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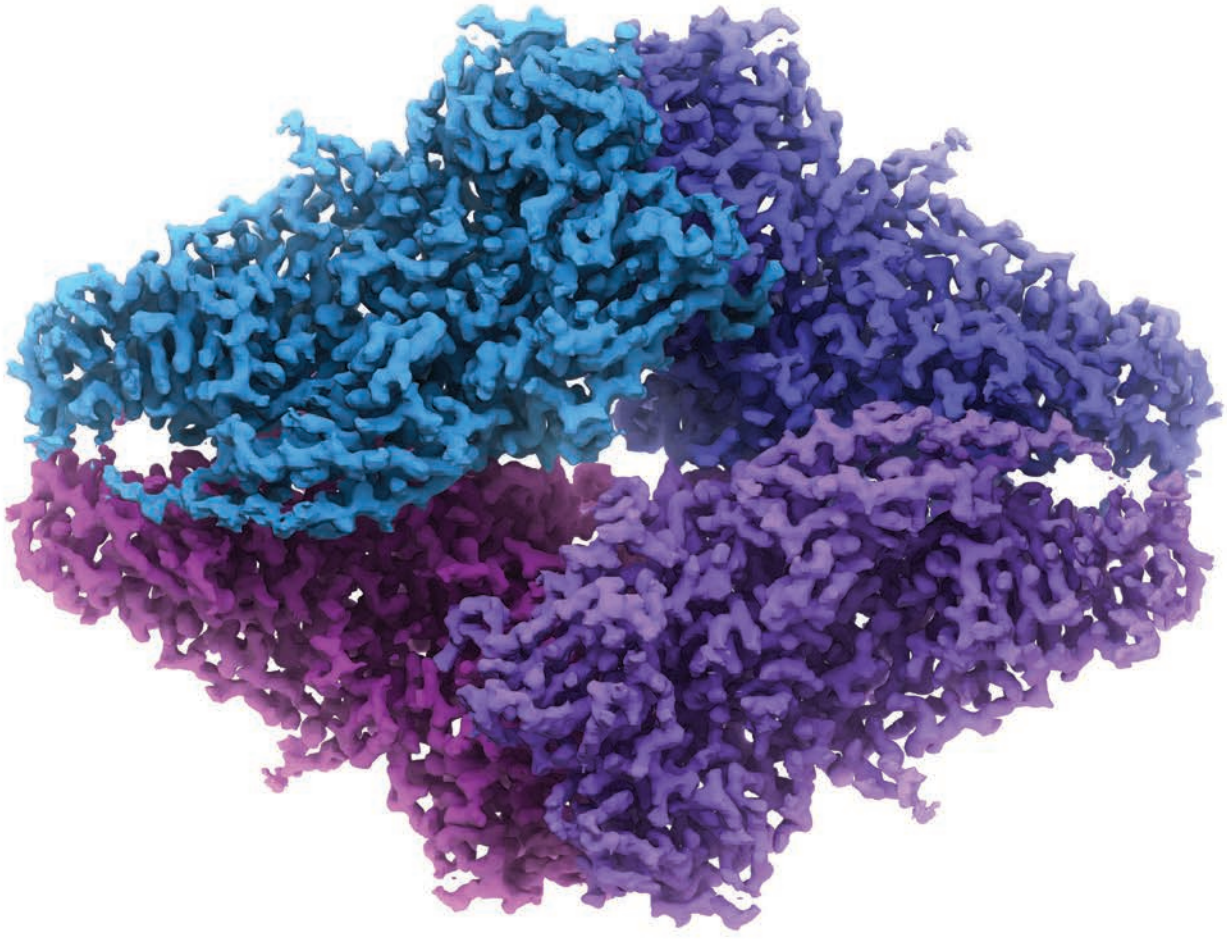
ASBMB TODAY

THE MEMBER MAGAZINE OF THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY



CRAZY FOR CRYO-EM

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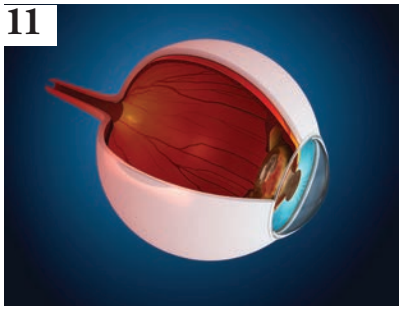

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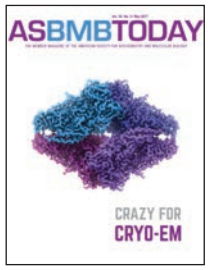

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ASBMB TODAY
THE MEMBER MAGAZINE OF THE AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

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Seeking: photographic evidence of scientific artifact

By Angela Hopp

Readers, we have a minor mystery on our hands. Well, it's not exactly a mystery, but the situation piqued my interest and might pique yours as well.

Twenty-four hours before going to press for this issue, I received an email from a couple in Oklahoma. They were hoping the society could help them find a photograph of a scientist with a typewriter – but not just any scientist and not just any typewriter.

The scientist is William C. Rose, one of the early presidents of the American Society for Biological Chemists, which eventually became the ASBMB. Rose, after whom one of our society awards is named, discovered, among other things, threonine, the last of the 20 amino acids universally present in proteins to be identified.

Born in South Carolina in 1887, Rose was the son of a Presbyterian minister and home-schooled before attending Davidson College in North Carolina. He earned his Ph.D. at Yale University, had a short stint around 1911 as an instructor at the University of Pennsylvania before heading to work in Germany, then ran a department at the University of Texas's medical branch in Galveston and finally ended up in 1922 at the University of Illinois, where he worked until retirement in 1955. He died in 1985.

The typewriter is a Hammond No.

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Standing up for science

By Benjamin Corb

Last month, scientists from across this country and around the world marched in support of science. For many, it was their first time to advocate and make the case for the critical role that science plays in everyone's lives. It was exciting to see such an impressive turnout.

Those of us involved in advocacy work at professional scientific societies, like me and the members of the American Society for Biochemistry and Molecular Biology's Public Affairs Advisory Committee, want to encourage you to let last month's actions be a first step on your journey to being a proud voice for science. There are many opportunities at the ASBMB for your continued involvement, and we'd like to share some of them with you.

First, to keep abreast of policy events, we encourage you to subscribe to our blog at policy.asbmb.org. Several times a week, the ASBMB staff updates the blog with analyses of the science-policy issues being discussed in Washington. We also host the occasional piece explaining not only what is happening but why we think it's happening. Every Friday, we publish our weekly roundup, which lists news from the week as covered by media. It's a great way to stay informed!

Second, the ASBMB advocacy website, www.asbmb.org/advocacy, has an online toolkit that offers the resources you need to take your advocacy efforts to the next level. Our toolkit has tips on how to invite your elected officials to tour your lab, sample letters or phone scripts that provide you with talking points, and suggestions on how to write an op-ed for your local paper. Also, our help doesn't stop there. The public affairs staff in Maryland is ready and willing to help you use these resources, so just reach out to me and ask!

A third way to get involved is to watch this space and our blog for advocacy opportunities organized by the ASBMB. Every spring, we bring researchers to Capitol Hill to advocate for biomedical research funding. Every summer, while the U.S. Congress is in recess, we organize ASBMB members to meet with their elected officials in their home districts. We provide meeting materials and even offer to schedule the meetings for you. These events happen annually, and we always are looking for participants!

Standing up to be a voice for science is an exciting and rewarding experience. But we understand that, at first glance, taking the stand can appear daunting. We're here to help. Please do not hesitate to reach out and let me and my colleagues know how we best can serve you and help you continue standing up for science!

Benjamin Corb (bcorb@asbmb.org) is director of public affairs at the ASBMB. Follow him on Twitter at twitter.com/bwcorb.

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12, manufactured in 1911 or 1912. In its day, the machine was pretty nifty, as it was designed to allow users to change out its shuttle to create special characters, including those necessary in scientific papers.

The Oklahoma couple, Mark and Christina Albrecht, tell me they have the largest antique typewriter collection in their region. They acquired the Hammond No. 12 from an antiques store in Dallas. In light of certain artifacts that came with the machine, including a few articles about Rose, the Albrechts are pretty certain the machine belonged to the scientific pioneer.

"It was probably purchased by him right after he left Yale. Maybe even a graduation gift, but almost certainly something purchased for use at his first job," Mark Albrecht said. "I am highly confident that he used this in Europe — maybe even purchased it there. And, also, he would have for sure used it in Galveston and very likely Illinois."

In other words, if there ever was a photo of Rose and his Hammond, it could have been taken in one of at least four U.S. states or in or around Germany sometime after 1911. That narrows it down! (I jest, obviously.)

But, seriously, I figure that, as unlikely as it is that we'll find such a photo for the Albrechts' collection, recruiting our readers would give us the best shot.

So, if you are harboring this sought-after piece of history and are willing to get in touch with the Albrechts, email us at asbmbtoday@asbmb.org. We'd be glad to publish the photo!

Angela Hopp (ahopp@asbmb.org) is executive editor of ASBMB Today and communications director for the ASBMB.

We would like to recognize the scientists who have been ASBMB members for more than 50 years.

Maurice Bessman
Anatoly Bezkorovainy
Clive Bradbeer
Robert Brockman
Donald Brown
Enrico Cabib
Benedict Campbell
Michael Chamberlin
Donald Clarke
Melvin Cohn
James Darnell
John DeMoss
S. Englander
Gary Felsenfeld
Carl Frieden
George Fujimoto
Martin Gellert
John Gerhart
Erwin Goldberg
Peter Goldman
Maurice Green
Paul Greengard
Gordon Hammes
Philip Hanawalt
Louis Hass
George Hauser
Murray Heimberg
Jerard Hurwitz
Jeremias Kagi
Akira Kaji
Julian Kanfer
William Konigsberg
Edward Korn
Leonard Laster
William Lawson
Paul Maurer
Richard McCaman
Elizabeth McFall
Elizabeth Neufeld
William Norton
Henry Paulus
Harvey Penefsky
Robert Post
Charles Richardson
Michael Rossmann
Lawrence Rothfield
Milton Schlesinger
Robert Sinsheimer
Marion Smith
Alkis Sophianopoulos
John Speck Jr.
Ian Spenser
George Stark
Herbert Tabor
P. Roy Vagelos
Martha Vaughan
Peter von Hippel
James Watson
Daniel Wellner
William Wells
Charles West
Takashi Yonetani
Morris Zimmerman
Steven Zimmerman

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Hart is inaugural recipient of Englund professorship

Gerald Hart, professor and director of the department of biological chemistry at the Johns Hopkins University School of Medicine, has been selected as the inaugural recipient of the Paul and Christine Englund professorship in biochemistry.

This professorship honors the legacy of Paul Englund, professor emeritus of biological chemistry, and his wife, Christine Schneyer Englund, longtime Hopkins endocrinologist.

Hart is being recognized for his significant contributions as a researcher and educator. Among his many research accomplishments, Hart discovered the crosstalk between O-linked β-N-acetylglucosamine and phosphorylation.

Hart founded the Journal of Glycobiology in 1989 and served as editor-in-chief until 2001. He currently serves as an associate editor for the American Society for Biochemistry and Molecular Biology's Journal of Biological Chemistry and Molecular & Cellular Proteomics.

Research!America honors Sharp

SHARP

Phillip Sharp, professor at the Massachusetts Institute of Technology, is the 2017 recipient of the Raymond and Beverly Sackler Award for Sustained National Leadership by Research!America.

This award recognizes outstanding leaders in medical and health research who have made significant contributions toward advocacy for medical or other health-related research.

Sharp was honored for his advocacy efforts for cancer research, which he has demonstrated in his role as chairman of Stand Up to Cancer's scientific advisory committee.

The award is part of the Research!America Advocacy Awards, established in 1996 to honor advocates for medical, health and scientific research.

Among his numerous honors, Sharp received the 1993 Nobel Prize in physiology or medicine for his role in the discovery of RNA splicing.

Leimkuhler Grimes wins Sloan Research Fellowship

GRIMES

Catherine Leimkuhler Grimes, assistant professor in the department of chemistry and biochemistry at the University of Delaware, won a Sloan Research Fellowship.

This fellowship is awarded annually to 126 early-career scholars in eight fields who have demonstrated the potential to make significant contributions to their areas of study. The fellowship lasts two years and grants fellows $60,000 to be used for research.

This fellowship will aid Leimkuhler Grimes in her research as she investigates how chronic inflammatory diseases arise in response to pathogenic and commensal bacterial cell wall fragments.

Leimkuhler Grimes previously was honored for her research with a Pew Scholarship in the Biomedical Sciences, the Cottrell Scholar Award and the National Science Foundation's Faculty Early Career Development Award. She joined the University of Delaware faculty in 2011.

Bertozzi elected to Eli Lilly board of directors

BERTOZZI

Carolyn Bertozzi, the Anne T. and Robert M. Bass professor of chemistry and professor of chemical and systems biology and radiology at Stanford University and an investigator at the Howard Hughes Medical Institute, was elected to the Eli Lilly and Co. board of directors. In her new role at the pharmaceutical company, Bertozzi will serve on the science and technology and public policy and compliance committees.

Bertozzi's research interests lie in both chemistry and biology, with a specific emphasis on studies of cell-surface glycosylation pertinent to disease states. In 1999, Bertozzi received an award from the MacArthur Foundation, which is known as a

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"genius award." She has been elected to the National Academy of Sciences as well as the National Academy of Medicine.

Diamandis receives award for excellence in education


DIAMANDIS

Eleftherios Diamandis, professor and head of clinical biochemistry in the department of laboratory medicine and pathobiology at the University of Toronto, will receive the 2017 Award for Excellence in Education by the American Association for Clinical Chemistry.

This award recognizes an outstanding individual who has contributed significantly to enhancing the practice and profession of clinical chemistry through education. Diamandis will receive the award at the AACC's annual conference, which will be held this summer in San Diego.

Diamandis' research interests include the discovery and validation of cancer biomarkers, proteomics, mass spectrometry and translation research. Diamandis has garnered numerous awards throughout his career for his excellence as an educator, including the 2013 Excellence in Graduate Teaching Award and the 2014 JJ Berry Smith Award for Excellence in Doctoral Supervision from the University of Toronto.

In memoriam: Mitchel Theodore Abbott

Mitchel Theodore Abbott, former professor and research scientist at San Diego State University, passed away Jan. 28 due to complications related to Alzheimer's disease and chronic lymphocytic leukemia.


ABBOTT

Abbott joined SDSU in 1964 as one of the founding faculty members of the Molecular Biology Institute. He also served as a member of the Ph.D. programs in chemistry and in cell and molecular biology.

Abbott left a profound impact upon his community through the many relationships and collaborations he formed throughout his career. He is survived by his sister, Camille; his three children, Valerie, Mark and Bruce; and his six grandchildren.

In memoriam: Wallace Brockman

Wallace Brockman, former head of the drug-resistance section at the Southern Research Institute in Maryland, passed away in April 2016. He was 91.

