TOOLS IN BRIEF

SENSORS AND PROBES

Brighter and more photostable red and green fluorescent proteins

Fluorescent proteins are widely used in biology. Two very important photophysical properties of these proteins are their brightness and their photostability, which influence the signal-to-noise ratio and duration of imaging experiments. Bajar *et al*. developed improved monomeric fluorescent proteins for enhanced protein labeling and molecular sensing by Förster resonance energy transfer (FRET). They subjected the green fluorescent protein mClover and the red fluorescent protein mRuby2 to mutagenesis and then screened for brightness and photostability. The resulting mClover2 was 60% more photostable than mClover, and mRuby3 was 200% more photostable and 35% brighter than mRuby2, making it the brightest and most photostable monomeric red fluorescent protein. The authors demonstrated that mClover2 and mRuby3 perform well as fusions in mammalian cells and are an efficient FRET pair.

Bajar *et al*. *Sci. Rep*. **6**, 20889 (2016).

CHEMICAL BIOLOGY

A super-splicing split intein

Protein engineers exploit naturally occurring split inteins in order to splice together two polypeptide sequences in a traceless manner, leaving behind a normal peptide bond. Stevens *et al*. used protein design to create an improved split intein, designated Cfa, that has unprecedented fast splicing activity, thermal and chaotropic stability, and high expression yields. To generate Cfa, they first performed a mutational analysis of two DnaE-family split inteins, one fast and one slow, in order to understand which residues were important for fast splicing activity. They then carried out a multiple-sequence alignment of DnaE inteins, filtering for those sequences containing residues important for fast splicing activity. From this result, they derived and generated the consensus sequence, Cfa, which has 2.5-fold faster splicing activity than the parent fast splicing sequence.

Stevens, A.J. *et al. J. Am. Chem. Soc.* **138**, 2162–2165 (2016).

NEUROSCIENCE

A transformative tool for trans-synaptic tracing

Rabies-virus-based tools have become invaluable for analyses of neural connectivity, as they retrogradely label input neurons. However, the commonly used rabies virus strain infects synaptically connected neurons inefficiently and also exerts neurotoxic effects. Reardon *et al*. revisited other laboratory strains of rabies virus and selected a strain called CVS-N2c, which has high neuronal affinity and reduced neurotoxicity. After introducing the same modifications that converted the commonly used strain into a retrograde-labeling tool, the researchers found that the CVS-N2c–based tool was more efficiently transmitted to connected neurons and strongly reduced neurotoxicity, allowing experiments over longer periods of time than possible with the traditional strain. The researchers used the improved retrograde-tracing tool to express fluorescent proteins, channelrhodopsin-2 or the calcium sensor GCaMP6f in neurons upstream of the neurons of interest.

Reardon, T.R. *et al*. *Neuron* **89**, 711–724 (2016).

SENSORS AND PROBES

Improved protein labeling for super-resolution microscopy

Super-resolution microscopy can be used to image cellular structures at nanometer resolution. At such resolution, the use of large tags such as antibodies to label proteins can obscure real structural details, creating a need for better tools for protein labeling. Chamma *et al*. describe an improved strategy for labeling membrane proteins for super-resolution imaging. They genetically tagged a membrane protein of interest with a short tag that was enzymatically biotinylated. This biotin was then bound by purified fluorescently labeled monomeric streptavidin (mSA). mSA is small tag, and the researchers showed that it does not perturb tagged proteins. The team demonstrated that the labeling strategy worked for µPAINT, STED and dSTORM imaging of several proteins, including synaptic adhesion proteins, and that the method works in two-color imaging applications with a GFP nanobody.

Chamma, I. *et al. Nat. Commun.* http://dx.doi.org/10.1038/ncomms10773 (2016).