BioNEXUS

THE MAGAZINE OF THE INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH

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TRAPPED IN ICE

Complex Biomolecules Reveal Their Structures

ALSO INSIDE

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STANDARDIZING ANTIBODY DRUG MEASUREMENTS

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IBBR Fellow Saif Hasan places a sample into the new cryo-EM instrument
Photo by John T. Consoli

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Left side: flask icon by Gabriele Malaspina from the Noun Project, solar panel icon by Laymik from the Noun Project; *Right side:* photos by John T. Consoli

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IBBR exterior photo by Vicki Buckholz; *Circle photos (clockwise):* images 1–3 by Dave Romero, photo by John T. Consoli, photo by Silvia Muro

FROM THE DIRECTOR

WELCOME TO THE INAUGURAL ISSUE OF BIONEXUS, the magazine of the Institute for Bioscience and Biotechnology Research (IBBR). "Nexus" refers to a connection or central point, making it a particularly apt term to describe the various ways that IBBR creates interdisciplinary links between basic and applied sciences.

IBBR connects federal scientists from NIST and academic researchers from across the University of Maryland's premier research institutions as partners in innovative biotechnology research spanning a broad scope of biological sciences. In addition, IBBR serves as a nexus of scientific discoveries and their translation into real-world healthcare and technology applications through collaborations that unite industry, government agencies, and academic stakeholders.

This issue of BioNEXUS highlights the recent addition of an exciting new instrumentation platform to the IBBR structural biology toolbox, cryo-electron microscopy. Cryo-EM complements our long history of expertise in determining the structure of biomolecules and understanding how they function in biological processes.

You will also read about the efforts of our researchers who work on biomedical projects aimed at preventing and treating disease—from developing new antibacterial drugs and designing vaccines against viruses of significant medical and public health importance, to understanding and harnessing the biology of autoimmune diseases and cancer. There is also a feature story about how a new NIST reference material is enabling global collaborations toward innovative measurement technologies that support the development of powerful new classes of protein-based therapeutics.

To learn more about IBBR research, I invite you to visit us at **www.ibbr.umd.edu** or at our beautiful Rockville campus in the heart of Maryland's biotechnology corridor.

Thomas R. Fuerst, PhD
Director

About IBBR

IBBR is a joint research enterprise of the University of Maryland, College Park (UMCP), the University of Maryland, Baltimore (UMB), and the National Institute of Standards and Technology (NIST). IBBR is also financially supported in part by the University of Maryland Strategic Partnership: *MPowering the State*, an initiative designed to achieve innovation and impact through collaboration.

The Institute sits at the nexus of academic research and commercial application, bringing together critical elements necessary to inspire transformative discoveries in the field of biotechnology that provide innovative solutions to major scientific and engineering challenges important to society. IBBR researchers seek to advance the fields of disease pathways and biomolecular targets, biomolecular measurement sciences, and biomolecular engineering, including structure-based design of vaccines and therapeutics. The Institute also serves to expand the economic base of science and technology in the state of Maryland.

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TRAPPED IN ICE

Complex Biomolecules Reveal Their Structures

Capturing pictures of proteins at atomic resolution

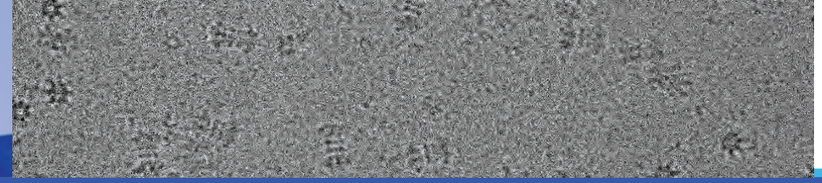
PROTEINS ARE INVOLVED in every task of every cell in every living thing. The antibodies that attack invading pathogens, the enzymes that digest food, the collagen that supports tissues, and the hemoglobin that carries blood oxygen are all proteins. Since function is determined by structure (think of a Phillips' head versus a flathead screwdriver), having a clear picture of a protein's structure is critical to understanding its role in the molecular world. Cryogenic electron microscopy (cryo-EM) is a powerful new method for determining a protein's structure, and it has just been added to the suite of tools available at IBBR.

