



Customer Insights

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Ultra high resolution imaging techniques allow a leading laboratory at Doshisha University to unravel the complexities of neurological disorders



Working with Bruker

The Department of Medical Life Systems at Doshisha University, Japan, houses a leading laboratory using MALDI Imaging mass spectrometry to advance its research across the genomics and proteomics field. Professor Masaya Ikegawa leads the laboratory, and describes how Bruker supports its work:

“For the work we do, particularly our neurological research, the rapifleX instrument is vital. Without its superior resolution we could not obtain the highly detailed images necessary for visualizing peptides in the brain.”

Department of Medical Life Systems, Doshisha University, Kyoto

Doshisha University in Kyoto, Japan, has a long-standing history as one of the oldest private schools in the country. The Department of Medical Life Systems was founded in 1998 and leading the laboratory for Genomics and Proteomics for Human and Model Organisms is Professor Masaya Ikegawa. The department was initially founded based on the membership of well-known scientists, primarily in Neurology and Molecular Cell Biology. Now the president of the department is Professor Noriko Noguchi, from University of Tokyo, who is studying on oxidative stress in biology and clinical settings. In addition, Professors Tomoyuki Takahashi and Yasuo Ihara were the initial members of the current department, both having moved from University of Tokyo. They are top leaders of Molecular Neuroscience and Neuropathology in Japan.

There are currently 17 staff and nine laboratories at the Department of Medical Life Systems, with 39 staff overall at the faculty. The main research projects include the clarification of various epidemiological mechanisms arising from im-

balances in systemic control systems and the prevention and treatment of such problems. Prior to his position at Doshisha University, Professor Ikegawa was an Associate Professor in the Division of Genomic Medical Sciences at Kyoto Prefectural University of Medicine.

The Work

Professor Ikegawa's laboratory focuses on genomic, proteomic and metabolomic approaches to understand, diagnose and cure intractable diseases, such as Alzheimer's Disease (AD), diabetes, cardiovascular diseases, cancers and autoimmune diseases. He started his omics studies at Professor Tasuku Honjo's laboratory at Immunology and Genomic Medicine, Graduate School of Medicine, Kyoto University and then moved to Professor Kei Tashiro's laboratory at the Division of Genomic Medical Sciences, Kyoto Prefectural University of Medicine.

Proteomics for the study of neurological diseases

Before joining Doshisha University, Professor Ikegawa completed a range of investigations on neurological diseases, including biomarker studies to differentiate between multiple sclerosis (MS) and neuromyelitis optica (NMO) – an MS-related

disorder – in Japan [1]. MS is an inflammatory and degenerative disease of the central nervous system (CNS) with diverse clinical presentations and heterogeneous histopathological features. Understanding the neuropathology of MS is essential to developing improved therapies. In recent advances of proteomic technologies, Professor Ikegawa employed a cerebrospinal fluid (CSF) proteomic pattern analysis by using magnetic bead-based enrichment of peptides and proteins, followed by matrix-assisted laser desorption/ionization time-of-flight (MALDI) mass spectrometry. In their study, CSF protein profiles were analyzed from patients with either MS-related disorders, and amyotrophic lateral sclerosis, or other inflammatory neurological diseases, used as controls. As a result, MS-related disorders differed considerably in terms of CSF protein profiles. The similarity of proteomic patterns between selected neurological diseases were demonstrated by pattern matching analysis. Their findings suggest that computational CSF proteomic pattern analysis can increase the accuracy of disease diagnosis of MS-related disorders and will aid physicians in appropriate therapeutic decision-making even for expanding neurological disease entities.

Another study investigated the differentiation of Parkinson's disease and multiple system atrophy (MSA) – a rare atypical Parkinsonian disorder – from the CSF proteome of patients diagnosed with one of the two diseases. MALDI mass spectrometry enabled the laboratory at Kyoto Prefectural University of Medicine to separate Parkinson's disease from MSA, and apply proteomic pattern analysis to the clinical diagnosis of the two neurological disorders. Recently, a genome-wide association study (GWAS) of MSA clearly showed a metabolic aspect of the pathogenesis such as coenzyme Q10 (COQ10) metabolism. The laboratory at Doshisha University has been looking at a metabolic approach, which in the future could be connected to the pathogenesis of Parkinson's disease and/or MSA [2].

