“Let’s Fish Mass ! ”

ms3d
FIRST Program

- **“ms3d” Project**

  Koichi Tanaka

- **Flexible Antibody**

  Dr. Daniel Capon

“ms3d” Project

ms3d
FIRST Program

Development of the Next Generation Mass Spectrometry System and Contribution toward Drug Discovery and Diagnostics <http://www.first-ms3d.jp/>

mass **s**pectrometer for **d**rug **d**iscovery and **d**iagnostics

--- Towards diagnostics, drug discovery
and treatment from a single drop of blood ---

ms3d? <http://www.first-ms3d.jp/english/>

Funding Program for World-Leading Innovative R&D on Science and Technology(FIRST Program)

Development of the next generation mass spectrometry system, and contribution toward drug discovery and diagnostics

Development of the next generation mass spectrometry system, and contribution toward drug discovery and diagnostics

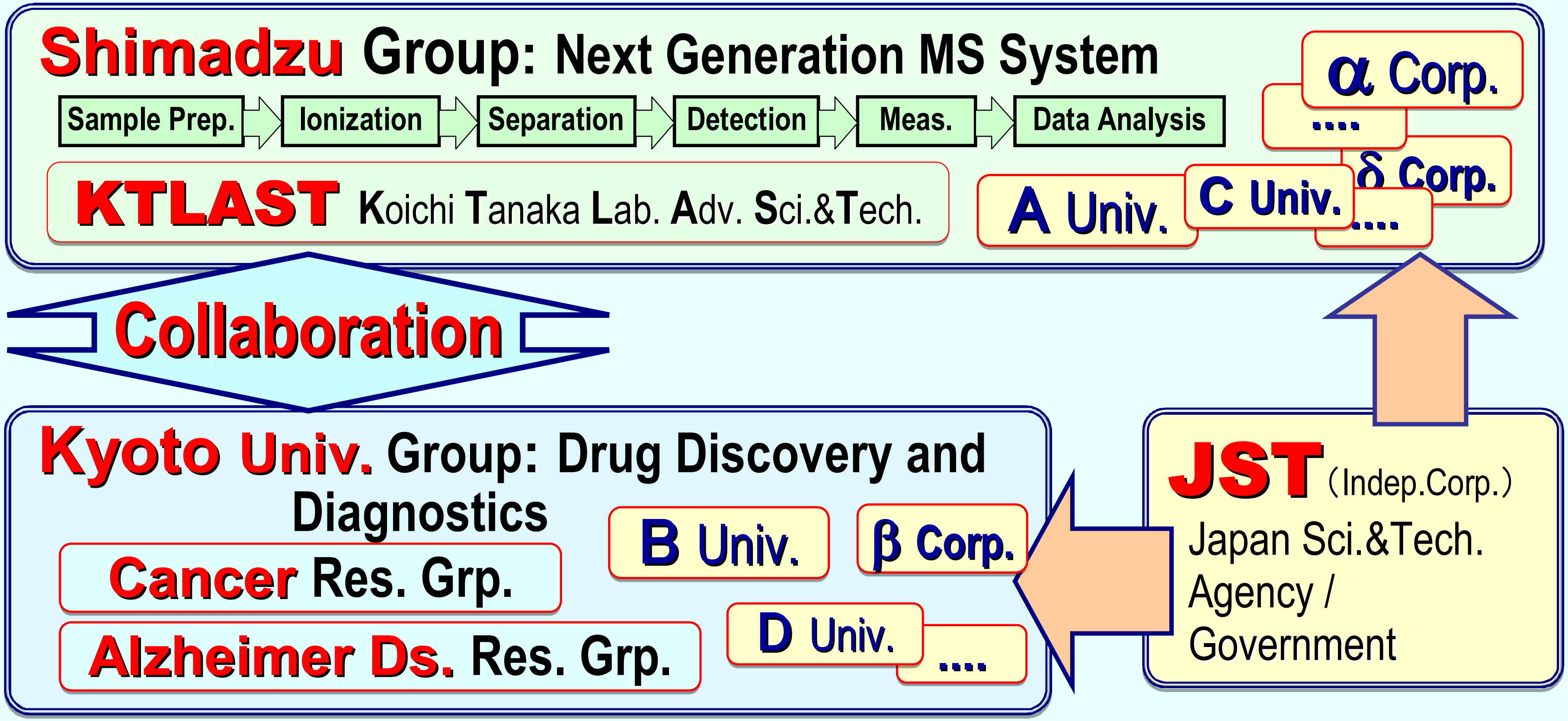
Funding Program for World-Leading Innovative R&D on Science and Technology(FIRST Program)

