TOOLS IN BRIEF

NEUROSCIENCE

A rad tool for detecting synapses and protein–protein interactions

The reconstitution of split proteins such as split GFP can be harnessed to detect protein–protein interactions; however, the resulting fluorescence is dim when the interaction occurs in the extracellular space, such as at synapses. Martell *et al.* developed split horseradish peroxidase (sHRP), a reporter that exhibits high sensitivity because of enzyme-mediated signal amplification. sHRP survives formaldehyde fixation, although it may be necessary to supply its cofactor heme when analyzing extracellular protein–protein interactions. Because sHRP, like its parent enzyme HRP, accepts a variety of substrates, its activity can be detected by different means, including electron microscopy and fluorescence microscopy. The researchers applied the sHRP tool to detect synapses in the mouse visual system.

Martell, J.D. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3563 (2016).

SENSORS AND PROBES

Labeling nonprotein biomolecules for CLEM

Correlative light and electron microscopy (CLEM) is a powerful tool for studying the precise organization of structures in cells. However, most labeling strategies for CLEM have focused on labeling proteins of interest, rather than nonprotein biomolecules. Ngo *et al.* addressed this gap by developing the Click-EM strategy for labeling nucleic acids, lipids and glycans. In Click-EM, metabolic analogs containing bio-orthogonal functional groups are incorporated into the target molecule by the cellular machinery. Singlet-oxygen-generating dyes, which can be used for fluorescence imaging, are then added to these analogs via click chemistry. These dyes are also capable of polymerizing diaminobenzidine (DAB) to generate contrast in electron microscopy for correlative imaging. The group used their labeling strategy to target DNA, RNA and lipids in mammalian cells and to label peptidoglycans in Gram-positive bacteria.

Ngo, J.T. *et al. Nat. Chem. Biol.* **12**, 459–465 (2016).

SENSORS AND PROBES

A bright orange fluorescent protein for enhanced *in vivo* imaging

Orange fluorescent proteins (OFPs) are valuable tools for multicolor imaging and also perform well in *in vivo* imaging as a result of their red-shifted emission spectra. Chu *et al.* developed CyOFP1 for use in diverse biological imaging applications. CyOFP1 has a long Stokes shift, meaning that there is a relatively wide spectral gap between its excitation and emission maxima. This allows CyOFP1 to be excited with cyan light. One benefit of this cyan excitation is that one wavelength can be used to excite both GFP and CyOFP1, enabling experiments such as simultaneous two-photon imaging of green calcium sensors and CyOFP1 in living mouse brains. The team also showed that CyOFP1 can be fused to nanoluciferase to create a bright probe for *in vivo* bioluminescence imaging.

Chu, J. *et al. Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3550 (2016).

CELL BIOLOGY

Painting and sorting cells of interest

For cells with distinctive behaviors or morphologies to be singled out from a population, they need to be individually marked before downstream analyses are carried out. Two related methods have been developed to achieve single-cell selection. Binan *et al.* incubate cell cultures with a biotin-conjugated fluorophore that releases reactive oxygen species upon targeted illumination with a laser beam and results in biotin cross-linking to the cell. Kuo *et al.* label cells with photoswitchable semiconducting polymer dots (Pdots) via antibodies directed to cell surface markers. Individual cells can then be selected on the basis of Pdot fluorescence, switched on with a laser beam. Neither approach interferes with cell viability. They both result in stable labeling, which the research teams harnessed to sort the labeled cells and to analyze their transcriptional profile or their genome.

Binan, L. *et al. Nat. Commun.* **7**, 11636 (2016).
Kuo, C.-T. *et al. Nat. Commun.* **7**, 11468 (2016).