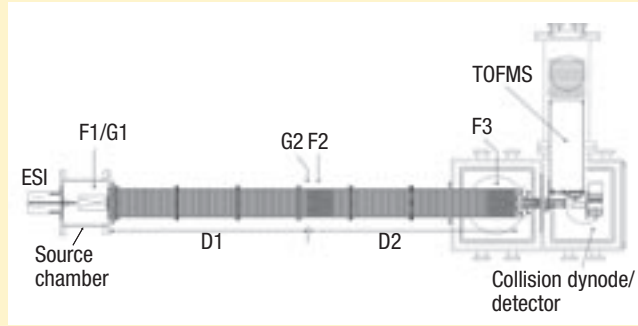
Multidimensional IMS

Ion mobility spectrometry (IMS) is known for its millisecond analysis times, but its resolving power is nothing to write home about. To improve the resolution, David Clemmer and colleagues at Indiana University and Pacific Northwest National Laboratory (PNNL) have introduced another dimension of IMS. Multidimensional IMS could be particularly useful for applications such as proteomics, in which low levels of peptides are analyzed in plasma and other complex bodily fluids.

IMS/IMS can be thought of as an analog of MS/MS. Ions are isolated and fragmented after the first IMS region. The fragment ions that are produced are then dispersed into a second IMS region before additional activation and MS analysis. The main difference is that in IMS/IMS, an ion is resolved according to its shape-to-charge ratio (mobility) rather than its *m*/*z*.

Multidimensional IMS makes it possible to observe small signals in complex mixtures by dispersing the noise of the system. The technique was facilitated by Richard Smith's work at PNNL on ion funnels, which essentially provide a way to overcome diffusion. Ion funnels allow the ion cloud to diffuse into a large sphere. Then, rf and dc fields can be used to collapse the cloud back into a point.



Into the next dimension. Multidimensional IMS can be performed in a modified IMS/TOFMS instrument. Drift regions (D1 and D2), ion funnels (F1–F3), and ion gates (G1 and G2) are labeled.

Clemmer and colleagues have used IMS/IMS to separate fragment ions of insulin chain B and ubiquitin that were produced by collision-induced dissociation (*Anal. Chem.* **2006**, *78*, 2802–2809). The resulting spectra were similar to those obtained in previous work, in which the researchers used IMS/MS methods to analyze fragments generated by MS/MS in an ion trap. The multidimensional IMS system, which does not include a mass spectrometer, had higher resolution and sensitivity than an IMS/MS system. IMS/IMS allowed the researchers to observe several features that were not readily discernible by MS analysis. (*Anal. Chem.* **2006**, *78*, 4161–4174)

What's in that organelle?

One way to "encourage" people to interact is to lock them in a room. The cell does something similar: To encourage particular proteins to interact, the cell tucks them away in the same membrane-bound compartment, or organelle. This compartmentalization also prevents the proteins from interacting with other sets of proteins at the wrong time or the wrong location.

To get a global view of the proteins that reside in various mammalian organelles, two groups have undertaken proteomics approaches. Andrew Emili and co-workers at the University of Toronto, the Hospital for Sick Children, and McGill University (all in Canada) performed comprehensive shotgun proteomics analyses of four mouse organellar compartments in six organs. In another study, Matthias Mann and coworkers at the University of Southern Denmark, the University of British Columbia (Canada), the Max Planck Institute for Biochemistry (Germany), the Chinese Academy of Sciences, the Broad Institute, and Harvard Medical School used a proteomics method called protein correlation profiling (PCP). With this method, the researchers mapped the subcellular locations of 1404 mouse liver proteins.

Emili and co-workers isolated cytosol, membrane-derived microsomes, mitochondria, and nuclei from mouse brain, heart, kidney, liver, lung, and placenta. Proteins were identified by multidimensional protein identification technology (known as MudPIT). About 8 million spectra were acquired in replicate experiments. Of the 4768 proteins that were identified, 3274 were localized to various organelles. Surprisingly, almost half of the identified proteins had not been localized to an organelle in previous studies. The researchers also compared their results with those obtained by two mRNA expression studies and found 1758 gene products in common. For most of these gene products, transcript expression levels were similar to protein expression levels. Several proteins also were localized by immunofluorescence. (*Cell* **2006**, *125*, 173–186)

In the Mann et al. study, the components of a homogenized mouse liver were separated by gradient centrifugation. Organelles with different densities were found in different fractions of the gradient. Proteins within the fractions were digested with a protease, then identified by LC/MS/MS. The researchers used PCP to pinpoint the positions of organelle-specific marker proteins in the density gradient. With this method, they used MS intensity profiles to locate proteins within 10 compartments. Of the 2197 unique liver proteins that were identified, they localized 1404 to organelles with the PCP method. These organelle assignments were compared with UniProt and Gene Ontology data; they were correct 87% of the time. The whereabouts of selected proteins were verified by immunofluorescence methods. The researchers also used genomics methods to find transcriptional regulators and sequence motifs that were involved in the biogenesis of particular organelles. (*Cell* **2006**, *125*, 187–199)

TOOLbox

Metabolite projection analysis

Manual interpretation of multivariate models and NMR spectra is a time-consuming and difficult task. So, Hans Senn and co-workers at Hoffman La Roche, Ltd., have developed metabolite projection analysis (MPA) to automate the process. Without human intervention, MPA identifies significantly changed metabolites in NMR spectra. Statistical techniques such as principal component analysis (PCA) and partial least squares (PLS) are used with the new method. Metabolites are visualized as dots on a graph; those that are unchanged between conditions appear as a cloud, and those that differ lie outside the cloud.

