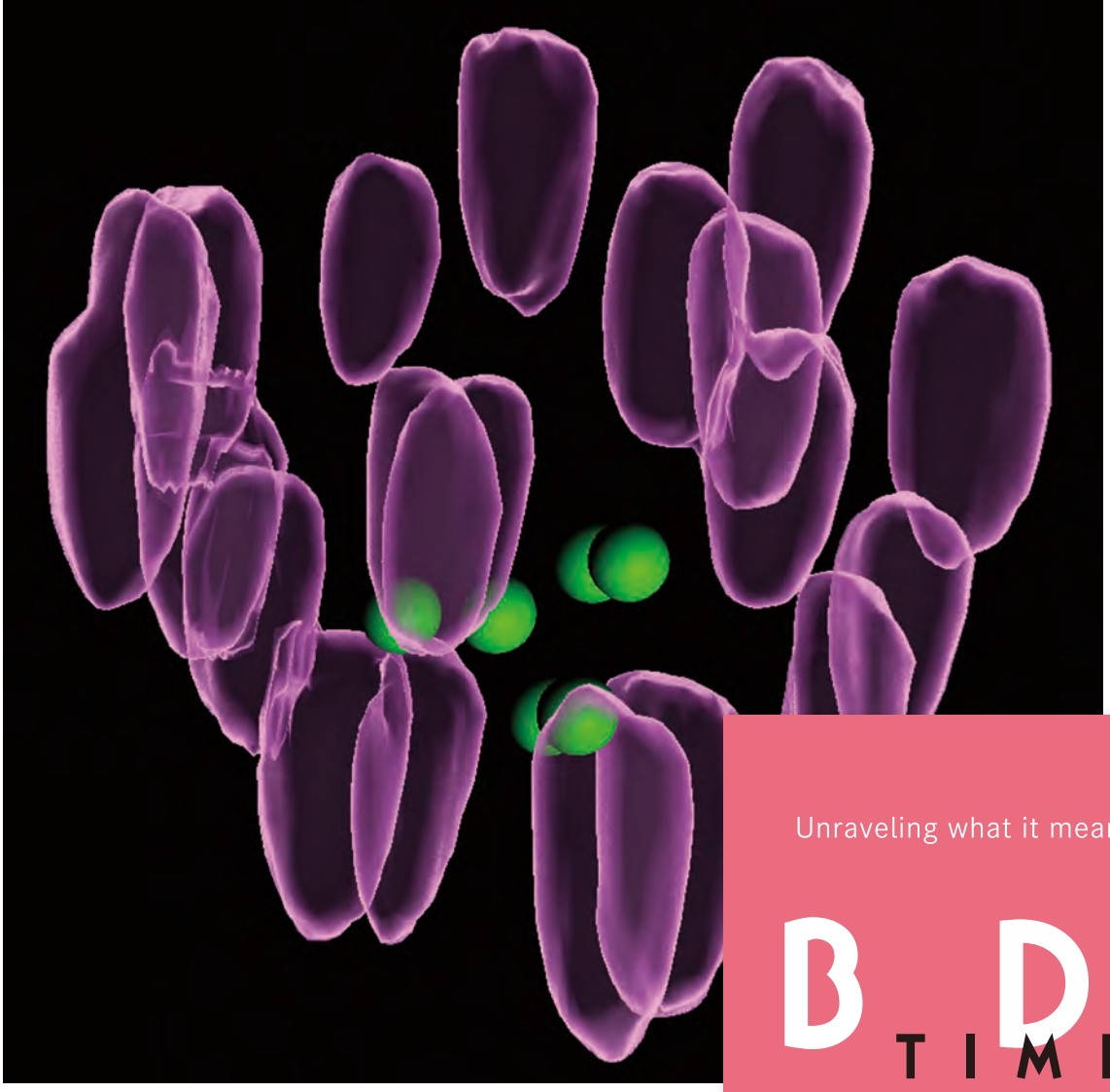

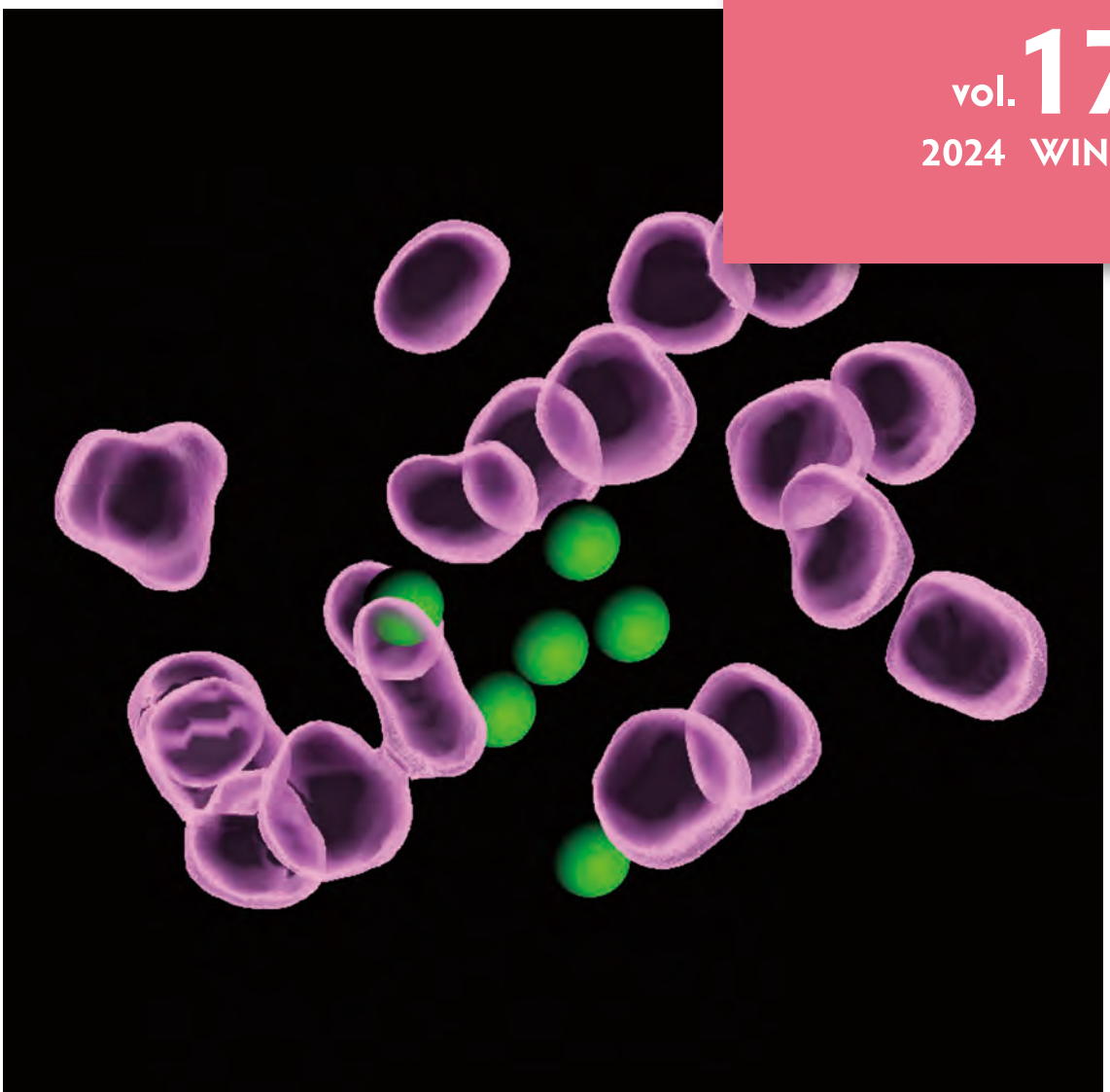

RIKEN Center for
Biosystems Dynamics Research

Unraveling what it means to be alive

BDR TIMES

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Dive into BDR's intriguing research

Measuring Various Things with Mass Spectrometers

Dr. Hina Kosakamoto from whom I learned about nutrition research using fruit flies, introduced me to my next interviewee, Dr. Keiko Masuda. Dr. Masuda is an expert in mass spectrometry. Through this interview series, I've heard a lot about genes and cells, but this is the first time mass spectrometry has come up. I wonder what kind of research we'll talk about today.

