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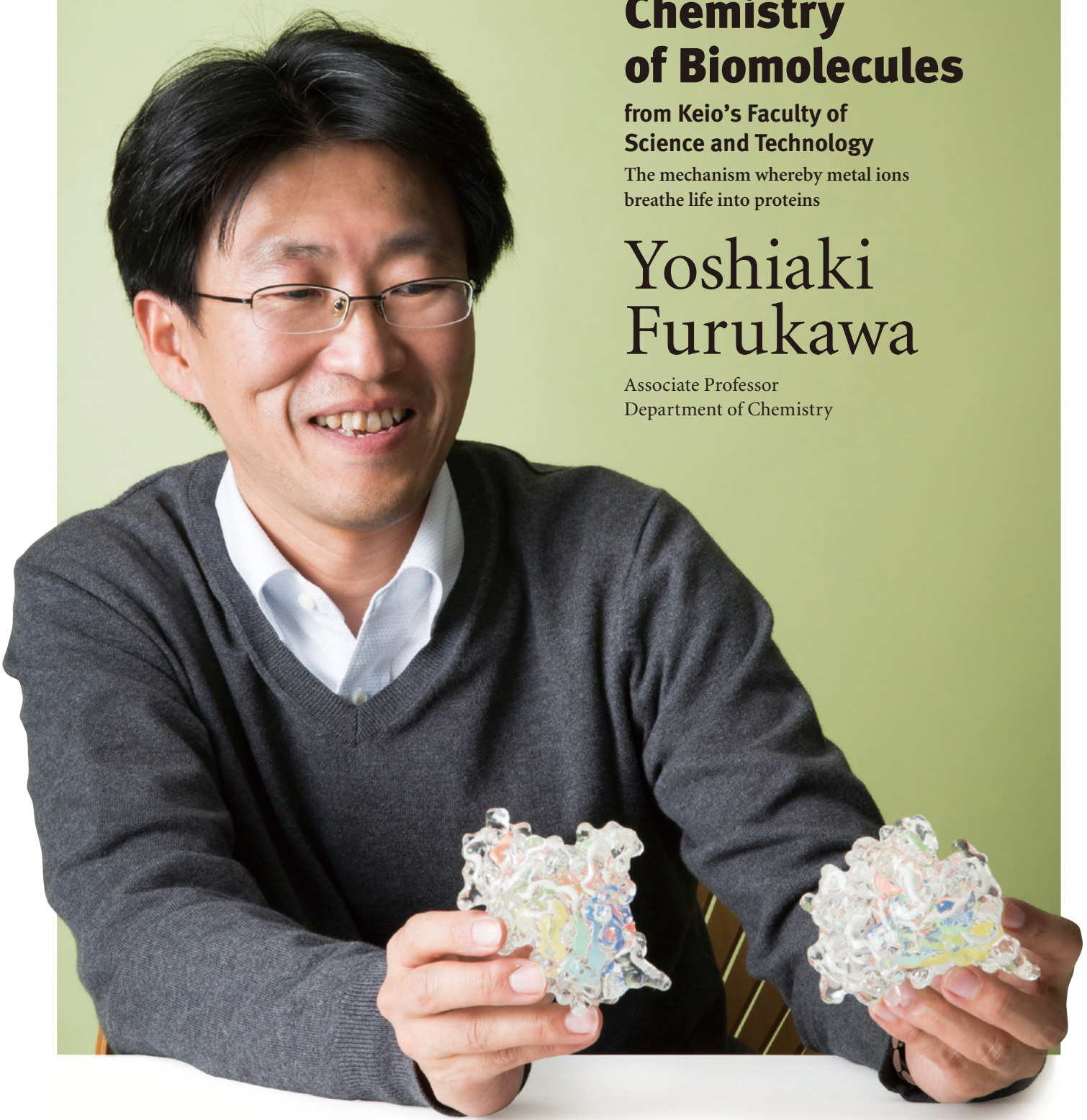
Mechanistic Chemistry of Biomolecules

from Keio's Faculty of Science and Technology

The mechanism whereby metal ions breathe life into proteins

Yoshiaki Furukawa

Associate Professor
Department of Chemistry



The Research

Associate Professor Yoshiaki Furukawa is featured in this issue, whose field of research focuses on mysteries of life phenomena through the metalloprotein formation process.

