in which selected antibodies are spotted on to a substrate and are then exposed to the sample. Detection can be performed directly with a labelled sample, although this can increase the background noise. Many researchers prefer 'sandwich' formats in which a second, tagged antibody is applied that recognizes a different epitope from the capture antibody. This considerably improves sensitivity and the signal-to-noise ratio, but also requires two high-quality antibodies for every target.

Antibody arrays have been commercially available for some time, but companies have taken different approaches to them. Sigma-Aldrich of St Louis, Missouri, for example, has several arrays in its Panorama product family, each containing 100–200 antibodies targeting proteins involved in processes such as signal transduction or gene regulation. The company also plans to release a broad-content array with more than 700 antibodies. "Our current pathway arrays will be included, in addition to various other signalling and regulatory gene-product antibodies," says market-segment manager Richard Pembrey.

TeleChem, on the other hand, leaves antibody selection to the users for its ArrayIt microarrays. "For now, it's custom work just because a full proteomic complement of antibodies is still expensive for most researchers," says Schena. "But our printing technology scales pretty nicely, and our products can span a pretty wide spectrum, between 100 elements and 50,000."

After encountering limited success with capture arrays in their clinical research, Liotta and Petricoin developed the reverse-phase protein array, in which samples are spotted on the array and then probed with detection antibodies, requiring only one antibody per analyte. Much of their work has centred on signalling pathways in human disease, and this technique has worked well with their research, they say. "From a few thousand cells obtained by laser microdissection, we can look at hundreds of phosphorylation endpoints quantitatively, and look at a target and all the downstream signalling around it," says Liotta.

Such sample-specific arrays are difficult to commercialize, although Zeptosens is attempting to address the needs of this community with its cell lysate array (CeLyA) product line, which provides users with protocols, reagents and equipment to prepare chips for reverse-phase experiments. The company also has an active service division. "We deposit roughly the content of one or two cells per spot," says Ehrat, "and we can monitor changes of 15% from control to a treated sample."

But both approaches face a key limitation: antibodies. "A lot of users have two problems," says Mathias Uhlén, a researcher at the Royal Institute of Technology in Stockholm, Sweden, and chair of the Human Antibody Initiative of the Human Proteome Organisation (HUPO). "One is that you buy reagents, and half of them don't work in your application, and the second is that it's not easy to buy 200 antibodies that work on a single platform." Part of the solution lies in thorough validation, but other problems arise from the feature density of today's arrays and the broad range of protein expression. "Even if you have an antibody with picomolar affinity for your target, where the background is micromolar; if you have cross-reactivity against a protein that is 10^6 times more abundant, you will see that protein first," Uhlén says. Some are exploring alternative affinity reagents, such as recombinant single-chain antibodies or nucleic-acid aptamers (see 'An apt solution?'), but most in the field still see the limits of antibodies as secondary to their strengths. "So far, the good old antibody is still going strong," says Uhlén.

All together now

High-content protein microarrays have brought the classic protein–protein interaction assay to levels of throughput previously only possible with two-hybrid assays. These 'proteome chips' originated in Snyder's lab, in the form of arrays composed of protein products from nearly 6,000 yeast open reading frames. These chips are now available from Invitrogen of Carlsbad, California, which has continued to develop these and other arrays as part of its ProtoArray product line. Invitrogen sells both human and yeast proteomic arrays in a variety of formats, designed for use in protein–protein interaction assays as well as functional studies.

Developing arrays of soluble — and ideally, functional — protein at this scale poses considerable challenges, says Paul Predki, vice-president of proteomics at Invitrogen. "Our latest array product has more than 8,000 human proteins, and you can imagine the challenges

(ALMOST) NO ASSEMBLY REQUIRED

Each stage in the building of a protein chip — expression, purification, immobilization — adds a layer of experimental complexity, as each feature may need its own optimization process to ensure consistent quality. "We thought we needed a better way to do this," says Joshua LaBaer, director of the Harvard Institute of Proteomics.

The solution that he and his team arrived at was the 'nucleic acid programmable protein array' (NAPPA), in which cDNAs encoding GST fusion proteins are arrayed on chips alongside antibodies that recognize GST. The array is then subjected to cell-free transcription and translation; as protein is produced, it gets bound by an antibody and presented for analysis. According to LaBaer, NAPPA has simplified his group's research. "You don't have to purify proteins — you just purify DNA, so it's pretty easy, and it's been successful for printing about 95% to 96% of the things we make," he says. "And when we do protein–protein interactions, we're getting interactions that make sense and not a lot of false positives."

Joshua LaBaer uses self-assembling arrays to bypass problems with proteins.

Although initial arrays were limited in size, LaBaer and his team have since generated NAPPA arrays with up to 2,000 features, and they hope to surpass this soon.

Other techniques even bypass DNA immobilization. In the protein *in situ* array (PISA) developed by Michael Taussig of the Babraham Institute in Cambridge, UK, cDNAs are amplified *in situ* with primers that encode polyhistidine tags, so that proteins can be captured on a nickel–NTA-coated surface.

More recently, Philipp Angenendt of the German Cancer Research Center in Heidelberg transferred the PISA principle to a microarray set-up, integrating cell-free production of histidine-tagged protein from unpurified PCR fragments with a multiple spotting technique (MIST) previously developed by his group. MIST uses automation to apply array reagents precisely and sequentially to specific spots. As a result, each transcription/translation reaction is confined to a tiny, sub-nanolitre droplet, allowing greater density — up to 13,000 spots at present. "The nice thing about it is that the proteins expressed remain in a liquid environment," says Angenendt, "and the structure should be as intact as it can be with a solid-phase immunoassay format."

Both Angenendt and LaBaer are now fine-tuning their processes. "We're really working on large-scale screens, doing biomarker and protein interaction studies," says LaBaer. "We've also got some preliminary enzymatic data that look promising." M.E.

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