Metabolic modeling and synthetic biology for the renewable production of fuels and chemicals

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Research background

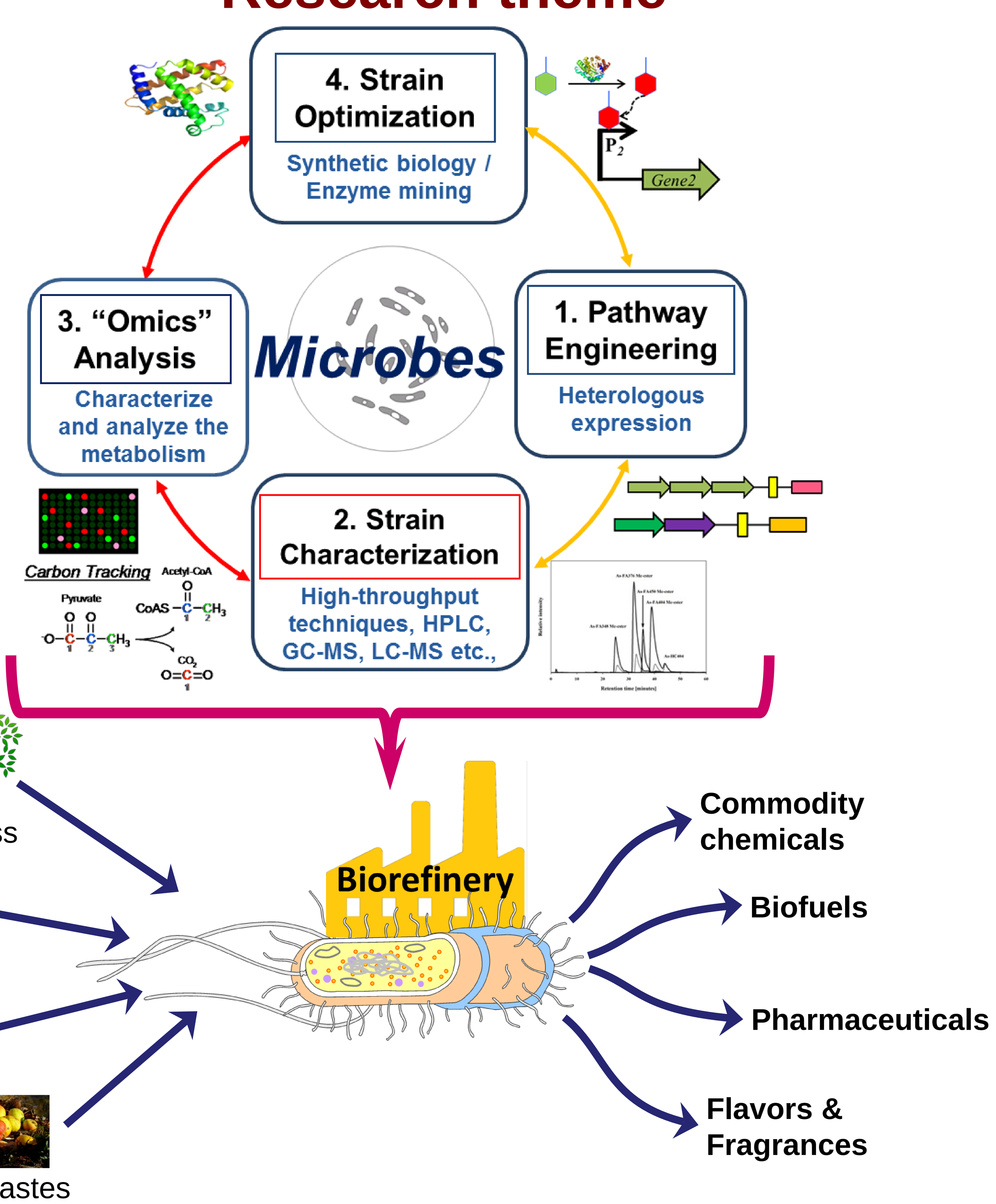
Global warming and decreasing fossil fuel reserves have prompted great interests in the synthesis of advanced biofuels and value-added chemicals from renewable resources. Lignocellulosic biomass and carbon dioxide represents two of the most abundant renewable carbon resources on the earth. Numerous efforts are underway for the production of renewable chemicals from the cellulosic and hemi- cellulosic portion of the biomass. However, economic analysis suggest that for the bio refinery to be cost competitive with the petroleum industry, more value needs to be arrived from lignin. Towards achieving this goal, I have worked on (a) engineering microbes for ligninolysis; (b) understanding the central metabolism of a soil bacterium, *Sphingobium* sp. SYK-6 via metabolic flux modeling and systems analysis; (c) hybrid promoter engineering for developing promoters inducible by lignin derived phenolics. Also, in an effort to produce renewable chemicals from CO_2, a model cyanobacterium *Synechocystis* sp. 6803 was engineered for the production of isobutanol and D-lactate. I have also employed data mining and machine learning to predict yield in engineered microbes.

