KHUPO

6th Annual International Proteomics Conference

" From Bench to Clinical Application "

March 30th – 31ST, 2006

New Millennium Building, Konkuk University, Seoul, Korea

CONTENTS

6th Annual International Proteomics Conference

Invitation

On behalf of the Organizing Committee of the Korean Human Proteome Organization (KHUPO), it is my great pleasure to welcome all the speakers and participants from the different part of the world and Korea. This will mark the 6th Annual Meeting of KHUPO and focus on the theme, "From Bench to Clinical Application". I am very much confident that this meeting will be a useful vehicle for disseminating information and knowledge pertaining to the human proteome project, which are now well moving under the leadership of directors of each initiative. For this meeting, The leaders of HUPO and distinguished scientists were invited and their presentations will provide an excellent opportunity to introduce the current status of HUPO initiatives. This meeting will also help us jump one big step towards the development of proteomics core technology and cooperation among the scientists in this field.

As proteomics is staged at rapid growth phase with respect to technology, training and infrastructure in Korea, we will continue working towards our ultimate goals to map and elucidate all human proteome, to understand mechanisms whereby many proteins are involved in the pathogenesis of human disease.

Finally, KHUPO wishes to be a key part of world proteome societies and will continue to function in its unique role under the auspices of HUPO. I hope that 6th Annual Meetings of KHUPO serves as a major communication vehicle for the high-quality research of proteome scientists between Korea and the rest of the world.

Thank you,

Young Mok Park
President, Organizing Committee of KHUPO Annual Conference
President, KHUPO
Council Member, Human Proteome Organization

Organizing Committee

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General Information

2006 scientific conference of Korean Human Proteomics Organization will be held at New Millennium Hall of Konkuk University in March 30 (Thur) - 31 days (Fri). The scientific conference which it sees ' From Bench to Clinical Application' has 7 plenary lectures, 7 field symposia, poster presentation, and workshops.

To this scientific conference the internationally prominent proteomics scholars will attend and the scientific level of Korean proteomics will be upgraded.

Your cooperation and interest are asked.

Organized by

The Korean Human Proteomics Organization (KHUPO)

Theme

"From Bench to Clinical Application"

Period and Place

March 30 (Thursday) – 31 (Friday), 2006

New Millennium Building, Konkuk University, Seoul, Korea

Deadline for Abstract Submission

March 18, 2006

Online Pre-registeration

March 24, 2006

Deadline for Exhibition and Advertisement in the Abstract Book

February 15, 2006

"이 발표논문집은 2005 년도 정부재원(교육인적자원부 학술연구조성사업비)으로
한국학술진흥재단의 지원을 받아 발간되었음."

Schedule

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(8:00-09:00)</td></tr>
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<tr><td>11:00</td><td colspan="2" rowspan="2">Poster & Exhibition Ⅰ
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<tr><td>18:00</td><td>Symp 2
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(17:40-18:20)</td><td>Symp 3
(16:40-17:40)</td><td rowspan="2">Symp 6
(16:50-18:05)</td><td rowspan="2">Symp 7
(16:50-18:05)</td></tr>
<tr><td>19:00</td><td colspan="2" rowspan="2">Welcome Reception
(18:30-20:30)</td><td colspan="2">Young Scientist Award & Closing Remarks
(18:10-18:30)</td></tr>
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Plenary Lectures

Plenary Lecture 1

March 30 (Thu) 10:00-10:40, Hall A
Chair: Chi Bom Chae (Konkuk Univ.)

Dr. Raymond J. Deshaies *(Howard Hughes Medical Institute & Caltech, **U.S.A**)*
Development of Mass Spectrometry-Based Methods to Identify Enzyme-Substrate and Ligand-Receptor Networks in the Ubiquitin-Proteasome System.

Plenary Lecture 2

March 30 (Thu) 14:10-14:50, Hall A
Chair: Young Ki Paik (Yonsei Univ.)

Dr. John J. M. Bergeron *(McGill Univ., **Canada**) : **BPRC- Sponsored Speaker***
A Systems Biology Approach for Proteomics as Illustrated for Liver Subcellular Fractions

Plenary Lecture 3

March 30 (Thu) 14:50-15:30, Hall A
Chair: Chun Sik Park (Soonchunghyang Univ. Bucheon Hospital)

Dr. Peipei Ping *(UCLA School of Medicine, **U.S.A**)*
Myocardial Infarction and The Stressed Mitochondrial Proteome

Plenary Lecture 4

March 30 (Thu) 17:40-18:20, Hall A
Chair: Young Mok Park (Korea Basic Science Institute)

Dr. Helmut E. Meyer *(Ruhr-Univ. Bochum., **Germany**)*
The HUPO Brain Proteome Project: The Pilot Studies

Plenary Lecture 5

March 31 (Fri) 10:00-10:40, Hall A
Chair: Kyung Soo Hahm (Chosun Univ.)

Dr. Jan E. Schnitzer *(Sidney Kimmel Cancer Center, **U.S.A**) : **BPRC- Sponsored Speaker***
Proteomic Mapping & Immunotargeting of Endothelium and Its Caveolae for Improved Tumor Penetration, Imaging and Therapy

Plenary Lecture 6

March 31 (Fri) 14:00-14:40, Hall A
Chair: Pann Ghill Suh (Pohang Univ. of Science and Technology)

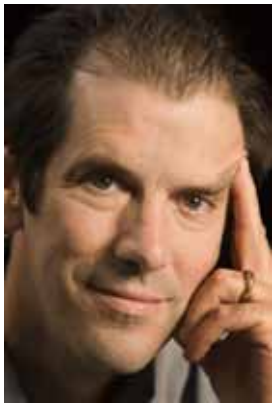
Dr. Rolf Apweiler (*EMBL Outstation European Bioinformatics Institute, **U.K**)*
Annotating the Human Proteome

Plenary Lecture 7

March 31 (Fri) 14:40-15:20, Hall A
Chair: Kil Lyong Kim (Sungkyunkwan Univ.)

Dr. Richard J. Simpson *(Ludwig Institute of Cancer Research & Walter and Eliza Hall Institute for Medical Research, **Australia**)*
Identification of New Diagnostic and Prognostic Markers in Colorectal Cancer

PL-1



Development of Mass Spectrometry-Based Methods to Identify Enzyme-Substrate and Ligand-Receptor Networks in the Ubiquitin-Proteasome System.

Raymond J. Deshaies

The ubiquitin-proteaosme system (UPS) comprises a large number of gene products in human cells, including upwards of 500 ubiquitin ligases, 100 deubiquitinating enzymes, several dozen ubiquitin-conjugating enzymes, and 1-2 dozen receptors for ubiquitinated substrates. This complexity reflects the fact that literally thousands of proteins are substrates for the UPS, and the degradation of many of these substrates is tightly regulated in time and space. The large number of components in the UPS reflects its broad role in biology; components of the UPS have been implicated in diverse processes including nutrient sensing, circadian rhythms, snyaptic plasticity, and cell division. To fully understand the biology of the UPS, it will be necessary to understand the biological functions of its enzymatic components. The physiological role of any one enzyme of the UPS is presumably determined by its substrates. Thus, a key challenge facing researchers who study the UPS is to work out the networks that connect the various enzymes of the UPS to its cellular substrates. In my talk, I will describe a general approach that we are taking to use mass spectrometry to identify substrates whose turnover depends on specific components of the UPS.

PL-2

A Systems Biology Approach for Proteomics as Illustrated for Liver Subcellular Fractions

John J.M. Bergeron

Hierarchical clustering of microarray data has been a useful way to discover hidden patterns in gene expression leading to biological insight. Protein expression data tools are currently under implementation and operative following different strategies for protein quantitation. One method is the redundant peptide method utilized previously to elucidate the protein composition of clathrin coated vesicles and which was sufficiently accurate to predict the 1:1 stoichiometry of the clathrin heavy and light chains. The efficacy of the method was extended to an evaluation of proteins spiked into yeast lysates with a linear correlation between redundant peptide counts and protein abundance over 2 orders of magnitude. We have applied this methodology to the characterization of over 2,500 different proteins from 5 subcellular fractions of rodent liver. Profile mining was then used to analyze the clusters of the distribution of peptide abundances as deduced from the tandem mass spectrometer. The postulate of biochemical homogeneity posits that the proteins and their relative abundance in any subcellular structure are constant. Hierarchical clustering enables the assignment of such proteins to compartments regardless of the degree of purity of the isolated compartment.

PL-3

Myocardial Infarction and The Stressed Mitochondrial Proteome

Jun Zhang, Jeong In Yang, Thomas Vondriska, Xiaohai Li, Manuel Myer, Peipei Ping
Departments of Physiology and Medicine, Cardiology, University of California, Los Angeles, USA.

Ischemic injury to the myocardium induces mitochondrial permeability transition (MPT), a loss of mitochondrial membrane potential and disruption of the outer membrane that leads to cardiac cell death. Several cardioprotective interventions have been shown to prevent MPT, and recent studies have divulged members of the MPT pore (transmembrane protein complexes implicated as causative agents in MPT) in the heart. The consequences of MPT, that is, how it kills cells, remain unclear in the cardiac setting. One proposed mechanism is through the release of damaging proteins, such as cytochrome c, which initiate detrimental signaling cascades leading to cell death. However, the full repertoire of proteins released during MPT has never been examined in the heart. We validated a model of mitochondrial injury via calcium overload. This injury caused mild swelling and disruption of the outer membrane of cardiac mitochondria (as evaluated by separation of the released proteins via SDS PAGE); furthermore, the injury was significantly milder than blunt destruction of the outer membrane with hypotonic treatment. With this approach, we isolated the molecules released from injured mitochondria, separated them by SDS PAGE and identified proteins by mass spectrometry. To focus our analyses on proteins that could conceivably be released from a reversibly-injured mitochondrion (i.e. one in which the outer membrane is not completely destroyed) we systematically evaluated proteins below the molecular weight of 60 Kda on the 1D gel. Criteria for LC/MS/MS protein identification were >2 unique peptides, SEQUEST Xcorr +1, 2.0; +2, 2.5; +3, 3.75 and DeltaCN>0.1. We have thus far identified ~600 mitochondrial proteins, including a subset of which that appear to be released concomitant with MPT. These studies advance our understanding of the cardiac mitochondrial proteome and highlight an interesting functional class of proteins that may serve as small molecule markers of cardiac mitochondrial dysfunction in the setting of ischemic injury.

PL-4

The HUPO Brain Proteome Project: The Pilot Studies

Helmut E. Meyer
Medizinisches Proteom-Center
Ruhr-Universitaet Bochum, ZKF E.143
Universitaetsstrasse 150
D-44801 Bochum

The Human Proteome Organisation (HUPO) was launched on February 9, 2001 in consequence of the need of an international proteomic forum to improve the understanding of human diseases. Presently there are seven scientific initiatives analysing a distinct organ each within an international consortium. The initiative dealing with the brain is the Brain Proteome Project (HUPO BPP).
In order to estimate the existing approaches in brain proteomics as well as to establish a standardized data reprocessing pipeline, pilot studies have been initiated including both mouse and human samples. Participating groups were free to analyse the samples according to their own approaches. In these studies brain from normal mice of three age stages as well as two human samples from an autopsy and a biopsy brain, respectively, had to be analyzed by quantitative proteomics techniques. Data had to be submitted to a Data Collection Center (DCC) for a central re-processing and will be public accessible at the PRIDE data base serving as reference data for future analysis. More than 755.000 spectra for the human samples and around 170.000 spectra for the mouse tissue have been generated, with an overall distribution of approx. 4.600 peptide mass finger prints (PMF) and approx. 920.000 peptide fragmentation finger prints (PFF) spectra. Half of all spectra originate from gel-based or LC-based approaches, respectively. In addition, more than 200 2-D gels were generated by 8 participating labs.
In the course of these studies and the subsequent central re-processing, a data collection, submission and storage pipeline has been established, a bioinformatics interpretation strategy has been elaborated and a very interesting insight into proteomics approaches today could be gained and will be presented in this lecture.

PL-5

Proteomic Mapping & Immunotargeting of Endothelium and Its Caveolae for Improved Tumor Penetration, Imaging and Therapy

Oh, P., Li, Y., Testa, J., Yu, J., Valadon, P., Borgstrom, P., Schnitzer, J.E.
Sidney Kimmel Cancer Center, San Diego, CA 92121

New targeting strategies are needed to fulfill the promise of molecular medicine. Proteogenomic technologies provide major analytical power to assess molecular expression and to generate a variety of diagnostic and therapeutic opportunities. Most targets are expressed by specific cell types, such as tumor cells, that reside deep within tissue compartments that have limited access in vivo. Tissues are very complex with extensive molecular diversity over a wide concentration range that creates challenges for target discovery via proteomic analysis. To discover targets inherently accessible to agents circulating in blood and to reduce data complexity and overcome dynamic range limitations, we use colloidal silica nanoparticles to coat luminal surfaces of endothelia in major organs & solid tumors in order to isolate this membrane and its caveolae. The proteins identified either by digestion, HPLC of peptides and tandem mass spectrometry or after protein separation by 1-D or 2-D gel electrophoresis. This multi-modal approach along with multiple measurements per sample expands protein coverage and detection several fold. We integrate subtractive proteomic mapping with bioinformatic interrogation and molecular imaging in vivo to identify and validate tissue- and tumor-induced endothelial targets that are accessible to antibodies injected intravenously. Endothelial cell surfaces and caveolae in vivo have quite distinct molecular signatures in solid tumors and major organs. The tissue microenvironment modulates this expression. Dynamic live imaging techniques demonstrate rapid tissue-specific vascular targeting in vivo, including within seconds (normal lung) and minutes (solid tumors) of intravenous injection the transendothelial transport in vivo of antibodies targeting caveolae. This pervasive access improves the effectiveness of radioimmunotherapy in destroying both stromal and tumor cells and in treating a wide variety of solid tumors. Targeting endothelial caveolae may be a worthwhile novel strategy to enhance molecular and functional imaging as well as drug, nanoparticle, and viral delivery in vivo.

PL-6

Annotating the Human Proteome

Rolf Apweiler

The completion of the human genome has shifted the attention from deciphering the sequence to the identification and characterization of the encoded components. The identification and functional annotation of the proteome is here of special interest and starts with the identification of genes and transcripts as a prerequisite of proteome annotation. Ab initio gene predictions are very powerful in predicting most of the exons in a genome, but reliable gene structure predictions of both known and novel genes are dependent on existing transcript and protein information. An enormous amount of data already exists on the function of many human proteins but this is scattered over many resources. Public domain databases are required to manage and collate this information and present it to the user community in both a human and machine readable manner. My talk will concentrate on the current status of annotating the human proteome, achievements and shortcomings, and future prospects.

URLs:
http://www.uniprot.org
http://www.ebi.ac.uk/integr8/
http://www.ebi.ac.uk/interpro/
http://www.ebi.ac.uk/intact/
http://www.ebi.ac.uk/pride/
http://www.ebi.ac.uk/GOA/
http://www.ebi.ac.uk/seqdb/

References:

Orchard S., Hermjakob H., Apweiler R.;
Annotating the human proteome.
Mol. Cell. Proteomics 4:435-440(2005).

