Kyurizukai

新版 窮理図解

2023 August
no.
37

Bulletin of Keio University Faculty of Science and Technology
https://www.st.keio.ac.jp/education/kyurizukai/

English versions are also available:
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Man-made Proteins

from Keio's Faculty of Science and Technology

Invention and creation through researching self-assembling proteins

Norifumi Kawakami

Senior Assistant Professor
Department of Biosciences and Informatics

This issue features Senior Assistant Professor Norifumi Kawakami and his ongoing research about creating soccer ball-shaped protein nanoparticles.

Self-Assembling Soccer Ball-Shaped Protein Nanoparticles

Towards next generation's nanomaterials

Proteins: a fundamental building block of all living organisms. Norifumi Kawakami has harnessed the properties of proteins in his creation of soccer ball-shaped nanoparticles. These nanoparticles can be broken down and still return to their original shape, meaning that they are expected to have incredible utility as nanocapsules for delivering medications inside the human body.

Creating a name and legacy through a molecule

When Professor Kawakami was hired to his current position in April 2014, he set to work on a research project that would "create a molecule that's never existed before in our world." In explaining his motivation for this project, Kawakami says, "I've done research in so many different areas that you could say that there's been no real consistency to my work. I realized that in order to survive as a researcher, I would need to make a name for myself, some achievement that would automatically be associated me, almost like a research 'calling card.'"

Because of Kawakami's frequent encounters working with proteins in the past, he decided to use them as his focus when creating the new type of molecule. For his research "motif" he landed on the idea of a soccer ball. "When I was a student, I was fascinated by the beauty of fullerenes. A fullerene is a form of sixty carbon atoms arranged in a pattern like a soccer ball. (Fig. 1) I later learned that this soccer-ball shape occurs naturally in everything from plants to outer space, so I thought there might be something special about it and some reason why it forms so readily. This was my inspiration in wanting to design a soccer ball-shaped molecule."

Fig.1 "Soccer ball" patterns appearing in various settings
A fullerene composed of 60 carbon atoms (left), newly budding pincushion flowers (center), and Kronberger 61, a planetary nebula shaped like a soccer ball (right). Rightmost photo courtesy of the International Gemini Observatory/AURA

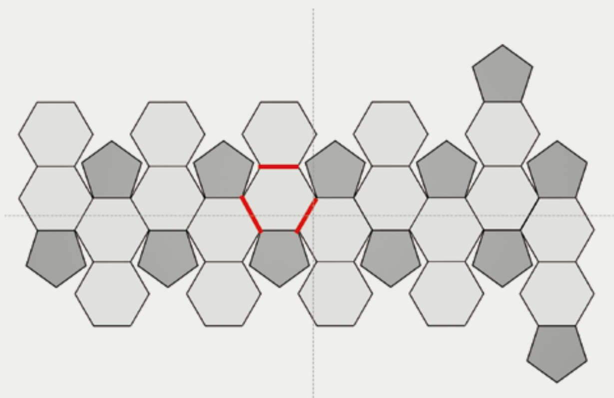


Fig.2 The geometric net of a soccer ball
Soccer balls are made up of 12 pentagons and 20 hexagons. The vertices of connected pentagons (red lines) naturally form hexagons.

Making a soccer ball-shaped molecule using fusion proteins

So how exactly is it possible to take proteins and make them into a soccer ball-esque molecule? Kawakami's chosen molecular design method was to use fusion proteins.

Inside our bodies, proteins are at constant work, spontaneously and automatically collecting into complex structures. Around the year 2000, researchers began to investigate ways that they could use this property to manipulate proteins into man-made shapes.