First step into protein research

Yakushiji (Y) ▶ When did you first come across protein research?

Masuda (M) ▶ The department at Hiroshima University where I did my studies is a bit unique. Students were allowed to take half of their credits in humanities subjects and the other half in the sciences. I remember hearing the dean of the department at the time saying, "It is important to foster experts who specialize in specific areas, but we also need people who can serve as a bridge between specialists in various areas." He also said, "It is important to foster human resources who have developed a wide human network and also have their antennae up in different directions."

Y ▶ So, your department was one that could be called "interdisciplinary."

M ▶ I always had an interest in biology, and when I was taking the university entrance exams, I had my heart set on pursuing pharmaceutical sciences. But when I was looking into Hiroshima University, I became intrigued by the degree programs of the School of Integrated Arts and Sciences.And I decided that I wanted to advance my studies with a broader perspective rather than deciding on a specific field.

Y ▶ Exactly what your dean was aiming for.

M ▶ After studying a range of different subjects, I found that my interests still laid close to the life sciences and in my third year, I chose to major in the life science program. The laboratory that I joined in my fourth year was doing research on genes whose functions had not yet been identified. Genes are the blueprints of proteins, so we can't understand the function of a gene until they become proteins. Thus, we were working as a team to synthesize proteins, injecting them into experimental animals and then examining their phenotypes. This was my first foray into protein research.

Common occurrences with protein synthesis

M ▶ Finding out DNA sequences can be done fairly easy, but when it comes to actually making proteins, it is quite difficult. We were struggling to obtain a specific protein. This protein was about 80 to 88 amino acids long and was highly hydrophobic. It also contained post-translational modifications such as disulfide bonds, making the protein hard to purify and isolate.

Y ▶ That's a common occurrence when looking at protein expression. But if you can't obtain the proteins, you can't start your experiments...

M ▶ Exactly. We tried to get *E. coli* to express the protein, but the yield was low. It was difficult to artificially synthesize the full length of the peptide using conventional chemical synthesis, so we even tried requesting a company that offered protein synthesis services to make the protein for us.

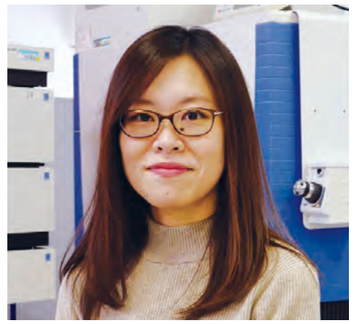
Y ▶ I imagine that because the peptide is pretty long and you also need a fair amount of the protein, it can become a bit costly.

M ▶ The quote was apparently a few million Japanese yen. Because we needed to synthesize the protein continuously, not just once, we abandoned the idea to outsource the protein synthesis part. Instead, we ended up installing a protein synthesis machine that uses microwaves. The machine allowed us to apply microwaves to the proteins that tended to aggregate during the synthesis process to stretch the protein as it was being made and allowed us to obtain the amount of protein we needed.

Y ▶ Were you able to confirm the phenotype in the end?

M ▶ Yes. We found when overexpressed, it can lead to fat accumulation.

Y ▶ Something that we all don't like.