During World War II, Brockman served as a meteorologist in the U.S. Army Air Corps in India and China. He subsequently attended Vanderbilt University, where he obtained his doctorate in organic chemistry.

Brockman later joined the cancer research team at Southern Research Institute and eventually became head of the drug-resistance section. His research focused on exploring why some cancer cells were resistant to chemotherapy drugs.

Brockman is survived by his wife, Jean Early Brockman; two daughters, Alison and Anne; and two granddaughters, Liza and Meredith.

In memoriam: James Hamilton

James Hamilton passed away in June. He was 93.

Hamilton volunteered to serve in World War II, aiding in the reconstruction efforts in Japan near the end of the conflict. After obtaining his doctorate from the University of Minnesota, Hamilton began his career as a professor and researcher, working at medical institutions in both Texas and Louisiana.

Hamilton's research focused on the study of lipids. He later continued this research at the pharmaceutical company Hoffmann–La Roche.

Hamilton is survived by his wife, Carol; his three children; and his two siblings.

In memoriam: William J. Williams

William J. Williams, former dean of the College of Medicine at the State University of New York Upstate Medical University, died Nov. 4.

Born in Bridgeton, New Jersey, Williams served in the Navy during World War II, during which he was assigned to the hematology laboratory at the U.S. Navy Hospital in Philadelphia. He transferred to and later graduated from the medical school at the University of Pennsylvania.

Williams later joined the SUNY Medical University in 1969, where he stayed for 33 years, serving as the Edward C. Reifenstein professor of medicine and chairman of the department of medicine in addition to being the dean of the College of Medicine.

Williams was an expert in the field of hematology, serving as the founding editor of Williams Hematology, one of the leading English-language textbooks on hematology.


Erik Chaulk (echaulk@asbmb.org) is a peer-review coordinator and digital publications web specialist at the ASBMB.

Upcoming ASBMB events and deadlines

JUNE

June 5: The Art of Communication online course begins
June 8: Special Symposium: Evolution and Core Processes in Gene Expression registration deadline
June 14: Building your C.V. to get "know"ticed webinar
June 15: Special Symposium: Transforming Undergraduate Education in the Molecular Life Sciences registration deadline
June 22: Special Symposium: Membrane-Anchored Serine Proteases oral abstract deadline
June 22–24: IMAGE Grant–Writing Workshop
June 29: Special Symposium: Membrane-Anchored Serine Proteases early registration deadline

JULY

July 13–16: Special Symposium: Evolution and Core Processes in Gene Expression, Kansas City, Mo.
July 20–23: Special Symposium: Transforming Undergraduate Education in the Molecular Life Sciences, Tampa, Fl.
July 20: Special Symposium: Membrane-Anchored Serine Proteases poster abstract deadline

AUGUST

Aug. 3: Special Symposium: Emerging Roles for the Nucleolus oral abstract deadline
Aug. 8: Special Symposium: Emerging Roles for the Nucleolus early registration deadline
Aug. 9: Special Symposium: Membrane-Anchored Serine Proteases registration deadline
Aug. 30: Special Symposium: Emerging Roles for the Nucleolus poster abstract deadline

ASBMB
American Society for Biochemistry and Molecular Biology

The Marion B. Sewer Distinguished Scholarship for Undergraduates

Benefits: $2,000 toward tuition for one academic year. Scholarship recipients are eligible to apply for an additional scholarship in subsequent years.

Requirements: Must be a U.S. citizen, U.S. national or permanent resident. Students with DACA status also are eligible. Must be a full-time student at an accredited two- or four-year institution located in the U.S. or U.S. territories. Must have completed a minimum of 60 credit hours or equivalent, have a GPA of 3.0 or higher, and have faced significant educational, social, cultural or economic barriers in pursuit of education. Must also be committed to diversity on campus and in the scientific community as a whole and be an ASBMB member (membership can be processed at time of application).

Application deadline: May 15

Learn more at
www.asbmb.org/MinorityAffairs/UndergraduateScholarship/

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American Society for Biochemistry and Molecular Biology

Carolyn W. Slayman (1937–2016)

By Rajini Rao

On Dec. 27, Carolyn Slayman died while undergoing treatment for cancer. We lost a pioneering scientist, a superbly accomplished academic leader and a warmly humorous human being. She was Sterling professor at Yale University, where she served as deputy dean for academic and scientific affairs, and previously chaired the department of genetics. She leaves behind her husband of 57 years, Yale professor Clifford Slayman; two grown children; scores of former trainees; and countless colleagues who miss her terribly. Much of her research career, spanning nearly half a century, was devoted to the study of a proton-pumping ATPase in the plasma membrane of fungi, initially in the bread mold Neurospora crassa and later in the baker's yeast Saccharomyces cerevisiae.

I may have first met Carolyn Slayman at a Gordon conference; it was such a long time ago. But I remember precisely when and where I later asked if I could join her lab for postdoctoral training: It was in the ladies' room at the University of Rochester after her seminar. With all the talk of men's locker rooms, I've always found this encounter both amusing and empowering. She said yes. I visited Yale and discovered that her lab opened into a sunny rose garden. We would sit out there on the white, wrought-iron patio furniture that Carolyn had purchased and drink tea. Carolyn's lab was filled with strong, independent women scientists. I was in heaven.

There is a lot that I can say about Carolyn Slayman that has been said already. A 1958 graduate of Swarthmore (Phi Beta Kappa, highest honors in biology and chemistry), she went on to The Rockefeller University to do her doctoral work with Nobel laureate Edward Tatum as the only woman in her class. Imagine what it was like when she started as a Yale faculty member in the 1960s: The school only just had become co-ed, and the president famously had announced that more men had to be admitted to make up for the loss of future leaders in a class diluted with women. Once Carolyn had to be brought in to a men's-only Yale club for an evening meeting through the fire escape.

Despite all this, or perhaps even because of it, Carolyn was a consummate diplomat, exquisitely serene and as unreadable as a sphinx when it came to professional critique of a colleague or their science. Where I was outspoken, she was measured. When I rushed to publish, Carolyn would suggest running that gel one more time. Her deceptively mild and all-too-familiar phrase "Wouldn't it be nice …" probably still evokes a good-humored groan from her former trainees, but our perfected manuscripts would pass peer review easily. Indeed, many years later while I was on a study section that reviewed her grant, the (un)scientific consensus was that Carolyn "walked on water." Despite her busy administrative schedule (she was the first woman to chair a medical school department at Yale), Carolyn made time for science over lunch or tea, always ready with her trademark stack of index cards and Pilot pen to sketch out an experiment with impeccable neatness and clarity.

Carolyn's warmth and graciousness encompassed our families. She listened intently while my mother explained the cultural significance of my daughter Anjana's cradling ceremony and carted my parents off to a vegetarian restaurant for dinner. She initiated my husband, Ananth, into the anatomical intricacies of consuming a Maine lobster (while I, also inconveniently vegetarian, contemplated how to deal with my corn cooked in their silent screams). When Ananth snagged his first job interview in New Haven, she declared that he must have a power tie. So off we went, the entire lab in tow, to J. Press, where we purchased a bold red (and very expensive) silk tie. Although Ananth still prefers his muted blues and dull grays, I absolutely wear red for those power occasions in honor of that memory.

Carolyn was a natural with children. She plied my daughter and son with piles of books that easily became their childhood favorites: Anjana's was Robert McCloskey's "Make Way for Ducklings," and Arjun's was "The Story of Ferdinand." When I gave my first big talk at an international conference in Cordoba, Spain, Carolyn snuck out of the audience just before I started, returning with a little girl who perched right next to her to hear mom's talk. Much to my chagrin, Anjana was the first to raise her hand for questions, to the amused delight of my scientific colleagues. A decade later, when we stopped by New Haven to visit the Slayman home en route

to dropping off our now-freshman to college, Carolyn was still entertained by the recollection of Anjana's innocent question to her at the decidedly carnivorous dinner in Spain: "Aunty, are you a cannibal?"

A veteran globetrotter, Carolyn was also a skilled raconteur, regaling us with anecdotes of her flight on the Concorde, a spin around a Formula 1 racetrack or a camel ride with a Saudi prince. Indeed, we took for granted how sought after she was on advisory boards, councils and committees. One morning, just as I had made up the alkaline SDS solution to extract bacterial DNA (this was in the pre-kit days), Carolyn stopped by my bench with one of her famous stories. As I recall, with some irreverence, by the end of the anecdote, my solution was no longer "freshly prepared," as the SDS had crashed out as a precipitate.

There is, however, a conversation that Carolyn probably forgot but one that profoundly influenced my career. I share it often and strive hard to pay it forward. Many of my colleagues know of my "unrelenting passion" (as it was recently described) for promoting women in science. I was not always so confident. Exhausted, sleep-deprived and drained of morale after what seemed like the hundredth sick call from Anjana's daycare, I finally confessed to Carolyn that I was no good to her as a scientist. I was very sorry for her (and for myself, clearly) that I was no longer pulling my weight in the lab.

Carolyn took my announcement with her usual equanimity. She put my experience in perspective: For the next few years, family would be all-consuming. Naturally, there would be fewer evenings in the lab, and I would be home on weekends. In time, my children would grow up and move away to college (just like her own Andrew and Rachel), and I might well find more time for science, much like she had. She made me feel like she was investing in me for the long term and that my worth would play out in the future. I never forgot that sense of belonging that she gifted me that day, and, just as she predicted, my child-rearing years are now past chapters, albeit precious and memorable, in the story of my life.

PHOTO COURTESY OF YALE SCHOOL OF MEDICINE

Carolyn Slayman's scientific expertise was in ion pumps.

My memory of Carolyn endures: Tall and poised, in her wool skirt and sensible shoes, she was the epitome of New England grace and strength, a matriarch among mentors. Simply by being herself, she surely and skillfully shaped my career and my life, and for that I am truly grateful. I hope she knew that.

Rajini Rao (rrao@jhmi.edu) is professor of physiology at the Johns Hopkins University School of Medicine.

Serpins in wasp venom block host immune response

By Mariana Figuera–Losada

In May 1860, a bewildered Charles Darwin wrote a letter to the botanist Asa Gray to say, "I cannot persuade myself that a beneficent and omnipotent God would have designedly created the Ichneumonidae with the express intention of their feeding within the living bodies of caterpillars."

PHOTO COURTESY OF GONGYIN YE

P. puparum lays its eggs in a butterfly pupa.

Darwin was referring to parasitoid wasps. This group of insects is estimated to have up to 600,000 species, according to Gongyin Ye at the Institute of Insect Science in Zhejiang University in China. The number makes parasitoid wasps the most abundant and diverse group of insects on Earth. The study of some of the molecular mechanisms of the relationship between one of these parasitoid wasps and its host, a butterfly, is the subject of Ye's recent paper in the **Journal of Biological Chemistry**. Ye and colleagues described the discovery of a splicing isoform of a serine proteinase inhibitor found in the venom of the wasp that can inhibit its host's immunity.

Endoparasitoid wasps lay their eggs inside the body of their hosts, while ectoparasitoid wasps do so on the body surface of their hosts. Eggs hatch and feed on the hosts, killing them.

Most of the known pest insects that attack crops are preyed upon by parasitoid wasps, which is why they regularly have been deployed to protect crops. Pteromalus puparum is a gregarious endoparasitoid wasp that attacks the pupal stage of several butterfly species, including Pieris rapae, the cabbage white butterfly, which can be a problem in commercial farming of cabbage, broccoli and cauliflower.

Ye explains that to increase the chance of survival of its offspring, these wasps inject venom along with their eggs to suppress host immune defenses. Melanization, one of the processes of host immunity, is a conserved and highly regulated process. It consists of a series of serine proteinase activation reactions that culminate in the production of prophenoloxidase-activating proteinases, which further catalyze the conversion of prophenoloxidase into phenoloxidase. In turn, phenoloxidase oxidizes tyrosine and o-diphenols to quinones, which polymerize to form melanin that can cover the surface of pest invaders and keep them from spreading or hatching and completing development.

In a previous study, using a combination of transcriptomic and proteomic approaches, Ye and colleagues found that the venom of P. puparum contains at least 70 different putative proteins that include proteases, inhibitors, and recognition and binding proteins.

In the JBC paper, Ye and colleagues show that the P. puparum venom blocks melanization by both the host pupae and larvae. By using a number of protein fractionation steps followed by sequence analyses of the isolated proteins, the authors identified a serine protease inhibitor or serpin. They named this inhibitor PpS1V.

Serpins are a family of inhibitors that react with their target proteinase via a reactive center loop to form an inactive complex. Ye and colleagues found that PpS1V is one of the 16 predicted putative splicing isoforms that differ in their eighth and last exon, which contains the reactive center loop. They found that PpS1V binds and inhibits the activity of a previously unknown host protein, which they called P. rapae prophenoloxidase-activating proteinase 1, which is possibly involved in melanization by the host.

Ye thinks that uncovering the mechanisms by which parasitoid wasps inhibit host immunity will yield potential candidates for novel insecticides. Considering the parasitoid wasps' diversity, these animals are an untapped source of bioactive compounds for pest control and drug discovery.

Mariana Figuera-Losada (fmariana@hotmail.com) is an associate scientist at Albert Einstein College of Medicine.

Sphingolipids connected to corneal disease

By Stefan Lukianov

Keratoconus is a progressive eye disease that results in the thinning, bulging and scarring of the cornea, which is the clear layer at the front of the eye. The disease typically begins at puberty and stops around age 30, with significant vision impairment potentially occurring at later stages. Keratoconus affects about one in 2,000 people worldwide, and about 25 percent of patients undergo corneal transplantation to treat advanced symptoms. The underlying cause of keratoconus is unknown, but a recent report in the **Journal of Lipid Research** elucidates a potential role for fats known as sphingolipids and suggests a way to treat the disease.

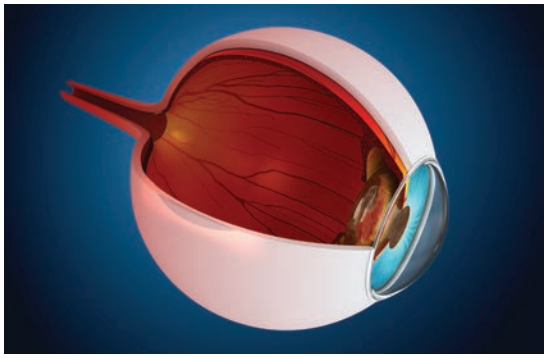


IMAGE COURTESY OF NAWAJES A. MANDAL

Keratoconus is a corneal disease.

Nawajes A. Mandal of the University of Tennessee Health Sciences Center, a senior author on the JLR report, says, "Keratoconus is a very challenging disease to work with because we do not know how or where the disease is initiated, how it progresses and how to stop it." There is no animal model for the disease, so studies rely on cells from normal and keratoconus corneas.