Then, in 2014, an American team of researchers successfully constructed a polyhedral molecule by using fusion proteins which combined two types of proteins together. However, a problem emerged; the proteins could take multiple different polyhedral shapes. Mixed types in a product create a major roadblock to industrial applications. Kawakami saw this issue and began to explore whether

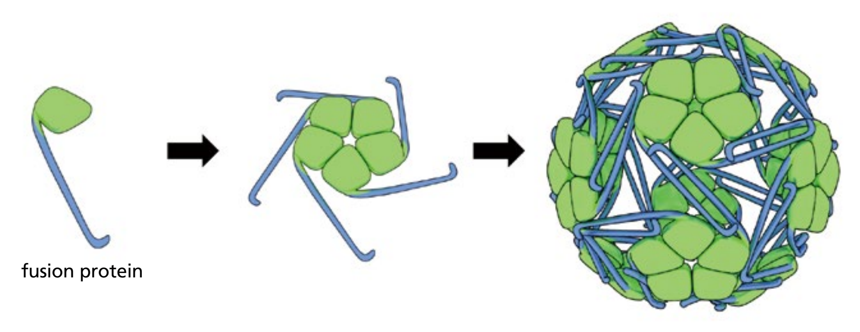


Fig.3 Design concept for creating the soccer ball-shaped molecules.
The scientists fused two types of natural proteins so that when 60 of them were collected, they would form the shape of a soccer ball. The green and blue proteins have properties that are attracted to each other. Furthermore, the blue proteins are hooked, meaning that when they latch to each other, they stabilize the molecule's structure. In practice, the fusion proteins are created when their genes are introduced to bacteria from the large intestine (E. coli). The bacteria synthesize the proteins, which then spontaneously gather and form into the shape of soccer balls.

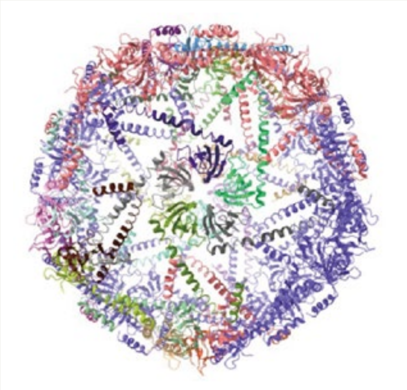


Fig.4 The model (photo) and actual structure of the molecule as revealed through cryo-EM (above)
Just as intended, the 60 individual proteins ended up forming into a soccer ball-shape. The photographed piece of the model shows how five proteins are amalgamated into a pentagonal piece. The final 60-molecule nanoparticle is a truncated icosahedral protein, which led to the name "TIP60."

there was a way of only producing soccer ball-shaped molecules.

"Soccer balls are essentially just polyhedrons made up of 12 pentagons and 20 hexagons, so my first thought was to figure out some sort of pattern that combined these shapes. The problem was that I had no idea how to arrange them together in a way that made sense and no clue how to make their edges line up." However, as Kawakami stared down the blueprints for his molecule model, he realized something. "I was stuck on the idea that hexagons were essential to building a soccer ball, but then I realized that if you simply use lines to connect pentagons to each other at their vertices, inevitably you will end up with hexagons." (Fig. 2)

Using Euler's theorem on polyhedrons—(number of vertices) - (number of edges) + (number of faces) = 2—it follows that a polyhedron consisting of hexagons and pentagons must include 12 pentagons. In other words, it is impossible to create any shape other than that of a soccer ball if relying on a mechanism which connects the lines extending from pentagons' vertices. "This is it!" Kawakami was confident he had found the solution.

The project researchers then designed a fusion protein (Fig. 3) and were able to confirm that the only types of molecules that should result from their experiments would be shaped like soccer balls. However, these particles were microscopic—only 22 nanometers in diameter (a nanometer is one billionth of a meter)—meaning that any tests to categorically determine whether the molecules were structured like soccer balls would require advanced techniques and equipment. Thanks to the help of the joint researchers on his team, Kawakami was able to conduct an analysis using cryo-electron microscopy (cryo-EM), a technique for which the inventors were awarded the 2017 Nobel Prize in Chemistry. Five years after the particles were first constructed, Kawakami was finally able to demonstrate conclusively that the molecules were, in fact, shaped like soccer balls(Fig.4)

Potential applications for nanocapsules and nanomaterials

While Kawakami originally started this research so that he could have an identifiable accomplishment associated with his name, the response to his soccer ball-nanoparticles in academic papers and at conferences was enormous. Because the centers of the nanoparticles were hollow, it was possible to fill them with medicine, allowing them to be used as "nanocapsules" or for other futuristic applications.

