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Title:	Lighting the way to bioproducts: Smart microbial biosensors for conversion pathway design
Author(s):	McDonald, Rebecca E. Jha, Ramesh Kumar
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Lighting the way to bioproducts: Smart microbial biosensors for conversion pathway design

For several years, Los Alamos scientists have been taking advantage of the molecular machinery within microbial cells to produce fuel precursors and bioproduct building blocks. Today, they are improving this process by adding a biosensor to the microbes that will use fluorescence to tell them how efficiently the product is made—thus enabling high throughput screening to increase overall yield.

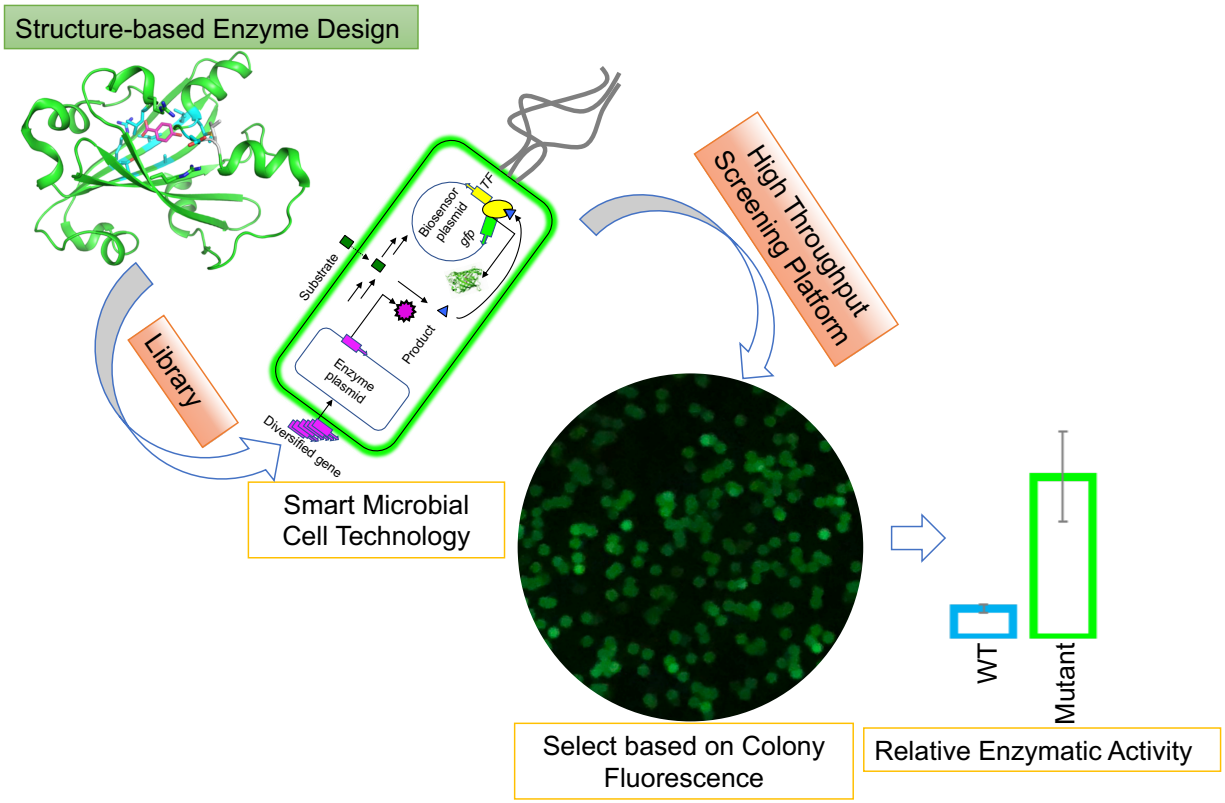
Due to a versatile metabolism housed in relatively simple cell structure, engineered microbes are poised to be the workhorses of the future bioeconomy. One of the challenges, however, is that when microbes are engineered for making a new product, it is difficult to pin-point the best performer in a pool of microbial cells with variable product formation efficiencies. Furthermore, testing the efficiency of microbial cells has always been a low throughput process and ultimately a bottleneck in the Design-Build-Test-Learn (DBTL) cycle for bio-based products.

In order to advance this, Los Alamos scientists have leveraged longstanding capabilities in protein design and computational modeling of ligand binding pockets in proteins to engineer custom biosensors that detect intracellular concentrations of a product of interest. These protein-based biosensors for small molecules detect the presence of the desired target molecule and respond via the accumulation of a fluorescent protein reporter, ultimately providing a convenient method for visualizing the productivity of the target molecule. Furthermore, by coupling this “smart microbial cell” to the high throughput efficiency of flow cytometry and fluorescence-activated cell sorting, they can successfully evaluate a large number of metabolic designs formulated for improved production of the target molecule.

With enhanced throughput in testing the designs, this approach can accelerate the DBTL cycle for biomanufacturing. Specifically, the technology is crucial for relieving the bottleneck in the Test step of the DBTL cycle. Not only does the increased throughput in the Test step help match the ‘Design’ and ‘Build’ steps, but the capability to produce enormous amount of data by high throughput evaluation is also critical as input for enhanced Learning for subsequent DBTL cycles.

Overall, LANL’s Smart Microbial Cell technology is an advanced platform for high throughput screening for enzyme discovery, design, and evolution. The approach can be translated to screening of metagenomic samples, rational enzyme design, or directed evolution of known enzymes. The technology is adaptable to a single enzyme, or a pathway, or global optimization of an industrial strain.

Microbial biosensor team includes Los Alamos scientists Ramesh Jha (photo), Niju Narayanan, and Taraka Dale (lead). The work was performed under the Agile BioFoundry, a multi-national laboratory effort to expedite biomanufacturing processes (https://agilebiofoundry.org).



(Left to right) Structure based design of an enzyme is used to create a library of diversified genes. Smart microbial cell technology demonstrates the efficiency of enzyme variants by producing a readable fluorescence signal. High throughput screening enables visualization of colonies with improved efficiency. Relative *in vitro* enzymatic activity measurement of the variants shows that colonies with brighter fluorescence (Mutant) correspond to higher enzyme activity (WT). WT= Wild Type.