Customer Insights

Breaking barriers with enhanced gene therapy

Harnessing Bruker's timsTOF Pro to advance biopharmaceutical research

The Uchiyama Laboratory

Professor Susumu Uchiyama
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Working with Bruker

Researchers at the Uchiyama Lab are studying immune-related proteins and the formulation of therapeutic proteins and gene therapy products using Bruker's timsTOF Pro for high-sensitivity peptide mapping. Professor Uchiyama explains:

"At our lab, we need to characterize therapeutic antibodies as well as the capsid proteins of the viral vectors used to deliver the antibody. We introduced Bruker's timsTOF in 2020 to help us with this routine work. It is a powerful instrument with high sensitivity and has the robustness we need."

A brief history

The idea of gene therapy emerged as early as the 1960s when scientists speculated that introducing DNA sequences into patients' cells could hold the key to curing genetic disorders. However, it was quickly realized that 'naked' nucleic acid material was rapidly eliminated from the body. It was the 1980s that witnessed the first notable advancements in molecular biology and genetic engineering following the discovery of retroviruses – which proved a much more efficient tool for gene transfer.

This new 'delivery method' paved the way for the first attempts at gene therapy in humans and in 1989, the rDNA Advisory Committee of the National Institutes of Health proposed the first guidelines for the clinical trials of gene therapy candidates , marking a pivotal moment in the history of biopharmaceutical research. One of the earliest clinical trials involved treating patients with adenosine deaminase (ADA) deficiency, a rare immune disorder. Despite initial setbacks, including the death of a patient in 1999, subsequent trials demonstrated promising results, heralding the potential of gene therapy for treating genetic diseases.

Gene therapy is a type of treatment designed to modify the expression of genes by introducing genetic material into a patient's cells to correct abnormal genes or regulate cellular function. As a therapeutic approach, it holds immense theoretical potential for treating a wide array of genetic disorders, ranging from conditions like cystic fibrosis and muscular dystrophy, to many types of cancer. Over the last few decades, significant strides have been made in the field of gene therapy, propelled by advancements in biotechnology, genomics and molecular biology. All of this has delivered progress in the development of new treatments for diseases that were previously considered untreatable.

As noted above, a crucial aspect of gene therapy is the need for a delivery system to protect the nucleic acid (usually siRNA, sometimes DNA) as it travels through the body to reach the target cells. Today, researchers are exploring a variety of delivery options, including viral vectors such as Adeno-Associated Virus (AAV) and lentiviruses, and non-viral vectors (such as nanoparticles and liposomes) to improve gene delivery efficiency and specificity.

Researchers continue to refine AAV vectors to enhance their targeting specificity and reduce unwanted immune response, in an effort to further improve the efficacy of gene therapy treatments. Advancements in vector design and engineering to provide reliable and robust delivery look set to advance targeted gene delivery, while minimizing adverse effects.

The Uchiyama Laboratory

In Professor Susumu Uchiyama's laboratory at the Department of Biotechnology, Graduate School of Engineering, research is focused on four primary areas; biophysical chemistry-based biotechnology for biopharmaceuticals, biochemistry-based biotechnology of macromolecules in food, yeast biotechnology and methods development. The Uchiyama Lab uses reliable biophysical and biochemical methods, including mass spectrometry (MS), to develop a comprehensive understanding of proteins including the study of protein aggregates and higher-level protein structures, as they play an important role in diseases.

For the last 20 years, Professor Uchiyama, whose background is in biophysical characterization of proteins, has been engaged in therapeutic protein research with an emphasis on therapeutic antibodies. Most recently, in the last 10 years, his research has been more focused on the final formulation and 'production' of a gene therapy product. For example, by understanding viral vectors he hopes to reveal a complete picture of protein structure through the chemistry, manufacturing and controls (CMC) approach for quality control (QC) testing of protein therapeutics.

Characterization of AAV capsid proteins

Viral vectors are vehicles derived from viruses that are modified to protect and deliver genetic material into target cells. They can be engineered to infect specific types of cells and tissues and are often used in research and clinical trials to develop new treatments for genetic disorders, cancer and other diseases. Professor Uchiyama began to study viral vector characterization from around 2016, and his work typically focusses on AAV which is extensively used for in vivo gene therapy. Recent advancements in developing clinically desirable AAV capsids have contributed substantially to the growth of the gene therapy field.

About Professor Susumu Uchiyama.

Professor Uchiyama has a background in biophysical characterization of proteins having studied at Nagoya University in the Department of Chemistry and completed his PhD at Osaka University in the Pharmaceutical Department. At that time, Professor Uchiyama's study was not directly related to pharmaceutical or medical purposes, but instead focused on the fundamentals of biophysical analysis of proteins and peptides, such as protein stability and how proteins can interact and assemble into a 3D-complex or higher order structure.

Following his PhD, Professor Uchiyama worked as a post-doctorate researcher at the RRF institute Inc. in Japan, and then joined as assistant professor at the Department of Biotechnology in Osaka University in the early 2000s, where he studied proteomics analysis including the identification of a whole set of proteins in cells and biological substances using MS.