About this project

Under the Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program), this project aims to develop the world's highest performance mass spectrometry system. The system will be used to find biomarkers and candidate target molecules in order to establish new diagnostic and treatment methods for breast and esophageal cancer and for Alzheimer's disease.

When practical technologies become available in near future, the discovery research of new biomarkers for diagnostic and therapeutic use will be greatly accelerated to achieve healthier and longer life for all.

Structure of ms3d project



Shimadzu Group: Next Generation MS System

Sample Prep. → Ionization → Separation → Detection → Meas. → Data Analysis

KTLAST Koichi Tanaka Lab. Adv. Sci.&Tech.

α Corp.

....

δ Corp.

A Univ.

C Univ.

....

Collaboration

Kyoto Univ. Group: Drug Discovery and Diagnostics

Cancer Res. Grp.

Alzheimer Ds. Res. Grp.

B Univ.

β Corp.

D Univ.

....

JST (Indep.Corp.)
Japan Sci.&Tech. Agency / Government

ms3d
FIRST Program

mass **s**pectrom. for
drug **d**iscov. & **d**iagnos.

To develop the world's highest performance ultra-high-sensitivity and ultra-high-selectivity mass spectrometry system under the **industry-university-government** cooperative research program

~4-year project

Goal: >10,000 times higher sensitivity and selectivity

Current Status of Life Science and MS Technique

Our approach to drug discovery and diagnosis

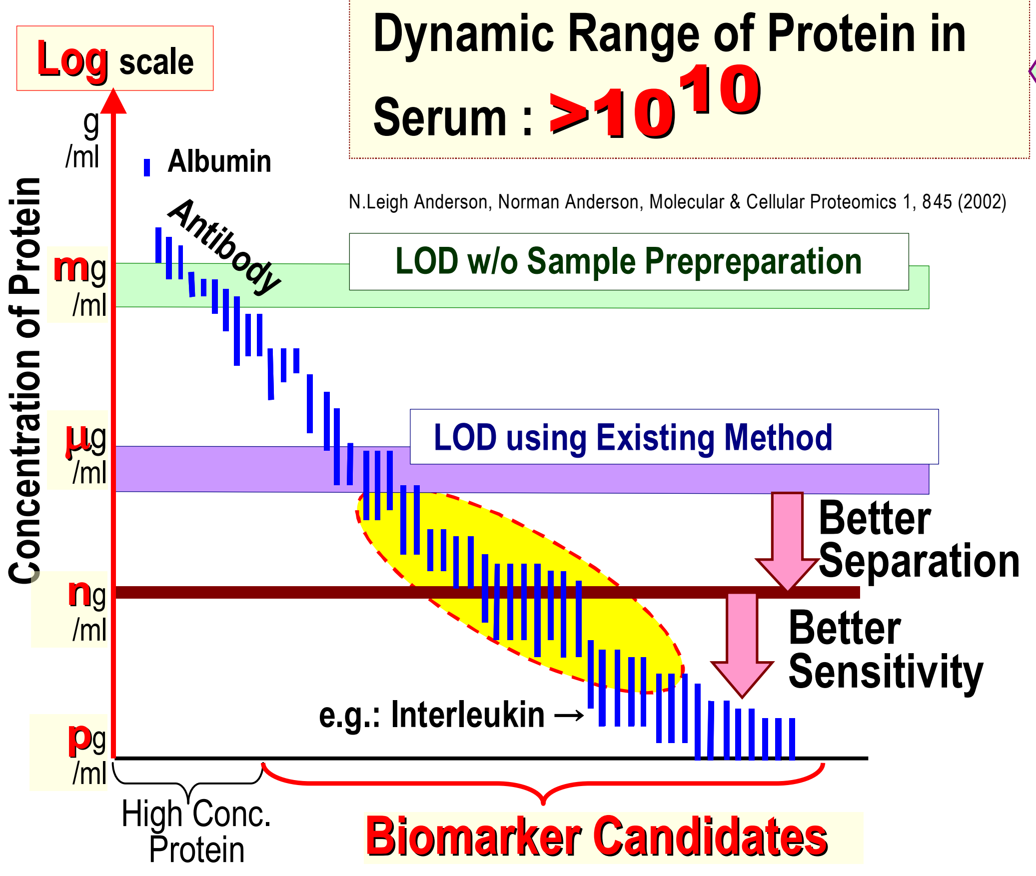
Proteomics to date, simply interpreted . . .

