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A dynamic picture of protein behavior in cells

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Abstract

Protein dynamics in living cells can now be studied by fully automated, high-throughput fluorescence correlation spectroscopy.

Understanding of the complex interplay among the thousands of protein species that make up a cell has been constrained by a lack of technologies to measure protein dynamics and interactions. An advance in fluorescence correlation spectroscopy (FCS), described in this issue by Wachsmuth *et al.*¹, sets a new standard in the field. The authors present a high-throughput (HT) FCS workflow that allows measurement of the dynamic properties of proteins in thousands of cells and conditions while retaining the ability to investigate specific subcellular locations and specific phases of the cell cycle (Fig. 1). This work introduces a powerful approach with a unique ability to measure the behavior of proteins—and potentially other biomolecules—in their natural cellular context in an unbiased, high-throughput manner.

Currently there are two main groups of methods that scientists use to study protein dynamics and interactions. The first group includes approaches such as fluorescence recovery after photobleaching, fluorescence resonance energy transfer and FCS, which are based on imaging fluorescently labeled molecules at high spatial resolution². These methods provide detailed information on the biochemical and biophysical properties of specific proteins in their cellular context, but they are very labor intensive and cannot be applied to more than a handful of proteins or conditions in a reasonable time frame. The second group consists of high-throughput methods, such as mass spectrometry, that can take snapshots of a broad range of proteins³ but in general have very low spatial and temporal resolution and require averaging over large populations of cells.

Among the microscopy-based methods, FCS is an ideal tool for gaining insight into the dynamic properties of proteins in living cells⁴. FCS measures the fluctuations in photons resulting from fluorescently labeled molecules diffusing in and out of a defined femtoliter-sized observation volume. By recording the fluctuations over time and fitting them with an appropriate correlation function, it is possible to calculate multiple parameters, including diffusion coefficient, local concentration and states of aggregation. The method can be further extended to fluorescence cross-correlation spectroscopy (FCCS) experiments, which

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measure co-fluctuation of the fluorescence intensity of differently labeled molecular species, providing information about multimolecular interactions⁵.

Despite its capabilities, FC(C)S has not been widely applied. It is labor intensive and requires subjective decisions to determine when and where to acquire data as well as considerable manual downstream data processing to interpret the intensity measurements. Now, exploiting recent progress in high-throughput screening microscopy and computer vision, Wachsmuth *et al.*¹ have fully automated the entire FC(C)S workflow. This enables for the first time routine FC(C)S measurements in at least 100 single cells per protein of interest, which is what is needed to be able to distinguish the contributions of biological and statistical variation.

To achieve this capability, the group customized the functionality of a high-throughput screening confocal microscope. Using an open-source software called ‘Micropilot’, they programmed the microscope to automatically acquire high-resolution images of cells at regular grid positions in a multiwell format and to subsequently identify and carry out FC(C)S measurements on suitable cells and subcellular regions. The new platform can also conduct ‘time-lapse’ FC(C)S by adding three-dimensional live cell tracking after cell identification. Finally, the group developed the ‘Fluctuation Analyzer’ software package to automatically carry out downstream data analysis that incorporates rigorous mathematical corrections of experimental artifacts, such as photobleaching, fluorophore cross-talk and background signals.

Wachsmuth *et al.*¹ applied their method to two specific examples of protein dynamics: the interaction of nuclear proteins with chromatin and the interaction dynamics of cell-cycle proteins through different phases of the cell cycle. In both sets of experiments, the results were consistent with previous findings and identified novel protein interactions to be examined in more detail in future studies.

It should be noted that HT-FC(C)S does not resolve all of the drawbacks of traditional FC(C)S. For example, slow-moving molecular species are still difficult to measure without significant photobleaching effects. In addition, the data must be routinely checked for potential artifacts and systematic errors resulting from non-ideal imaging conditions or imperfect microscope setup. Although HT-FC(C)S fully automates data collection, proteins to be measured have to be fluorescently labeled. Preparing a library of correctly labeled proteins can be challenging as a fluorescent tag may cause protein mislocalization, changes in degradation and other differences in behavior. Finally, given the nature of cross-correlation as a pairwise measurement, extension of the method to protein complexes containing more than two proteins increases analytic complexity substantially.

Despite these limitations, the capacity of HT-FCS to measure protein interaction dynamics in large numbers of single living cells will allow researchers to tackle questions about the cell-to-cell heterogeneity of protein dynamics with sufficient statistical power to distinguish between biological and statistical noise⁶. Pairing HT-FCS with other high-throughput methods such as LC-MS/MS will enable experimental pipelines that can take advantage of large, low-resolution experiments to screen globally for interesting protein interaction

candidates and feed them directly into an experimental format that can extract detailed biophysical information. The potential to conduct all these measurements on tagged endogenous proteins (as demonstrated by the authors) by incorporating new genome-editing technologies would also be an exciting avenue to explore in the future. Finally, this method could be combined with more specific chromosomal or other subcellular fluorescent labeling and optogenetic perturbations. We envision HT-FCS developing into a widely used microscopy-based platform to both perturb and measure the biochemical and biophysical properties of endogenous proteins of interest over time with precise spatial and temporal control.

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Figure 1.

HT-FCS: fluorescence correlation spectroscopy in a high-throughput setup. **(a)** By measuring and correlating the fluctuations of the fluorescence intensity of fluorescent particles in solution, FCS can be used to obtain quantitative information on protein behavior. **(b)** Wachsmuth *et al.*¹ developed a workflow to automatically carry out HT-FCS on hundreds of cells per well. **(c)** By adding 3D cell tracking after cell identification, time-lapse

experiments can be carried out to extract FC(C)S data automatically from several tens of single cells throughout the cell cycle in a 24-hour period.

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