The researchers tested the method with data obtained from a study of the effects of the drug amiodarone, which induces phospholipidosis, on rats. MPA generated fewer false positives with non-equidistant binned spectra than with equidistant binned spectra from PCA models. Similar results were obtained when MPA was used on PLS-derived models. According to the researchers, MPA also could be applied to other spectroscopic methods, such as LC/MS, MS/MS, and IR. (*Anal. Chem.* **2006**, *78*, 3551–3561)

2DDB for proteomics data

Lars Malmström and co-workers at Lund University (Sweden) have developed a bioinformatics solution that allows users to store and analyze proteomics data from multiple types of experiments. Called 2DDB, the information platform handles quantitative data from 2DE and LC/MS/MS experiments. Instead of accession numbers, multisequence identifiers (MIDs) are used to keep track of proteins in the system. Whereas accession numbers are database-specific and refer to only one form of a protein, MIDs are more general in scope. MIDs are based on protein sequences, and many forms of the same protein (i.e., polymorphisms and fragments) are grouped under the same MID. In addition to models for data storage, 2DDB includes several bioinformatics tools. Experiments entered into 2DDB also can be compared. 2DDB can be accessed at www.2ddb.org. (*BMC Bioinformatics* **2006**, *7*, 158)

Variables that affect serum and plasma profiling

Sen-Yung Hsieh and co-workers at Chang Gung Memorial Hospital (Taiwan) have studied several variables that could alter the low-molecular-weight profiles of human serum or plasma samples. Of these variables, sample-collection procedures had the greatest effect. The researchers found that identical serum samples collected in two types of tubes, with or without a gel separator, had different profiles. Identical samples treated with the anticoagulants heparin, EDTA, and sodium citrate to produce plasma samples had different profiles; these profiles were also different from serum profiles. Mechanical hemolysis, or cell breakdown, also caused measurable changes.

Surprisingly, other factors that have been suspected of altering protein and peptide profiles were found to have only minor effects. For example, eating a meal a few hours before sample collection or the subject's sex or age did not cause drastic changes. In addition, several sample-handling methods, such as repeated freeze/thaw cycles and centrifugation speeds, had little or no effect on protein and peptide profiles. (*Proteomics* **2006**, *6*, 3189–3198)

Which cows are on steroids?

Farmers sometimes give their cows growth-promoting agents (GPAs), such as steroids, to produce muscle-bound animals. Enhancing meat production with GPAs is controversial in the U.S. and illegal in the EU; reports in the literature suggest that GPAs in meat may be harmful to humans. However, these agents are difficult to detect by conventional techniques because doses given are often low and because molecules with unknown structures and properties are occasionally used.


PHOTODISC

Have you been working out? Calves treated with steroids can be differentiated from control calves by proteomics techniques.

Instead of trying to detect the presence of GPAs directly, Carlo Nebbia and colleagues at the Università degli Studi di Torino, Università degli Studi "G. d'Annunzio" Chieti e Pescara, Fondazione Università "G. d'Annunzio," and Bioindustry Park Canavese (all in Italy) took a different approach. They used proteomics to find proteins that were differentially regulated as an effect of illicit GPA treatment. They found two potential biomarkers that could be incorporated into tests for GPAs.

Nebbia and colleagues obtained liver tissue from six control and six GPA-treated veal calves. In the study, treated calves were injected with 17β-E, clenbuterol, and dexamethasone, which are commonly used GPAs. When they ran whole-liver samples on 2DE gels, the researchers found that these low-resolution gels were difficult to analyze. So, they isolated subcellular fractions of the liver tissue and ran cytosolic and microsomal fractions from each calf on separate 2DE gels. The gels were stained with Coomassie Blue and quantitatively analyzed.

Only one spot in each of the two fractions differed significantly between the control and treated calves. The protein in the cytosolic spot was identified as adenosine kinase (AK); this spot was decreased in intensity by more than twofold in treated animals. The researchers verified by western blotting that the level of AK was lower in treated calves than in control calves. The protein in the microsomal spot was tentatively identified as reticulocalbin. According to the researchers, results suggest that proteomics is a promising method for discovering proteins that are differentially regulated in response to GPA treatment. (*Proteomics* **2006**, *6*, 2813–2822)

Plastic chip for 2D protein separations

Steven Soper and Hamed Shadpour of Louisiana State University have developed a plastic microfluidic chip on which proteins are separated in two dimensions: SDS microcapillary gel electrophoresis (SDS-μCGE) and micellar electrokinetic chromatography (MEKC). The new chip can separate a mixture of proteins ranging from 38 to 110 kDa in ~12 min.