It was at this point that Kawakami then invented a way for the nanoparticles to disassemble and reassemble themselves into their soccer ball-form. If a manufacturer adds a substance when the particles are reassembling into their original shape, this substance will be trapped inside the ball.

In a more recent development, one of the students working on the research project figured out how to produce large quantities of the soccer ball nanoparticles at a low cost. The team is also working to produce a soft, gel-like material where the nanoparticles form large structures that store water around them. This mechanism would make it so that when the gel is activated, the soccer-ball particles will "collapse" allowing whatever they carry inside to be released.

When asked about his thoughts and aspirations for the future of nanoparticles, Kawakami says, "My goal is to have people all over the world use these soccer ball nanoparticles. That way, when other researchers see how we were able to package other substances inside them, turn them into a gel, and find different uses for them, they might look back on our work making the soccer balls when exploring materials and chemicals of their own. That would feel incredible."

(Interview and text writer: Chisato Hata)

Finding the fun in the trials and tribulations of research

Professor Kawakami has conducted research in a variety of specialties dating back to his time at a Japanese college of technology, or "*kosen*." From strategies on how to survive as a researcher, experience on how to get out of tight spots, and an understanding of how to produce results, Kawakami's skills are on constant display in his work at Keio University.

After middle school, you ended up going straight to a "college of technology," or "*kosen*," right? (For our international readers, *kosen* refer to five-year programs or engineering schools that middle school graduates can attend to get an associate degree.)

Honestly, my first preference was to continue my education at a high school, but one of my middle school teachers told me that my grades would make that difficult, so somewhere along the way I ended up choosing to go to a *kosen*. I didn't really know what type of place it would be, but after I started attending, it ended up fitting me perfectly. There were a lot of atypical students, so I managed to fit in pretty easily. I was at home with them.

Also, the *kosen* teachers were like college professors. They each had their own specialties, and whenever they talked about things in their personal area of expertise, you could really tell that they had fun explaining what they were teaching us. Whenever I expressed confusion, they would take my questions seriously when answering. If there was no clear answer, they would suggest potential thought strategies about the topic so that I could get my head around everything. It was also a lot of fun watching the teachers interact with each other. I remember thinking "this is how science gets done, by people working like this."

That was a big moment for me. Up through middle school, I always thought that "studying" meant memorizing the information in textbooks and then filling it out on answer sheets. But as I listened to my teachers' conversations, I was able to come to terms with the idea that the things being taught might be useful. As I kept working to understand the things that interested me, my grades in those subject areas steadily increased.

Why did you decide to pursue research as a career?

Kosen are designed so that their main programs last for five years, but for students who are interested in pursuing a higher level of education, they also offer 2-year majors. A lot of *kosen* students end up entering the workforce after they've finished their core coursework, but I felt like if I had come this far, I might as well continue to do a major course.During my time at the school, I studied ceramic water filtration and other water purification methods and was greatly influenced by my then-advisor, Dr. Shiro Kubuki (who now works as an associate professor at Tokyo Metropolitan University.) When I was unsure about what I should do after graduation, especially since I had realized how much I enjoyed research, he was the one who recommended that I go to graduate school.

At the time, I remember him nonchalantly saying that he would like to collaborate on a project together if I made it as a researcher, but it felt like a bit of a pipe dream to me back then. As it turns out, in addition to my soccer ball nanoparticle project, I'm also working on some research on biological evolution using Escherichia coli which seems like a good match for Dr. Kubuki, so I think that a joint research collaboration might be a very real possibility in the near future.

