METHODS IN BRIEF

SEQUENCING

Dark matter translation

Evidence is accumulating that most of the mammalian genome is not only transcribed but more transcripts than anticipated are actively translated. Ingolia *et al*. recently adapted established methods to study the dynamics and complexity of mammalian proteomes. By deep-sequencing RNA fragments protected by ribosomes, they obtained a map of genome-wide protein synthesis; stalling the ribosomes at sites of initiation followed by run-off elongation allowed them to investigate the kinetics of translation. In mouse embryonic stem cells the researchers found many unannotated open reading frames and short translation products in the vicinity of long noncoding RNAs. A catalogue of these translation products is the first step toward exploring their function.

Ingolia, N.T. *et al. Cell* **147**, 789–802 (2011).

MICROSCOPY

Two-color STED gets easier

Super-resolution fluorescence imaging using stimulated emission depletion (STED) microscopy is experiencing increased application to living systems. But because of the need to both stimulate and deplete each fluorophore, extending STED imaging to multiple colors is more complex than it is with conventional fluorescence microscopy. Several methods for two-color STED imaging have been reported, but each has particular drawbacks. Tønnesen *et al*. now show that the use of fluorophores with similar spectral properties—such as YFP and GFP—coupled with conventional spectral unmixing methods, allows STED using a single pair of lasers—as in single-color STED—and leaves plenty of spectrum available for other dyes. They demonstrated the method's capabilities with time-lapse two-color super-resolution imaging of axonal boutons and dendritic spines in living brain slices labeled with YFP and GFP as well as other fluorophore pairs.

Tønnesen, J. *et al. Biophys J*. **101**, 2545–2552 (2011).

LAB ON A CHIP

Sub-attoliter volume mixing

High-throughput screening can benefit from miniaturization, requiring smaller amounts of reagents and allowing more complex assays to be performed. Christensen *et al*. describe a nanofluidics platform for handling and mixing sub-attoliter volumes of reactants, in a reproducible, parallelized manner. They make arrays of small unilamellar vesicles (SUVs) by extruding the SUVs through polycarbonate membrane pores and immobilizing the SUVs on functionalized glass surfaces. By functionalizing 'target' and 'cargo' SUV reactors with lipids of opposite charge, this platform allowed them to mix volumes as small as 1×10^{-19} liters. As a proof-of-principle demonstration, the researchers mixed SUVs containing the enzyme alkaline phosphatase with SUVs containing the substrate fluorescein diphosphate and observed the formation of the fluorescent produce fluorescein.

Christensen, S.M. *et al. Nat. Nanotechnol*. advance online publication (30 October 2011).

SYNTHETIC BIOLOGY

Using bacteria to generate ubiquitylated proteins

Cells use ubiquitylation as an important way to regulate proteins, but degradation and deubiquitylation make it difficult to isolate the modified isoforms for studies. Keren-Kaplan *et al*. code the entire eukaryotic ubiquitylation apparatus in two plasmids and transport it to *Escherichia coli*, where ubiquitylated proteins are stable. One plasmid contains affinity-tagged ubiquitin and ubiquitin-activating and ubiquitin-conjugating enzymes, and the other includes an affinity-tagged protein of interest and its cognate ubiquitin ligase. The researchers show that the reconstituted system produces proteins with native, stable ubiquitin modifications, and generate several plasmids with ligase and substrate combinations from different organisms. Affinity pulldown using both tags produces milligrams of highly pure ubiquitylated protein for downstream biochemistry or crystallography studies.

Keren-Kaplan, T. *et al. EMBO J*. advance online publication (11 November 2011).