Proposal experience

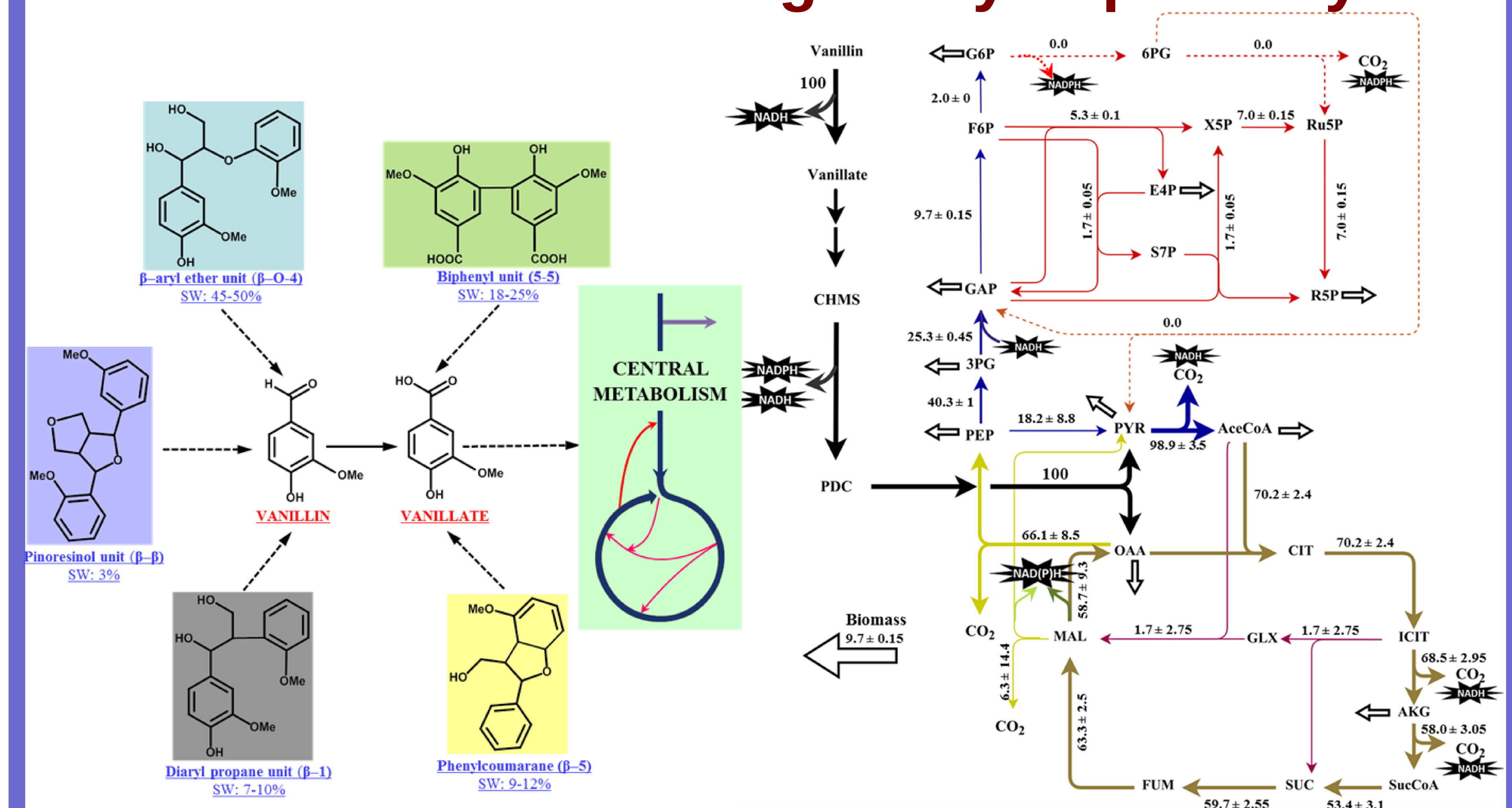
Substantive contribution in writing proposals for

