METHODS IN BRIEF

NEUROSCIENCE

Two-photon properties of pharmacogenetic tools

Photoswitchable tethered ligands (PTLs) in conjunction with their engineered receptors can activate neurons in response to optical stimulation. Typically, these PTLs are switched by UV or blue light, which is not efficient in highly scattering tissue such as the brain. Carroll *et al.* characterized the two-photon properties of the glutamate analogs L-MAG0 and L-MAG0$_{460}$ in solution and when bound to their cognate receptor LiGluR. L-MAG0 deactivates upon illumination with near-infrared light, whereas L-MAG0$_{460}$ can be efficiently and quickly activated under the same conditions. Using two-photon digital holography to switch L-MAG0$_{460}$, the researchers could achieve currents almost as high as with one-photon illumination in cultured hippocampal neurons expressing LiGluR. Furthermore, two-photon activation of these tools is compatible with calcium imaging using red calcium sensors such as R-GECO1.0.

Carroll, E.C. *et al. Proc. Natl. Acad. Sci. USA.* **112**, E776–E785 (2015).

EPIGENETICS

Single-cell bisulfite sequencing for populations

Treating DNA with bisulfite before sequencing enables the detection of methylated cytosine residues across the genome. The method is powerful but also damages DNA, making its application to small samples very challenging. Farlik *et al.* ligate sequencing adaptors after chemical treatment for whole-genome bisulfite sequencing of small samples (μWGBS) and single cells (scWGBS). The researchers gear the method toward profiling large numbers of cells at low sequencing coverage and include a computational tool for extracting epigenetic states from sparse methylation data. They studied *in vitro* differentiation of human blood and mouse stem cells, as well as the effect of the drug azacytidine on an erythroleukemia-derived cell line, and showed that their bioinformatic approach can gain statistical power by combining signal from multiple genomic regions such as regulatory elements.

Farlik, M. *et al. Cell Rep.* **10**, 1386–1397 (2015).

STRUCTURAL BIOLOGY

Electron crystallography reveals amino acid charges

Electron crystallography of three-dimensional protein crystals is an emerging technique in structural biology. Electron crystallography may be useful for studying weakly diffracting or thin crystals, as electrons are scattered 4–5 orders of magnitude more strongly than X-rays. Yonekura *et al.* describe a method for electron crystallographic analysis of ultrathin protein crystals. They developed a new diffractometer for data collection on thin protein crystals as well as a set of programs for data analysis. Using this method, they solved the atomic structures of a Ca^{2+}-dependent ATPase and catalase. Because electron crystallography yields Coulomb potential maps rather than electron density maps, the charged states of amino acids in the Ca^{2+}-dependent ATPase active site and the iron atom in catalase could be determined, yielding new insights into these proteins.

Yonekura, K. *et al. Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.1500724112 (17 February 2015).

PROTEOMICS

Cell-specific proteomics in the worm

Proteomics information is most useful when it comes from specific cell types. In small organisms such as the worm, it is hard to isolate cell types by sorting or microdissection methods. As an alternative, Yuet *et al*. present a method to tag proteins from specific cell types in *Caenorhabditis elegans*, allowing the proteins to be specifically enriched for proteomic analysis. The researchers express an engineered phenylalanyl-tRNA synthetase in a cell type of interest, permitting proteins in those cells to be labeled with the unnatural phenylalanine analog *p*-azido-L-Phe (Azf) by feeding bacteria labeled with Azf to the worms. This allows tagged proteins to be visualized through conjugation to probes that label the bioorthogonal Azf or to be enriched and subjected to quantitative mass spectrometry profiling. The approach could be used to build a cell-specific proteomic atlas of the worm and could potentially be adapted for other organisms.

Yuet, K.P. *et al. Proc. Natl. Acad. Sci. USA*. **112**, 2705–2710 (2015).