Sphingolipids are specialized fat molecules important in cell-membrane structure, cellular processes and molecular signaling. They also are involved in disease mechanisms, such as tissue inflammation and fibrosis; they are known to interact with other profibrotic, proinflammatory molecules in the cell. Given that keratoconus is a fibrotic disease that involves dense protein deposition on the cornea, there is a significant possibility that sphingolipids are involved.

Mandal, his fellow senior author, Dimitrios Karamichos, and colleagues first sought to determine whether sphingolipid levels were altered in keratoconus corneal cells compared with normal corneal cells. They observed that two sphingolipids, ceramide and sphingosine-1-phosphate, were more abundant in the keratoconus cells, suggesting they may contribute to the disease.

Another class of molecules involved in corneal healing and keratoconus are the proteins known as transforming growth factors, with TGF-β being the main factor. Mandal and colleagues previously had investigated TGF-β in keratoconus but now wanted to determine if the growth factor was connected to sphingolipids. The investigators found that three types of TGF-β, known as 1, 2 and 3, differentially regulated sphingolipid levels in keratoconus and normal cells. Moreover, the investigators discovered that TGF-β treatment also changed the expression of sphingolipid-production genes in keratoconus and normal cells.

At this point in the research, several molecular players that change in keratoconus had been identified. The results, however, did not yet show that these molecules were potentially causative in keratoconus. The final set of experiments performed by Mandal, Karamichos and colleagues demonstrated just that.

The researchers treated normal cells with ceramide and sphingosine-1-phosphate. The treatment caused the cells to become more like keratoconus cells by expressing higher levels of profibrotic genes. Finally, when ceramide synthesis was blocked in keratoconus cells using a potent inhibitory drug, the cells had lower levels of profibrotic gene expression and became more like normal cells.

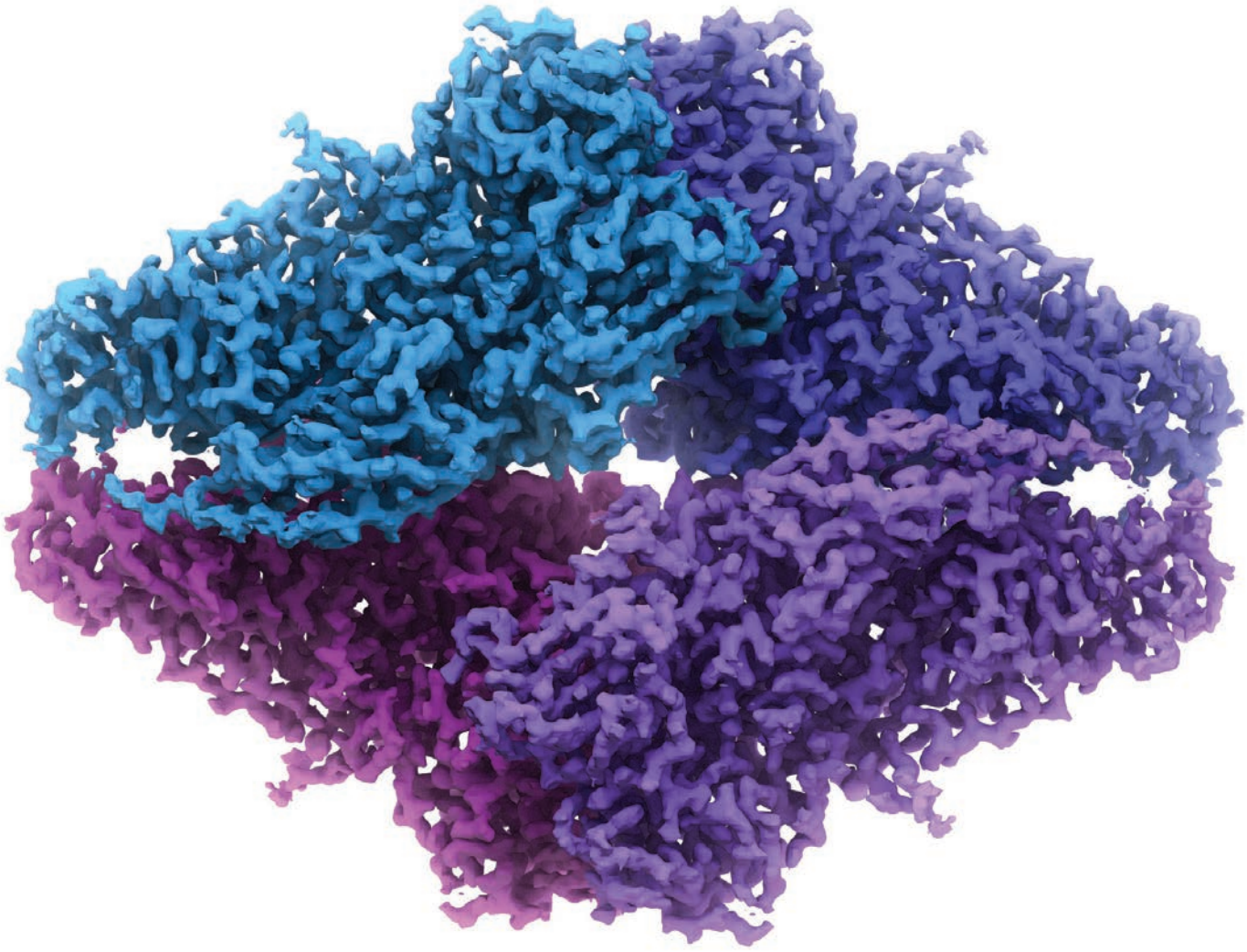
These results open the door to possible treatments for keratoconus based on the sphingolipid pathway. Mandal explains, "These findings are very exciting, as we are not only discovering a novel mechanism of human keratoconus development but also generating some clues about how keratoconus could be reversed by manipulating sphingolipids and thus leading to development of novel therapeutics."

Stefan Lukianov (stefanlukianov@gmail.com) is a Ph.D. candidate at Harvard Medical School.

Crazy for cryo-EM

The National Institutes of Health will fund national cryo-electron microscopy facilities to give biologists better access to the method

By Rajendrani Mukhopadhyay



Cryo-electron microscopy is coming to a corner relatively near you. The National Institutes of Health has decided to fund national facilities with the latest cryo-EM instrumentation.

Although NIH-funded facilities won't be springing up like Starbucks, the NIH, through a Common Fund initiative, is hoping to start off with three national service centers and eventually expand to having more around the U.S. The initiative aims to give researchers more opportunities to do molecular structure determination by high-resolution cryo-EM.

Why cryo-EM? The method, which has been around for 30 or so years, has had such a dramatic makeover recently that the journal Nature Methods bestowed it with the title "Method of the Year" in 2015. The once-fringe method is now beating the usual darling of structural biology, X-ray crystallography, at its own game, thanks to advancements in technology.

"We think — and this has been supported by many researchers in structural biology — that cryo-EM most likely will overtake or equal crystallography in the coming decade," says Jon Lorsch, the director of the National Institute of General Medical Sciences. "The time seems right for a major investment to allow researchers in the U.S. to access these important new technologies."

The NIGMS is administering the funding opportunity announcement for the Common Fund. The earliest date for submitting an application to host a service center is May 30. The earliest start date for the centers is projected to be April 2018.

For the veterans of cryo-EM, the move by the NIH is long overdue. Their microscopes have been in constant use, and they can't keep up with the demand from other structural biologists who want to get in on the action. "People desperately want to get onto these instruments," says Bridget Carragher at the New York Structural Biology Center. "If you don't have access to one and your competitor does, you're blown out of the water."

Freeze!

To understand cryo-EM, think of the Mannequin Challenge of 2016. The social media phenomenon, which even had the Dallas Cowboys and Michelle Obama participating, involved people remaining stock-still in the middle of an action shot while a camera rolled and the song "Black Beatles" played.

The idea is more or less the same for cryo-EM: Researchers flash-freeze their samples at liquid-nitrogen temperature so that the molecules remain frozen in action, and then researchers use high-end cameras to capture the images of the molecules. (The song is missing.) By looking at the frozen action shots of the molecules, researchers can discern their structures and functions.

The method of freezing molecules to study them by electron microscopy made its way into biological applications in the 1980s. In the early 1980s, Jacques Dubochet, then at the European Molecular Biology Laboratory and later at the University of Lausanne in Switzerland, described the plunge-freezing method of sample preparation (the "cryo" part of "cryo-EM"). The rapid freeze, going from room temperature to –195.79 °C (the temperature of liquid nitrogen) in fractions of a millisecond, causes water to form vitreous ice, which is an amorphous solid form of water that doesn't have ice crystals. The paper is noted as the work that helped microscopists embrace cryo-EM.

The thin layer of vitreous ice holds molecules more or less in the way they would be held in aqueous solution, so the freezing process allows researchers to see molecules practically in their native state. Cryo-EM doesn't require the protein to be forced into a crystal,

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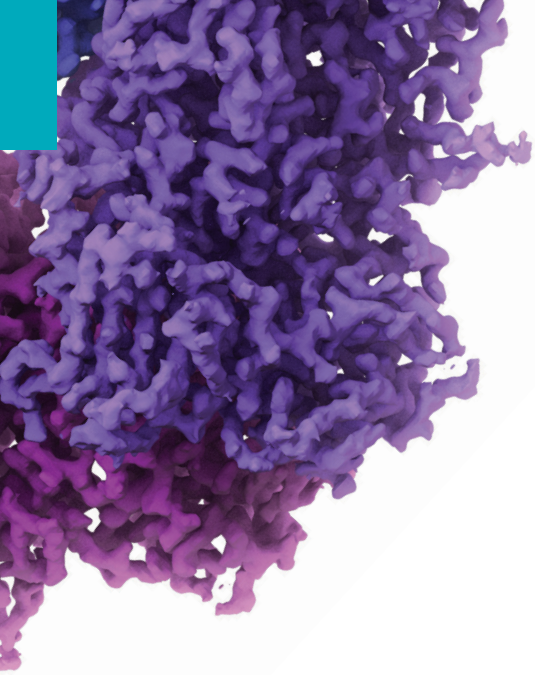
THE STRUCTURE OF β-GALACTOSIDASE BY CRYO-EM. IMAGE, WHICH IS ALSO ON THE COVER, IS COURTESY OF VERONICA FALCONIERI AND SRIRAM SUBRAMANIAM.



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which is a bottleneck in X-ray crystallography. The method also doesn't require as much purified sample as does X-ray crystallography. Richard Henderson at the Medical Research Council points out that the protein sample "doesn't need to be particularly pure … 90 percent pure would be perfectly fine." The method also can handle proteins as small as 100 kilodaltons and ones as large as viruses.

Large molecules contain more signals and so are easier to detect than small molecules, says Erhu Cao at the University of Utah, adding that "there is no upper limit" on what can be viewed by cryo-EM. That's why large complexes, such as ribosomes and viruses, have been among the first structures to be solved by cryo-EM. For example, the structure of the Zika virus was solved in 2016 by two independent groups using cryo-EM.

Most importantly, cryo-EM can visualize protein complexes and give indications about protein flexibility. As the freezing process captures the molecules in action, cryo-EM permits researchers to look at multiple conformations of molecules within a single field of view and get an appreciation for the dynamics of a protein. When looking at protein complexes, explains Jeff Lengyel at the company FEI, low-affinity interactions are easier to discern by cryo-EM. FEI, which was bought out by Thermo Fisher Scientific, is a vendor of cryo-EM instrumentation,

See clearly now

Instead of using light to image molecules, as is done in optical microscopes, researchers use beams of electrons in cryo-EM. The way the electrons bounce off a molecule gives a picture of the molecule. The scattering of electrons as they hit the frozen molecules is picked up by detectors.

The detectors are the reason for cryo-EM's sudden surge in popularity. For most of cryo-EM's existence, researchers used film. "We collected data on film that we would have to develop the old-fashioned way in darkrooms," recalls Eva Nogales at the University of California, Berkeley. "That made it very difficult to automate the data collection and (achieve) high throughput."

Charge-coupled device cameras came along next. "They were great for high throughput but they never got to high resolution," notes Nogales. The CCD cameras detected electrons in a roundabout way. The cameras were coated with a film of phosphoscintillator, which picked up only photons, not electrons. The electron signal had to be converted into photons of light, detected and then made into an image. The roundabout way didn't help to get very crisp and clear images.

The introduction of direct electron detectors in 2013 changed the game. Their name says it all: "You are literally counting electrons, and that's really transformative," says Sriram Subramaniam at the NIH. "It essentially eliminates the noise that was present before in CCD images."

The elimination of noise means crisper, clearer pictures of molecules, to the point of being able to see amino-acid side chains at resolutions as high as two or three angstroms.

Taking over structural biology

In the past four years, with the introduction of the direct electron detectors as well as advances in imaging software and instrument automation, it seems as though every week brings with it a new structure of a molecule solved by cryo-EM. (See box on "Tackling sample preparation.") Several experts point to the structure of a mammalian TRP channel, TRP V1, as the structure that made structural biologists sit up and take notice.

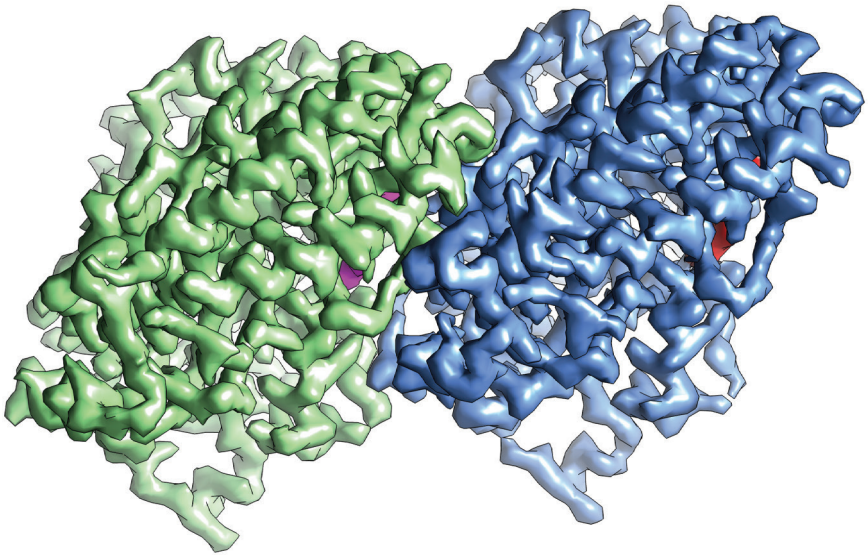

IMAGE COURTESY OF EVA NOGALES

The structure α-tubulin (green) and β-tubulin (blue) determined by cryo-EM

TRP V1 is the protein that allows us to feel the heat, in the form of both temperature and the molecule capsaicin, found in hot peppers. The membrane protein resisted X-ray crystallography attempts for years, says Cao, who was tasked with solving the structure when he was a postdoctoral fellow in David Julius' laboratory at the University of California, San Francisco.

