

 PROTEIN STABILITY

GPS for proteomes

Stephen Elledge and colleagues have developed a new technology to analyse global protein stability (GPS) in live cells, in real time and with single-cell resolution.

The fluorescence-based system relies on a reporter construct that permits the translation of two fluorescent proteins from one mRNA transcript. The red fluorescent protein serves as an internal control, whereas the green fluorescent protein is expressed as a fusion with the protein of interest (X). The ratio of red to green fluorescence can be quantified by fluorescence-activated cell sorting (FACS) and represents the stability of protein X.

By coupling flow cytometry with DNA microarray technology, the authors then evaluated protein abundance on a global scale. They used ~8,000 cDNAs to generate a GPS library of cultured human cells in which each cell expresses the red fluorescent protein and a variable green fluorescent fusion protein. The stability of fusion proteins was monitored by FACS and the identity of the open reading frames they express could be established by a polymerase chain reaction-based microarray approach.

In an accompanying paper, the authors used the library to screen for substrates of the SKP1–cullin–F-box (SCF) ubiquitin ligase in cells with abrogated cullin function. Of the 359 putative SCF substrates, 66 were tested and 31 were verified by flow cytometry and western blotting. With this proof-of-principle screen, the authors identified 25 new SCF substrates, with a wealth of candidate substrates still to be characterized.

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ORIGINAL RESEARCH PAPERS Yen, H.-C. S. *et al.* Global protein stability profiling in mammalian cells. *Science* **322**, 918–923 (2008) | Yen, H.-C. S. & Elledge, S. J. Identification of SCF ubiquitin ligase substrates by global stability profiling. *Science* **322**, 923–929 (2008)