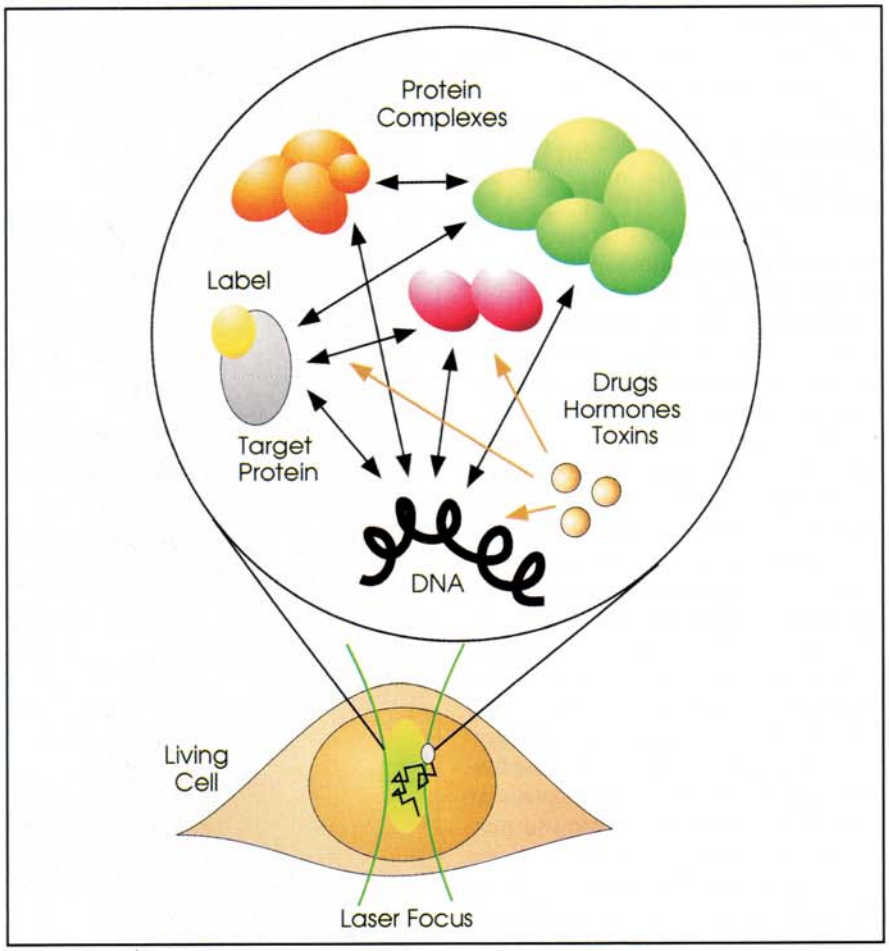
Fluorescence correlation spectroscopy technique adds quantitative details

New method bolsters monitoring of biochemical networks in living cells

Researchers developing treatments for diseases such as diabetes and cancer have begun to focus on nuclear receptors. These receptors set transcription in motion and interact with regulatory proteins in response to signaling molecules — such as hormones — and to drugs and pollutants. Because these receptors control many aspects of cell metabolism, understanding how they work could lead to new therapies, but scientists still have learned few quantitative details about nuclear receptor-mediated signaling.



A fluorescence correlation spectroscopy technique incorporates diffusion-time distribution analysis, which enables quantification of protein-protein interactions and molecular complexes in vivo, contributing to improved monitoring of biochemical networks in living cells.

Recent studies have demonstrated the feasibility of using optical microscopy and selective fluorescence labeling for imaging proteins and molecular complexes in vivo to provide access to the biochemical networks in which nuclear receptor-mediated signaling plays a major role. In a *Biochemistry* paper published online Aug. 11, Hanna Jankevics, a graduate student with Horst Vogel at the Swiss Federal Institute of Technology in Lausanne, reported a fluorescence correlation spectroscopy technique that provides quantitative data on the mobility and interactions of the human estrogen nuclear receptor and the nucleus of breast cancer cells.

"We were looking for a way to quantify protein interactions in situ in living cells at native expression levels," said Michael Prummer, one of the principal investigators. "We wanted to avoid artificial interactions caused by overexpression of the target protein, and we wanted to catch even weak interactions, which are easily lost by harsh treatment."

