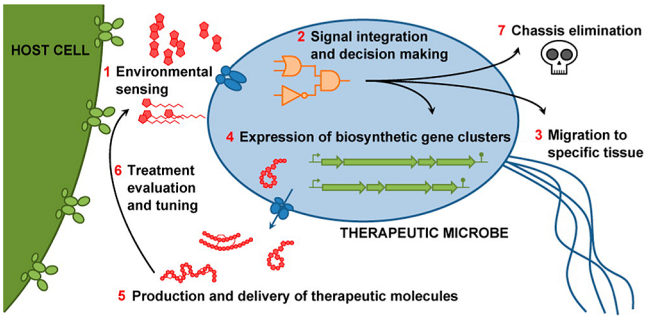
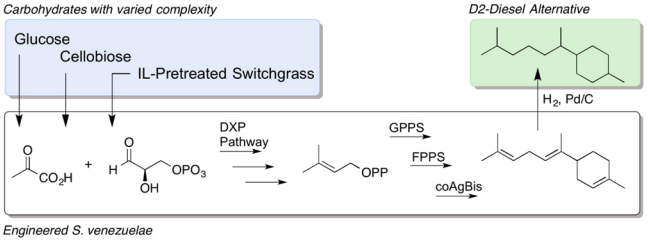
SYNTHETIC MICROBES AS DRUG DELIVERY SYSTEMS



Synthetic biology involves the design and construction of biological circuits for use in a variety of applications. A subfield of synthetic biology is synthetic cell therapy, which has broad potential for applications in the treatment of human disease. *In vivo* synthesis and delivery of therapeutic agents has several advantages over systemic treatment, including less invasive routes of administration and reduced dosage requirements.

In this review, Claesen and Fischbach (DOI: 10.1021/sb500258b) envision that the next generation of bacterial cell therapy systems will be able to diagnose human disease, make decisions on, and execute appropriate treatment, ultimately self-eliminating from the human host when the condition is alleviated. They highlight examples of modules that are currently employed in each of these functions, in a synthetic system, and speculate about future directions for their implementation.

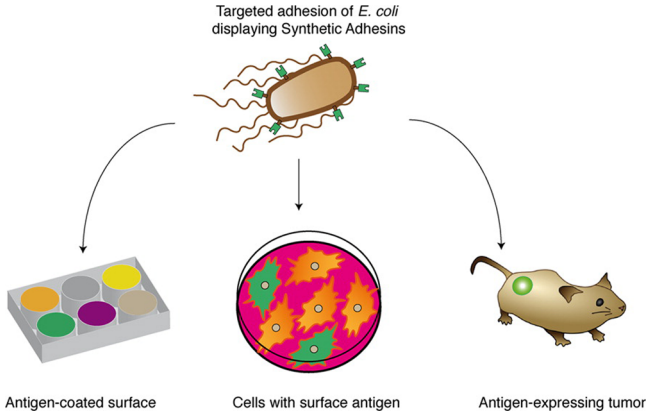
ENGINEERING TERPENE BIOSYNTHESIS IN *STREPTOMYCES* FOR PRODUCTION OF BISABOLENE



As geopolitical and sustainability issues place increasing hardships on fossil fuel use, the generation of alternate, environmentally friendly energy sources is of increasing importance. One possible solution is the biosynthetic production of next generation fuels. While large advances have been made toward high-titer, scalable, and cost-competitive production of petroleum alternatives, efforts have been largely restricted to improving production in common hosts such as *Escherichia coli* and *Saccharomyces cerevisiae*. Here, Phelan *et al.* (DOI: 10.1021/sb5002517) make an effort to expand the range of known hosts for fuel production, exemplifying the many benefits *Streptomyces* offers as a production host.

The authors mapped the biosynthetic path responsible for terpene production in a model actinobacterium, *Streptomyces venezuelae*, and altered secondary metabolism to produce the advanced biofuel precursor bisabolene. Information gained from these biosynthetic inquiries enabled the authors to boost titers well above those realized in the first-generation strain. Finally, the authors assessed the ability of *Streptomyces venezuelae* to catabolize glucose, cellobiose, and ionic-liquid pretreated switchgrass, defined the host's native capacity for cellulose catabolism, and established it as a microbe capable of consolidated bioprocessing.

PROGRAMMING CONTROLLED ADHESION OF *E. COLI*



In the effort toward rational design of micro-organisms for use in biotechnological and clinical applications, the ability to program the adhesion properties of engineered micro-organisms is crucial. Here, Piñero-Lambea *et al.* (DOI: 10.1021/sb500252a) show, for the first time, that the adhesion capacities of *E. coli* can be finely tuned against distinct target surfaces and cells using synthetic adhesins (SAs) of different specificities.

