SAND2016-4622PE



# Building the Biorefinery of the Future

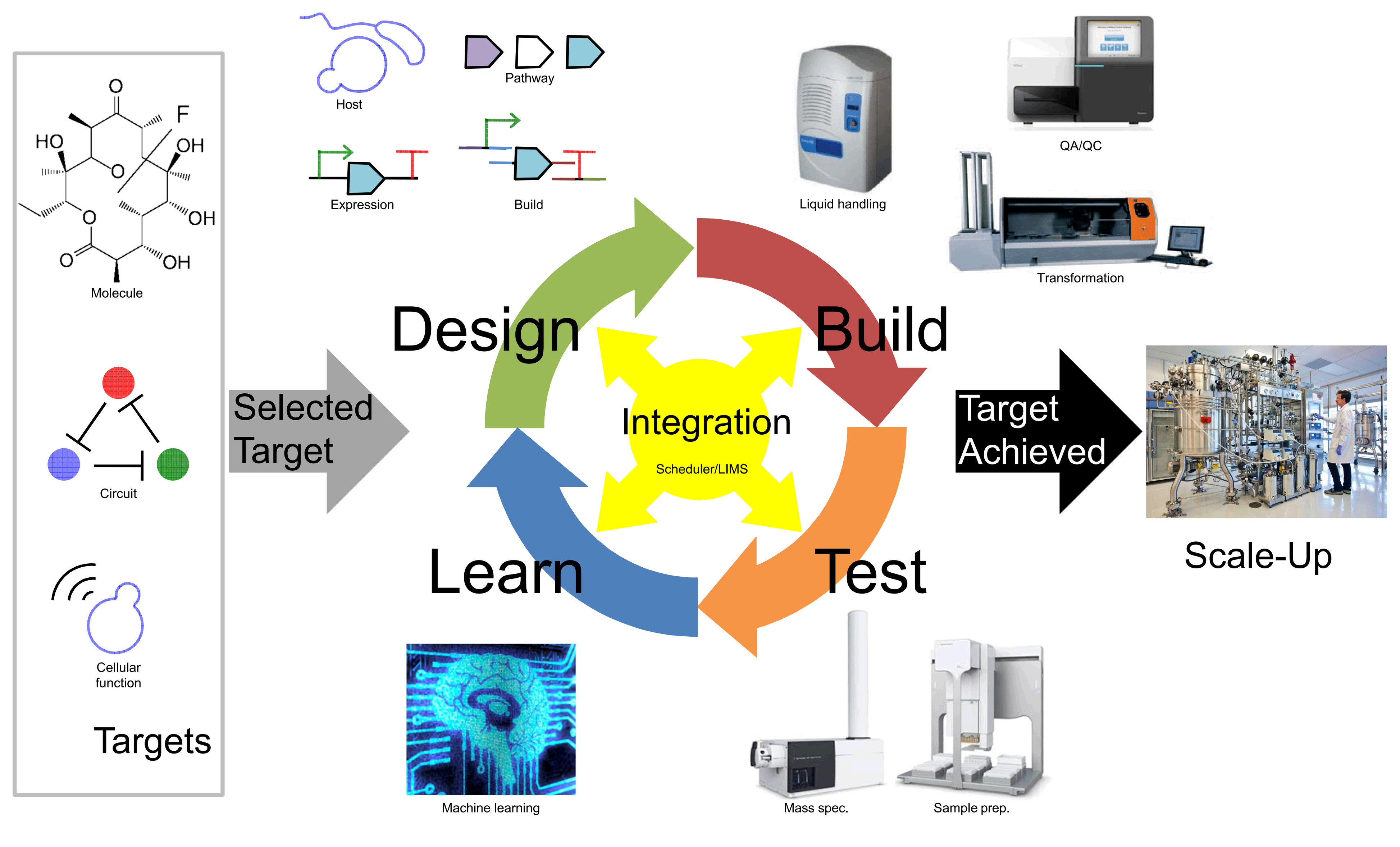
John Gladden*, Nathan Hillson, Gregg Beckham, and Mark Butcher