PL-7

Identification of New Diagnostic and Prognostic Markers in Colorectal Cancer

Richard J. Simpson, Hong Ji, David Greening, Justin Lim, Robert Goode & Robert L. Moritz
Joint ProteomicS Laboratory (JPSL), Ludwig Institute for Cancer Research & The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia

Colorectal cancer (CRC) is a leading cause of cancer death in the Western World. Early detection is the single most important factor influencing outcome of CRC patients. If identified while the disease is still localized CRC is treatable. To improve outcomes for CRC patients there is a pressing need to identify biomarkers for the early detection (diagnostic markers), prognosis (prognostic indicators), tumor responses (predictive markers) and disease recurrence (monitoring markers). Despite recent advances in the use of genomic analysis for risk assessment, in the area of biomarker identification genomic methods have yet to produce reliable candidate markers for CRC. For this reason, attention is now being directed towards protein chemistry or proteomics as an analytical tool for biomarker identification. Here, we discuss various proteomics technologies with reference to how they may contribute to CRC biomarker discovery. One such strategy uses a combination of continuous free flow electrophoresis (FFE) in the first dimension, a liquid-based IEF technique, followed by rapid RP-HPLC (1-6 min/analysis) in the second dimension. Imaging software has been developed to present the FFE/RP-HPLC data in a virtual 2D format. Demonstration of the method is presented using proteome analysis of human plasma and urine specimens. Additionally, we describe strategies for analyzing the 'secretome' of human CRC cell lines, the human platelet membrane proteome, and a proteomics approach for analyzing the effect of non-steroid anti-inflammatory drugs on LIM1215 cells.

Symposia

Symposium 1 Post-translational Modification

March 30 (Thursday) 9:00-10:00, Hall A

Chair : **Helmut E. Meyer**, *Ruhr-Univ. Bochum., Germany*

(SY1-1) 09:00-09:15 Unbiased SILAC Analysis Reveals Phosphorylation Sites Regulating Neuronal Ion Channel Gating
Kang Sik Park , *School of Medicine, Univ. of California*

(SY1-2) 09:15-09:30 Mass Spectrometric Analysis of of Phosphoproteome with Phosphospecific Tagging and Site Localization
Jong Shin Yoo , *Korea Basic Science Institute*

(SY1-3) 09:30-09:45 Comprehensive Identifications of Post-translational Modifications for the Biological Functions of Proteins: Proteomic and Systematic Approaches
Kong Joo Lee , *Ewha Womans Univ.*

(SY1-4) 09:45-10:00 MODi : A Tag Chain Method to Identify Multiple Post-translational Peptide Modifications from Tandem Mass Spectra
Eun Ok Paek , *Univ. of Seoul*

Symposium 2 Disease Proteomics 1

March 30 (Thursday)16:40-17:40, Hall A

Chair : **John J. M. Bergeron**, *McGill Univ., Canada*

(SY2-1) 16:40-16:55 Differential Expression of Vitreous Proteins in Proliferative Diabetic Retinopathy
Young Soo Kim , *Seoul Nat'l Univ.*

(SY2-2) 16:55-17:10 Development of Biomarkers for Osteoporosis from Patient Urine
Jin Kyu Im , *Kyungpook Univ.*

(SY2-3) 17:10-17:25 Cytoprotective Role of Heat Shock Protein 27 in NSAID-Induced Gastrophathy
Marie Yeo , *Ajou Univ. Genome Research Center for Gastroenterology*

(SY2-4) 17:25-17:40 Systems Biology and Signaling Pathways in Stem Cell Research
Bong Hee Lee, *Cheju Nat'l Univ*

Symposium 3 Plant/Microbial Proteomics

March 30 (Thursday) 16:40-17:40, Hall B

Chair : Chan Wha Kim, *Korea Univ.*

(SY3-1) 16:40-16:55 Proteomic Approach to Elucidate Biological Functions of PR-Proteins
Kyu Young Kang , *Gyeongsang Nat'l Univ.*

(SY3-2) 16:55-17:10 Microbial Proteomics and its Applications
Byoung Chul Park , *Korea Research Institute of Bioscience and Biotechnology*

(SY3-3) 17:10-17:25 Proteomic Approaches to Cell-to-Cell Protein Trafficking in Plants.
Jae Yean Kim, *Gyeongsang Nat'l Univ.*

(SY3-4) 17:25-17:40 Analysis of the Phosphoproteome in Arabidopsis
Ohk Mae K. Park , *Korea Univ.*

Symposium 4 Proteomics Technologies

March 31 (Friday) 9:00-10:00, Hall A

Chair : Jin Won Cho, *Yonsei Univ.*

(SY4-1) 9:00-9:15 Development of Non-gel Based 2-Dimensional Protein Separation
Myeong Hee Moon , *Yonsei Univ.*

(SY4-2) 9:15-9:30 Developments of Ultra High Performance Multi-dimensional LC Separations in Proteomics
Zee Yong Park , *Gwangju Institute of Science & Technology*

(SY4-3) 9:30-9:45 Extended Range Proteomic Analysis and its Application
Jeong Kwon Kim , *Chungnam Nat'l Univ.*

(SY4-4) 9:45-10:00 An Approach to Identify Peptides by Manual Evaluation of Tandem Mass Spectra
Sung Won Kwon , *College of Pharmacy, Seoul Nat'l Univ.*

Symposium 5 Protein Chip

March 31 (Friday) 9:00-09:45, Hall B

Chair : Soo Ik Chang, *Chungbuk Nat'l Univ.*

(SY5-1) 9:00-9:15 Nanomaterials for Label-free Biochips
Jong Man Kim , *Hanyang Univ.*

(SY5-2) 9:15-9:30 Near-field Optical Studies on Protein Microarrays and Nanoarrays
Yong Hoon Cho , *Chungbuk Nat'l Univ.*

(SY5-3) 9:30-9:45 Photoreaction-Based Protein Array Technology
Min Gon Kim , *Korea Research Institute of Bioscience and Biotechnology*

Symposium 6 Disease Proteomics 2

March 31 (Friday) 16:50-17:50, Hall A

Chair : Jan E. Schnitzer, *Sidney Kimmel Cancer Center, U.S.A*

(SY6-1) 16:50-17:20 Identification of Novel Cancer Diagnostic and Therapeutic Candidates Using Multi-dimensional Proteomics Strategies
David Speicher, *The Wistar Institute, U.S.A*

(SY6-2) 17:20-17:35 Proteomic Study on Human Stomach Cancer : Overexpression of GALECTIN -1 in GASTRIC Cancer
Jae Won Kim , *Gyeongsang Nat'l Univ.*

(SY6-3) 17:35-17:50 TiO2 Particle Induced Protein Expression Pattern Changes in Epithelial Cell Line
Tai Youn Rhim , *Soonchunhyang Univ.*

(SY6-4) 17:50-18:05 Development of New Cancer Diagnosis Method Based on Proteome Pattern Recognition Analysis
Mi Young Han , *Green Cross Reference Lab/Bio-Infra Inc.*

Symposium 7 Bioinformatics

March 31 (Friday) 16:50–17:50, Hall B

Chair : Rolf Apweiler, *EMBL Outstation European Bioinformatics Institute, U.K*

(SY7-1) 16:50-17:05 High-End Bioinformatics Technology in Bioscience & Biomedical Research
Hyun Joo , *Inje Univ.*

(SY7-2) 17:05-17:20 Data Analysis Strategies for the Accurate Protein Identification and Quantitation
Jin Young Kim , *Korea Basic Science Institute*

(SY7-3) 17:20-17:35 Integrative Analysis of Ubiquitination Network
Gwan Su Yi , *ICU*

(SY7-4) 17:35-18:05 Tandem MS Protein Biomarker Discovery: An Evaluation and Comparison of Search Algorithms False-Positive Error Rates at the Protein Level Based on a HUPO-PPP Dataset
Eugene Kapp , *Ludwig Institute for Cancer Research*

SY1-1

Unbiased SILAC Analysis Reveals Phosphorylation Sites Regulating Neuronal Ion Channel Gating

Kang-Sik Park
Department of Pharmacology, School of Medicine, University of California, Davis 95616, USA

Dynamic modulation of ion channel function by phosphorylation is crucial to brain and heart function. Voltage-dependent gating of Kv2.1 delayed rectifier potassium channels in mammalian neurons is regulated by calcineurin-dependent dephosphorylation induced by excitatory synaptic activity, ischemia and neuromodulation, leading to homeostatic/neuroprotective suppression of neuronal firing. A mass spectrometric approach employing SILAC labeling identified sixteen in vivo Kv2.1 phosphorylation sites, of which seven are regulated by calcineurin in vivo. Mutation of individual calcineurin-regulated sites was sufficient to shift channel gating properties incrementally towards those of dephosphorylated Kv2.1 channels; mutations at other sites had no effect. The phosphorylation-dependent changes in Kv2.1 activation occurred without affecting gating currents, suggesting that cytoplasmic phosphorylation affects coupling of voltage sensor movement to opening of the channel pore. Such an unbiased proteomic strategy to identify complex sets of dynamically regulated phosphorylation sites is a powerful approach to pinpoint specific sites controlling physiologically important protein function.

SY1-2

Mass Spectrometric Analysis of of Phosphoproteome with Phosphospecific Tagging and Site Localization

Jong Shin Yoo
MS Instrumentation Division, Korea Basic Science Institute, Daejeon, Korea 305-333

Protein phosphorylation plays an important role to control many cellular processes, and therefore considerable efforts have been devoted to characterize the phosphorylation states. Here a phosphospecific chemical tagging method was introduced for site localization and quantification of phosphoproteome. In order to identify the phosphorylated states of phosphoproteins separated by 2D gel electrophoresis, phosphoproteins immobilized in the gel was labeled with guanidinoethanethiol and in-gel digested for mass spectrometric (MS) analysis. The phosphospecific enzyme digestion at the labeled site provided a powerful tool to identify phosphorylation site. On the other hand, as a high throughput phosphoproteome analysis, the phosphopeptide enrichment and the subsequent chemical labeling with guanidinoethanethiol was efficiently coupled. The labeled peptides showed an increase of MS sensitivity and a unique fragment ion of MS/MS spectra, which enables highly sensitive analysis for the site localization of phosphoproteome. Differentiation of phosphorylation states between proteome samples could also be accomplished by the chemical tagging with stable isotope-coded guanidinoethanethiol.

SY1-3

Comprehensive Identifications of Post-translational Modifications for the Biological Functions of Proteins: Proteomic and Systematic Approaches

Jawon Seo, Jaeho Jeong, Kong-Joo Lee
From the Center for Cell Signaling Research, Division of Molecular Life Sciences and College of Pharmacy, Ewha Womans University

Recently produced information on post-translational modifications makes it possible to interpret their biological regulation with new insights. Various protein modifications finely tune the cellular functions of each protein. Understanding the relationship between post-translational modifications and functional changes is another enormous project, not unlike the human genome-like project. Proteomics, combined with separation technology, mass spectrometry and informatics, makes it possible to dissect and characterize the individual parts of post-translational modifications and provide a systemic analysis. Systemic analysis of post-translational modifications in various signaling pathways has been applied to illustrate the kinetics of modifications. Availability will advance new technologies that improve sensitivity and peptide coverage. The progress of "post-translatomics", novel analytical technologies that are rapidly emerging, offer a great potential for determining the details of the modification species and sites. The obtained information makes it possible to understand the cellular functions of various proteins. [Supported by KOSEF through 21C Frontier Functional Proteomics Project (FPR05A2-480) and through the CCSR and by BK21 program]

SY1-4

MOD^i : A Tag Chain Method to Identify Multiple Post-translational Peptide Modifications from Tandem Mass Spectra

Eunok Paek
Dept. of Mechanical and Information Engineering, University of Seoul

Identification of post-translational modifications (PTMs) is important to understand cellular functions of proteins. Various computational methods have been developed to interpret MS/MS spectra for PTM identification, but they are applicable only if the number of PTM sites and types being considered are limited. As the number of PTM sites and PTM types increase, the execution time and the space consumption required by the current software algorithms grow exponentially. To identify multiple PTMs in MS/MS spectra, we present here an innovative algorithm called MOD^i that makes use of a tag chain, which is a list of sequence tags and in-between gaps. The tag chain method differs from former tag-based techniques in that it uses multiple adjacent and nonadjacent tags to interpret MS/MS spectra while the former techniques use either only one tag or adjacent tags. The proposed method performs well even when more than a hundred modification types are considered and multiple potential PTMs exist in a peptide, as in the case of proteins such as histones and GAPDH. MOD^i manages the computational complexity of PTM identification innovatively and therefore can serve as an effective software platform for identifying multiple PTMs.

SY2-1

Differential Expression of Vitreous Proteins in Proliferative Diabetic Retinopathy

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The purpose was to identify vitreous proteins which were differentially expressed in patients suffering from proliferative diabetic retinopathy with active neovascularization. The vitreous samples of 15 active proliferative diabetic retinopathy patients were analyzed by two-dimensional gel electrophoresis and mass spectrometry, and the results were compared with those from age-matched patients with macular hole. 25 protein spots were identified in the two-dimensional gel electrophoresis gels. Eight proteins (pigmented epithelium derived factor, serine protease inhibitor, apolipoprotein A-IV precursor, prostaglandin–H2 D-isomerase, alpha-1-antitrypsin precursor, ankyrin repeat domain 15 protein, alpha2-HS-glycoprotein, and beta V spectrin) in the 25 spots were expressed significantly differently between the macular hole and proliferative diabetic retinopathy patients (p value < 0.05). Five proteins were up-regulated in the proliferative diabetic retinopathy patients, and three were down-regulated (p value < 0.05). We constructed vitreous protein profiles for the proliferative diabetic retinopathy patients, and identified eight candidate proteins believed to be involved in the pathogenesis of proliferative diabetic retinopathy.

SY2-2

Development of biomarkers for osteoporosis from patient urine

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Identification of biomarkers for diseases from body fluids is a challenging task because of the application to diagnosis and prognosis of diseases, and the laborious processes to achieve it. Urine, as a filtrate of blood, can be used to monitor the physiological conditions of body by analyzing the changes of its proteomic entity or other metabolites. In this study, we analyzed urine proteomes from normal and osteoporotic patients to find osteoprosis specific markers. Due to the individual variation of human urine proteome, we first needed to establish a standardized normal human urine proteomic map. Proteins from pooled urine from over 30 normal patients were prepared by desalting and TCA precipitation without any fractionation. The separated protein spots on 2-D gels were identified by peptide mass fingerprinting by MALDI-TOF MS analysis. As one of the limitations of the method is that the low molecular weight proteins cannot be resolved on regular gels, proteins whose molecular masses under 15 kDa were obtained by separation on 18% SDS-PAGE and cutting the region. The low molecular weight proteins were identified by ion-trap ESI mass spectrometry. To compare the normal and osteoporotic proteomes over 15 kDa, we employed the 2D-DIGE method to analyze the differentially expressed proteins statistically. From this dual approaches and increased patient urine samples, we are anticipating to identify biomarkers for the diagnosis of osteoporosis.