Binding of metal ions to proteins sustains our lives

Mechanistic chemistry of biomolecules sheds light on life phenomena on the molecular level

With the help of metal ions, proteins control numerous vital reactions. For example, a protein known as superoxide dismutase 1 (SOD1) begins to protect cells against reactive oxygen species only after it has bound copper and zinc ions. However, mutant forms of SOD1 incapable of metal binding form abnormal aggregates, which is considered to be a cause of amyotrophic lateral sclerosis (ALS). By focusing on an *in vivo* process that supplies metal ions to proteins, Dr. Yoshiaki Furukawa strives to shed light on various life phenomena and wishes to apply the results of his research to the prevention of incurable diseases and the development of remedies.

Metal ions indispensable to biological activities

Inside organisms, a variety of metal ions, such as iron, zinc, copper, molybdenum, cobalt, etc., exhibit their functions upon binding to proteins. Each and every one of these ions is indispensable to sustaining our lives. An environmental shift of tremendous magnitude that occurred on Earth in remote antiquity is believed to have much to do with the question why we humans came to need such a wide variety of metal ions.

Dr. Furukawa explains, "For example, hemoglobin, which is contained in our red blood cells, is a protein with an iron (II) ion. Hemoglobin can carry oxygen molecules because an oxygen molecule is bound to the iron (II) ion. Iron (II) ion is considered to be the first metal ion used by living things. This is presumably because this ion was abundant in oceans of the primeval Earth." Proteins that have metal ions (hereinafter metalloproteins) caught Dr. Furukawa's special interest, making him intent on studies of metalloprotein structures and functions.

Concentration of molecular oxygens in the atmosphere increased sharply as photosynthetic cyanobacteria appeared on Earth. As a result, most of the iron (II) ions were oxidized, which later accumulated on the sea bottom as insoluble iron(III) oxide. The use of iron ions suddenly limited, it is assumed that living things adapted themselves to environmental changes by smartly using copper and various other metal ions instead.

Protecting lives against reactive oxygen species by using metal ions

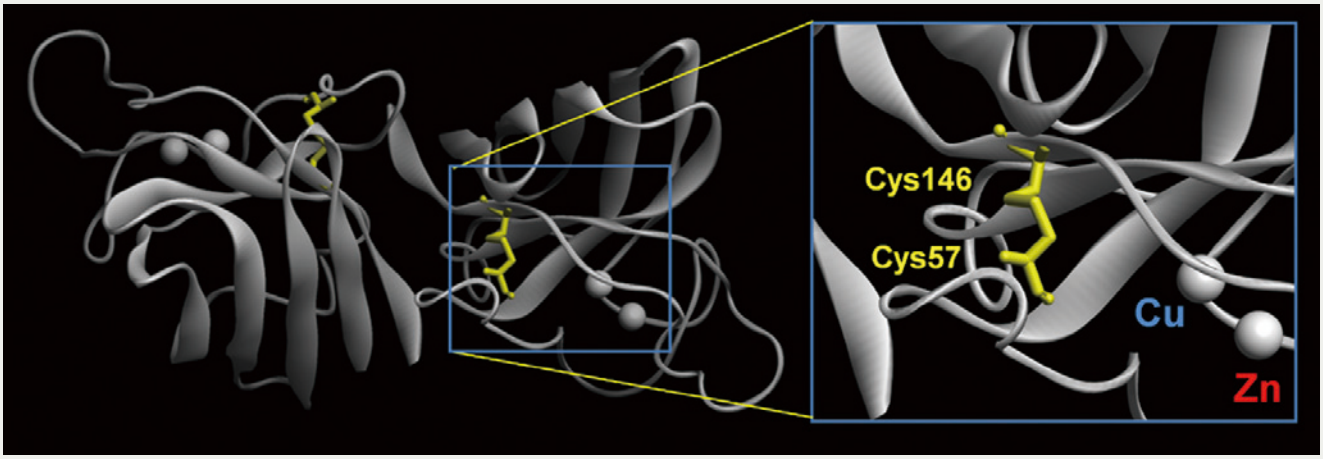
Of all proteins, about one third are said to bind some sort of metal ions. Why do living things need metal ions this much?

"Various chemical reactions are taking place inside organisms to sustain their lives. Characters behind these chemical reactions are proteins. Proteins are polymers consisting of amino acids, but proteins as such can produce only limited chemical reactions. By binding metal ions, however, the number of chemical reactions that proteins can afford increases in a single swoop." Metal ions thus came to play vital roles indispensable to maintain highly advanced, sophisticated life phenomena.

