NEUROSCIENCE

Highlighting active neurons

Moeyaert, B. et al. *Nat. Commun.* **9**, 4440 (2018).

Active neurons have traditionally been identified by their expression of immediate early genes. More recently, an alternative tool called CAMPARI has become available. CAMPARI is a coincidence detector that photoconverts from a green to a red fluorescent protein in the presence of calcium and blue light. Thus, this tool allows specific labeling of neurons that are active at a particular time. Moeyaert et al. have developed CAMPARI2, which exhibits brighter fluorescence in both the green and the red form, as well as faster calcium unbinding kinetics and higher contrast between the two forms. Furthermore, variants with a range of calcium affinities are available, which provides options that can be chosen according to the properties of the cells of interest. The researchers demonstrate the improved performance of CAMPARI2 in vitro, in neuronal cell culture, in brain slices, in larval zebrafish, and in the mouse visual cortex. As a bonus, the researchers have also generated an antibody that specifically binds to the red form of CAMPARI2 and its precursors, facilitating the detection of red CAMPARI in fixed tissue. *NV*

https://doi.org/10.1038/s41592-018-0281-y

PROTEOMICS

Proteomics data reuse with MassIVE-KB

Wang, M. et al. *Cell Syst.* **7**, 412–421 (2018).

Mass-spectrometry-based proteomics research has yielded huge amounts of data made publicly available via several dedicated repositories. Despite such wealth, these data have largely not been leveraged for reuse by the proteomics community. To facilitate this, Wang et al. report the MassIVE Knowledge Base (MassIVE-KB), a tool that continuously aggregates proteomics data as they become available, in an open, reusable format. They distilled spectral libraries from 31 TB of human proteomics data available in the MassIVE repository; statistical controls and provenance records are imposed to ensure that the libraries are of high quality. Such libraries enable researchers to leverage previous discoveries for protein identification and quantification in their own mass spectrometry experiments, using either data-dependent or data-independent acquisition modes. Wang et al. themselves found MassIVE-KB library evidence for 430 'missing' human proteins with scant previous experimental support. *AD*

https://doi.org/10.1038/s41592-018-0283-9

IMAGING

Detecting protein glycosylation

Li, J. et al. *J. Am. Chem. Soc.* **140**, 16589–16595 (2018).

Advances in mass spectrometry have helped scientists detect protein glycosylation in vitro; however, such approaches have practical limitations for in vivo applications. Meanwhile, fluorescent proteins have been used to monitor glycosylation in a protein-specific manner, but this approach lacks sensitivity. To enhance sensitivity, Li et al. use an amplification approach that relies on a proximity-induced hybridization chain reaction (HCR). The approach includes two DNA probes that either attach to glycans or recognize target proteins. The latter consists of an aptamer domain for protein recognition, as well as a spacer domain and initiator domain to trigger HCR. When both probes are bound, HCR occurs, resulting in an amplified fluorescent signal. The researchers apply this method to visualize tyrosine-protein-kinase-specific sialic acid states on CCRF-CEM cell surfaces. They also visualized glycosylation in zebrafish larvae that were injected with CCRF-CEM cells. *LT*

https://doi.org/10.1038/s41592-018-0284-8

NEUROSCIENCE

Two-component optogenetic inhibition

Bernal Sierra, Y. A. et al. *Nat. Commun.* **9**, 4611 (2018).
Alberio, L. et al. *Nat. Methods* **15**, 969–976 (2018).

Channelrhodopsin is a widely used optogenetic activator, but despite the availability of a few inhibitory optogenetic tools, a clear consensus on the most suitable tool remains to be reached. Bernal Sierra et al. report a two-component system for optogenetic inhibition. For their PAC-K tool, the researchers combined the photoactivatable nucleotidyl cyclase bPAC with the cyclic nucleotide-gated potassium channel SthK. These microbial proteins are small and express well in mammalian cells. PAC-K and the recently described BLINK2 channel (Alberio et al.) modulate the potassium gradient across the cell membrane, which provides advantages over other inhibitory tools that change intracellular chloride or proton levels and can cause paradoxical effects. Bernal Sierra et al. found that they could elicit strong outward currents upon expression of the PAC-K tool in cardiomyocytes, and that inhibition depended on illumination intensity. PAC-K also efficiently silenced neurons in the mouse hippocampus and in zebrafish larvae. *NV*

https://doi.org/10.1038/s41592-018-0285-7

Allison Doerr, Rita Strack, Lei Tang and Nina Vogt