NREL
Transforming ENERGY

DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Synthetic C1 Condensation Cycle for Formate-Mediated ElectroSynthesis

March 9, 2019

Technology Session Area Review: **CO_2 Utilization**

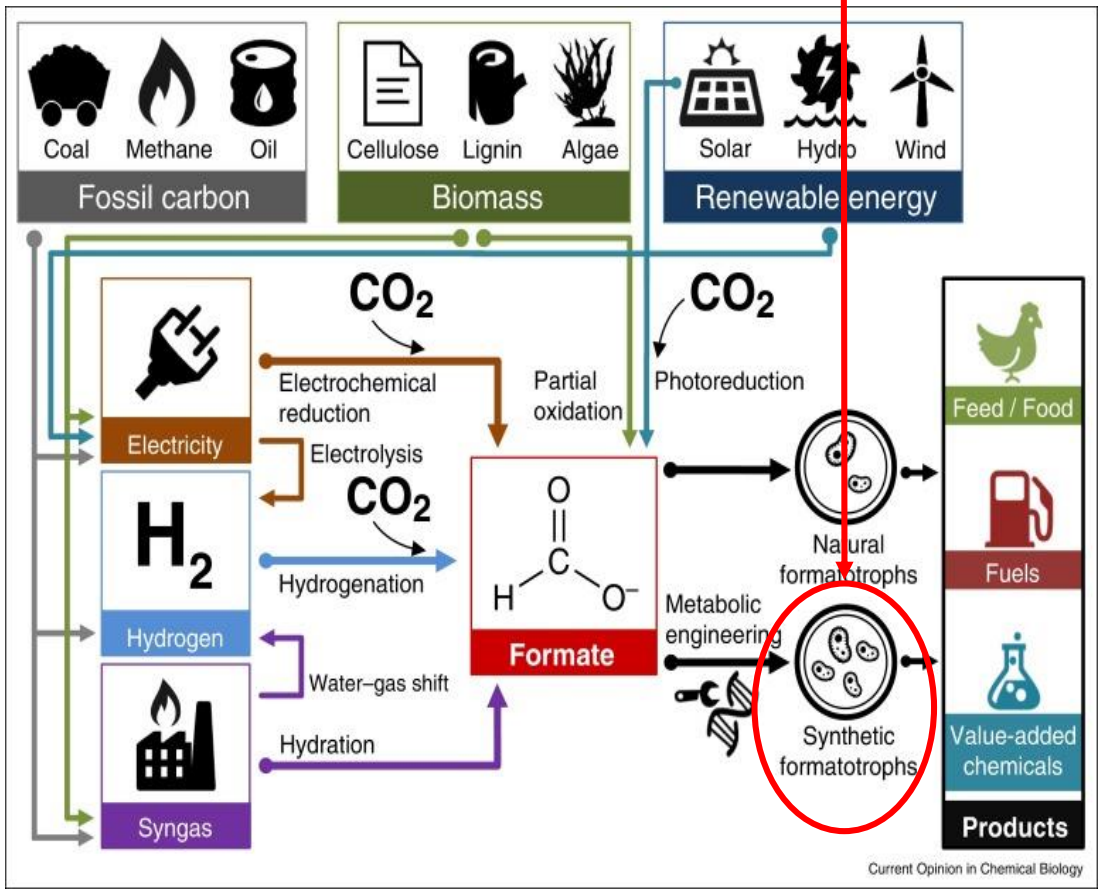
Principal Investigator: **Wei Xiong**

Organization: **National Renewable Energy Laboratory**

Goal Statement

The goal of this project is to develop novel pathways for advanced biological upgrading of **formate** to bio-commodities by investigating efficient and rapid formate utilization, high carbon efficiency, cost effective processes.

Focus of this project



Yishai, O. et al., 2016. **The formate bio-economy**. *Current Opinion in Chemical Biology*, 35, 1-9.