The metalloprotein SOD1 (Cu/Zn-superoxide dismutase) that Dr. Furukawa focuses on is an enzyme capable of removing the superoxide O_2^-, a highly toxic reactive oxygen species. Malfunction of SOD1 increases the intracellular concentration of O_2^-, which would damage DNA and/or membranes so much that living things won't be able to live any longer. SOD1 consists of two subunits, each having a structure to which one each of a copper and zinc ion are bound (Fig. 1). The copper ion functions

Fig.1 Structure of SOD1 protein

One each of copper (Cu) ion and zinc (Zn) ion is bound to each of the two subunits. Two cysteine residues (Cys57, Cys146) form a disulfide bond, which serves as something like a "lid" to prevent the Cu and Zn ions from getting dissociated.



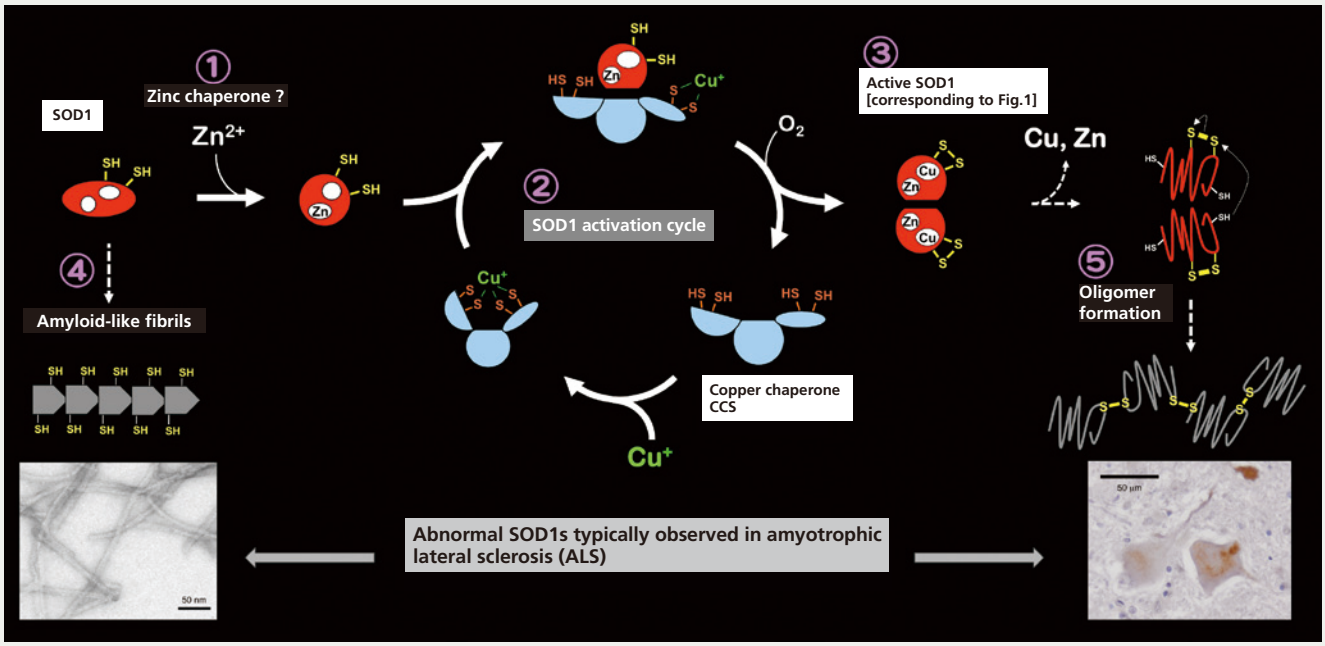


Fig.2 Dr. Furukawa-suggested mechanism of SOD1 activation and aggregation

① SOD1 binds a zinc ion first (mechanism unknown); ② then a copper ion is supplied by a copper chaperone, CCS; ③ SOD1 with enzymatic activity is produced as a result; metal-dissociated SOD1s aggregate into abnormal structures like ④ amyloids (photo: electron microscopic image) and ⑤ oligomers (photo: neurons of an ALS patient, the brown-colored area being oligomer). Dr. Furukawa suggests that this mechanism concerns the development of ALS.

as the active site for removing O_2^- while the zinc ion serves for stabilizing the SOD1 structure. Curiously enough, SOD1 can function within organisms by selectively binding copper and zinc ions only from among the diverse range of metal ions.

How do proteins capture metal ions?