"IBBR has long been on the cutting edge of structural biology research, which helps us understand how biological entities work. This new equipment will significantly enhance our research contributions to this important field," says IBBR Director Thomas Fuerst.

Ed Pozharski monitors data collection from the new 200kV Talos Arctica instrument.

PHOTO BY JOHN T. CONSOLI

APOFERRITIN IMAGES BY ED POZHARSKI

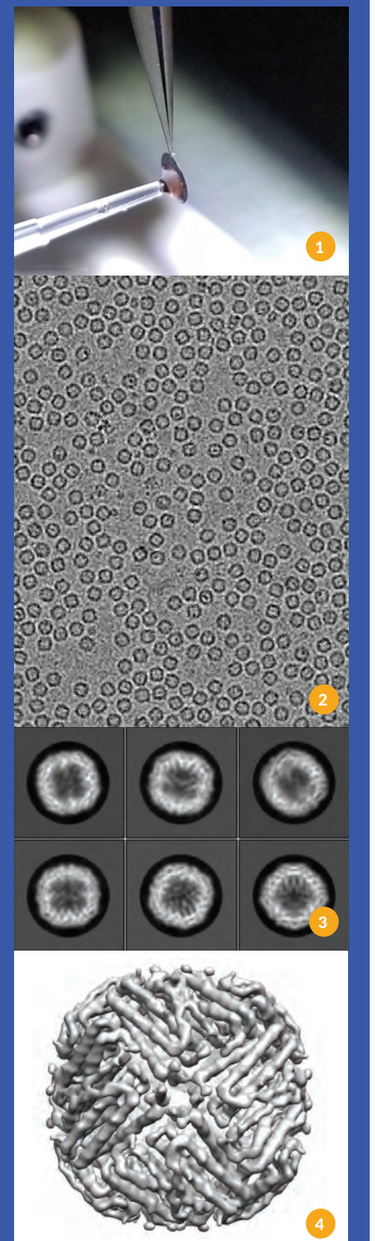


"Freezing samples is both highly technical and a bit of an art form. Getting a good sample frozen in 'perfect' ice takes a lot of practice!"
—THOMAS CLEVELAND, NIST RESEARCH PHYSICIST

What is Cryo-EM?

INSTEAD OF beams of light, electron microscopy uses a beam of electrons to produce images of matter. This allows for higher resolution—the ability to see smaller particles—than traditional light microscopes. Researchers have been improving the resolution of electron microscopy since its invention in the 1930s. Three scientists won the Nobel Prize in Chemistry in 2017 for advancing this method to determine atomic-resolution structures of large, complex proteins.

1. Cryo-EM involves flash freezing a sample of protein in thin, clear ice. This -180°C cryogenic freeze holds everything still, which prevents the constant jiggling of molecules from blurring the picture.
2. An electron beam is passed through the sample and a specially designed camera creates thousands of images from the patterns of electrons that scatter off the individual proteins in the sample.
3. Researchers use computer algorithms to sort through the images for those that show proteins in the same orientation and group these images together into "classes."
4. Images in classes representing different protein orientations are combined to produce high-resolution, three-dimensional pictures that show where each atom is located in the sample protein.



Cryo-EM researchers at IBBR (left to right: David Weber, Thomas Cleveland, Ed Pozharski, and Saif Hasan)

Why Cryo-EM?

Proteins are measured in nanometers. For illustration, a sheet of paper is about 100,000 nanometers thick, but a mid-sized protein is only 10 nanometers wide! Their tiny size means that proteins cannot be viewed using traditional light microscopes.