## Overview

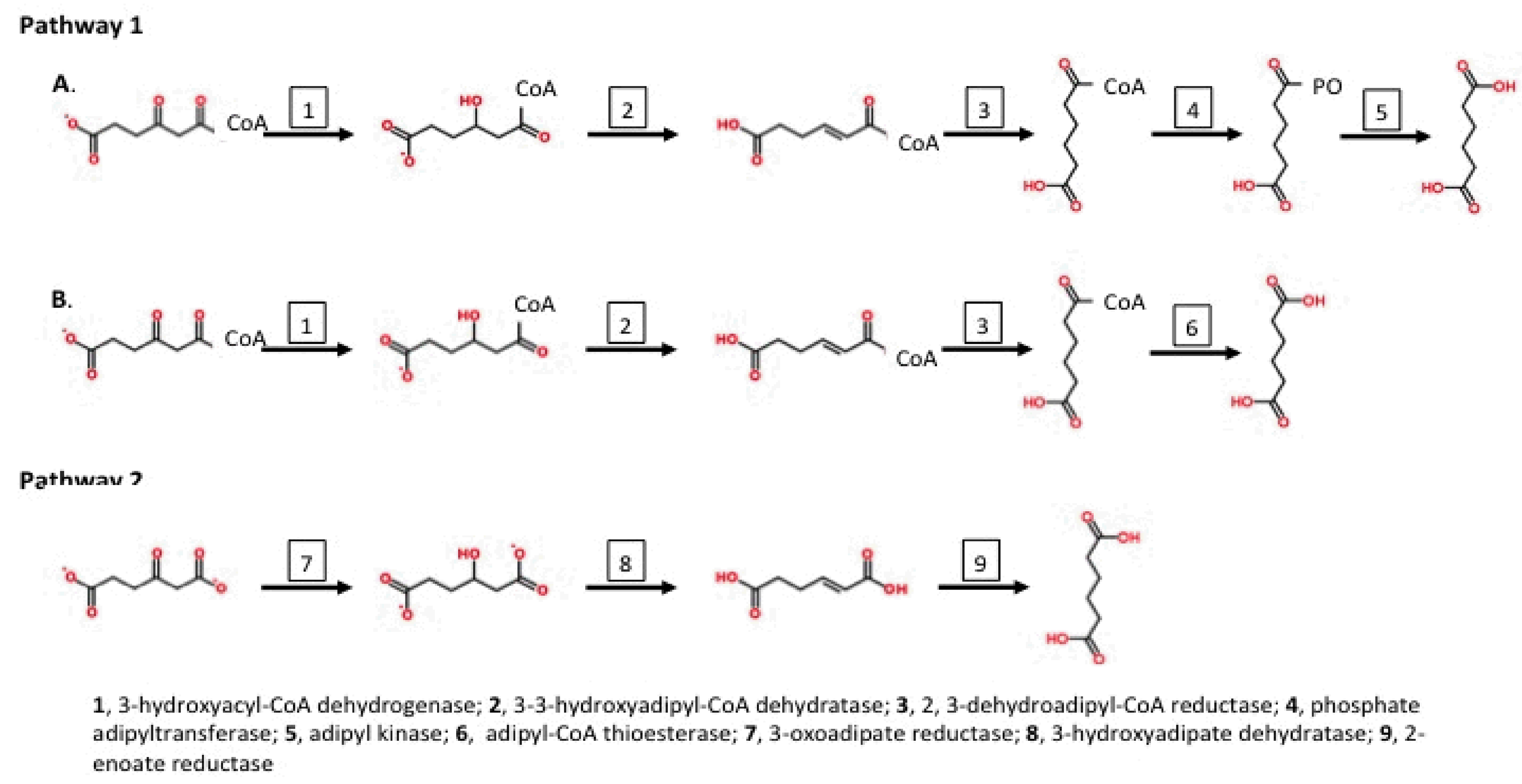
The US chemicals industry, in particular those market sectors that matured and deployed in support of essential DoD missions, is a ~$770 billion enterprise based almost entirely on the manipulation of traditional chemistry and the inter-conversion of petroleum feedstocks from specific inputs and intermediates to finished products. Biological chemistry and the conversion of sustainable, affordable, and scalable sugar feedstocks could enable the displacement of the entire portfolio of currently available products with renewable and chemically identical compounds, with the added benefit of enabling advanced biofuels production by diversifying the products generated from a biorefinery and enhancing the cost-competitiveness of these business models. Unfortunately, biological chemistry as it is realized today is difficult to reliably engineer, requiring long development times and significant financial investments incompatible with the low-margin chemicals and fuels business. The successful development of a robust biomanufacturing strategy and technology platform, based on the latest advances in synthetic biology and chemical catalysis would decrease both the cost and time to market by half based on previous industry experience. We aim to establish a Foundry to provide solutions that greatly improve the time to market for renewable fuels and chemicals at the enterprise level for industry (Figure 1).