In 2005, Professor Uchiyama was given the opportunity to study native MS at the University of Cambridge, England, for one year in Professor Carol Robinson's laboratory.

Professor Uchiyama describes the lab's use of MS:
"We had to use a mass spectrometer when looking at a protein or protein complex, because it will be ionized and go to a very high vacuum condition. In the high vacuum condition, the water surrounding the proteins will evaporate, resulting in the protein being unable to maintain its higher order structure and the interactions between proteins will be interrupted. If we reduce to a low vacuum condition after ionization, the evaporation will occur gradually instead and result in maintained high order structure. This means that we can monitor protein interactions via MS. We developed a new technique by modifying the mass spectrometers we were using."

Back at Osaka University, Professor Uchiyama continued his research into native MS and uses the same technology and applications, but instead focused on antigen-antibody complexes and therapeutic proteins rather than the general ones previously studied.

He also launched his company U-Medico Inc. in 2006 to provide analytical services on proteins and viral vectors, including AAVs, for pharmaceutical companies in Asia and US and Europe. His research interests encompass gaining insights into the energetic properties of immune-related proteins and developing formulations for therapeutic proteins and gene therapy products. In addition, he is investigating container closure systems for biopharmaceuticals to enhance quality and safety standards.

Professor Uchiyama discusses his work with AAV:
"AAV is the leading platform for gene therapy viral vectors, they are small viruses (approximately 20-25 nanometer in diameter) that can carry a small payload of single-stranded DNA (ssDNA) which can be used for therapeutic purposes. The ssDNA is encapsulated in viral proteins and transported to the site of action in the body. Typically, 60 viral proteins constitute a capsid. Our lab is focused on the manufacturing and quality control of the AAV."

The Uchiyama Lab is currently manufacturing AAV using human culture cells and adding plasmid to force cells to produce viral vectors. The viral vectors are then purified using a combination of chromatography and centrifugation. Professor Uchiyama explains:

> *"We collaborate with medical doctors who want to carry out clinical trials in hospitals, therefore, it is crucial that we understand and monitor the quality of the produced viral vectors and ensure that they meet the criteria of regulatory agencies.*

"For example, the viral vector capsid proteins should be produced according to the designed sequence, so we also need to confirm if the viral proteins have the amino acid sequence as designed and that the full length is translated. We need to be sure that we confirm the identity and purity of the produced viral vectors. Even after high purification, there can be contamination, meaning that the host cell protein (HCP) will be included in the viral vector product, so we need to be able to identify how many and which kind of proteins are included in addition to the product."

For the quality control steps, Professor Uchiyama is using MS to identify the HCP and also the stoichiometry of viral proteins. Typically, AAV is composed of three different viral proteins, VP1 VP2 and VP3 so the lab needs to be able to identify the stoichiometry as they have different functions. A higher number of VP1 proteins usually results in higher efficacy, so that number needs to be precisely determined for quality control. The viral vector output of the Uchiyama Lab is then used by a clinical group who adds the genetic material and takes it into a clinical trial.

Professor Uchiyama describes the split between his academic research and work at U-Medico:
"We use the same techniques at U-Medico as the University, but we apply them to the pharma industry. Firstly, the method is developed in the University lab, with the final goal of publication and we are more focused on the scientific work and developing methods. The result is high quality, but not always robust and reproducible, therefore not suitable for providing as a service. The Uchiyama Lab then transfers the developed method to U-Medico where we will further modify and improve the method so that it can be offered to pharma companies based on high level operational protocols.

> *This allows us to offer advanced methods to pharmaceutical companies, including the analytical service of proteins, antibody therapeutics and AAVs – capitalizing the science and spreading the use of the research into the commercial world."*

In recent years, Professor Uchiyama has published more than 250 peer-reviewed papers and reviews and edited a book of Analytical Ultracentrifugation published by Springer. He has also been on the advisory board for Coriolis Pharma since 2012, a globally operating contract research and development organization (CRDO) and leader in formulation research and development of biopharmaceutical drugs, including cell and gene therapy products and vaccines, which has helped him to develop connections with US and EU companies in the academic field.

Impact of the timsTOF

While MS is a powerful tool for proteomics research, alone it lacks the ability to provide complete sequence coverage of proteins and give definite measurements of the molecular interactions and downstream effects of protein complexes. Trapped ion mobility spectrometry (TIMS) has emerged as a novel technique for the separation of peptides and proteins as it provides new levels of sensitivity, selectivity and speed to proteomics research. It is particularly beneficial for those low abundant, difficult-to-isolate species involved in many diseases.

TIMS involves an electrical gradient which prevents each ion from moving beyond a certain position in the TIMS tunnel, and the passage of the ions is defined by the ion's shape in gas phase. This allows the selective release, fragmentation, identification, and quantification of ions from the TIMS tunnel in a process called parallel accumulation-serial fragmentation (PASEF). With TIMS and PASEF, the ion mobility measurements can be used to determine ion specific collisional cross section (CCS) values. The incorporation of CCS provides an additional fourth dimension to the analyses, which allows researchers to transition from 3D-proteomics (retention time, mass to charge (m/z) and tandem MS (MS/MS) fragment ion spectra) to 4D-proteomics. The addition of CCS values further increases the system's selectivity, resulting in more reliable quantitation in complex samples.

