Probing the proteome from single cells to living animals

RAYMOND MOELLERING SEARCHES FOR NEW CONNECTIONS BETWEEN PROTEINS AND DISEASE
Proteins are genetic transcripts rewritten in amino acids, macromolecules responsible for a variety of functions as signals, structures, catalysts, motors, and more. Yet how the environments in which they operate influence their individual and emergent properties is poorly understood. “Biochemists typically study the structure and chemistry of a single protein in isolation,” says assistant professor Raymond Moellering. “But you can’t just take one out and say, ‘OK, in a test tube I see this protein does this—that means that’s what it does in the cell.’” Instead, by using intricately engineered chemical probes, Moellering and his lab have made strides in understanding the complexities of how proteins work in living cells and animals by studying them in the context of the proteome—the entire complement of proteins produced by a genome.

The scope of the proteome is staggering—far greater than the genetic information alone would indicate. “The central dogma of science says that we have genes transcribed to mRNA that then become proteins,” says Moellering. “Well, we have many ways of identifying proteins because they have a select sequence based on their gene, but even after a protein is made, the cell has complex machinery to change its structure through posttranslational modifications. My group has discovered a method they call ADPL (activity-dependent proximity ligation), which we are using to identify proteins that interact with proteins selectively, depending on whether they are on or off—which can be used in live cells, tissue samples, and even live animals.”

“Before, all this was inferred using complementary approaches—measuring where the protein was in the cell, measuring the activity of the protein in isolation, putting that information together, and hoping it was right.”

“The biochemistry of the past forty or fifty years has taught us a lot, but we’re missing a lot of the action if we can’t perform the experiments in complex biological environments,” Moellering remarks. He and his lab thus create molecules that interact with proteins selectively, depending on whether they are on or off—which can be used in live cells, tissue samples, and even live animals. “We’re building new technologies to ask many questions at the same time in complex environments. If we want to understand regulation in the proteome, and we want to understand how it relates to disease, we need to be able to do that.”

In his recent study, Moellering focuses on serine hydrolases and cysteine proteases, enzyme families that include proteins that are upregulated in aggressive cancers. Beyond showing their activity in cancer cell lines, Moellering collaborated with University of Chicago Hospital oncologist Ernst Lengyel to investigate enzyme activity in ovarian cancer spheroids, small clusters of cells that bud off from primary tumors. “Spheroids are unique to studying the proteome because they are comprised of only a few hundred cells, a thousand times less than what we need to run a typical proteomics experiment using existing technologies,” says Moellering. However, using a method they call ADPL, Moellering and coworkers were able to demonstrate high levels of the enzymes associated with metastasis in single cells harvested from the spheroids. “We think that’s going to be broadly useful for many types of diseases where we need to ask questions with spatial resolution or with few cells,” he says.

Moellering’s perspective as a chemist has also enabled him to elucidate commonly studied biological processes, such as metallophilism. “If you’re...”

The proteins that get modified in you and me are exactly the same that got modified billions of years ago. a cell, the most important thing probably is whether you have nutrients to survive. So you need mechanisms to know if you have enough sugar, if you should bring more in, if you should store it, and so on. We found that these modifications occur in humans, mice, and other mammals. Then we went to the other end of the spectrum, and we found that they’re present in different kinds of bacteria, and they’re more abundant the further you go back in evolution. In other words, the proteins that get modified in you and me are exactly the same that got modified billions of years ago. They are hard encoded, and we’re just beginning to look at what their signaling functions are. With an eye to understanding how these minute changes affect protein structure and function, Moellering suggests that they play a role in many diseases, including diabetes and neurodegenerative disorders. “Are these signaling pathways and these little modifications part of pathology? If we manipulate them, can we use that as a therapeutic angle? Many of the molecules we’re making in this lab and some I’ve made previously in my training could have clinical potential,” he says, noting that some of his work has formed the basis for startup companies or has been licensed to pharmaceutical companies. Yet in addition to the use of his molecules as drugs or diagnostic tools, Moellering remains motivated by fundamental questions in the chemistry and biochemistry of biological systems. “You have things happening in every cell on the planet—are there interactions we don’t know about? Glucose metabolism is the one pathway we have probably studied the most, yet there were really fundamental things we didn’t know about it. I would say that’s still true for all areas of biology.” (ICH)
Hisashi Yamamoto converses with Irene C. Hsiao in the Members Lounge at the Art Institute of Chicago, 4 November 2017

Hisashi Yamamoto, Arthur Holly Compton Distinguished Service Professor Emeritus at the University of Chicago and currently on the faculty at Chubu University in Japan, is the author of more than 540 articles and 140 reviews. A pioneer in the design and development of chiral catalysts, he was the 2017 recipient of the ACS Roger Adams Award in Organic Chemistry and has also been recognized with the Japanese Purple Medal of Honor, the Prelog Medal, the Tetrahedron Prize, and many other distinctions. The impact of Yamamoto’s work in Lewis acid catalysts is reflected in his citations: of more than 56,845 articles published on Lewis acids since 1980, 61 is the first.

