TOOLS IN BRIEF

CHEMICAL BIOLOGY

Chemical decaging in live cells

The ability to 'cage' the activity of a protein in a living cell and then rapidly turn it 'on' by decaging is useful for studying its function. Several photoactivatable caging groups are available, but the wavelength of light needed for decaging can cause unwanted cellular effects, and photodecaging is not suitable for animal or deep tissue experiments. Chemical decaging offers an alternative strategy that may provide advantages in many situations. Li *et al*. report a bioorthogonal, biocompatible approach that utilizes the inverse electron-demand Diels-Alder reaction for chemically decaging a protected lysine, enabling them to turn on enzyme activity in live cells. As demonstrated for firefly luciferase, the decaging reaction is much faster than previously reported chemical decaging methods, comparable in speed to photodecaging methods, and may be extended to amino acids beyond lysine and to *in vivo* settings.

Li, J. *et al. Nat. Chem. Biol.* **10**, 1003–1005 (2014).

MICROSCOPY

A microscope with two arms

To understand the functional relationship between different brain areas, researchers must obtain information on the electrical activity of neurons in these areas at the same time. Lecoq *et al.* developed a two-photon microscope with two arms, which the researchers can independently direct to the areas of interest. These microscope arms harbor miniaturized objectives, and a mirror diverts the light path for one of the objectives to allow even closer juxtaposition of the areas to be analyzed. With this microscope, Lecoq *et al.* performed simultaneous calcium imaging in awake, head-fixed mice in brain regions that were about 1 millimeter apart. They discovered that the activity in the lateromedial visual area depends on the activity of the primary visual cortex.

Lecoq, J. *et al. Nat. Neurosci.* **17**, 1825–1829 (2014).

SENSORS AND PROBES

Finding new RNA mimics of GFP

RNA mimics of GFP, such as Spinach, allow RNAs to be tagged analogously to protein tagging with GFP. These RNA aptamers function by binding and activating fluorescence of an analog of the GFP chromophore. The discovery of such aptamers has proven difficult because not all RNAs that can bind the chromophore can also activate its fluorescence. Filonov *et al.* describe a FACS-based method for discovering bright aptamers. In this approach, RNAs that can bind the chromophore are expressed in *Escherichia coli* and sorted for brightness. The approach yielded Broccoli, which is brighter than Spinach2 in both bacteria and mammalian cells. This method for identifying new aptamers and the addition of Broccoli to the RNA imaging toolbox can potentially improve live-cell imaging of RNAs.

Filonov, G.S. *et al. J. Am. Chem. Soc.* **136**, 16299–16308 (2014).

GENE EXPRESSION

Controlled protein degradation in bacteria

Tuning protein levels post-translationally in bacteria is challenging. Cameron and Collins developed a new strategy for targeting any protein in *Escherichia coli* for degradation using a modified transfer-messenger RNA (tmRNA) system. tmRNAs add a protein-degradation tag ('pdt') to targeted proteins that subjects them to degradation. The *Mesoplasma florum* tmRNA system is unique because the pdt is degraded by a specific protease called Lon but not by cellular proteases in *E. coli*. By tagging genes with this pdt and coexpressing Lon, the researchers were able to tunably degrade target proteins. The team then tagged 238 of the 305 essential genes in *E. coli* to create the Essential Protein Degradation library. They show that modulation of essential protein levels can be used to discover antibiotic targets and to identify new targets for antibiotic development.

Cameron, D.E. & Collins, J.J. *Nat. Biotechnol.* **32**, 1276–1281 (2014).