- NSF-CAREER (cyanobacterium engineering)- funded $500K
- GATES Grand Challenges Explorations (waste-to-biofuel) – funded $100K
- NSF-MCB (machine learning) – funded $500K
- EMSL user facility (Lignin valorization) – funded $100K
- LDRD Sandia – denied $600K
- ASM and MAGEEP travel grants - accepted

Research theme

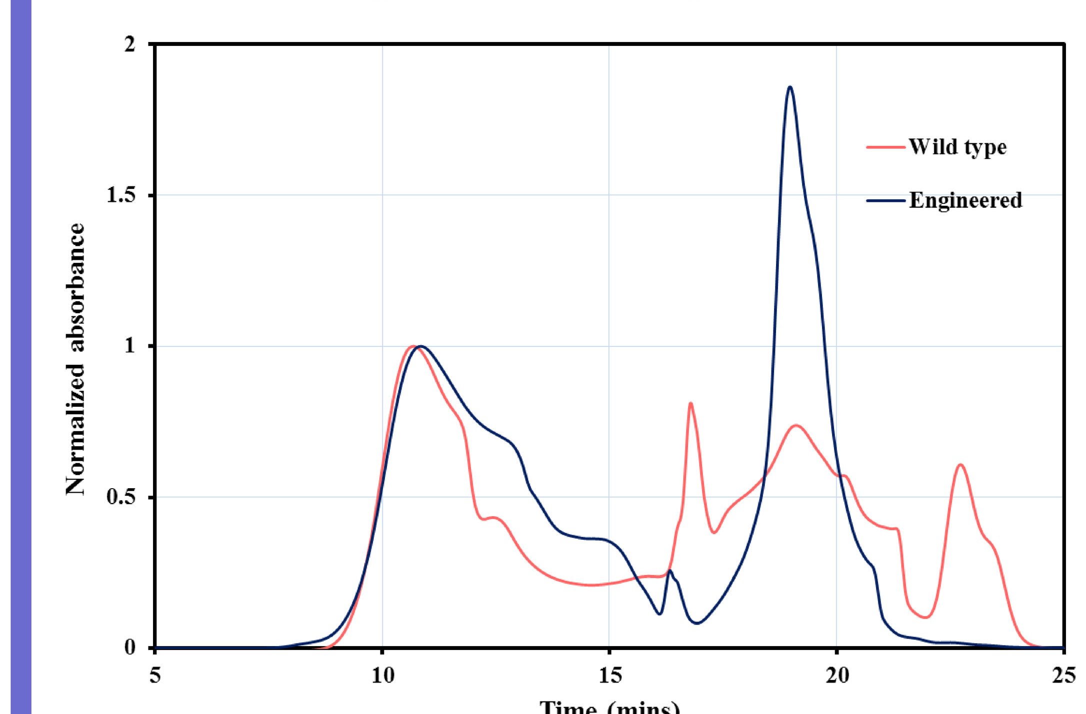


