ACS SyntheticBiology

In This Issue

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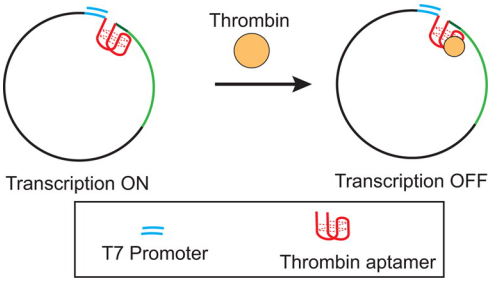
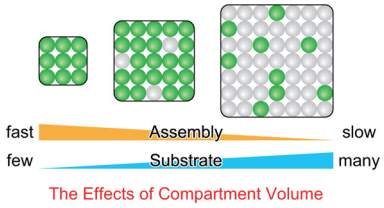
THROMBIN-MEDIATED TRANSCRIPTIONAL REGULATION



Harnessing biological functions for practical applications requires the engineering of biological systems and their components. Although living systems can be adapted for new purposes, cell-free systems offer greater design flexibility by allowing the use of non-native molecules and conditions. Realizing the potential of cell-free systems requires the development of ligand-sensitive gene promoters that control gene expression in response to a ligand of interest. However, only a limited number of gene promoters have been employed for cell-free transcription, and the design of ligand-sensitive promoters is far from routine. Here, Iyer and Doktycz (DOI: 10.1021/sb4000756) describe an approach to designing ligand-sensitive transcriptional control in cell-free systems that is based on the combination of a DNA aptamer and the T7 viral promoter.

The ability for ligand binding to repress transcription was evaluated after placing the aptamer in different structural contexts, double or single stranded, and locations relative to the T7 polymerase binding site. Placement of the aptamer near the T7 polymerase binding site, and using a primarily single stranded template, results in up to a 5-fold change in gene expression in a ligand concentration dependent manner. Since the molecular recognition properties of aptamers can be engineered to bind various molecular targets, these results pave the way for modular regulation of transcription to a wide variety of ligands.

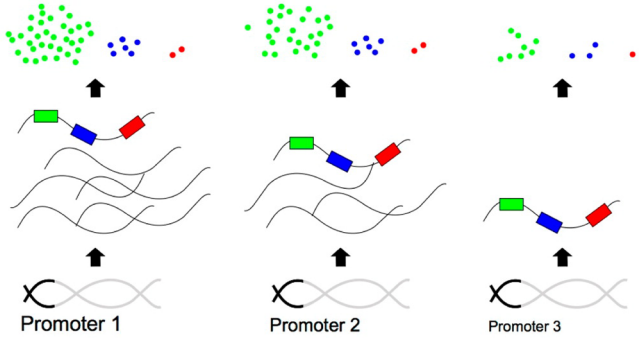
AN OPTIMUM COMPARTMENT VOLUME FOR HIGH-ORDER REACTIONS



The application of microelectromechanical systems to chemistry and biochemistry allows various reactions to be performed in microscale compartments. Here, Okano *et al.* (DOI: 10.1021/sb400087e) use the glass microchamber to study the role of compartment size on protein synthesis, one of the most important reactions in the cell.

By encapsulating the cell-free protein synthesis system with different reaction orders in femtoliter microchambers, chamber size dependency of reactions initiated with a constant copy number of DNA was investigated. The authors observed properties specific to the high order reactions in microcompartments with high precision, and report the presence of an optimum compartment volume for a high-order reaction using real biological molecules.