So, after that, you completed your program at Hiroshima University and started your postdoc at Nagoya University.

That's right. In graduate school at Hiroshima University, I studied chrysanthemums and ascidian. After that, I was hired as a postdoctoral fellow at Nagoya University in the Laboratory of Bioinorganic Chemistry, which focuses on proteins. However, I spent all my time at Hiroshima University researching biological systems, so I had not been involved in chemistry research since I was in my *kosen* program. I didn't even know where to start to get involved in a research topic. I decided to ask the other students what was the most difficult or interesting chemical reaction they knew about at the lab. If I was somehow able to produce a chemical reaction that everyone else thought was impossible, I figured it would benefit both the lab and my ability to survive as a researcher.

As luck would have it, we did manage to produce exactly such a chemical reaction. The only problem was that there was a German research lab which had submitted a research paper on the same topic at the same time. If our paper's submission was even slightly later than theirs, the chemical reaction would be credited to the German researchers. If that happened, I feared that I might reach the end of the road in my research career.

And this research experience is what later led to your success in creating the soccer ball-shaped nanoparticles?

Well, actually, after I came to Keio, I was working on another molecular design project before the soccer-ball nanoparticles, but I just couldn't get it right. It was one failure after another. I had a professor at Tohoku University analyze some of the molecules I had created so that I could learn about their structures. They told me that "it's pretty much garbage" after their observations revealed that the particles were all a bunch of different sizes.

This happened after putting a little over a year of work into that project, so it was really discouraging. My contract at the time could only last up to three years. I remember thinking that I had finally reached the end of my time as a researcher, but as soon as I came to this conclusion, I realized that I might as well go out on a high and work on the molecular design I wanted to do. On the bullet train ride home from Tohoku University, I turned to one









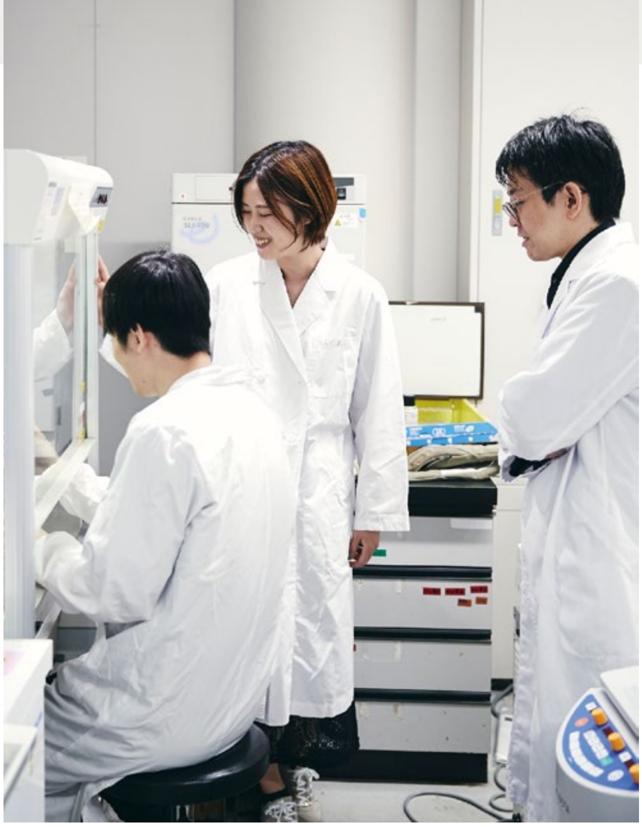
of my students and said enthusiastically, “We’re going to make a soccer-ball shaped molecule.” I’m not really the type to dwell on things. I think that’s one of the reasons I’ve been able to come this far.