"Living things are unable to create metal ions themselves. Naturally, they have to take in metal ions from food. Since some metal ions are toxic, however, they need to capture specific metal ions only, bring them into their cells, and supply the metal ions to specific proteins. This is a very intriguing phenomenon, but much remains unknown about it," says Dr. Furukawa, admitting that relationships between metal ions and proteins are still shrouded in mystery. It is likely that various biomolecules are scrambling for metal ions within cells, which leads to assumption of the existence of "metallochaperones" tasked with conveying metal ions to specific proteins. Prof. Valeria Culotta of Johns Hopkins University and Prof. Thomas O'Halloran of Northwestern University discovered copper chaperones for the first time in 1997. This accomplishment was followed by recent reports of discovery of iron and nickel chaperones. By the way, the chic-sounding term "chaperone" is a French word originally meaning a senior lady who teaches refined manners to young ladies about to make their debut in society.

For SOD1, a copper chaperone called CCS supplies copper ions. Dr. Furukawa found that SOD1 receives copper ions from CCS via the cycle shown in Fig. 2, and figured out that such copper ions protect the body against toxic reactive oxygen species. Furthermore, Dr. Furukawa determined that failure of this cycle hampers the supply of copper ions from CCS to SOD1. If that is the case, SOD1 cannot function as an enzyme and maintain its proper structure, causing numerous SOD1 molecules to aggregate – an abnormal phenomenon. Yet, Dr. Furukawa confesses he is still totally in the dark about how SOD1 captures a zinc ion. His challenge continues toward identifying the world's first "zinc chaperone."

Failure of metal binding causes neurodegenerative diseases

"What I'd like to know is how proteins secure metal ions. The importance of this process can be clearly understood from the fact that proteins that failed to bind metal ions are observed in various diseases," remarks Dr. Furukawa. While he asserts that his research is purely directed to basic studies, his research also attracts the attention of the medical circles given that SOD1 aggregates are a phenomenon observed in some amyotrophic lateral sclerosis (ALS) patients.

ALS is an incurable disease, with which nerves (motor neurons) that control body muscles are affected. This, in turn, causes muscles necessary for moving legs or breathing to atrophy. Many cases of hereditary ALS involve mutations in the SOD1-coding gene. As of now, more than 150 kinds of mutations have been reported in SOD1-coding gene.

What's more, mutant SOD1s are found aggregating in motor neurons of the ALS patients. By controlling the binding of copper and zinc ions to SOD1, Dr. Furukawa succeeded in a test tube experiment to reproduce the process of SOD1 aggregation that develops within motor neurons of ALS patients.

"The question yet to be solved is whether SOD1 aggregation causes ALS or whether the development of ALS causes SOD1 to aggregate. This is the point we need to watch for. Even if we have successfully identified a substance that controls SOD1 aggregation, therefore, we cannot definitely say it will become an ALS remedy," says Dr. Furukawa cautiously. Nevertheless, he continues to publish his own research results, convinced that clarifying the detailed mechanism of metal ion-controlled SOD1 activation and aggregation will eventually lead to a full understanding of ALS.

We may see the day come before long, when Dr. Furukawa's genuine interest in metalloproteins will lead to the discovery of an innovative drug for this incurable disease.

(Reporter & text writer : Akiko Ikeda)

Associate Professor Yoshiaki Furukawa

Entering into the world of research, fascinated by the lifestyle of my respected mentors who enjoyed pure pursuit of sciences

So far Dr. Furukawa has met a number of wonderful people and enjoyed engaging in research in various places. He sincerely wishes that his students never forget the "joy of thinking" no matter how small or trivial the theme in question may seem. His experience of continuing research on metalloproteins, an interdisciplinary field between biochemistry and inorganic chemistry, makes him keenly aware of the importance of maintaining an interest in various fields of study.

What was your childhood like?

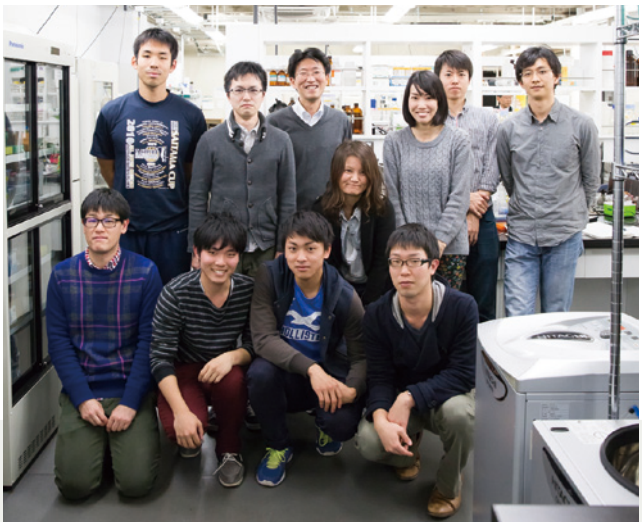
As an elementary schoolboy, I spent almost everyday playing baseball with my friends till dark immediately after coming back home from school. I wanted to become a professional star player at the Hanshin Tigers team in the future. I really meant it. I was also fond of insects and other living things. After school, I would go to a nearby open space to catch grasshoppers or to a rice paddy or irrigation canal to catch tadpoles and crayfish.

