# Illuminating protein networks in one step

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A new assay capable of examining hundreds of proteins at once and enabling new experiments that could dramatically change our understanding of cancer and other diseases has been invented by a team of University of Chicago scientists.

Described today in the journal Nature Methods, the new micro-western arrays combine the specificity of the popular "Western blot" protein assay with the large scale of DNA microarrays. The technique will allow scientists to observe much of a cell's intricate protein network in one experiment rather than peeking at one small piece at a time.

"The proteins are the actual machines that are doing everything in the cell, but nobody's been able to examine them in depth because it's been too complicated. Now, we can begin to do that with this new method," said Richard B. Jones, senior author and assistant professor at and the University of Chicago's Ben May Department for Cancer Research and the Institute for Genomics and Systems Biology.

Since the 1970's, laboratories have used Western blots to measure proteins. Cellular material is loaded into a gel and proteins of different sizes are separated by an electric field. A protein is then targeted by an antibody, allowing scientists to measure the amount present in the cells.

The method has led to numerous findings across the field of cell biology, but is limited by a need for large amounts of cell material and expensive antibodies, and the inability to measure more than a handful of proteins at a time. With hundreds or even thousands of proteins involved in

cellular networks, scientists were restricted to observing only a small fraction of protein activity with each experiment.

"When you walk into a dark room and don't have much information, it's difficult to predict where everything is going to be," Jones said. "If someone can simply turn on the light, you don't have to progress one step at a time by bumping into things. With this new technology, you can potentially see everything at the same time."

Micro-western arrays adapt the technology of the micro-array, typically used to assess the expression of thousands of genes in a single experiment, to proteins. With pre-printed micro-western array gels, essentially comprising 96 miniature Western blots, scientists can compare the levels of hundreds of proteins simultaneously, or compare dozens of proteins under dozens of treatment conditions in one shot. Mere nanoliters of cell material and antibodies are needed for the experiments, reducing cost and maximizing the information obtained from a single sample.

To demonstrate the potential of the micro-western array, Jones and colleagues from the University of Chicago and the Massachusetts Institute of Technology looked at the behavior of proteins in a cancer cell line with elevated amounts of epidermal growth factor receptor (EGFR).

"We started asking questions about what we could do that no one else could previously do," Jones said. "We could actually reproducibly see 120 things at a time rather than looking at 1 or 2 or 5."

The experiments found that activating EGFR simultaneously activated several other receptors in the cell - a new discovery that may explain why some tumors become resistant to cancer therapies.

With more information, the method may potentially be used clinically for more precise diagnoses of cancer and other diseases that can direct individualized treatment.

"In the clinic, you're limited by the fact that typically most cancers are diagnosed by one or two markers; you're looking for one or two markers that are high or low then trying to diagnose and treat an illness," Jones said. "Here, we can potentially measure a collection of proteins at the same time and not just focus on one guess. We've never been able do that before."

Other scientists in the field of systems biology said that micro-western arrays would make possible experiments that were previously beyond the scope of laboratory methods.

"I think this is really a breakthrough technology that allows us to monitor in close to real time the activity profiles of modified signaling proteins, which is essentially impossible right now," said Andrea Califano, professor of biomedical informatics at Columbia University. "This opens up a completely new window in terms of the molecular profiling of the cell."

"One of the biggest hurdles for systems biology is the struggle for high density, dynamic and quantitative data, and the micro-western array method will go a long way to address this problem," said Walter Kolch, director of Systems Biology Ireland and Professor at University College Dublin. "It is a fine example of generating exciting new technology from applying a new idea to an old method."

**More information:** The paper, "Systems analysis of EGF receptor signaling dynamics with micro-western arrays," will be published online in *Nature Methods* on Sunday, January 24th.

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