Decoding the central metabolism of a bacterium with ligninolytic pathways



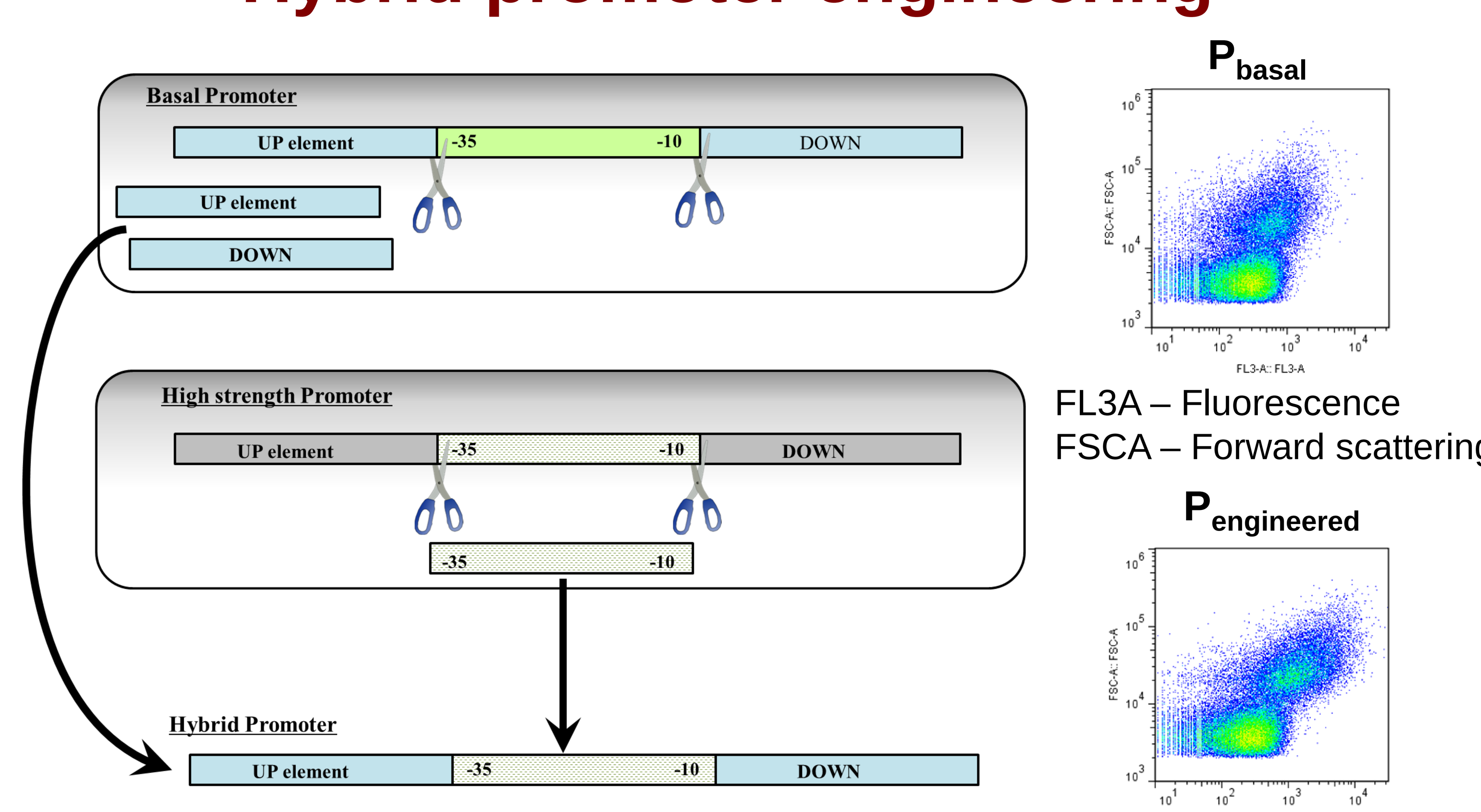
- Vanillin catabolic pathway is the major contributor for NAD(P)H synthesis and therefore, is essential for SYK-6 to obtain sufficient reducing equivalents and maintain the redox balance within the cell.