I have a strong desire for my research to be useful to other people, but when it comes down to it, I create what I want to because it brings me a sense of self satisfaction. For me, molecular design is like the feelings a person might get from working on a painting. While it is demanded that research be functional in our day and age, I also want to prioritize my personal interests and the internal inquisitiveness that research elicits.

Since coming to Keio University, what is your impression of the atmosphere here and in your laboratory?

Compared to the national universities, I would say students are given a large degree of freedom. Students are very honest and upfront with the teachers, so it’s easy to talk with them, get an idea of their personalities, and provide guidance as necessary.

I’ve experienced quite a few different laboratories and the atmosphere in the lab can drastically influence the level of motivation everyone feels towards the research. This is why I want to prioritize making the workspace as inviting as possible. I make a point of talking to the students regularly as a part of my teaching philosophy.



◎ Some words from students . . . ◎

● I am working on the E. coli biological evolution research project. I chose this laboratory largely because of Professor Kawakami’s personality. He’s enthusiastic about answering any questions I have and keeps things light by joking around with us a lot of the time, so it’s really easy to approach him for advice. He’ll discuss things with us until we’re all satisfied with things, so I feel like I can be proactive in making progress on the research (2nd year master’s student).

(Interview and text writer: Chisato Hata)

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

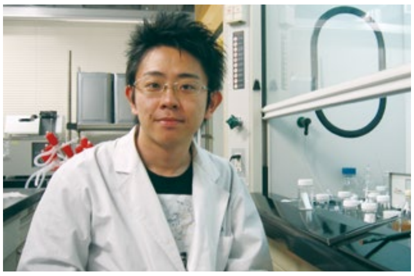
It is important that research be useful, but the ultimate goal is for it to bring personal satisfaction.

Norifumi Kawakami

Specializations in protein science, enzyme engineering, and the bio-metal science . 2004 graduate of the National Institute of Technology, Ube College (NITUC) with a degree in Chemical and Biological Engineering. In 2009, he completed the coursework for a Ph.D. at the Department of Biological Science, Graduate School of Science, Hiroshima University. Ph.D. in Science. After working as a postdoctoral researcher at the International Research Center for Materials Science and at the Department of Biological Science at Nagoya University, he became a project assistant professor at Keio University’s Department of Biosciences and Infomatics in the Faculty of Science and Technology in 2014, and a senior assistant professor in 2017, a position he still holds.

Norifumi Kawakami's ON and OFF

Expressing and
Exploring One's "Self"



At the lab

This photo was taken over ten years ago during an experiment I was conducting when I was a doctoral student. Back then I used to call myself the "visual kei postdoc," so my hair made quite the scene. One of the international students was from Germany and told me that he couldn't comprehend why I would style my hair that way, so in a way my unconventional hairdo actually helped me gain a more global perspective and the interactions I had because of it turned into a great cultural learning experience.

Giving a speech at my old workplace

This is a photo from a lecture I gave at Nagoya University during an event that other alumni organized in conjunction with Dr. Yoshihito Watanabe's final lecture. He is currently the director general at the Institute for Molecular Science at Nagoya University. At the time of this photo, it had been about six years since I had left Nagoya University. Between being selected to speak during one of the limited time slots and people expressing their excitement for my current research, it was a great experience.

Introducing my research

This was from a discussion about the UN's Sustainable Development Goals at the Pre-Keio Techno-Mall. The online format really reflects the time. The video was also uploaded to YouTube. I haven't had that much experience discussing these topics in the past, so I learned a lot from the event.

Kappa-kun dreams too!

This is Kappa-kun. I first grew interested in making computer generated (CG) art and animations around 2011. For the first two years I used POV-Ray. This program became really complicated when dealing with more complex figures though, so I switched to Blender in 2014. The photo above is "Kappa-kun the 3rd." I use it as an icon on my social media.

A researcher dreaming

Depending on how much alcohol I consume, you can see a progression of my drunken state. This is my final form. By being "on-the-job" at work parties, at some point it feels like my plug gets pulled. When my battery is completely gone, I don't drink at all.

