TOOLbox

PROMPT for comparisons

After identifying proteins by MS, researchers often want to know whether the list includes proteins with certain functions. To perform this analysis, they must integrate sequence and annotation data from many sources that may not be compatible. So, Dmitrij Frishman and Thorsten Schmidt at the Munich University of Technology have developed a software system called protein mapping and comparison tool (PROMPT). The tool uses sequence information to match protein entries with different accession numbers. Protein properties, such as p*I* or functional categories, are compared, and statistical analyses are conducted. For example, researchers used PROMPT to examine the structural differences between two sets of *E. coli* proteins. Results are visualized as plots or spreadsheets and can be exported in many formats. Academic users can access PROMPT for free at http://webclu.bio.wzw.tum.de/prompt. (*BMC Bioinformatics* **2006**, *7*, 331)

msInspect

Martin McIntosh and co-workers at LabKey Software, the Fred Hutchinson Cancer Research Center, and the University of Washington have developed a bioinformatics software platform for the visualization and quantitation of LC/MS data that is called MS in silico peptide characterization tool (msInspect). With the new platform, data from experiments involving isotope labeling or label-free methods can be analyzed and compared.

The graphical interface allows users to view their LC/MS data as a heat map, with retention time on the *x* axis and *m*/*z* on the *y* axis. The brightness of the color on the heat map indicates the signal intensity. Users can zoom in and out of the map and view 1D cross-sectional slices of the data. The researchers applied the algorithms to the quantitative analyses of human serum samples that were labeled with the isotope-coded affinity tag (known as ICAT) method or that were unlabeled. In addition, the programs in the msInspect platform (available at http://proteomics.fhcrc.org) were used to distinguish two bacterial strains. (*Bioinformatics* **2006**, *22*, 1902–1909)

Phosphoproteomics of a signal transduction pathway

Her2, a tyrosine kinase, is overexpressed in 20–30% of human breast cancer tumors. To better understand the Her2 signaling pathway, Akhilesh Pandey, Philip Cole, and co-workers at the Johns Hopkins University School of Medicine and the Institute of Bioinformatics (India) have used stable-isotope labeling with amino acids in cell culture (known as SILAC). The researchers compared cells that overexpressed Her2 with control cells and found ~200 proteins with increased phosphorylation and 81 proteins with decreased phosphorylation.

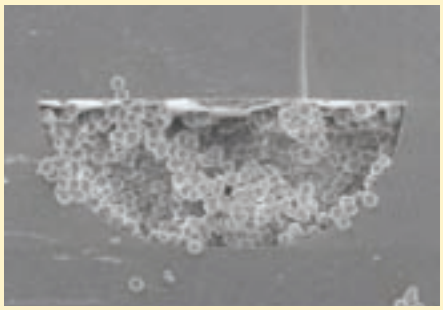
The researchers grew 3 sets of NIH 3T3 cells in different media. Control cells that had been transfected with a vector were grown in regular arginine-containing media, cells that overexpressed Her2 were grown in "heavy" arginine media ($^{13}C_6{}^{15}N_4$-Arg), and cells that overexpressed Her2 and had been treated with a Her2 inhibitor were grown in "medium" arginine media ($^{13}C_6$-Arg). To discover whether Her2 overexpression or inhibitor treatment affected the phosphorylation of proteins, the researchers mixed the cell lysates and isolated the phosphotyrosine-containing proteins with antibodies against phosphotyrosine. The proteins were run on a 1DE gel and then digested and analyzed by LC/MS/MS.

A total of 462 proteins were identified and quantified. Of the 198 proteins that had increased phosphorylation in the overexpressing cells, many were known to be involved in Her2 signaling. In addition, several proteins, such as Stat1, Dok1, and δ-catenin, were identified as Her2 signaling proteins for the first time. The phosphorylation changes of some of these proteins also were verified by western blotting.

The researchers used 2 methods to figure out the roles of the identified proteins in the Her2 pathway. In one approach, they obtained protein–protein interaction data from the Human Protein Reference Database (known as HPRD) and found that 2 of the proteins directly linked the Her2 and epidermal-growth-factor receptor pathways. The researchers also used Bayesian networks to model the Her2 pathway on the basis of the data obtained in this study and 3 additional studies. The modeled network was similar to the known pathway and enabled the researchers to predict how other proteins may fit into the pathway. (*Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 9773–9778)

A step toward proteomics on a chip

Iulia Lazar and colleagues at the Virginia Polytechnic Institute and State University have developed a microfluidic LC system for proteomics. The new device includes all of the components necessary for separations, including a separation channel, micropump, valve, and ESI interface for MS analyses.



Small separations. Scanning electron microscopy image of a cross section of a microfluidic LC channel packed with 5-μm particles.

Lazar and colleagues compared the performance of the chip with that of a benchtop micro-HPLC system by running a strong-cation-exchange fraction of an extract from breast cancer cells on both and analyzing the separated peptides by MS. In an initial pass, the researchers identified ~10× fewer proteins with the microfluidic system. But when the column length, buffer composition, and sample-injection volumes of the benchtop system were adjusted to match those used with the chip, a similar number of identifications were made. All five potential biomarker proteins that were previously identified with the benchtop system also were found with the LC chip. The researchers plan to develop the microfluidic system into a disposable lab-on-a-chip device. (*Anal. Chem.* **2006**, *78*, 5513–5524)