Feeling defeated by his lack of success in solving the structure of TRP V1 by X-ray crystallography, Cao decided to give cryo-EM a shot. He got together with another postdoctoral fellow, Maofu Liao, in the laboratory of Yifan Cheng at UCSF in 2009. They spent almost three years optimizing sample preparation and imaging conditions for cryo-EM.

At that time, when Liao and Cao were working on the TRP V1 channel, others in the Cheng laboratory, along with the group of David Agard, also at UCSF, and the company Gatan were developing direct electron detectors and the software to accompany them. In 2013, Liao and Cao tested their sample preparation with the new detector.

The collaborators got a structure of TRP V1 at 3.4 angstrom resolution, "breaking the side-chain resolution barrier for membrane proteins without crystallization," as they noted in the abstract of their 2013 Nature paper.

Cryo-EM had achieved similar or higher resolutions on other samples, but in all those cases there were structures of the same or similar proteins already solved by X-ray crystallography. TRP V1 was the first cryo-EM-solved structure that X-ray crystallography had failed to deliver. The TRP V1 structure proved that cryo-EM was catching up to the capabilities of X-ray crystallography.

Given some of the advantages that cryo-EM has over X-ray crystallography, many structural biologists started to dive into their freezers and to dig around for samples that they had given up on solving by X-ray crystallography. Now structures of ribosomes, viruses, ion channels and membrane proteins have been solved by cryo-EM.

And it's not just fundamental science that benefits. Clinically important proteins now can be tackled by cryo-EM. Subramaniam's group recently solved the structure of p97, which is a cancer target. "It's a relatively dynamic protein," says Subramaniam. "It's been almost impossible to

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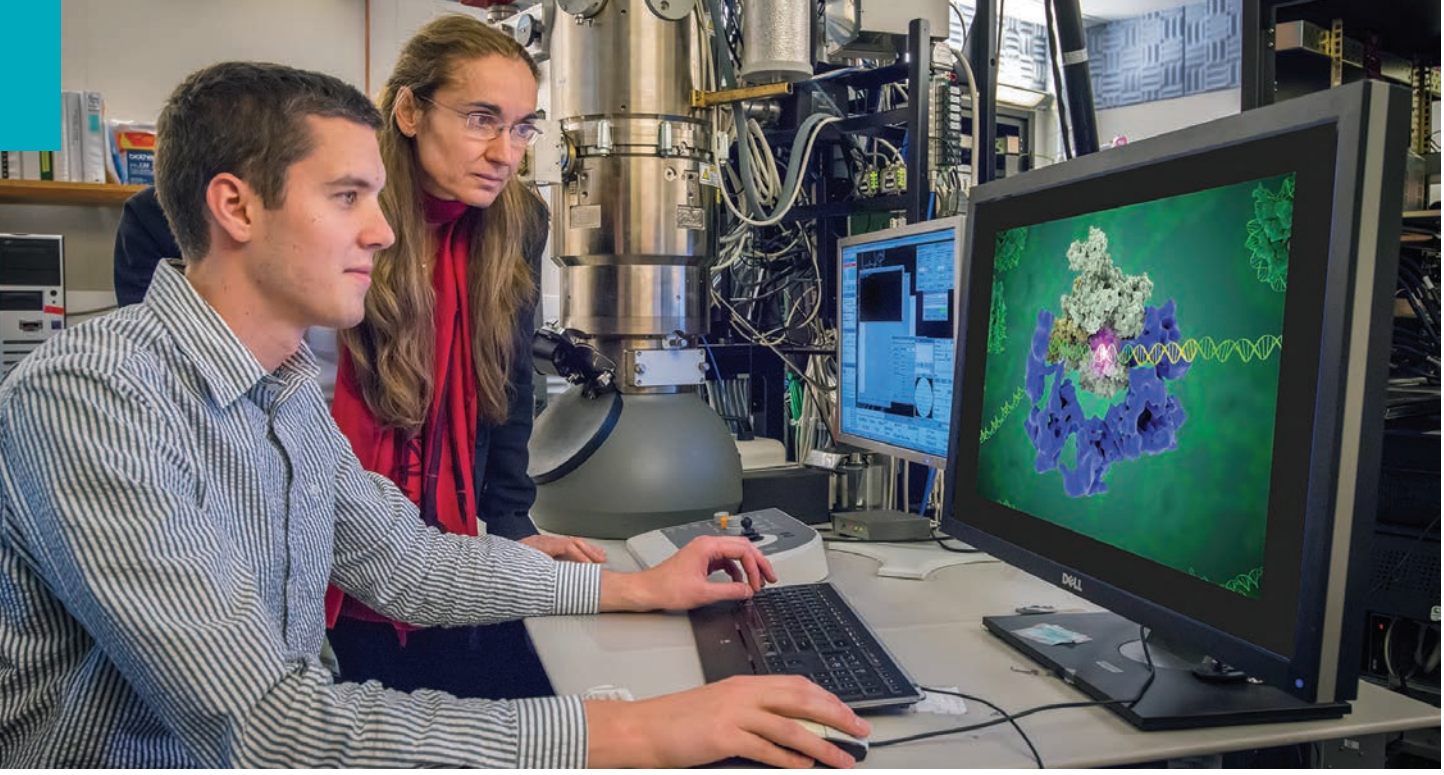

PHOTO COURTESY OF EVA NOGALES

Eva Nogales and Robert Louder review a structure by cryo-EM.

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get structures of it by X-ray crystallography simply because of this fact. But we showed that, by classifying the images into different states, we could tease out three simultaneously present conformations, which is basically what made it difficult to get to by crystallography."

Indeed, the ability of cryo-EM to help researchers deduce dynamics is a strong draw. Elizabeth Stroupe's group at Florida State University is working on sulfite reductase. It's an 800-kDa complex that can come together several different ways. She says, "The fact you now can do really accurate data collection means that, with enough biochemistry to back up our structure, even if we can't get to atomic resolution, we should be able to start to understand these heterogeneous modes of action for this enzyme in a way that we couldn't have done with crystallography."

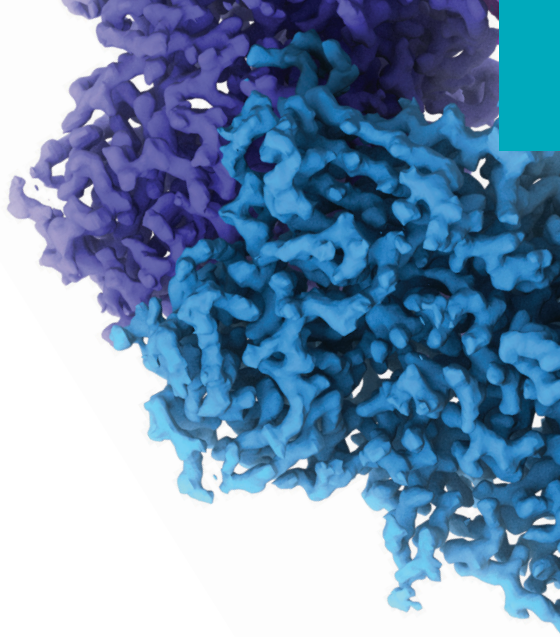
A more recent advent in cryo-EM has been phase plates, which have experts giddy with excitement. These are new bits of hardware, explains Lengyel. Phase plates help researchers overcome problems with sample damage and get enough information from their images with low-dose electron beams. "While it's still very new, several labs are beginning to generate extremely promising results," says Lengyel, whose employer, Thermo Fischer Scientific, sells the technology. "That will be the next advance in the field."

Indeed, a paper showed up in bioRxiv in November that described the determination of the structure of hemoglobin at 3.2 angstroms with the use of phase plates. At this resolution, the investigators were able to see amino-acid side-chain densities and prosthetic heme groups. Hemoglobin has a size of 64 kDa, which dramatically drops the lower limit of the size of molecules that can be determined by cryo-EM.

Experts also are expecting cryo-EM to tackle samples more complicated than purified proteins. The method is now moving into the realm of visualizing proteins in their native environments inside cells.

Economies of scale

With all the advances in cryo-EM, it's not surprising there now is a clamor for access to the instruments. Experts interviewed for this story all expressed enthusiasm for the NIH



funding opportunity announcement for the national facilities for cryo-EM but, in the same breath, said the NIH is playing catch-up. Lorsch accepts the criticism openly.

"The U.S. has been falling behind countries in Europe and Asia in terms of having access for researchers to these cutting-edge cryo-EM technologies," he says. "We were very concerned about that and felt that something needed to be done. Francis Collins, as he was made aware of these issues, was very supportive of doing some significant investment in this area through the Common Fund."

The Howard Hughes Medical Institute and the Wellcome Trust already have funded cryo-EM facilities to give researchers access to the instrumentation. The NIH has funded several cryo-EM consortia, but those are composed of cryo-EM experts. The National Cancer Institute opened a cryo-EM facility under the leadership of Subramaniam. But more are needed.

"Universities are coming to the conclusion if they want to maintain their standing in structural biology, they need to acquire this technology," says Lengyel. "It's getting to the point where X-ray crystallographers are either not accepting jobs or threatening to leave if they don't get access to cryo-EM because it's become so essential for their research."

The investment into a cryo-EM setup isn't trivial. As Carragher says, "It can't be taken on as if it's a Sunday-afternoon affair."

Just the cost of a high-end instrument that can do high-resolution imaging is about $6 million. To run one instrument for one year costs half a million dollars. Then there are the costs for all the software, computers, hard drives for storage, electricity, air conditioning (so the computers and instrumentation don't overheat) and people.

Some research institutions haven't bothered to wait for the NIH and have gone ahead and set up their own cryo-EM facilities. But, as Lorsch points out, "Even if the institutions have enough capital to purchase an instrument, most of them will realize that, once they do so, the cost of maintaining it really becomes a major burden."

The reason for the funding opportunity announcement is to, as Lorsch says, "create economies of scale. The old model is one in which each institution tries to buy its own major piece of equipment for everything, but we could really find a way to get more for the taxpayer's money by creating these nationally shared facilities and get access for more people for the same amount of money."

The expectation is that each center will have four microscopes, two that are high-end and two that are dedicated to screening samples for viability. There will be computational resources and data storage.

The precedent for this shared facility comes from the national synchrotrons that X-ray crystallographers use. Most crystallographers will make their crystals at their home institutions but then book a day or two at a national synchrotron facility, such as the ones at the National Institutes of Standards and Technology and Lawrence Berkeley Laboratory, to use the radiation source to collect data from their crystals.

The goal of the shared facilities is to give "biologists from all walks from structural biology access to this equipment and, very importantly, to give them training to become expert cryo-EM users," says Lorsch. The NIH has noted that there is a dearth of cryo-EM experts to help other structural biologists enter the field, so the national facilities will work to train people to become experts in doing cryo-EM.

Even the instrument manufacturers are taken aback by the craze over cryo-EM. When FEI developed the Titan

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Rajendrani Mukhopadhyay (raj.mukhop@gmail.com) is the former managing editor of ASBMB Today.

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Krios, a high-end instrument, Lengyel says the company expected to sell 35 total worldwide. They sold more than 100. "The last couple of years have been the biggest explosion in the field, given the advent of the new detectors," says Lengyel. "We expect that to continue."

Lengyel isn't the only one optimistic about the future of cryo-EM. "Very soon, there's going to be very few labs that just do crystallography," says Nogales. "That's my prediction."

Tackling sample preparation

Several experts point out that sample preparation for cryo-EM remains largely unchanged since the 1980s. "It's not a very good method, because you waste a lot of protein," says Yifan Cheng at the University of California, San Francisco.

To analyze a protein, researchers pipette a drop of about 3 microliters of their purified protein solution onto a carbon grid, wick off much of the drop with blotting paper and then plunge-freeze the grid with the thin layer of sample into liquid ethane that has been cooled by liquid nitrogen. "Everything in cryo-electron microscopy has improved since 1980 or so when it started except for the specimen preparation," says Carragher. "We make the darn grids more or less the same way."

Most of the sample ends up on the blotting paper that gets thrown in the trash can. To prevent waste of precious samples, Carragher's group has developed a device called the Spotiton. The device uses piezoelectric dispensing to drop a few nanoliters, rather than microliters, onto a grid. The grid itself is self-blotting and wicks the sample into a thin layer. The device is en route to commercialization.

"If you can use tiny amounts of samples, many things become feasible," says Cheng. "One thing limiting us is that biochemically, we still need to purify our protein sample. Purification is a major hurdle." If researchers can get away with a few nanoliters of sample, it will make cryo-EM even more attractive.

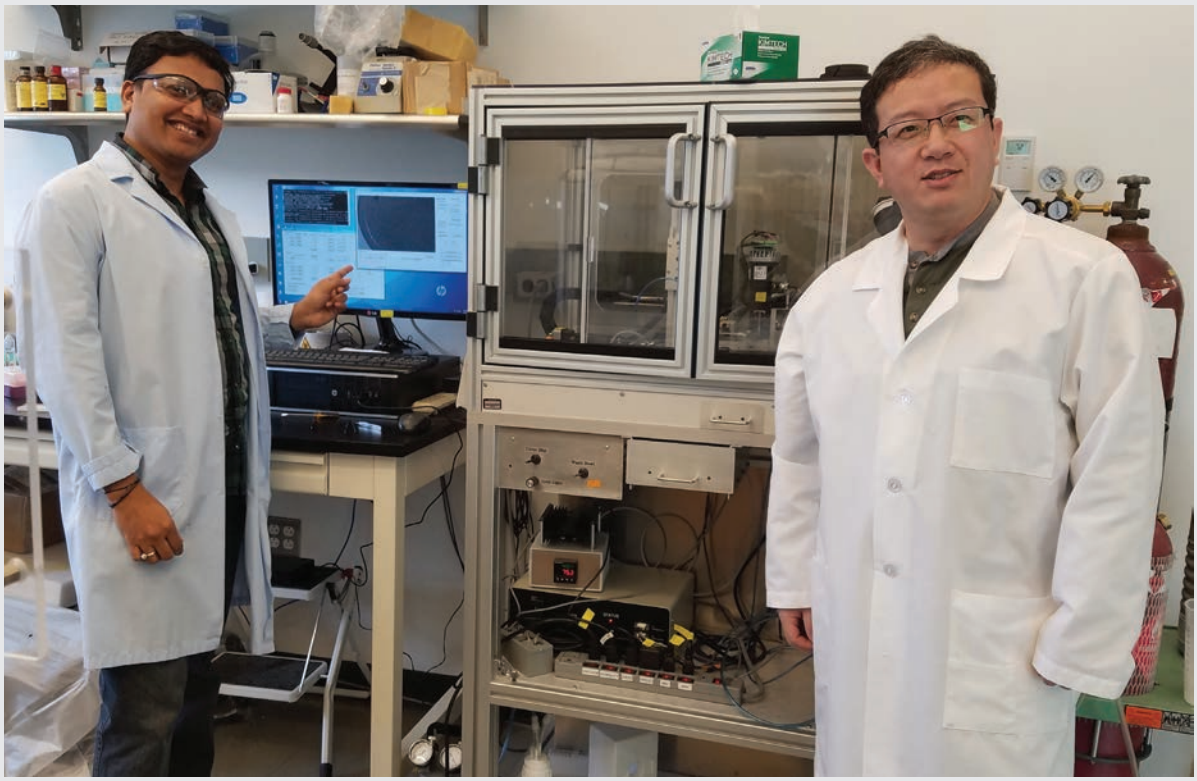

PHOTO COURTESY OF BRIDGET CARRAGHER

The Spotiton robot operated by Venkata Dandey (left) and Hui Wei (right) in the Carragher lab.

