PROTEOMICS

Interactomes by mass spectrometry

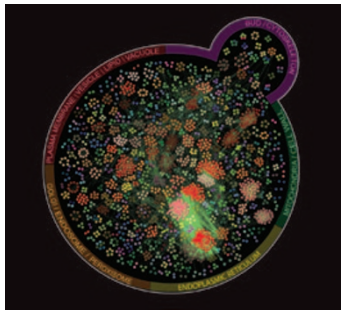
Two different mass spectrometry–based approaches are applied to analyze protein complexes on a global scale.

Proteins rarely work in isolation; rather, it is the supramolecular organization of proteins into complexes and molecular machines that is crucial for carrying out biological function. Detecting protein complexes on a global scale is therefore one of the most important quests in proteomics. Recently, two reports were published describing the use of mass spectrometry–based approaches for profiling membrane-protein complexes in yeast (Babu *et al.*, 2012) and soluble protein complexes in human cells (Havugimana *et al.*, 2012).

University of Toronto researchers Jack Greenblatt and Andrew Emili have previously used affinity purification–mass spectrometry (AP-MS) to map the soluble protein interactome in yeast. In this approach, an affinity purification tag is systematically inserted in the yeast chromosome to produce fusion protein 'baits'. Gentle purification conditions allow proteins interacting with the bait to be pulled down and identified by mass spectrometry.

But because purification conditions are optimized for soluble complexes, membrane-protein complexes are usually sorely underrepresented in AP-MS data sets. Greenblatt, Emili and their colleagues decided to tackle this recalcitrant group of proteins by modifying their AP-MS pipeline, namely by utilizing one of three nondenaturing detergents for membrane-protein extraction in yeast (Babu *et al.*, 2012).

The researchers identified 1,726 high-confidence membrane protein-protein interactions, many of which were previously unknown, including 501 putative membrane protein complexes. The data are available in a database (http://wodaklab.org/membrane/) maintained by Shoshana Wodak's lab, also of University of Toronto. The team was unable to detect interactions for about 550 yeast membrane-protein open reading frames,



The yeast membrane-protein interactome map. Image courtesy of M. Babu.

but as Emili explains, they did not expect all membrane proteins to be expressed under standard growth conditions. "We were actually pleasantly surprised that we achieved the coverage that we did," he says. Still, detection of low-abundance proteins is also tightly tied to the sensitivity of the mass spectrometry instrumentation, which is continually increasing as newer instruments become available. "If we were to go back and redo all these purifications," Emili says, "we could probably double or triple the map density."

Although the plethora of yeast genetics tools allows researchers to affinity-tag such proteins with relative ease, in contemplating the more complex human interactome, Emili's team knew early on that they would need a different strategy. "The motivation ultimately was not to have these big [affinity-tagging] pipelines, which is laborious and tedious," he says. Moreover, the tag-based approaches his lab has used for mammalian cells have involved overexpression, which could possibly lead to artifactual interactions.

The tagless strategy that Emili and Edward Marcotte of the University of Texas at Austin came up with together was to perform systematic, highly extensive biochemical fractionation of the soluble human protein interactome using multiple separation techniques including nondenaturing high-performance multibed ion-exchange chromatography, sucrose gradient centrifugation and isoelectric focusing (Havugimana *et al.*, 2012). By mass spectrometric analysis of the fractions, they detected a total of 13,993 high-confidence interactions between 3,006 proteins, many of them novel, including 622 putative protein complexes. Key to the study was the development of a bioinformatics strategy for filtering the data to determine which detected interactions were reliable. The data are available at http://human.med.utoronto.ca/.

With regards to the tagless approach, "the beauty is we can simultaneously look at thousands of proteins interacting," says Emili. "Even though [this] study took a considerable amount of time to come together, now we're actually cranking through interactome maps in a couple of months for different organisms." The method also allows researchers to look at protein–nucleic acid and protein–small molecule interactions, areas his lab is also pursuing.

The major benefits of the tagless approach are speed and scale-up potential. "I'm very optimistic that the tagless approach will really generalize to look at many different conditions or many different species," Emili says; with AP-MS, looking at multiple species or conditions is not practical. However, the tagless approach is biased towards high-abundance proteins. The benefit of AP-MS is that it has higher sensitivity and is less prone to false positives. "The tag is very effective for low-abundance proteins and gives high-quality interaction data," says Emili.

To tag or not to tag… With these complimentary methods in hand, researchers interested in performing interactome studies by mass spectrometry can now choose which approach will work best, depending on the type of question they want to ask.

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RESEARCH PAPERS

Babu, M. *et al.* Interaction landscape of membrane-protein complexes in *Saccharomyces cerevisiae*. *Nature* **489**, 585–589 (2012).

Havugimana, P.C. *et al.* A census of human soluble protein complexes. *Cell* **150**, 1068–1081 (2012).