METHODS IN BRIEF

CHEMICAL BIOLOGY

Binders of O-glycosylated proteins

Many intracellular proteins are modified with O-linked β-N-acetylglucosamine (O-GlcNac). Although it is known that this modification is essential in mammals, there is still much to be learned about the details of its functional roles. To help in this endeavor, Yu *et al.* describe a method for identifying intracellular binding partners of O-GlcNac–modified proteins. The researchers designed a photo-cross-linking approach in which they engineered cells in culture to incorporate a diazirine-modified form of GlcNac into O-glycosylated proteins; UV-light irradiation of cells then results in specific cross-linking only of O-GlcNac–modified proteins to nearby molecules. After confirming that the approach detects known interactions of O-linked proteins, the researchers used it to identify nuclear transport factors as binding partners of Phe-Gly repeat–containing nucleoporins in mammalian cells.

Yu, S.H. *et al. Proc. Natl. Acad. Sci. USA* **109**, 4834–4839 (2012).

IMAGING

A better look at the nuclear pore

The nuclear pore is a complex structure that is intimately connected with the organization of information in the cell. Field emission scanning electron microscopy (FESEM) can provide high-resolution, three-dimensional images of the pore surface, but the harsh treatment of nuclear membrane preparations precludes the imaging of pores in their native state. Shaulov & Harel optimize FESEM preparations by anchoring chromatin to silicon chips before applying *Xenopus laevis* egg extracts in a classic nuclear membrane reconstitution system. They also avoid detergents by repeated hypotonic treatment and pipetting of mammalian cells to expose the outer nuclear surface. Images from these preparations showed expanses of intact pores and membrane, robustness to antibody labeling and intermediate stages of pore assembly visualized using an assembly inhibitor.

Shaulov, L. & Harel, A. *Structure* **20**, 407–413 (2012).

GENETICS

In vivo protein targeting with chemical genetics

There is increased interest in the development of methods that allow direct regulation of protein activity *in vivo* and monitoring of the process. Rodriguez & Wolfgang achieve this by using a chemical-genetic approach. The team targeted the metabolic enzyme malonyl-CoA decarboxylase by fusing its gene to that encoding FK506 binding protein 12, rendering the protein fusion stable only in the presence of the small molecule Shield-1. They tagged the protein fusion with YFP to monitor stabilization of the protein. In addition, the researchers engineered the entire transgene so that it could be conditionally regulated by Cre recombinase and included two additional fluorescent proteins: CFP as a marker of transgene expression, and monomeric (m)Cherry as a marker of recombination. They used the approach to alter fatty acid metabolism in live mice.

Rodriguez, S. & Wolfgang, M.J. *Chem. Biol.* **19**, 391–398 (2012).

MICROSCOPY

Fast two-color structured-illumination microscopy

Structured illumination is a resolution-doubling alternative to higher-resolution super-resolution methods and can image larger areas at greater speed with lower irradiation intensities. But whereas other super-resolution methods have been used for two-color time-lapse imaging of living cells, imaging of more than one color by structured-illumination microscopy has been limited to fixed cells. Replacement of a mechanically translated diffraction grating with nonmechanical light modulators recently allowed structured-illumination microscopy to achieve fast, time-lapse imaging of living cells, but the wavelength dependence of the method precluded imaging more than one color. Fiolka *et al.* adapted this setup for wavelength-dependent adjustment of the illumination. This allowed them to image an entire living cell in three dimensions and two colors at 22 seconds per frame. Reducing the imaged volume to a 1.25-micrometer-thick slice of the cell increased the speed to 8.5 seconds per frame.

Fiolka, R. *et al. Proc. Natl. Acad. Sci. USA* **109**, 5166–5169 (2012).