Professor Uchiyama describes his relationship working with Bruker's timsTOF to characterize antibodies and viral vector capsid proteins:

"The Bruker timsTOF is commonly used for the identification of proteins, however we use it for peptide mapping and confirming the protein sequence and for PTM state of capsid proteins. We can identify impurity proteins simultaneously if we use the timsTOF."

"High sensitivity is vital for our work. Typically, 5g or 10g of protein can be obtained from one culture, however with viral vectors we can only obtain somewhere 1mg from 1L culture in the best-case scenario. That's a much smaller yield than that for therapeutic antibodies. With viral vectors being expensive and the amount produced limited, having a highly sensitive instrument is crucial. The Bruker timsTOF is the ideal solution for this."

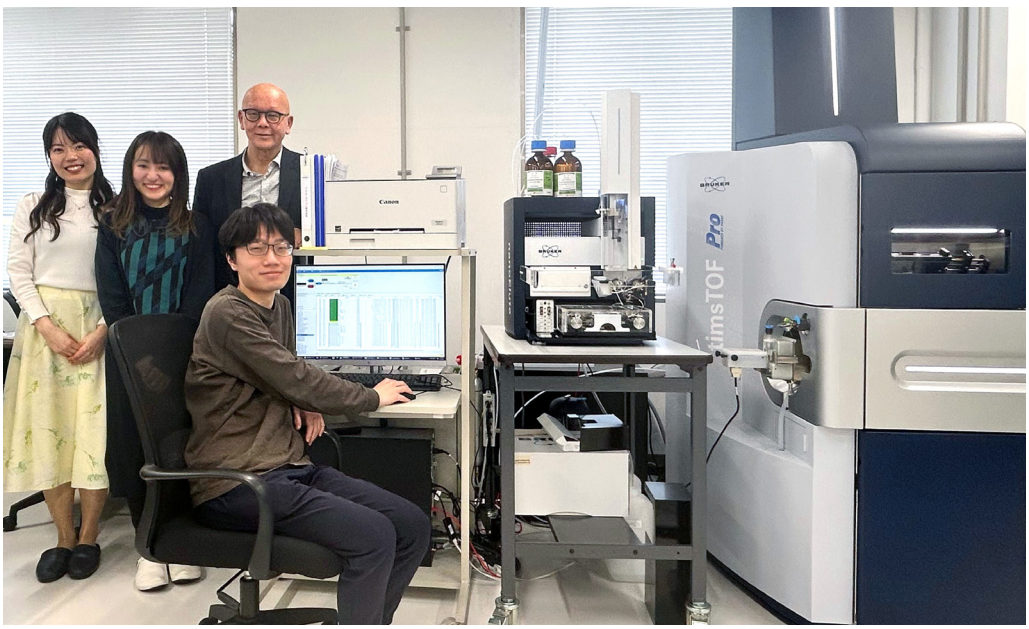
"With a limited quantity of material, analysis was almost impossible before we had the timsTOF."

What's next?

Professor Uchiyama will soon be involved in a national project in Japan for the development of characterization and quality control of virus vectors for gene therapy. The goal is to offer clinical grade viral vectors, manufactured in quality-controlled conditions for hospitals to use in trials. Professor Uchiyama describes this move to become one step closer to patients:

"I am hoping to become more involved in clinical aspects of gene therapy in Japan, to create viral vector methods in my laboratory that can be manufactured by CDMOs under good manufacturing practice (GMP) conditions. To do this will require high-sensitivity characterization, so Bruker's timsTOF will be a key instrument to establish control strategies."

Looking ahead, gene therapy holds immense promise for transforming the treatment landscape across a wide spectrum of diseases. The future of biopharma research and gene therapy has the potential to transform the landscape of medicine, offering hope for patients grappling with genetic diseases. With continued innovation, collaboration, and ethical consideration, gene therapy stands poised to usher in a new era of personalized and precision medicine, where treatments are tailored to the unique genetic makeup of each individual, ultimately improving health outcomes and enhancing quality of life for patients.



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About The Uchiyama Laboratory

Prof. Dr. Susumu Uchiyama is a biophysical chemist with over 25 years of experience. Since 2017, he has held a professorship in the Department of Biotechnology, Graduate School of Engineering at Osaka University, Japan. His lab studies the biophysics of proteins, protein complexes and protein-nucleic acids complexes using a variety of methods including mass spectrometry, especially native MS and HDX-MS. He launched the company U-Medico Inc. in 2006 which has provided analytical services for pharmaceutical companies since 2008. His research interests include understanding immune-related proteins and the formulation development of therapeutic proteins and gene therapy products.

For more information, please visit: **macromolecularbiotechnology.com/en/**

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