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Structural physiology of membrane proteins by cryo-electron microscopy

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Q: What motivated you to develop the cryo-electron microscope?

A: The starting point of my research came out of a simple quest to understand the molecular mechanisms that form cognitive ability and personality. Membrane proteins are important for the function of nerve cells, but lipid membranes are only about 5nm (50Å) in thickness. To analyze the molecular structures of such thin samples, electron beams are more suitable than X-rays, because their atomic scattering factors are larger. For this reason, I started to learn about electron microscopy.

The pioneers of electron crystallography are Richard Henderson and Nigel Unwin, and they first determined the structure of a membrane protein, bacteriorhodopsin (bR), at 7Å resolution in 1975. When I began researching electron microscopy in the late 1970s, I learned that biological samples could be severely damaged by the electron beams. Radiation damage of the biological samples made high-resolution analysis extremely difficult. After struggling through many attempts, we found that if the temperature was lowered to 8K or less, the damage to samples caused by electron beams could be reduced to about 1/20th compared with the damage at room temperature. Therefore, we set out to develop a cryo-electron microscope in 1983, and perfected a helium stage that could be built into an electron microscope in 1986. Since then, we have continued to make improvements and our eighth generation is currently under development. In 1997, using this type of cryo-electron microscope, we successfully analyzed the structure of bR at 3Å resolution.

Q: In electron crystallography, you have demonstrated the structure of many membrane proteins in addition to bR. What are the most impressive proteins in your research so far?

A: One example is the aquaporin (AQP) family of proteins, which are known to work as water channels. The AQP family proteins exist in all organisms, from bacteria to humans. A human has 13 types of AQPs that function in various parts of the body and are involved in numerous physiological processes.

An AQP1 channel passes 3 billion water molecules per second, but excludes any ions and even protons (H^+). If ions pass through a water channel, then ion channels cannot function, and if protons pass, the pH in the cell will change and cause dysfunction of the cell. However, since water molecules are connected by hydrogen bonds, protons could easily pass through the hydrogen bonds of a water wire formed in the channel. The mechanism of such rapid water permeation and high water-selectivity of AQP proteins gave us the puzzling questions.

In 2000, based on an analysis of the structure using electron crystallography, we proposed that there exists a hydrogen-bond isolation mechanism, which explained how AQP1 can block ion- and proton-permeation while maintaining rapid permeation of water molecules (Fig. 1). In 2009, we succeeded in individually observing eight water molecules in the channel of AQP4 (Fig. 2), and substantiating the proposed mechanism. This achievement was made possible by electron crystallography, which allows analysis of membrane proteins that are embedded in lipid membranes. The water molecules were not discriminated in the channel structure analyzed by X-ray crystallography even at higher resolution. This kind of counterintuitive notion could be attributed to the difference of surround atmosphere for structure analyses achieved in lipid bilayer or not. When X-ray crystallography is used to examine membrane proteins, the lipid membrane must usually be removed.

By the way, AQP2, which among AQPs plays an important role in the kidney, was discovered by Prof. Sei Sasaki, who was at that time a professor in the Department of Nephrology at TMDU (currently emeritus professor). Prof. Sasaki discovered the following mechanism: When a human is thirsty, a hormone called vasopressin is secreted from the postpituitary, and when it reaches the kidney, AQP2 in the cell comes to the cell surface and absorbs water, which otherwise would be excreted as urine. APQ2 inhibitors have potential for use in the treatment of heart failure and edema in cirrhosis, and we have started collaborative researches with Prof. Sasaki.

Q: Cryo-electron microscopy also plays an important role in single-particle analysis.

A: Yifan Cheng, who once was a postdoc in my lab, and his group analyzed the structure of the TRP (Transient Receptor Potential) channel at high resolution by single-particle analysis using a cryo-electron microscope and a high-performance camera that can detect electron beams directly with CMOS (complementary metal oxide semiconductor). That was in December 2013. Their work triggered an explosion in the study of structural biology by single-particle analysis.

In 2017, three scientists received the Nobel Prize for their work on the development of cryo-electron microscopy and single-particle analysis. One of them, Richard Henderson, explicitly cited our contributions at a press conference for the Nobel Prize when he said "Contributed people in Japan — Yoshi Fujiyoshi" (Fig. 3). The JEM-Z300CF cryo-electron microscope developed by JEOL Ltd. using my patent is starting to be widely used for single-particle analysis.

Q: You coined the term "structural physiology." What does this mean?

A: This term represents a discipline that seeks to understand physiological functions of membrane proteins from a structural point of view. For example, when we see an apple and judge that it is edible, then we reach out and bring it to our mouth — for each action, various molecules come into play, such as rhodopsin in the retina, ion channels in nerve cells, acetylcholine receptors at neuromuscular junctions, and so on. I have been attempting to determine the structures of all of these molecules and to understand human capabilities at the molecular structure level.

