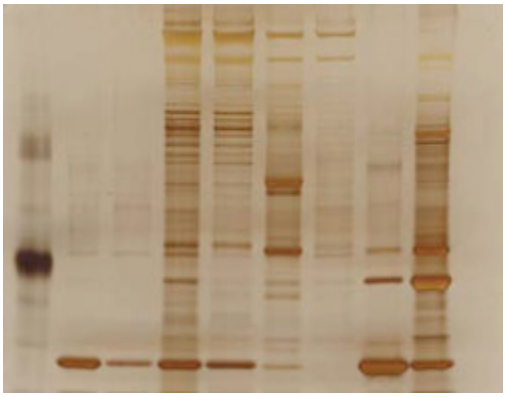
The Scientist

Volume 22 | Issue 10 | Page 61

By Jeffrey M. Perkel

Close Encounters

Protein-protein interaction assays for all occasions.



For tandem affinity purification, purified proteins are visualized on a silver-stained gel, then analyzed by liquid chromatographytandem mass spec.

Tilmann Buerckstuemmer and Giulio Superti-Furga / Center for Molecular Medicine of the Austrian Academy of Sciences

Proteins almost never act alone. In a molecular version of "guilt by association," identifying the function of novel proteins often requires pinning down the proteins with which they interact. But yeast two-hybrid assays and coimmunoprecipitation, the two main techniques for generating "interactomes," maps of protein interactions on a proteome-wide scale, can leave gaping holes.

The yeast two-hybrid (Y2H) assay is genetically simple and amenable to genome-scale analyses. It involves two fusion proteins, called "bait" and "prey," coexpressed in yeast; each is coupled to one half of a transcriptional activator such that their association tethers a transcriptional activation domain to DNA, inducing expression of a reporter gene. The technique has some restrictions, however: The fusion proteins must be overexpressed and localized in the nucleus, and can not include membrane proteins, transcriptional activators, mammalian post-translational modifications, or multiprotein complexes.

Fishing proteins out of cell lysates via coimmunoprecipitation (coIP) and analyzing them using mass spectrometry (MS) avoids some of these concerns. Yet this approach requires antibodies to a protein of interest, tends to overlook weak or low abundance interactions, and can only identify which

proteins are present in a complex, not who binds to whom.

The Scientist asked four researchers to describe their preferred method for mapping protein interaction while avoiding these shortcomings. Here's what they said:

Complex ID

Researcher: Giulio Superti-Furga, Director, Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna

The project: Mapping protein complexes in yeast (Nature, 440:631-6. 2006) and mammalian cells (Nature Methods, 3:1013-9, 2006)

The problem: Y2H measures only binary interactions, not multiprotein complexes. But coIP /MS requires high-quality, specific antibodies, few of which are available. Superti-Furga needed a generic way to isolate intact complexes from cells.

The Solution: Superti-Furga and his team adapted a process called tandem affinity purification (TAP) that is akin to standard coIP /MS, but doesn't require a proteome's worth of different antibodies.

Unlike in traditional coIP , the TAP tag enables sequential purification, first on immunoglobulin-coated beads and then, following protease digestion, on calmodulin-coated beads. Finally, the purified complexes are eluted and analyzed by MS.

Like coIP /MS, and unlike Y2H (and LUMIER , described below), TAP tagging offers no information on binary proteinprotein interactions. It can, however, reveal complexes other methods may overlook.

"As [the tagged protein] comes off the ribosome, it can assemble with its natural binding partners," explains Superti- Furga. "This allows TAP to capture effects like order of addition, where one protein must bind before a second can bind, or posttranslational modifications." The method "gives you two things," he adds: "The members participating in a particular process, and the 'organizational chart' of how these members are organized."

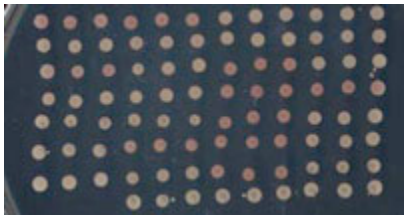
But, he cautions, "What you cannot be sure [of] is whether that measurement is due to some spurious contamination because some protein may have a very high concentration, for instance."

Costs: TAP plasmids and strains from EuroSCARF cost from €15 to €30.

Membrane bound

Researcher: Igor Stagljar, Professor, Donnelly Center for Cellular and Biomolecular Research, University of Toronto

The project: Interactive proteomics of integral membrane proteins (PNAS 94:5187-92. 1998)



Growth of blue-color yeast reveals protein-protein interaction.

Saranya Kittanakom and Igor Stagljar / University of Toronto

The problem: Existing biochemical and genetic approaches either don't work with membrane proteins (Y2H) or destroy the complexes (coIP / MS). Stagljar wanted a genetic alternative.

The Solution: Stagljar's technique, called MY TH (membrane yeast twohybrid), takes the benefits of Y2H - chiefly easy genetics and amenability to high-throughput analyses - and applies them to membrane proteins. According to Stagljar, "this is the only assay so far demonstrated to work as a screening system to find protein interactors of full-length, integral membrane proteins."

MYTH employs a so-called "split-ubiquitin" approach. The bait (an integral membrane protein) is fused at one end to the C-terminal half of ubiquitin linked to a transcriptional activator; potential prey are fused to the other half of ubiquitin. Interaction between bait and prey reconstitutes ubiquitin, which is then cleaved by a specific protease to release the transcriptional activator. The activator then migrates to the nucleus and turns on reporter gene expression.