Outcomes

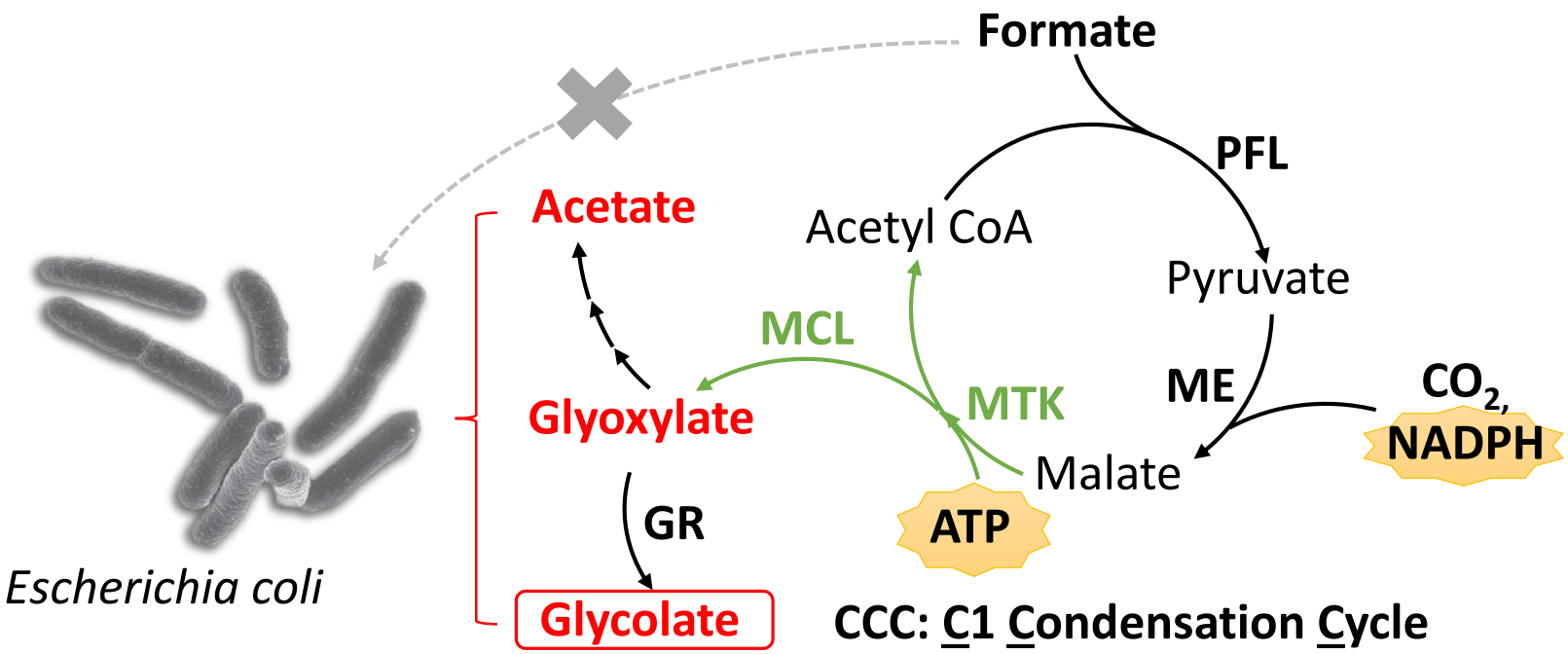
- *Build up a novel biological pathway upgrading formate to glycolate at **1 g/L/d production rate**.*
- *Analyze the economic feasibility of the hybrid system by TEA.*
- *Construct a synthetic **formatotroph** capable of utilizing formate/CO_2 as the sole carbon source.*

Relevance

- *Success could enable chemicals production from CO_2*
- *Low cost electricity reduces CO_2 to formate electrochemically*
- *Scalable strategy could be a stand-alone process or value add to existing industry*

1 - Project Overview

Working with a model organism



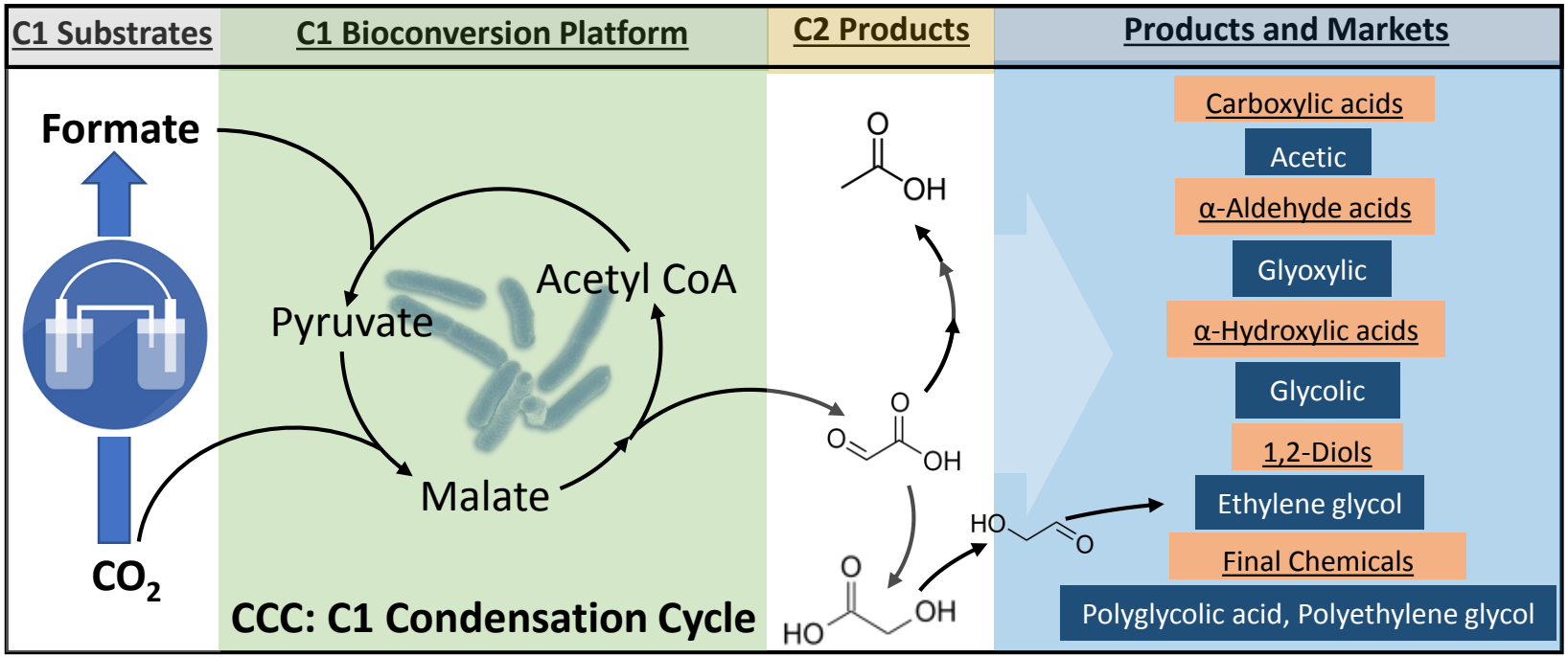
Why *E. coli*?

