Team 20: Synthetic Minimal Cell for Carbon Sequestration

Justhine Kaye Placio [1,2], Alex Lorang [1,2]
Yo Suzuki[2], Licha Tatiyaratana[1], Immo Burkhardt[3]

1: Shu Chien-Gene Lay Department of Bioengineering, University of California, San Diego
2: Synthetic Biology and Bioenergy Group, J. Craig Venter Institute
3: Scripps Institute of Oceanography, University of California, San Diego

UC San Diego
JACOBS SCHOOL OF ENGINEERING
Shu Chien-Gene Lay Department of Bioengineering

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Motivation

- **CO_2 emissions** are a major culprit of the **climate crisis (global warming)**
- **38.1 billion metric tons** of fossil CO_2 was emitted in 2025, record high
- ***In vitro*** carbon fixation is being explored but:
  - **large-scale** fixation requires self-sustaining systems
  - ***in vivo*** carbon capture needs further testing
- Growing need for **climate change solution:**
  - **optimized cellular carbon capture**

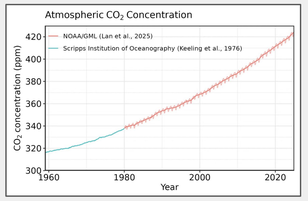


Figure 1: Global CO_2 concentration from 1960-2025[1]

Background

- **POAP Cycle:** theoretically simplest cycle for carbon sequestration
- **4 enzymes** from unique organisms
  - pyruvate synthase (PFOR)
  - pyruvate carboxylase (PYC)
  - oxaloacetate acetylhydrolase (OAH)
  - acetate-CoA ligase (ACS)
- More **metabolically efficient** than the Calvin cycle
- Captures **2 CO_2 molecules** per cycle
- Produces **oxalate** as a byproduct
- **Bottleneck**:
  - some function optimally at **>40℃**

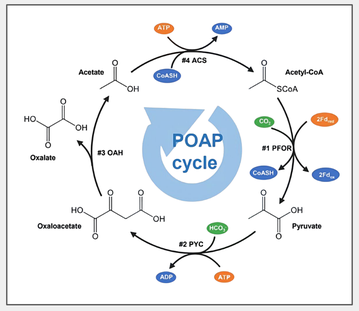


Figure 2: Synthetic *in vitro* cycle[2]

Previous Work

- **Thermal Adaptive Laboratory Evolution (TALE)** to create minimal cells that survive at the higher temperatures required for enzyme function
- Creation of **two-enzyme cell constructs (A1 & B1)** to be the foundation for the four-enzyme construct and the minimal cell transformation with it

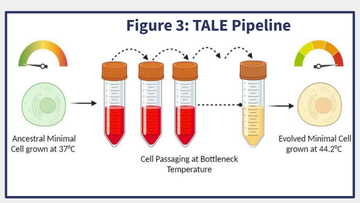


Figure 3: TALE Cell Pipeline

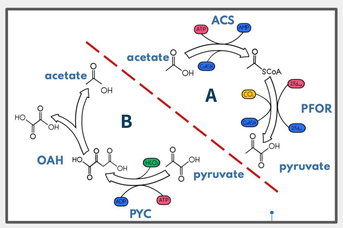


Figure 4: Division for A1 & B1 pathways

Predicting Cell Growth Period

Previously, variable GC-MS data were attributable to cultures in different phases.
Goal: Reproducibly prepare cultures in similar stages

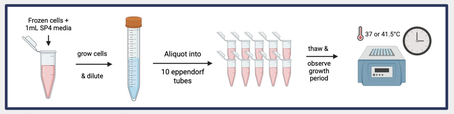


Figure 5a: Cell culture growth & observation pipeline

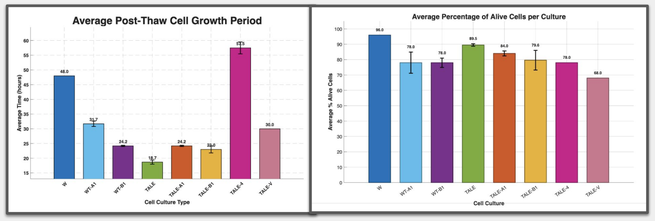


Figure 5b: Average time needed for each culture to reach stationary phase & percentage of alive cells