Although I was not so much interested in school studies, as an elementary school fifth or sixth grader, I was unexpectedly awakened to the "fun of thinking." Mr. Goto in charge of our class at the time was a teacher of a somewhat peculiar type, who let us elementary school students think about the relationship between the brightness of stars and their distances to the Earth, or taught us about various properties of atoms using a periodic table of the elements. What's more, he would often take us outdoors to let us understand the importance of observing and experiencing things in the field. Looking back at those days, I think these experiences might have been too advanced in content for us, but we could feel the fun of learning firsthand.

Why did you find an interest in chemistry?

During my junior and senior high school days, I was fortunate enough to be in an environment surrounded by bright people who did not try to rush at solving problems, but solved problems exquisitely after thinking them through. This, I think, led me to awaken to the fun of learning.

I still remember one day. During a chemistry class on the electronic theory of organic chemistry, I was fascinated by its clear-cut approaches. I was greatly motivated to study this simple, beautiful field – chemistry – more deeply and chose to learn at Kyoto University with a good reputation for chemistry.



At Kyoto University, however, I did not attend classes so diligently because the university in those days allowed its students to graduate only if they pass exams. So, I learned chemistry mainly by reading a variety of textbooks. Instead, I would actively take part in reading circles of Latin and German language seminars while also attending other seminars on the campus as an audit student. Thus I exposed myself to studies other than chemistry, which later proved to be very good experiences. By doing so, I was also able to make a number of good friends, with whom I fully enjoyed my campus life. In retrospect, however, it's a pity I missed many of the classes by prominent professors representing Japan's chemistry learning.

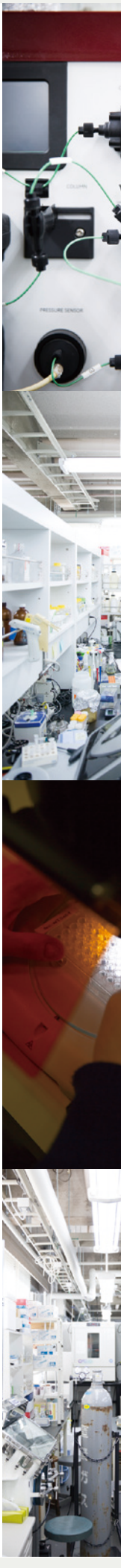
What motivated you to enter into the world of research?

The origin of my academic career is Prof. Isao Morishima's lab, to which I was assigned as an undergrad. Back in those days, I already became interested in both biochemistry for molecular-level understanding of life phenomena and coordination chemistry that describes reactivity of molecules using molecular orbitals (MO's). I was happy joining the Morishima lab and was able to satisfy both of my interests because the lab focused on the structure-function relationship of hemoproteins – an interdisciplinary area between proteins and metal complexes. In addition, the figure of Prof. Morishima that I saw when I visited the lab to look at it for the first time was so impressive I cannot forget it even today. Comfortably sitting on a large chair and smoking a pipe, he eagerly explained hemoglobin reactivity by the molecular orbitals of heme, which was truly inspiring.

Despite some difficulties and distress experienced in the course of research, every day was really fulfilling – developing hot discussions with all lab members while writing this and that on white boards set up here and there within the lab. I'd also like to mention the then assistant professor Koichiro Ishimori, who thoroughly taught me how to communicate one's idea to others, including how to write (not to mention scientific papers) and how to make a presentation at scientific meetings. I owe much to him for what I am today as a researcher.

What course of life did you have in mind after graduation?

So devoted to experiments, discussions and presentations, finding employment with a business was the very last thing I could think of. I suddenly found myself in the third year summer of the doctoral course without any plan for the future – a crisis situation. Thanks to recommendations from many professors, however, I was able to study under Prof. Thomas O'Halloran of



Understanding of metalloproteins requires knowledge of sciences across the board. It's, therefore, important to expose yourself to as many fields of learning as possible from your earlier years.