- A model industrial microorganism;
- Metabolic adaptability;
- Successful synthetic C1 pathways exemplified by using *E. coli.*

Why C1 Condensation Cycle?

- Bioenergetics feasibility;
- Pathway simplicity;
- Intermediates of industrial interest.

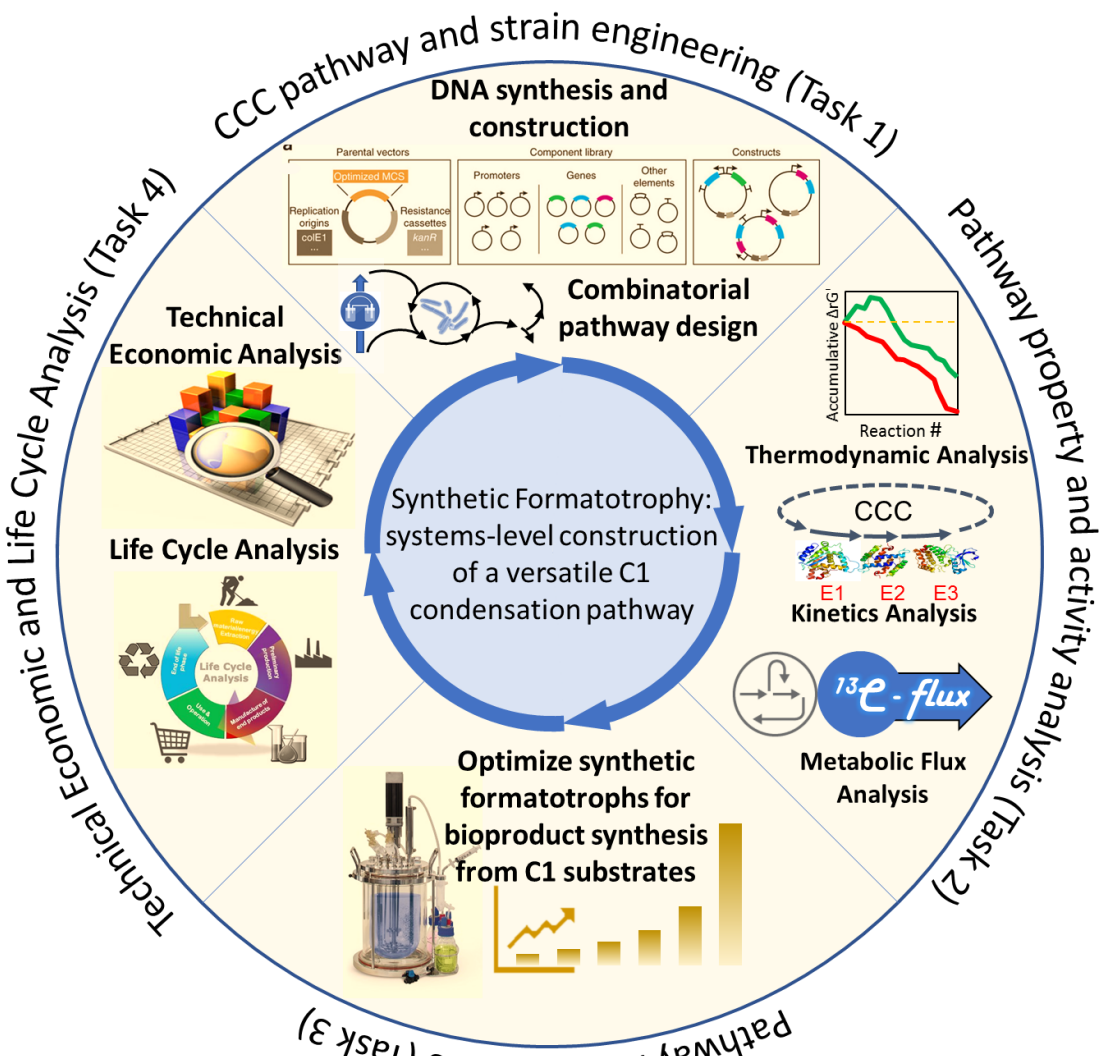
1 - Project Overview



Why Glycolate?

- A representative C2 compound;
- Applicable in energy, textile, food and pharmaceutical industry
- Good market size; (*The glycolate market was USD **93.3 million** in 2011 and is expected to reach USD **203 million** in 2018)

2 – Approach (Management)



Strain Engineering and Analysis

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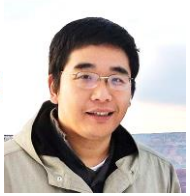
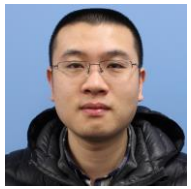


Wei Xiong, PI

Xiang Gao,
Strain and Pathway engineering



Chao Wu,
Thermodynamics and ^{13}C flux analysis

Automatic DNA Synthesis

Automated and high-throughput gene synthesis

Nathan Hillson

BERKELEY LAB
Lawrence Berkeley National Laboratory

TEA and LCA

TEA and LCA

Ling Tao

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2 – Expertise of the Team

1. **Expertise in microbial C1 pathway (Wei)**

- Wei Xiong, et al. The plasticity of cyanobacterial metabolism supports direct CO_2 conversion to ethylene. ***Nature Plants***. 2015.53.
- Wei Xiong, et al. Phosphoketolase pathway contributes to the carbon metabolism in cyanobacteria. ***Nature Plants***. 2015, 2: 15187
- Wei Xiong, et al. CO_2-fixing one-carbon metabolism in a cellulose-degrading bacterium *Clostridium thermocellum*. ***Proceedings of the National Academy of Sciences***. 2016, 113 (46): 13180-13185