Engineering microbial lignolysis



- Gel permeation chromatography shows the presence of lignin depolymerization by engineered microbes.

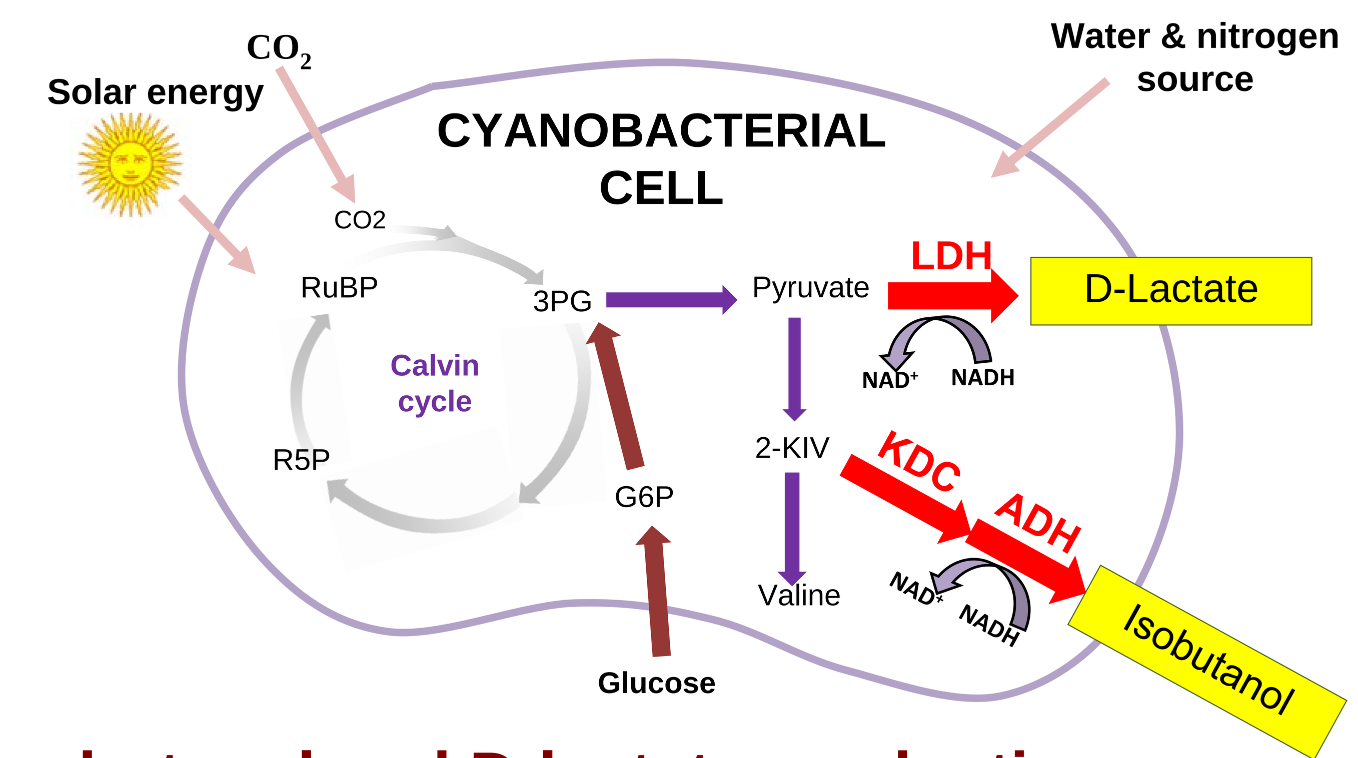
Hybrid promoter engineering



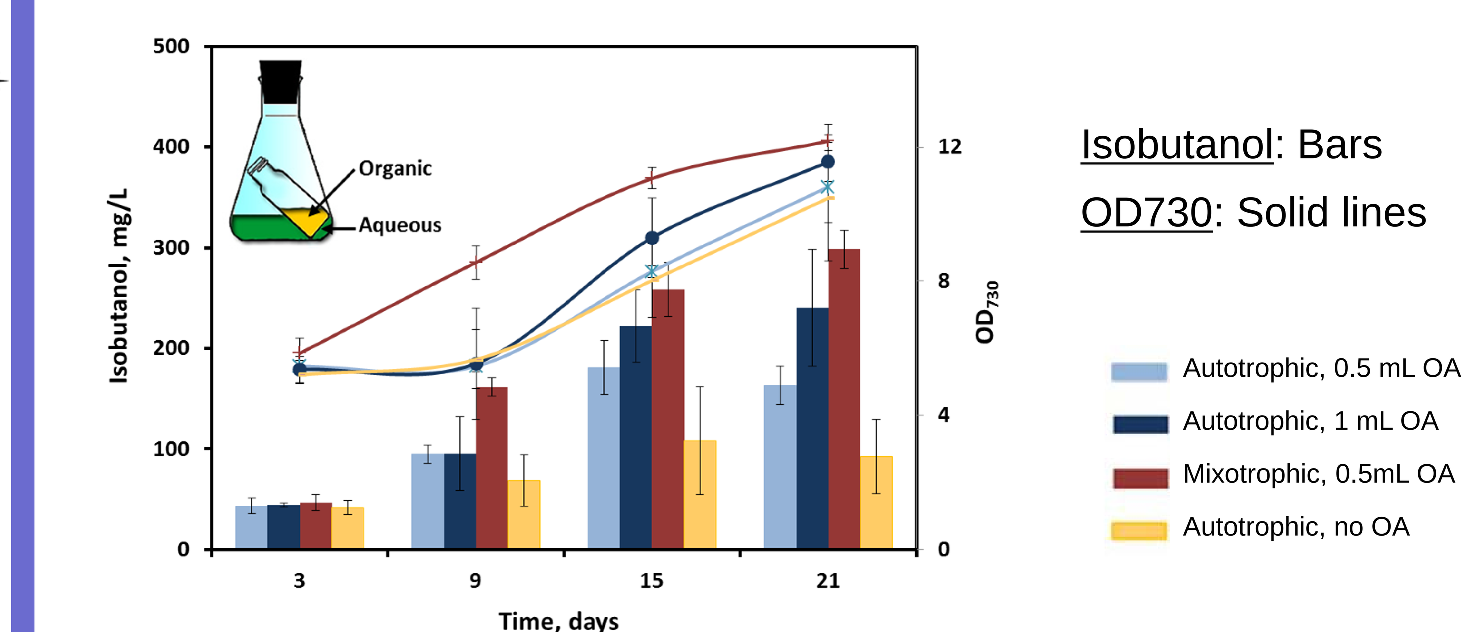
FL3A – Fluorescence

FSCA – Forward scattering

Engineering cyanobacteria for isobutanol and D-lactate



Isobutanol and D-lactate production

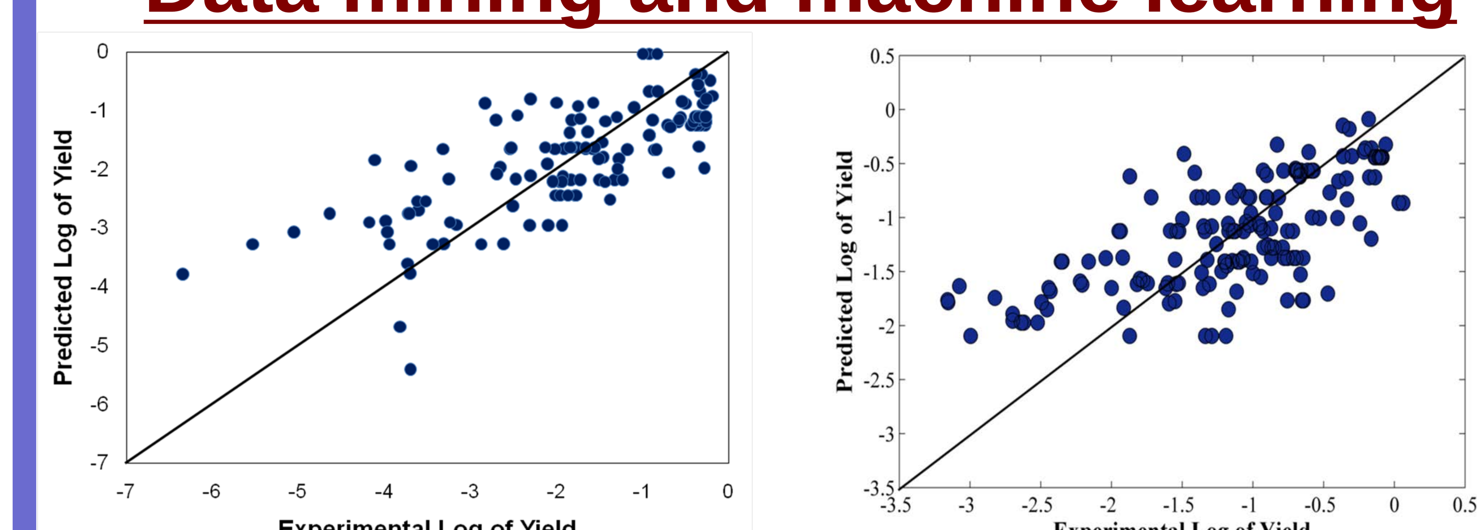


Isobutanol: Bars

OD730: Solid lines

- Isobutanol production increased by over 3 folds (298 mg/L) with the utilization of *in situ* removal of isobutanol using oleyl alcohol (OA) and by growing the cyanobacteria under mixotrophic conditions.