Amyloid- β species in Alzheimer's disease

The current primary focus of the laboratory's neurology studies is the deposition of amyloid- β (A β) – the main component of the amyloid plaques found in the brains of patients with Alzheimer's disease [3]. This work was supported in part by the Grant-in-Aid for Scientific Research

on Innovative Areas (Brain Protein Aging and Dementia Control) in collaboration with Assistant Professor Tomohiro Miyasaka, Doshisha University. A β deposition in the brain is an early and consistent feature of Alzheimer's disease and the A β peptides, composed of approximately 40 amino acids, are generated from amyloid precursor proteins (APP). The laboratory aimed to characterize the distribution of individual A β peptides in the autopsied brains of elderly subjects, and those suffering from Alzheimer's disease and cerebral amyloid angiopathy (CAA), using the Bruker rapifleX MALDI TissueTyper (Figure 1).

“We have succeeded in publishing Alzheimer's disease amyloid pathology data using Bruker's rapifleX”

explains Professor Ikegawa, continuing: “We usually use human autopsy brain samples and a mouse model of Alzheimer's disease for our research.”

In typical Alzheimer's neuropathy, immunohistochemistry (IHC) methods have been used in the past to determine the localization of A β peptides in brain tissues. However, this technique can introduce bias because it cannot discriminate between different variants when a number of epitopes are used simultaneously. Mass spectrometry-based proteomic analysis has gained popularity as an alternative method for characterizing the variety of A β species in the brain and most recently, MALDI imaging mass spectrometry has emerged as an important tool for investigating protein and small molecule distribution within biological systems. MALDI imaging can individually track the whole distribution of complex molecules having multiple modifications, which is an advantage over IHC.

The laboratory was able to characterize a broad range of A β species deposits in brains with Alzheimer's disease and CAA using MALDI imaging, and found that A β structure determines the deposition location in brains with Alzheimer's disease. Characterizing and visualizing the broad A β species is necessary for the understanding of A β -production (metabolism and deposition) and may help elucidate the pathogenesis of Alzheimer's disease and CAA.