Protein mixtures are often separated in two dimensions on slab gels by isoelectric focusing followed by SDS-PAGE. However, this method is labor-intensive. In their chip-based approach, Soper and Shadpour used SDS-μCGE, which separates proteins by size in a gel inside a capillary, and MEKC, which separates proteins according to how they interact with micelles. The combination of gel- and CE-based methods increased the orthogonality, resolution, and efficiency of the separation compared with conventional techniques.

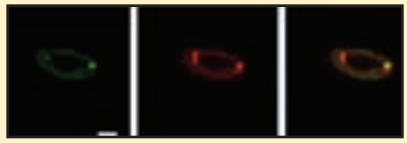
The investigators labeled >10 commercially available proteins with the Alexa Fluor 633 fluorophore and used laser-induced fluorescence for detection. The results demonstrated that the 2D separation had a peak capacity (estimated to be 1000) significantly higher than that of the corresponding individual 1D separations. But because modifying proteins with fluorophores is time-consuming, Soper and Shadpour are now looking to detection methods that don't require labels, such as conductivity and MS. (*Anal. Chem.* **2006**, 78, 3519–3527)

The lipid droplet subproteome

Observed under a microscope, lipid droplets may look like spots of oil, but researchers have recently learned that these greasy drops are not just passive balls of fat; the droplets may be actively involved in several cellular processes, such as vesicular transport and lipid trafficking. Lipid droplets are coated with proteins that could direct these processes. So, Ronald Kühnlein and co-workers at the Max Planck Institute for Biophysical Chemistry (Germany) and the German Research Center for Biotechnology (known as GBF) conducted a proteomics study to get a comprehensive picture of the proteins associated with lipid droplets. They identified several proteins in the lipid droplet proteomes of four *Drosophila melanogaster* larvae genotypes.

Lipid droplets were obtained from larval fat body cells and purified by sucrose-gradient density centrifugation. Proteins were released from the droplets by delipidation and precipitation. The proteins were run on 1DE gels and analyzed by LC/MS/MS.

The researchers analyzed proteins from wild-type flies and 3 mutants: *adp*^{60} and induced *Lsd-2*:EGFP (enhanced green fluorescent protein), both of which are genetically predisposed to obesity, and *Lsd-2*^{51}, genetically predisposed to leanness. A total of 248 proteins were identified from all 4 genotypes. Of these proteins, 60 were common to all larvae. Only a few proteins were expressed exclusively in one genotype. For example, regucalcin was specifically identified in *adp*^{60} flies. According to the researchers, a mouse homologue of this protein was recently found to affect lipid storage and body weight.



Oil droplets with a protein coating. The short-chain dehydrogenase CG2254 (green) surrounds a subset of lipid droplets (red) in fat cells. Scale bar = 4 μm. (Adapted with permission. Copyright 2006 American Society for Biochemistry and Molecular Biology.)

To validate their findings, Kühnlein and co-workers labeled three of the identified proteins with EGFP in cultured *Drosophila* cells or in flies. One of the proteins was localized to a subset of lipid droplets. The other two were found in the cytoplasm and in a subpopulation of lipid droplets. The researchers will use mutants to determine whether some subpopulations of lipid droplets have different functions. (*Mol. Cell. Proteomics* **2006**, doi 10.1074/mcp.M600011-MCP200)

TOOLbox

Real-time peptide–spectrum matching

Spectra are typically matched to peptides after LC/MS/MS data are collected. One program, such as Sequest or Mascot, is used to predict the identity of the peptide, and another program, such as PeptideProphet, is used to evaluate the likelihood that the peptide is correct. Real-time analysis of peptide–spectrum matches (PSMs), however, would enable on-the-fly identifications. So, Guy Poirier and colleagues at Laval University Medical Research Center and the University of Saskatchewan (both in Canada) developed RT-PSM, an algorithm that identifies peptides and determines the statistical significance of those matches in real time for 3D ion trap or Q-TOF mass spectrometers. Unlike other PSM programs, RT-PSM searches spectral data against a sorted database of tryptic peptides instead of a database of proteins. The researchers tested RT-PSM on two sets of MS/MS data and obtained good sensitivity and specificity. (*Rapid Commun. Mass Spectrom.* **2006**, *20*, 1199–1208)

Integrator

Ram Samudrala and colleagues at the University of Washington have developed Integrator, a web-based search tool for retrieving and analyzing protein–protein interaction (PPI) data. Interaction data are provided by the Bioverse database, which is composed of experimentally derived and predicted PPIs for >50 genomes. Users can search for information about a single protein or many proteins at the same time. For single-protein queries, the program produces an interaction map for the protein. Nodes (proteins) and edges (interactions) can be added or subtracted from the map. When the user moves the cursor over a node, a tool tip with hyperlinked annotations is accessible; over an edge, tool tips with database source information and confidence values are accessible. The data also are displayed in tables of node and edge information. For multiple-protein queries, only the queried PPI searched is displayed. Integrator is freely available at http://bioverse.compbio.washington.edu/integrator. (*BMC Bioinformatics* **2006**, *7*, 146)