METHODS IN BRIEF

IMAGING

Super-resolution imaging of neuronal circuits

Fluorescence microscopy is a valuable tool for volumetric neural circuit reconstruction, but small structures such as synapses can be difficult to identify when imaged at diffraction-limited resolution. To address this issue and image neurons and synapses at high resolution over a large volume, Sigal *et al*. combined stochastic optical reconstruction microscopy (STORM) with serial ultra-thin sectioning. STORM was used to provide super-resolution images of neurons and synaptic proteins in each section, and the images were then aligned to generate 3D volumetric reconstructions using automated image-analysis tools. The authors used their platform to study on-off direction-selective ganglion cells in the mouse retina and were able to gain new insights into the structural basis of crossover inhibition in these cells.

Sigal, Y.M. *et al*. *Cell* **163**, 493–505 (2015).

SYNTHETIC BIOLOGY

Efficient *in vivo* mutagenesis in bacteria

Introducing random mutations into DNA with *in vitro* techniques is easy, but getting this mutagenized DNA back into cells often is not. Performing mutagenesis directly in cells allows researchers to avoid this bottleneck, but current *in vivo* methods show only modest mutation rates. To improve these rates, Badran and Liu created and tested several plasmids that include genes affecting mutation frequency. The final plasmid tested, MP6, encodes dominant negative variants of a DNA-proofreading enzyme, a protein that impairs mismatch repair, a cytidine deaminase that increases base transitions and a transcriptional repressor. *Escherichia coli* carrying MP6 showed a 322,000-fold increase in the mutation rate of their chromosomal DNA compared to wild-type *E. coli*. MP6 enabled the rapid evolution of an *E. coli* strain with resistance to many common antibiotics.

Badran, A.H. and Liu, D.R. *Nat. Commun.* **6**, 8425 (2015).

NEUROSCIENCE

In silico neocortex

Attempts to understand brain function benefit from both experimental and computational approaches. Markram *et al*. report on the progress of the Blue Brain Project, a European initiative that has resulted in reconstruction of a piece of rat neocortex based on morphological characterization of about a thousand neurons distributed among different layers as well as more than 14,000 electrical recordings and other information such as synaptic composition. Although it is considered a draft reconstruction, the *in silico* neocortex can exhibit spontaneous activity and reproduce activity evoked by whisker stimulations. Future drafts of the neocortex simulation will require further refinement based on additional data, but the current implementation of the simulation may nevertheless be useful for testing hypotheses or for analytical purposes.

Markram, H. *et al*. *Cell* **163**, 456–492 (2015).

CHEMICAL BIOLOGY

Optogenetics gives drug screening an assist

Optogenetic tools are being used increasingly for photocontrol of biological systems with high spatiotemporal precision. Inglés-Prieto *et al*. now expand the domain of optogenetics to high-throughput drug screening. Specifically, they study the mitogen-activated protein kinase/extracellular signal–regulated kinase (MAPK/ERK) pathway, which is stimulated by the activation of receptor tyrosine kinases (RTKs). In their screen, they use RTKs fused to light-oxygen-voltage–sensing domains ('opto-RTKs') that are activated by light-dependent dimerization; light thus stimulates the MAPK/ERK pathway activity and downstream reporter expression. Because activation and readout are both optical, fewer steps and no additional activation reagents are necessary, enabling screening with low variability. The authors demonstrate the power of their technique for several opto-RTKs, including 'orphan receptors' for which activating ligands are unknown.

Inglés-Prieto, Á. *et al*. *Nat. Chem. Biol*. doi:10.1038/nchembio.1933 (12 October 2015).