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Passionate parents catalyze research

Serendipitous meetings and basic biochemistry accelerate the push for a cure for the rare and fatal Lafora disease

By Kim Rice & Matthew S. Gentry

In 2007, at the University of California, San Diego, School of Medicine's white coat ceremony for entering medical students, a young man and woman met. Jesse Dixon is the son of Jack Dixon, a prominent biochemist who studied a rare condition called Lafora disease. Katie Rice had a younger sister, Kristen, who suffered from the disease. Little did the two aspiring physicians know when they first laid eyes on one another at that ceremony that soon their futures would intertwine in a manner that would have a profound effect not only on their own lives but on those of Lafora patients around the world.

The story of the research into Lafora disease highlights the importance of both human connections and defining the basic biochemistry of a disease. We are part of the story: Matthew Gentry performed fundamental research into the disease while he was a postdoctoral fellow in Jack Dixon's lab at UCSD. (Dixon is a former president of the American Society for Biochemistry and Molecular Biology.) Gentry's lab continues to work on LD at the University of Kentucky. The human connections were facilitated by Kim Rice (Katie's mother) and Jack Dixon.

LD is an autosomal recessive neurodegenerative epilepsy that is fatal. Its onset is in adolescence in apparently healthy teenagers, who begin to suffer headaches, seizures that look like bouts of staring, and visual auras. Patients then typically experience generalized seizures and insidious decline in cognitive function.

LD initially is difficult to distinguish from other juvenile epilepsies. But as time goes on, LD patients also develop highly frightening epileptic and nonepileptic visual hallucinations.

The initial response LD patients have to antiepileptic drugs is lost within three years, and constant bouts of myoclonic epilepsy begin. The patients then develop dementia, often disinhibited, and seizures with increased frequency despite any number or combination of antiepileptic drugs. The patients become bedridden. Death comes after a protracted decade of unceasing epilepsy in the form of a particularly massive seizure or pneumonia.

Biochemistry reveals mechanism

LD first was described in 1911 by Gonzalo Rodriquez–Lafora. Lafora described results from an 18-year-old patient. Among other things, Lafora noted in the paper he published in the journal Virchows Archiv "amyloid bodies in the protoplasm of the ganglion cells."

Amyloid originally referred to

substances that stained in a manner similar to starch rather than the protein inclusions now associated with Alzheimer's disease. These deposits that Lafora described in LD patients were later shown to be inclusions comprised of water-insoluble glucose chains more similar to plant starch than human glycogen. The deposits are named Lafora bodies. More than 100 years later, Joan Guinovart's lab at the Institute for Research in Biomedicine Barcelona demonstrated that LBs are the pathogenic cause of LD.

LD research was revolutionized in 1995 when Jose Serratosa and Antonio Delgado–Escueta at the University of California, Los Angeles, located the first chromosome locus for LD. Subsequent papers described the discovery of the EPM2A gene, which was led by Berge Minassian at University of Toronto and the Hospital for Sick Children Research Institute and by Jose Serratosa at the Laboratory of Neurology Jimenez Diaz Foundation in Spain. Mutations in EPM2A account for about 50 percent of LD cases. Subsequently, Minassian, Delgado–Escueta and colleagues identified the EPM2B/NHLRC1 gene as the second LD locus in 2003.

Once the affected genes were known, the focus turned to defining the function of the protein product of each gene. In Dixon's lab at UCSD, Gentry and Carolyn Worby demonstrated that malin, encoded by EPM2B, is an E3 ubiquitin ligase that ubiquitinates proteins involved in glycogen metabolism. They also showed that laforin, encoded by EPM2A, is not a protein phosphatase but that it dephosphorylates carbohydrates. They proposed that loss of laforin results in hyperphosphorylated glycogen that disrupts branching and leads to longer glucose chains that develop into LBs.

This hypothesis was corroborated by the labs of Peter Roach and Anna Depaoli–Roach at Indiana University and Minassian using LD mouse models to show that LD

PHOTOS COURTESY OF CHERYL BIGMAN

Kristen Rice suffered from Lafora disease.

mice developed LBs, that these LBs were hyperphosphorylated, that the glycogen branching was aberrant, and that recombinant laforin could remove the phosphate. Subsequently, the Roach, Depaoli–Roach and Minassian laboratories demonstrated that reducing glycogen synthesis by 50 percent abolished LD in mouse models.

Determined parents

Katie and Jesse began to date as first-year medical classmates in 2007. Their relationship progressed, and they decided that it was time to introduce one another to their parents. By this time, the strange coincidence had surfaced that Katie's sister suffered from the same rare disease that was of paramount interest to researchers in Jesse's father's lab.

It was not until the families actually met that the implications for Lafora research would begin to emerge. Jack Dixon had studied the disease for many years, but Katie's sister Kristen was the first patient he ever had met. Seeing the ravages of the disease firsthand drove home the potential of his research to impact lives profoundly.

Along with Katie's father, Jim Rice, Jack began to think outside the box about steps that could be taken to accelerate progress in Lafora research. They came up with the idea of an international workshop. Katie's mother, Kim Rice, a member of the

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Chelsea Gerber, for whom the foundation Chelsea's Hope is named, suffered from Lafora disease.

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board of directors of Chelsea's Hope, the North American Lafora advocacy group, took responsibility for fundraising and organizing the event. Kim worked closely with Linda Gerber, the founder of Chelsea's Hope and mother of an LD patient, to coordinate the event. Jack agreed to invite each of the scientists personally and to host the workshop. Under the sponsorship of Chelsea's Hope, it all came together as the 1st International Lafora Workshop in June 2014 at the Sanford Consortium for Regenerative Medicine in La Jolla, California.

Scientific momentum

The workshop allowed each research group to present its latest findings. All were in agreement that the identification of the genetic basis for LD had ushered in a new era in understanding the cause, leading to rapid progress in the field and opening up the possibility of a cure. Collectively, data from the laboratories presented at the workshop definitively demonstrated that eliminating LBs and reversal of this starchlike accumulation wholly abolished LD in mouse models. Thus, we and other researchers found ourselves positioned to realize the dream of treating and curing LD patients.

During the workshop discussions, the idea nucleated to write a collaborative U54 center grant to request funding from the National Institutes of Health. The grant had three main foci: 1) personalizing diagnosis of LD patient mutations at the biochemical and atomic levels, 2) establishing methods to inhibit glycogen synthesis, and 3) defining the window of opportunity when LB progression could be stopped and reversed.

While the group was excited about these goals immediately, the deadline for the grant was just four months away. Reality started to settle in. The group members felt they likely could not put together a successful large mechanism grant in such a short time. Jim Rice, Katie and Kristen's father, stood up from the back of the room and stated, "I'm going to play the parent card and ask that you guys pull together and get this done."

The group could not resist his plea. The members of the group wrote a U54 grant that received the best score, but the NIH did not fund any U54 grants in that cycle. The group was disappointed but not defeated. They split the clinical component of the grant away from the basic and transla-

Biochemist Jack Dixon gives a talk at the Lafora disease meeting.

tional portion, wrote a grant focusing on the basic and translational components, and subsequently obtained a P01 program project grant entitled "Lafora epilepsy: basic mechanisms to therapies" that established the Lafora Epilepsy Cure Initiative. They now are applying for funding for the clinical aspects so that the patient cohort is available when therapies are ready.

The LECI P01 is a unique collaboration between labs that once were competitors. It comprises four scientific projects and three core facilities. Members of the collaboration include Gentry at the University of Kentucky as the P01 director along with Craig Vander Kooi, Sylvie Garneau–Tsodokova and Dave Watt; Peter Roach, Anna DePaoli–Roach, Tom Hurley and Steve Johnson (Indiana University); Carolyn Worby (UCSD); Pascual Sanz (Institute of Biomedicine, Valencia); Gino Cingolani (Thomas Jefferson University); Guinovart; Minassian and Serratosa. Jack Dixon and Antonio Delgado–Escueta have provided invaluable advice and direction throughout the process.

A blueprint

The story of LD provides an excellent blueprint for how to attack a genetically inherited disease through basic research: identify the genes, generate mouse models, define the function of the proteins, and target specific biochemical pathways to alleviate or ablate disease outcomes. Yet without the personal interactions, it is extremely unlikely that clinical trials now would be on the horizon.

The group is pleased with recent successes but is not satisfied. Real work lies ahead: It is a unique opportunity to demonstrate how basic research combined with personal interactions can be translated into therapies and a cure.

The group has made dramatic progress over the past decade, but the hoped-for cure will not come soon enough for many. Kristen Rice and Chelsea Gerber both succumbed to the disease. Kristen died in 2014. Chelsea died in December. But their deaths have not in any way lessened the hope that is growing stronger every day as researchers get ever closer to their goal of curing the disease.

Katie and Jesse are now married. Katie practices ob-gyn in San Diego, and Jesse runs his laboratory at the Salk Institute. They have two children: Kristen (named after Katie's sister) and Jack (named after Jesse's dad).



Kim Rice (kimballw@mac.com) is a retired diagnostic radiologist in Seattle serving on the board of directors of Chelsea's Hope and liaison to the Lafora Epilepsy Cure Initiative. Matthew S. Gentry (matthew.gentry@uky.edu) is a professor of molecular and cellular biochemistry at the University of Kentucky College of Medicine and director of the Lafora Epilepsy Cure Initiative.

Meet Thomas Neubert

The new associate editor for the journal Molecular & Cellular Proteomics has his fingers in many signaling pies

By John Arnst

Thomas A. Neubert, a professor of cell biology at New York University's Skirball Institute of Biomolecular Medicine, joined the ranks of associate editors at the journal Molecular & Cellular Proteomics in June. Neubert, who is the director of the institute's mass spectrometry core for neuroscience and is involved in a wide variety of collaborations utilizing mass spectrometry, spoke with John Arnst, ASBMB Today's science writer. The interview has been edited for clarity and length.

What is your group focused on?

We use mass spectrometry to study cell signaling, mostly in neurons. We do most of that in collaboration with neuroscientists through a grant from the National Institute of Neurological Disorders and Stroke that funds the NYU mass spectrometry core for neuroscience. Most of our projects are collaborative, and we spend quite a lot of time developing methods to do these projects. We have, I would say, about 30 different collaborative projects going on at any time.

For example, when we worked with Paul Mathews and Stephen Ginsberg at the Nathan Kline Institute and NYU, we found some very interesting and specific changes in phosphorylation of proteins in hippocampal neurons in monkeys with a form of Type I diabetes. The changes may help explain why people with diabetes are more susceptible to Alzheimer's disease.

We helped NYU neuropathologists Matija Snuderl and David Zagzag by identifying the molecular composition of an interesting structure that helps tumors get blood supply without forming normal blood vessels.

I like to try to figure out how cells make decisions and work on a molecular level. For example, how do neurons know how to connect to the correct neurons when forming a brain, and how do these connections change when we learn? Mass spectrometry can be very good at getting a relatively comprehensive view of how cell signaling works if you do the right kind of experiment. A colleague at NYU, Moosa Mohammadi, has found that one type of growth factor ligand can correct a metabolic defect in cells, while another ligand can correct metabolism but also cause the cells to proliferate. We are now doing experiments to find out how these ligands exert their different effects. The ultimate goal would be to find a treatment for diabetes without causing cancer as a result of the diabetes treatment.

What is your background and research training?

My Ph.D. is from Johns Hopkins in immunology and infectious disease. After that, I did a postdoc with Jim Hurley at the University of Washington in Seattle, where I was studying vision signal transduction, and another short postdoc with Lubert Stryer at Stanford (University).

While I was studying the post-translational modification of transducin, which is the G-protein involved in vision signal transduction, I was lucky enough that another postdoc, Richard Johnson, was working two floors down in Ken Walsh's lab in the biochemistry department at the University of Washington. Rich trained with Klaus Biemann, and Rich was a pioneer in using mass spec to study proteins. He helped me characterize post-translational modifications on transducin, and I realized then how powerful mass spectrometry was for protein chemistry. I learned as much as I could from Rich and have focused on using mass spectrometry to study proteins since then.

After working for a company in Germany for a few years, I took my current job in 1998 at New York University School of Medicine in the Skirball Institute, and I've been doing mass spec ever since.

What company did you work for?

It was Fournier Pharma in Heidelberg, a French family-owned company. While I was there, I met Matthias Mann, who was at the European Molecular Biology Laboratory at the time. I learned a lot from him about mass spectrometry and did a sabbatical in his lab at the Max Planck Institute in Martinsried near Munich in 2012.

Heidelberg is also where I met my wife. My kids and wife speak German at home in New York, so, when I'm speaking with the in-laws or visiting her family and friends, I have to dust off the German a little. When I was living in Germany, I was speaking German, but I was born in Ohio, so I'm not a native German speaker.

After three years, the family decided to sell the company, and they closed down the institute in Heidelberg where I was working. That's when I moved to NYU.

What does it mean to you to be an associate editor of MCP?

It's a great honor. MCP has a leadership position in the field of proteomics in the kind of articles they publish, and they institute guidelines. Whenever the field of proteomics struggles with a quality-control issue, MCP comes up with guidelines they require for publication. Those (guidelines) tend to be adopted by the rest of the proteomics community. MCP is a very important journal, and I'm very honored to be on as an associate editor.

MCP tries to get manuscripts reviewed very quickly, and the AEs are very important in the process. It requires setting aside time every day to work on (MCP). It can be a challenge when I get very busy at my day job running a lab at NYU. It requires developing good time-management skills, which I think I'm finally doing.

Do you have any words of wisdom or a favorite motto?

I think it's important to keep in mind why we decided to become scientists in the first place. We want to make discoveries or cure diseases. The day-to-day struggles to get funding and publish papers can wear you down, and you lose sight of why it is you became a scientist.

PHOTO COURTESY OF THOMAS NEUBERT

Thomas A. Neubert

John Arnst (jarnst@asbmb.org) is ASBMB Today's science writer. Follow him on Twitter at twitter.com/arnstjohn.

Meet Pierre Thibault

The new associate editor for the journal Molecular & Cellular Proteomics discovered mass spectrometry's power while tracking a deadly toxin

By John Arnst

Pierre Thibault at the University of Montreal's Institute for Research in Immunology and Cancer joined the ranks of associate editors at the journal Molecular & Cellular Proteomics in June. Thibault is a former senior research officer with the National Research Council of Canada's Institute of Marine Biosciences in Halifax and a founding director at Caprion Proteomics. His research interests include investigating post-translational modifications by mass spectrometry-based proteomics. Thibault spoke with John Arnst, ASBMB Today's science writer. The interview has been edited for clarity and length.

