Desorption Electrospray Ionization (DESI)

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DESI is a powerful new ionization method that combines features of desorption ionization (DI) with those of spray ionization, especially ESI. It allows MS to be performed in the ambient environment on samples that are fully accessible to manipulation and allows additional physical and chemical processing during analysis. DESI requires no sample pretreatment whatsoever and responses are almost instantaneous. Ionization is effected by spraying the sample with an electrically charged aqueous mist. The ions released from the surface are transported through air at atmospheric pressure to any mass spectrometer equipped with an atmospheric pressure interface.

Initial results on the applicability of DESI to metabolomics give evidence for high sensitivity, repeatability and long-term signal stability using urine samples examined on paper without de-salting or pre-separation.

The direct analysis of unmodified biological tissues at atmospheric pressure under ambient conditions has been demonstrated. For example, the results from mouse pancreatic tissue show characteristic signals due to the presence of phospholipids of the cell membrane. DESI mass spectra collected on two different regions of metastatic human liver adenocarcinoma tissue show the phospholipid distributions in the diseased tissue to be shifted towards more unsaturated fatty acid containing phospholipids. It is very simple to recognize the diseased and non-diseased tissues.

High throughput experiments are possible, for example on the active ingredients in pharmaceutical samples formulated as tablets, ointments and liquids. Compounds of a wide variety of chemical types are detected in these complex matrices. A variable speed moving belt allowed high throughput sampling. This experiment gave qualitative and semi-quantitative information on drug constituents in tablets. Sampling rates as high as 3 samples per second were achieved. Relative standard deviations of relative ion abundances for major components in the mass spectra were in the range of 2–8%. Impurities and components present at levels as low as 0.1% were also identified. No significant carryover effects were observed in high throughput on-line analysis of the pharmaceutical samples.

Protein/ligand affinity studies can be done in air, at high rates. This is achieved by reactive desorption, an experiment in which reagents are added to the solvent spray and when they bind strongly adduct ions are released and can be mass analyzed.

Bio-ion/Ion Reactions and Their Application to Protein Mixture Analysis

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Ion/ion reactions are particularly useful within the context of a tandem mass spectrometry experiment applied to peptides and proteins. Electrodynamic ion traps are uniquely well-suited as reaction vessels for ion/ion chemistry because both ion polarities can be stored simultaneously in overlapping regions of space. Furthermore, ions traps are well-known for their capabilities for multi-stage mass spectrometry experiments. Therefore, it is straightforward to design a mass spectrometry experiment that combines one or more ion/ion reaction steps with other types of reactions, such as collision-induced dissociation.

A variety of applications to the analysis of bio-polymer mixtures have been demonstrated involving ion/ion reactions in electrodynamic ion traps. These include, for example:

Bio-polymer mixture analysis: Electrospray ionization is typically not applied directly to mixtures of large biomolecules due to a high degree of spectral congestion associated with the overlap of charge state distributions arising from each mixture component. Ion/ion proton transfer reactions have been used to minimize spectral congestion by reducing charge states to as low as +1 or -1 so that the mass of each component can be determined.

Product ion charge state determination: Product ion spectra derived from the dissociation of multiply charged ions can be difficult to interpret when the charge states of the product ions cannot be determined directly from the spacings of the isotope peaks. Ion/ion proton transfer reactions have been used to convert all product ions largely to singly charged species thereby allowing for the straightforward determination of product ion masses.

Parent ion concentration and charge state purification: By taking advantage of the selective ion acceleration capability of ion traps, it has been demonstrated that ion/ion reaction rates can be inhibited in a selective fashion. This has allowed for almost all ions initially dispersed by electrospray over a range of charge states to be concentrated into a single charge state for subsequent ion activation. The process just mentioned has been termed "ion parking." Two consecutive steps of ion parking have been shown to be capable of charge state purifying a parent ion for subsequent ion activation. This capability has proved to be particularly useful when mixtures of proteins are subjected to electrospray.

Structural characterization: It has been demonstrated that electron transfer reactions lead to fragmentation that is highly analogous to that noted for electron capture dissociation. This form of ion dissociation is highly complementary to that obtained via collision-induced dissociation and significantly enhances the protein identification and characterization capabilities of tandem mass spectrometry.

Charge inversion: When multiply charged reagent ions are used for manipulate charge states, the polarity of the analyte ion can be inverted. Two such charge inversion steps in sequence can, with the use of appropriate reagents, lead to an increase in the net charge of an ion. This allows for the increase of the charge state of an ion in the gas-phase.