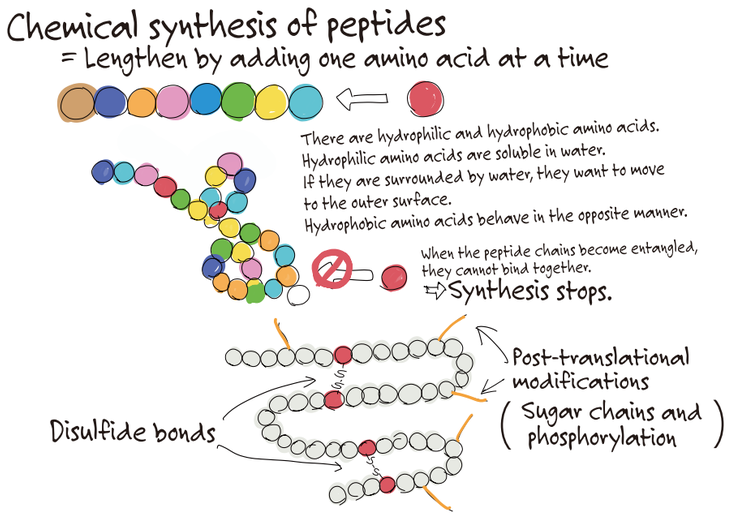
Keiko Masuda

Research Scientist in the Laboratory for Cell-Free Protein Synthesis

She received her PhD from the Graduate School of Integrated Arts and Sciences, Hiroshima University. She then moved to join RIKEN where she becomes involved in the research and development to mass spectrometry related technology. She says that she tends to get bored easily, but research work is the one thing that she has never found boring. To wind down, she enjoys partaking in good food and drinks, and scrolling through short movies of chinchillas on social media. She currently shares her home with a rescue dog.

Hideki Yakushiji

Business developer based in Kobe, and a RIKEN alumni. He has a broad background in areas such as analytical chemistry, optics, biotechnology and IT. He is involved in a wide range of activities to assist in commercializing technologies and ideas born from academia, including setting up opportunities for idea sharing, finding investors, and strategic planning.



Development of mass spectrometry techniques

Y ▶ Is that when you were first introduced to mass spectrometry?

M ▶ Yes. But in the beginning, I was not actually doing it myself; rather, I was asking a technical specialist to do the analysis using the mass spectrometer at the university's common facilities. It was not until after moving to RIKEN that I began to seriously work with mass spectrometry, and it was Dr. Tsutomu Masujima (deceased) who led me to this path. It's because of this connection that I now work with mass spectrometry and cell-free protein synthesis.

Y ▶ I think mass spectrometry is often used to identify substances. But it can also be used to quantify substances, right?

M ▶ Our team developed a technique called MS-QBiC, which we are currently working to improve. It's a method that involves using a peptide with the same sequence as the sample, but that is labeled with a stable isotope as an internal standard before measuring the sample with the mass spectrometer.

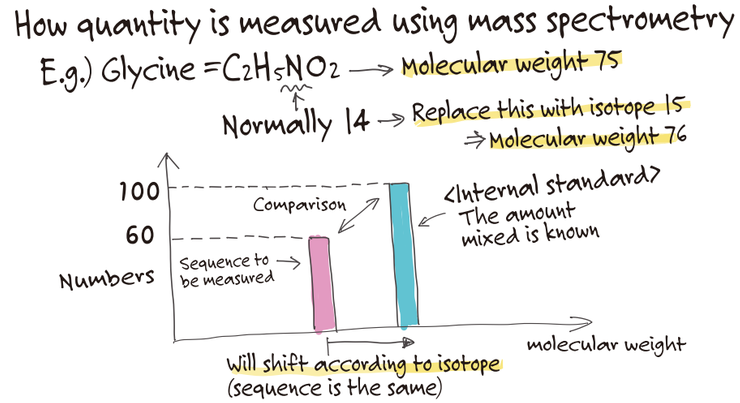
For example, the molecular weight of nitrogen is normally 14, but if we use the nitrogen 15 isotope, the molecular weight shifts by one. If you add a fixed amount of this labeled peptide, we can measure the amount of the target peptide.

Y ▶ So, you are basically mixing something like a ruler into your sample. But I imagine that using stable isotopes can be quite costly.

M ▶ MS-QBiC can actually resolve this issue. It is based on a reconstituted cell-free protein synthesis system, called the PURE system. This system allows us to make very small amounts of proteins. The cost of the ruler is basically the cost of the stable isotope, so if you only need a small amount, the cost of the experiment drops accordingly.

Y ▶ You can measure many peptides at once using mass spectrometry. Does that mean that you need a lot of rulers as well?

M ▶ We've solved that issue as well. MS-QBiC includes a tag sequence, and if you make a lot of variations of the tag sequence, you can still distinguish between peptides even if the sample is a mix of different peptides. We were also able to improve the measurement throughput, and we are now working beyond that. Our next goal is to be able to measure post-transcriptional modifications to proteins, such as phosphorylation, which is important for regulating protein activity. It's still in the developmental stages, however, we would like to be able to synthesize peptides with post-transcriptional modifications by modifying the enzymes and genetic codes used in the PURE system.