What is your research group studying?

Our group is studying changes in the proteome in the context of cancer and immunology. We're interested in profiling changes in protein modifications involved in cell signaling and how these modifications affect protein function. We're also using mass spectrometry to uncover changes in what we call the immuno-peptidome of malignant cells to identify antigens that can be used for cancer immunotherapy. By profiling the repertoire of peptides presented by the major histocompatibility complex class I of lymphocytes from different patients, we can identify hundreds of minor antigens that share optimal features for immunotherapy and can be used in hematological cancers. We can use these minor antigens to prime allogeneic T cells against hematologic cancers without causing any side reactions to the patients.

What was your academic background and research training?

I'm a chemist by training, and I conducted my Ph.D. in bioanalytical mass spectrometry at the Université de Montréal. My Ph.D. with Michel Bertrand provided me with training in protein chemistry and what we call de novo peptide sequencing. My postdoctoral training with Bob Boyd at the National Research Council of Canada's Institute for Marine Biosciences in Halifax allowed me to extend these skills to develop new methods to analyze protein extracts. It was then that I became fascinated with protein modifications and the idea of using mass spectrometry to identify post-translational modifications and decipher how they regulate protein function.

What made you choose science as a career?

After I did my bachelor's in chemistry at Université de Montréal,

I worked for close to two years as an occupational hygienist, visiting different companies and evaluating the risk and health hazards that were affecting workers. I was first exposed to mass spectrometry when we sent chemical adsorbent tubes to the lab after sampling the air in some of the sites we were visiting. It was then that I learned (about) the analytical merits of this tool and wanted to pursue research in bioanalytical mass spectrometry.

During my postdoc at the Institute for Marine Biosciences in Halifax, I had the opportunity to be part of a multidisciplinary team tasked to identify an unknown marine toxin found in mussels from Prince Edward Island. The mussels were loaded with domoic acid. (But) there was no clue that this was a toxin or that this ever had been found in mollusks before. Unfortunately, they were distributed to the market. Four people died and about 140 were admitted to intensive care after eating the contaminated mussels.

It was an intense effort that led to the rapid identification of the new toxin, for which no record of human illness or mortality ever had been reported until this incident. However, it took another three months before additional sampling and collecting efforts confirmed that contaminated mussels were loaded with the toxin-producing diatom Pseudo-nitzschia pungens that was blooming on the east coast of PEI at that time. This collective effort made me aware of the significance and impact of mass spectrometry and how it can be used to identify trace-level toxins from biological specimens.

What does it mean to you to be an associate editor?

I think it's a great honor. I'm certainly flattered to be asked to serve MCP as an associate editor. I was previously a board member for about 10 years, and I've regularly contributed papers to this journal since its inception, so MCP has always occupied a very special place in my scientific life. To me, MCP represents the flagship journal in the proteomics community, and I look forward to serving this journal in my best capacity.

How is the new role going so far?

It's great to be able to tap into the collective knowledge of other associate and deputy editors when you're handling a difficult situation and provide words of advice to the authors so that you can improve their submissions. Overall, you want the best science to be published, so, if you can work along with the authors and reviewers to make sure that this happens, it's a great feeling.

Do you have any advice for balancing life in the lab with life outside it?

My family is a great source of joy. I have two daughters, one with two daughters of her own. It certainly brings life into a different perspective, and spending quality time with the family is important. Starting the day with a morning jog or playing squash with colleagues once a week has also been quite therapeutic.

Do you have any words of wisdom or a favorite motto?

My two cents of wisdom would be: Embrace research with passion. This is an amazing adventure, never boring, and filled with surprise. Where else can you have the latitude to venture into unknown territories and have fun doing it?

PHOTO COURTESY OF PIERRE THIBAULT

Pierre Thibault

John Arnst (jarnst@asbmb.org) is ASBMB Today's science writer. Follow him on Twitter at twitter.com/arnstjohn.

Combatting compression

By Kaoru Sakabe

I love reading blog posts about what the next generation will never experience because of changes in technology. Isn't it crazy that, at one point in time, data storage used to mean using floppy disks holding less than 1 MB? Or connecting to the internet involved using a phone line to dial into a server? Nowadays, cloud-based servers and online collaborative programs allow researchers to share large amounts of raw data quickly. External hard drives that can store terabytes of data can be purchased cheaply.

With the decreased cost of storing digital data and the ability to rapidly share files electronically, minimizing file size should no longer be a factor when deciding in what format to store your data. What you should keep in mind is that your electronic data should be stored in a universal format that does not alter its original information in any way, thus preserving your high-quality image data. In other words, you should be saving your files in a way that uses a lossless compression. For images, your go-to should be TIFF, or tag image file format, and

not JPEG, or joint photographers expert group format. Although there are other lossless file types, such as RAW, BMP or PNG, ideally you should save as a TIFF, because it is uniformly supported across different software platforms.

Because disk space and transfer speed were great limitations many years ago, scores of authors chose to save their images as JPEG files. But beware: JPEG files can compromise your hard-earned data. Technically, JPEG is not a file format but rather a method that specifies how the image will be compressed. You will see the extension JPG or JPEG when you save files this way, but there is no difference between these two extensions. When an image is saved using JPEG compression, it is broken up into 8x8 pixel blocks, and a transformation then is applied to each block independently of the rest of the image to reduce the file size. This transformation also separates the color information from the brightness and discards more of the color information. Ultimately, JPEG is a lossy compression method (see the Due Diligence column in the January issue of ASBMB Today), which means that every time you save the file, you are discarding information. I'll demonstrate the reasons why you should avoid this format, and hopefully I can convince you to avoid using JPEGs altogether.

First, saving as a JPEG fundamentally alters the image in a way that cannot be restored. Take, for example, the original TIFF image shown in Figure 1. In last month's column, we discussed how informative histograms can be. Looking at the histogram of the TIFF image, we can see that the image contains many white pixels, some black pixels and a few pixels of various shades of gray. For JPEGs,

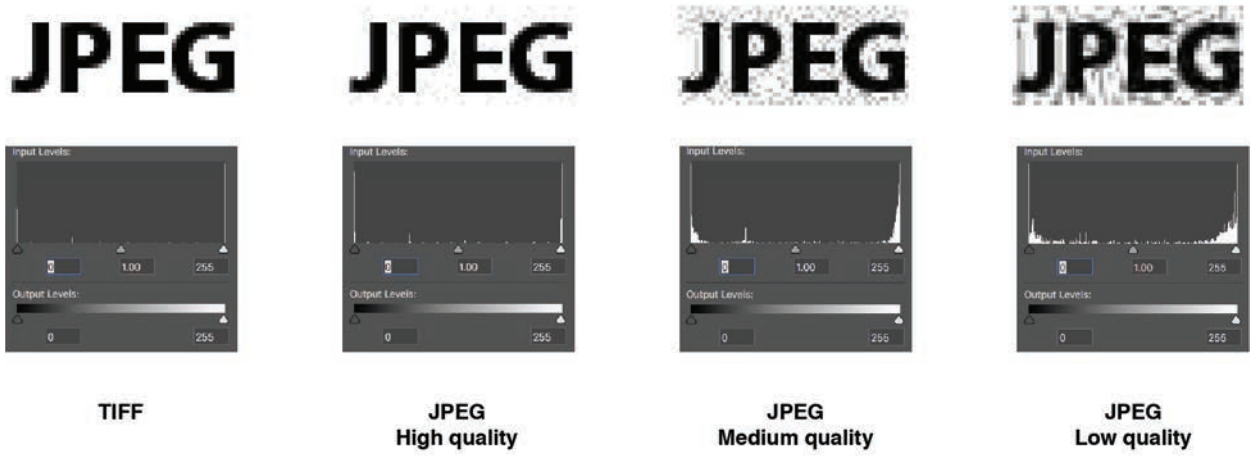

Figure 1. Saving your data as a JPEG changes the pixels in your image.

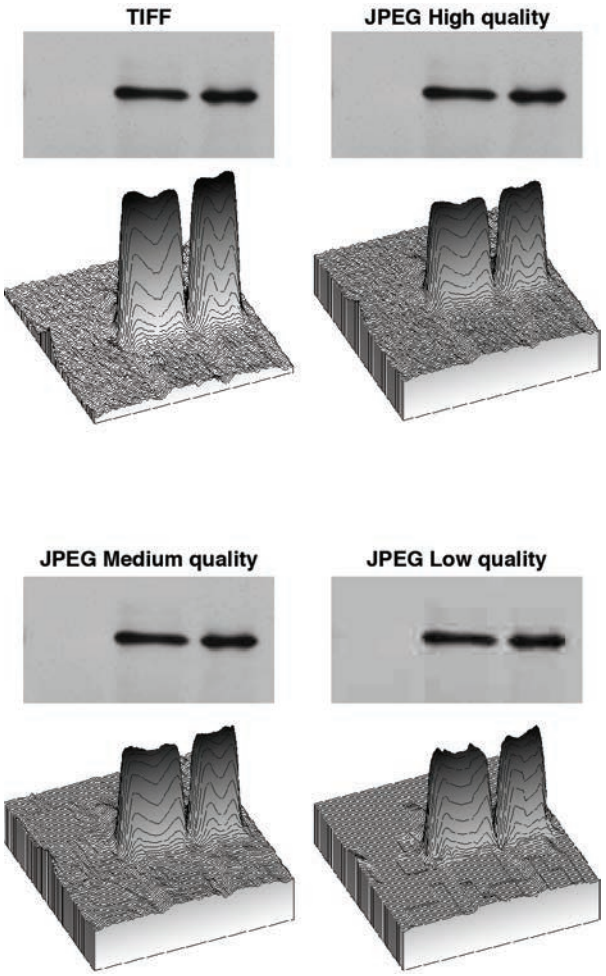

Figure 2. JPEG compression introduces artifacts.

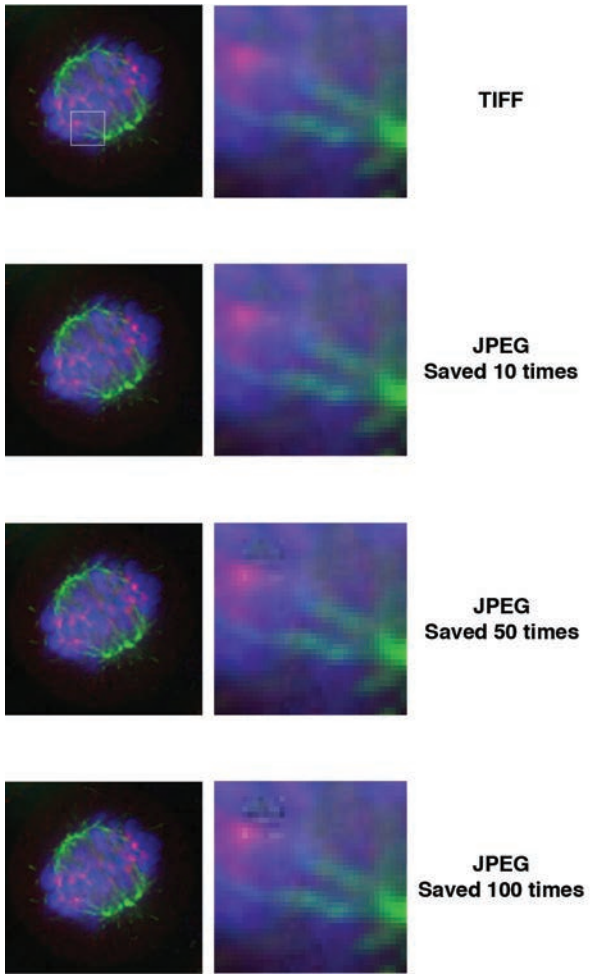

Figure 3. Repeatedly saving as a JPEG introduces artifacts.

high quality means little compression and a larger file size; low quality means high compression and smaller file size. Saving the same image as a JPEG at different quality levels introduces pixels that were not present in the original, creating a distorted image.

Now how does this translate into a scientific image? In Figure 2, I've taken a TIFF image and saved it at three different JPEG qualities. Visually, there doesn't appear to be a huge difference between the TIFF and the high-quality JPEG; however, if you analyze the image with a surface-plot analysis, you'll notice appreciable differences between the two images. As you compress the image further, blocks start to appear, the background looks less like a real experiment and the bands seem pasted in. These artifacts occur especially in areas of high contrast, such as a dark band on a clean background.

Another issue is that each time a JPEG image is saved, the compression is applied. Repeatedly saving an image during editing can introduce artifacts. For example, in Figure 3, I've taken an image of a dividing cell and saved it 100 times at maximum quality. By the 100th save, several anomalies have appeared, and it no longer looks the same as the original. While this exercise is almost certainly an exaggeration of what's happening in the lab, it illustrates that each time you save in the JPEG format, you are changing your data.

Finally, remember that by snapping the picture of the cell or scanning your film, you are recording the results of your experiment. Saving the image in a lossy file format, such as JPEG, distorts the actual results you obtained. Don't get stuck assembling a figure with muddled data. By saving your image initially in a lossless format, such as TIFF, you will be doing your due diligence in preserving your data.

Kaoru Sakabe (ksakabe@asbmb.org) is the data integrity manager at the ASBMB.

Transforming glycoscience

By Natasha E. Zachara

Sugars, carbohydrates, saccharides and glycoconjugates, collectively known as glycans, permeate every kingdom of life, where they play essential structural and functional roles. Interest in glycans has lagged behind interest in other biomolecules, such as proteins and nucleic acids. This ambivalence has been attributed in part to the challenges of working with glycans — they can be complex and require highly specialized methodology to study. This leads to a critical question raised by the National Research Council in 2012: If we are understudying glycans, what advances in areas as diverse as medicine, energy generation and materials science are we missing? To address this concern, in 2015, the National Institutes of Health started the Common Fund Glycoscience Initiative to fund projects whose goal was to transform glycoscience by building tools and resources that easily can be accessed and applied by the broader research community. The first generation of new tools is now available, and it is critical to get the word out!

Glycans are critical in several ways: They define self in both eukaryotes (blood types) and prokaryotes (serotypes); protect from pathogens and dehydration; provide structural integrity; and, in multicellular organisms, act as ligands for cell–cell and cell–extracellular-matrix interactions. Glycan-binding proteins play important roles in development, inflammation, hemostasis, transformation and metastasis by regulating both cell adhesion and signal transduction. Inside the cell, glycans play key roles in protein folding, targeting and turnover. Intracellular glycosylation, O-GlcNAcylation, regulates thousands of proteins in a manner analogous to protein phosphorylation. Disruptions in glycan biosynthesis cause hundreds of congenital disorders and contribute to the etiology of numerous diseases that include cancer, diabetes, pancreatitis and muscular dystrophy.