Yoshiaki Furukawa

Born in Hyogo Prefecture, Dr. Furukawa specializes in bioinorganic chemistry, focusing on the roles metalloproteins play in physiological and pathological phenomena. He completed the doctorate course at Kyoto University Graduate School of Engineering and obtained a doctorate degree (Dr. Eng.). After serving Northwestern University of the United States and the RIKEN Brain Science Institute as a postdoctoral fellow, he joined Keio University Department of Chemistry as an associate professor (non-tenured) in April 2010, then assumed the current post in April 2015.

Northwestern University who was active and world-renowned for his research in copper chaperone. I thought I might be able to shed some light on life phenomena and the development of various diseases if, in the course of my research in copper chaperone, I could clarify interactions between proteins and metal ions. This idea continues to be the source of energy behind my current research activities.

"Everything should be as simple as possible, but not simpler." Prof. O'Halloran often cited this remark of Albert Einstein. While Prof. O'Halloran is currently engaged also in other projects, I'm overwhelmed by his ability to come up with one innovative idea after another. He advised me, saying "Don't be afraid of creating new ideas, but just enjoy it." Thanks to this encouraging advice, I'm coming to enjoy my research work even more.

Later, I joined Dr. Nobuyuki Nukina's research team at RIKEN Brain Science Institute in Japan. Dr. Nukina was one of the few neurologists, who were attempting to understand the pathologies of neurodegenerative diseases by focusing on structural changes in proteins. Even today, he sticks to basic studies. As a member of his team, I was privileged to freely use cultured cells and experimental models like mice and rats I had never handled before, as well as costly experimental equipment. These experiences helped me a lot in honing my experimental skills. In this sense, I may call it the most fulfilling period of my life.

In retrospect, I have been truly blessed with good mentors. Each and every one of my mentors was enjoying their scientific pursuits and their own lives, thus providing me with good role models for my life.

What do you expect of your current students?

I joined Keio University in 2010. Fortunately, I was able to set up my own lab soon thanks to enthusiastic support of Department of Chemistry professors who voiced, "Keio needs to introduce a new field of research."

With alumni of many graduates who are active at the forefront of society, I feel Keio University is a driving force of Japan, so to speak. On one hand, it is wonderful. On the other hand, I'd advise our students not to become too dependent on the Keio brand and its extensive human network. I'd like my students to develop and exhibit their own individual colors.

Universities are where you should think about your future paths, not a mere waypoint to find employment with big businesses. Prof. Morishima once taught me the phrase "noblesse oblige." This means the noble have their obligations to perform for society. Our students are privileged to enjoy such an enviable environment as Keio. Therefore, they should think more seriously about what they should do to contribute to society, and live their lives with pride.

How do you refresh yourself when you have time to spare from research?

I like languages and letters/characters of the world, so I often take my family to exhibitions and events that interest me. The catalyst for my liking were the ancient Tangut characters that I knew in the novel "Dunhuang" authored by Yasushi Inoue that I read when I was a junior (or senior ?) high school student.

I like to travel overseas with persons with whom I can share joy and distress. In fact, I involved my wife to visit various overseas destinations even before our marriage.

As for daily breathers, the best is playing with my daughters, an elementary school girl and a kindergarten student. Simply looking at them is really fun!

◎ Some words from students . . . ◎

● Dr. Furukawa came to Keio in 2010. It just happened to be when I entered Keio. Eager to challenge a research theme dealing with substances like proteins with large molecular weight, I jumped at the Furukawa lab engaged in research in metalloproteins. I'm now in the first year of the doctoral course. Dr. Furukawa puts faith in me and allows me to carry out research as freely as I like, which is encouraging as well as comfortable.

(Reporter & text writer : Akiko Ikeda)

For the full text of this interview
http://www.st.keio.ac.jp/kyurizukai

Traveling Overseas in Search of Characters

It is said there are more than 100 different kinds of characters and letters in the world. I set off on overseas journeys driven by an ambition to "conquer" all of these foreign characters and letters sometime in the future.

Luxor (Egypt)

When asked about ancient characters, the first thing that comes to your mind may be hieroglyph. Shown in photo is the Mortuary Temple of Rameses III in Luxor. It was so huge and magnificent I was simply overwhelmed by it. Even today, it is possible to see hieroglyph in various parts of Egypt, impressing the visitor with the "strength" of characters that have survived countless years. Do you know hieroglyph was the model for the alphabet?