CONTROL OF CELL-FREE OPERON EXPRESSION

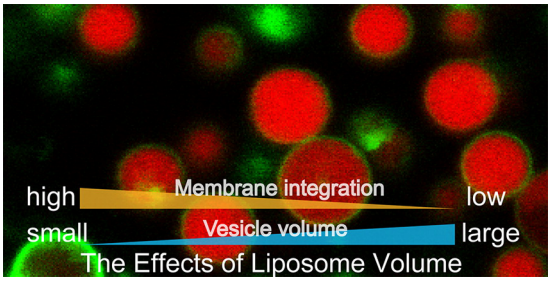


Despite the increasing popularity of the cell-free methodology in synthetic biology, almost no effort has been expended in deciphering the rules needed for assembling genetic devices that function *in vitro*; protein levels are usually modulated simply by titrating a DNA template. In this study, Chizzolini *et al.* (DOI: 10.1021/sb4000977) sought to better understand how RNA and protein synthesis proceed *in vitro* so that synthetic genomes, which control the activity of artificial cells built from scratch, can be assembled in the future.

The authors characterize and exploit a series of transcriptional promoters to control both RNA and protein levels *in vitro*. RNA levels were quantified through the real-time detection of Spinach fluorescence, whereas protein levels were determined through the use of different fluorescent proteins.

While the authors were able to regulate protein expression, the desired effect was found to taper off as the distance between the transcriptional promoter and the gene increased. A strong metabolic load effect was not observed; ribosome instability was the limiting factor in protein yield.

DEPENDENCE OF MEMBRANE PROTEIN INTEGRATION ON VESICLE VOLUME



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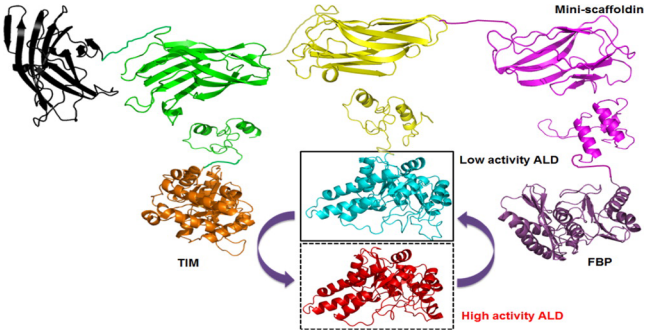
Many studies have shown that membrane protein can be synthesized and integrated in the vesicle membrane using *in vitro* transcription−translation systems. While studies until date have described protein synthesis on the outside of the vesicle, synthesis inside the vesicle has rarely been investigated. Here, Soga *et al.* (DOI: 10.1021/sb400094c), using a reconstituted *in vitro* transcription−translation system called PURE, show that the bacterial transporter EmrE can be synthesized in its functional form inside cell-sized vesicles.

By analyzing the relationship between membrane integration and vesicle volume using flow cytometry, the authors found that the fraction of EmrE integrated into the membrane increased with decreasing vesicle volume; this finding was explained quantitatively by the effect of the increased surface-area-to-volume ratio in smaller vesicles. These results indicate that the experimental setup described can be used to study the effect of negative curvature on protein localization *in vitro*. This allows for the development of a method for *in vitro* evolution of membrane proteins, which, in turn, has applications in the field of membrane protein engineering.

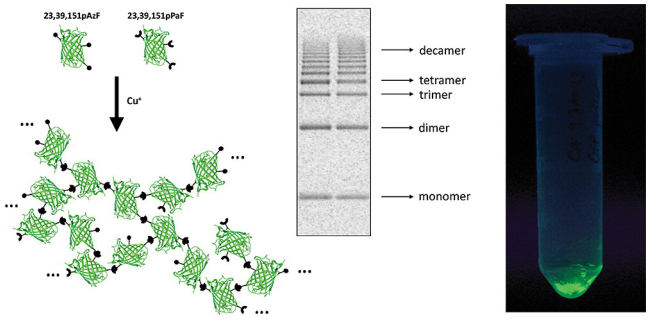
ANNEXATION OF A HIGH-ACTIVITY ENZYME IN A SYNTHETIC THREE-ENZYME COMPLEX



Synthetic enzyme complexes, containing multiple cascade enzymes, are a powerful tool in synthetic biology research. These complexes can be constructed by gene fusion, coimmobilization, coentrapment and scaffold-mediated assembly. Here, You and Zhang (DOI: 10.1021/sb4000993) study the self-assembled three-enzyme complex containing triose-phosphate isomerase (TIM), aldolase (ALD), and fructose 1,6-biphosphatase (FBP).

