1.1 Global Analysis of Small Molecule Interactions with Proteins

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Extensive effort has been made to characterize the interactions of proteins with other proteins, and for the case of transcription factors, their interaction with DNA. Natural small compounds comprise the majority of the cellular molecules, and have been shown to bind proteins as substrates, products, cofactors and regulatory ligands. Although they participate in such diverse processes, a large scale investigation of protein-small molecules has never been performed. We have developed a mass spectrometry assay for the global analysis protein-natural small molecule interactions in yeast, and applied this method to the analysis of molecules that bind lipid pathway biosynthetic proteins and protein kinases. We find that a large number of proteins bind small molecules. Some enzymes bind substrates, others bind their products, and many key regulatory proteins such as protein kinases bind small molecules. One common ligand bound by many proteins is ergosterol suggesting a general role for this compound in regulation. We further explore this by demonstration that the activity of the high conserved AKT protein kinase homolog of yeast depends upon the presence of ergosterol. Overall, our study helps define potential key regulatory steps in biosynthetic pathways and demonstrates that small molecules bind many proteins in a variety of biochemical and regulatory roles and suggest they can serve as regulators of protein activity.

1.2 Membrane-assisted Sample Preparation for Online ESI-MS Analysis of Biomolecules

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In addition to further improvements in resolution, mass accuracy, sensitivity and throughput, advances in sample preparation are essential in order to fully unveil the potential of any mass spectrometer. A technology based on ion-selective membranes has been developed in order to perform online sample preparation before electrospray ionization. The technique permits to change the chemical composition of the solution and manipulate analytes a few microliters upstream from the electrospray process. Compounds can be removed or injected from an adjacent channel to the main flow stream through an ion-selective membrane that separates both channels. In this manner, protons can be injected into the flow stream to carry out rapid pH scans; deuterons can be delivered to perform deuterium/hydrogen exchange experiments in order to study protein structural changes; and metal ions can be delivered into the flow stream to study -for example- metal-binding peptides. Furthermore, a device with two membrane sections can be used to introduce an electric field into the main channel in order to immobilize ions and perform pre-concentration, clean-up, multi-step micro reactions, solvent exchange and separation prior to mass analysis. Examples of each application will be shown as well as preliminary data of other applications.

1.3 Two-Dimensional Liquid Chromatography Coupled with ESI-MS for Protein Identification and Quantification

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Mass spectrometry is widely accepted as an essential tool to better understand protein function, facilitating both the identification and quantification of proteins in complex samples. Mass spectrometry based protein identification strategies have previously been described [1–3] that facilitate the simultaneous acquisition of qualitative and quantitative information, Indiana, USA a data independent fashion.

We have extended this approach to generate precise relative quantitation values for proteins contained in biological systems [4–5], and have constructed protein abundance curves for specific tissues, cell lysates and biofluids. This has been shown to be transferrable between different laboratories and independent of instrument type. An important aspect of this quantification approach is that it allows sample loading onto a given analytical column to be determined and optimized, to ensure that ideal chromatographic and mass spectrometric performance is obtained. This results in the maximum number of peptide and proteins being determined from the sample, whilst maintaining maximum accuracy for quantitative measurements. More recently this approach has been extended to cover a wider range of protein abundance by implementing a 2-dimensional reverse phase-reverse phase separation strategy, using differential pH. In this manner wide quantitative proteome coverage can be obtained.

Experimental information obtained from such studies will be compared to theoretical models of the given proteome; considering complexity, dynamic range and the inherent physiochemical properties of tryptic peptides in solution and the gas phase.

References

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Viruses have evolved finely tuned interactions with their hosts to manipulate and adapt complex cellular processes for their own use. The study of virus-host interactions has therefore emerged as a key driving force in the research of infectious disease during the post-genomic era. Despite these efforts, our understanding of the protein interactome remains, Indiana, USA large part, unknown. The development and incorporation of new approaches that can reveal the dynamics of virus-host protein interactions is a necessity. Modern proteomic techniques have the ability to provide access to such interactions, and the ever increasing sensitivity of mass spectrometry allows the identification and quantification of relatively low levels of proteins. This presentation will describe targeted proteomic approaches for studying virus-host macromolecular assemblies. Highlights will be shown from our studies on infections with human immunodeficiency virus (HIV) and human cytomegalovirus (HCMV).

We employed targeted genetic-proteomic approaches to study the virus-host interface either from the virus or host perspective. Using a library of tagged replication competent HCMV and HIV mutant viruses, we infected primary human fibroblasts (for HCMV) and CEM T cells (for HIV), and employed cryogenic cell lysis and rapid immunoaffinity purifications on magnetic beads to isolate virus-host assemblies. For studies on histone deacetylases (HDAC) during viral infections, we generated cell lines stably expressing green fluorescent protein tagged HDACs and probed their interactions and deacetylation activity. Isolated protein complexes were analyzed using MALDI LTQ Orbitrap (Thermo Fisher Scientific) and the specificity of observed interactions was confirmed by immunofluorescence, reciprocal immunoprecipitation and metabolic labeling with stable isotopes (I-DIRT).

Two interesting findings will be highlighted: 1) studies on pUL32, pUL99, pUL83 and pTRS1 HCMV proteins demonstrated that parallel processes occur at distinct cellular sites during the assembly of HCMV virions, and 2) chromatin remodeling complexes, including histone deacetylases, are targeted by viruses, possibly in part to gain control over host gene expression and modulate the outcome of an infection.