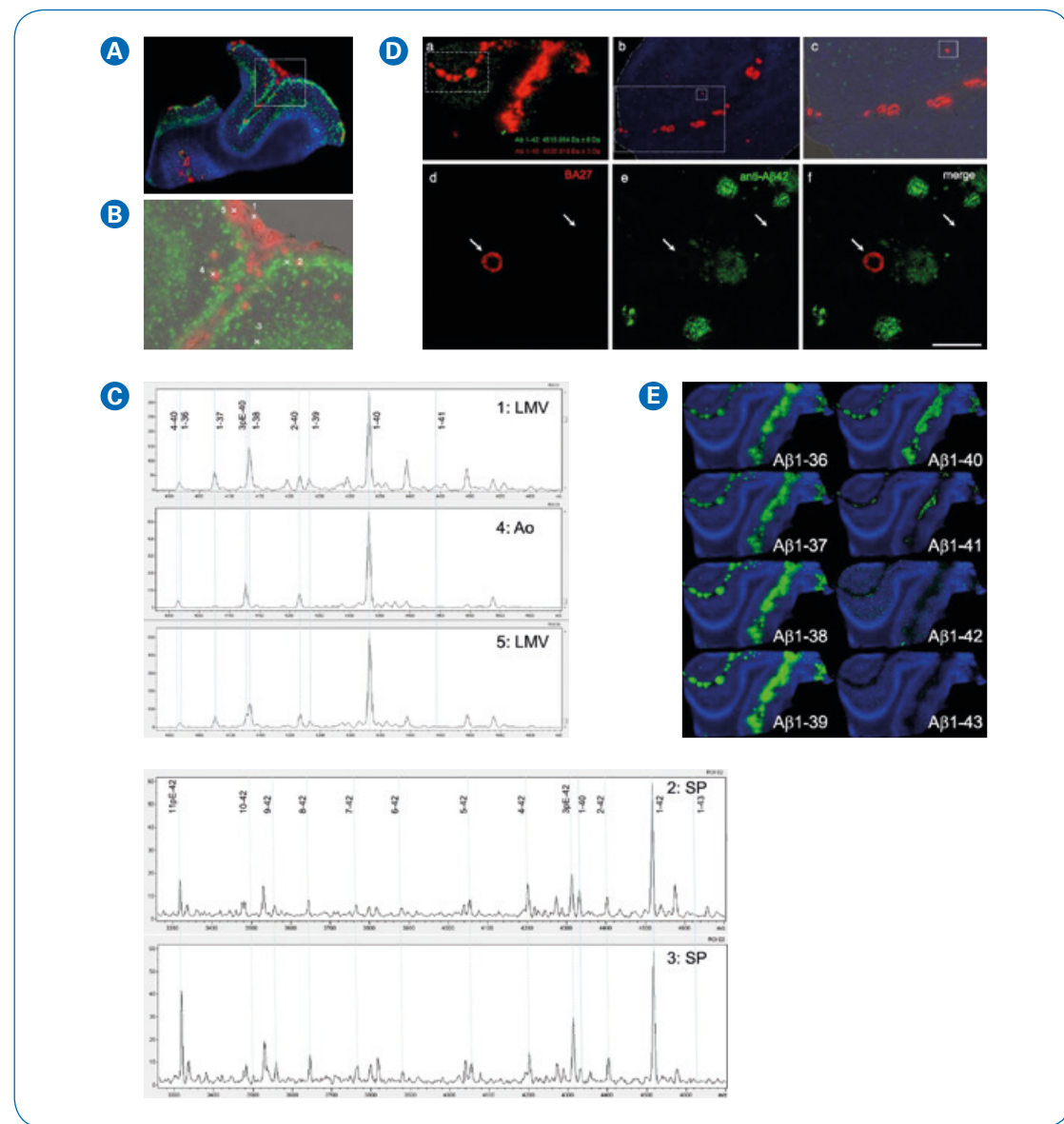


Figure 1: MALDI imaging for frozen Alzheimer's disease (AD) brain sections. **A**: Aβ1-40 deposits in the leptomeningeal blood vessels and arterioles (red) and Aβ1-42 deposits in cerebral parenchyma (green). The m/z 4939.9 was used to detect the tissue structure and shows an unknown biomolecule (blue). **B**: Optical density for MALDI imaging. This figure is a magnification of the region within the dotted square in Figure 1A. Aβ1-40 is deposited in leptomeningeal blood vessels (1 and 5) and arterioles (4) shown in red. Aβ1-42 is deposited in cerebral parenchyma as senile plaques (2 and 3) shown in green. **C**: MALDI mass spectrum in leptomeningeal blood vessels (LMV), arterioles (Ao), and senile plaque (SP) of Figure 1B. Aβ1-40 and N-terminal truncated Aβx-40 are located in Ao, while Aβ1-36 to Aβ1-41 are in LMV. Aβ1-42, Aβ1-43, and N-terminal truncated Aβx-42 are preferentially located in SP. **D**: MALDI imaging and IHC of various C-terminal truncated Aβ peptides in AD with severe CAA. (A) MALDI imaging 100 μm resolution imaging for Aβ1-40 (red) and Aβ 1-42 (green). (B) Highlight 20 μm resolution LMV and cortex imaging in dotted square (a). (C) Highlight of an arteriole in solid square (b). Adjacent sections of the occipital cortex from AD brains were immunostained and focused on arteriole and cerebral parenchyma (c) using antibodies against Aβ40 (D: BA27) or Aβ42 (E: anti-Aβ42 polyclonal) and merged view (F). Both analyses demonstrated that Aβ40 is preferentially deposited in LMV and arterioles in the subarachnoid space and the cerebral parenchyma forming CAA. In contrast, Aβ42 is mainly deposited in SP. IHC analysis also demonstrated the differential distribution of Aβ40 and Aβ42, which were CAA dominant and SP dominant deposition, respectively. Solid rectangles indicate the area illustrated in the panel. Scale bars = 100 μm. **E**: MALDI-IMS of various C-terminal truncated Aβ peptides in AD with severe CAA (NO. 3). Aβ1-36 to Aβ1-41 are preferentially deposited in LMV, while Aβ1-42 and Aβ1-43 are deposited in the cerebral parenchyma as senile plaques.