SY2-3

Cytoprotective role of heat shock protein 27 in NSAID-induced gastrophathy

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Heat shock proteins (HSPs) are crucial for the maintenance of cell integrity during normal cell growth as well as during pathophysiological conditions, withafter named as molecular chaperone. While functioning mainly as molecular chaperones through folding activities, heat-shock proteins also appear to be involved in diverse biological functions, such as apoptosis, carcinogenesis, and cytoprotection from cytotoxic and damaging stress. Biological activity of the HSP proteins is regulated elaborately by their cellular localization, phosphorylation status, oligomerization as well as expression level of the protein. In the stomach, induction of HSP27 in addition to HSP70 is revealed to play a very determining cytoprotective role in several gastric damages and rescue gastric epithelial cells from the injuries. However, relationship between its phosphorylation status and pathophysiological function in gastric diseases remains unknown. We therefore evaluated phosphorylation of HSP27 in NSAID-induced gastritis of rat using the proteomic analysis. We found that rat administrated indomethacin (40 mg/kg, *ip*) significantly decreased phosphorylation of HSP27 compared to control. While mice administrated revaprazan, novel acid pump antagonists prescribed for treatment of acid related gastric diseases including duodenal ulcer, prior to indomethacin exposure showed the restoration of its phosphorlyation which is well correlated with prevention of the gastropathy. In conclusion, these results provide important novel insights into phosphorylation of HSP27 in response to NSAID-induced gastropathy and highlight the cytoprotective role of posttranslational modification of HSP27 in NSAID-induced gastric injury, leading to newer application of novel APA, revaprazan, for the treatment of NSAID-induced gastropathy.

SY2-4

Systems biology and signaling pathways in stem cell research

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Systems Biology Systems biology is the study of an organism, viewed as an *integrated* and *interacting network* of genes, proteins and biochemical reactions which give rise to life. Instead of analyzing individual components or aspects of the organism, systems biologists focus on all the components and the interactions among them, all as part of one system using computer-based or special algorithms-based analysis. The individual function and collective interaction of genes, proteins and other components in an organism are often characterized together as an interaction network, and this mapping can be used as an effective tool in identifying the target genes of certain biological processes.

Wnt Pathway Wnt/ -catenin signalling has been known to promote self-renewal in a variety of tissue stem cells including neural stem cells. However, recent research shows that activation of the Wnt/ -catenin pathway promoted and inhibition of the pathway prevented differentiation of neuronal precursor cells.

Stem Cell Research With systems biological analysis of the gene and protein expression profiles in neural stem cells and progenitor cells, the key target genes which involved in the control of the differentiation process can be estimated. After systemic molecular biological experiments for target genes with western blotting, real time PCR, immunohistochemical staining, siRNA and Chromatin Immunoprecipitations(ChIP) technique to determine protein binding sites on DNA, the gene regulatory mechanism can be investigated.

Today's Seminar One example of the research for defining stemness and differentiation in neural stem cells with above-mentioned techniques will be presented and discussed.

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SY3-1

Proteomic approach to elucidate biological functions of PR-proteins

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We have isolated and characterized elicitor and pathogen induced PR proteins from rice suspension cells and leaves using 2DE-based differential proteomic analysis from the sample of resistant and susceptible interactions. One PR-10 protein and 6 different isoforms of PBZ proteins displayed on 2DE-gel have induced earlier and more in resistant interaction than in susceptible interactions. Both PR-10 family fusion proteins were purified and their RNase activity was identified. Immunohistochemistry revealed that PR-10 has been localized in phloem tissues but PBZ in cytosol surrounding pathogen infected area. PBZ has been closely associated with and found in tissues in PCD. We generated transgenic lines of Arabidopsis harboring PR-10 mutants which had no 2 cys-residues to prevent dimmer formation through disulfide bonds. We also have generated overexpression lines of PR-10 and PBZ transgenic plants to analyze changes in proteomic patterns between wild type and mutants. The differential biological functions of PR-10 family proteins will be discussed.

SY3-2

Microbial proteomics and its applications

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Genome-wide analysis of microbial proteome and molecular regulation processes has become a considerable area of Proteomics. These studies have been made possible by several advances, including completion of the microbial genome sequencing, striking appearances of powerful bioinformatics tools, and high-throughput proteomics, and maturation of Metabolomics. Despite these advances, relatively little effort has been expended in the bacterial engineering arena to develop and use integrated research platforms in a systems biology approach to enhance our understanding of industrial processes. This review discusses progress made in exploiting microbial proteomics with an integrated genome-wide research platform to gain new knowledge about how the industrial application of microbe group. Several Streptomyces produce many kinds of secondary metabolites. Results of these systemic researches have provided many new avenues for basic genome structures to metabolic engineering of the polyketide synthesis. One goal in summarizing this line of study is to bring exciting new findings to the attention of the industrial applications. In addition, we hope this research will stimulate investigators to consider using analogous approaches for analysis of the molecular combinatorial biosynthesis of other microbes.

SY3-3

Proteomic approaches to cell-to-cell protein trafficking in plants.

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Intercellular communication is a critical biological process for the orchestration of position-dependent cell differentiation, organ development and whole plant physiology. The intercellular trafficking of proteins and RNAs has emerged as a novel mechanism of cell-to-cell communication in plants. As a strategy to efficient intercellular communication, plant cells evolved plant specific symplasmic communication networks: plasmodesmata and phloem. Plasmodesmata are symplasmic channels connecting neighboring cytoplasm and responsible for local molecule exchange. Plasmodesmata have a capacity to mediate the cell-to-cell trafficking of macromolecules including proteins and RNA as well as small signaling molecules. Plasmodesmal connection enabled macromolecules to move several cell layers from the cytoplasm of origin cells to that of distant cells, consisting of supracellular symplasmic networking. In addition to local symplasmic networking through PD, plant evolved a long distance informational highway called phloem. Plasmodesmata and phloem conduit have been shown to allow the transport of proteins and RNA non-selectively or/and selectively. Here, proteomic approaches to identify plasmodesmal components involved in the cell-to-cell trafficking of proteins are presented. In addition, we used proteomic approach to isolate non-cell autonomous proteins in phloem sap in various plants. Phloem is composed of companion cells(CCs) and sieve elements(SEs). Protein exchange between CCs and SEs seems to be controlled tightly and selectively and a limited number of proteins found in phloem sapare capable of dilating the size exclusion limit (SEL) of PD. Here we describe a proteomic analysis of lettuce and cucumber phloem proteins. Using a nano-high-performance liquid chromatography/mass spectrometry (nano-HPLC/MS) system, we constructed a comprehensive peptide maps for lettuce and cucumber phloem proteins. In this study a total of over 600 different proteins from lettuce and cucumber phloem proteins were identified. These proteins were grouped into more than 15 categories based on their predicted functions: HSPs, eIFs, eEFs, metabolic enzymes, RNA-binding proteins, nuclear transporting proteins, protein kinases, cytoskeleton-associated proteins and protein degradation-associated proteins etc. The comprehensive analysis of them will greatly enhance our current knowledge of phloem functions and help us uncover new physiological and intercellular communication processes, coordinating nutrient allocation, defence and development in higher plants.

SY3-4

Analysis of the Phosphoproteome in *Arabidopsis*

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Protein phosphorylation is a dominant mechanism of information transfer in cells, and a major goal of current proteomic efforts is to generate a system-level map describing all the sites of protein phosphorylation. Here we describe a method of phosphoprotein enrichment from *Arabidopsis thaliana* total proteins, which involves chemical replacement of the phosphate moieties by affinity tags. The weakness of phosphates at high pH allows chemical replacement by biotinylated moieties. These biotin groups can then be used as affinity handles for immobilized avidin enrichment of the previously phosphorylated proteins from complex mixture of proteins. The phosphorylated proteins can be enriched with immobilized avidin and separated by two-dimensional gel electrophoresis. We have identified the isolated phosphorylated proteins by matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).

SY4-1

Development of Non-gel Based 2-Dimensional Protein Separation

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Proteome analysis requires comprehensive and systematic approaches including high performance separation methods, mass spectrometric analysis, bioinformatics, and etc. First of all, separation of proteins/peptides should be done properly prior to the mass spectrometric analysis and therefore, the need of high performance separation technique for proteins is increasing due to the importance of low abundant proteins which expressed at extremely low levels. While 2-dimensional gel electrophoresis (2DE) has a widespread usage in proteomic study, it is labor intensive and difficult in handling hydrophobic proteins due to the difference in solubility of proteins. Moreover, isolated proteins are not in their intact forms since they are denatured and trapped in gel matrix which can make it difficult to collect proteins in intact form for further biological applications when needed. Due to these reasons, a non-gel based multidimensional liquid separation technology could be ideal for the direct integration to MS. In this presentation, a non-gel based two dimensional protein separation (pI & Mw) method will be introduced by developing on-line capillary isoelectric focusing and hollow fiber flow field-flow fractionation (CIEF/HF FlFFF) for the separation of proteins in intact forms. Capillary isoelectric focusing (CIEF) is a high resolution separation method based on pI of proteins and HF FlFFF, a subtechniqe of FFF family, is an elution-based technique that is capable of separating proteins, DNAs, aqueous polymers, and even cells according to sizes or molecular structure. By miniaturizing flow field-flow fractionation (FlFFF) channel systems by utilizing a microbore hollow fiber membrane, it became possible to operate HF FlFFF at microflow rate regime and can be coupled to CIEF for proteomics application. The developed method has several advantages such as collecting protein fractions at certain pI and Mw ranges as their intact forms which can be utilized for other biological or analytical usages, on-line removal of ampholyte during HF FlFFF separation, and significant reduction of total separation time. Application of the method has been made with human urinary proteome and the collected protein fractions were digested into peptide mixtures and followed by shotgun analysis using nanoflow LC/tandem mass spectrometry for protein/peptide identification.

SY4-2

Developments of Ultra High Performance Multi-dimensional LC Separations in Proteomics

Zee-Yong Park
GIST

"Shotgun Proteomics Approach" is a combined method of peptide LC separation and tandem mass spectrometry (MS/MS). Proteolytically digested peptides are analyzed to infer amino acid sequences from their tandem mass spectrometric data. Shotgun proteomics approach became the method of choice for protein mixture analysis due to its simplicity and rapid analysis speed. Shotgun proteomics approach has benefited greatly from recent advances in capillary scale multidimensional peptide LC separation techniques and the protein coverage was greatly expanded. Yates and his coworkers at Scripps Research Institute developed a well-known proteome analysis technique, multi-dimensional protein identification technique (MudPIT). It uses single capillary column constructed with a serial packing of strong cation exchange (SCX) and C18 reverse phase (RP) column material to fractionate and separate peptides sequentially. In depth protein profiling analysis are routinely carried out with MudPIT technique. One limitation of this technique is that separation capability of conventional capillary RPLC is not sufficient enough for the direct analysis of whole cell/tissue protein extract samples. Smith and his coworkers at Pacific Northwest National Laboratory developed a ultra high pressure capillary RPLC system and demonstrated remarkable improvements in peak capacity and detection sensitivity of RPLC based peptide separations. Later they reported successful identifications of over 1000 plasma proteins using an off-line based multidimensional peptide LC separation with ultrahigh pressure system.

In this study, we introduce a newly developed ultrahigh performance multi-dimensional peptide LC separation system which combined a ultra high pressure RPLC system with a MudPIT system. Significant improvements in terms of peptide detection sensitivity and peak capacity of peptide LC separation were made over conventional MudPIT system. Very complicated proteome samples, whole cell lysates and abundant protein depleted plasma samples were investigated. Using this new method, we were able to identify many new proteins, previously undetected by conventional MudPIT system, and to observe dynamic changes of protein abundances between two different samples. It appears that our newly developed ultra high performance multidimensional peptide LC separation system can be a successful solution to the target protein screening system or biomarker discovery platform by achieving precise determination of in-depth protein profiles and quantitative analysis of proteome samples under various patho-/ biological conditions.

for following tandem mass spectrometry analysis. one Currently In this study, we have developed a highly confident target protein discovery system which combined shotgun proteomic approaches capable of delivering sensitive identifications of hundreds of proteins contained in individual samples with non labeling quantification technique for rapid discovery of disease related target proteins..

SY4-3

Extended Range Proteomic Analysis and its applications

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While trysin is the most commonly used enzyme in a typical proteomic analysis for digesting proteins, the advanced development of mass spectrometry and liquid chromatography made it possible to use an enzyme which produces larger peptides than trypsin. In this presentation, an analysis method with larger peptide fragments - Extended Range Proteomic Analysis - will be discussed. This new platform uses (1) an enzyme, such as Lys-C, which cleaves less frequently than trypsin, (2) a hybrid mass spectrometer that combines a linear ion trap and a Fourier transform ion cyclotron resonance cell, and (3) high performance LC separation with narrow bore (20 and 50-μm i.d.) polymer based monolithic columns for separating large peptides and achieving high sensitivity. Comprehensive characterization of post-translational modifications was achieved with this approach in the analyses of Lys-C digested samples of bovine beta-casein and epidermal growth factor receptor at the level of 4 fmole and 20 fmole, respectively. The examples of detecting large peptide fragments with phosphorylation or glycosylation will be presented. A peptide with molecular weight of ~10 kDa was also successfully detected.

SY4-4

An approach to identify peptides by manual evaluation of tandem mass spectra

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Accurate protein identification is a crucial step in proteomic research. Currently automated searching programs are employed to search a sequence database with tandem mass spectra of peptides for protein identifications. When these spectra of peptides contain limited information, however, a computer assisted search program may not lead to correct peptide identifications. This addresses the need to validate the peptide identifications by careful manual verification. Here, we introduced a systematic approach to evaluate peptide identifications derived from automated search programs. To validate our approach, tandem mass spectra obtained from tryptic digests of E.coli and HeLa cells were identified with the automated search program and then subjected to the manual verification. We established rules for the evaluation of peptide identification based on the basic principles, i.e., the candidate peptide sequence should explicate the observed fragment ions and the mass errors of neighboring fragments should be similar. We considered peptide identifications that can meet the rules as correct identification. As a result, our method showed that correct peptide identifications were not always consistent with the scores from automated search algorithms. Our systematic approach comprehensively detected false-positive peptide identifications and found peptide identifications which were missed in the search algorithms. Therefore our approach to incorporate manual inspection into the automated searching algorithms could enhance the accuracy of protein identification, which might help to develop a better searching algorithm for protein identification.