- Sequential investigation **from the most abundant** proteins out of, at least, hundreds of thousands
- **Pool of knowledge** obtained by world's leading researchers
- Ability to **focus on targets** / **formulate** appropriate **hypotheses** based on them

We started our "MS" project based upon above interpretation

ms3d
FIRST Program

Current Status of Life Science and MS Technique



Dynamic Range of Protein in Serum : >10^{10}

N.Leigh Anderson, Norman Anderson, Molecular & Cellular Proteomics 1, 845 (2002)

Dynamic Range of MS: $10^{3\sim4}$

It is well known that proteins are linked to almost all diseases.

Detecting very low abundance proteins is the key to unraveling unknown phenomena.

< To solve this problem >

It is necessary to develop methods to "**ignore**" abundant known compounds, and to **detect** candidates at very low amount.

What were developed to construct the best MS system in ms3d project up to Aug/2012

MS : at first, desired compound(s) is(are) selected, ionized, separated, detected, measured and analyzed

Sample Prep. → Ionization → Separation → Detection → Meas. → Data Analysis

Sample Prep. #1

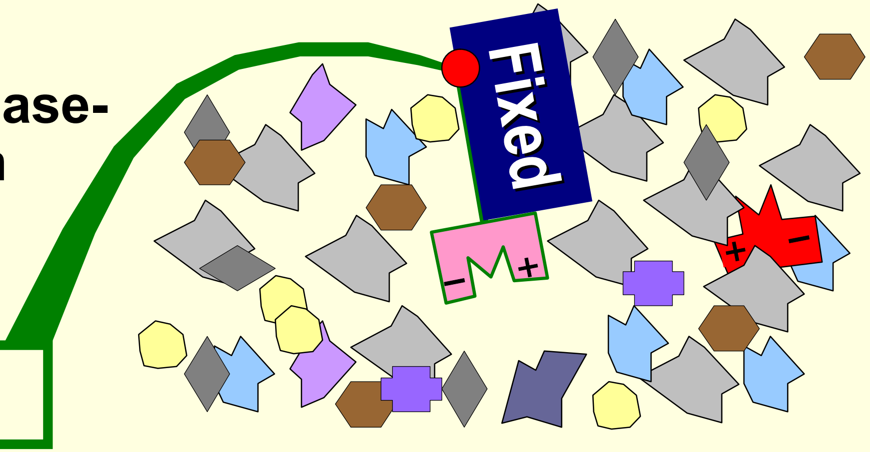
MS : at first, desired compound(s) is(are) selected, ionized, separated, detected, measured and analyzed

Sample Prep. → Ionization → Separation → Detection → Meas. → Data Analysis

Sample Prep.