The development of novel methodologies can transform a field of research by providing greater molecular insight into physiological processes, increasing the rate at which information is collected and analyzed; if made accessible enough, the methodologies can boost the number of researchers working in that field. To this end, the Common Fund Glycoscience Initiative has funded 26 projects that will generate glycoscience tools. They include techniques that facilitate the synthesis and purification of glycans and their conjugates, improve the detection and analysis of glycans, develop new glycan-binding molecules, build expanded glycoconjugate arrays for characterizing glycan-binding molecules, develop synthetic sugars that will enable the determination of glycan function, and engineer cells and mice in which specific glycans or their modifications can be modulated for functional studies.

The fruits of this initiative already are appearing in the literature. Highlights include the development of a novel oxidative release method, which uses nothing more complex than household bleach to release N-linked and O-linked sugars as well as the glycan component of glycolipids. This approach, published in Nature Methods (1), will enable researchers to identify many of the glycans expressed by a cell, tissue or organism and rapidly identify developmental, physiological and disease-associated changes in glycans. Several groups have reported significant improvements in the stereospecific synthesis (2) and the purification and analysis of glycans (3, 4). These studies will facilitate the production of much-needed standards. Finally, the Glyco-Seek technology will enable researchers to detect and quantify O-GlcNAc modified proteins in a polymerase chain reaction machine using proximity ligation PCR (5).

These and other enabling technologies are anticipated to change the faces and landscape of the glycoscience field and, as a result, develop a more comprehensive understanding of the roles that glycans play in physiology and disease.

Find news about developing technologies and recent publications in glycoscience at the NIH Common Fund website (https://commonfund.nih.gov/Glycoscience) or on Twitter (@Glyco_PM).

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Natasha E. Zachara (nzachara@jhmi.edu) is an assistant professor at the Johns Hopkins University School of Medicine whose work includes the development of tools for studying the O-GlcNAc modification (funded by the Common Fund Glycoscience Initiative).

Be specific in emails!

By Meghan Duffy

A few years ago, I asked a senior colleague for feedback on something I'd written. He agreed and, a couple of days later, sent an email saying, "Is there a good time to discuss this?" I thought it must mean he hated what I'd written. I replied, suggesting a few times in the next couple of days. In his reply, he chose the latest of those times, saying he needed more time to mull it over. That confirmed my worst fears — it was so bad he needed extra time to figure out how to tell me how bad it was! After spending some time getting no other work done because I was so distracted, I decided to write to say that, based on his emails, I was worried that there was a major problem with what I'd written. He replied immediately saying not to worry — that it read very well and that he just had a few ideas that he thought would be easier to discuss in person.

I was thinking of this situation recently when I was emailing a student in my lab. She'd emailed about a proposal she was working on, laying out two options for a fellowship proposal. My thinking was that both of them could work but that there might be other options and it would be best to discuss the options in person. Looking at my schedule and comparing it with hers, I could see that we wouldn't be able to meet until the end of the week, so I initially wrote a reply that said, "Can we meet Friday at 11 to chat about this?"

In the brief pause before hitting send, I realized that if I were in her shoes, I would spend the rest of the week trying to interpret what that email meant, most likely assuming it meant something bad. I realized that easily could be addressed by instead

saying something like, "Both of these ideas look good to me, but there might be other options worth considering too. Are you free to meet Friday at 11 to discuss the options more?"

After writing about being a scientist who deals with anxiety on my blog "Dynamic Ecology," one question I've been asked repeatedly is what faculty can do to make their labs friendlier to students with mental-health issues. I'm unsure of how to respond to this — so much depends on each situation. But avoiding unnecessary vagueness in emails is one straightforward thing that people can do to make academia friendlier to everyone, especially those with anxiety issues.

I tweeted about this, and it was clear from the response that I'm not alone in finding the vague "Let's chat" or "Come see me" emails (or notes) anxiety-inducing. So please take a little more time to explain what you want to talk about and, if it's not something major, indicate that. Another advantage of giving the person a heads-up about what you want to discuss is that it allows them to arrive more prepared for the discussion.

Similarly, another thing that can help reduce some of the unnecessary uncertainty is to be specific about when you'll get feedback to folks. Especially earlier in my career, when I was waiting on feedback from a mentor or collaborator, it would be easy to check my email obsessively to see if anything had arrived. So, when possible, I try to be specific about when I'll give someone feedback. Usually, I block off time on my calendar for it so I can let the person know when to expect feedback (e.g., "I think I'll have time to work on this Monday morning").

Neither of these things, on their own, will address the problem of mental-health issues in academia. But they will make things a little friendlier for academics who struggle with anxiety and for those who do not.

Meghan Duffy (duffymeg@umich.edu) is an associate professor in the department of ecology and evolutionary biology at the University of Michigan. This piece originally appeared on the blog "Dynamic Ecology" on Jan. 23.

Tips for going into a career outside of academia

By Rita-Marie T. McFadden

When I was a graduate student at the University of North Carolina in Chapel Hill, I moved to complete my dissertation research with a co-mentor at the University of Arizona in Tucson. It was wonderful to experience another university and to see a beautiful part of the country. However, I felt isolated from career-development resources and opportunities, as I was not officially a student at UA. As I neared graduation, I was curious about careers in industry. I applied to positions but to no avail. It wasn't until a few years later, during my postdoctoral research, that I learned much more about how to apply effectively and do a job interview.

I am now a scientist at PRA Health Sciences, a contract research organization, as well as a member of the American Society for Biochemistry and Molecular Biology Education and Professional Development Committee. I have some tips that I hope fellow scientists, especially those early in their careers or without many career-planning resources, will find helpful.

Before the interview

Tip 1: Develop a strong résumé.

Before you get your dream job, you must apply! One of the most useful career seminars I attended featured a successful and well-paid science businesswoman who started as a dog-food tester. You can and should honestly and openly explain the details of your job experience at the interview. However, you must first get to the interview through word-search software programs and human resource personnel who may not have a science background. Instead of listing "dog-food tester," the businesswoman listed the experience as "research associate" on her résumé. When all that represents you initially is words, words matter.

Another tip is to keep your résumé to no more than two pages and have someone review your résumé, such as someone in your university's career services department or a guest speaker on resume writing. Come with your resume printed. Are you running out of room on your résumé? The C.E.O. of a huge company told me that few outside of academia focus on publications, so include only publications that are directly applicable to the job for which you're applying.

Lastly, employ SMART goals when writing your résumé. SMART stands for specific, measureable, actionable, relevant and time-bound. Utilizing SMART goals is an excellent way to be concise and informative about your experiences. For example, you could write "designed experiments and wrote protocols during grad school." Or, you could write, "created and implemented seven research plans about bone-marrow transplants that contributed significantly to three federally funded grants each worth over $40,000 over the course of two years." The latter is more specific, measureable, relevant and time-bound and shows you attained something. Using SMART goals as a guide will make your résumé bullet points much stronger.

Tip 2: Get leadership experience.

There are endless leadership opportunities for graduate students and postdoctoral scholars that will help you stand out to future employers. For example, you could take a leadership role (or establish one) in your university's postdoctoral association or graduate student organization. Or you could start a diversity and equity committee within your postdoctoral association. These are just some ideas to help your local community while gaining leadership experience.

Tip 3: Seek professional career counseling.

Career counselors give you structure to think constructively through your strengths and goals and formulate a strategy, and they also can help you to connect your job to your other life goals. They are especially helpful in preparing for career transitions, interviews and job negotiations.

Getting the interview

Tip 1: When in doubt, dress to impress.

Business formal attire, such as a black pants or dress suit, is recommended for most research position interviews in industry. Dressing appropriately shows that you take the interview seriously and can be professional.

Tip 2: Think of how you'll answer questions.

Remember it's not just about your technical knowledge. The company

also is looking for a decent colleague and team member — someone they would want to work with.

If you are asked what your greatest weakness is, don't say that you are too passionate, too meticulous about your work or something along those lines. Be honest and show that you have thought about how to improve. For example, you could say, "I have found it difficult to communicate with people who are indirect. I have started to repeat back in my own words what they are saying in order to clarify the communication and make sure I understand them correctly."

If you are asked to explain a time you failed, don't tell an extremely personal sob story. They are looking for a succinct (no more than five sentences) example of how you handled and learned from the situation. For instance, "My first graduate student seminar was not successful. I was nervous, spoke haltingly, couldn't remember my information and had spelling mistakes on the slides. Before the next presentation, I made sure to complete it in advance, practice in front of friends and colleagues and check for grammar and spelling mistakes. I learned the value of good preparation, seeking guidance and being professional. It may be a small thing, but it has made all the difference."

If they really throw you a curve ball and you have no idea how to answer, don't panic. You can start an answer with, "Well, I have never been in that situation, but if I were, this is how I would handle it …"

When your interviewer asks if you have questions, make sure you do. Prepare these in advance, but don't ask about salary just yet. The following are a few examples of good questions to ask at your interviews: 1) When is the last time an employee failed to meet a deadline, and how did you handle it? 2) What led you to work at this company? 3) If I want to transition to a position with x, y and z opportunities, is in-house training available, or do you have continuing-education benefits?

Lastly, if you have a perfect interview, you will be the first person in history to do so. Just do your best. Prepare by reading about possible questions and practicing with colleagues and the career-center staff at your university.

After the interview

Tip 1: Don't forget to say "thank you."

Emailing a thank-you note to the main interviewer or hiring manager after the interview will show you have good manners.

Tip 2: Bear down!

This phrase is from my Arizona days. It's a Wildcat fight song. But it just so happens to be good life advice. There's no stronger message you can send to yourself and others than simply working hard (bearing down) and doing your best. Sometimes, good things really do take time. Bear down in your quest for jobs with all those job applications and résumé modifications. Never give up! With a little elbow grease, your dreams will be a "when," not an "if."

Rita-Marie T. McFadden (ritamcfadden@gmail.com) is a scientist at PRA Health Sciences and a member of the ASBMB Education and Professional Development Committee.

Early support goes a long way

By Austin Maduka

While growing up in Prince George's County in Maryland, I had many peers who aspired to careers in either sports or entertainment. For young black males in my community, these were our stereotypes: We were expected to become professional football players, basketball players or rappers. I played football in high school. For a short time, I too was convinced that I had a future as a professional football player. My peers and I weren't expected to have other aspirations; that expectation was difficult to overcome without role models or mentors to say otherwise.

I eventually found my aspiration to become a physician-scientist and can attribute much of my development to this point to my two incredible mentors — my mother and my older brother. My mother always shares stories of her trials of coming from Nigeria to the U.S. in the 1980s. She attended Gallaudet University to study mathematics as an undergraduate student and achieved success as a deaf woman despite the doubts of others. She now is a business professor who teaches accounting at the university. She raised my brother and me on her own and instilled strong values in us and inspired us through her commitment to education.

My conversations with my brother, who is a medical student at the University of Pennsylvania, often were centered on science and medicine and their implications for human health. Combined with my innate curiosity about how the human body works, my discussions with my brother ignited my passion for research and medicine, which steered me to pursue my own path in the field. The examples set by my mother and my brother paved the way for me to set high and clear goals and helped me understand that any adversity I faced provided opportunity for growth.

Throughout the years, comments like these motivated me to dispel the stereotypes of young black males and prove that students from any background have the capability to succeed.

Having my mother and brother as a support system gave me the confidence to challenge the stereotypes. The stereotypes are perpetuated in many avenues throughout a young black man's development, whether it be through the media or the school system. During my school years, I encountered different types of teachers. Some were encouraging and inspiring, telling me that I was more than just a misbehaving kid. However, others were demeaning of my abilities.

When it came time to matriculate into high school, I applied to a competitive science program at the Eleanor Roosevelt High School. The process required a standardized test and a review of a school transcript. As I was a bit doubtful of my chances, I was elated when I received admission! My confidence was boosted; however, others were skeptical. A teacher told me, "I guess they don't choose students as selectively as they used to." Another teacher asked me, "Did you cheat on the exam to get into that program?"

Initially, these comments caused more doubt within myself, causing a feeling of imposter's syndrome. However, down the line, I viewed this opportunity and others like it as a way to success. Throughout the years, comments like these motivated me to dispel the stereotypes of young black males and prove that students from any background have the capability to succeed.

During high school, I took advantage of advanced classes and internships in fields like chemistry, physics and biology. Now, as an undergraduate student at the University of Maryland, Baltimore County, I participate in the Meyerhoff Scholars and MARC U*STAR Programs. Both provide me a larger network of support with mentors and passionate peers who have increased my confidence in my abilities as a scholar, scientist and future physician. With this confidence, I have chosen to serve as a tutor and teaching assistant for courses in chemistry and biology, using my role to encourage students who may lack confidence that they too can succeed.

With strong interests in medicine and science, I also pursued research and clinical experiences at Johns Hopkins University School of Medicine. I have worked with Natasha Zachara, elucidating a novel pathway with implications for cardiovascular diseases. I also have shadowed Anne Murphy, a physician-scientist in the pediatric cardiology clinic at Johns Hopkins Hospital. Interacting with patients as well as doing research allows me to connect my bench work

PHOTO COURTESY OF AUSTIN MADUKA

Austin Maduka (center) devotes a lot of time mentoring and helping underserved teenagers in Baltimore City.

to the community I am serving. These experiences have solidified my choice to pursue the combined M.D.-Ph.D. degree, because I find that working at the interface between medicine and research provides a synergistic enhancement to both careers.

In reflecting on my path, I recognize that my successes are not purely my own. My mother, brother and other mentors have done so much to help me realize my potential. I now have dedicated myself to helping others as I have been helped. I cofounded a UMBC organization called Achievement and Inspiration through Mentorship, known as AIM. The organization's mission is to create long-term, high-quality mentoring experiences for underserved adolescents in Baltimore City that strengthen their drive to pursue a quality education. We currently are mentoring 7th-grade students at the Commodore John Rodgers School. We organize activities related to mental health, money management and other areas to which these middle-schoolers may not have proper access. Through learning about their interests, we try to help cultivate their passions in science, medicine, mathematics, literature and other subjects. We also serve as coaches, tutoring them in classes and helping them set goals for the week and into the future.

It is crucial to realize that support can change a person's trajectory in life, especially for people in minority, inner-city communities. I have seen that they may not have this support. Although I had a positive outcome, many like me do not. Whenever possible, be a source of support for others. It may have a bigger impact than you would expect.

Austin Maduka (amaduka1@umbc.edu), a recipient of a Marion B. Sewer Distinguished Scholarship for Undergraduates in 2016, is studying at the University of Maryland, Baltimore County.

Exploring a new scicomm approach in Uruguay

By Ana Inés Zambrana

On Jan. 27, a study published in the journal Science showed that girls as young as 6 years old believe that they are not suited or smart enough for careers in science, technology, engineering and mathematics. Stereotypes and unconscious bias undermine women's self-confidence and put them at the risk of imposter syndrome, which makes them feel they cannot or should not be good at STEM. So I, as part of a group of like-minded scientists and science communicators in Uruguay, have come up with a way to challenge the stereotypes and biases that women can't excel at STEM. We believe our project, "Feminencias," will help to change those regressive tendencies in our societies while popularizing the lives and work of inspiring women scientists.

