ACS SyntheticBiology

In This Issue

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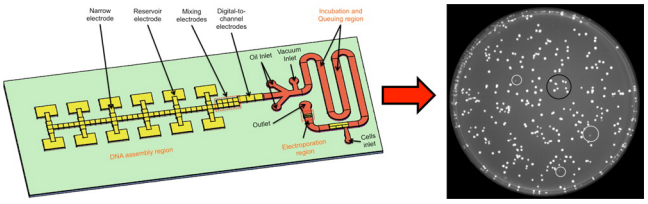
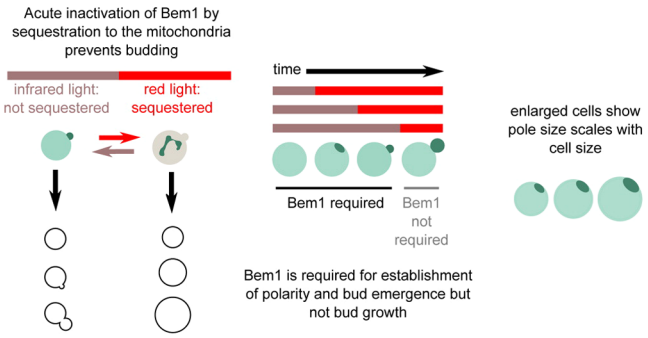
## A VERSATILE MICROFLUIDIC DEVICE FOR AUTOMATING SYNTHETIC BIOLOGY




A field with potential applications in diverse fields such as pharmaceuticals and biofuels, synthetic biology currently seeks to manipulate the structure and function of DNA to create new biological systems that can produce a desired output. This typically follows an iterative process of specification, design, build, and test. Increased automation is needed as current state-of-the-art physical tools are relatively underserved in terms of build and test processes. In this paper, Shih et al. (DOI: 10.1021/acssynbio.5b00062) design the first automated microfluidic method that will help accelerate the build and test processes for synthetic biology.

The authors integrated three different DNA assembly methods (Golden Gate, Gibson, TAR cloning) on a microfluidic device with on-chip transformation, and demonstrate the power of their approach by engineering two sets of 16 DNA plasmid combinations—a total of 32 plasmid combinations.

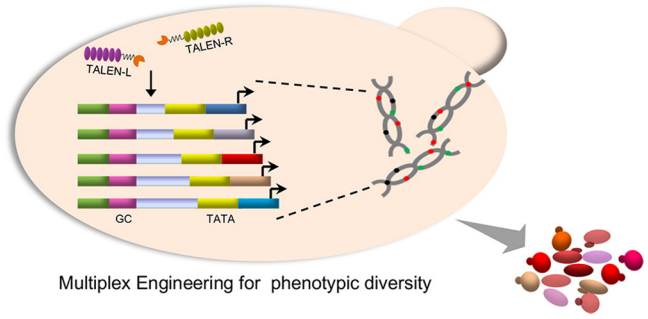
## PROBING YEAST POLARITY WITH ACUTE, REVERSIBLE, OPTOGENETIC INHIBITION OF PROTEIN FUNCTION




Bem1 is a scaffold protein involved in budding yeast polarity. In this paper, Jost and Weiner (DOI: 10.1021/acssynbio.5b00053) adapt a previously described technique for rapidly and reversibly inhibiting protein function through light-inducible sequestration of proteins, for inducible inactivation of Bem1.

The authors demonstrate that acute inhibition of Bem1 produces greater defects in cell polarization and cell viability than cells lacking Bem1. By disrupting Bem1 activity at specific points in the cell cycle, they also show that Bem1 is required for the establishment of polarity and bud emergence but is not essential for the growth of an emerged bud. The authors further report that pole size scales with cell size, and that this scaling is dependent on the actin cytoskeleton. The experiments described here detail how rapid reversible inactivation of protein function complements traditional genetic approaches, a strategy with potential applications in a variety of biological contexts.

## TALENS-ASSISTED MULTIPLEX EDITING FOR ACCELERATED GENOME EVOLUTION




Classical strain engineering has contributed significantly to strain improvement but lacks the ability to rapidly engineer complex phenotypes. While several methods for introducing mutations and modifying microbial genomes are available, there are relatively few methods for the simultaneous generation of multiple defined mutations and modifications. Transcription activator-like effector nucleases (TALENs) enable the generation of double-strand breaks and integration of site-specific editing into genomes. Here, Zhang et al. (DOI: 10.1021/acssynbio.5b00074) describe a novel method for TALENs-assisted multiplex editing (TAME) and demonstrate the ability of this method to accelerate genome evolution and improve cellular phenotypes in yeast.

This new TAME toolbox is based on multiplex interactions of TALENs with DNA sequences between the critical and conserved TATA box (TATAAA) and GC box (GGGCGG), developed after functional analysis of different TALENs and genomic sequence mapping via a self-programmed script. The TAME approach described in this study could be used to achieve multiplex genome modifications and generate genetic diversity, thereby improving complex phenotypes of living cells in short amounts of time.

