ANALYTICAL SEMINAR

Thursday
October 14
12:15 p.m.
Room 1315

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Electrostatic Repulsion-Hydrophilic Interaction Chromatography (ERLIC) in Proteomics

ERLIC is a variation of HILIC performed on an ion-exchange column with the same charge as most of the analytes. This combination features unusual selectivity compared with other methods and has been applied to peptides, amino acids, nucleic acids and small oligonucleotides.

Isolation of peptides with PTM's:

ERLIC can be used for the selective isolation of phosphopeptides from tryptic digests. The same conditions also serve for isolation of glycopeptides; from an extract of mouse brain membranes, 41% of the phosphoproteins identified were also glycosylated. In total, 544 unique glycoproteins and 922 glycosylation sites were identified. Many but not all contained sialyl- groups. ERLIC, which is easy to implement and use, identified 3-5x more glycosylation sites and glycoproteins than hydrazide covalent chromatography. Analysis of the same fractions led to identification of 383 phosphoproteins and 915 phosphorylation sites.

ERLIC identifies 3x more phosphotyrosine sites than do other methods, and there is minimal overlap with the phosphopeptide set identified by SCX-IMAC. Thorough coverage of the phosphoproteome requires a combination of both methods.

Fractionation of peptides in general:

ERLIC of a tryptic digest of rat kidney tissue led to identification of 4821 proteins and 30,659 unique peptides with high confidence from two replicates. This was 36% and 64% higher, respectively, than was obtained using SCX fractionation. ERLIC identified over 120% more highly hydrophobic and basic peptides than did SCX. ERLIC could be implemented with totally volatile mobile phases, and afforded perhaps the most uniform distribution reported to date of tryptic peptides among the collected fractions. It is promising as a first dimension of multidimensional LC for fractionation of peptides.