Bardo Científico

It was a cold and rainy August in 2015 when a group of 50 scientists, science communicators, teachers and journalists gathered in the old town of Montevideo in Uruguay. We were there to attend the "Science Slam Festival 2015," a four-day workshop on science-communication skills. And, to be honest, we were eager to see "BigVan," the renowned Spanish science-outreach group. Besides their workshops and blog, they regularly perform scientific monologues and have published several science communication books. We were there to learn how to communicate science in an innovative way: performing scientific monologues.

Monologues are oral communications with almost no props, gadgets or audiovisuals (including PowerPoint). They combine solid scientific rigor with accessibility for audiences with no science backgrounds. The goal is to attract the audience's attention with nonexpert language and to connect with them emotionally. In this way, the structure of a molecule may be described as a combination of colorful paper clips or the concept of nuclear fusion may end up being explained by drawing comparisons to oranges in the supermarket.

The workshop included training in improvisation, storytelling and scientific monologues and ended with a science slam where some of the participants presented monologues. This experience sealed the deal for a new way of science communication in Uruguay.

After the workshop, some of the attendees gathered to decide what to do with this newly found enthusiasm for science communication. Long story short, "Bardo Científico" was born. The monologues improved so much that we started to get invitations to perform in many science fairs, scientific gatherings and congresses in Montevideo.

"Feminencias"

Over the past few years, there have been initiatives to popularize women's contributions to society. In 2016, the United Nations declared Feb. 11 the International Day for Girls and Women in Science.

Addressing those gender issues, Bardo Científico started a new project dedicated specifically to women in science, which we called "Feminencias."

The hourlong show, co-organized with UNESCO and initially financed by the University of the Republic of Uruguay, began with the aim to share the discoveries and life experiences of women in STEM, with the belief that the role of women in science has been long hidden. Our school and university textbooks mainly were written by men and about men, so we organized those monologues around the life stories of the forgotten women.

Each member of the group chose a scientist with remarkable achievements. Monologues were created to show the time in which those scientists worked and describe their contributions and their pioneering roles. Some of the scientists that we highlighted were neuroscientist Rita Levi–Montalcini, who discovered the neural growth factor; nuclear physicist Lise Meitner, who discovered nuclear fission and inspired the name of meitnerium; chemist and physicist Rosalind Franklin, who characterized the DNA molecule by X-ray diffraction and whose results led to the description of the double-helix structure; and the neurophysiologist Linda Buck, who discovered the hundreds of genes for odorant sensors located in the sensory neurons of our noses.

At the same time, our group is concerned with an egalitarian look at scientific knowledge, taking to heart what Mae Jamison, who was the first

PHOTO COURTESY OF SEBASTIÁN PARENTELLI

Bardo Científico after one of their events

black woman in space, once said: "We look at science as something very elite, which only a few people can learn. That's just not true. You just have to start early and give kids a foundation. Kids live up, or down, to expectations."

Knowing about the existence of more women scientists than just Marie Curie is a small intellectual addition, but it goes in the right direction. Children of all ages, regardless of their gender, will be guided by their own desires and drives if they grow up in a supportive environment that is inclusive and stimulating with positive role models. Moreover, adults also can take stock of their negative biases. The contribution of "Feminencias" is that it promotes change at both the individual and the community levels.

That the presence of women in science is no longer an exception and that both women and men can contribute equally to the production of knowledge are indisputable. However, discoveries made by women continue to be a big surprise to audiences, probably due to the fact that history, prizes and awards mostly acknowledge men.

Since its first presentation last March, "Feminencias" has steadily increased its audience. We started with small groups but now have grown into a sold-out theater. Some attendees have approached us after the presentations to ask questions and to learn where they could find reliable information about more women in science. Teachers, educators, museum curators and the general public have contacted us frequently on our Facebook fan page to request our presentations at high schools, small theaters or workshops. We strongly believe that, with every show we do, we fight gender inequalities by showing people who are the real pioneers of science.

Connecting with our audience

The popularity of our group has been growing after each show, and the experience of sharing those hidden stories with the public has been increasingly rewarding.

To continue with our work, we find plenty of inspiration in the Spanish outreach group that introduced the monologue method in Uruguay. We also find inspiration in the many outstanding scientists, philosophers and thinkers who have promoted scientific education and communication, such as Levi–Montalcini.

But there is also great strength drawn from our audiences. At the end of each presentation, there usually is a Q&A session. The Q&As are a display of our basic instinct to seek knowledge, an instinct we all share as humans and one that sets us apart from other species.

Despite the differences in scientific training between us — the scientists and science communicators on the one hand and the audience on the other — we all end up sharing knowledge and thinking. We share the views and push forward the ideas of Rosalind Franklin, who once said, "Science and everyday life cannot and should not be separated."

Ana Inés Zambrana (zanaines@gmail.com) is a science educator, outreach promoter and researcher focused on Type I diabetes.

An appreciation of uncertainty

By Ulli Hain

I don't have numbers on how many perfectionists go into Ph.D. programs, but I have a feeling that many fewer come out the other side — not because they drop out but because perfection is irreconcilable with exploring the outer reaches of knowledge.

This was certainly the case for me. In high school and college, perfection was attainable, quantifiable and highly rewarded. Though my first love was writing, I assumed early on that a career in writing was way too risky. What if I wasn't any good? What if I failed?

So I pursued my second interest: science. In my naivete, I assumed a career in research would be more secure and straightforward, though to be fair, this was soon after the budget of the National Institutes of Health had doubled. Plus, graduate school, with its humble yet guaranteed stipend, appeared to be the least risky choice I could make.

But anyone who has conducted research, from a summer intern to a Nobel laureate, knows that failure is part and parcel with science. For proof, look no further than the number of student presentations, including my own for three years, titled "Attempts at Crystallizing Protein X." Crystallizing a protein is an early and not trivial step in determining the three-dimensional structure of a protein in X-ray crystallography. But matrix screening is built on the premise that more than 99 percent of chemical conditions will fail to yield diffracting crystals.

Uncertainty is found not only in the day-to-day failure of experiments but in choosing a hypothesis that may be completely wrong. For some, pursuing a reoccurring band on a Western blot leads to the discovery of ubiquitin; for many others, that band really is just a contaminant.

But as researchers, we must embrace that uncertainty, much as a jazz musician does not know where the music will end when he begins playing. Jonas Salk, inventor of the polio vaccine, said as much a 1991 interview: "Risks, I like to say, always pay off. You learn what to do, or what not to do. I like to say 'nothing ventured, nothing gained.'"

Though I had heard similar quotes throughout my life, it wasn't until graduate school that I learned the process of failure in practice and that what makes science so risky is also what makes it so exciting.

Eventually I left research to try my hand at science writing. People sometimes ask me if I regret not pursuing writing sooner. But I can't imagine a different path, since it was precisely this newfound appreciation for uncertainty that empowered me to ask, "What if I left bench science?"

Yes, failure was possible, but so was success. While I wouldn't achieve my dream job as a science writer immediately, if the career path remotely resembled the sinusoidal valleys and peaks of research, I could persist and persevere.

Ulli Hain (hain.ulli@gmail.com) received her Ph.D. from the Johns Hopkins University. She is a science writer at Palladian Partners, Inc.

Reminiscing scientist

By Michael R. Murphy

As a Brit from the smoke chimneys and cobble streets of northern England, I often wonder what life would have been like for me if I hadn't moved to America. I currently am in the fourth year of my Ph.D. studies in molecular biology at Hunter College in the heart of New York City.

In the time-traveling device of my mind, I have returned to 2012 — the year I completed my undergraduate studies at the University of Glasgow in Scotland. You see, in this particular academic year, I believed I had sacrificed enough to get me that 1st (the academic equivalent of an A grade) I had been dreaming about, the surefire ticket to graduate school.

I had spent the year lobbying the estimable professors of the University of Glasgow's genetics department into believing I was capable of achieving such an academic feat as an A and that they should pass on this incredible information to prospective universities looking for students on findaphd.org. (Yes, there is such a site.)

Having successfully sold myself to them, I turned to the task of sending out those applications. A good 12 months of having those poor professors send reference letter after reference letter yielded nothing but rejection emails. And to top it off, I finished the year with a 2:1 (the equivalent of a B). My time-traveling self would have tried to stop the self-pity that resulted in drinking a 24-can pack of Carlsberg (our version of Budweiser) that night.

Not one to be disheartened by a classic reality check, I threw myself at two labs that summer at the University of Glasgow, working for nothing but the time the principal investigators could give me. I also happened to meet someone who lived stateside and was visiting for the summer. We fell in love, and we promised that one of us would end up in the other's country. Would I have made that promise knowing what I do now? There's not a doubt in my mind that I would.

After the summer was up, I moved back home to England, broke and unemployed, with the cat as my senior adviser. Looking for cash to scrape together, I got a job packing items in a warehouse for a business that sold everything from shoes to dish cloths during the Christmas season. Long hours and throbbing feet. The only good part was that it counted my performance and ranked me against my colleagues, which was mildly entertaining. I hated this job more than anything, but I would do it again for the cash.

In the months leading up to Christmas, I had emailed effectively every single PI in New York City who worked on something even remotely related to genetics to ask to be a lab technician. Strangely, after emailing one such person a second time, he got back to me, or, more precisely, his postdoctoral fellow did.

"Dr. _____ forwarded your résumé to me to investigate the possibility of considering you for a position in our lab," the email read. That day at the warehouse, I broke my own record for picking items off shelves and putting them in boxes.

I was visiting my girlfriend in New York just after Christmas, and I scheduled an interview. The PI, based at New York University, just happened to be the provost of the newly opening NYU facility in Abu Dhabi, UAE. He seemed much keener on hiring me for the new facility, a possibility I hadn't considered leading up to the interview.

My commitment, however, was to get hired in New York and keep my promise to my girlfriend.

I got offered the technician job in New York. But I turned it down. At the same time, I had been applying for Ph.D. programs in the U.S. and had been accepted at Hunter College. The relationship didn't work out.

I don't know much about Abu Dhabi or even money for that matter. But I suspect I would know a lot more about both if I had moved there to work for the provost. I wouldn't be so pale; that's for sure. I even could be writing papers for the UAE-based company Bentham Science Publishers' journals!

This potential do-over would have changed a lot of things: where I've been over the past three years, the work that I did, the people I helped and hurt.

But some of it wouldn't have changed. I would still be very far from my family. I've already lost one elderly grandparent in that time; the other has limited time left. Perhaps the best do-over I could do would be to stay in England. Be near my family and friends. I don't regret moving to America, but I do regret leaving home. It's mixed emotions that no amount of time traveling can solve. It's why it's right that we can only move forward.

Michael R. Murphy (m.r.murphy456@gmail.com) is a Ph.D. student at Hunter College.

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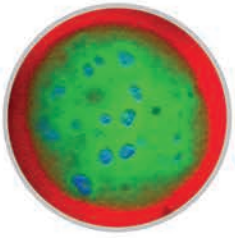

Emerging Roles for the Nucleolus
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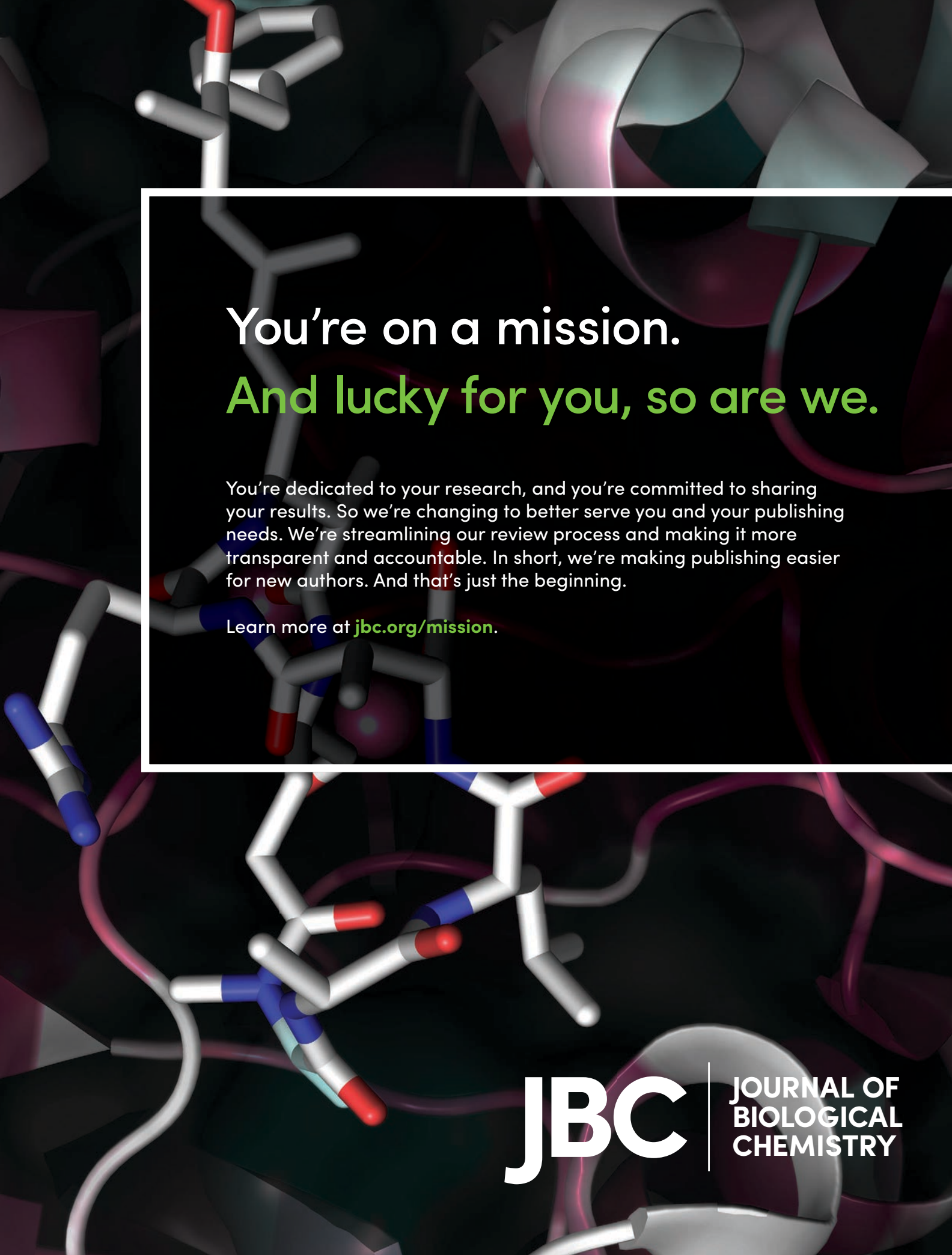
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