PROTEOMICS

Protein dynamics for the masses

Automation tools make large-scale fluorescence correlation spectroscopy experiments feasible for a wide range of users.

Fluorescence correlation spectroscopy (FCS) allows scientists to study the dynamic behavior and interactions of proteins in their natural environment. However, data analysis requires a daunting level of effort and expertise, and performing FCS on large numbers of different proteins in parallel remains particularly challenging.

An automated pipeline developed by at the European Molecular Biology Laboratory in Heidelberg takes away a lot of the pain, bringing high-throughput FCS (HT-FCS) capabilities within reach of a far larger community of users. FCS experiments entail laser excitation of fluorescently tagged molecules within a sample volume, which are then profiled on the basis of properties such as brightness and diffusion rate. The laboratories of Rainer Pepperkok and Jan Ellenberg have developed a pair of software tools to control this entire process. "Micropilot" analyzes labeled samples to identify regions suitable for FCS analysis, with the capacity to collect thousands of individual readings; these data are then passed along to "FluctuationAnalyzer," which extracts relevant biological information.

The researchers used HT-FCS to profile 53 different nuclear proteins, collecting over 60,000 measurements from more than 10,000 individual cells. For each protein, the rate of diffusion indicated whether the target was generally free in solution or bound in a complex, whereas the brightness of the labeling indicated whether the protein was monomeric or prone to multimerization. The cells expressed tagged histones in addition to the various fluorescently labeled target proteins, making it possible to detect proteins that physically associate with chromatin.

HT-FCS can also collect time-course data. Over the course of the cell cycle, the researchers monitored various components of the chromosomal passenger complex (CPC), which helps coordinate multiple steps of cell division. After collecting numerous measurements at 10- or 20-minute intervals from 71 different cells, the researchers determined that CPC components are relatively scarce and remain dissociated from each other during G1 and S phases but that they grow more abundant and begin forming precursor complexes in G2 before finally assembling into mature CPCs at the onset of mitosis.

"Our technology makes live-cell proteomics accessible to nonspecialist users and [is] sufficiently robust to be offered as a service by high-level imaging facilities," the authors conclude.

Michael Eisenstein

RESEARCH PAPERS

Wachsmuth, M. *et al.* High-throughput fluorescence correlation spectroscopy enables analysis of proteome dynamics in living cells. *Nat. Biotechnol.* **33**, 384–389 (2015).