Protein synthesis machinery from bacterial consortia in one shot

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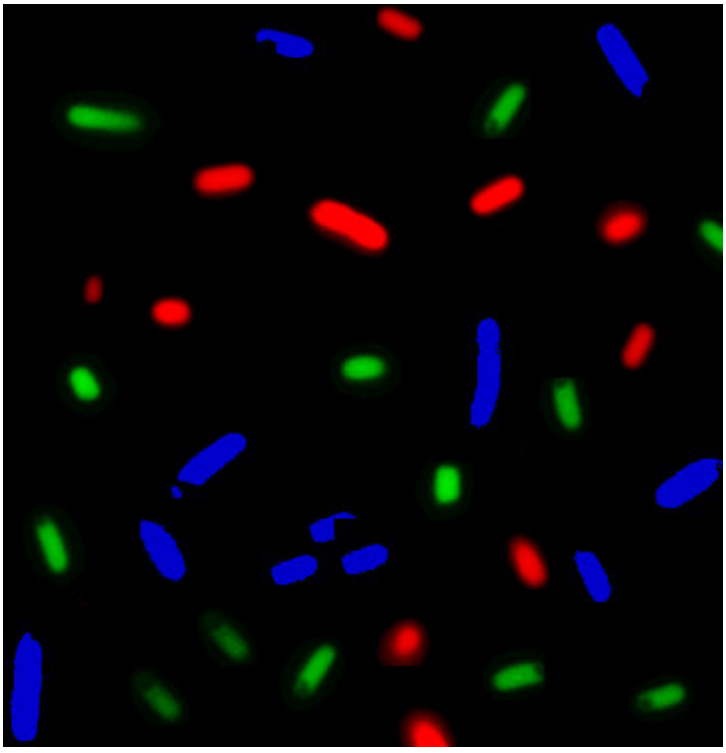

These *E. coli* bacteria tagged with different colors produced different mixtures of proteins. Together, the bacterial consortium makes all the proteins needed for mRNA translation/protein synthesis. The new method developed at UC Davis could speed development of cell-free biological systems. Credit: Fernando Villarreal, UC Davis

A new technique developed at UC Davis may have broken the barrier to rapid assembly of pure protein synthesis machinery outside of living

cells.

In order to reconstitute cellular reactions outside of biological systems, scientists need to produce the proteins involved. Rapid yet high purity reconstitution of the cellular reactions is critical for the high-throughput study of cellular pathways and cell-free diagnostic tests for various diseases. Reconstituting cellular reactions outside cells, however, requires the separate expression and purification of each protein required to execute the reactions. This process is expensive and time consuming, making the production of more than several proteins at once extremely challenging.

In a paper published in *Nature Chemical Biology*, Fernando Villarreal and colleagues in Professor Cheemeng Tan's lab in the Department of Biomedical Engineering at UC Davis describe the production in a single culture of all 34 proteins required for mRNA translation—the process of synthesizing protein from genetic code—in the correct proportions.

Currently, proteins are extracted from whole cells and used directly for in vitro translation. Proteins extracted by this method can contain cytoplasm and other elements of the original cell, and are undesirable for some applications. Another method involves purifying each of the 34 proteins separately and blending them to approximate the mixture, or "machinery", required to start the mRNA translation.

The Tan lab circumvented these limitations by synthetically engineering strains of Escherichia coli bacteria to produce the required proteins of correct quantity within a single mixed culture. By manipulating transcription rates, translation rates and relative strain densities, the group found that they could induce the bacterial consortia to produce correct quantities of the translation machinery.

"I believe the work will open doors to fundamental improvement in the

protein yield of pure cell-free transcription-translation systems and throughput of studying disease-relevant pathways outside of living cells," Tan said.

The team call their method TraMOS, for Translation Machinery One Shot. They used the proteins produced by TraMOS in a test that screens for the presence of peptides that inhibit a protease. Because proteases are commonly involved in the life cycle of parasites and cancer development, a test that could locate and identify many of the protease inhibitors all at once will be useful for drug development.

By reducing the time and cost associated with preparing multiprotein systems, the Tan lab's approach enables high-throughput applications of TraMOS without having to invest in additional purification equipment. Unlike existing approaches, scientists can customize the expression and control of proteins using the TraMOS approach. Most labs that routinely perform protein purification already have the equipment to use the TraMOS approach, making it easy to implement, and democratizing access to the system. The microbial consortia-based approach may be generalized for the synthesis of other multi-protein systems, making it a potential game changer for high-throughput cell-free applications.

More information: Fernando Villarreal et al, Synthetic microbial consortia enable rapid assembly of pure translation machinery, *Nature Chemical Biology* (2017). DOI: 10.1038/nchembio.2514

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