SY5-1

Nanomaterials for Label-Free Biochips

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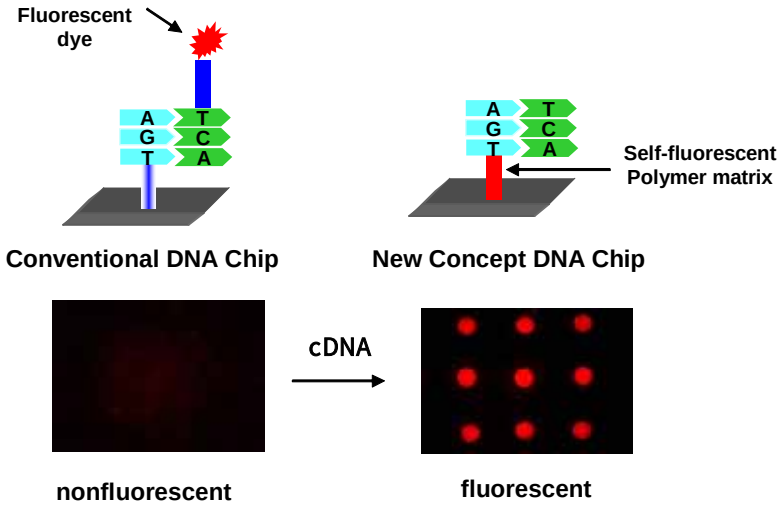
Recently, development of efficient label-free sensor systems has been a topic of great interest in both fundamental and applied research areas. Among materials which can be utilized as sensor matrixes, polydiacetylene (PDA)-based chemosensors are unique in terms of method of preparation, molecular structure, and output signal. The unique property of nanostructured polydiacetylenes that leads to their application as label-free sensing elements is the occurrence of a fluorescence change from nonfluorescent to red fluorescent that takes place in response to ligand-receptor interactions. In this symposium, recent advances in the area of fluorescence-based polydiacetylene sensor systems will be presented.



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SY5-2

Near-field Optical Studies on Protein Microarrays and Nanoarrays

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Microarray technology has recently proved to be a powerful tool for high throughput rapid and simultaneous detection and analysis of a large number of biological samples with high sensitivity and precision. They serve as a useful platform for ultraminiaturized bioanalysis. The major success of this technology can be attributed to biophotonics since optical methods are implemented for the detection and readout of signal from microarrays. In this work, we prepared protein microarrays printed on a surface treated glass substrates using the microchip arrayer and the photomask lithographic techniques. For fabrication of protein nanoarrays, dip pen nanolithographic technique using an atomic force microscope was used. The advantage of using photomask technique for printing protein microarrays is that the protein spot size can be reduced to nearly 50 microns as compared to protein spots made by a microarrayer (200-300 m diameter). We characterized optical properties of these microarrays and nanoarrays by means of photoluminescence (PL), confocal microscope, and near-field scanning optical microscopy (NSOM). An argon ion laser (488 nm) was used as an excitation source and the emitted light was dispersed by a 300 mm triple grating monochromator and detected by photomultiplier tube. The NSOM topography and optical imaging was conducted in a transmission mode to study the known 'doughnut effect' in protein spots. We have tried to correlate and explain the reduction in the doughnut effect with decrease in protein spot size fabricated by the three different techniques. These results as well as the future scope of this kind of study will be presented.

SY5-3

Photoreaction-Based Protein Array Technology

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The protein microarray is one of the most dynamic parts studied in the biochip technology at present [1]. Addressing proteins on a particular position of surfaces with resolutions from the micron to the nanometer scale is necessary for the development of proteins chips. Various patterning methods of proteins, such as micromachining, microwriting, electrochemical stripping, photopatterning and microcontact printing, have been developed. Photochemical methods have at least two advantages, addressing of proteins in a microfluidic system [2] and reducing the pattern size [3]. Two methods of protein photopatterning was developed. One of the methods used a caging chemistry. Terminal carboxylic acid group of glass surface was esterified with 1-(4,5-Dimethoxy-2-nitrophenyl)diazoethane (DMNPD). The ester linkage was photolyzed at < 360, and the exposed carboxylic surface could be used to immobilize proteins by a general amine coupling method [4]. The protein photopatterning method was applied to fabricate micron-size array of proteins.

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SY6-1

Identification of Novel Cancer Diagnostic and Therapeutic Candidates Using Multi-dimensional Proteomics Strategies

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The complexity and dynamic ranges of human cancer cell, tumor, and plasma proteomes greatly exceed the protein profiling capacities of conventional 2-D protein profiling methods such as 2-D PAGE, 2-D DIGE, and LC/LC-MS/MS methods. In order to examine more than the “tip of the iceberg” in these cancer proteomes, additional protein separations must be incorporated into protein profiling strategies. To dig deeper into these proteomes, we have developed several alternative 3-D, 4-D, and 5-D protein/peptide separation strategies that greatly expand the numbers of proteins detected and extend detection to lower abundance proteins. For example, a powerful plasma protein profiling method utilizes three orthogonal protein separations (immunoaffinity depletion, microscale solution IEF (MicroSol IEF), and 1-D SDS PAGE) followed by pixelation of the 1-D gels and LC-MS/MS analysis on a linear ion trap mass spectrometer. This method can detect more 3,000 proteins spanning more than nine-orders-of-magnitude in human or mouse plasma samples including proteins in the ng/ml and pg/ml range. This method is being used to discover serological biomarkers of cancers and other human diseases in mouse models. A similar 3-D method has been developed for comprehensive analysis of human cancer cell lines with differing metastatic potential. An alternative and complementary 3-D analysis strategy for cell and tissue samples combines DIGE fluorescent tags with MicroSol IEF followed by slightly overlapping very narrow pH range 2-D gels. Far more protein changes can be detected using this approach compared with conventional 2-D gels. The protein changes implicated in development of the metastatic phenotype include many proteins that have been previously associated with tumor progression as well as novel proteins. The majority of protein changes map to networks known to play key roles in tumorogenesis.

SY6-2

PROTEOMIC STUDY ON HUMAN STOMACH CANCER : OVEREXPRESSION OF GALECTIN -1 IN GASTRIC CANCER

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Recently we have established a gel image database comprising the images of two-dimensional gel electrophoresis of tumor tissues and paired normal tissues of one hundred and fifty two gastric cancer patients (refer to the accompanying presentation by Chang-Won Lee). The analysis of the database for the screening of differentially expressed proteins revealed that the spot 3023 is overexpressed in gastric cancer tissues with a high statistical singnificance. The spot was identified as galectin-1 by peptide mass fingerprinting using MALDI-TOF mass spectrometry, with 8 matching peptides that corresponds to a sequence coverage of 74%. Galectin-1 is a 14.5-kilodalton protein which have been found in a variety of normal and malignant cells and have been implicated in the regulation of cell growth, cell adhesion, and metastasis. The higher expression of galectin-1 was further confirmed by Western blotting of tissue proteins after one- or two-dimensional gel electrophoresis and also by immunohistochemistry. This is the first report to present the evidences of higher expression of galectin-1 in gastric cancer tissues than in normal ones.

SY6-3

TiO2 Particle induced protein expression pattern changes in epithelial cell line

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Inhalation of particles aggravates respiratory symptoms including mucus hyper-secretion in patients with chronic airway disease and induces goblet cell hyperplasia in experimental animal models. However, the underlying mechanisms remain poorly understood. To better understand this phenomena, we examined the effect of a TiO2 (titanium dioxide) particle on protein expression in epithelial cell line (BEAS-2B) by using 2-DE. As a result, 20 protein spot were changed in TiO2 particle treated epithelial cells and were identified by LC-MS/MS. They can be classified into four groups according to the time-course of expression pattern. These include ATP binding proteins, cell cycle related proteins, cytoskeleton proteins, as well as proteins involved in signal transduction. Among them, the macrophage migration inhibitory factor (MIF) expression changes were validated by using various conventional experiments. Levels of mRNA for MIF were higher in TiO2 treated cells than in control cells with fold change ranging from 3 to 7. These results agreed with those of immunoblot analysis and 2-D PAGE. Using several well known particles, such as carbon black and diesel exhausted particle (DEP), induction of MIF was also occurred. These data suggested that MIF may be a marker for particle exposure.
In conclusion, we demonstrated that the different protein expression in BEAS-2B cell lines in response to TiO2 particles. The increased expression of MIF may be involved in modulating the inflammatory response within the lung of TiO2-instilled rats. These data suggest that some of these proteins may serve as markers for stress during particle exposures.

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SY6-4

Development of new cancer diagnosis method based on proteome pattern recognition analysis

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Gastric, lung, liver cancers are the major causes of cancer death in all of countries. Recently, proteomics approaches have been used in attempt to identify new diagnostic markers. Characterizing the Proteome will be complicated because it exists in many different states and thus enters the issue of differential protein expression. We have focused on the development of strategies for identifying sera markers. Serum proteins are useful diagnostic tools and alteration of the expression of serum proteins is an early sign of an altered physiology and may be indicative of disease.

Advances in proteomics technology, particularly in mass spectrometry, are now providing an excellent opportunity to develop high throughput and accurate testing tools that can aid in disease diagnosis and prognosis. The Protein-Chip system uses surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS) to perform separation, detection and analysis of proteins directly from unprocessed biological samples. Using a case-control study design, 210 serum samples from patients with gastric cancer (n=105), and normal controls (n=105) were analyzed on strong anion-exchange surfaces. By comparing with normal and gastric cancer serum samples, we found that at least 5 proteins were significantly changed. The result shows that SELDI profiling of serum could be used to accurately distinguish patients with gastric cancer from normal controls. These protein markers can also be used as targets for further study in diagnostic and prognostic markers of stomach cancer therapy. In the future, we are going to validate that the proteins ca be used as diagnostic markers.

SY7-1

High-End Bioinformatics Technology in Bioscience & Biomedical Research

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Although the most powerful biocomputing centers in the world devoted to biomedical research, many scientific endeavors need successful implementation of key data acquisition, disease-oriented curation, and modeling of biological data. Bio-Grid computing is an emerging area that utilizes geographically distributed commodity computers as a virtual supercomputer. With the rapidly growing amount of data, it becomes impossible to analyze massive biological genes or protein sequences manually. The biggest performance bottleneck in "Biological Sequence Alignment and searching" is the involvement of streaming massive jobs. Based on a clustal algorithm, we firstly developed ClustalX-MPI tool for more convenient and powerful approach, which allows user-friendly GUI interfaces in the genome-wide sequence and function searching within billions of nucleotide or amino acid sequence space. An important criterion on the design of ClustalX_MPI was the implementation of a fully automated distributed computation and an intelligent load balancing algorithm (ILBA). ClustalX_MPI is a task benchmark for the multiple alignments of DNA, RNA, and protein sequences arisen in the biosciences and biomedical areas. The machine was also applied for implementing a LINUX-based high performance MSA algorithm, which aligns huge protein or genome sequences (at least seven terra bit computation memory). Our new program also provides an efficient compensation for various mutations in order to identify sequences that are closely related, but not identical.

SY7-2

Data analysis strategies for the accurate protein identification and quantification

Jin Young Kim[1], Kyung-Hoon Kwon[1], Gun Wook Park[1], Jeong Hwa Lee[1], Kun Cho[1] Young Ki Paik[2] and Jong Shin Yoo[1]

1. Korea Basic Science Institute, 2. Yonsei Proteome Research Center and Biomedical Proteome Research Center

Recent successes illustrate the role of mass spectrometry-based proteomics as an indispensable tool for molecular and cellular biology and for the emerging field of systems biology. An explicit goal of proteomics is the identification and quantification of proteins expressed in a cell or tissue, where proteomic studies frequently depend on MS analysis of peptides generated by proteolysis of protein sample. In general, complex protein mixtures are digested with proteases and the resulting peptide samples separated by one or multi-dimensional liquid chromatography (LC) and analyzed by MS and MS/MS to sequence the peptides. However, the complexity and wide protein concentration range of protein mixture make the identification and quantification of low amount proteins very difficult. Multidimensional analytical processes are required to overcome this problem, in which multiple separations or data filtration can reduce the complexity and help analysis protein correctly. To correctly identify as many proteins as possible from protein complex, we used multiple separation methods for protein and peptide mixtures and optimized the searching strategy by combining accurate MS with FT/MS and MS/MS analysis with LT/MS. In this study, we propose data analysis strategies for the accurate protein identification and quantification in high throughput proteome analysis.

SY7-3

Integrative analysis of ubiquitination network

Gwan-Su Yi
Information and Communications University

Analysis of protein network data is an inevitable process to bridge proteomics and systems biology. This process comprises distinctive steps such as collection, integration, knowledge inference and system modeling. Here, we propose a comprehensive strategy enabling an integrative analysis of protein network. First, the initial model of interested biological system should be outlined. Second, individual protein data of the system are collected from publicly available protein databases and literatures. The salient bio-functional data corresponding to those proteins and their interaction partners should also be collectively included in the same manner. Third, those data are integrated based on ontology so that the system could infer new information from the relationships among protein functions. Finally, a more realistic protein network model could be constructed based on the ontologically structured data. Using this model, we can further discover and validate novel knowledge of the system. In our research we apply aforementioned approach to ubiquitination network as a model system. Ubiquitination network, in this context, refers a complex network of ubiquitination associated proteins which are well known to be involved in degradation of the most cellular proteins and protein signaling processes.

SY7-4

Tandem MS protein biomarker discovery: An evaluation and comparison of search algorithms false-positive error rates at the protein level based on a HUPO-PPP dataset

Eugene A Kapp
Deasy C Djatmiko, Desmond Lim and Richard J Simpson

Tandem mass spectrometry using triple-quadrupole type instruments and SRM/MRM scan functions are important clinical tools for detecting inborn errors of metabolism (neonatal screening). For example, in patients with phenylketonuria (PKU) who are not treated, delayed development becomes noticeable between 6 and 12 months of age, by which time up to 50 IQ points will have been lost. Quantitation is based on internal standards and a balance between sensitivity and specificity of the assay is achieved by defining cutoffs as multiple of median (MoM) to minimize batch variation and shifts. Similarly, the process of identifying proteins (discovery), which are biologically interesting, using shotgun tandem MS techniques entails using similar statistical approaches. Based on a HUPO-PPP dataset, a comparison and evaluation of popular tandem MS database search algorithms will be made specifically in terms of reported false-positive or false-discovery protein error rates and how these error rates impact on pushing proteomic biomarkers beyond the discovery phase and into the validation phase.

Workshop

Workshop 1 Applied Biosystems Korea

March 30 (Thu) 12:10-13-50, Hall A

Frank Rooney *(Applied Biosystems Australia)*
Advanced MS Based Systems and Workflows for Protein Biomarker Discovery and Validation

Sang Hwa Kim *(Applied Biosystems, Korea)*
Next Generation Mass Spectrometry Solution for Proteomics.

Workshop 2 Becton Dickinson Korea Ltd.

March 30 (Thu) 12:10-13:00, Hall B

Min Jin Song *(Becton Dickinson Korea Inc., Korea)*
Recent Approach to Intra-Celluar Study Using Flow Cytometry

Workshop 3 SCINCO Co.,Ltd. & Thermo Electron Corp.

March 30 (Thu) 13:10-14:00, Hall B

Ken Miller *(Thermo Electron Corp., U.S.A)*
Latest developments in mass spectrometry for protein disease diagnostic biomarker discovery and validation

Workshop 4 Dong-il SHIMADZU Corp.

March 30 (Thu) 15:50-16:40, Hall A

Masaki Yamada *(SHIMADZU, Japan)*
AXIMA QIT, MALDI-QIT-TOF mass spectrometry and its practical use for glycoprotein analysis

Masaru Furuta*(SHIMADZU, Japan)*
New Approaches in Proteomics Using the Chemical Inkjet Printer

Workshop 5 Waters Korea Limited

March 30 (Thu) 15:50-16:40, Hall B

Mark A. Ritchie *(Waters Asia Ltd, UK)*
A Quantitative Proteomic Study on the Effects of Osmotic Stress on of a Cell Membrane Fraction of Salmonella Enterica Serovar Typhimurium

Workshop 6 GE Healthcare Bio-Scineces

March 31 (Fri) 12:10-13:00, Hall A

Richard Simpson *(Ludwig Institute, Austria)*

Workshop 7 GenoProt Inc.