Y ▶ And that is why you are currently working on mass spectrometry and cell-free protein synthesis.

It's fun to be able to work on different themes

Y ▶ Do you have a lot of research collaborations where you identify and quantify proteins?

M ▶ Yes. It's fun to be involved with a lot of different research topics. There is a lot of data that you can acquire only by using mass spectrometry. Dr. Kosakamoto, who introduced me to you, is also one of my research collaborators.

Y ▶ I see.

M ▶ I can operate a mass spectrometer, but what is ultimately important is knowing what you want to measure. Nothing will emerge from me alone, but the conversations I have with different researchers can lead us to measure something where we think mass spectrometry might prove useful. Of course, I am happy when we get the results we wanted, but even if the results are not what we expected, there is something new to be learned from that as well. While I may not be focusing and delving into a specific research theme, I am happy and also enjoy being able to help out with a variety of research. There are many researchers around me who have a wide range of interests, and so I find that each new round of discussions, data acquisition, and then getting feedback on the data from those researchers is refreshing as I am learning something new.

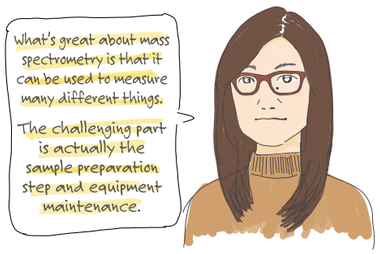
Y ▶ It's nice that there are also many types of researchers.

M ▶ Mass spectrometry is very sensitive, and there is a lot of information that you can extract from it. People sometimes think that we just push a button and voila! the machine will spit out the results. But it is a job that requires knowledge and experience in order to optimize the workflow to produce the type of results that meets the aim for a particular project. It can be an intricate process. For example, depending on the sample, the sample preparation process or figuring out the measurement parameters may turn out to be unexpectedly complex. Or how the data that is obtained is analyzed may be important. This is what makes it both challenging and interesting.

Y ▶ Mass spectrometers are vacuum systems, so their maintenance must be difficult.

M ▶ To ensure that we can collect good data at any time, we clean or replace parts at appropriate times and also run quality control measurements to check that there are no irregularities. To maintain the vacuum inside the instrument, we basically leave the power on. It is a real concern when there are planned power outages or when typhoons are approaching. We don't want to turn the power off unnecessarily as it can place a heavy load on the instrument when you release the high-vacuum pressure and then reinstate it again. Repair work is costly if the machine breaks down and it's not unusual to have to wait several months for some parts.

Y ▶ I guess the analogy, 'The more troublesome a child is the more endearing they become,' fits perfectly in this case.



Postscript

During the interview, it felt like the energy she was projecting screamed, "I love mass spectrometry!" When I mentioned this to her, she told me that was not her intention. Mass spectrometers are sometimes shortened and called "mass spec" in English or *massu* in Japanese in the industry. During the interview, Dr. Masuda often referred to the instrument as *massu* but because it maybe difficult to understand, I decided to use the full term "mass spectrometer" in this piece. I also recalled the sadness I felt in my ownpast experience when the turbo pump or some other part of the vacuum systems broke down...

BDR Research Highlights

Research highlights articles and press releases between December 2023 to July 2024.
Read these and other articles on the BDR website.

01

Researchers model blood-brain barrier using "Tissue-in-a-CUBE" system

The blood-brain barrier is a strict gatekeeper around the brain that prevents foreign substances in blood from entering the brain. Although protective, the barrier poses challenges when treatments need to affect the brain in order to work. Now, Masaya Hagiwara and Isabel Koh of the Human Biomimetic System RIKEN Hakubi Research Team have succeeded in establishing a model of the blood-brain barrier using modularized tissue derived from human cells. The "Tissue-in-a-CUBE" is a small cubic structure that could provide a boost in the drug discovery field and be used as an alternative to animal models in pre-clinical studies.