My Kappa-kun construction cave

This is the space in my home where I make Kappa-kun. Over the weekend, you can usually find me here using Blender.

Recommended videos

I have picked out three of my favorite videos from my YouTube channel, KachupaTube, which you can watch using the QR code. In order, they are, "Kappa-kun Hits an Isosceles Triangle," "Kappa Castle," and "Kappa Evolution." While the titles seem strange, once you've watched the videos, I think that they'll make a lot more sense. Make sure to leave a like and subscribe to the channel!

私の本棚

My favorite books

Today’s Art by Tarō Okamoto (Kobunsha)

I picked this book up when I was wondering how people in the art world decide what has value and if it was something anyone could actually understand in the first place. Very early on, it said that “everyone should be free to judge art based on their own capacity.” It left quite the impression. I read this for the first time 20 years ago. All I knew about the author was that he had exploded in popularity back in the day, but as I read, I grew to understand why his career trajectory went the way it did. While my talents have never really manifested in the artistic fields, I have been able to pursue research that interests me, work on Kappa-kun on my YouTube channel, and other hobbies while recognizing that their “value” isn’t something for me to decide. This has let me work on these projects with a certain ease of mind, so I would say that this book has made quite a large impact on my life.

Patterns in Nature by Philip Ball (University of Chicago Press)

When I was looking for insight into how to design protein molecules, I skimmed over quite a few books on design, but none of them really captured my interest. For my situation, I followed the adage, “nature is the best teacher” and came across this book. This book introduces the reader to an array of patterns that appear in various scales in the natural world. I found myself staring at the beautiful images and patterns at the store, and ended up buying the book on the spot. While the book didn’t directly lead to my research’s success, I personally enjoyed imagining what it would be like to create some of the patterns it talked about. It’s relatively short, but there are captions and explanations for the different designs. Even now, on days when I’m tired, I will page through this to relax.

Osama no Restaurant, Script by Kōki Mitani (Fuji TV)

“If there is no kindness in your (the owner’s) heart, even if your restaurant is called ‘first-class’, it’ll never be more than third-rate.” (translated from Japanese) This quote left a strong impression on me. Right before this scene, the characters are discussing what “first-class” means and if there can even be some type of universal standard. This train of thought made me think hard about how arrogant it is to try to rate humans on some sort of universal standard. Whether it’s about trying to learn how to become a better leader or just the basic idea that even when you think an issue is trivial, it could mean the world to someone else, this show demonstrates how important it is to be considerate of others and their backgrounds. No matter how many times I watch this series, I am so moved.

Molybdenum and Tungsten Enzymes, Russ Hille, Carola Shulzke, Martin L. Kirke, eds. (Royal Society of Chemistry)

I felt like I needed to include at least one specialized book to look good. I started my research on evolution and the elements because I had been involved in research on life and elemental science since I was a graduate student and wanted to produce my own theories. This book gives a thorough summary of how molybdenum is used in living organisms and how tungsten, which is rarely used by regular organisms, can be used. I think that a reader needs to be somewhat familiar with how respiration works, but I was able to get a much clearer understanding of how electrons transfer during anaerobic respiration. I was especially moved by the inherent beauty of living systems when reading about how elaborately coenzymes are laid out and set up to form pathways for electron transfers.

Fukoku Kyohei: An Introduction to Geopolitical Economy by Takeshi Nakano (Toyo Keizai)

The title translates roughly to “Rich Nations and Strong Militaries,” which makes it seem a bit extreme, but it’s an incredibly serious analysis of history and economics. It’s not what would be called an “economics” book in a traditional sense, but rather it tackles the very ideas of “nations,” “taxes,” and “money.” I found it especially interesting that while current mainstream economics argues from the commodity money theory, in which money has value, the author argues from the credit money theory, in which money is a type of debt. Looking at things from the credit money theory, I was forced to think about whether our current understanding of monetary value is inherently flawed despite being easy to understand. I think this is a topic, and which theory best explains reality, is something that young people should consider since they will be the ones living in the future.