March 31 (Fri) 12:10-13:00, Hall B

Sung Chun Kim *(Genoprot Co., Ltd., Korea)*
High Throughput Analysis for Diagnosis and Biomarker Discovery Using Aptamer-Based Biochip

Workshop 8 Agilent Technologies Korea. Ltd.

March 31 (Fri) 15:50-16:40, Hall B

John Chakel *(Agilent Technologies, Inc., U.S.A.)*
Advances in Expression Profiling and Protein Characterization

WS 1-1

Advanced MS Based Systems and Workflows for Protein Biomarker Discovery and Validation

Frank Rooney
Applied Biosystems

Many candidate protein biomarkers are currently being discovered today but their subsequent validation will require analysis of very large sample sets and represents a major bottleneck in the biomarker pipeline. Practical, technical limitations in the detection of low abundance proteins and high-throughput, high precision quantitation are often the limiting factors in biomarker discovery and validation. These problems are especially acute in serum/plasma, which because of its clinical accessibility and utility remains the single most commonly used sample type. The focus of this presentation will be on discussing mass spectrometry based workflows and systems optimized to move protein biomarker research through discovery to validation.

The discovery workflows take advantage of the multiplexing capabilities of iTRAQ™ reagents and provide high confidence identification of putative protein biomarkers, and relative quantitation in a single run. High resolution, quantitative instruments such as TOF/TOF or QqTOF mass spectrometers along with sophisticated software analysis provides high confidence in the results. Examples of use of this strategy will be discussed. Once candidate biomarkers have been identified in discovery, a verification/validation step where many candidates can be screened and quantitated at low levels is required. A targeted, hypothesis driven verification/validation workflow, using the unique quantitative and qualitative capabilities of the hybrid triple quadruople linear ion trap mass spectrometer combined with high precision LCMS for high throughput, high precision quantitation of large panels of candidate markers will be shown.

WS 1-2

Next generation mass spectrometry solution for proteomics.

Sanghwa Kim
Applied Biosystems Korea

There's no doubt that mass spectrometry has become one of essential tools to those who work either in proteomics or in pharmaceutical industry now. Since the first MALDI TOF/TOF platform, 4700, was introduced into proteome research world, Applied Biosystems leads the way as the solution provider for LC-MALDI workflow, which has been widely accepted as one of the standard workflows along with LC-ESI-MS/MS. In the chemistry side, cICAT and iTRAQ are also used as de facto standard tagging chemistry for biomarker discovery.

Applied Biosystems launched 4800 and Qstar Elite as the next generation MALDI TOF/TOF and QqTOF, and quite recently iTRAQ plasma kit, the optimized version for the plasma proteomics. 4800 has x10 sensitivity increase by using on-axis laser and improved capability of precursor selection compared to its predecessor 4700. Qstar Elite shows improvements in sensitivity, resolution and accuracy by using autocalibration feature, and at least one order increase in dynamic range. During this talk, the performance improvement and key benefits will be discussed with reference data in proteomic research

WS 2

Recent approach to Intra-celluar study using Flow cytometry

Min-Jin Song
Becton Dickinson Korea Inc.

Flow cytometry is a technology that simultaneously measures and then analyzes multiple physical characteristics for single particles, usually cells, as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence.

The most common application of flow cytometry has been in the study of the differentiation and function of the immune system. Extensive research studies in experimental animals and humans have resolved the pathways and processes of lymphocyte differentiation from the pluripotent hematopoietic stem cell and the phenotype of the mature, functional, peripheral lymphocyte populations. More recent studies have demonstrated the usefulness of flow cytometry in assessing lymphocyte activation or the cytokine profile of cells following either antigen-specific or mitogenic stimulation.

Flow cytometry, however, is not limited to these applications. Flow cytometry is used in cell biology research to study such things as enzyme function, biochemical activity, metabolism, and gene function and expression. Especially, using BD phosflow protocol enables phosphorylation-state analysis in single cells under the most native conditions possible in vitro, without involving time consuming and disturbing cell purification procedures. More and more researchers are now also interested in Multiplexed Bead application for quantitative detection of multiple analytes in a single serum. plasma, tissue culture supernatant, or cell lysate sample. Therefore, we'd like to introduce about Phosflow and Multi-plex bead array system(BD CBA^{TM}) using flow cytometry which are advanced technologies BD currently provides.

WS 3

Latest developments in mass spectrometry for protein disease diagnostic biomarker discovery and validation

Speaker : Dr. Ken Miller
Thermo Electron Corp.

Methods for systematic discovery and verification of biomarkers will be discussed, including detailed aspects of study design, sample preparation, sample analysis and data reduction. Discovery workflows involve the use of the Finnigan LTQ FT and LTQ Orbitrap for detailed and sensitive sample analysis and SIEVE software for label-free differential quantitation. Verification workflows use quantitative assays based on the TSQ Quantum or Finnigan LTQ with stable isotope labeled peptide standards to quantify target protein markers in large sample populations

WS 4-1

AXIMA QIT, MALDI-QIT-TOF mass spectrometry and its practical use for glycoprotein analysis

Masaki Yamada
SHIMADZU, Japan

AXIMA QIT™, the matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS) which can perform MS^n (n □ 3) has been developed with several features as follows;

- High resolution precursor ion selection (selection resolution=1000)
- True MS^n capability: Complete fragmentation
- High sensitivity (1 fmol)
- High Mass Accuracy
- MS/MS of high molecular weight ion

AXIMA QIT™ has enabled us more easily and surely protein identification and sugar chain characterization. We have already report that glycoprotein structural analyses including protein identification, sugar chain sequence and determination of glycosylation site was achieved using a single MS instrument (Fukuyama, Y., Wada Y., Yamazaki, Y., Ojima, N., Yamada, M. and Tanaka, K. *J. Mass Spectrom. Soc. Jpn.,* 2004, 52(6), 328 – 338.). Furthermore, the technology needs only protease digestion for sample preparation.

Here we will present that the application of the glycoprotein analytical method for an aberrant cryoglobulin analysis. An aberrant N-glycosylation site and the N-glycan structure on the light chain of cryoglobulin observed in a myeloma patient were determined by the following MALDI-QIT-TOF MS^n and MALDI-TOF MS analyses. MS spectra of the sample before and after treatment with Glycopeptidase F (GPase F) clearly showed glycopeptide ions and the corresponding peptide ion, respectively. The N-glycan structure was identified as A2G2 type by MS/MS of a glycopeptide ion. MS^n analyses of the peptide ion at m/z 1958 revealed the sequence consisting of 18 residues including N-glycan consensus sequence. Homology search of the obtained peptide sequence resulted that the peptide was located on N-terminus moiety of IgG kappa chain. Unexpected formation of the consensus sequence might be caused to the N-glycation at IgG kappa chain.

WS 4-2

New Approaches in Proteomics Using the Chemical Inkjet Printer

Masaru Furuta
SHIMADZU, Japan

The Chemical Inkjet Printer (CHIP-1000) delivers pico-liter volumes of reagent to a target surface using piezo-electric technology. It is a unique platform for micro-scale proteome analysis of 2-D electrophoresed samples on the membrane, and also for MS analysis of tissue sections.
Using this device, we have developed a new procedure “Western MS” to identify proteins using mass spectrometry (MS) after the detection by immunostaining on the membrane. For the first time, this methodology has enabled the whole procedures from Western blotting to MS identification to be completed directly on the identical membrane. Effectiveness of this technology was confirmed using the two-dimensionally separated protein blot on a polyvinylidene difluoride (PVDF) membrane, which was immunostained, subjected to on-membrane digestion using the chemical inkjet printer, and successfully identified by direct MALDI-TOF MS from the membrane. This strategy could be adopted to the first development of direct identification of counterpart proteins under molecular interactions on the membrane, taking the lectin staining of glycoprotein as a model system. This novel methodology described here opens a new phase on current proteomics, making direct connections to well-established "classic" protein sciences. In addition, the application of the chemical inkjet printing to MALDI tissue analysis will be discussed.

WS 5

A quantitative proteomic study on the effects of osmotic stress on of a cell membrane fraction of *Salmonella enterica* serovar Typhimurium

M. Ritchie[1], P.Skipp[2], J I. Langridge[1], I. Campuzano[1], T. McKenna[1], P., D. O'Connor[2], B. Cochrane[2]
[1]Waters Corporation, Manchester, United Kingdom, [2]University of Southampton, Southampton, United Kingdom

Salmonella enterica serovar Typhi is the causal agent of typhoid fever in humans, affecting a wide sector of the world population and causing an estimated 600,000 deaths per year. A further three million fatalities per year are caused due to acute gastroenteritis and diarrhoea. . *S. enterica* serovar Typhi invades and survives within macrophages, with typhoid fever a systemic infection, characterised by the presence of bacteria in the liver, spleen, and bone marrow. Specific immune responses are mounted against *Salmonella* outer membrane proteins (OMPs). In addition, *S. enterica* serovar Typhimurium double *ompC* and *ompF* porin mutants have shown attenuated virulence, and *S. enterica* serovar Typhimurium porins have been observed to trigger signal transduction in host cells. Hence, studies on the molecular features and regulation of *Salmonella* OMPs and porins should aid in further understanding their role during bacterium-host interactions. The ompR gene controls regulation of certain OMP porins. In this study we have investigated the response of *Salmonella* to osmotic stress. Specifically, we have looked at the change in protein expression profiles when *Salmonella* is exposed to high concentrations of NaCl. In addition we have looked at a mutant strain deficient in ompR. Cell membrane extracts were obtained from the wild type and ompR mutant *Salmonella* after exposure to NaCl and also from a control grown under normal conditions. These extracts were digested with sequencing grade trypsin and analysed by replicate LC-MS experiments on a Q-Tof mass spectrometer. Quantification and identification of peptides were carried out simultaneously using specialised bioinformatic algorithms. Protein fold changes were observed between control and salt shocked cultures. The mutant strain exhibited differences in its response to salt shock when compared to the wild type, especially in their expression of Porins and other Outer Membrane Proteins (OMPs).

WS 6

Richard Simpson
Ludwig Institute, Austria

WS 7

High throughput analysis for diagnosis and biomarker discovery using aptamer-based biochip

Ph. D. KIM Sung-Chun
Genoprot Co., Ltd., Seoul Korea 152-841.

Aptamers are small molecules that can bind to another molecules, nucleic acids, proteins or small organic compounds. This made them particularly attractive to us as biomarker. These aptamers have been selected from vast populations of random sequences, through modification of a combinatorial approach known as systematic evolution of ligands by exponential enrichment (GP-SELEX). The specificity and affinity of aptamers for their cognate ligands are comparable to those of antibodies for antigens. To analyze various analytes with aptamers, we developed a new high-throughput technique. Our strategy have been that the aptamer was split into two DNA phase and allowed to reassemble into the functional aptamer by the cognate ligands. We have named this method "aptamer-based biochip assay". As proof-of-principle, we used the microarray containing oligonucleotides derived from the aptamer library against proteins pool, human cancer serum, cancer cell line and microorganisms. We actually constructed 1K and 3K aptamer-based biochip and analyzed a pattern of proteins, such as breast, colon, gastric, liver, lung cancer, cardiovascular disease, normal healthy control serum, surface of cancer cell line and microorganisms. According to the disease, the age and the stage of cardiovascular disease, surface of cancer cell line and microorganisms, our biochip showed different protein profiling patterns. Consequently, our biochip analysis of complex protein mixtures says that we can use an aptamer-based biochip for biomarker discovery.

WS 8

Advances in Expression Profiling and Protein Characterization

John A. Chakel
Agilent Technologies, Inc

Protein identification and expression profiling in complex samples presents significant challenges to scientists. New strategies and tools will be discussed for addressing these challenges, including enhancements to the innovative HPLC-Chip/MS system and Spectrum Mill database search software. A two-step approach to expression profiling will be described that combines rapid differential analysis of samples using accurate mass MS data with profile-directed MS/MS of those differentially expressed potential markers from the MS step. Informatics approaches to facilitate rapid differential analysis of samples for proteomic profiling applications will also be discussed. In addition, the latest strategies for multidimensional separations and PTM analysis will be presented.

Colloquium

March 31 (Friday), 15:50-16:50, Hall A

Chair : **Richard J. Simpson** *(Ludwig Institute of Cancer Research & Walter and Eliza Hall Institute for Medical Research,* ***Australia****)*

Colloquium 1

March 31 (Friday), 15:50-16:05, Hall A

Ah Young Lee, *Korea Research Institute of Bioscience and Biotechnology*
Interactome analysis of annexin A4

Colloquium 2

March 31 (Friday), 16:05-16:20, Hall A

Hyun Jung Kim, *Korea Univ.*
Proteomes Analysis of Serum from Type 2 Diabetic Nephropathy Patients

Colloquium 3

March 31 (Friday), 16:20-16:35, Hall A

Sang Yoon Park, *Yonsei Univ.*
O-GlcNAc modification is involved in final differentiation of myoblast C2C12

Colloquium 4

March 31 (Friday), 16:35-16:50, Hall A

Sang Woo Kim, *Daegu Univ.*
Differentially expressed plasma proteins in streptozotocin-induced diabetic rats before and after administration of fungal polysaccharides

Colloquium 1

Proteome analysis of hypothermophilic bacterium *Thermotoga neapolitana*

Ah Young Lee[1], Chang Won Kho[1], Mi Jang[1], Youra Lee[1], Sung Goo Park[1], Do Hee Lee[1], Sayeon Cho[2], Kwang-Hee Bae[1], Sunghyun Kang[1], Byoung Chul Park[1]

[1]Protein Therapeutics Research Center, Korea Research Institute of Bioscience and Biotechnology Daejeon, South Korea; [2]College of Pharmacy, Chung-Ang University, Seoul, South Korea

Thermotogales utilize a wide variety of feedstocks, including complex carbohydrates and proteins. Interestingly, all members of the order *Thermotogales* have been confirmed for the ability to produce hydrogen. *Thermotoga neapolitana* have demonstrated the potential capacity to utilize a diversity of organic wastes and to cost-effectively produce hydrogen in the batch reaction. Although the *T. neapolitana* proteins responsible for the production of hydrogen have been studied individually, systematic analysis in the proteome level has not been made. Here we present a proteome reference map displaying intracellular proteins of *T. neapolitana*. From this map, we detected ~800 protein spots and identified ~130 different proteins after searching in-house and public database. Notably, we observed that Fe-hydrogenase protein was specifically up-regulated under the conditions for enhanced hydrogen production suggesting that its cellular level is closely related to the regulation of microbial production of hydrogen.