Starting in the 1940s, researchers have used a method called x-ray crystallography (the same method used to solve the structure of the DNA double helix) to develop models of over 100,000 protein structures. But x-ray crystallography requires very stable proteins that can organize into repeating patterns, called crystals, so it isn't applicable to all proteins, especially the more flexible ones.

Beginning in the 1990s, a new method called nuclear magnetic resonance (NMR) spectroscopy began to provide high-resolution structural data on small- to medium-sized proteins dissolved in solution.

For very large and flexible proteins, and complexes of proteins, cryo-EM is needed. Like NMR, cryo-EM doesn't require the protein sample to be in a crystallized state. Instead, proteins are literally frozen in near-natural states, giving researchers clearer insight into their functions. In addition, recent technological advances have dramatically improved researchers' ability to resolve these structures. Previously, subtle drift of the specimen reduced the resolution of single cryo-EM images, making protein molecules look like blobs of matter. Now, thousands of images (400 per second) are snapped and recorded like a movie, allowing researchers to track and subtract the drift for a much clearer resolution.

IBBR now has all three, complementary methodologies co-located at its site in Rockville. "Taken together, x-ray crystallography, NMR, and cryo-EM, along with the computational biology and biochemical expertise of IBBR scientists, allow our researchers to develop accurate models of biomolecules, regardless of their size, complexity, or flexibility," says David Weber, IBBR Associate Director and Director of M-CAMA. Those models can be used to better understand diseases and design drugs to stop them. ■

Behind the Scenes

A cryo-EM instrument is an incredibly sensitive piece of equipment requiring a very particular environment. In addition to having tightly controlled temperature and humidity, the space must be free of electromagnetic interference (EMI) and vibration. EMI can come from something as common as the electrical wires in the room's wall. And, any large metal object moving nearby—like an elevator or even a metal door outside the room's wall—can disrupt the EMI flux.

"We worked closely throughout the renovation and installation process with structural, electrical, and mechanical engineers from the manufacturer, as well as with the research faculty who will use the instrument," says Jim Johnson, IBBR's Director of Facilities and Lab Services. "All sources of EMI in and adjacent to the cryo-EM room were relocated when practical and shielded when necessary." There is now so little EMI and vibration that the stabilized electron beam drifts less than one billionth of a meter during each minute of data collection!

While Facilities worked on the environment, IBBR's IT team prepared to handle the collection, storage, and analysis of huge amounts of data. The cryo-EM's special, rapid-fire camera churns out thousands of high-resolution images for each sample, which requires a tremendous amount of file storage. To meet the demand, the IT team designed a 750 terabyte (TB) parallel file system storage array that will cover the first year of operation and is expandable for the future.

The IT team also built new GPU-based image processing nodes to analyze the acquired cryo-EM data, and they configured and tested common software for image processing. "IBBR's high performance computing cluster had been configured for solving small, independent problems at high throughput. Cryo-EM requires both more and different resources to solve each problem. The required shift to provide a solid workflow for cryo-EM data has been a fun and satisfying challenge," says Christian Presley, IBBR's Director of Information Technology.

Installation of the new instrument was completed in spring 2019; another instrument will be installed in 2020. The instruments comprise the Maryland Center for Advanced Molecular Analysis (M-CAMA).

TOP PHOTO BY JOHN T. CONSOLI, BOTTOM PHOTO BY AN NGO

IBBR researchers are developing new cryo-EM methodologies and using the technology to advance both basic research and applied science.

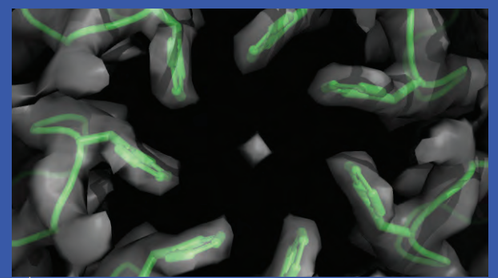
Basic Research: Disease Mechanisms

Many cellular processes depend on communication and coordination among specialized internal compartments called organelles. The transfer of information and biological materials between organelles occurs through distinct "molecular pipelines." Viruses exploit these pipelines for replication, and the disruption of biological transport is implicated in various metabolic diseases, including metastatic cancers.