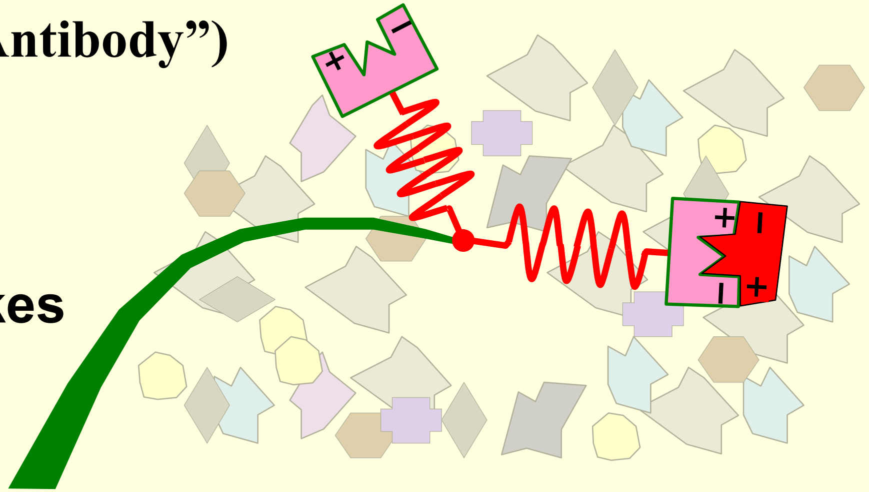
(Conventional fishing by antibody)

- >100k types of human proteins
- Endless variety of abundances
- Especially low abundance in disease-related compounds, "To catch an unidentified fish out of the sea from a billion others"
- Selection of optional needle and bait for **fishing**