2. **Expertise in ^{13}C-metabolic flux analysis (Wei & Chao)**

- Wei Xiong, Chao Wu, et al. Isotope-Assisted Metabolite Analysis Sheds Light on Central Carbon Metabolism of a Model Cellulolytic Bacterium *Clostridium thermocellum*. ***Frontiers in Microbiology***. 2018.01947

3. **Expertise in synthetic biology (Nathan, Wei & Xiang)**

4. **Expertise in TEA/LCA (Ling)**

Strain Engineering and Analysis

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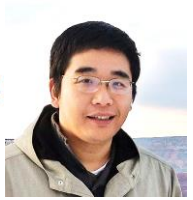

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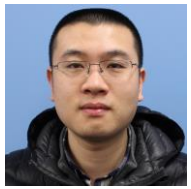

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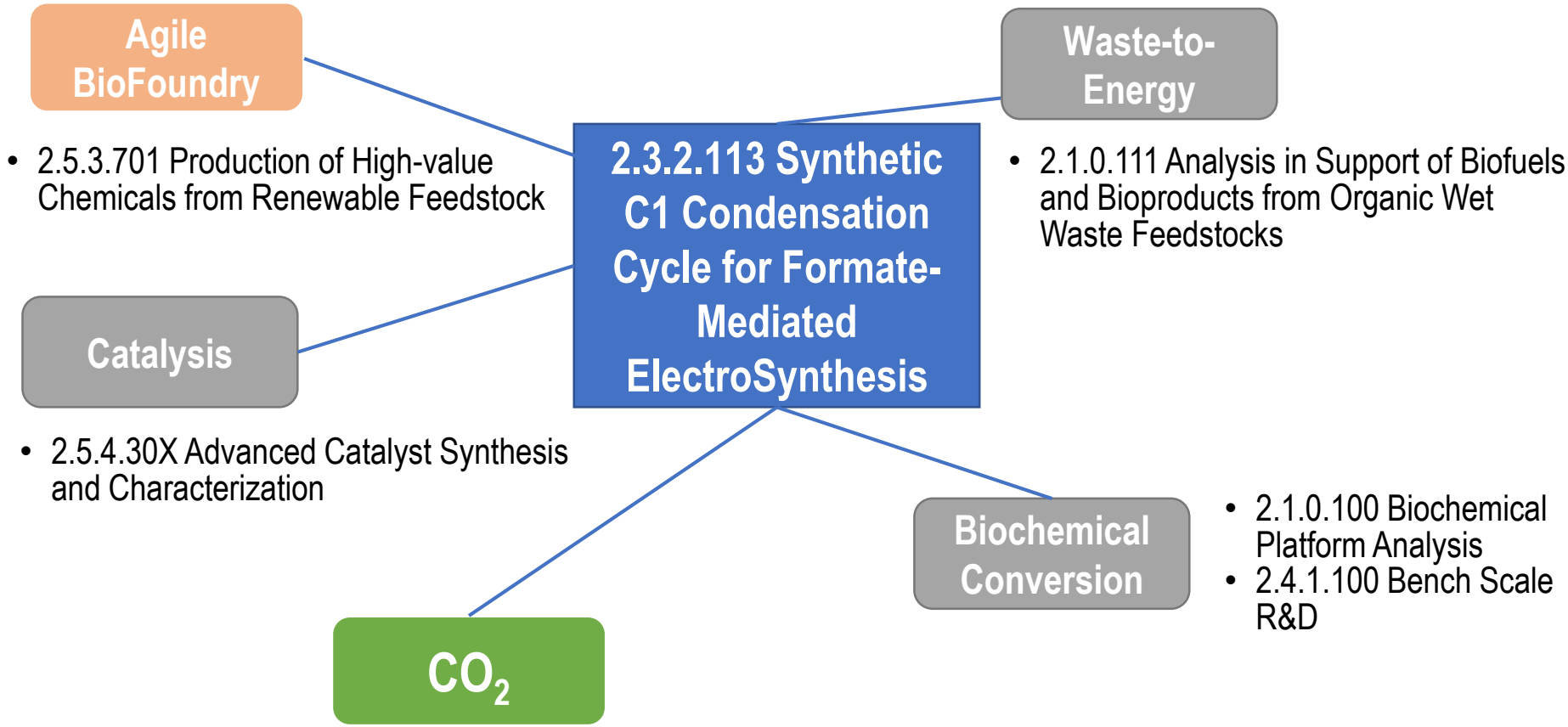
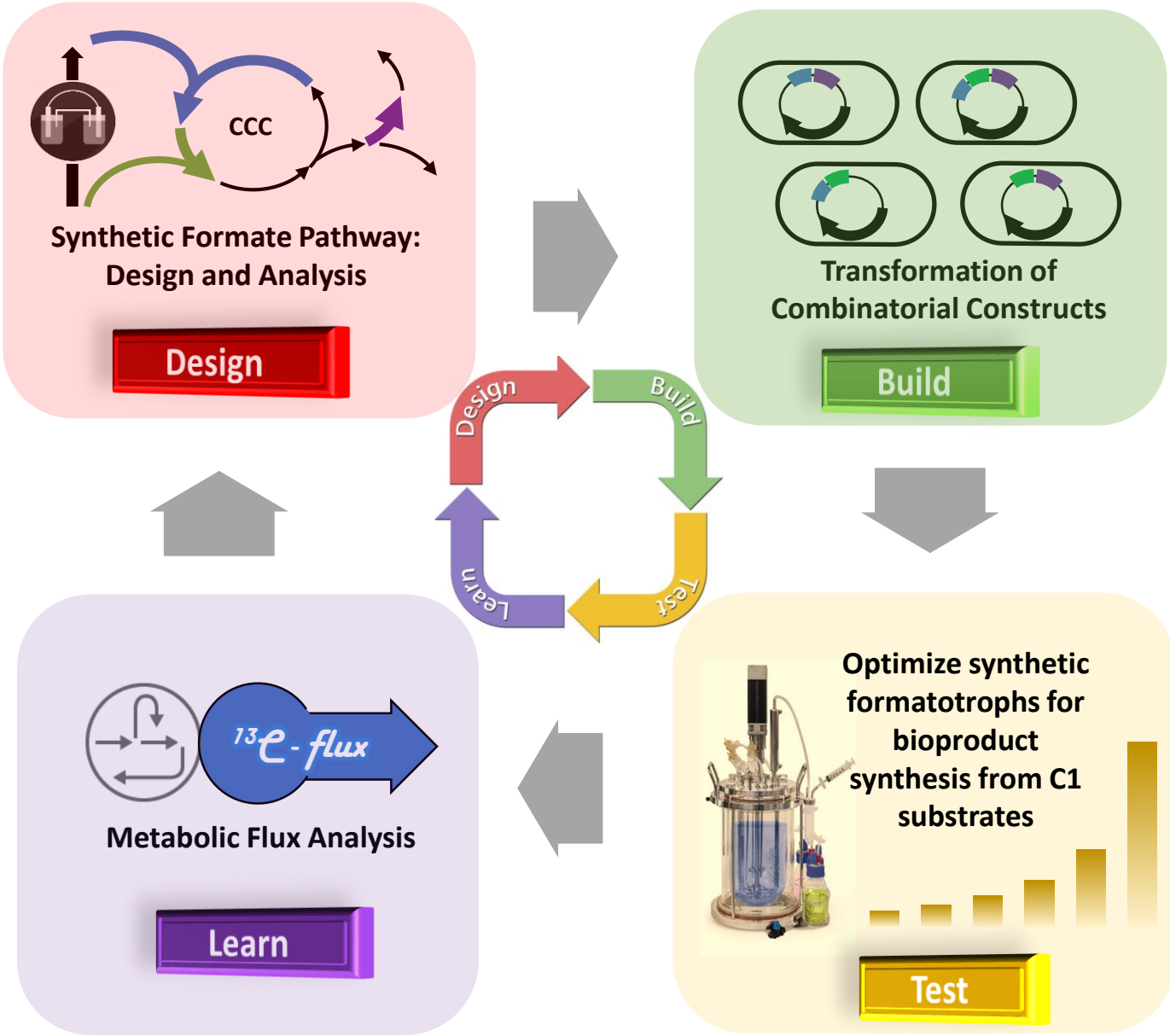
2 – Approach (Management): Interface with other projects