IBBR Fellow **Saif Hasan** studies how biological signals are communicated and interpreted. The Hasan lab uses cryo-EM to determine the structures of complex protein components of biological transport systems. "Our ongoing investigations will shed new light on key aspects of diseases such as cancer and viral infections that place an enormous burden on human health," says Hasan.

Applied Science: Rational Drug Design

Drugs usually target and bind to proteins. Using an understanding of protein structure and function to guide drug discovery is called *rational drug design*. Cryo-EM enables researchers to determine the structures of large, complex drug targets that were previously impossible to figure out. IBBR Associate Director and Director of M-CAMA David Weber adds, "Being able to 'see' the entirety of a large protein complex allows us to identify several potential drug target sites from the start."



Surface rendering model of phi-gate area of *C. difficile* binary toxin as revealed by cryo-EM (data collection at New York Structural Biology Center)

Weber is using cryo-EM to determine the structure of a dangerous toxin produced by an emerging strain of *Clostridioides difficile*, in collaboration with IBBR Fellow **Ed Pozharski**, who also manages M-CAMA. *C. difficile* is an opportunistic bacterial pathogen that infects nearly 500,000 people in the US every year, causing severe diarrhea and potentially fatal dehydration. Understanding the macromolecular assembly of this large toxin is uncovering new details about *C. difficile* infection and advancing drug design efforts at IBBR.

Methods Development: Biopharmaceutical Applications

"In addition to enabling fundamental insights into biomolecular structure and function, IBBR's new cryo-EM capabilities complement NIST's mission of developing advanced measurements to support biopharmaceutical development and regulation," says IBBR Associate Director John Marino.

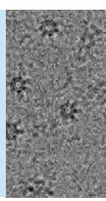
Cryo-EM promises to yield unprecedented structural details of biopharmaceuticals and other complex therapeutics that could inform drug development and lead to better patient outcomes.