Traditionally, investigators use techniques such as mass spectrometry, yeast two-hybrid screen and immunoprecipitation to detect protein-protein interactions. However, although these techniques provide molecular details about the interactions, they cannot be used in situ, and they often miss transient interactions. Also, high protein concentrations can cause artificial interactions to be recorded.

Other optical techniques, including fluorescence recovery after photobleaching (FRAP), Förster resonance energy transfer (FRET), fluorescence cross-correlation spectroscopy, fluorescence intensity distribution analysis and photon-counting histogram analysis enable researchers to detect protein-protein

interactions. FRAP provides the mobility of labeled proteins, which is indirectly related to interactions (similar to fluorescence correlation spectroscopy), but it requires higher expression levels, has a lower spatial resolution and is less well suited to discriminate multiple diffusing components. FRET and fluorescence cross-correlation spectroscopy offer a more direct measure of protein-protein interactions than fluorescence correlation spectroscopy, but the information they yield is limited to pairs of proteins.

The conventional approach to fluorescence correlation spectroscopy enables researchers to acquire one diffusion time (diffusion coefficient) of a labeled protein per condition — a particular ligand concentration, for example — and associate it with bound or unbound species. Extensive time and ensemble averaging are required to estimate the mean diffusion time. The researchers instead used a method called diffusion-time distribution analysis, which allows analysis diffusion times over very short time scales.

"With our approach," Prummer said, "we are avoiding time averaging as much as possible in order to retrieve most of the distribution of the diffusion times present in the sample. In this way, we are able to investigate the whole population comprising free and different bound states, rather than just the average value."

To demonstrate the benefits of the method, the investigators used it to track the mobility and interactions of human estrogen receptor-α in the nuclei of living cells, applying it to compare the diffusion of YFP fused to the receptor and YFP fused to the receptor's cofactor in breast cancer cells. (They determined that constructing fusion proteins with YFP provided the best results, after testing several other methods, including using dye-modified ligands.)

For fluorescence correlation spectroscopy, they used a Zeiss confocal laser scanning microscope outfitted with a fluorescence correlation spectroscopy unit and a 40×, 1.2-NA water-immersion objective. An argon-ion laser excited the YFP at 488 nm. Fluorescence was detected after passing through a 70-µm pinhole and a 530- to 600-nm bandpass filter. Typically, the researchers recorded 200 individual intensity time series in the nucleus of 10 cells, each over a period of 5 s, at a time resolution of 0.2 µs.

Interactions observed

The experiments revealed a number of simultaneous yet discrete interactions between the receptor and nuclear components in living breast cancer cells, demonstrating that the technique could be used to investigate the distribution of diffusion times in a highly heterogeneous and dynamic sample. Specifically, the researchers noted that the nature and population of these states was concentration-dependent and ligand-specific and, furthermore, that about 15 percent of the receptors appeared to interact with the receptor interaction domain in the absence of the female sex hormone estradiol, which naturally activates the receptor. They would not have been able to see the latter with conventional techniques, Prummer said.

He noted that FRET, FRAP and fluorescence cross-correlation spectroscopy also could benefit from the concept of diffusion-time distribution analysis, "to determine the whole distribution of an observable, and possibly even its time-evolution and spatial map, rather than only its average value."

Ultimately, diffusion-time distribution analysis could enhance the potential of fluorescence correlation spectroscopy for drug screening and diagnostics applications. More generally, Prummer added, it could contribute to improved monitoring and quantification of biochemical networks in living cells, "a major goal of functional proteomics with broad applications in biomedical research and screening of active compounds."

For now, the researchers are continuing their investigations of the estrogen receptor's interaction network and planning comparative studies of the mobility distributions of estrogen receptors and other labeled cofactors. They also hope to explore the mobility distribution of estrogen receptors after deletion of one or more interacting proteins.

Beyond this, they have begun computer simulations to learn more about the possibilities and limitations of the technique. "To prove its broad applicability, it is essential to apply diffusion-time distribution analysis to other protein interaction networks much different from estrogen receptors," Prummer said. □

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