- 2.5.3.701 Production of High-value Chemicals from Renewable Feedstock
- 2.1.0.111 Analysis in Support of Biofuels and Bioproducts from Organic Wet Waste Feedstocks
- 2.5.4.30X Advanced Catalyst Synthesis and Characterization
- 2.1.0.100 Biochemical Platform Analysis
- 2.4.1.100 Bench Scale R&D

- 2.1.0.304 Feasibility Study of Utilizing Electricity to Produce Intermediates from CO_2 and Biomass
- 2.3.2.111 Improving formate upgrading by *Cupriavidus necator*
- 2.3.2.112 Enhancing Acetogen Formate Utilization to Value-Added Products
- 2.3.2.106 CO_2 Valorization via Rewiring Metabolic Network
- 5.1.3.101 Integration of CO_2 Electrolysis with Microbial Syngas; Upgrading to Rewire the Carbon Economy

2 – Approach (Technical)



Quad Chart Overview

Timeline

Start Date: Oct 1, 2018

End Date: Sep 30, 2021

Percent complete: <10%

	Total Costs Pre FY17**	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded	$0	$0	$0	$ 740,000
Project Cost Share*	$0	$0	$0	$0

•**Partners:** Lawrence Berkeley National Laboratory (LBNL)

Barriers addressed

Ct-D. Advanced Bioprocess Development

Ct-H. Efficient Catalytic Upgrading of Gaseous Intermediates to Chemicals

Objective

This project aims to design and engineer one-carbon substrate utilization in *E. coli*. A synthetic and orthogonal **C1 Condensation Cycle** (CCC) will be constructed for the conversion of formate to C2 carboxylates (e.g., glyoxylate and acetate) which can serve as the sole carbon source for *E. coli* growth. Leveraging this innovative C1 bioconversion platform, we propose to enable **high yield production of value-added glycolate**.

End of Project Goal

Achieve 2 g/L glycolate production from formate in 5-liter benchtop bioreactor within 48 hours. This productivity goal will serve as the input in final TEA and LCA report which will further shed light to the feasible way towards industrialization.

