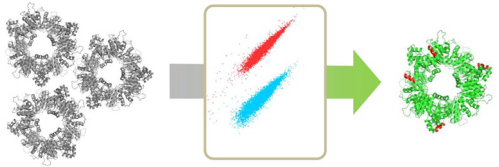
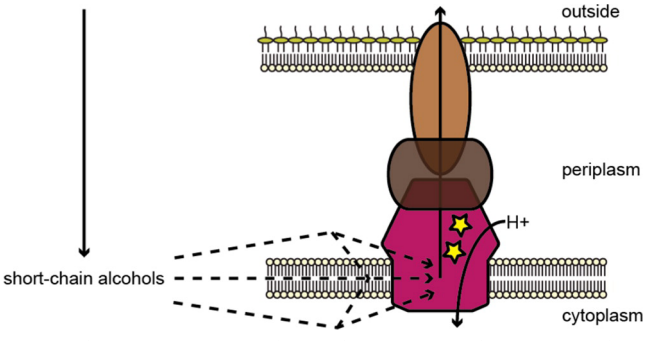
PRODUCT SENSING TO REMOVE FLUX CONTROL



Several million tons of important small molecules, such as pharmaceutical intermediates and biofuels, are produced annually from microorganisms. The increased demand for these molecules necessitates an increased efficiency in the conversion of renewable sources to the valuable small-molecule. However, enzymes initiating the biosynthesis are frequently inhibited by the end-product of the respective pathway. Now, Schendzielorz *et al.* (DOI: 10.1021/sb400059y) present a methodology that provides a new means to overcome such inhibition.

The authors use a sensor to monitor the concentration of a specific small-molecule within bacterial cells and screen large libraries of key enzymes for increased small-molecular production at the single-cell level, using high-throughput sorting via FACS. This approach provides a tool to overcome flux control in pathways by key enzymes that are controlled by feedback mechanisms.

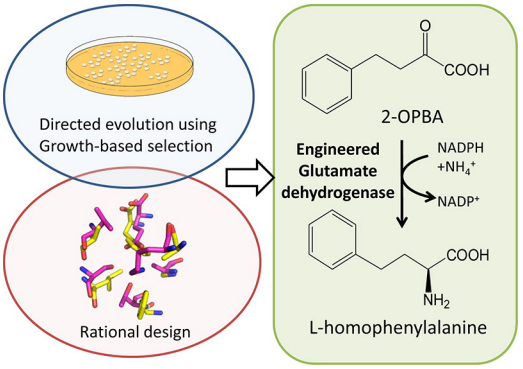
ENHANCING BACTERIAL TOLERANCE TO SHORT-CHAIN ALCOHOLS



As an alternative to petroleum, biomass can be converted to chemicals by a variety of methods. One particularly promising method is the degradation of biomass to sugars which are then fed to engineered microbes that enzymatically synthesize desired chemicals. A challenge is that, in addition to sugars, compounds that are toxic to microbes enter the cells. The chemical product that is synthesized is also often toxic, reducing cell growth and overall chemical production. To meet this challenge, Fisher *et al.* (DOI: 10.1021/sb400065q) have engineered new substrate specificity into a membrane protein that transports small molecules out of bacteria.

The new transporters secrete n-butanol, isobutanol, and other short-chain alcohols, all of which have applications as fuels and industrial chemicals. This work also provides proof-of-concept that transporters can be engineered for the secretion of non-native substrates, and that cell growth in the presence of inhibitory compounds can be improved by relying on the engineered transporters.

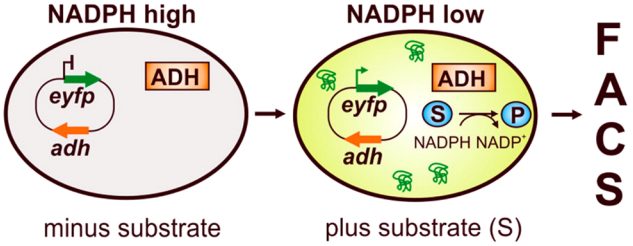
NEW TOOL FOR METABOLIC ENGINEERING OF WHOLE-CELL BIOCATALYSIS



The nonproteinogenic amino acid, L-homophenylalanine is a useful pharmaceutical intermediate. However, current biocatalysts involved in its production are not optimal for the metabolic engineering of whole-cell biocatalysis. To circumvent this, Li and Liao (DOI: 10.1021/sb400093x) now report the engineering of the *E. coli* NADPH-dependent glutamate dehydrogenase (GDH) to develop a NADPH-dependent homophenylalanine dehydrogenase, a new tool for *in vitro* catalysis and *in vivo* metabolic engineering.

The authors used a stepwise substrate walking strategy to develop a selection scheme for directed protein evolution based on growth rescue. Compared to wild type GDH, the final GDH variant demonstrated a catalytic efficiency ~100 fold higher for the precursor of L-homophenylalanine and ~3000 fold lower for the original substrate, 2-ketoglurate.

SOXR AS A SINGLE-CELL BIOSENSOR



Enzymes play an increasing role in the industrial synthesis of chiral compounds required in the production of pharmaceuticals and chemicals. A particularly important enzyme class is that of the NADPH-dependent alcohol dehydrogenases, which convert achiral ketones to chiral alcohols. For commercial applications, these enzymes need to be evolved to have specific properties—a process that requires the screening of large libraries of mutated derivatives. Here, Siedler *et al.* (DOI:

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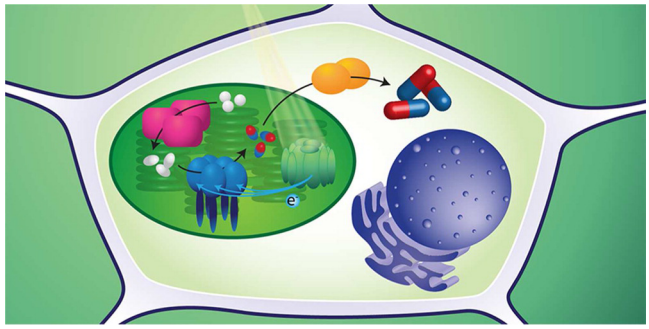
10.1021/sb400110j) establish a universal ultrahigh-throughput screening system for NADPH-dependent enzymes.

This system makes use of fluorescence-activated cell sorting and is based on the transcriptional regulator SoxR of *E. coli*. This manuscript provides a novel method for protein engineering of NADPH-dependent enzymes.

REDIRECTING PHOTOSYNTHESIS TOWARD THE SYNTHESIS OF ALTERNATIVE PRODUCTS



In this review, Lassen *et al.* (DOI: 10.1021/sb400136f) briefly discuss photosynthesis and photosystems in plants, green algae and cyanobacteria, how photosystem I has been used in conjunction with hydrogenases and how initial optimization of electron transport toward the hydrogenase has been achieved. They also describe specialized metabolites made by plants and the synthetic approaches used to synthetize these high-value compounds, including bioengineering approaches involving the cytochrome P450s tried in the past in different biological systems. Finally, the authors detail potential challenges with this novel use of photosynthetic systems in synthetic biology approaches.

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