Technology watch

TESTING TOPOLOGY

In *Nature Methods*, Lippincott-Schwartz and colleagues now describe a new fluorescence-based technique — fluorescence protease protection (FPP) — that can be used to study protein topology and to localize protein subpopulations in living cells.

The new method involves attaching a fluorescent protein to the N or C terminus of a protein of interest and then exposing cells that are expressing this fusion protein to trypsin, either before or after the cells have been treated with digitonin to permeabilize the plasma membrane. The fluorescent protein will be degraded and the fluorescence signal lost if trypsin can access the fluorescent protein, and this gives information on protein topology. For example, a protein of interest that has its fluorescent tag located inside an intracellular organelle will continue to give a fluorescence signal following the addition of digitonin and trypsin. As proof of principle, the authors used FPP to confirm the topology of proteins that localize to various intracellular organelles. In addition, they proved its applicability to localizing protein subpopulations. By adding digitonin and trypsin to cells expressing a fluorescently labelled cytosolic enzyme, they showed that most of the fluorescence signal was immediately lost. However, small signals persisted as punctae in the cytosol, which indicated that this enzyme is a substrate for autophagosomes. This simple technique could be applied to large-scale analyses in the future.

REFERENCE Lorenz, H. *et al.* Fluorescence protease protection of GFP chimeras to reveal protein topology and subcellular localization. *Nature Methods* **3**, 205–210 (2006)

PLAIN SAILING

NMR is routinely used to determine three-dimensional solution structures of proteins, but it has its limitations. Protein size is a problem, with increased size resulting in more complex, crowded and overlapping spectra that are difficult to interpret. In addition, increased protein size leads to broader linewidths. However, in *Nature*, Kainosho and colleagues now describe a new protein-labelling technique — stereo-array isotope labelling (SAIL) — that can help to overcome these problems.

In SAIL, amino acids are chemically and enzymatically synthesized to contain a complete stereospecific and regiospecific pattern of stable isotopes, a pattern that is optimal with respect to the quality and content of the resulting NMR spectra. The efficient incorporation of these amino acids into a protein of interest is achieved using a cell-free protein-expression system. As proof of principle, the authors used SAIL to determine the structures of calmodulin (17 kDa) and maltodextrin-binding protein (41 kDa). Their approach resulted in sharpened lines and simpler spectra, without the loss of information. In addition, they could collect data rapidly to solve a structure — that of maltodextrin-binding protein — that is twice as large as those usually determined by NMR.

REFERENCE Kainosho, M. *et al.* Optimal isotope labelling for NMR protein structure determinations. *Nature* **440**, 52–57 (2006)