Why Not Start Small?

Norifumi Kawakami

Right before I was approached to be interviewed for this issue, I had the opportunity to reconnect with an old friend who studies plant ecology and listen to them give a talk about their current research project. The subject my friend discussed was about trees that are planted on the borders of private properties and how to preserve them. The research documentation detailed 2001 individual trees from 177 different locations, numbers that left us all dumbfounded. But, if you think about it, the researchers did not magically collect this data all at once. They started with a single tree and, from there, painstakingly gathered example upon example to produce these results. Simply put, they found a project that interested them and stuck with it. However, I understand that this can be a difficult thing to do. Many people drop projects after a few days or don't even pursue new ideas in the first place.

When I look back to our time as graduate students, my friend was the person who told me that "science is all about pursuing the things you love!" I think that this research is an embodiment of that idea. I remember how shocked I was back then because it confronted my fundamental understanding of science. I never doubted the idea that there was no "meaning" to research that had no direct application. And yet, somehow, I ended up going into the sciences too.

After the twists and turns in my career that led me to Keio University, I realized that if I started working on a project that I didn't find interesting, I would inevitably quit somewhere down the road. This is why I chose to begin trying to create a new molecule even though the only thing this project had going for it was my personal interest. Luckily, through seemingly endless time, patience, and perseverance, this research led to the creation of TIP60. It would be presumptuous of me to put myself in the same league as my friend, but in the end, the key to both of our success was to start and to never give up.

Looking around the world, there are myriad ways to define "success," and different approaches to getting there, so I can't say that blind repetition is always the right answer. However, no matter what you're working on, by continuing to build on your previous efforts, the likelihood of you stumbling across something important only increases, just like how my friend whose single data point of one tree eventually became 2000. Even if you think you lack talent at something, your understanding of the topic will increase, you might come up with new ideas, and somewhere along the way you might realize that you're having fun. This is what will motivate you to persevere. This is one of the reasons I started my YouTube channel. There's no guarantee that any job will go well. If there is something that you think you can do, some occasion where you want to raise your hand and volunteer, why not try? Start small, put aside profits, and even if it's just something you work on casually, why not at least take the first step?

理 工 学 Information

Videos of Research Findings from the Faculty of Science and Technology (Sci-Tech TV)

The website for the Faculty of Science and Technology (https://www.st.keio.ac.jp) has set up a page for each of the different laboratories to introduce their research projects (https://www.st.keio.ac.jp/rikou-tv/).

Providing a unique perspective into the ambience and energy at Yagami Campus, these videos allow viewers to get an inside look at the equipment and general atmosphere in the laboratories as well as up-to-date information on the researchers' most recent projects. Explore the latest in science and technology from the comfort of your own screen!

Editor's postscript

This issue has featured Senior Assistant Professor Norifumi Kawakami and his research on artificial protein nanoparticles. We hope that these collections of articles prove useful to a wide readership.

We believe that the vitality and positivity Professor Kawakami has demonstrated strongly embodies Keio's principle of *Jiga Sakko*, or "Creating History to Define the Future," embarking to understand unexplored fields of study, and overcoming challenges or adversity that stand in the way. We look forward to seeing how his resiliency in facing problems inspires his students.

(Midori Nakayama)

新版 窮理図解

New Kyurizukai
No. 37 August 2023

Editing: "New Kyurizukai" Editing Committee
Photographer: Keiichiro Muraguchi
Designers: Hiroaki Yasojima, Yukihiko Ishikawa (GRiD)
Cooperation for editing: Sci-Tech Communications, Inc.
Publisher: Toshiyuki Murakami
Published by : Faculty of Science and Technology, Keio University
3-14-1, Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522
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For inquiries (on industry-academia collaboration) :
kll-liaison@adst.keio.ac.jp
Website version:
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