"For me there is no other choice except to use the rapifleX for targeting Aβ" explains Professor Ikegawa, adding: "the ultrafleXtreme has great capabilities for this kind of work, but when it comes to resolution, nothing comes close to the rapifleX. This instrument can travel rapidly over all the targeted samples, so the imaging from our previous immunohistochemistry work is not able to cover the images we're achieving now."

In situ proteomics for the study of cardiovascular diseases

In parallel to neurology research, Professor Ikegawa's laboratory carries out projects in cardiovascular diseases. Ongoing works are on: 1) Myocardial Allograft Rejection 2) Hypertrophic Dilated Cardiomyopathy (DCM) and 3) Cardiac Amyloidosis – which links to the laboratory's Alzheimer's research – and is currently being explored. Once the laboratory discovered the ability of Bruker's rapifleX to produce top-level results in the Alzheimer's disease Aβ study, it used these parameters to verify proteomics for the cardiovascular system. Preparation of Formalin-Fixed Paraffin-Embedded (FFPE) sections for *in situ* proteomics is challenging, but with the experience from the Alzheimer's research the laboratory was able to overcome this and will achieve typing amyloidosis using imaging data.

Mass Spectrometry Collaboration

Japan-France collaborative network

Professor Ikegawa works with a number of scientists to tackle diverse research questions. For example, he works closely with a wide range of scientists on cell biology, neurology, and pathology all over Japan, most especially at Kyoto University and Kyoto Prefectural University of Medicine. Professor Ikegawa explains how his research focus has evolved:

"Our university is very rich in distinguished researchers, and our geographical location allows us to collaborate with many clinically important groups. Many scientists and clinicians are working with us on the amyloid-β project, and the influence of researchers such as Professor Ihara is very valuable."

"When I moved to Doshisha University, Professor Ihara was interested in the upcoming study of MALDI imaging mass spectrometry. Within the department, now we want to develop modern life science techniques."

The Department of Medical Life Systems is working with the National Cerebral and Cardiovascular Center – the largest national center for the cardiovascular system in Japan – situated near Doshisha University. Professor Hatsue Ishibashi-Ueda, the leader of the Japanese current cardiovascular pathology, united the department with a group in Paris at the Hôpital Européen George Pompidou, Paris, France in collaboration with Professor Patrick Bruneval where one of Professor Ikegawa's students is working to learn about cardiac pathology:

"We are exchanging information with the group in Paris and are now targeting cardiovascular diseases using imaging mass spectrometry-oriented research. The four institutes (Hôpital Européen George Pompidou, National Cerebral and Cardiovascular Center, Kyoto University and Doshisha University) make a very good collaborative network, and our main theme is to target biomarkers for the diagnosis of cardiovascular diseases."

Japan Agency for Medical Research and Development (AMED)

Professor Ikegawa and his colleague, Assistant Professor Nobuto Kakuda, work closely with the Japan Agency for Medical Research and Development (AMED), which is aiming to launch a biomarker study to screen out the pre-clinical situation of Alzheimer's disease. AMED aims to merge cutting-edge basic neuroscience with clinical research, and elucidate the molecular pathophysiology of dementia and develop diagnostic and therapeutic methods, e.g., analyzing metabolic and inflammatory stress, Aβ degradation in Alzheimer's disease, developing novel immunotherapies, diagnostic and therapeutic drugs against dementia with Lewy bodies and molecular targeted therapies in frontotemporal lobar degeneration (FTLD), thereby finding cure for dementia by 2025. Professor Ikegawa's laboratory was working collaboratively with AMED on three key themes: The first theme involved establishing the very high sensitivity ELISA system to detect Aβ peptide in plasma (with Assistant Professor Kakuda), the second

was forming a novel therapeutic strategy to inhibit production of A β (with Associate Professor Funamoto). The third theme is centered around the laboratory's key research project of using imaging mass spectrometry for detecting A β peptide in the circulation of patients affected by Alzheimer's disease. Professor Ikegawa explains the relationship:

"AMED encouraged the work we were carrying out. They asked us what we needed to progress the project and we requested Bruker's rapifleX as the number one system for that purpose."

