**Outrunning radiation damage**

The very bright X-ray beams at synchrotron facilities have enabled high-resolution crystal structure determination for scores of proteins. However, X-rays generate reactive species that cause radiation damage to protein crystals, even when held at cryogenic temperatures. This has prevented researchers from resolving key structural details for many especially radiation-sensitive proteins. Ago, Yoshikawa and colleagues report an approach that allowed them to obtain a radiation damage–free structure for the large membrane metalloprotein bovine cytochrome c oxidase (CcO). The crystal structure of this protein has been previously solved using synchrotron radiation, but the arrangement of the peroxide ligand at the O₂-reduction site could not be observed. The authors resolved the ligand structure details at high resolution by utilizing large CcO crystals and taking advantage of the ultrafast, femtosecond pulses of an X-ray free-electron laser to outrun the onset of radiation damage.

*Brief Communication p734*

**Whole-animal imaging of neural activity**

Methods for fast, non-invasive and three-dimensional imaging of neural activity are of much interest to understand brain and nervous system function. Vaziri, Boyden and colleagues extend light-field microscopy (LFM), a previously reported method, to imaging neural activity *in vivo*. They combined the fast signal-acquisition capabilities of LFM with three-dimensional deconvolution to achieve high-speed imaging of calcium signals in neurons in whole *Caenorhabditis elegans*, as well as in entire larval *Danio rerio* brains, at single-cell resolution.

*Brief Communication p727*

**CRISPR-based repression**

Repression devices, defined as regulators that implement an inhibitory relationship between input and output, can be used to build any computational circuit. Weiss and colleagues focus on the clustered, regularly interspaced, short palindromic repeats (CRISPR) system to enlarge the toolbox of biological repression devices: small guide RNAs (gRNA) directed to a promoter and coupled to a catalytically inactive Cas9 nuclease block transcription. The authors express gRNAs from both polymerase II and III promoters and show multilevel control by directing one gRNA-Cas9 complex to regulate the expression of another gRNA, which in turn regulates the output of the circuit. After characterizing the repression efficiencies of a small library of gRNAs, the authors concluded that a range of regulatory properties can be achieved with these devices.

*Brief Communication p733*

**Western blots from single cells**

Cell-to-cell heterogeneity is important in many biological processes. Although genomic and transcriptomic approaches to detect this heterogeneity are available, measuring the proteomes of single cells presents a much greater challenge. Herr, Schaffer and colleagues describe a western blotting approach for single cells, allowing the multiplex detection of up to 11 proteins per cell for ~2,000 cells in less than four hours. Their platform uses a glass slide coated with a photoactive gel containing an array of 6,720 microwells. Single cells are allowed to settle into the microwells, where they are lysed. The proteins are separated by electrophoresis; they are then immobilized by photo-cross-linking of the gel and, finally, detected with antibodies. The authors used the approach to study targeted protein expression variability during differentiation of rat neural stem cells.

*Article p749*

**Multifeature targeting of specific cells**

In order to study specific cell types *in vivo*, methods are needed to target relevant tools—optogenetic modulators or fluorescent reporters, for example—to the cell or the group of cells of interest. This is challenging to do: specific cell types are typically defined by the intersection of several features such as marker-gene expression or promoter activity, location and, in the case of neurons, connectivity. Deisseroth and colleagues describe an intersectional strategy based on engineered introns and orthogonal recombinases for targeting specific cell types *in vivo*. The system is contained in a single adeno-associated virus vector. Applying this approach, they targeted genetically encoded tools to mouse neuronal populations defined by the intersection of multiple features.

*Article p763*