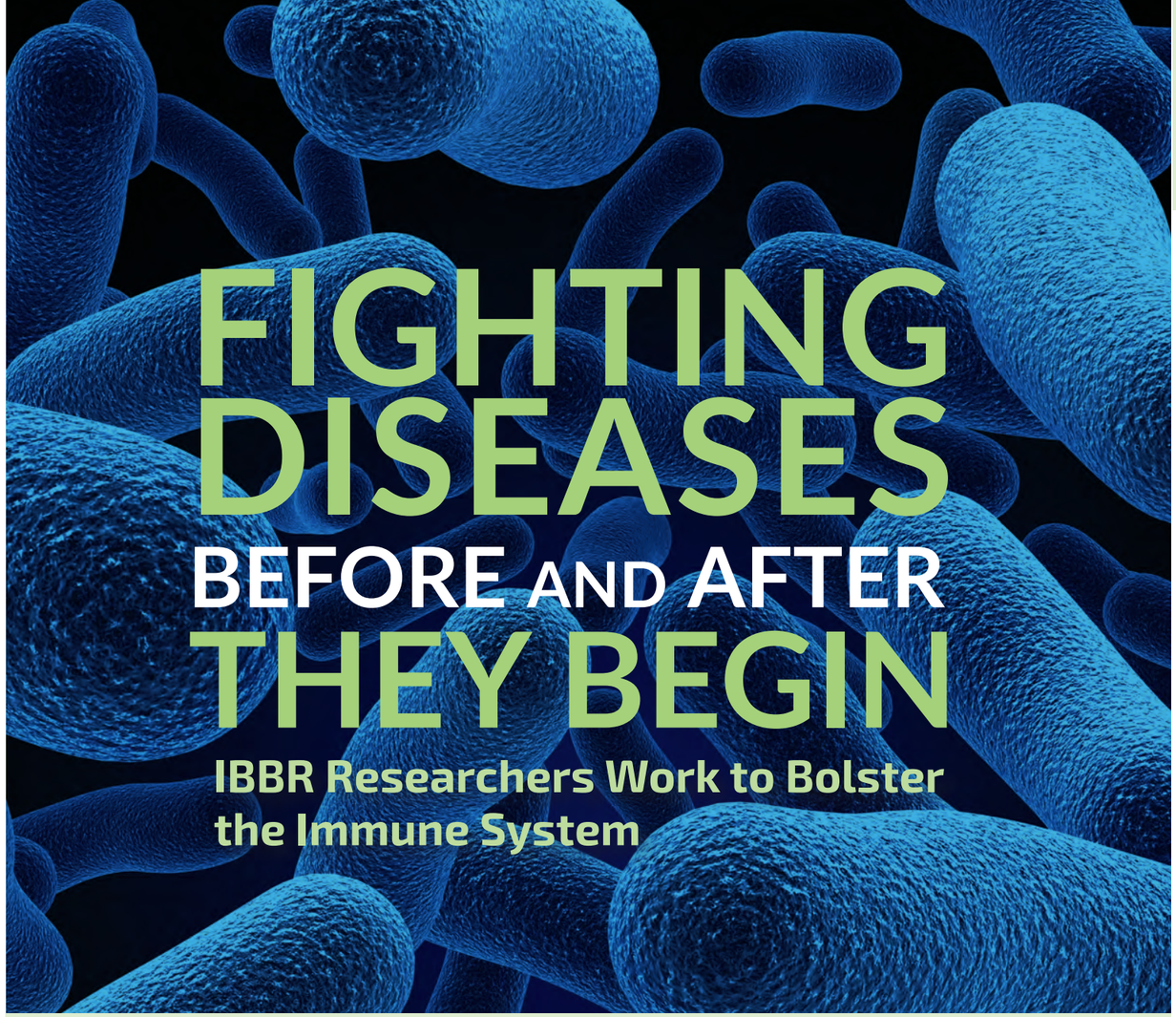
IBBR researcher **Thomas Cleveland** is leading an effort to develop molecular "scaffolds" to hold particularly small or flexible biopharmaceuticals in place in order to improve image alignment. The group expects their work to expand the range of cryo-EM applications.

Thomas Cleveland: *Research Physicist, NIST Biomolecular Structure and Function Group*
Saif Hasan: *Assistant Professor, UMB Department of Biochemistry & Molecular Biology*
Ed Pozharski: *Assistant Professor, UMB Department of Biochemistry & Molecular Biology*

IMAGE BY ED POZHARSKI

Bringing cryo-EM to IBBR required new levels of collaboration and funding. In addition to the three IBBR partner institutions, funding was provided in part by the University of Maryland Strategic Partnership: *MPowering the State*, and the Greenebaum Comprehensive Cancer Center helped to cover the costs of bringing on faculty and staff with cryo-EM expertise. Thanks to these efforts, investigators throughout the University System of Maryland, NIST, and the whole region now have local access to this state-of-the-art capability.





FIGHTING DISEASES BEFORE AND AFTER THEY BEGIN

IBBR Researchers Work to Bolster the Immune System

BACTERIA BY IROCHKA_T/ISTOCK

If you're not sick right now, it's due to the wonders of your immune system, not the absence of foreign invaders. But sometimes your immune system could use some backup.

THE HUMAN IMMUNE SYSTEM is a complex marvel. At every moment, its sentinels are scanning the body for unwelcome entities: bacteria, viruses, cancer. Once spotted, troops are rallied and equipped for battle as information about the enemy is disseminated. If the enemy is new, novel weapons are innovated and deployed, and the blueprints stored away for next time. By understanding these processes, scientists hope to manipulate, mimic, and bolster the immune system to develop preventative vaccines and new treatments for disease.