Why did you come to Chicago?
I was at Chicago officially for ten years, from 2002 to 2012. I left Nagoya University at the age of 58. I didn’t want to retire. That was a major reason I came to Chicago. After ten years, I began to think about going back to Japan. I was not particularly satisfied with the food in the United States. I am now a professor at Chubu University. Even at a private university, most must retire by 70. I have very nice funding, so even now I have five more years of funding from the Japanese government. I have nine postdocs. That’s good enough for me.

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Why do you want to be famous?
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Isn’t fame one of the hardest ways to get that satisfaction?
When I was young, that desire to do something special, something new, something completely different from others was the reason I did chemistry. Previously in Japan, everybody could have a certain amount of money without asking from the government. That was a good system to do pure chemistry. Now it’s no more. Writing a proposal that said, “This is an important drug for cancer; this is important for the development of materials,” this kind of thing, was relatively difficult for me. Then I decided to do amide synthesis—using carboxylic acids and amides to generate amides. This is simple, but people couldn’t do it truly efficiently. Very simple but very difficult is a good target, and amide synthesis also uses the same reaction as synthesizing peptides. Peptides are a little more complicated because they involve various amino acids.

Have you been successful?
There are so many possibilities. There is a medicine developed in Japan recently, a peptide synthesized biochemically, that cures cancer in even late-stage patients. It has no side effects. It costs about a quarter of a million dollars for a single patient. The peptide has 150 amino acids, but I feel that the most important portion is relatively small, maybe 10 to 20 amino acids. But nobody knows which portion it is. I think biology will find out. And then how will we make it? That’s a challenge for synthetic organic chemistry.

Did you start working on this kind of chemistry with the intention of building particular molecules?
What we are doing is methodology, not total synthesis. Still, if we have a nice technology at hand, then we can make the molecule.

How does this work fit into the trajectory of your career?
I have worked on the chemistry of Lewis acids for more than forty years. When I started, I didn’t expect it would become so huge. For the first 20 years of my research, I thought we were doing something new, something interesting. I wanted to be famous. I was interested in developing new methodologies, and I didn’t ask how important or useful they were. I gradually changed to seeking applications because, without target-oriented research, I could not get good funding.

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Why did you come to Chicago? I was at Chicago officially for ten years, from 2002 to 2012. I left Nagoya University at the age of 58. I didn’t want to retire. That was a major reason I came to Chicago. After ten years, I began to think about going back to Japan. I was not particularly satisfied with the food in the United States. I am now a professor at Chubu University. Even at a private university, most must retire by 70. I have very nice funding, so even now I have five more years of funding from the Japanese government. I have nine postdocs. That’s good enough for me. Wherever I can work, I am satisfied. I don’t care where—Japan, the US, another place. I have set up a new lab five times in my life. Every time, after moving, I could do good chemistry. After moving, my adrenaline was up, and I had to do something.

What are you working on right now? I think I am doing one of the best projects of my life, the development of catalytic peptide synthesis. Peptide chemistry has had a long history, more than 70 or 80 years. Forty or fifty years ago, everyone thought it was finished. There are almost 30,000 papers on the synthesis between the amine and carboxylic acid of amino acids that produces peptides. But none of them works catalytically. That’s unfortunate. About three years ago, I found a new simple amidation reaction system using Lewis acid catalysts. It solves most of the existing problems for peptide synthesis. For example, if you buy a peptide of seven amino acids, one gram, pure, you have to pay at least ten million yen in Japan. No pharmaceutical company uses such expensive materials for drugs, so that’s the reason why peptide chemistry has not progressed. I wanted to prepare such a peptide of seven amino acids for less than a thousand yen. That’s still pretty expensive, but it’s acceptable for pharmaceutical companies, and the use of peptide drugs is increasing. Most drugs will be changed to peptides if peptides become more economically accessible. Most of the small molecule chemical drugs, aromatic and heteroaromatic compounds, are not good for your body. The body needs lots of energy to digest them, leading to lots of side effects. Peptides are not so foreign to the body.

How does your system work? My chemistry is based on Lewis acids. If you have a molecule, and the molecule has Lewis base centers, a Lewis acid approaches such a Lewis base center. If you have a Lewis acid and substrate complex, and then another reagent comes, the Lewis acid brings the substrate and the reagent near enough to do the reaction. We call this a substrate-controlled reaction. For example, the carpenter does his business with a hammer and nails. He holds the lumber with his hand, and the other hand uses the hammer. Here we bring two or more hands of Lewis acids together to hold the substrate and do the reaction.