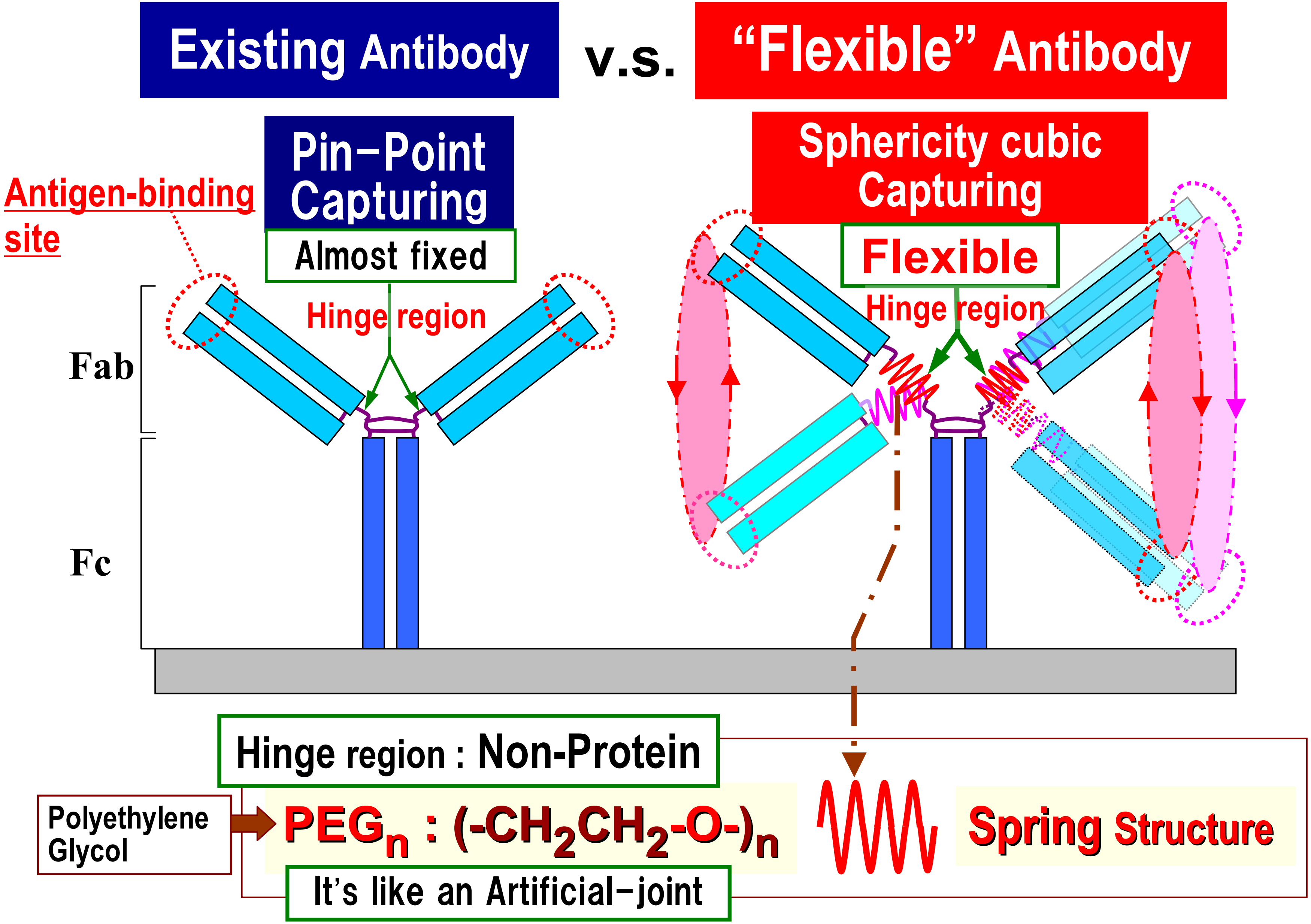


(Fishing by "Flexible Antibody")

Rather than "ice fishing" . . .
rotating, **expanding** and
contracting the fishline makes
fishing more **practical !**



Sample Prep. #2



Existing Antibody v.s. **“Flexible” Antibody**

Pin-Point Capturing

Almost fixed

Sphericity cubic Capturing

Flexible

Hinge region : Non-Protein

Polyethylene Glycol → PEG_n : $(-CH_2CH_2-O-)_n$ Spring Structure

It’s like an Artificial-joint

Ionization

MS : at first, desired compound(s) is(are) selected, ionized, separated, detected, measured and analyzed

Sample Prep. → Ionization → Separation → Detection → Meas. → Data Analysis

Liquid Matrix

Phosphopeptide : Max. x10k
Glycopeptide : Max. x100

Liquid matrix:
- Softer ionization
- More Hydrophilic

Neutral Glycan : Max. x100

Hydrophobic peptide : Max. x100

Development of matrix capable of high-sensitive detection of hydrophobic peptides (weak area in conventional MALDI)

Cys Labeling Reagent : Max.: x200

C-Term Labeling

Development of chemical processing method for simple detection of protein C-terminus sequence information that was conventionally challenging

MS Hardware

MS : at first, desired compound(s) is(are) selected, ionized, separated, detected, measured and analyzed

Sample Prep. → Ionization → Separation → Detection → Meas. → Data Analysis

World's First

High Speed MS + High Performance MS^n = MALDI - Digital Ion Trap - TOFMS

Linear/Reflectron TOF for MS

&

Digital Ion Trap TOF for MS^n

Digital Ion Trap

ms3d FIRST Program

MS Software

MS : at first, desired compound(s) is(are) selected, ionized, separated, detected, measured and analyzed

Sample Prep. → Ionization → Separation → Detection → Meas. → **Data Analysis**

ms3d
FIRST Program

Workshop 4 "Mass++ and MassBank" Tue Evening

"Mass++" :