Three types of cryo-electron microscopy are available for studying three dimensional structures — electron crystallography, single-particle analysis and electron tomography. These are listed in order of the resolution they can achieve, but their order of importance in a biological sense might be the reverse. I've been conducting analysis using electron crystallography for many years; indeed, it took as long as 18 years in one case to analyze just one receptor. Therefore, recently we have also been using single-particle analysis, a technology in which there has been remarkable progress.

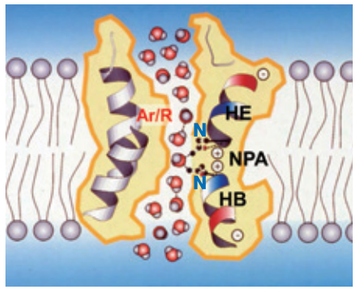

Fig. 1: Proposed hydrogen-bond isolation mechanism based on the structure of APQ1 at 3.8Å resolution. The middle of the channel is narrow enough to allow only one water molecule to pass. The two asparagine residues (N in the figure) form hydrogen bonds with the oxygen (red sphere) of the water molecule that has arrived, breaking the hydrogen bonds between the upper and lower water molecules.

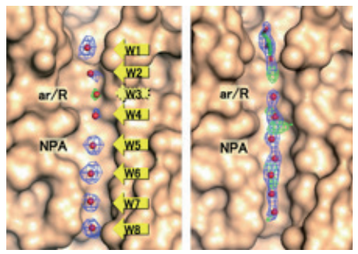

Fig. 2: APQ4 structure determined at 2.8Å resolution by electron crystallography (left), and structure analyzed at 1.8Å resolution by X-ray crystallography (right). Electron crystallography analyzed at lower resolution, but the eight water molecules (W1 to W8) in the channel are clearly discriminated unlike X-ray.

Fig. 3: Prof. Fujiyoshi (left) and Dr. Richard Henderson (MRC LMB) (right) at the Nobel Prize ceremony in Stockholm in 2017.

Currently, we are studying the mechanisms of acquired synaptogenesis, ion-channel gating and tight junctions formed by claudins, in addition to water channels. A typical example of single-particle analyses is structural analyses of gap-junction channels, which are the central feature of the electrical synapse. In a rather short period, we have been able to effectively analyze structures that are helping us understand the unique gating mechanism of the channel.

Q: Recently, you have been advocating the concept of "drug rescuing." Could you explain what this is?

A: Since around 2000, "evidence-based drug development." which identifies target proteins based on functional analyses of them and screens for their ligands, has become the mainstream method of drug discovery. However, even if promising compounds are found thorough screening, preclinical and clinical trials often encounter adverse effects and the success rate is as low as 1 in 30,000.

For this reason, "drug repositioning" to find other medicinal effects of already approved drugs is being actively pursued. However, there is a risk that the stores of these drug targets will be depleted. Also, in Japan, variations on the same compound will command the same price, so pharmaceutical companies do not make much profit from repositioning their drugs.

To overcome these problems, I came up with a strategy called "drug rescuing." The idea is to "rescue" compounds as well as drug target proteins that are discarded during the drug-development process. The promising way to implement this strategy is by determining a structure of the target protein binding the compound that was discarded. The structure will tell us detailed information about pharmacologic action, and allow us to improve the interaction of the compound. Importantly, the structure will also tell us the part of the compound that has no impact on binding. We can modify the compound at that specific location in various ways without affecting the optimized binding activity.

Drug rescuing is possible because single-particle analysis has accelerated structural analysis, but cryo-electron microscopes, which are essential for single-particle analysis, are expensive and difficult to maintain. For this reason, we have launched a business venture offering structural analysis services, and have started operation in cooperation with TMDU. Specifically, we will set up our equipment at Tokyo University of Agriculture and Technology as a hub center and plan to build remote operation systems in TMDU via fiber-optic connections. We will also conduct joint research with pharmaceutical companies and others, and hold workshops to share know-how for the method based on cryo-electron microscopy.

Q: Lastly, what are your future ambitions?

A: In addition to the contributions at TMDU I have already mentioned, I hope to use the network for other research groups as well as pharmaceutical companies. We have already succeeded in building up an effective remote operation system in TMDU, same of which could be installed in other groups.

There is another reason I founded the business venture: If we can be profitable enough, I would like to contribute to establishing an institute similar to The Medical Research Council, Laboratory of Molecular Biology (MRC LMB) in Cambridge, UK, where the number of Principal Investigators is small but already 19 researchers have been awarded the Nobel Prize. Although there are many reasons why MRCLMB is so successful, one important reason might be that the environment is conducive — a place where researchers can do their work without worrying about money. I therefore have a tremendous dream of creating a system that allows researchers to conduct research by using the income we will earn through venture-business sales and patent revenues.