Chichén Itzà (Mexico)

Another ancient character as great as hieroglyph is the Mayan script. Though the Mayan script is very complex in design, some of its meaning can be understood if you come face-to-face with it patiently and character by character with a manual in your hand, which is exciting. Photo shows the Kukulcan Pyramid in the prehistoric city of Chichén Itzà. Though it is forbidden to climb it now, the steep stone steps were very fearful.

Chicago (U.S.A.)

I once studied in the City of Evanston in the suburbs of Chicago, so I frequented Chicago to hang out there. This metropolis is skylined by numerous skyscrapers, each with its individual beauty. Its population consists of many immigrants from Poland, Ireland and Israel, hence various languages can be heard as you walk along the streets. Its winter is as cold as freezing Lake Michigan shown in my background.

Machu Picchu (Peru)

It is said that the Inca civilization did not have characters for writing, but superb masonry was breathtakingly marvelous as it created structures by heaping up rocks so precisely as not to allow even a sheet of paper to come through. Set among precipitous mountains, Machu Picchu unfolds remains of Inca rock works here and there. It is indeed a site where ancient history is still alive. The City of Cusco near Machu Picchu is a very beautiful town and one of the most beautiful cities I've ever visited.

Phnom Penh (Cambodia)

Southeast Asia is a treasure-trove of characters, most of which have their origins in Brahmi script, the first ever characters that ancient India had. Brahmi script was also introduced into Japan many centuries ago as Sanskrit characters (Bon-ji) and contributed significantly to the completion of the table of the Japanese syllabary. By the way, the vitality of local Southeast Asian people was amazing! Following suit with the locals, I also traveled around Cambodia on the rear seat of a motorbike.

Xinjian-Uygur Autonomous Region

This region is where Chinese and Arabian characters meet. Even today, we can feel the atmosphere of a cross-cultural intersection along the ancient Silk Road. In the town of Kashgar, once perceived to be the farthest land, the streets are flooded with signboards using Chinese characters as phonetic symbols for Uighur words. It looked like a world of another dimension as if surrounded by ancient Japanese words written in "man'yo-gana" – Chinese characters used as phonetic symbols for Japanese words. The sand storm in the Takla Makan Desert was also furious.

私の本棚

My favorite books

The Great White Tower

I'm sure this long novel will arouse the interest of any university professors. Things may vary largely according to faculty, but the things written in this book were felt real, bringing us into that world in a single swoop. It is well known as a social-oriented novel criticizing vicious aspects of the medical world through corruptions of Prof. Zaizen, the main character. Depictions of Zaizen's internal conflict between his burning desire for high social status and his pure enthusiasm for pursuit of medicine are very impressive. Incidentally, the actor Toshiaki Karasawa, who played the role of Zaizen in the TV drama, was cool.

Sherlock Holmes

Needless to say, this is a great detective story masterpiece. The image of Sherlock Holmes, who solves criminal cases with his keen observant eye and enormous knowledge, somewhat overlaps with that of researchers who strive to shed light on mysteries of natural phenomena. Viewing a TV drama series produced in England, I found the starring actor Jeremy Brett cool and impressive in all his movements. In the Japanese-dubbed version, Shigeru Tsuyuguchi played the role of Holmes marvelously with his nice low voice. As such, this TV drama series captivated me. Sherlock Holmes as performed by Jeremy Brett is the only idol I admire.

The Family That Couldn't Sleep

If you are tired of reading stiff textbooks, I'd recommend science nonfictions as alternative sources of knowledge. This book is a nonfiction about fatal familial insomnia which is a dreadful disease (even just to image it) making the patient become sleepless gradually and steadily to death. Just like amyotrophic lateral sclerosis (ALS), which is one of the targets of my research, this disease falls into the category of neurodegenerative diseases and is considered to be caused by abnormal structures of proteins (prion). At the present time, there is no remedy or means of prevention for this disease.

Stag Beetles of the World

I'm somehow attracted by the peculiar form of stag beetles. From the moment I open this book, before I know it I forget the time and find myself returning to childhood. It is said that there are some 1,500 varieties of stag beetles in the world, each having distinctive large mandibles. Not only their appearances, but there are also large differences in the degree of their violent temper and habitats. As such, they deserve being called creatures that exemplify genetic diversity. To tell the truth, I'm still enjoying keeping several stag beetles at home. Someday in the future, I may take up a challenge of clarifying stag beetle's biology at the molecular level (?).