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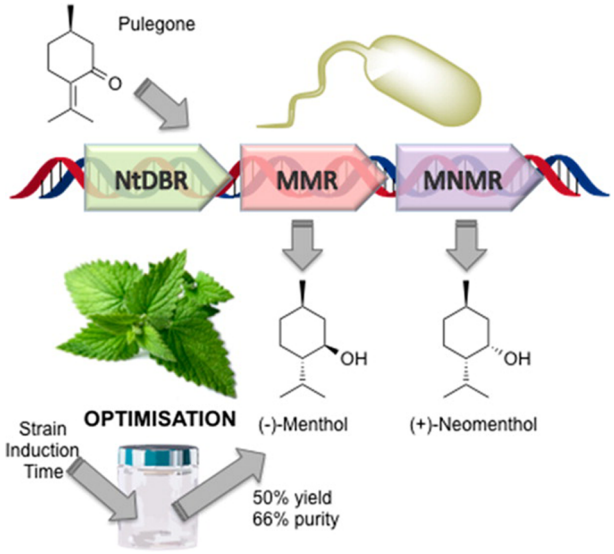
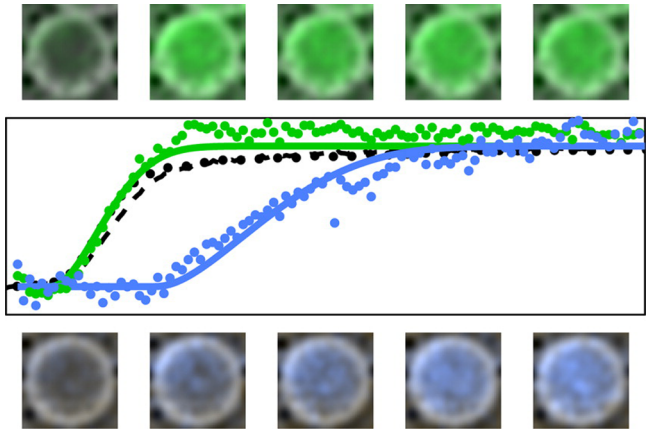
## ENZYMATIC MENTHOL PRODUCTION: ONE-POT APPROACH USING ENGINEERED *ESCHERICHIA COLI*




Menthol isomers are produced naturally by mint plants, Mentha spp. They are high-value monoterpenoid commodity chemicals, and thus alternative clean biosynthetic routes to them are commercially attractive. Here, Toogood et al. (DOI: 10.1021/acssynbio.5b00092) report the incorporation of modified *M. piperita* biosynthetic pathways within *E. coli* to enable the synthesis of individual menthol isomers from pulegone.

This work demonstrates that successful one-pot biosynthesis of monoterpenoids can be accomplished by generating enzyme pathways from multiple organisms (Mentha and Nicotiana), rather than simply transplanting whole gene clusters from the original source organism. The best performing enzymes (e.g., catalytic rate, enantiospecificity, and soluble expression) can be combined into functional cascades of activity (operons), including the biosynthesis of non-natural products. The approach described here combines enzyme selection and validation, followed by seamless PCR-based operon assembly and one-pot biotransformations within *E. coli* extracts. The importance of host enzymes (epimerases) in driving the enantiopurity of target reaction products is also demonstrated.

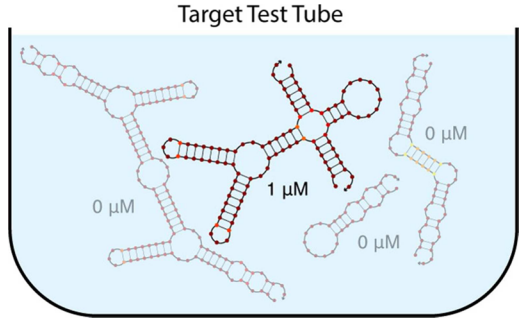
## PARTITIONING VARIABILITY OF A COMPARTMENTALIZED *IN VITRO* TRANSCRIPTIONAL THRESHOLDING CIRCUIT



Encapsulation of biochemical reactions into microcompartments is of considerable interest both for fundamental biochemical studies and for technological applications. Previous investigations of the effect of compartmentalization of an *in vitro* transcriptional oscillator into microdroplets found a large variability in the oscillator dynamics, which was attributed mainly to the variability of enzyme activity after compartmentalization. In this paper, Kapsner and Simmel (DOI: 10.1021/acssynbio.5b00051) work with a subcircuit of the oscillator and provide a thorough quantitative analysis of the effect of compartmentalization on the dynamics of this simple *in vitro* "genelet" circuit.

The results described here suggest that, upon compartmentalization, a seemingly simple reaction circuit composed of only three DNA species and one enzyme can display large statistical fluctuations which depend on the exact "operation point" of the circuit. In future experiments, understanding such dependences will be helpful to deliberately operate synthetic reaction circuits in "clean" or "noisy" regimes.

## SEQUENCE DESIGN FOR A TEST TUBE OF INTERACTING NUCLEIC ACID STRANDS




The programmable chemistry of nucleic acid base-pairing serves as a versatile medium for the rational design of self-assembling molecular structures, devices, and systems. Over the last three decades, analysis algorithms have been developed to enable calculation of the equilibrium base-pairing properties of a test tube of interacting nucleic acid strands, yielding predictions for the equilibrium concentration and base-pairing probabilities for an arbitrary number of complex species that form from an arbitrary number of strand species. However, no analogous sequence design algorithm exists for engineering the equilibrium base-pairing properties of a test tube of interacting nucleic acid strands. Here, Wolfe and Pierce (DOI: 10.1021/sb5002196) formulate nucleic acid sequence design in the context of a test tube of interacting nucleic acid strands at equilibrium.

The design objective function and optimization algorithm described here represent the state-of-the-art in nucleic acid sequence design, providing a crucial tool for nucleic acid engineers working in diverse settings. The algorithm serves as a key part of the sequence design engine for NUPACK (www.nupack.org), which is widely used to engineer synthetic nucleic acid molecules, devices, and systems for use *in vitro*, *in situ*, and *in vivo*.



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