## Objective and Results

The primary objective of this multi-lab effort is to establish and demonstrate the positive impact of a Biofoundry in the production of adipic acid in two industrially relevant hosts: *Pseudomonas putida* and *Streptomyces venezuelae*. This project will establish a working Foundry that leverages the expertise and capabilities within the four lab partners (LBNL, NREL, PNNL, and SNL). The Foundry will center around a Design, Build, Test, Learn architecture to rapidly engineer organisms to produce bioproducts. The team will focus on routes to optimize the production of adipic acid in the two hosts, both of which have been established as viable and compelling biofuel hosts with a goal of demonstrating significantly improved titers and yields in both that could only be realized through the Foundry approach. To date we have designed and are in the process of building and testing four adipic acid production pathways, with the goal of producing 10g/L of adipic acid through implementing multiple DBTL cycles to improve titer, rate and yield of these pathways.

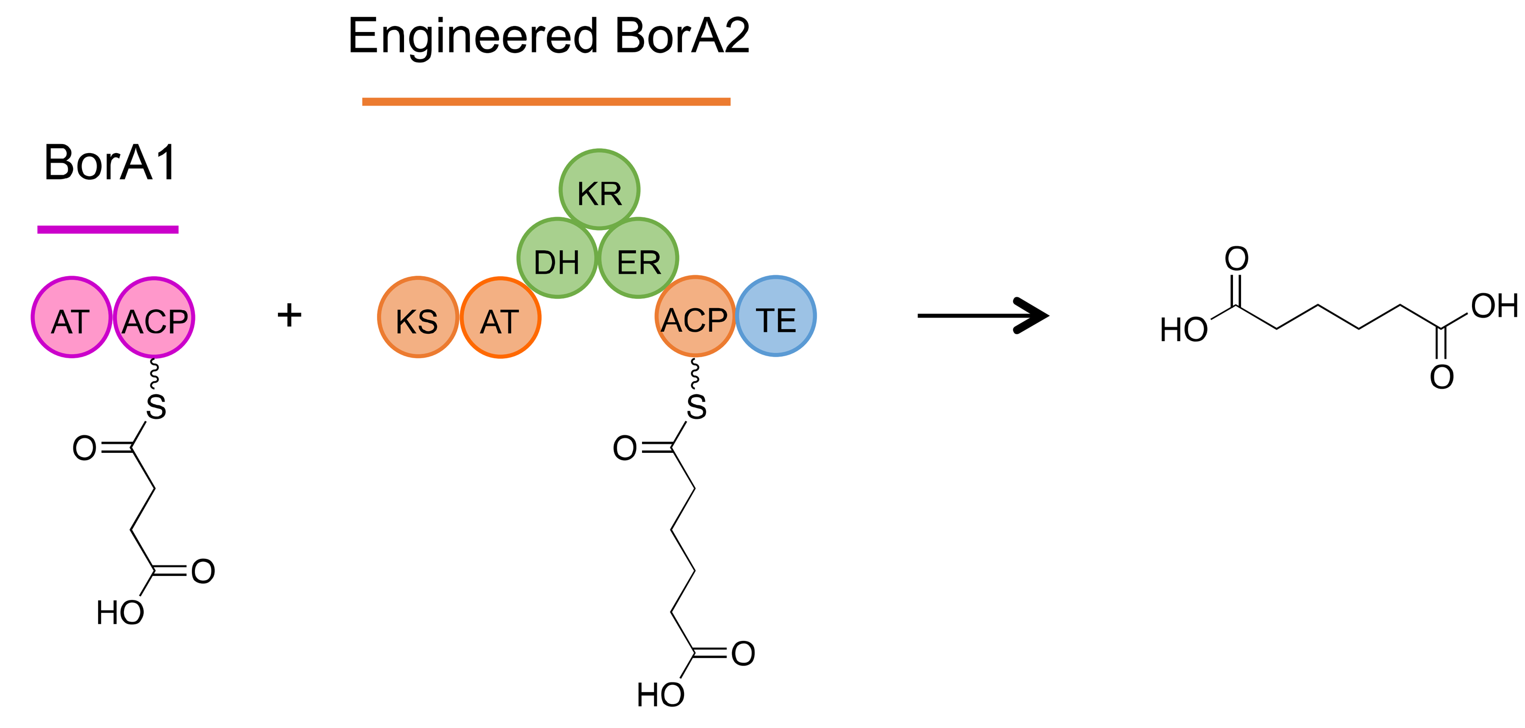



## *P. putida*




Three adipic acid pathways were designed to utilize intermediates from the native B-ketoadipate pathway. Strains were developed that can generate these intermediates from either aromatics or sugars derived from lignocellulose.

## *S. venezueale*




An engineered PKS system to produce adipic acid. Circles of the same color mean that the domain was taken from the same natural source. Pink circles are domains from the BorA1. Orange circles are domains from the BorA2. Green circles are domains from the BorA5. Light blue circles are domains from the DEBS3.

*Exceptional service in the national interest*

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