Ancient Characters

Although I haven't the slightest idea of what ancient characters mean, through characters I can somehow feel ancient people's intentions or eagerness to communicate to others. Whatever the ancient characters may be, each and every one is refined in terms of design, indicating ancient people's good sense. Deciphering of ancient characters is a field of highly advanced science as it involves estimation of the language used, meticulous analysis of the appearance frequency of phonemes, and so forth. Even with hard and miserable time deciphering a short sentence written in cuneiform script some 4,000 years ago, it was found to be simply meant, "Give the money back!" Once deciphered, the illustrations contained in this book may possibly be found meaning total nonsense or something horrible.

Pursuit of Research

Yoshiaki Furukawa

I can proudly say I have been engaged in research on wide-ranging themes from biology of bacteria to pathogenic mechanisms of human diseases, centering on metalloprotein structures and functions. Therefore, when I'm asked, "What kind of research are you engaged in?" I usually respond with a variable answer, trying to see what the questioner really wants to know. The case of the interview for this issue was no exception; I felt a bit undecided which research to talk about. Nevertheless, if asked about why I pursue research, my only, definite answer is "Because it's fun."

Pursuit of research – what do you think of it? Given difficulties inherent in research, some of you may have the impression that the more difficult a research theme is, the more exciting it is. It is true we have to make preparations (preliminary studies) to understand the content of a research; you actually don't have to regard research itself as something invincibly difficult. For instance, why don't you regard research as an extension of amusement?

Just remember the days when as a child you played hide and seek. It must have been fun for you to look for a new hiding place or think up a crazy idea for fleeing from the tagger. Exactly the same can be said of research. Desires to discover new things and surprise all others are the very motives for research. No matter how small a discovery may be, only the first researcher who has published it can be the winner. This drives researchers around the world to aim at winners' positions with a vigilant eye. That said, this rule is the same as that of hide and seek. Everyone being serious about the game makes research endeavors all the more fun.

Despite the nature of research, which is supposed to be fun, we often hear of gloomy screams over the decreasing number of students who aim to choose researchers' careers. There may be various reasons for this trend. For one thing, to my great regret, negative aspects of researchers' careers (such as unstable employment) are bandied about as the use of the Internet and SNS spread. In my early years, there were no terms like "network society". Information was practically limited to "live" success stories about our seniors and professors who were around, which helped us young ones devote ourselves to research in a situation of "Ignorance is bliss", so to speak. Instead of aiming only at finding employment with big businesses, why don't you shut yourself off from worldly noises and come to grips with the world of experiments and research? Your future will somehow take care of itself only if you enjoy your researcher's life in earnest. I'm sure you will find a life of amusing yourself in the "fun" of research much better and rewarding than perceived from outside.

Science and Technology **Information**

Introducing Keio's organization for industry-academia collaboration

By establishing an organization for close collaboration with industry and local governments, Keio University's Faculty of Science and Technology strives to return its research results to society and foster up-and-coming human resources. We are also intent on strengthening our research organization by supporting government initiatives such as the Program for Promoting the Enhancement of Research Universities and the Top Global University Project.

Yagami Campus
(Yokohama City)

[National & local governments]

[Private businesses]

[Universities & research organizations]

Shin-Kawasaki Town Campus

Sosokan Bldg.

Keio Leading-edge Laboratory of Science and Technology (KLL)
[Support of collaborative research; Fostering of human resources]
Returning research results to society

Design Center / Manufacturing Center
[Support of research for manufacturing]
Equipped with the most advanced machine tools

Technology Center Bldg.

Keio Innovation Foundry (KIF)
[Large-scale research projects]
Promotion of projects in collaboration with industry

Keio-Kanagawa Manufacturing Technology Verification & Evaluation Center
[Commercialization support for local manufacturers]
Equipped with nano-fabrication machines

Shin-Kawasaki Frontier Research & Education Collaborative Square
[Research & Education on advanced technology]
Functioning as a base of industry-academic collaboration

Editor's postscript

In the process of their evolution, living things had acquired a smart mechanism of using metal ions as metalloproteins. In recent times, significant progress has been made in research, particularly shedding light on mysteries of life sciences at molecular and atomic levels. This progress is expected to contribute to human society in a wide range of fields. We sincerely hope Keio University will also bear an active part by offering its most advanced research results in science and technology including medical and pharmaceutical sciences. Dr. Furukawa's lab is filled with various chemical test apparatuses, where we found member students devoted to research, which was an impressive sight. (Kenji Kobayashi)

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