MYTH accommodates both cytosolic and integral membrane prey, as well as interactions occurring at any cellular membrane (for instance, endoplasmic reticulum, Golgi, and mitochondria). "If you work with yeast membrane proteins, we are almost 100% successful, because you are expressing a yeast membrane protein in its natural milieu," Stagljar says. Mammalian proteins are more problematic, though, because their targeting often requires posttranslational modifications that do not occur in yeast.

Costs: Stagljar cofounded DualSystems Biotech of Switzerland to commercialize MY TH. The DUAL membrane kit 3 costs $3,900.

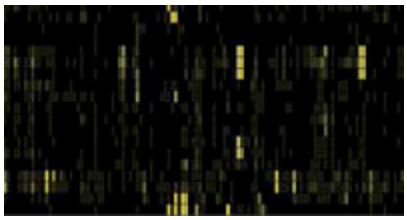
Signal specs

Researcher: Jeff Wrana, Senior Investigator, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto

The project: Mapping the dynamics of protein-protein interactions in mammalian cell signaling systems (Science, 307:1621-5, 2005)

The problem: Signaling events often involve membrane proteins and posttranslational modifications, both of which are incompatible with Y2H, and low-abundance proteins, which are difficult to detect by coIP /MS.

The Solution: Wrana's approach, called LUMIER (luminescence-based mammalian interactome mapping), combines elements of Y2H and coIP /MS in a high-throughput format.



A LUMIER dataset (bait, Y axis; preys, X axis). The color intensity (yellow) reflects the magnitude of the interaction.

Jeff Wrana / Samuel Lunenfeld Research Institute

Head to head

Researcher: Mike Snyder, Professor of Molecular, Cellular, and Developmental Biology & Director, Yale Center for Genomics and Proteomics, Yale University

The project: Searching for direct calmodulin (CaM)- and calmodulin-like (CML)-binding proteins in the Arabidopsis thaliana proteome (PNAS 104:4730-5, 2007)

The problem: Existing techniques often overlook relatively weak interactions. The interactions they do detect can often be either direct or mediated by an intermediary protein. Snyder wanted a way to exclusively map direct protein-protein interactions.

A section of a protein microarray: glutathione-S-transferase (GST) fusion proteins probed with a GST antibody (GST is a commonly used fusion moiety).

PNAS 104:17494-17499, 2007 / © 2007 National Academy of Sciences, U.S.A.

The Solution: Snyder's team made protein microarrays by expressing 1,133 plant proteins and arraying them in duplicate on nitrocellulose-coated glass slides. They then probed those arrays with seven fluorescently labeled CaM and CML proteins, detecting 173 partners.

Snyder's lab pioneered protein microarrays, having first described a yeast proteome chip in 2001. According to Snyder, arrays offer several advantages. First, because it is in vitro, observed interactions must occur without intermediaries. Second, arrays enable direct comparison of binding strengths - data other techniques do not provide. Finally, because all proteins on the array are present in equal amounts, "You can detect interactions amongst proteins that might be present at very low copy numbers."

On the other hand, because arrays probe interactions in vitro, results must be validated in vivo. And, not insignificantly, "someone has to make [the chips]," he says. "They are fairly expensive and labor-intensive to set up."

Costs: Commercial protein (as opposed to antibody) arrays are rare. Snyder's lab licensed its technology to Invitrogen. The 8,000- protein ProtoArray human microarray V4.1 lists for $1,700.

Four steps for improving your protein interaction studies

1. Use at Least Two Complementary Methods

It always pays to validate at least a subset of interactions using some alternative method, preferably involving endogenous proteins expressed in vivo at normal levels. "That's very important because sometimes, a certain number of interactions can be seen as proteins are overexpressed that you wouldn't see looking at endogenous proteins," says Jeff Wrana of Mount Sinai Hospital Toronto. But don't just pick any method; if your first approach is in vitro, make your second in vivo, and visa versa.

2. Cross-check Against Orthologous Data Sets

If two proteins interact, they must by definition be found in the same place. They should also yield similar knockout phenotypes. If you cannot generate such data yourself, at least cross-check your results against localization and/or RNAi datasets (if available). "If you have a protein interacting with a receptor, then you should be able to demonstrate that the protein is not, say, exclusively nuclear, because that would suggest the interaction may not be physiologically relevant," says Wrana.

3. Be Quantitative

Some interaction methods provide yes/no answers; others provide quantitative data. If possible, have one of your techniques provide quantitative information, says Yale University's Mike Snyder. "It might come in useful." Because all proteins on a microarray are present at roughly equal levels, for instance, Snyder's lab can directly compare the binding strengths of different probe proteins. "You could very easily see which proteins interact with which partners, and you can quantify that, get a sense of relative binding strengths, because it is done in parallel," he says.

4. Archive Your Data

Just as with sequence data, community online databases exist to archive interaction data. Such data benefit the entire interaction community, including your lab. Two of the most popular resources are the BioGRID (General Repository for Interaction Datasets, www.thebiogrid.org) and the Database of Interacting Proteins (DIP, http://dip.doe-mbi.ucla.edu/). The MIPS Mammalian Protein-Protein Interaction Database (http://mips.gsf.de/proj/ppi/) web site lists more than 20 additional archives, as well.