SYNTHETIC BIOLOGY

Protein circuit engineering

Gao, X. J. et al. *Science* **361**, 1252–1258 (2018).

Bestowing new functions on cells is a key goal of synthetic biology. Researchers most commonly seek to engineer new circuits at the transcriptional level, for example, by expressing transcription factors; cells, in contrast, often regulate their pathways at the protein level. Gao et al. decided to follow nature's lead and designed CHOMP (circuits of hacked orthogonal modular proteases), which uses viral proteases for circuit design. They first showed that three different viral proteases can be used to increase or decrease the expression of reporter genes, by cleaving a degradation signal and by exposing a destabilizing domain after cleavage, respectively. Then they used these principles to design two-input logic OR, AND and NOR gates, analog filter processing and regulatory cascades. They engineered a circuit that responds to upstream activators of Ras by activating a protease that in turn switches on caspase-3 and thus induces death in cells with elevated Ras levels, a hallmark of many cancer cells. *NR*

https://doi.org/10.1038/s41592-018-0201-1

MICROBIOLOGY

Mechanistic microbiota models

Medlock, G. L. et al. *Cell Sys.* **7**, 245–257 (2018).

Gut microbes have a profound effect on human metabolism, but the complexity of interactions among microbial species and metabolites makes it extremely challenging to extract mechanistic insights. Medlock et al. took a synthetic approach to the problem, by culturing six bacterial strains from the well-characterized altered Schaedler flora (ASF). The researchers first measured growth rates and profiled the metabolomes of ASF monocultures and co-cultured strain pairs. They then fed these data into their constant yield expectation (ConYE) analytical model, which identifies potential metabolic interactions that contribute to growth modulation in co-culture. They validated one predicted cross-feeding interaction between *Clostridium* and *Parabacteroides* strains, based on Stickland fermentation of amino acids, with in vitro supplementation experiments. *TN*

https://doi.org/10.1038/s41592-018-0202-0

SENSORS AND PROBES

Blinking in the cold

Dahlberg, P. D. et al. *J. Am. Chem. Soc.* **140**, 12310–12313 (2018).

The resolution that can be achieved in single-molecule localization microscopy measurements is dependent on the number of photons emitted by individual fluorophores. Dahlberg et al. show that carrying out localization microscopy at cryogenic temperatures can improve the localization precision by a factor of four, which they find is largely due to increased photon counts from reduced photobleaching. To enable these studies, they first had to identify a probe that was suitable for localization microscopy at cryogenic temperatures. They screened several fluorescent proteins and found that the photoactivatable fluorescent protein PAmKate could be activated at temperatures as low as 77 K. Using this probe, the researchers carried out cryogenic super-resolution microscopy to study the localization of a bacterial protein to microdomains at the cell poles in *Caulobacter crescentus*. This finding should help enable super-resolution correlated light and electron microscopy. *RS*

https://doi.org/10.1038/s41592-018-0203-z

SEQUENCING

Improving long-read accuracy

Volden, R. et al. *Proc. Natl. Acad. Sci. USA* **115**, 9726–9731 (2018).

Long-read sequencing technology offers an opportunity to decipher full-length RNA transcript isoforms. However, long-read sequencers typically suffer from high error rates, low throughput and high cost. To improve read accuracy, Volden et al. harnessed rolling-circle amplification to generate concatemeric cDNAs for sequencing by a nanopore sequencer. They split the raw reads into subreads and applied custom algorithms to obtain full-length cDNA consensus reads. The principle is similar to the circular consensus sequencing applied by a PacBio sequencer. The researchers termed this method Rolling Circle Amplification to Concatemeric Consensus (R2C2) and demonstrated that it can generate more accurate reads of RNA transcript isoforms in bulk or single-cell samples. They applied R2C2 to characterize the transcriptomes of single B cells and identified multiple isoforms of the CD19 receptor, a target for CAR-T cell therapy. Although R2C2 still cannot achieve both high throughput and high accuracy, it provides a way to analyze full-length transcriptomes at a reasonable cost. *LT*

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