Koh I, Hagiwara M. *Commun Biol* 7, 177 (2024)

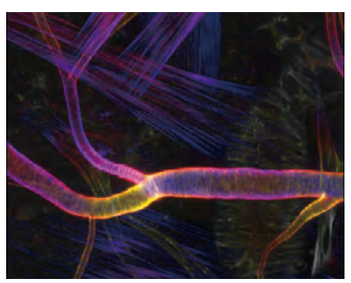
Illustration by Hiroko Uchida

02

How tubular tissues form uniform channels

BDR researchers led by Sayaka Sekine and Mitsusuke Tarama developed a model explaining the cytoskeleton patterning that stabilizes tubular structures in the body. Focusing on the formation of actin nanoclusters in the fruit fly's trachea, they discovered that uneven stress distribution drives the reorganization of these nanoclusters into stable, regularly spaced cables. This process, driven by a protein called DAAM, is crucial for the smooth development of tubular lumens. The team's model paves the way for understanding similar mechanisms in the formation of more complex human tissues, such as the primitive heart tube.

Sekine S, Tarama M, Wada H, et al. *Nat Commun* 15, 464 (2024)

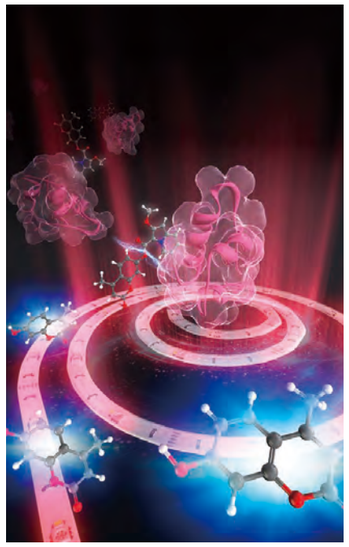


03

Killing cancer with a trick of the light

A team of researchers led by BDR's Kenji Watanabe have created a novel method to potentially improve cancer treatment using gold nanoclusters as a carrier system. Their gold-nanocluster system carries two components: an indolizine-masked anticancer drug and a photosensitizer. The anticancer drug is released when exposed to red light, minimizing harm to nearby healthy cells. The team overcame challenges in achieving precise ratios of the indolizine and photosensitive components by combining them into a single chemical entity and developing a method to attach it to gold nanoclusters under mild conditions. Future developments will include adding targeting mechanisms for more selective cancer treatment.

Watanabe K, Mao Q, Zhang Z, et al. *Chem Sci* 15, 1402-1408 (2024)



04

Flies fed restricted diet in early adulthood live longer

Many studies have suggested that the life expectancy of animals can be extended by a lifelong diet of calorie restriction, but for many people this road to longevity is unpalatable. However, similar benefits may be possible by a much more targeted dietary intervention, research by Fumiaki Obata and co-workers in the Lab for Nutritional Biology now suggests. They found that fruit flies live considerably longer when fed a diet that limits consumption of a certain amino acid during early adulthood.

Kosakamoto H, Obata F, Kuraishi J, et al. *Nat Commun* 14, 7832 (2023)

05

Comparing how limbs develop in chicks and frogs

To shed light on the principles that govern tissue dynamics across species, Yoshihiro Morishita and his colleagues in the Lab for Developmental Morphogeometry have developed a new method for analyzing the development of vertebrate limbs. They proposed a space–time coordinate system that enables tissue dynamics to be compared directly across species that differ in size, shape and developmental time scale. "This system could reveal an organ-specific design principle that is independent of species, which will lead to a deeper understanding of developmental biology," Morishita concludes.

Morishita Y, Lee SW, Suzuki T, et al. *Nat Commun* 14, 8199 (2023)

06

Dynamic view of opioid receptor could refine pain relief

A BDR research team led by Ichio Shimada and Shunsuke Imai has uncovered a way to enhance the pain-relieving effects of the μ-opioid receptor (MOR) without the harmful side effects of traditional opioids. They found that allosteric modulators enhance MOR activity by shifting the dynamic structural equilibrium toward a more active form without directly altering its structure. This dynamic modulation allows the body's natural opioids to bind more effectively, potentially leading to pain relief with fewer side effects. Their findings not only offer hope for safer opioid alternatives but also provide a framework for developing new drugs targeting other signaling proteins in the body.