This presentation emphasizes the applications of these reactions to biopolymer mixture analyses. Particular emphasis is placed on recent work related directly to the identification and characterization of proteins in mixtures.
3.3 Methodology Advances in Ease of Use in Protein ID and Expression Analysis
Applied Biosystems, Foster City, CA

The complexity of current informatics tools is currently such that a scientist doing protein ID or protein expression analysis must have a substantial amount of experience in order to attain good results. Expert users frequently construct complicated multi-stage processing workflows using one or more software tools, various filtering steps, data reduction steps or transformation steps, and data resources in order to attain optimum results. Even with all this effort, as substantial amount of manual inspection is generally still necessary. Because of these complexities, proteomics as a field remains largely the realm of experts in mass spectrometry and bioinformatics. One key to the maturation of this field is the creation of methodologies that do not necessitate this level of expertise; we need methods that enable the average biologist to focus on asking interesting scientific questions, rather than the methodology.

The creation of Pro Group software has taken a significant step toward this goal in several ways. The most significant benefit is a protein confidence methodology that has a protein grouping analysis at its core. This has produced a substantial reduction in false positive protein identifications and meaningful assessment of the detection of multiple related protein forms. This eliminates one of the major reasons for substantial manual inspection. The application also offers advances in visualization of results that substantially aids the process of manual inspection, should it still be necessary. It also offers advances in protein expression analysis that are only possible with a protein identification approach that includes a protein grouping treatment.

We will present new methodology advances designed to improve ease of use with a focus on enabled biological applications such as the analysis of the natural peptidome in serum.

3.4 Application of Quantitative Phosphoproteomics in Drug Discovery Research
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Protein phosphorylation plays a key role in signal transduction. Deregulation of the process may lead to diseases such as cancer and metabolic disorders. Good understanding of the phosphorylation events will help dissect disease mechanisms and thus in rational drug design.

Due to the wide dynamic range of protein concentration in cells and tissues, enrichment is a prerequisite in order to detect proteins of low abundance. Different enrichment strategies have been developed for protein phosphorylation studies. In this report, we will present our effort in developing and implementing a quantitative phosphoproteomics strategy with the combination of proteolysis, peptide esterification, immobilized metal affinity chromatography and inverse labeling. The strategy has been successfully applied to mammalian cell and animal tissue analysis. Summary of the results will be presented. Discussions will cover the aspects of work flow, MS platform, data analysis, current challenges, and potential areas of application.

3.5 Quantitative Analysis of Cyclin B Ubiquitination Catalyzed by the Anaphase-promoting Complex
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In the current study, quantitative mass spectrometry has been coupled to classical biochemistry to build an emerging model for the ubiquitination and degradation of cyclin B by the anaphase-promoting complex and the 26S proteasome. The anaphase-promoting complex (APC) is a multi-subunit E3 ubiquitin-ligase that regulates cell cycle progression through the ubiquitination of many critical regulatory proteins. Ubiquitination of substrates by the APC stimulates their rapid degradation by the 26S proteasome. To characterize the ubiquitination of cyclin B by the APC, the Ubiquitin-AQUA (Absolute QUAntification of Ubiquitin) method was developed. AQUA utilizes isotope labeled internal standard peptides and selected reaction monitoring (SRM) experiments to quantify proteins and post translational modifications by mass spectrometry (MS). Here, by performing multiplexed SRM analyses in the presence of internal standard peptides corresponding to the various forms of ubiquitin, it was possible to determine the total amount of ubiquitin and the abundance of each ubiquitin-ubiquitin linkage within samples. The coupling of MS-based peptide quantitation with the law of conservation of mass allowed for the elucidation of structural features of ubiquitinated proteins including the average length of poly-ubiquitin chains, the frequency of each ubiquitin-ubiquitin linkage, and the number of modification sites per substrate. Using Ubiquitin-AQUA, we demonstrated that APC catalyzed ubiquitination of cyclin B by chains linked through multiple lysine residues within ubiquitin including K48, K63, K11, K33 and K6, but not through K27 or K29. For APC catalyzed reactions, the frequency of each poly-ubiquitin chain linkage differed depending on the E2 ubiquitin-conjugating enzyme utilized. Multiply mono-ubiquitinated forms of cyclin B represented a significant proportion of the small and intermediate conjugates produced by the APC, while high molecular weight forms of mult ubiquitinated cyclin B are modified by a series of short poly-ubiquitin chains instead of a single long chain. In conclusion, we propose that systematic application of the Ubiquitin-AQUA method should be undertaken in a variety of cellular systems to fully characterize the functional biochemistry which drives the ubiquitin-proteasome system as well as proteasome independent ubiquitin-signaling. In combination with biochemical and genetic approaches, Ubiquitin-AQUA should enable thorough dissection of protein ubiquitination.