizing pathway sequence

Transplanting in natural/artificial cells

The concept of synthetic metabolism

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