Most reactions are not substrate-controlled. They are reagent-controlled or catalyst-controlled—so there’s only a hammer. No holding, no selectivity, and a lot of side reactions.

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Weren’t you always interested in chemistry? Yes. We are synthetic chemists; we make molecules, like making houses. Architecture is designing and making a house. We do exactly the same thing, design and make a molecule. The only difference is that architects can see. We cannot see. Molecules are so small. But it may have interesting biological properties or material and physical properties—everything comes from the molecule: color, stiffness. We can design everything.

We now have lots of theories, so we can think about how a molecule can catalyze many reactions, and so on. So there are endless possibilities. AI is now getting popular. People believe AI can solve everything, but from a chemical point of view, AI has limitations. AI is effective, for example, in chess or go. Now the computer can win. They made a chess game relatively quickly because the possibilities are limited. That is exactly the same situation as peptides. It’s also limited to only

Disruptive Innovation
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20 amino acids. We can use AI efficiently in peptide chemistry. I’d like to introduce AI systems for peptide chemistry. I asked someone in Japan to collaborate. But that needs more money!

Do you have any advice for young chemists? One chemist I respect very much is Professor Eugene van Tamelen—he was at Stanford University. He did nitrogen fixation and pioneered biomimetic synthesis. When he was 55, he simply resigned. He said, “I did everything, so I’m finished. It’s getting boring.” And then he sold real estate for 15 years. Then he moved to Hawaii and bought a lot of paintings. He was so talented. When you visited his office, you found only a table and chair, nothing more than that. When he received a journal, he read it, and then he threw it away. He didn’t file anything. One of my friends asked him, “Why do you throw things away?” And he said, “When I keep things, I am somehow influenced by that knowledge, so I throw everything away.” So his office was completely empty. That was how he was. That kind of science has completely disappeared. Now everything is digital.

Digital photography is a disruptive innovation that completely stopped the industry of silver-type photography. I would like to tell young people that they should develop disruptive innovations for target-oriented science, and, for that purpose, you have to throw away classical ideas and classical papers. In the US, people can make disruptive innovations more easily than in Japan. In the US, everybody thinks you should be a different person from other people. In Japan, you should be the same person as other people.

In Japan, we had almost 100 national universities where everyone was equally funded, and everyone in math, chemistry, and physics was very happy. Then one day the government thought, “Maybe some professors are doing something useful, so why don’t they get a patent and use it for industry?” The government invested a huge amount of money in that, and nothing succeeded. People weren’t starting with a target. They were starting from basic science. But target-oriented is starting from the target and then coming back—that’s much more successful.

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Dear friends,

I have now had the pleasure of editing five issues of the Chemists Club since Spring 2016, with the essential contributions of Physical Sciences Division Graphic Arts Assistant Director Sean Hernandez, who is responsible for the beautiful look of these pages, as well as many of the posters and programs you may have seen at departmental events. We have been honored to share stories about the Department of Chemistry, and we would love to have your thoughts on what you like and what we can do better. Are there stories you would like to read? Stories you would be interested in contributing? Types of features you would like to see? Please let us know at chemistsclub@gmail.com!

Also, I would like to devote the Summer 2018 to issue to stories about alumni and would love to hear your suggestions—please don’t be shy about nominating yourself!

With bright wishes for the new year,

Irene C. Hsiao, Editor
Dear friends,

Happy New Year! Welcome to the winter issue of the Chemists Club. In this issue, we feature two members of the Department of Chemistry who are pioneering work on proteins and peptides. Raymond Moellering, who joined the faculty in 2015, is leading a burgeoning investigation into how proteins work in living cells, ranging from minuscule posttranslational modifications to a broad view of how the entire proteome functions in living cells—and malfunctions in disease. Also in this issue, Professor Hisashi Yamamoto, a pioneer in the development and use of Lewis acid catalysts in organic synthesis, shares his thoughts on science and his love of chemistry. The latest challenge that he is tackling is the development of a practical method for the large-scale synthesis of peptides for biomedical purposes.

We also celebrate some of the many recognitions that our faculty have received over the past year and express our deep appreciation for the generous support that you have given to the Chemistry Department. Our commitment to research, collaboration, and the training of the next generation of scientists could not be achieved without your help. Our faculty and students strive to match the aspirations you have for us, and we look forward to sharing new discoveries with you in the coming year.

We wish you good health and cheer in 2018.

Best regards,

Viresh Rawal
Professor and Chair