To investigate the role of the rate-limiting enzyme (ALD) in this complex, the authors replaced low-activity ALD from *Thermotoga maritima* with a similar-size ALD from *Thermus thermophilus* with over 5 times greater specific activity. Their results suggest that the degree of substrate channeling in synthetic enzyme complexes depend on enzyme choice. This study implies that the construction of synthetic enzymes in synthetic cascade pathways could be a very important tool to accelerate rate-limiting steps controlled by low-activity enzymes.

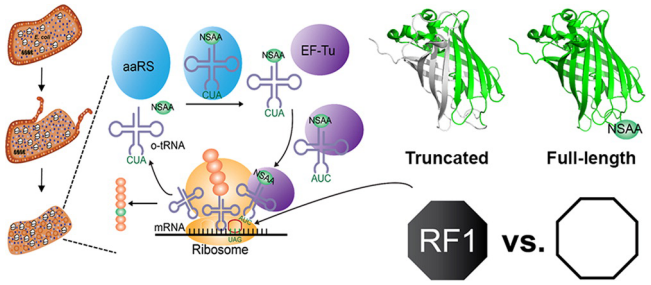
DIRECT POLYMERIZATION OF PROTEINS



Existing bioconjugation methods using natural amino acids lack precision due to the presence of multiple surface-exposed copies of modifiable amino acids, such as lysines and cysteines, on most proteins. Covalent coupling of proteins *via* these residues could result in an undesirable, heterogeneous mixture of products as well as in the elimination of the biological activity of the conjugated protein. This problem can be circumvented by the site-specific incorporation of non-natural amino acids (nnAAs), which introduces unique chemical handles at specific positions on the protein to be conjugated. In this paper, Albayrak and Swartz (DOI: 10.1021/sb400116x) demonstrate the synthesis of linear and branched protein polymers, in which each monomer is a folded and active protein.

Superfolder green fluorescent proteins (sfGFP) containing multiple (up to six) copies of nnAAs were synthesized *in vitro*, using a previously published method, and were then covalently coupled by Click chemistry to form sfGFP polymers in one step. The proteins were seen to maintain most of their specific fluorescence activity after coupling. Since nnAA incorporation sites can be determined precisely and inserted into any protein by standard genetic manipulation, the method described here can be used to synthesize polymers of virtually any protein or mixture of proteins, or polymers comprising both proteins and small molecules.

CELL-FREE PROTEIN SYNTHESIS FROM A RELEASE FACTOR 1 DEFICIENT *E. COLI*



In recent years, cell-free protein synthesis has emerged to help satisfy a growing demand for simple, affordable, and efficient protein expression technologies. With bacterial protein synthesis yields now exceeding 1 g/L for simple proteins, new applications have been inspired. One such application is the incorporation of nonstandard amino acids (nsAAs) into proteins, which enables the creation of biopolymers and proteins with new chemical properties, structures, and functions. To achieve this, amber (TAG codon) suppression has been widely applied. However, the suppression efficiency is limited because of the competition with translation termination by release factor 1 (RF1), which leads to truncated products.

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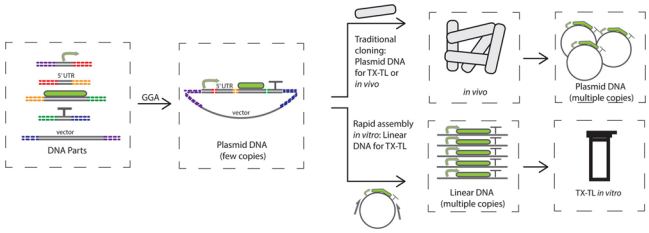
Here, Hong *et al.* (DOI: 10.1021/sb400140t) describe the development and characterization of a novel *Escherichia coli* based platform for cell-free protein synthesis from a genomically recoded organism lacking RF1.