Colloquium 2

Proteomes Analysis of Serum from Type 2 Diabetic Nephropathy Patients

Hyun-Jung Kim, Eun-Hee Jo, Shin-Ae Yu, Mi-Ryung Kim and Chan-Wha Kim*

School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea

In diabetes mellitus (DM), a high proportion of individuals are found to have microalbuminuria and overt nephropathy, shortly after the diagnosis of their diabetes. Without specific interventions, 20~40% of type 2 diabetic patients with microalbuminuria progress to overt nephropathy. In addition, by 20 years after onset of overt nephropathy, about 20% will have progressed to end-stage renal disease (ESRD). Although, there are many studies on the cell biological mechanism of diabetic nephropathy (DN), the pathomechanism are still not fully understood. In addition, there is no specific diagnostic biomarker for type 2 diabetic nephropathy except microalbuminuria and macroalbuminuria. Therefore, early identification of patients prone to progress to terminal renal failure is important. In this study, to find a specific biomarker proteins suitable for diagnosis of DN, the proteins in serum of patient groups were analyzed by two-dimensional electrophoresis (2-DE): DM without albuminuria (DM, n=30) and with albuminuria (MA, n=30) and chronic renal failure (CRF, n=28). The efficiency of removing six major high abundant proteins (albumin, transferrin, IgG, IgA, haptoglobin, and antitrypsin) from human serum were also investigated using multiple immunoaffinity resins. As a result, albumin and five high abundant proteins were depleted specifically. The proteins which differentially expressed with statistical significance ($p<0.05$) in microalbuminuric and CRF patient groups compared to DM were selected. They were identified by ESI-Q-TOF MS/MS and among the identified proteins, three proteins which have a possibility to be used as biomarkers of type 2 DN were confirmed with western blotting.

***Corresponding E-mail: cwkim@korea.ac.kr**

Colloquium 3

O-GlcNAc modification is involved in final differentiation of myoblast C2C12.

Sang Yoon Park[1], Hyung Wook Nam[2], Suena Ji[1], Yu Sam Kim[2,*] and Jin Won Cho[1,*]
[1] Department of Biology Yonsei University, Seoul 120-749, Korea ; [2] Department of Biochemistry Yonsei University, Seoul 120-749, Kore*a*

β-O-linked N-acetylglucosamine(*O*-GlcNAc) is dynamic post-translational modification in nucleus and cytosol. Transcription factors, cytoskeletal proteins and variable enzymes are modified with *O*-GlcNAc at their Ser/Thr residues and their functions are regulated by *O*-GlcNAc. It is reported that this modification has an important role on differentiation of various cell types involving epithelial cells.

Thus, we studied how *O*-GlcNAc modification is involved in differentiation using myoblast C2C12 cell lines. Myoblast C2C12 is finally differentiated to myotube in differentiation media for 5 days. We confirmed dynamic changes of total *O*-GlcNAc modification levels during myogenesis. Especially, total *O*-GlcNAc modification is decreased gradually during first 12 hours after induction of myogenesis. Also, we identified that the increment of total *O*-GlcNAc modification by the treatments of STZ or PUGNAc, specific *O*-GlcNAcase inhibitors, and the addition of glucose inhibited final differentiation of myoblast C2C12.

From these results, we could conclude that *O*-GlcNAc modification might be involved in final differentiation of myoblast at early time period.

Colloquium 4

Differentially expressed plasma proteins in streptozotocin-induced diabetic rats before and after administration of fungal polysaccharides

Sang Woo Kim, Hye Jin Hwang, and Jong Won Yun
Department of Biotechnology, Daegu University

To search out novel biomarkers for monitoring diabetes prognosis, we examined the influence of hypoglycemic fungal polysaccharides (EPS) on the differential levels of plasma proteins in streptozotocin-induced diabetic rats. The 2-DE analysis demonstrated that nineteen proteins from about 500 visualized spots were differentially regulated, of which seventeen spots were identified as principal diabetes-associated proteins and two spots were unidentified proteins. The distinct effect of diabetes induction on the pattern of rat plasma proteins includes the downregulation of albumin, α_1-inhibitor-3, hemopexin, haptoglobin, Gc-globulin, vitronectin, and transthyretin, and the upregulation of ceruloplasmin, α_1-antitrypsin, serine protease inhibitor III. Two unidentified proteins of interest (tentatively named as Protein I and Protein II) showed distinctly with opposite trends: Protein I was significantly downregulated and Protein II was significantly upregulated after diabetes induction, both of which were also normalized to those of healthy groups upon EPS treatment. Furthermore, the changes in the concentration of plasma proteins of interest were monitored from 0 d to 14 d in the three experimental groups. Consequently, there was no clear trend in concentration variation of plasma proteins during monitored time periods. However, irrespective of time duration, several protein levels were also restored to those of healthy rats by EPS treatment, although the order of magnitude of the changes differed widely.

Scientific Programs

Thursday, March 30, 2006

Topics : Post-translational Modification, Disease Proteomics, Plant/Microbial Proteomics

08:00-09:00 **Registration & Poster Set-up/display**

Symposium 1 **Post-translational Modification**
Chair: **John J. M. Bergeron**, *McGill Univ., Canada*

09:00-09:15 (SY1-1) Unbiased SILAC Analysis Reveals Phosphorylation Sites Regulating Neuronal Ion Channel Gating
Kang Sik Park, *School of Medicine, Univ. of California*

09:15-09:30 (SY1-2) Mass Spectrometric Analysis of of Phosphoproteome with Phosphospecific Tagging and Site Localization
Jong Shin Yoo, *Korea Basic Science Institute*

09:30-09:45 (SY1-3) Comprehensive Identifications of Post-translational Modifications for the Biological Functions of Proteins: Proteomic and Systematic Approaches
Kong Joo Lee, *Ewha Womans Univ.*

09:45-10:00 (SY1-4) MODi : A Tag Chain Method to Identify Multiple Post-translational Peptide Modifications from Tandem Mass Spectra
Eun Ok Paek, *Univ. of Seoul*

Plenary Lecture 1

Chair: **Chi Bom Chae**, *Konkuk Univ.*

10:00-10:40 (PL-1) Development of Mass Spectrometry-Based Methods to Identify Enzyme-Substrate and Ligand-Receptor Networks in the Ubiquitin-Proteasome System.
Raymond J. Deshaies, *Howard Hughes Medical Institute & Caltech, U.S.A*

10:40:12:10 **POSTER and EXHIBITION Session Ⅰ**
(Visit Exhibition Booths To Receive Stamps for Entering the Special Gifts Competition)

Parallel Session A (HALL-A)

Workshop 1 **Applied Biosystems Korea Workshop**

12:10-13:00 (WS 1-1) Advanced MS Based Systems and Workflows for Protein Biomarker Discovery and Validation
Frank Rooney, *Applied Biosystems Australia*

13:00-13:50 (WS 1-2) Next Generation Mass Spectrometry Solution for Proteomics.
Sang Hwa Kim, *Applied Biosystems, Korea*

Parallel Session B (HALL-B)

Workshop 2 **Becton Dickinson Korea Workshop**

12-10:13-00 (WS 2) Recent Approach to Intra-Celluar Study Using Flow Cytometry
Min Jin Song, *Becton Dickinson Korea Inc., Korea*

Workshop 3 **SCINCO Co.,Ltd. & Thermo Electron Corp. Workshop**

13:10-14:00 (WS 3) Latest developments in mass spectrometry for protein disease diagnostic biomarker discovery and validation
Ken Miller, *Thermo Electron Corp., U.S.A*

14:00-14:10 **Opening Ceremony**
Special Welcome Remarks, by Dr. **Kyeong Ho Lee**
President, KHIDI, Ministry of Health & Welfare

Plenary Lecture 2

Chair: **Young Ki Paik**, *Yonsei Univ.*

14:10-14:50 (PL-2) A Systems Biology Approach for Proteomics as Illustrated for Liver Subcellular Fractions
John J. M. Bergeron, *McGill Univ., Canada:* ***BPRC- Sponsored Speaker***

Plenary Lecture 3

Chair: **Chun Sik Park**, *Soonchunghyang Univ. Bucheon Hospital*

14:50-15:30 (PL-3) Myocardial Infarction and The Stressed Mitochondrial Proteome
Peipei Ping, *UCLA School of Medicine, U.S.A*

15:30-15:50 **Coffee Break**

Parallel Session A (HALL-A)

Workshop 4 **Dong-il SHIMADZU Workshop**

15:50-16:15 (WS 4-1) AXIMA QIT, MALDI-QIT-TOF mass spectrometry and its practical use for glycoprotein analysis
Masaki Yamada, *SHIMADZU, Japan*

16:15-16:40 (WS 4-2) New Approaches in Proteomics Using the Chemical Inkjet Printer
Masaru Furuta, *SHIMADZU, Japan*

Parallel Session B (HALL-B)

Workshop 5 **Waters Korea Workshop**

15:50- 16:40 (WS 5) A Quantitative Proteomic Study on the Effects of Osmotic Stress on of a Cell Membrane Fraction of Salmonella Enterica Serovar Typhimurium
Mark A. Ritchie, *Waters Asia Ltd, UK*

Parallel Session A (HALL-A)

Symposium 2 **Disease Proteomics 1**

Chair: **Peipei Ping**, *UCLA School of Medicine U.S.A*

16:40-16:55 (SY2-1) Differential Expression of Vitreous Proteins in Proliferative Diabetic Retinopathy
Young Soo Kim, *Seoul Nat'l Univ.*

16:55-17:10 (SY2-2) Development of Biomarkers for Osteoporosis from Patient Urine
Jin Kyu Im, *Kyungpook Univ.*

17:10-17:25 (SY2-3) Cytoprotective Role of Heat Shock Protein 27 in NSAID-Induced Gastrophathy
Marie Yeo, *AjouUniv., Genome Research Center for Gastroenterology*

17:25-17:40 (SY2-4) Systems Biology and Signaling Pathways in Stem Cell Research
Bong Hee Lee, *Cheju Nat'l Univ.*

Parallel Session B (HALL-B)

Symposium 3 **Plant/Microbial Proteomics**

Chair: **Chan Wha Kim**, *Korea Univ.*

16:40-16:55 (SY3-1) Proteomic Approach to Elucidate Biological Functions of PR-Proteins
Kyu Young Kang, *Gyeongsang Nat'l Univ.*

16:55-17:10 (SY3-2) Microbial Proteomics and its Applications
Byoung Chul Park, *Korea Research Institute of Bioscience and Biotechnology*

17:10-17:25 (SY3-3) Proteomic Approaches to Cell-to-cCell Protein Trafficking in Plants.
Jae Yean Kim, *Gyeongsang Nat'l Univ.*

17:25-17:40 (SY3-4) Analysis of the Phosphoproteome in Arabidopsis
Ohk Mae K. Park, *Korea Univ.*

Plenary Lecture 4

Chair: **Young Mok Park**, *Korea Basic Science Institute*

17:40-18:20 (PL-4) The HUPO Brain Proteome Project: The Pilot Studies
Helmut E. Meyer, *Ruhr-Univ. Bochum, Germany*

18:30-20:30 **Welcome Reception**

Friday, March 31, 2006

Topics : Proteomics Technologies, Protein Chip, Disease Proteomics 2, Bioinformatics

8:00-9:00 **Registration & Poster Set-up/display**

Parallel Session A (HALL-A)

Symposium 4 **Proteomics Technologies**
Chair**: Kil Lyong Kim**, *Sungkyunkwan Univ.*

9:00-9:15 (SY4-1) Development of Non-gel Based 2-Dimensional Protein Separation
Myeong Hee Moon, *Yonsei Univ.*

9:15-9:30 (SY4-2) Developments of Ultra High Performance Multi-dimensional LC Separations in Proteomics
Zee Yong Park, *Gwangju Institute of Science & Technology*

9:30-9:45 (SY4-3) Extended Range Proteomic Analysis and its Application
Jeong Kwon Kim, *Chungnam Nat'l Univ.*

9:45-10:00 (SY4-4) An Approach to Identify Peptides by Manual Evaluation of Tandem Mass Spectra
Sung Won Kwon, *College of Pharmacy, Seoul Nat'l Univ.*

Parallel Session B (HALL-B)

Symposium 5 **Protein Chip**
Chair: **Soo Ik Chang**, *Chungbuk Nat'l Univ.*

9:00-9:15 (SY5-1) Nanomaterials for Label-free Biochips
Jong Man Kim, *Hanyang Univ.*

9:15-9:30 (SY5-2) Near-field Optical Studies on Protein Microarrays and Nanoarrays
Yong Hoon Cho, *Chungbuk Nat'l Univ.*

9:30-9:45 (SY5-3) Photoreaction-Based Protein Array Technology
Min Gon Kim, *Korea Research Institute of Bioscience and Biotechnology*

Plenary Lecture 5

Chair: **Kyung Soo Hahm**, *Chosun Univ.*

10:00-10:40 (PL-5) Proteomic Mapping & Immunotargeting of Endothelium and Its Caveolae for Improved Tumor Penetration, Imaging and Therapy
Jan E. Schnitzer, *Sidney Kimmel Cancer Center, U.S.A*

10:40:12:10 **POSTER and EXHIBITION Session** II

Parallel Session A (HALL-A)

Workshop 6 **GE Healthcare Bio-Scineces Workshop**

12:10-13:00 (WS 6) **Richard Simpson**, *Ludwig Institute, Austria*

Parallel Session B (HALL-B)

Workshop 7 **GenoProt Inc. Workshop**

12:10-13:00 (WS 7) High Throughput Analysis for Diagnosis and Biomarker Discovery Using Aptamer-Based Biochip
Sung Chun Kim, *Genoprot Co., Ltd., Korea*

12:10-14:00 **Lunch**

13:10-14:00 KHUPO General Assembly Meeting (HALL-A)

Plenary Lecture 6

Chair: **Pann Ghill Suh,** *Pohang Univ. of Science and Technology*

14:00-14:40 (PL-6) Annotating the Human Proteome
Rolf Apweiler, *EMBL Outstation European Bioinformatics Institute, U.K*

Plenary Lecture 7

Chair: **Moon Hi Han**, *Proteogen Inc.*

14:40-15:20 (PL-7) Identification of New Diagnostic and Prognostic Markers in Colorectal Cancer
Richard J. Simpson, *Ludwig Institute of Cancer Research & Walter and Eliza Hall Institute for Medical Research, Australia*

15:20-15:50 **Coffee Break**

Parallel Session A (HALL-A)

Colloquium Chair: **Richard J. Simpson**, *Ludwig Institute of Cancer Research & Walter and Eliza Hall Institute for Medical Research, Australia*

15:50-16:05 (Colq -1) Interactome analysis of annexin A4
Ah Young Lee, *Korea Research Institute of Bioscience and Biotechnology*

16:05-16:20 (Colq -2) Proteomes Analysis of Serum from Type 2 Diabetic Nephropathy Patients
Hyun Jung Kim, *Korea Univ.*

16:20-16:35 (Colq -3) O-GlcNAc modification is involved in final differentiation of myoblast C2C12
Sang Yoon Park, *Yonsei Univ.*

16:35-16:50 (Colq-4) Differentially expressed plasma proteins in streptozotocin-induced diabetic rats before and after administration of fungal polysaccharides
Sang Woo Kim, *Daegu Univ.*

Parallel Session B (HALL-B)

Workshop 8 **Agilent Technologies Korea. Ltd. Workshp**

15:50-16:50 (WS 8) Advances in Expression Profiling and Protein Characterization
John Chakel, *Agilent Technologies, Inc., U.S.A.*

Parallel Session A (HALL-A)