IBBR researchers are on the cutting edge of these efforts. Some are designing vaccines for diseases including hepatitis C, Ebola, and HIV. Others are working to develop next-generation protein-based drugs and advancing the science behind cell-based therapies against diseases for which prevention is not yet feasible. Still others are engineering better ways to target delivery of these new vaccines and therapeutics within the body.

An effective vaccine introduces the body to particular **antigens**, priming the immune system to respond quickly and robustly if it encounters the real **pathogen**. One of the immune system's primary weapons is the **antibody**, a protein that specifically "sticks to" or *binds* a particular **epitope** on an antigen, and signals other parts of the immune system to respond to an infection. The best antibodies are broadly-neutralizing antibodies, or **bNAbs**, those that signal strongly and bind tightly to an epitope that is shared across multiple strains of a pathogen in a way that inhibits infection. The best vaccines contain antigens that stimulate the production of bNAbs.

GLOSSARY

Antibody: a Y-shaped immune system protein (yellow)

Antigen: a molecule recognized by the immune system as foreign (green)

bNAb: broadly-neutralizing antibody

Epitope: the part of an antigen bound by a particular antibody (red)

Pathogen: a disease-causing entity such as a virus or bacterium

The binding of an antibody to an epitope is like fitting together two pieces of a three-dimensional puzzle. By determining the 3D structure of the interface between an antibody and an epitope, researchers can understand which parts of each piece are important for binding. This approach enables structure-based vaccine design (SBVD).

THE NOUN PROJECT: DIAGRAM ICONS BY GRAPHIC TIGERS, ANTIBODY BY KELSEY ARMSTRONG; ISTOCK: PUZZLE BY FILO

Heading Off Hepatitis C Infection

Chronic hepatitis C affects approximately 2.4 million Americans and over 70 million people worldwide. The causative agent is hepatitis C virus, which can slowly and silently damage the liver over years and is the leading cause of liver cancer. A curative treatment was discovered a few years ago, but it's very expensive and doesn't prevent reinfection or reduce the risk of developing liver cancer from a previous infection.

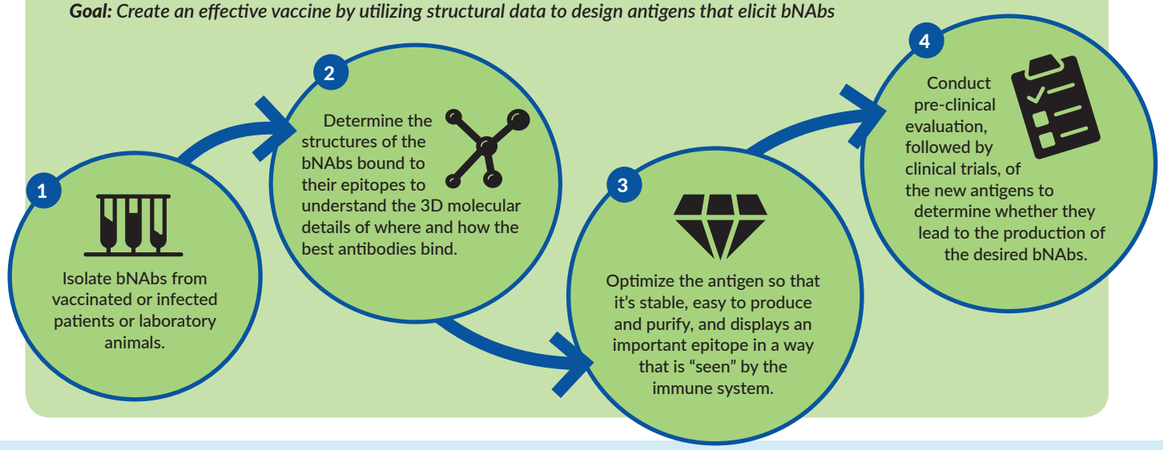
Vaccines exist for hepatitis A and B, but the hepatitis C virus is much more difficult to grow or model in the lab and even more variable than HIV. These factors make hepatitis C vaccine development a challenge and a good candidate for SBVD.