As compared to the parent *E. coli* strain with RF1, the authors demonstrated a protein synthesis improvement of over 250%. They also incorporated multiple identical nsAAs at levels not previously possible. The results described here hold promise to impact efforts to produce protein therapeutics, to make novel materials, and to synthesize large libraries of proteins in high-throughput for protein evolution, functional genomics, and structural studies.

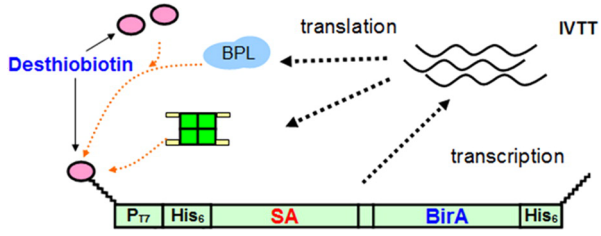
LINEAR DNA FOR RAPID PROTOTYPING OF SYNTHETIC BIOLOGICAL CIRCUITS



In conventional engineering, each part of an instrument to be designed is assembled and tested with multiple cycles of prototyping in a simplified environment. However, for synthetic genetic circuits the norm has been to assemble and test purely in the end environment, which takes several days to weeks. In this paper, Sun *et al.* (DOI: 10.1021/sb400131a) describe a way to rapidly test or prototype genetic circuits in a simplified *in vitro* environment, the biological breadboard, to serve as an alternative to testing *in vivo*.

To speed up iteration time, the authors used linear DNA instead of plasmid DNA. To do so, they first characterized methods to protect linear DNA strands from exonuclease degradation in an *E. coli* based transcription–translation cell-free system. Using this rapid prototyping approach the authors reduced prototyping cycles to 4–8 h, which is relatively invariant to circuit complexity.

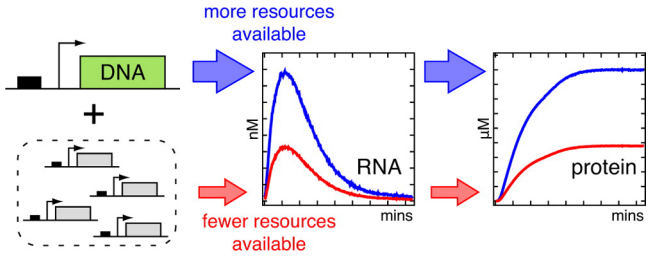
DESIGN AND SELECTION OF A SYNTHETIC OPERON



Cell-free systems are being used increasingly for the synthesis of both proteins and small molecules. While *in vitro* transcription and translation reactions until date have been primarily used for the production of single proteins, Lu and Ellington (DOI: 10.1021/sb400160m) now demonstrate the possibility of coupled reactions.

The authors show that synthetic operons composed of wild-type or evolved biotin ligases and streptavidins could function following *in vitro* transcription and translation. A biotin acceptor peptide was conjugated to the DNA and could be biotinylated by a translated biotin ligase and then captured by a translated streptavidin. Biotin ligases with different substrate specificities could be discriminated *via* emulsion-based selection. These experiments open the way to the *in vitro* co-optimization of parts for genetic circuits.

GENE CIRCUIT PERFORMANCE CHARACTERIZATION AND RESOURCE USAGE



The field of synthetic biology involves the assembly of biological parts into modestly complex circuits with wide-ranging applications. However, designed circuits often fail to perform as expected. This is largely due to the lack of the prototyping stage prevalent in all other engineering disciplines. In this work, Siegal-Gaskins, Tuza, Kim *et al.* (DOI: 10.1021/sb400203p) carry out an in-depth characterization of their recently developed cell extract-based testing environment for the purpose of improving the synthetic circuit prototyping pipeline.

The authors developed an *in vitro* "breadboard" prototyping platform based on *E. coli* cell extract that allows biocircuits to operate in an environment considerably simpler than, but functionally similar to, *in vivo*. Here, they use real-time and simultaneous measurements of transcriptional and translational activities of a small set of reporter genes and a transcriptional activation cascade to characterize this breadboard. It is the simplicity of this cell-free breadboard that makes it a promising tool for probing fundamental aspects of gene circuit operation that are normally masked by cellular complexity.

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