GC-MS Optimization Using Pure Solutions

- Optimize GC-MS settings using pure solutions before cell samples
- Lactate, oxalate, oxaloacetate, pyruvate **(LOOP)** compounds
  - pyruvate is converted to **lactate** in anaerobic respiration
- **Lactate** and **oxalate** as primary indicators for enzyme activity

Compound	Retention Time (min)	Characteristic Ion Peaks (m/z)
Lactate	6.65	117, 191, 219
Oxalate	8.13	73, 147, 190
Oxaloacetate	6.60	89, 115, 174
Pyruvate	6.75	89, 115, 174
Acetate	7.71	61

Table 1: Expected GC-MS Results

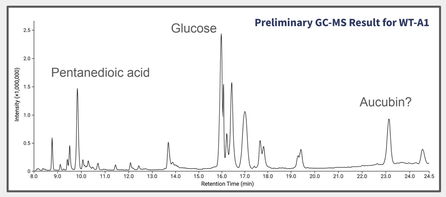
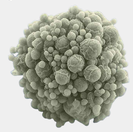


Figure 6: Early GC-MS trial on WT-A1

- Early trials showed mainly unexpected peaks which might be due possible error during derivatization or sample/machine contamination.
- Experimented on various GC-MS settings and derivatization protocol.

Future Directions

- Continue GC-MS analysis on cell samples to quantify **oxalate** and **lactate** on our cell cultures and verify the **enzyme and cell functionality**
- Track **carbon sequestration efficiency** and improve protocol to increase efficiency of carbon sequestration
- Modify the protocol to **scale up** the process



4-Enzyme Plasmid Transformation

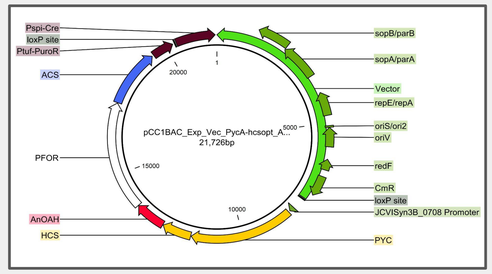


Figure 7: Expected T4 DNA sequence

- Fragment from the B1 plasmid containing enzyme genes was transferred to the A1 plasmid
- **Transformed** TALE cells with vector and 4-enzyme plasmid; amplified and sequenced the integrated construct

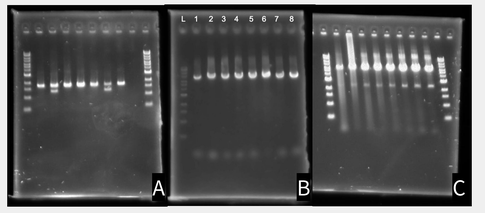


Figure 8: Gel bands result after PCR of (A) TV samples; (B) T4 samples; (C) T4 samples with optimal primer

- **Extracted 8 colonies** from T4 and TV plates
- During PCR of the T4 samples, two sets of primers were used to amplify two halves of the construct

Expected Bands
TALE Vector (TV) samples → 1.8 kbp
TALE 4-Enzyme (T4) samples → 13 kbp

TV Samples

5/8 samples produced a **single band** of the expected size, while **2/8** samples showed **doublets**.
All 7 had the correct sequence.

T4 Samples

First half: 5/8 samples were PCR positive with correct sequence.
Second half: 8/8 samples were PCR positive with correct sequence.

Conclusion

80%
Cell Growth Period
Missing WT-4 cell cultures; collect more data

60%
GC-MS Analysis
Issues detecting acetate; more cell sample testing needed

100%
4-Enzyme Transformation
Sequencing verified the successful transformation

References

[1] Kerlin, Katherine E. "Fossil Fuel CO2 Emissions Hit Record High in 2025." *UC Davis*, 13 Nov. 2025, www.ucdavis.edu/climate/blog/fossil-fuel-co2-emissions-hit-record-high-2025.

[2] L. Xiao et al., "A Minimized Synthetic Carbon Fixation Cycle," ACS Catal., vol. 12, no. 1, pp. 799–808, Jan. 2022, doi: 10.1021/acscatal.1c04151.

Acknowledgement

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Shu Chien-Gene Lay Department of Bioengineering
U.S. DEPARTMENT OF ENERGY
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