The authors demonstrate that SAs can be constitutively and stably expressed in *E. coli*, and can direct robust, fast, and specific adhesion of the engineered bacteria to target antigenic surfaces and cells. The authors also demonstrate the functionality of SAs *in vivo*: efficient colonization of solid tumors expressing the target antigen was seen with engineered *E. coli* at a dose two-orders of magnitude lower than that required for tumor colonization with the wild type unmodified *E. coli*. Thus, SAs could increase the specificity of engineered bacteria for use in specific *in vivo* applications.

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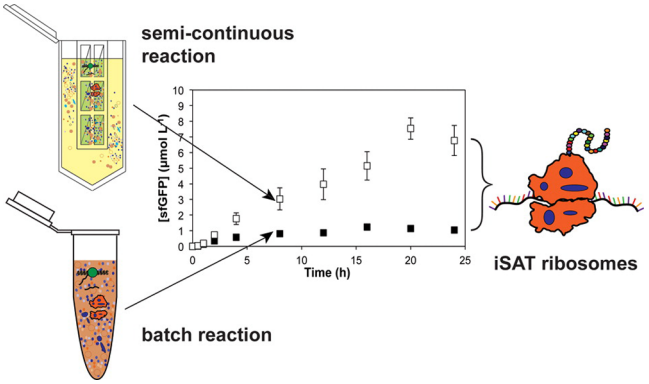
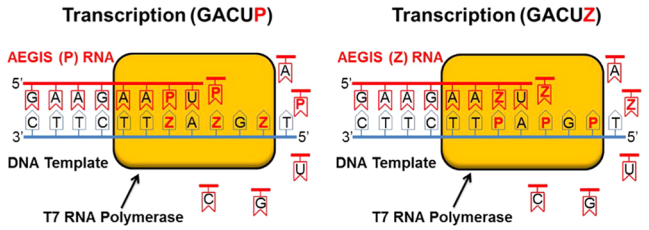
CHARACTERIZING AND ALLEVIATING SUBSTRATE LIMITATIONS FOR IMPROVED *IN VITRO* RIBOSOME CONSTRUCTION



In vitro ribosome construction could enable studies of ribosome assembly and function, provide a route toward constructing minimal cells, and permit the construction of ribosomal variants with new functions. Despite tremendous interest in making ribosomes *in vitro*, current construction efforts are limited. Here, Liu *et al.* (DOI: 10.1021/sb5002467) address a key limitation: namely, energy source depletion.

The authors previously reported on an integrated, one-pot rRNA synthesis, ribosome assembly, and translation technology (termed iSAT) for the *in vitro* construction of *E. coli* ribosomes. In the current study, they explored the causes of reaction termination in iSAT to improve efficiency and yield. They report that phosphoenolpyruvate (PEP), the secondary energy substrate, and nucleoside triphosphates (NTPs) were rapidly degraded during iSAT reactions, causing a significant drop in the reaction's energy charge, leading to eventual termination of protein synthesis. Adoption of a semi-continuous method, where passive diffusion enables substrate replenishment and byproduct removal, prolonged iSAT reactions and increased protein yield by 7-fold. The innovations reported have the potential to inspire larger ribosome construction and engineering efforts to push the limits of engineered biological systems.

ANALYSIS OF RNA CONTAINING ARTIFICIAL GENETIC COMPONENTS



One aspect of synthetic biology deals with the assembling of unnatural parts to create systems that reproduce natural behaviors of biology, including coding, replication and evolution. In this study, Leal *et al.* (DOI: 10.1021/sb500268n) describe the use of expanded DNA to encode RNA, and *vice versa*, to reproduce elements of "the central dogma" in natural biology, but with unnatural systems.

Before expanded genetic sets, such as the six letter set used here, can be implemented into living systems, it must first be determined whether these unnatural oligonucleotides can function in harmony with established enzymatic and biochemical tools. The authors have examined the transcription and reverse transcription of Artificially Expanded Genetic Information Systems (AEGIS) using their non-natural base pairs (Z and P). They tested specific incorporation of these non-natural base nucleotides into RNA and DNA by gel electrophoresis, mass spectrometry of the products, and HPLC and gel analyses of digested products. Results obtained demonstrated the enzymatic incorporation of the non-natural base nucleotides by pairing with their appropriate complementary pair on a template strand. The work described here is a step toward the biosynthesis of proteins containing additional amino acids.

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