- **Freeware**
- Manual(Tutorial) : English
- Users can develope "**Plug-In**" software
- Data format : mzxml, Applied Biosys., Thermo, Waters, Shimadzu/Kratos
- Mascot Search
- Batch Processing
- *De novo* sequencing "SIMSE"
- Quantitative Analysis
- Statistical Analysis
- Link to MassBank

<http://www.first-ms3d.jp/english/>

Some of MS Application

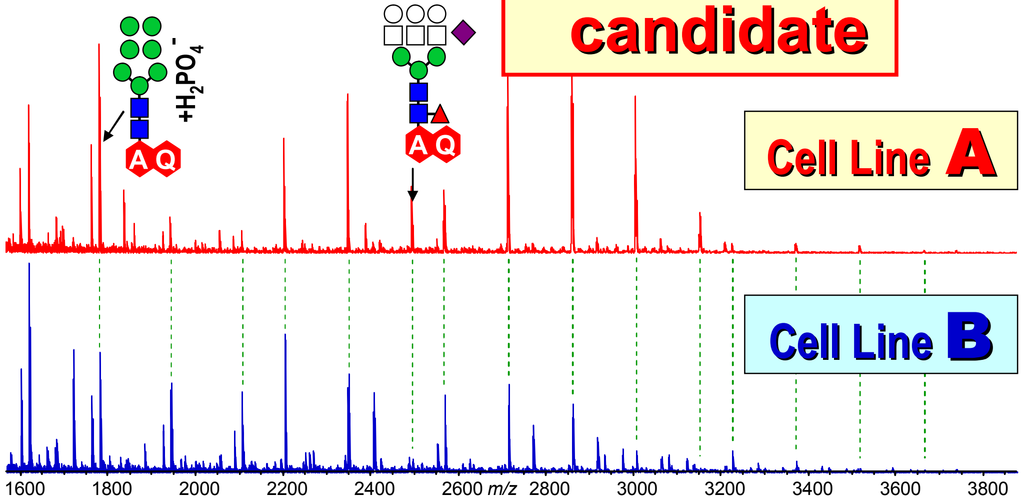
World's First

Oligosaccharide Profile of Glycoprotein HER2

MALDI-DIT-TOFMS Prototype Machine

Kratos Axima-Resonance

Biomarker candidate



Breast Cancer Imaging

HE stain : difficult to distinguish cancer area

Biomarker candidate

MS Imaging of a Lipid A

Imaging MALDI

ms3d Present. List MSSJ2011 1-12/12

	No.	First Author and/or Presenter	Co-author(s)	Title
1	1P-09	Yusaku Hioki /KTLAST	Yuko Fukuyama, Chikako Hamana, Shinichi Iwamoto, Koichi Tanaka/ KTLAST	Improving the Sensitivity of MALDI-MS Analyses by Small Droplet Deposition
2	1P-12	Kaoru Kaneshiro /KTLAST	Makoto Watanabe, Koichi Tanaka /KTLAST ; Kazuya Terasawa, H. Uchimura, K. Shimizu /Kyoto Univ.	N-glycan profiling of HER2 by AQ-labeling method with 3-AQ/CHCA liquid matrix
3	1P-40	Natsumi Funakoshi /KTLAST	Yuko Fukuyama, Shinichi Iwamoto, Koichi Tanaka /KTLAST	Highly sensitive MALDI analyses of glycopeptides using liquid matrices 3-AQ/CHCA and 3-AQ/CA
4	1P-49	Kei Kodera /KTLAST	K. Watanabe, M. Hazama, M. Furuta, S. Sekiya, K. Kinoshita, H. Takahashi, K. Hosoi, T. Kobayashi, Shinichi Iwamoto, Koichi Tanaka /KTLAST	MALDI-Digital-Ion-Trap-TOFMS with ion trap Pass-through TOF Mode