Kaneko S, Imai S, Uchikubo-Kamo T, et al. *Nat Commun* 15, 3544 (2024)

Peek-a-LAB

This time we hear from Dr. Makito Miyazaki, team leader of the Laboratory for Bottom-up Cell Biology, about his research and laboratory. The laboratory was established at the BDR in April 2023.

Laboratory for Bottom-up Cell Biology

Q **Before establishing your laboratory at RIKEN, where and what kind of research were you doing?**

A Up until my master's program, I was researching the movements of single molecular motors using the most advanced optical microscopes at the time to observe and manipulate them. Realizing that I needed a better knowledge of physics to understand the principles of molecular motor operation, I decided to pursue a PhD in theoretical research related to nonequilibrium statistical mechanics. During this period, I was also questioning my career path choice. Thinking back to my early childhood when I was infatuated with insects, I realized that I wanted to study something a little more related to living organisms, so from my postdoc I started working on reconstitution experiments which tie into my current research.

Q **What kind of research are you aiming to conduct at RIKEN?**

A Living organisms have complex hierarchical structures spanning molecular to individual scales, and our lab's research aims to bridge the molecular and cellular scales which are the boundary between matter and life. I think many of us have experienced taking apart and reassembling Lego blocks, cars, origami, or clothes with our own hands to get a clear picture of how they work or are put together. The challenge for us is to do the same thing with cells. It may seem like a wild challenge, but I believe that we can get closer to understanding the mechanisms of how life forms from molecules.

Q **Please tell us something that is unique to your laboratory.**

A The team's greatest strength is having the technology that allows us to mix purified proteins and encapsulate them in cell-sized liposomes, and in particular having the technology to reconstitute the actin cytoskeleton. Another strong point of our lab is having technology for extracting the cytoplasm from eggs of African clawed frogs. Recently, we have made optogenetic technology available for use in reconstituted systems. As for equipment and software, we try and build much of it ourselves when possible.This may take time, but making our own equipment and software will allow us to gain a deeper understanding of the apparatus or program and make it customizable to our needs leading to the creation of a one-of-a-kind experimental technology.

Q **What is your hobby?**

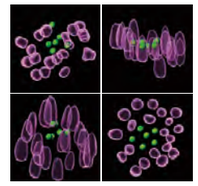
A I have many hobbies, but I'll mention a few that are somewhat related to my research. I enjoy both eating and cooking food. My first supervisor told me that someone who is a good cook is also good with experiments, and since hearing those words, I try to be in the kitchen whenever I have the chance.
I also like to stroll around town in a manner similar to *Bura Tamori**. It is fun to uncover interesting things from everyday life that might otherwise be overlooked, and to have them all suddenly connect together to reveal how that town came to be. I think this is an important attitude and useful skill to have, especially when your research feels stagnant because it is not yielding expected results.
Then there is drawing. I enjoy taking my sketchbook, pencils, and watercolors with me when I travel and draw the landscapes I see. The process of extracting the core elements from among the complex architecture, plants and trees of the landscape and drawing them as simple lines and colors is kind of similar to research.

Q **What type of people do you want to join your lab?**

A In a few words, someone who enjoys research. If one can't enjoy the daily grind of research, it's easy to fall into a negative mindset, like "Nothing worked out today either. It's a total failure." Instead, if you can find something interesting from the failed experimental data and come up with unconventional hypotheses—enjoying the *Bura Tamori* style guesswork—you are sure to find joy in the daily research routines even when the results you obtain are not what you were hoping to get.

* *Bura Tamori*: A Japanese TV series that was broadcasted on NHK, where the host Tamori strolls around cities or regions around Japan with local experts to explore and learn about the local history, culture, and how the landscapes were formed.

On the cover!



Mysterious stone circle?

These images show the behavior of chromosomes (purple) and artificial kinetochore beads (green) within the cell during metaphase, when they align on the same plane.

Credit: Lab for Chromosome Segregation

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