IBBR Director **Thomas Fuerst** leads the Institute's core SBVD program focused on hepatitis C vaccine design, in collaboration with Stanford University Professor of Pathology Steven Foung. The team has spent the last several years collecting structural data about an important hepatitis C virus antigen called E2 envelope protein, including where and how bNAbs bind to E2 epitopes shared across a range of viral strains. Armed with this information, they are now using computer modeling to guide them to new E2 designs that they hypothesize will be more stable and better able to elicit the production of bNAbs.

In particular, the team uses Rosetta modeling and glycoengineering to stabilize specific regions and adjust the carbohydrate content on the protein surface to make

Structure-Based Vaccine Design

***Goal:** Create an effective vaccine by utilizing structural data to design antigens that elicit bNAbs*

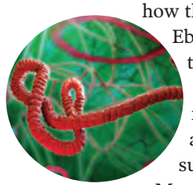


Hepatitis C team members (from left to right: Eric Toth, Khadija Elkholy, Ruixue Wang, and Kailyn Groisser)

these areas more immuno-prominent, and to mask epitopes that only "distract" the immune system from these important areas. With promising results in mice, the team is moving ahead with plans for studies in non-human primates.

A Two-Pronged Attack on Ebola

Perhaps no illness in current headlines elicits more fear than Ebola. The disease's often fatal fluid loss and bleeding are the stuff of nightmares. IBBR researchers are studying how the immune system recognizes and destroys Ebola viruses to better design vaccines and therapeutic antibodies.



The first steps of the Ebola virus replication cycle involve binding and entering a human cell—processes facilitated by a viral surface protein called glycoprotein, or GP. Many antibodies that protect against Ebola do so by binding GP and interfering with the entry process, but most only recognize the GP of a single species of the virus.

In 2017, IBBR Fellow **Yuxing Li** and her team isolated an antibody called CA45 that's cross-protective; it recognizes and neutralizes four of the five known species of Ebola.

IBBR Fellow **Gilad Ofek** recently published the structure of CA45 bound to GP. The structural insight into which binding sites confer the ability to recognize and neutralize a broad spectrum of Ebola viruses is informing Ofek's work toward developing a vaccine, as well as Li's work toward developing CA45 into a powerful treatment option for those who do get the disease.

Finding the Path to Optimized HIV Antibodies

As the immune system responds to a natural infection, better and better antibodies are produced. A successful HIV vaccine must recreate that process to elicit bNAbs that recognize and protect against many different HIV strains.

"Based on patients whose bodies have kept viral numbers low, we know that the best antibodies recognize particular epitopes of Env, a viral surface envelope protein," says IBBR Fellow **Yuxing Li**. But so far, using Env as a vaccine has not elicited potent bNAbs.

To study this paradox, researchers have used samples from HIV-infected individuals to trace the pathway by which effective antibodies are produced back to the beginning of the process. Li and IBBR Fellow **Brian Pierce** are currently using this information to inform their structural and computational biology efforts to design, produce, and test new variants of Env. Their goal is to learn which variants, at which moments in the immune response, will best shepherd the immune system through the steps needed for potent bNAb production that can stop HIV in its tracks.

The Enemy of My Enemy

When most of us think about viruses, we think about people getting sick. But IBBR Fellow **Daniel Nelson** studies viruses that could make people well—by killing disease-causing bacteria.

Bacteriophage (or *phage* for short) are viruses that infect bacteria. The Nelson group studies enzymes produced by phage that directly destroy bacterial cells within seconds upon contact. These enzymes, called endolysins, may prove to be alternatives to antibiotics, an important area of research given ongoing concerns about antibiotic resistance.