Working with Bruker

Doshisha University has purchased a number of Bruker mass spectrometry instruments over the years. Professor Ikegawa's laboratory uses the autofleX II TOF/TOF mass spectrometer, purchased in 2004, and the recently-installed rapifleX MALDI Tissue typer. Furthermore, adjacent to Doshisha University, Doshisha Women's College of Liberal Arts owns an ultrafleXtreme MALDI-TOF/TOF mass spectrometer, which the laboratory has been given access to by Professor Kiyoshi Kawasaki.

"We are mainly using the Bruker system – the rapifleX – because it is excellent for proteomic record imaging, and it is complementary to the other modality of the imaging, for example TOF-SIMS, laser ablation (LA)-inductively coupled plasma (ICP), and Fourier-transform ion cyclotron resonance MS (FT-ICR-MS)" explains Professor Ikegawa, adding: "I was very lucky to have the chance to collaborate with passionate applications staff from Bruker, for over 15 years! Bruker always introduce us to their new instruments and we're very grateful."

The relationship between Bruker and Doshisha University is active, with Professor Ikegawa taking samples to Bruker's Yokohama laboratory in addition to Bruker staff visiting the university laboratory. Professor Ikegawa comments on the use of the rapifleX in his laboratory:

"Even very junior students can follow the protocol for Bruker's machine. The most important factor is preparing good tissue sections: creating a pathological section is very challenging and laborious, but it is the most important step of the imaging process."

The rapifleX makes this much easier, so we can obtain some very nice data."

Challenges and Solutions

Professor Ikegawa explains some of the challenges facing the laboratory's work on brain protein aging affected by Alzheimer's disease:

"At first for our neural imaging, it was very hard to visualize the human A β in human autopsy samples. When we try to complete autopsy samples, it was difficult to ionize human amyloid data. When the rapifleX came to market, it took a bit of time but with the help of Bruker staff, we obtained nice signals from the autopsy samples when ionizing the human A β with a specified protocol. This protocol is now patented with Bruker and Doshisha University, using acute treatment for brain sections. For me there is no other choice of system other than the rapifleX."

The laboratory has been working with Bruker on its A β imaging project for two years, but the ultra-high speed modality of the rapifleX allows Professor Ikegawa's laboratory to tackle the clinical obstacles it previously faced.

Future

Although there is currently no cure for Alzheimer's disease, organizations across the globe are using novel techniques and instrumentation to research new treatments for this form of dementia. The laboratory at the Department of Medical Life Systems, Doshisha University, is using cutting-edge mass spectrometry equipment in neurological proteomics research.

MALDI imaging allows Professor Ikegawa's laboratory to reveal the distribution of various A β species within the same sections of human autopsied brains, without specific probes, at a high resolution. Professor Ikegawa explains how his laboratory's relationship with Bruker excels its research goals:

"The next step should be targeting tau protein using imaging mass spectrometry with an advanced strategy and fruitful network."

"The Bruker experts are very passionate. We will certainly be continuing to work closely with them in the future for our ongoing neurology research. The rapifleX can really substitute the conventional immunohistochemistry method by eliminating the blurry difference between signals. Previously, the signal could be quite vague but even with a muddy biological matrix at 100 μ m resolution, the rapifleX gives us great results."

For more information on Bruker's rapifleX, please visit <https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-tof-tof/rapiflex/overview.html>.

For more information on the Department of Medical Life Systems, please visit <https://www.doshisha.ac.jp/en/academics/undergrad/life/index.html>.

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For more information, please visit: www.bruker.com

Bruker Daltonik GmbH

Bremen • Germany
Phone +49 (0) 421-2205-0
Fax +49 (0)421-2205-103

Bruker Daltonics Inc.

Billerica, MA • USA
Phone +1 (978) 663-3660
Fax + (978) 667-5993

ms.sales.bdal@bruker.com - www.bruker.com/microbiology