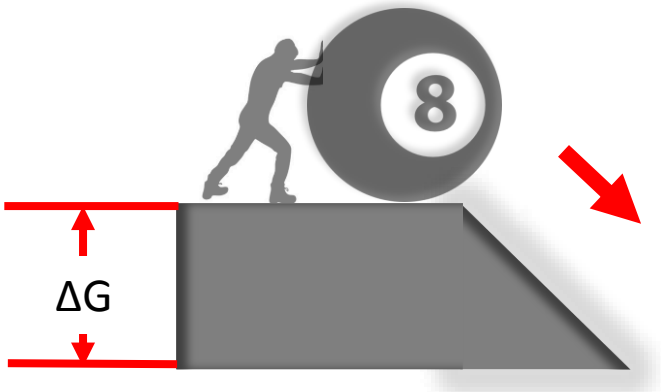
Technical Challenges

Name	Target Completion Date	Response	Description
CCC enzymes engineered are not active in E. coli host.	09/30/2019	We will make DNA constructs with combinatory expression components. We will test different strengths of RBSs and may use inducible promoters to control the expression levels of CCC enzymes.	Inappropriate heterologous expression of MTK, MCL and/or GR in E. coli might form insoluble proteins in inclusion body.
Engineered E. coli strains do not grow well on formate as the carbon source.	03/31/2020	We will adopt laboratory evolution strategy to enable E. coli's adaptability to formate by growing it on formate along with a preferable carbon source initially (e.g., glucose) and then transferring the cultures continuously by gradually decreasing glucose/formate ratio. In case, an absolute formatotrophy cannot be achievable, we will, at least, ensure C1 feedstocks (formate plus CO_2) to be the most dominant carbon source for the CCC strains, contributing over 50% carbon in glycolate production. This number will be evidenced and validated by ^{13}C-MFA.	In theory, E. coli engineered with functional CCC should grow on formate. But we cannot exclude the possibility of slow growth, poor productivity presumably caused by this new nutrient mode.

Go/No-Go Milestone

Name	Description	Criteria	Date
Construct E. coli formatotrophs grown on formate as the major carbon source.	E. coli engineered for formate utilization and conversion is the key to the success of this project. Thus, creating E. coli strains grown significantly on formate may be a GO/NO-GO milestone.	Measuring that formate and/or CO_2 are the dominant source for cell growth and glycolic acid production, contributing over 50% carbon in glycolate or cell biomass.	3/31/2020

3 – Results: Thermodynamics Feasibility of the CCC



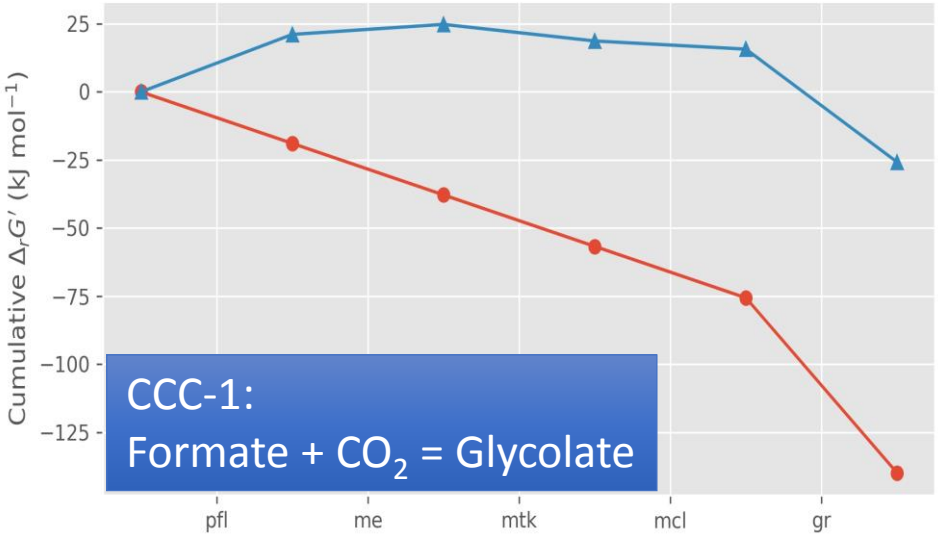
Thermodynamics Modeling:

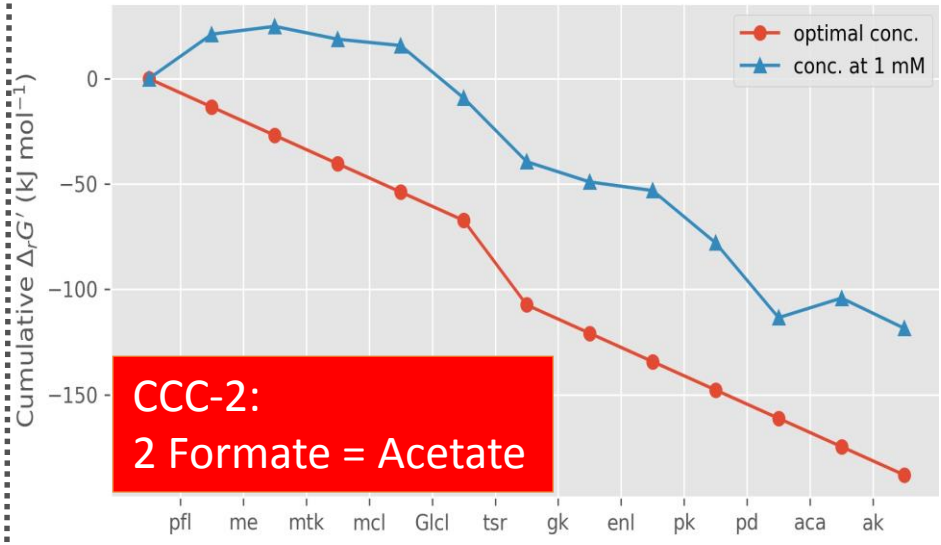
Maximize $\min_r(-\Delta rG')$

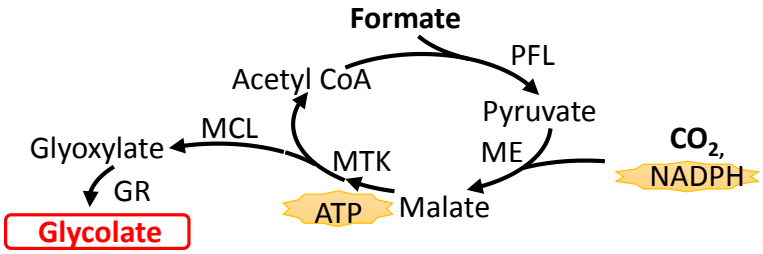
Where $\Delta rG' = \Delta rG'^0 + RT.\ S.\ \ln(C)$

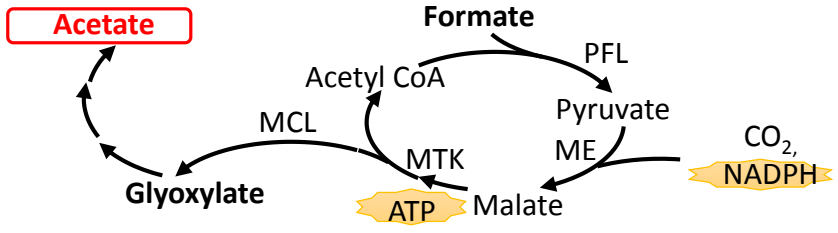
to satisfy $\ln(C_{min}) \leq \ln(C) \leq \ln(C_{max})$ and $\Delta rG' \leq 0$ for all r.

CCC: C1 Condensation Cycle



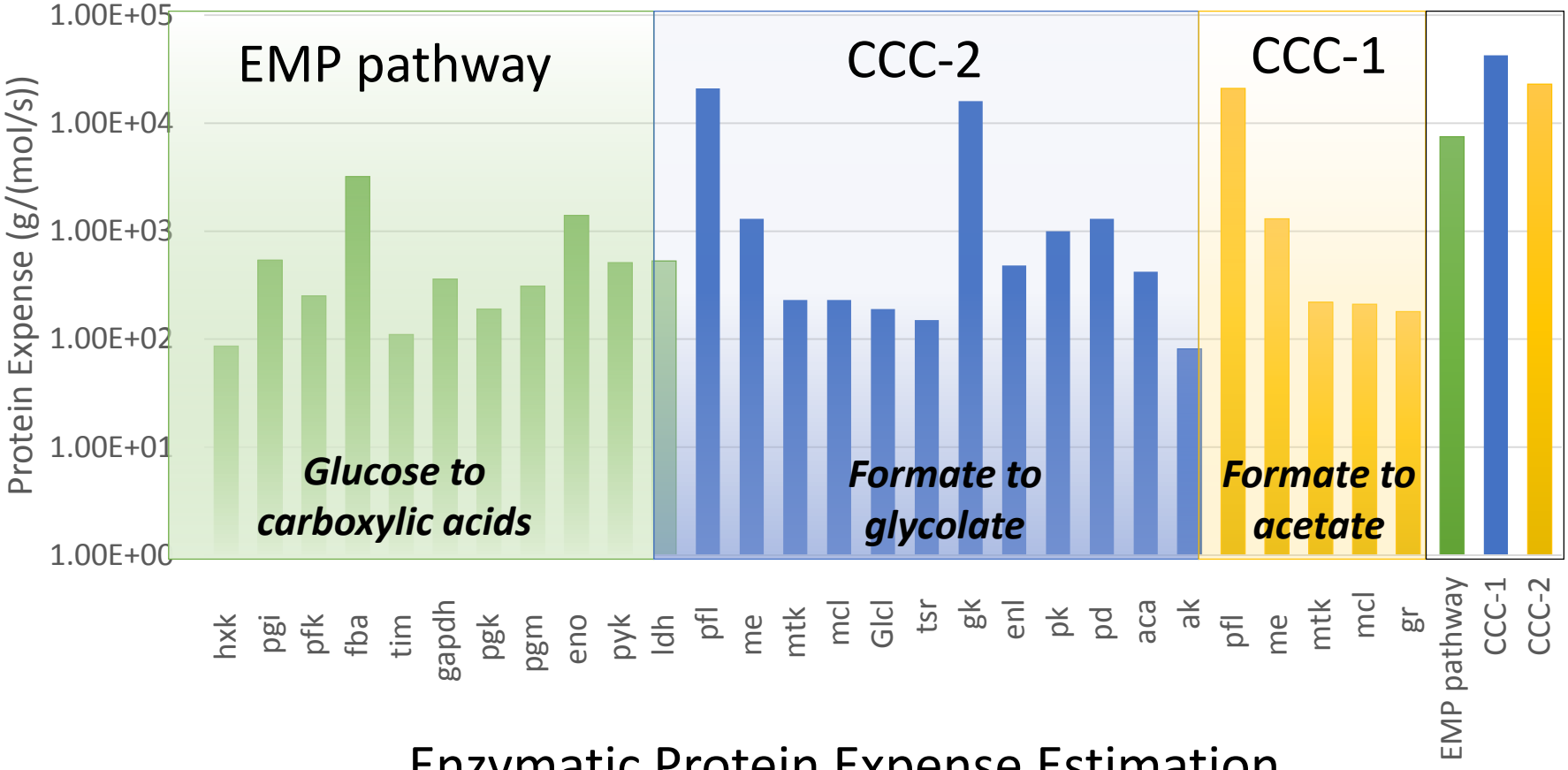






3 – Results: Reasonable Enzyme Expense for the CCC

- ***Protein expense*** is defined as the overall amount of enzymatic proteins utilized for each pathway to achieve certain flux for the product.
- ***Protein expense*** is determined by enzymatic kinetics, and thermodynamics parameter which has been analyzed as aforementioned.