- D-lactate titer of 2.17 g/L was achieved in a separate work.

Data mining and machine learning



$$\log_{10} Y = \beta_0 + \beta_{PRI}PRI + \beta_{SEC}SEC + \beta_{OVE,C2}OVE_{C2} + \beta_{OVE,C3}OVE_{C3} + \beta_{KNO,C2}KNO_{C2} + \beta_{NUT,C2}NUT_{C2} + \beta_{INT,C2}INT_{C2} + \beta_{CUL,C2}CUL_{C2} + \beta_{OXY,C2}OXY_{C2} + \beta_{TMP}TMP$$

Selected publications

1. **Varman AM**, He L, Follenfant R, et.al. Decoding how a soil bacterium extracts building blocks and metabolic energy from ligninolysis provides road map for lignin valorization. ***Proceedings of the National Academy of Sciences.*** 2016. 113 .
2. **Varman AM**, Yu Y, You L, Tang YJ. Photoautotrophic production of D-lactic acid in an engineered cyanobacterium. ***Microbial Cell Factories***. 2013. 12:117.
3. **Varman AM**, Xiao Y, Pakrasi H, Tang YJ. Metabolic engineering of *Synechocystis* sp. strain PCC 6803 for isobutanol production. ***Applied Environmental and Microbiology***. 2013. 79(3): 908-914.
4. **Varman AM**, He L, Tang YJ. "Chapter 7: Microbial metabolisms." Bioenergy: Principles and Applications, Editors: Yebo Li and Samir Kumar Khanal, **John-Wiley, 2016.**
5. **Varman AM**, Xiao Y, Leonard E, Tang YJ. Statistics-based model for prediction of chemical biosynthesis yield from *Saccharomyces cerevisiae*. ***Microbial Cell Factories***. 2011. 10:45.

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