TOOLS IN BRIEF

SENSORS AND PROBES

Expanding Spinach2's spectral properties

The RNA aptamers Spinach and its recently improved variant Spinach2 (Strack *et al.*, 2013) can be fused to an RNA of interest, allowing RNA tracking experiments to be performed in living cells. Spinach2 lights up RNA by binding the fluorogenic small molecule (*Z*)-4-(3,5-difluoro-4-hydroxybenzylidene)-1,2-dimethyl-1*H*-imidazol-5(4*H*)-one (DFHBI) and activating its fluorescence. However, DFHBI does not have ideal spectral characteristics: most microscopes have filters that are optimized for imaging GFP or fluorescein isothiocyanate. In order to adapt the spectral characteristics of Spinach2 for common microscope filters tuned for GFP or YFP, Song *et al.* present novel DFHBI chemical derivatives that serve as 'plug-and-play' fluorophores for Spinach2, allowing researchers to choose the optimal fluorophore for their experimental needs or swap out fluorophores with ease.

Song, W. *et al. J. Am. Chem. Soc.* **136**, 1198–1201 (2014).
Strack, R.L. *et al. Nat. Methods* **10**, 1219–1224 (2013).

PROTEOMICS

New proteases, greater proteome coverage

Trypsin has long been the workhorse protease for bottom-up proteomics, in which proteins are digested into peptides, the peptides are analyzed by mass spectrometry (MS), and the resulting spectra are identified by database searching. However, many peptides generated by trypsin digestion are of non-optimal length for MS analysis, meaning that important post-translational modifications can be missed. Several alternative proteases, such as LysC, are also in use, but like trypsin, most of these cleave at charged amino acids. Meyer *et al.* now report two new proteases for bottom-up proteomics that cleave at aliphatic side chains: wild-type alpha-lytic protease (WaLP) and an active site mutant, M190A alpha-lytic protease (MaLP). Meyer *et al.* combined proteomics data from separate digestions of the *Saccharomyces pombe* proteome with WaLP, MaLP, trypsin and LysC. This combination boosted sequence coverage of the proteome substantially compared to the coverage obtained with the use of trypsin alone and especially in hydrophobic transmembrane regions.

Meyer, J.G. *et al. Mol. Cell. Proteomics* doi:10.1074/mcp.M113.034710 (14 January 2014).

CHEMICAL BIOLOGY

Optimized optogenetic gene expression

Light can be a powerful tool for precise spatiotemporal control of transcription when used with optogenetic gene expression systems. Although several photosensitive proteins have been engineered into light-controlled transcriptional activators, the systems in current use have notable limitations, including toxicity, poor transcriptional activation, long deactivation times, the need for non-endogenous chromophores and/or the need for multiple protein components. Motta-Mena *et al.* introduce an optogenetic transcription system based on an engineered fusion of VP16, which is a transcriptional activation domain, and EL222, a bacterial protein that binds DNA when activated by blue light. Besides offering reduced toxicity, the new system has rapid activation and deactivation kinetics and a large dynamic range. Motta-Mena *et al.* demonstrated the system for light-controlled gene expression in mammalian cell lines and zebrafish embryos.

Motta-Mena, L.B. *et al. Nat. Chem. Biol.* doi:10.1038/nchembio.1430 (12 January 2014).

IMAGING

Activatable photoacoustic probes

Through the integration of optical excitation and ultrasonic detection, photoacoustic imaging is well placed to overcome some of the limitations of whole-body optical imaging techniques, such as their low spatial resolution and shallow tissue penetration. But although endogenous molecules can generate photoacoustic contrast, the signals are often too small to discern, and exogenous contrast agents must be used. Pu *et al.* report a near-infrared light–absorbing semiconducting polymer that shows ideal properties for *in vivo* photoacoustic imaging. The small size of the polymer nanoparticle and its brightness allowed the researchers to image lymph nodes in living mice. In addition, Pu *et al.* used the nanoparticles to develop an activatable version of the probes that can be used to sense reactive oxygen species *in vitro* and *in vivo* in real time.

Pu, K. *et al. Nat. Nanotechnol.* doi:10.1038/nnano.2013.302 (26 January 2014).