5	2P-01	Howell E Parry /KTLAST	Satoshi Tanaka, Shin-ichi Utsunomiya, Shigeki Kajihara, Koichi Tanaka /KTLAST ; T. Tabata, K. Aoshima, Y. Oda /Eisai Corp. ; Y. Nihei, T. Nishioka /Keio Univ.	Mass++ 2.0.0: New version of freeware mass spectrometry analysis software
6	2P-11	Kenichi Taniguchi /KTLAST	Yuko Fukuyama, Yutaka Ibuki, Koichi Tanaka /KTLAST	Highly sensitive analysis of glycopeptides using labeling technique by MALDI-MS
7	2P-24	Takashi Shimada /KTLAST	Taka-aki Sato, Koichi Tanaka /KTLAST	Development of novel MS probes for high-sensitivity proteomics
8	2P-40	Ritsuko Tanimura /KTLAST	Yuko Fukuyama, Shinichi Iwamoto, Koichi Tanaka /KTLAST ; Shunsuke Izumi /Hiroshima Univ.	Highly sensitive MALDI analyses of hydrophobic peptides using a novel additive ADHB with conventional matrices
9	3P-01	Kentaro Morimoto /KTLAST	Masaki Murase, Shigeki Kajihara, Koichi Tanaka /KTLAST ; T. Tabata, K. Aoshima, Y. Oda /Eisai Corp.	Evaluation of a novel database search algorithm using MSn spectra
10	3P-21	Akiyasu C. Yoshizawa /KTLAST	Shigeki Kajihara, Koichi Tanaka /KTLAST ; T. Tabata, T. Kimura, K. Aoshima, Y. Oda /Eisai Corp.	Application of post-translational modification information in database search
11	3P-26	Kazuya Terasawa /Kyoto Univ.	H. Uchimura, K. Shimizu /Kyoto Univ. ; Kaoru Kaneshiro, Makoto Watanabe, Koichi Tanaka /KTLAST	Estimation of HER2 N-glycosylation ratio using MALDI MS
12	3P-40	Yuko Fukuyama /KTLAST	Kohei Takeyama, Shinichi Iwamoto, Koichi Tanaka /KTLAST	Highly sensitive MALDI analyses of phosphorylated peptides using an optimized liquid matrix 3-AQ/CHCA

