PROTEOMICS

Everything is illuminated

A new luminescence-based assay allows scientists to assemble detailed protein-protein interaction maps that can reveal the dynamic workings of highly complex signaling networks.

Biologists have been tracking protein-protein interactions for decades now, but the scale of these studies has exploded since the early days of coimmunoprecipitation and the two-hybrid screen. Recent articles have showcased a variety of high-throughput interaction assays, assembling complex webs of association between hundreds or even thousands of proteins, considerably advancing our understanding of several cellular processes. At the same time, however, most of these approaches offer only a rapid snapshot of interaction states.

"The available techniques do not really provide the means to assess the dynamics of how protein networks form," explains Miriam Barrios-Rodiles, a postdoctoral fellow in the lab of Jeffrey Wrana at the University of Toronto. "And for signal transduction, this is very important, because we want to know what happens when the cells receive a signal... how new protein-protein interactions form while other interactions disappear."

In a recent article in *Science* (Barrios-Rodiles *et al.*, 2005), Barrios-Rodiles and her colleagues present their effort to remedy this situation, a new technique called luminescence-based mammalian interactome mapping (LUMIER). In a LUMIER assay, a luciferase-tagged 'bait' protein is screened against a series of Flag-tagged 'prey' proteins; an antibody against Flag is used to affinity-purify the prey, and the prey-associated luminescence reveals the extent of bait interaction (**Fig. 1**). Although the fundamental principle of LUMIER is not radically different from other interaction assays, the technique derives additional strength from its considerable sensitivity and the ease with which comparative measurements of interactive states can be conducted, allowing the quantification of dynamic shifts in complex networks.

Furthermore LUMIER has the benefit of considerable scalability. After several pilot experiments using a single bait against a handful of prey fusion proteins, the Wrana group conducted a much larger study with a customized robotics platform. Because much of the lab's work revolves around the study of signaling by the growth factor TGF-β, they designed bait fusions for 18 established participants in this signaling pathway, which were in turn screened against more than 500 prey fusions, chosen to represent a broad range of potentially relevant protein types. Using a fairly stringent standard for positive signals, the group identified nearly 1,000 interactions from these experiments, which they assembled into a detailed map.

The investigators then performed a before-and-after experiment to identify changes in the network interaction profile that result from TGF-β signaling. This revealed some striking changes, and the Wrana group created an animated movie (available as supplemental material for their article) that depicts some of the dramatic alterations in protein-protein association that take place downstream of this signaling event.

Although these initial results demonstrate the power of the technique, there are still some caveats. A primary concern is that the interaction data is relative rather than absolute, as the assay is not quantitative for the Flag-tagged prey. According to Barrios-Rodiles, "We are working towards developing a complementary assay that will allow us to measure the prey, [so] we will know exactly how much of the bait is expressed, and also how much of the prey is expressed." Another issue is the risk of false-positives, more or less inevitable for any overexpression-based assay, and the Wrana group estimates an approximate 20% false-positive rate when using a relatively conservative cutoff. At the same time, an overly strict cutoff increases the likelihood of missing weaker interactions. "We know that if we lower the cutoff, we may be able to detect transient interactions," says Barrios-Rodiles, "or maybe interactions that occur in an indirect manner." Nonetheless, the Wrana team experimentally confirmed several endogenous interactions, including some new and unexpected associations—one of these is the foundation for a second *Science* article (Ozdamar *et al.*, 2005).

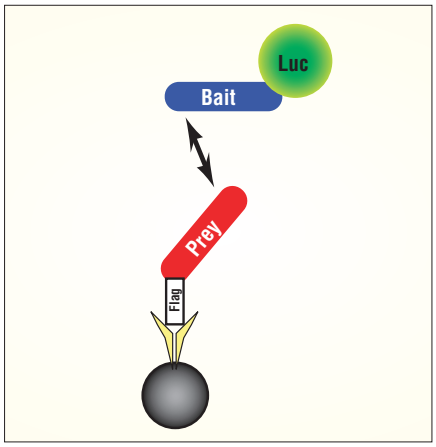

Figure 1 | LUMIER overview. Each assay measures the interaction between a luminescent, luciferase-tagged bait protein and a Flag-tagged prey, which can be captured by antibodies (yellow) immobilized on sepharose beads (black). An array scanner can be used to quantify the relative extent of interaction for large numbers of assays.

Beyond this initial windfall of data, Barrios-Rodiles foresees numerous other potential applications for this technique. "For future directions, we would like to see it as a tool for mapping domains," she says, and adds, "and I would [also] like to see LUMIER applied to chemical biology—for example, in drug discovery—and to finding small molecules that interfere with protein-protein interactions that are involved with disease."

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RESEARCH PAPERS

Barrios-Rodiles, M. *et al.* High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* **307**, 1621–1625 (2005).

Ozdamar, B. *et al.* Regulation of the polarity protein Par6 by TGF-β receptors controls epithelial cell plasticity. *Science* **307**, 1603–1609 (2005).