Enzymatic Protein Expense Estimation

4 - Relevance

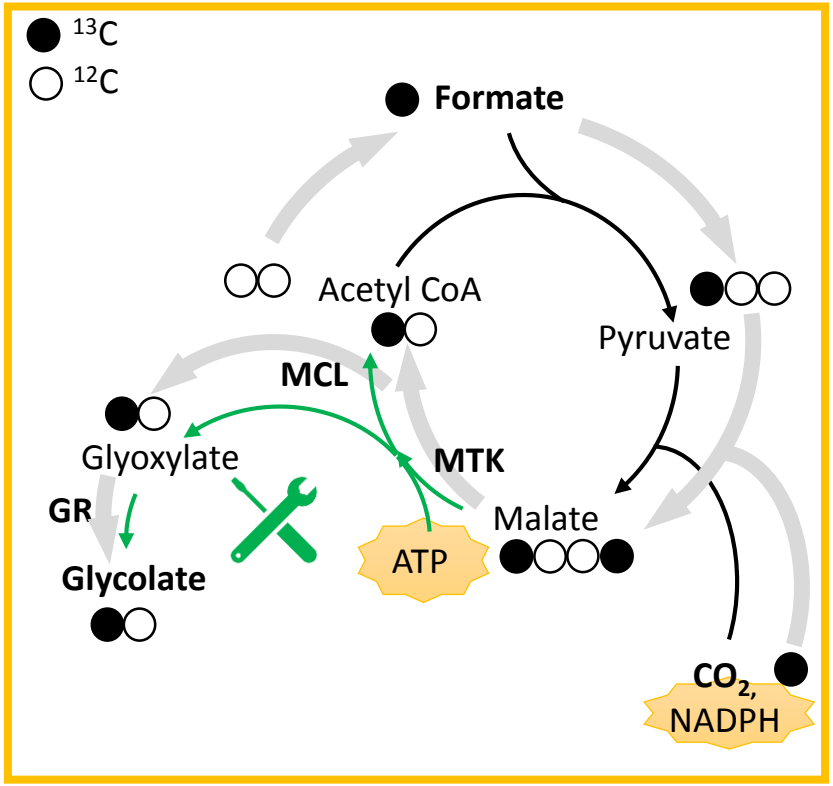
- **Biological formate utilization by *E. coli* has several merits:**
  - *E. coli* is a well-established industrial **chassis microbe**. It allows the use of more matured technologies to improve process efficiencies.
  - The project provides **understanding of pathways** for formate to C2, which could lead to follow-on work to achieve rates/titers that are economically relevant.
  - The pathway designed can allow *E. coli* to use **variety of substrates** like biomass sugars, formate and CO_2.
  - Formate conversion to **model C2 compounds** like acetate integrates with production of many chemicals which use acetyl unit as the precursor (*e.g.* alcohols and fatty acids).
  - **Systems biology** in this work can be integrated with **classic TEA and LCA**. New approaches can evaluate overall process economic potentials, key cost drivers, and define innovative and relevant pathways to achieve cost competitiveness in a new dimension.

- **Tech Transfer/Marketability:** The new conversion process using *E.coli* can be a fit to existing industry or stand alone. We will use TEA to determine how this pathway can get to acceptable productivity.

5 – Future Work in FY19

- **"Wet" experiments: Increase metabolic flux from formate to bioproducts**
  - **Metabolic engineering:** synthesize DNA constructs carrying MTK, MCL, and GR that are engineered with promoters, RBSs, and terminator; transform into *E. coli* host strains.
  - **^{13}C-fluxomics:** identify bottlenecks and test glycolate production from formate/CO_2.
- **TEA/LCA effort** will build high-level preliminary TEA models for establishing the productivity and yield targets needed for economic viability.

Engineering Formate/CO_2 Conversion to glycolate



FY19 Q4 Milestone: Measuring that **100 mg/L glycolic acid** production is from formate in the engineered mutants of *E. coli*.

Summary

- **Overview:** We just started this new BETO project for biological formate conversion, aiming to upgrade formate from electrochemical CO_2 reduction;
- **Approach:** We are engineering a pathway into *E. coli*; This synthetic C1 Condensation Cycle is designed to convert formate and CO_2 into glycolate, a model C2 molecule of industrial interest;
- **Results:** Systems biology analysis exhibited the feasibility of this synthetic pathway in thermodynamics and enzymatic protein cost.
- **Relevance:** Conversion of CO_2 to formate by an industrial microbe (*E. coli*) could fit into a variety of industrial processes as value add or stand alone
- **Future work in FY19:**

- To construct the *in vivo* pathway for formate upgrading.

- To understand native and engineered pathways by ^{13}C-fluxomics.

- To build high-level preliminary TEA models and explore glycolate upgrading strategies to products for commercial uses.

Acknowledgements

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

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