

TOOLbox

Network cartography

Luís Nunes Amaral and Roger Guimerà at Northwestern University have developed a new way to display biological network information. The cartographic method links components within a pathway into modules according to functional relationships.

In the new approach, nodes within modules are treated analogously to people within social networks. Just as many people socialize within a main group of friends, many proteins and metabolites typically interact with a limited number of other molecules. Some proteins and metabolites, however, interact with molecules within other modules.

The researchers classified molecules within a metabolic network into hubs and nonhubs for 12 organisms. The metabolites were then subclassified into different roles, depending on the participation coefficient that was calculated for each molecule. Most modules contained metabolites that function together within a particular pathway. Both new and previously known connections were found with the method. (*Nature* **2005**, *433*, 895–900)

Unexpected modifications

Although search algorithms can be used to identify modified peptides, most are only capable of searching a limited number of modified species, and the user must specify the modifications. To overcome these limitations, Wilfred Tang and colleagues at Applied Biosystems have developed Interrogator, a new search algorithm that can efficiently find numerous peptides with either known or unknown modifications.

According to the researchers, the new algorithm is 100× faster than conventional database search algorithms for comprehensive searches of a wide range of modified peptides. To demonstrate the power of the algorithm, Tang and colleagues used it to examine 473 MS/MS scans. Of those, 315 corresponded to unmodified peptides and 158 to various modified peptides (*Anal. Chem.* **2005**, *77*, doi 10.1021/ac0481046)

Isolating membrane proteins

Most researchers who study plasma membranes (PMs) disrupt cells by mechanical or osmotic methods, then separate subcellular organelles by centrifugation on the basis of size and density. But clinical samples are often frozen, and the freezing process can damage subcellular organelles. Therefore, Jacek Wiśniewski and co-workers at Protana (Canada), MDS (Denmark), and the University of Southern Denmark have developed a new way to isolate PMs. The new method does not rely on the isolation of subcellular organelles, so membrane proteins can be isolated from frozen samples.

Wiśniewski and co-workers isolated membranes by shearing brain tissues at high speed in solutions that removed soluble proteins. To isolate PMs from the remaining membranous material, the researchers performed density-gradient fractionation on the sample and treated the lower-density frac-

tions with digitonin. PM proteins were digested with endoproteinase Lys-C, and the peptides were separated on a reversed-phase column. The collected fractions were treated with trypsin. The researchers analyzed each fraction three times by LC/MS/MS with a Q-TOF instrument. They also adapted the method for use with smaller amounts of sample by using a linear ion trap Fourier transform MS instrument for MS/MS analysis.

The researchers used the new technique to identify 862 brain PM proteins from a sample of mouse cortex. They observed few contaminating proteins from other membranous structures. With the adapted method, they also analyzed a sample of mouse hypothalamus and identified >1600 proteins. Many expected proteins were isolated, including neurotransmitter receptors and gated channels, prion proteins, and a protein involved in Alzheimer's disease. (*Mol. Cell. Proteomics* **2005**, *4*, 402–408)

Low-abundance proteins in hemodialysis fluid

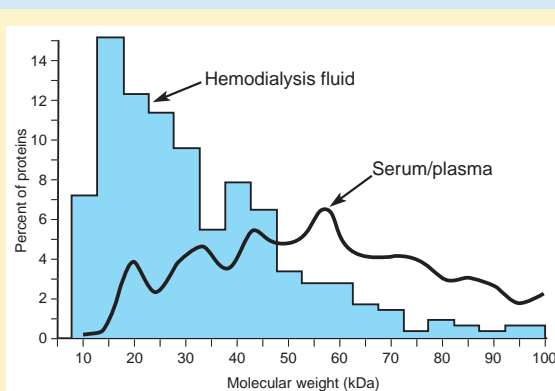
Although proteomics approaches have been used to extensively study human serum and plasma, a comprehensive analysis of the proteins in these fluids has proven difficult because of the overwhelming percentage of high-abundance proteins. To get a better view of low-abundance proteins, Akhilesh Pandey and colleagues at Johns Hopkins University, the University of Southern Denmark, and the Institute of Bioinformatics, International Tech Park Ltd. (India) have turned their attention to human hemodialysis fluid.

The kidneys filter out proteins with molecular weights <66 kDa, so the protein content of hemodialysis fluid is much less than that of blood. The concentration of albumin, for example, is 5000× lower in hemodialysis fluid than in serum. At the same time, hemodialysis fluid contains a higher concentration of small proteins and polypeptides. These low-abundance proteins can be analyzed without interferences from high-abundance proteins.

Using gel electrophoresis and LC/MS/MS, Pandey and colleagues identified 292 proteins in human hemodialysis fluid. Of these, >70% had not been previously identified in serum or plasma. More than 50% of the proteins had molecular weights <40

kDa. Several, including cytokines, were novel proteins that were only present in databases as predicted transcripts.

The lack of overlap between the proteins found in hemodialysis fluid and those found in serum or plasma points to the limitations of current proteomics strategies. The researchers believe that more sensitive methods will enable the detection of these proteins in serum or plasma. (*Mol. Cell. Proteomics* **2005**, *4*, 637–650)



High and low. Molecular weights of proteins identified in hemodialysis fluid compared with those of proteins previously reported in serum or plasma. (Adapted with permission. Copyright 2005 American Society for Biochemistry and Molecular Biology.)