chromatography (LC)–MS/MS is gaining in acceptance and is generally more amenable to automation. If quantification is required, the proteins or peptides can be labelled with stable isotopes to enable measurement of differential levels of protein expression. One option is the site-specific, covalent labelling of proteins with isotope-coded affinity tags such as the ICAT reagents from Applied Biosystems in Foster City, California.

Technologies for analysing biomolecular interactions, such as surface plasmon resonance (SPR), are also hitching themselves up to MS. BIAcore of Uppsala, Sweden, has integrated its BIAcore 3000 SPR-based instrument with MALDI–MS analysis so that proteins bound to the ligand on the sensor surface can be automatically recovered and deposited directly onto the MALDI plate for further analysis by MALDI-TOF or TOF/TOF–MS.

Good preparation

But no matter what the analytical method, good sample preparation is essential. Fractionation methods such as centrifugal ultrafiltration, immunoaffinity purification and free-flow electrophoresis (FFE) can be used to reduce the complexity of the sample and enrich rare proteins before processing by 2DE or LC–MS. Tecan in Männedorf, Switzerland, sells the ProTeam FFE workstation, a matrix-free FFE fractionation system for processing proteins, organelles, membrane fragments or whole cells. The sample flows continuously in a thin film of aqueous medium between two parallel plates. The application of a high voltage generates an electric field perpendicular to the laminar flow. Charged species are deflected, allowing fractions to be collected on a fast, preparative and continuous basis.

The Gyrolab MALDI SP1 from Gyros in Uppsala, Sweden, is a microlaboratory in the form of a compact disc on which sample preparation procedures (sample clean-up and concentration) for peptide mapping or sequencing by MALDI–MS are miniaturized and integrated. Gyros also sells the MALDI IMAC for concentrating, purifying and crystallizing phosphorylated peptides directly onto MALDI target areas on the CD. Gyrolab workstations and kits are available for use with MALDI mass spectrometers from Shimadzu-Biotech in Kyoto, Japan, and Bruker Daltonics in Billerica, Massachusetts. MassPREP target plates from Waters-Micromass of Milford, Massachusetts, are designed for use with the company's robotic protein-handling system (MassPREP Station) and the Waters MALDI-TOF family of mass spectrometers. They provide fast, simple 'on-plate' preparation of protein digests and are designed to offer a tenfold increase in sensitivity over conventional target plates.

Several companies now sell automated equipment for spotting proteins onto MALDI targets. The Microlab Star MALDI Spotting Workstation from Hamilton Bonaduz, Switzerland, for example, has a volume range of 0.5–1,000 microlitres and can spot a 384-well target in 1 or 2 hours, depending on the number of channels fitted. The Ettan Spot Handling Workstation 2.1 from Amersham Biosciences in Piscataway, New Jersey, automates the process from spot picking from 2D gels through to digesting, drying and spotting the resultant peptides onto the target plates. A sample loader that will automatically place the target plates into the mass spectrometer is in the works.

With many of the component techniques now automated, the next trick is to bring them together. Start-to-finish automated proteomics systems are beginning to appear. Last year, Proteome Systems launched ProteomIQ (see 'When the chips are down', below). Others hope to do likewise. Amersham Biosciences has joined forces with Thermo Electron in Waltham, Massachusetts, and Waters-Micromass has partnered with Bio-Rad of Hercules, California, to develop ProteomeWorks.

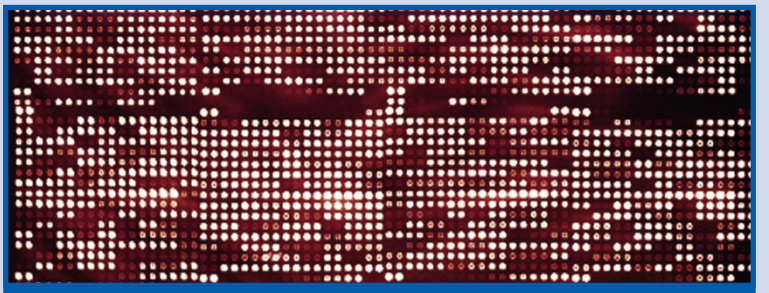
Alternative angles

A quite different approach to probing protein activity and function is the protein microarray, of which there are two basic types. The analytical microarray contains an ordered array of protein-specific ligands, typically antibodies, spotted onto a derivatized solid surface. They can be used to monitor differential protein expression, protein profiling and clinical diagnostics. But progress here is constrained by a lack of comprehensive sets of high-specificity, high-affinity antibodies.

UK-based Cambridge Antibody Technology is tackling this problem by using phage display to create large libraries of antibodies

WHEN THE CHIPS ARE DOWN

Off-the-shelf protein arrays are just beginning to trickle onto the market. But most have been low-density antibody arrays for profiling cytokines, secreted proteins that act as intercellular chemical messengers. Panomics of Redwood City, California, produces TranSignal Human Cytokine Antibody Array 2.0 for profiling the expression of 21 well-studied human cytokines. The array uses standard chemiluminescence detection and detects soluble cytokines at concentrations in the picogram per millilitre range. Zyomyx of Hayward, California, offers a human cytokine biochip for profiling 30 cytokines, which is sold for use with its Protein Profiling Biochip System, which includes an automated workstation, biochip reader and data-analysis software.



The Yeast ProtoArray from Protometrix contains some 5,000 proteins.

BD Biosciences Clontech in Palo Alto, California, sells an off-the-shelf antibody array for detecting a variety of cytosolic and membrane-bound proteins relevant to signal transduction, cell-cycle regulation, gene transcription and apoptosis. Its Antibody Microarray 500 contains over 500 antibodies printed on a standard-size (75 × 25 × 1 mm) glass slide and uses fluorescence-based detection. It is designed for qualitative rather than quantitative analysis but can detect differences in protein abundance between two samples with detection limits of 20 pg ml^{-1} for each protein target.

On the protein-function microarray front, Protometrix of Branford, Connecticut, will later this year roll out its first product, the Yeast ProtoArray, which stems from the pioneering protein microarray work of Michael Snyder's group at Yale University. This 'proteome' microarray will contain some 5,000 proteins in the *Saccharomyces cerevisiae* (yeast) proteome, including over 1,500 with significant homology to human proteins.

"The biggest bottleneck for everybody is still content," says Barry Schweitzer, Protometrix's senior director for technology. The company has now industrialized the cloning, expression and protein-purification process. "It's just a factory running 24/7," he says. "Everything that we clone we also have to sequence." The Yeast ProtoArray is targeted more towards the research market but Protometrix expects to follow this up with products of direct relevance to drug discovery. **D.G.**