ATHENS PROTEOMICS / METABOLOMICS WORKSHOP

April 19-20th, 2012

Hellenic Pasteur Institute,

Main Amphitheatre,

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Thursday, April 19th, 2012	
MORNING SESSION	
Chair: Prof. Anthony Tsarbopoulos, Chairman of the Hellenic Mass Spectrometry Society	
9:00 a.m. - 9:20 a.m.	**Arrivals & Registration** Main Amphitheatre
9:20 a.m. - 9:45 a.m.	**Mass Spectrometry in Greece** Prof. Anthony Tsarbopoulos
9:45 am - 10:15 am	**Coupling Mass Spectrometry with Ion Mobility** John Hoyes Ph.D. C.Phys, Technical Director, Waters Corporation Interest in ion mobility coupled with mass spectrometry has grown over the last decade or so largely due to its use in studying bio-molecules and the later part of the presentation will focus on the development of the SYNAPT G2 as a tool for bottom up Proteomics. It will be explained how the various components of the instrument were developed; IMS separation, high resolution TOF analyzer, high dynamic range detection system. Particular attention will be paid to the orthogonal nature of the nested IMS separation and its consequential increase in peak capacity and throughput for the proteomics workflow. Finally a comparison of TOF analyzers will be made to alternative mass spectrometer technologies along with predictions for the future performance of this dynamic technology.
10:15 am - 11:15 am	**Combining IM MS with Electron Microscopy to Determine Structures of Polydisperse Proteins** Dr. Justin LP Benesch, Department of Chemistry, Physical & Theoretical Chemistry Laboratory, University of Oxford We have investigated members of the small heat-shock proteins (sHSPs), an important family of molecular chaperones that act to prevent protein aggregation and deposition under conditions of cellular stress. Molecular level details of these proteins have been

	hard to come by due to their intrinsic heterogeneity and equilibrium fluctuations, properties that likely govern their function in vivo. Our method is widely applicable to homomeric protein assemblies, and highlights the important role MS can play in structural biology
11:15 am - 11:45 pm	**Increasing the System Peak Capacity of LC-MS/MS Work-Flows for Qualitative and Quantitative Protein Profiling by Incorporating Ion Mobility Separations** Mark Andrew McDowall, Strategic Development Manager, Mass Spectrometry, Waters Corporation Over the past decade the complexity of biological samples intended for qualitative and quantitative proteomics analysis has been significantly under estimated. Consequently the peak capacity of typical LC-MS/MS systems used for such analyses has been insufficient. We describe a novel approach to address the analytical challenge inherent in such sample complexity embodying the on-line combination three dispersive analytical techniques; HPLC, Ion Mobility Separation (IMS) and Time of Flight MS. Additionally the advantage of collecting LC-IMS MS data at high mass resolution and mass accuracy will be summarized. Our results illustrate how LC-IMS-MS successfully increases system peak capacity by a factor of 10 thus enabling more components of complex protein digests to be unambiguously identified and quantified per unit time.
11:45 am - 12:00 pm	***Coffee Break***
	AFTERNOON SESSION
Chair: Dr. George Th. Tsangaris, Chairman of the Hellenic Proteomics Society	
12:00 pm - 12:20 pm	**Proteomics in Greece** Dr. George Th. Tsangaris
12:20 pm - 13:20 pm	**A Data Independent LC-IM MS Strategy to Significantly Improve the Reproducibility of Protein Identification in Complex Proteomes** Dr. Yishai Levin, Head of the mass spectrometry and proteomics teaching unit at Weizmann Institute of Science We advocate a new Data-Independent strategy for the analysis of complex proteomes which combines liquid chromatography, ion mobility (IM) separation and exact mass analysis of both precursor

	and product ions. The resulting LC-IM MS protocol has the advantage of being able to interrogate multiple co-eluting peptide precursors in parallel, (i.e. increases sampling/coverage). IM separation frequently enables co-eluting isobaric peptide precursor ions to be differentiated (i.e. reduces co-fragmentation) resulting in an increase in the number of reproducibly confident peptide/protein identifications.
13:20 pm. - 13:50 pm	**Study of a newly discovered variant of Alpha 1-antitrypsin by IM MS** Dr. Konstantinos Thalassinos, Institute of Structural and Molecular Biology, Division of Biosciences, University College London Alpha 1-antitrypsin (A1AT) is the archetype of the serpin (serine protease inhibitor) superfamily of proteins. It is predominantly synthesized in hepatocytes and secreted into the circulation at the highest levels of any serum antiprotease. Under physiological conditions it protects the lungs from human neurophil elastase but upon aggregation it is implicated in chirosis of the liver and chronic obstructive pulmonary disease. We have used ion mobility mass spectrometry to study a newly discovered variant of A1AT, with the mutation K154N, termed A1AT Queens.
13:50 pm. - 14:20 pm	**Integrating mass spectrometric datasets into computational approaches for structure characterization of large protein assemblies** Dr. Argyris Politis, Department of Chemistry, University of Oxford, Chemistry Research Laboratory, Mansfield Road, Oxford, UK Integrative computational methods have added a whole new dimension to the study of protein-protein interactions within the functional modules of the cell. Classical structural biology methods are increasingly complemented by emerging experimental techniques and integrative hybrid approaches. This is especially important if no high-resolution structure can be attained due to the transient nature of macromolecular assemblies. Here, for the first time we integrate ion mobility mass spectrometry (IMMS) and chemical cross-linking coupled with mass spectrometry (CXMS) into a computational approach for structural determination of multi protein complexes.

14:20 pm. - 15:00 pm	**SMALL MOLECULES MINI SESSION** **Development of a Metabolomic/Lipidomic Platform Based on a Hybrid Quadrupole Time-Of-Flight (QTof) Ion-Mobility Mass Spectrometer.** John P. Shockcor, PhD, FRSC, CChem, Director Strategic Operations, Pharmaceutical and Life Sciences Division, Waters Technologies Corporation Metabolomics/Lipidomics represents a paradigm shift in metabolic research, away from approaches that focus on a limited number of enzymatic reactions or single pathways, to approaches that attempt to capture the complexity of metabolic networks. Additionally, the high-throughput nature of these approaches is well suited to biomarker discovery. Mass spectrometry is highly discriminatory for a large range of pathological processes, which makes it the principal tool for these omic studies. In this study we have examined a mouse model where Glucose Transporter 1 has been increased in expression selectively in the heart to examine heart function under conditions of glucose stimulation. Mice (3 month) were sacrificed and the heart tissue rapidly dissected and stored at -80C. A chloroform/methanol extraction was performed and the organic layer analyzed using a UPLC/Synapt G2 quadrupole time-of-flight (QTof) ion-mobility mass spectrometer. The data obtained from the samples was subjected to a series of multivariate statistical analysis method and those lipids which contribute to the variance between the wild-type and GM animals were identified.
15:00 pm - 16:00 pm	***Break for Lunch & Networking***
Friday, April 20th, 2012	
8:00 am - 17:30 pm	***Excursion to Peloponnese***