Symposium 6 **Disease Proteomics 2**

Chair: **Jan E. Schnitzer**, *Sidney Kimmel Cancer Center, U.S.A*

16:50-17:20 (SY6-1) Identification of Novel Cancer Diagnostic and Therapeutic Candidates Using Multi-dimensional Proteomics Strategies
David Speicher, *The Wistar Institute, U.S.A*

17:20-17:35 (SY6-2) Proteomic Study on Human Stomach Cancer : Overexpression of GALECTIN -1 in GASTRIC Cancer
Jae Won Kim, *Gyeongsang Nat'l Univ.*

17:35-17:50 (SY6-3) TiO2 Particle Induced Protein Expression Pattern Changes in Epithelial Cell Line
Tai Youn Rhim, *Soonchunhyang Univ.*

17:50-18:05 (SY6-4) Development of new cancer diagnosis method based on proteome pattern recognition analysis
Mi Young Han, *Green Cross Reference Lab/Bio-Infra Inc.*

Parallel Session B (HALL-B)

Symposium 7 **Bioinformatics**

Chair**: Rolf Apweiler**, *EMBL Outstation European Bioinformatics Institute, U.K*

16:50-17:05 (SY7-1) High-End Bioinformatics Technology in Bioscience & Biomedical Research
Hyun Joo, *Inje Univ.*

17:05-17:20 (SY7-2) Data Analysis Strategies for the Accurate Protein Identification and Quantitation
Jin Young Kim, *Korea Basic Science Institute*

17:20-17:35 (SY7-3) Integrative Analysis of Ubiquitination Network
Gwan Su Yi, *ICU*

17:35-18:05 (SY7-4) Tandem MS Protein Biomarker Discovery: An Evaluation and Comparison of Search Algorithms False-Positive Error Rates at the Protein Level Based on a HUPO-PPP Dataset
Eugene Kapp, *Ludwig Institute for Cancer Research*

18:00-18:30 **Applied Biosystems Young Scientist Award & Closing Remarks**

Poster Sessions

Poster Session Ⅰ

March 30 (Thu) 10:40-12:10

Poster Session Ⅱ

March 31 (Fri) 10:40-12:10

Session 1

Expression Proteomics , Functional Proteomics, Interdisciplinary, Proteomics and diseases 1, Signal transduction

P-1

A Proteome Profiling of *Rhodobacter Sphareroides KCTC 12085*

Yun Kyung Park[1,3],Youra Lee[1,3], Mi Jang[1,3], Ah Young Lee[1], Chang Won Kho[1], Sung Goo Park[1] Do Hee Lee[1], Sayeon Cho[2] , Pyung Keun Myung[3] , Kwang-Hee Bae[1], Sunghyun Kang[1], Byoung Chul Park[1]

P-2

A proteome Reference Map of *Enterobacter Aerogenes SNU-1*

Kyeong Sook Na[1, 2], Mi Jang[1], You Ra Lee[1], Yun Kyung Park[1], Ah Young Lee[1], Chang Won Kho[1], Sung Goo Park[1], Do Hee Lee[1], Sayeon Cho[3], Chong Kil Lee[2] , Kwang-Hee Bae[1], Sunghyun Kang[1], Byoung Chul Park[1]

P-3

Adaptative Response to Enhanced Oxidative Stress is Mediated by Glutathione Peroxidase 3 (GPx3) in *Saccharomyces Cerevisiae*

Pil Young Lee[1, 4], Chang Won Kho[1, 3], Ah Young Lee[1], Mi Jang[1], Youra Lee[1], Sung Goo Park[1], Do Hee Lee[1], Sa Yeon Cho[2], Seong Man Kang[3], Kwang Hee Bae[1], Sung Hyun Kang[1], Pyung Keun Myung[4] and Byoung Chul Park[1]

P-4

Analysis of Protein Expression Related with Tetracycline Resistance of *Salmonella Enteritidis* Using 2D DIGE

Min Young Park[2], Min Ah Park[2], Jae Yong Song[2], Min Seok Kim[1] and Jongsoo Chang[1]
[1]Korea National Open University and [2]Seoul National University

P-5

Differential Expression Profiling of Proteins by 2-DE in Human Cord Blood Mesenchymal Stem Cells Undergoing Osteoblast Differentiation.

Ji-Soo Kim[1], Hyun-Kyung Lee[1], Mi-Ryung Kim[1], Chan-Wha Kim[1]

P-6

Effects of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxins on Proteins Expression in Released Proteins of HepG2 Cell Lines

Hosub Im, Eunha Oh, Jin-Young Khim, Joohee Mun, Sohee Park, Eun-Kyoung Cho, Min-Young Bae, Sun-Young Choi, Donggeun Sul

P-7

Enhanced Proteome Profiling by Inhibiting Proteolysis with Small Heat Shock Proteins.

Jeong Wook Lee[1], Mee-Jung Han[1], and Sang Yup Lee[1,2]

P-8

Expression of Growth-Dependent Proteins in Chicken Oviduct

Myung Won Park[1], Yan Nan Shen[1], Tae Yoon Kim[2], Sung June Byun[3], Ik Soo Jeon[3] and Sang Hoon Kim[1]

P-9

Mapping and Comparative Study of Skeletal Muscle Mitochondrial Proteome

Nam-Kuk Kim[1], Jong-Hyun Lim[1], Min-Jin Song[1], Tae-Kyu Park[1], Oun-Hyun Kim[2], Beom-Young Park[3] and Chang-Soo Lee[1]

P-10

Oxygen Evolving Protein 1 as a Redox Pegulator of Thylakoid Lumen.

Young Min Kim, Yuna Jung[1], Gu Sang Hwang[1], Dong hyun Kim[2] and Dong bin Lim[1]

P-11

Play a Role of Chaperone Network in Psychrophilicity of *Bacillus Psychrosaccharolyticus*

Jong Bok Seo[1, 2], Hye Sook Kim[1], Joo Hee Chung[1, 2], Myung Hee Nam[1], Chan Wha Kim[2]

P-12

Protein Alternation after Intracerebroventricular (ICV) Injection of Corticotropin-releasing Factor (CRF) and Hippocampal Cholinergic Neurostimulating peptide (HCNP) into Rat Brain

Hong Gi Kim, Woo Ram Jung, Eun Young Lim, and Kil Lyong Kim

P-13

Protein Profiles of Mesenchymal Stem Cells Derived from Human Umbilical Cord Blood during Chondrogenesis

Hyun-Kyung Lee, Ji-Soo Kim, Mi-Ryung Kim, and Chan-Wha Kim

P-14

Proteome Analysis of Hypothermophilic Bacterium *Thermotoga Neapolitana*

Ah Young Lee[1], Chang Won Kho[1], Mi Jang[1], Youra Lee[1], Sung Goo Park[1], Do Hee Lee[1], Sayeon Cho[2], Kwang-Hee Bae[1], Sunghyun Kang[1], Byoung Chul Park[1]

P-15

Proteome Analysis of Neural Stem Cell using Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC)

Jeong Hwa Lee[1], Jin Young Kim[1], Ju Yeon Lee[1], Gun Wook Park[2], Kyung-Hoon Kwon[2], Kyung Hee Byun[3], Sung, Min Ahn[3], Bong Hee Lee[3], and Jong Shin Yoo[2]

P-16

Proteomic Analysis for Determination of Optimal Harvest Time by Storage Proteins in the Rice Endosperm

Pyung Gyun Shin, Sang Hyo Kim, An-Cheol Chang, Seong Chang Hong, and Ki Sang Lee

P-17

Proteomic Analysis in Bronchoalveolar Lavage Fluid (BALF) of Formaldehyde-Inhaled Rats

Kyong Hoon Ahn, Hyung Jun Jeon, Dong Hun Lee, Seok Kyun Kim, Jin Yi Han and Dae Kyong Kim

P-18

Proteomic Analysis of Bovine Skeletal Muscle Related with the Breeding Value in Hanwoo

Yan Nan Shen[1], Myung Won Park[1], Kang Seok Seo[2], Du Hak Yoon[2], , Hong-gu Lee[3], Sang Hoon Kim[1]

P-19

Proteomic Analysis of Lumenal Fraction of Secretory Vesicle from Adrenal Medulla

Jaeyoon Kim1, Kyungmoo Yea1, Moon-Chang Baek1, Byung-dae Lee1, Youndong Kim1, Sun kyu Choi2, Jong-in Kim2, Taehoon G. Lee3, Pann-Ghill Suh1, and Sung Ho Ryu1

P-20

Proteomic Analysis of Membrane Proteins in Erythritol–Producing Yeast *Candida magnoliae*

Ji-Hee Yu[1], Myoung-Dong Kim[2], Dae-Hee Lee[1], and Jin-Ho Seo[1]

P-21

Proteomic Analysis of Strain Specific Liver Proteins in Inbred Mice

Hye Suk Hwang, Il Yong Kim Sae Jin Oh, Kyung Jin Roh, and Je Kyung Seong
Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National

P-22

Proteomics of Human Umbilical Cord Vein-Mesenchymal Like Stem Cell

Jung KH, Oh SM, Park KS, Lee, JH, Kim KS[2], Choi MR[2], Park JY[2], Kim SH[1], Kim HT[1], Koh SH[1], Chai YG.

P-23

The Proteome of *Mannheimia Succiniciproducens*, a Capnophilic Rumen Bacterium.

Jeong Wook Lee[1], Sang Yup Lee[1, 2], Hyohak Song[1], and Jong Shin Yoo[3]

P-24

The Proteomic Analysis of Membranes from Adipogenic Differentiated Human Mesenchymal Stem Cells

Seung-Ah. Park, Ji-soo. Kim, Hyun-Kyung. Lee, Mi-Ryung. Kim, Chan-Wha. Kim*

P-25

The Role of a Transcriptional Regulator Involved in Cyanobacterial Photomovement

Joo Hee Chung[1,2], Young Hye Kim[1], Mi-Sun Cho[1], Young-Ho Chung[1], Ohoak Kwon[1], Myung Hee Nam[1], Jeong-Kook Kim[2], Chan Wha Kim[2], and Young Mok Park[1]

P-26

Transcriptome and Proteome Expression Profile during Acclimation from Low to High Light Intensity in the Cyanobacterium Synechocystis sp. PCC 6803

Soo-Jung Kim[1], Yong-Cheol Yoo[1], Kun Cho[1], Doo-Byoung Oh[2], Young-Ho Chung[1], Jong Shin Yoo[1], Hye Yeon Moon[2], Hyun Ah Kang[2], and Young Mok Park[1]

P-27

A differentially Expressed Proteomic Analysis in Placental Tissues in Relation to Pungency during the Pepper Fruit Development

Je Min Lee[1, 2 #], Seyoon Kim[3 #], Ji Young Lee[1 #], Eun Young Yoo[1, 2] Myeong Cheoul Cho[4], Min Rae Cho[5], Byung-Dong Kim[1, 2], Young Yil Bahk[3]

P-28

A *P*roteomic Approach for Dissecting H-Ras *S*ignaling Networks in NIH/3T3 Mouse Embryonic Fibroblast Cells.

Jung Wook Park[1, 2], Seyoon Kim[1, 2], Yu Sam Kim[1, 2], and Young Yil Bahk[2, *]

P-29

Caspase-7-Mediated Inactivation of Proteasomal Function during Apoptosis

Mi Jang[1, 3], Ah Young Lee[1], Chang Won Kho[1], Youra Lee[1,3], Sung Goo Park[1], Do Hee Lee[1], Sayeon Cho[2], Kwang-Hee Bae[1], Sunghyun Kang[1], Pyung Keun Myung[3] and Byoung Chul Park[1]

P-30

Comparison of Protein Expression of Rumen Anaerobic Fungus *Neocallimastix Frontalis* under Different Substrate Conditions Using 2D DIGE.

Min-Ah Park[2], Min-Young Park[2], Jae-Yong Song[2], Min-Seok Kim[1], Jong-Soo Chang[1]

P-31

Development of Affinity MALDI Plate

Mi Young Ha[1], Jeong Heon Lee[2], Hyoung Soon Park[3], and Yangsun Kim[1, 2]
[1]Microbiochip Center; [2]Proteonik Inc. Lab and [3]Probiond co. ltd

P-32

Functional Proteomic Analysis of Hippocampal Cholinergic Neurostimulating Peptide Precursor Protein (HCNPpp) Depletion Status in HT22 Cells Using si-HCNPpp RNA

Hong Gi Kim, and Kil Lyong Kim

P-33

Identification of Ca^{2+}-Dependent and Independent Interacting Proteins of Calmodulin from Human Proteome.

Joong Sup Shim, Eun Ju Yang, You Sun Kim, and Ho Jeong Kwon

P-34

Identification of Proteins in Response to Cholesterol Overfeeding in *Caenorhabditis Elegans*

Yun-Kyung Shin, Yu-Joun Yun and Yhong-Hee Shim
Department of Biological Sciences, Bio/Molecular Informatics Center, and Institute of Biomedical Science and Technology, Konkuk University

P-35

Interactome Analysis of Annexin A4

Young Ju Jeon[1,3], Ah Young Lee[1], Sun Young Lee[1], Mi Jang[1], Chang Won Kho[1], Youra Lee[1], Sung Goo Park[1], Do Hee Lee[1], Sayeon Cho[2], Kwang-Hee Bae[1], Sunghyun Kang[1], Pyung Keun Myung[3], Byoung Chul Park[1]

P-36

Possible Role of PTK6 in Modulation of Cell spreading by Interaction with ARAP1

Eun-Saem Lee and Seung-Taek Lee

P-37

Proteolysis of Aldolase A by caspase-3 and Caspase-7 during Apoptosis

Sun Young Lee[1,3], Ah Young Lee[1], Mi Jang[1], Chang Won Kho[1], Youra Lee[1], Sung Goo Park[1], Do Hee Lee[1], Sayeon Cho[2], Chong Kil Lee[3], Kwang-Hee Bae[1], Sunghyun Kang[1], Byoung Chul Park[1]

P-38

Inhibitory Effect of Resveratrol (3,4', 5-Trihydroxy-Trans-Stilbene) on Huh-7 and SK-HEP-1.

In Sok Hwang[1], Jun Hee Hong[1], Seon Ah Chong[1], Myeong Jin Nam[1]

P-39

Lack of Oxygen Enhanced Efficacy on Arsenic Trioxide-Induced Cytotoxicity in Human Hepatic Cancer Cell.

Jun Hee Hong[1], In Sok Hwang[1], Seon Ah Chong[1], Myeong Jin Nam[1].

P-40

퇴행성 뇌질환 의 진단을 위한 항체부착 **color coded bead** 키트 개발.

장중식[1], 김명래[1], 오소미[1], 김윤화[1], 이영하[1], 신정우[1], 최연식[1], 이종서[1], 강상원[2]
[1]㈜랩프런티어; [2]이화여자대학교

P-41

Acetaldehyde Dehydrogenase 1 is Down-Regulated in the Fibroblasts Derived from Atopic Dermatitis Patients

Yong-Doo Park[1,2], You-Jeong Lyou[2], Dong-Youn Lee[1], and Jun-Mo Yang[1,2,¶]

P-42

Analysis of Serum Glycoproteome of Type 2 Diabetics with Nephropathy

Mi-Yeon Kim, Shin-Ae Yu, Mi-Ryung Kim, and Chan-Wha Kim

P-43

Apolipoprotein E Is a Novel Substrate for Matrix Metalloproteinase-14

Jun-Hyoung Park[1], Sung-Min Park[1], Sun-Hyun Park[2], Kyung-Hyun Cho[2], and Seung-Taek Lee[1]

P-44

Cardioprotective Effects of KR33028, A Novel Na^+/H^+ Exchanger-1 Inhibitor Evaluated In Ischemia/Reperfusion Induced Rat Hearts by Comparative Proteomics.