ms3d Present. List IMSC2012 1-14/14

Shimadzu Group Present. : 80

	No.	First Author	Co-a	
1	PMo-031	Yusaku Hioki /KTLAST	Hiroki Kuyama, Chikako Hamana, Kohei Takeyama, Koichi Tanaka / KTLAST	Influence of Sample Preparation Techniques on the Sensitive Detection of Peptides by MALDI-MS
2	PMo-082	Masahiro Kawashima /Kyoto Univ.	Noriko Fukao, Minoru Suzuki, Taka-aki Sato, Koichi Tanaka /KTLAST ; Nobuko Kawaguchi-Sakita, Masahiro Sugimoto, Takayuki Ueno, Kazuya Terasawa, Kazuharu Shimizu, Masakazu Toi /Kyoto Univ.	Molecular detection of breast cancer-related phosphatidylinositol by high-resolution imaging mass spectrometry
3	PMo-094	Chihiro Nakajima /KTLAST	Hiroki Kuyama, Koichi Tanaka /KTLAST	Mass spectrometry-based sequencing of protein C-terminal peptide using alpha-carboxyl group specific derivatization and COOH capturing
4	PMo-120	Shinichi Iwamoto /KTLAST	Yusaku Hioki, Ritsuko Tanimura, Yuki Ohta, Koichi Tanaka /KTLAST ; Takashi Shimada /Shimadzu Corp.	Multidimensional LC-MALDI Workflow for High Sensitivity Detection of Low-Abundance Peptides from Complex Samples
5	PMo-200	Makoto Watanabe/ KTLAST	Rie Yamamoto, Shinichi Iwamoto, Yuko Fukuyama, Ritsuko Tanimura, Shin-ichiro Kawabata, Taka-aki Sato, Koichi Tanaka /KTLAST ; Shunsuke Izumi /Hiroshima Univ.	Direct detection of S-nitrosylated peptides with UV-MALDI MS using porphyrin and retinoic acid as a matrix additive
6	PTu-042	Takashi Nishikaze /KTLAST	Kaoru Kaneshiro, Shin-ichiro Kawabata, Koichi Tanaka /KTLAST	Negative-ion fragmentation of neutral N-glycans derivatized with 3-aminoquinoline and other non-acidic reagents
7	PTu-082	Kenji Nakayama / Kyoto Univ.	Takahiro Inoue, Hiroki Tsumoto, Kazuharu Shimizu, Osamu Ogawa /Kyoto Univ. ; Shin-ichiro Kawabata, Shinichi Iwamoto, , Sadanori Sekiya, Minoru Suzuki, Koichi Tanaka /KTLAST	Discovery of novel urinary biomarker candidates for diagnosis of prostate cancer
8	PTu-128	Kei Kodera /KTLAST	Masafumi Jinno, Kiyoshi Watanabe, Makoto Hazama, Masaji Furuta, Sadanori Sekiya, Kaori Kinoshita, Hidenori Takahashi, Kosuke Hosoi, Toshinori Kobayashi, Shinichi Iwamoto, Koichi Tanaka /KTLAST	A Novel Precursor Isolation Method using Digital Ion Trap Mass Spectrometer
9	S30-1640	Ken Aoshima /Eisai Corp.	K. Takahashi, K. Matsuura, H. Watanabe, Y. Sato, T. Uehara, T. Kimura, T. Nakamura, Y. Oda /Eisai Corp. ; K. Shigeru, F. Mitsuru /Ibio-Tech ; Parry Howell, Satoshi Tanaka, Shin-ichi Utsunomiya, Shigeki Kajihara, Koichi Tanaka /KTLAST	Ab initio peak identification for SRM/MRM data
10	PWe-058	Masaki Murase /KTLAST	Hidenori Takahashi, Yosihiro Yamada, Sadanori Sekiya, Shigeki Kajihara, Shinichi Iwamoto, Koichi Tanaka /KTLAST	Data-dependent acquisition system for N-linked glycopeptides using MALDI-DIT-TOF MS
11	S32-0940	Takae Takeuchi /Nara Women's Univ.	Ayaka Takahashi, Erika Sugawara, Tomoko Kimura, Yuka Kurosaki /Nara Womens Univ. ; Hiroko Morinaga /Shimadzu Corp. ;Shigeki Kajihara, Shinichi Iwamoto, Koichi Tanaka /KTLAST	Ab initio MO Study on the Fragmentation Mechanisms of Protonated Phosphopeptides in "On-Resonance" and "Off-Resonance" Pulsed Gas Introduction Collision-Induced Dissociations
12	S34-1040	Akiyasu C Yoshizawa /KTLAST	Tsuyoshi Tabata, Takayuki Kimura, Ken Aoshima, Yoshiya Oda /Eisai Corp. ; Shigeki Kajihara, Koichi Tanaka /KTLAST	MSPTM-DB: a known PTM database for high-speed and accurate search available on the "ProteoAnalysis" web site
13	PTh-024	Howell E Parry /KTLAST	Satoshi Tanaka, Shin-ichi Utsunomiya, Shigeki Kajihara, Koichi Tanaka /KTLAST ; Tsuyoshi Tabata, Ken Aoshima, Yoshiya Oda /Eisai Corp.	Rapid development of functional extensions for mass spectrometry using freeware Mass++
14	PTh-033	Tsuyoshi Tabata / Eisai Corp.	Takayuki Kimura, Tatsuji Nakamura, Ken Aoshima, Yoshiya Oda /Eisai Corp. ; Akiyasu C Yoshizawa, Shigeki Kajihara, Koichi Tanaka /KTLAST	JobRequest - an easy-to-use software platform for proteomic analysis - and ProteoAnalysis, its application for protein identification.