THE AUTHOR FILE

Philippe Bastiaens

Researchers watch proteins interact in real time.

When Philippe Bastiaens considers the logic of cell signaling, his thoughts turn to termites. When they build nests, these insects sense where their colony-mates have dropped a sand grain and are more likely to leave sand in the same space. Just as random movements and local interactions of insects create

a self-amplifying signal and help build a large self-organizing colony, Bastiaens reasons, diffusion and interactions of biomolecules can result in self-organization on a nanometer scale to allow cells to function. But to understand that process, such interactions must be studied in motion.

One common technique to study protein interactions is co-immunoprecipitation, where an antibody is used to pull out a protein of interest along with other cellular components bound to that protein. This approach can identify multiple interaction partners for a particular protein but says little about protein dynamics or function, and it is poorly equipped to capture crucial but short-lived enzymatic interactions that allow cells to move through the cell cycle or respond to environmental signals. To detect these kinds of interactions, Bastiaens and collaborators had previously developed a technique, dubbed visual immunoprecipitation, in which a protein of interest is studded onto micrometer-sized beads and placed into cell extracts containing fluorescently labeled binding partners of the protein of interest (Niethammer *et al.*, 2007). By imaging the beads under a confocal microscope, Bastiaens and colleagues could watch microtubule-associated proteins interact with each other and with tubulin and, thus, monitor a dynamic process without perturbations such as overexpression.

Of course, to really understand what happens in a real biological system, one must study such processes in the native environment. For this, the beads have a major drawback: they are too large to be injected into cells. As reported on page 295, Bastiaens and his colleagues at the Max Planck Institute, Dortmund replaced the beads with much smaller quantum dots and demonstrated that these can be used to quantify dynamic

protein interactions at nanomolar concentrations in living cells (Zamir *et al.*, 2010).

To test the new method, the researchers took a quantum dot that fluoresces red and studded it with a short peptide known to tightly bind and inhibit the regulatory domain of protein kinase A (PKA), which is important in several networks, including those involved in regulating metabolism and the cell cycle. Next, they genetically engineered a line of monkey cells so that the catalytic domain of PKA was labeled with enhanced yellow fluorescent protein. Finally, they injected the quantum dots into the cells and monitored them under a confocal microscope using a technique called fluorescence correlation spectroscopy, which can be used to count fluorescent molecules that diffuse in and out of a very small area (1 cubic micrometer). By looking at the correlation of red with yellow fluorescence, the researchers could tell whether the catalytic domain was bound to its inhibitory unit. The researchers then used a variety of techniques to activate PKA and found that they were able to consistently monitor the binding and release of the catalytic domain. “You don’t take snapshots,” Bastiaens says, “you look dynamically.”

And the technique can be applied beyond kinases, or even enzymes, says Bastiaens. “It is a very generic approach. You can imagine any interaction to be monitored,” he adds. Moreover, using several colors of quantum dots would allow multiple parameters of an interaction to be explored simultaneously, such as how interaction timing or partners change when a protein is phosphorylated or otherwise modified.

The hope, says Bastiaens, is that being able to look into protein interactions within the intact system of living cells will provide clues about how protein networks establish themselves and maintain homeostasis. Researchers observing termites have been able to deduce simple rules that yield complex nests; perhaps observations of molecular interactions can do the same, he says, adding: “I think these methods give us eyes to describe properties of the cells in this way.”

Monya Baker

Niethammer, P. *et al.* Discrete states of a protein interaction network govern interphase and mitotic microtubule dynamics. *PLoS Biol.* **5**, e29 (2007).

Zamir, E., Lommerse, P.H.M., Kinkhabwala, A., Grecco, H.E. & Bastiaens, P.I.H. Fluorescence fluctuations of quantum-dot sensors capture intracellular protein interaction dynamics. *Nat. Methods* **7**, 295–298 (2010).

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