Jina Kim[1], Kyu Yang Yi[2], Byung Ho Lee[2], Dong Cho Han[1], Kab Seog Yoon[1], Kwang Hee Son[1], and Byoung-Mog Kwon[1,]

P-45
Changes of Plasma Proteins by Treatment with Leukotriene Receptor Antagonist in Aspirin Induced Asthma
SO YOUN KIM, SEUNG-HA LEE, JONG SOOK PARK, TAIYOUN RHIM, CHOON-SIK PARK

P-46
Coagulation Factors Involved in Recurrent Pregnancy Loss (RPL)
Myung-Sun Kim[1], Yong-Soo Kim[1], Eung-Ji Lee[1], Brijesh S. Ajjappala[1], Sook-Hwan Lee[1], Bum-Chae Choi[2], Kwang Yul Cha[1], and Kwang-Hyun Baek[1]

P-47
Comparative Analysis of the Liver And Plasma Proteomes for Discovery of Biomarker of Hepatocellular Carcinoma
Eun-Young Lee, Hye-Young Kim, Min-Jung Kang, Hoguen Kim[1] and Young-Ki Paik

P-48
Comparative Analysis of The Serum Proteome in Type 2 Diabetes Mellitus Patients
Hyo-Shick Jun, Min-Jung Kim, Chan-Wha Kim

P-49
Comparative Proteome Analysis of Serum in the Patients with Wilson Disease for the Discovery Of Biomarkers.
Sun Hee Heo, Gu-Hwan Kim, Sang-Wook Park, Han-Wook Yoo

P-50
Comparison of Serum Proteomes for the Discovery of Biomarkers for Type 2 Diabetic Nephrophathy
Hyun-Syuk Yoo, Eun-Hee Cho, Mi-Ryung Kim, and Chan-Wha Kim*

P-51
Deciphering Synaptic Architecture at Type1 Glutamatergic Synapses in Drosophila: Molecular Basis Of Synapse Development, Formation, and Plasticity
Jong Bok Seo[1], Dae Won Lee[2], Young Ho Koh[2]

P-52
Differential Expression of Protein in Response To Ascochlorin in a Human Osteocarcoma Cell by 2-D Gel Electrophoresis and MALDI-TOF-MS
Jeong Han Kang[1], Hyun Ji Cho[1], Jung Hwa Woo[1], Cheorl-Ho Kim[2] and Young-Chae Chang

P-53
Differentially Expressed Plasma Proteins in Streptozotocin-Induced Diabetic Rats before and after Administration of Fungal Polysaccharides
Sang Woo Kim, Hye Jin Hwang, and Jong Won Yun

P-54
Effects of Thioredoxin on LPS-induced Inflammatory Response In Human Skin Melanoma Cells by Proteomic Analysis.
Gi-Yeon Han, Mi-Ryung Kim, Chan-Wha Kim*

P-55

A Proteomics Analysis Revealed PDEF Interacts with HER-2 Signaling Molecules in Breast Cancer Cells.

Minjung Lee[1], Seung-Jin Lee[1], Byung-Gyu Kim[1], Sun Hee Her[1], Youn–Jeong Kim, Hye-Jeong Park[1], Hyun-Mo Ryoo[2], Je-Yoel Cho[1]

P-56

Gene Expression Profiles of PBMC by Antigen Stimulation in Food Allergy of Atopic Dermatitis using DNA Expression Chip (IGEC-64, Immune Gene Expression DNA Chip-64)

G Noh[1], SY Choi[2], SY Lim[2], JW Oh[3], JH Lee[4]

P-57

Inhibitory Effect of Cisplatin on the Cell Proliferation and Identification of Cisplatin-Regulated Proteins in Mouse Osteoblast MC3T3-E1 Cells

Myung Hee Kim, Su-Ui Lee, Bum Tae Kim, Yong Ki Min, Seong Hwan Kim*

P-58

Inhibitory Mechanisms of Methylmercury on Retinoic Acid-Induced Differentiation of Human Neuronal Cells

Young-Seok Kim[1], Youn-Jung Kim[1], Mi-Soon Kim[1], Eu-Ju Choi[2] and Jae-Chun Ryu[1]

P-59

Modulation of Ca^{2+}-Activated K^+ Current by H-89 in Rabbit Coronary Arterial Smooth Muscle Cells

Won Sun Park, Nari Kim, Jae Boum Youm, Jae-Hong Ko, Sung Hyun Hang, Tae-Ho Kim, Jin Han[1]*

P-60

Proteomic Analysis of Differentially Expressed Proteins induced by IL-3 in Lymphocytes

Brijesh S. Ajjappala, Yong-Soo Kim, Myung-Sun Kim, Min-Young Lee, Jung-Mi Park, In-Soo Han and Kwang-Hyun Baek

P-61

Slowing of the Inactivation of Voltage-Dependent Sodium Channels by Staurosporine, the Protein Kinase C Inhibitor, in Rabbit Atrial Myocytes

Jae Hong Ko, Nari Kim, Jae Boum Earm, Won Sun Park, Yung E Earm

P-62

The mTOR Pathway Integrates the Glucose Sufficiency through an Interaction between Rheb and Glyceraldehyde-3-Phosphate Dehydrogenase

Mi Nam Lee, Jae Yoon Kim, Sang Hoon Ha, Jung Hwan Kim, Chang Sup Lee, A-Ra Koh, Pann-Ghill Suh, and Sung Ho Ryu

P-63

The Protein Kinase A Inhibitor, H-89, Directly Inhibits K_{ATP} and Kir Channels in Rabbit Coronary Arterial Smooth Muscle Cells

Won Sun Park, Nari Kim[1], Jae Boum Youm[1], Jae-Hong Ko[2], Sung Hyun Kang, Hyoung Kyu Kim, Jin Han[1]*

P-64

Two-Stage Identification of Proteome for Understanding BMP2-Induced Osteoblast Differentiation in C2C12 Premyoblast Cells

Byung-Gyu Kim[1], Minjung Lee[1], Seung-Jin Lee[1], Hye-Jeong Park[1], Sun-Hee Her[1], Won-Bong Lee[1], Hyun-Mo Ryoo[2], Je-Yoel Cho[1]*

Session 2

Biotechnology perspectives, High-throughput protein expression in vivo and in vitro, Post-translational modifications, Protein Informatics, Proteomics and diseases 2, Resources and Technology, Structural proteomics

P-65

A Proteomic Analysis of Chung-Kuk-Jang for the Identification of Proteins Related to Bio-Functional Activities

Il-Young Son, Hyunsoo Choi, Jong Sang Kim, Jinkyu Lim

P-66

Analysis Of Biomolecular Interactions On Protein Chips by a Novel Scanning Surface Plasmon Microscope Biosensor

Jong Seol Yuk, Jae-Wan Jung, Young-Myeong Kim, and Kwon-Soo Ha

P-67

Gene to Antibody in Proteomics.

Jong-Seo Lee

P-68

Plasma Proteome Analysis by Label Free Quantitation with Nano-UPLC/Q-tof.

Ju Yeon Lee[1], Jin young Kim[1], Jeong Hwa Lee[1], Gun Wook Park[2], Kyung-Hoon Kwon[2], Kun Cho[1], Young Ki Paik[3], Kyoung Wook Kim[4] and Jong Shin Yoo[2]

P-69

Protein Nanoarray on Prolinker Surface Constructed by Atomic Force Microscopy Dip-pen Nanolithography for Analysis of Protein Interaction

Minsu Lee[1], Dong-Ku Kang[2], Hyun-Kyu Yang[3], Keun-Hyung Park[3], Soo Young Choe[4], ChangSoo Kang[5], Soo-Ik Chang[2], Moon Hi Han[6], and In-Cheol Kang[1]

P-70

Proteomic Analysis of Human Serum Protein using an Aptamer-Based Biochip

KIM Young Mi, NOH Jaesang, YOO Hye Young, YOON Shine young and KIM Sung-Chun*

P-71

Quantification of MAP3K in Rice using a New Quantitative Technique, AQUA

Soo Jae Lee[1], Joo Young Bang[2], Ji Sook Park[3], Jung A Kim[4], Nam-Soo Jwa[4], Kwang Pyo Kim[1,2,3]

P-72

Differential Expression Analyses of *Saccharomyces Cerevisiae* KNU5377 Against Menadione-Induced

Oxidative Stress
Il Sup Kim[1], Hae Sun Yun[2], and Ing Nyol Jin[1]

P-73
Proteomic Identification of Human Brain Proteome by Biochemical Prefractionation Coupled with RPLC-ESI MS/MS method
Sang Kwang Lee[1], Jin Young Kim[1], Kyung-Hoon Kwon[2], Young Hye Kim[1], Se-Young Kim[1], Gun Wook Park[2], Jeong Hwa Lee[1], Bonghee Lee[3], Jong Shin Yoo[2], and Young Mok Park[1]

P-74
Structural Characterization of I2GdBN, a New Recombinant Factor VIII, by Mass Spectrometry Combined with Liquid Chromatography
Jong-il Yoo, In Young Song, Jong Wan Kim, Hun Taek Kim, and Yong Kook Kim[*]

P-75
Dynamic *O*-GlcNAc Modification in Response to Glucose Deprivation
Jeong Gu Kang[2] and Jin Won Cho[1,2]

P-76
High-Throughput Phosphoproteome Analysis by TiO_2 Bead-Mediated Enrichment and GET-Labeling
Kun Cho, Yeong Hee Ahn, Jin Young Kim and Jong Shin Yoo

P-77
Identification of Phosphoprotein of Human Plasma Protein using 2DE and Data-Dependent Neutral-Loss Scans on Linear Ion Trap
Min-Jeong Kang, Hyoung-Joo Lee, Eun-Young Lee, Hyon-Suk Kim[1], Young-Ki Paik

P-78
Identification of Phosphorylation Sites of a Rice ABF Family of bZIP Transcription Factor by Mass Spectrometry and Phosphopeptide Mapping
Jin Hee Lee[1], In-Sun Yoon[3], Ji-Yeon Hong[3], Kun Cho[2], Jong Bok Seo[1], Joo Hee Chung[1], Myung Hee Nam[1],

P-79
Identification of Protein Nitration on Tyrosine Residue using Electrospray Ionization (ESI) Tandem Mass Spectrometry
Soo Jae Lee[1], Jin Woo Jung[2], Keum Yong Kang[2], Yoon Shin Park[3], Sang Ick Park[3], Kwang Pyo Kim[2,4]

P-80
***O*-GlcNAc Modification is Involved in Final Differentiation of Myoblast C2C12.**
Sang Yoon Park[1], Hyung Wook Nam[2], Suena Ji[1], Yu Sam Kim[2,*] and Jin Won Cho[1,*]

P-81
Phosphoproteome Analysis Based on the In-Gel GET-Tagging/Digestion Approach
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Awards Information

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자격 및 선정	만 35 세 미만의 석박사 과정생 혹은 박사후 과정인 자 중 본 학술대회에 초록을 제출한 자.
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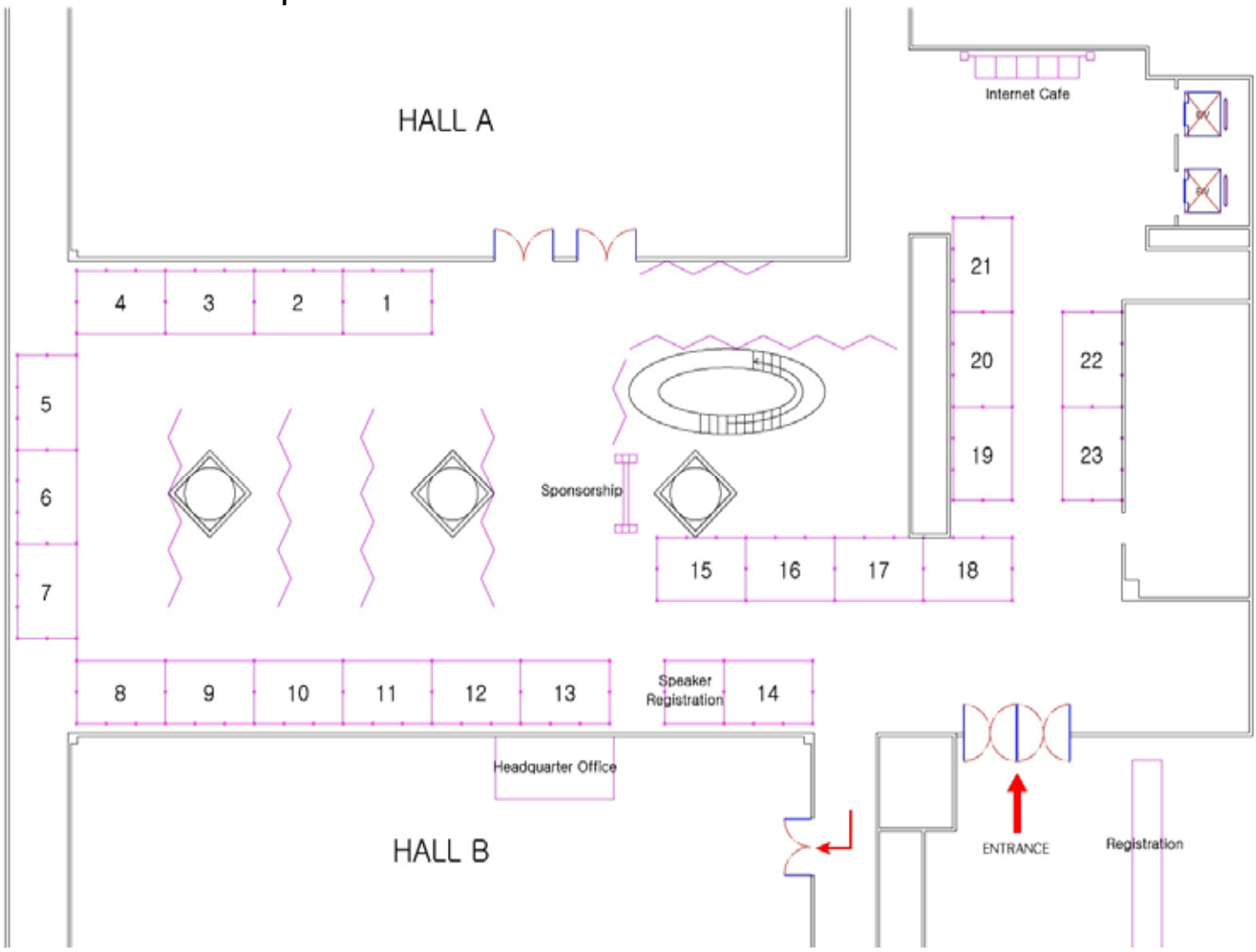

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