TOP PHOTO BY JOHN T. CONSOLI; EBOLA IMAGE BY NOPPARIT/ISTOCK; HIV BY DR_MICROBE; BOTTOM PHOTO BY VICKI BUCKHOLZ

TOP PHOTO BY JOHN T. CONSOLI; BOTTOM PHOTO BY WRAGG/ISTOCK

From Good to Better

Vaccine formulations include not just the antigen, but also an *adjuvant*, which stimulates the immune system more generally. Adjuvants bring the vaccine components to the attention of immune cells, which can increase vaccine efficacy and lower costs by minimizing the amount of antigen that must be administered.

IBBR Fellow **Alexander Andrianov** and his team have generated an array of potent adjuvants based on a class of molecules called polyphosphazenes (PPZ). PPZs are water-soluble and biodegradable and their protein-stabilizing properties can prolong vaccine shelf life. Certain PPZs can self-assemble into tiny, immune-stimulating particles that carry the vaccine antigen.

PPZ researchers Alexander Andianov and Alexander Marin

Recently, Andrianov's group and Fuerst's hepatitis C team have been working to add a third component to vaccine formulations: small molecules that will further modulate and fine-tune the immune response to the vaccine.

Nelson lab graduate students (left to right: Adit Alreja, Niels Vander Elst, and Daniel Kemboi)

"These enzybiotics (for ENZYme antiBIOTICS) break chemical ties in the bacterial wall. The high internal pressure within the bacterial cell then causes it to rupture and die," explains Nelson.

The Nelson group uses bioengineering approaches to optimize naturally occurring endolysins for use as therapeutics, striving for attributes such as high activity, expanded host range, better stability, and the ability to enter human cells to kill intracellular pathogens. Some of the pathogens in their sights include the bacteria that cause anthrax, methicillin-resistant *Staphylococcus aureus* (MRSA), and the streptococcal bacteria that infect dairy cow udders. They are even studying an endolysin against *Propionibacterium acnes* that could be used as a topical acne treatment.

Combining Forces: Stopping Bacteria and Their Toxins

In an exciting new application, the group has combined endolysins with another powerful protein therapeutic—an antibody against anthrax toxin.

Like landmines left behind by a retreating enemy, toxins secreted by certain bacterial species can continue to harm their victims even after the invading bacteria are killed. For such toxemias, including anthrax, current treatment involves antibiotics to kill the bacteria and antibodies to neutralize the toxin.

But what if one therapeutic could do both?

Nelson is working with Rockville-based Integrated BioTherapeutics Inc. to design such a molecule. They have engineered and begun testing a single immunotherapeutic with two working ends. One end is an antibody that binds anthrax toxin; the other is a piece of an endolysin that anchors onto the surface of the bacteria. The endolysin end directs the molecule to bacteria at the site of infection, where the antibody end can neutralize the toxin and signal the immune system to kill the bacteria.

With the support of National Institutes of Health awards aimed at facilitating cooperation between small businesses and research institutions, Nelson and his collaborators are performing additional pre-clinical optimization and validation. Hopefully, this exciting new platform will one day be expanded to address other toxemias, including botulism and *Staphylococcus* and *C. difficile* infections.

Unlocking the Secrets of T Cells

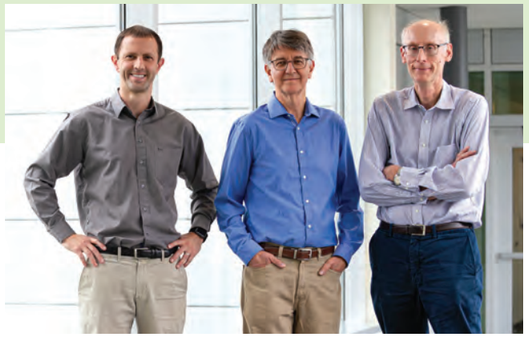
T cells are a family of cells of central importance to the immune system. They are activated to perform multiple functions by a team of proteins called the *T cell receptor complex*. Some of IBBR's structural and computational biologists are striving to understand how these proteins work in order to harness the disease-fighting powers of the cells they control. »

Getting SMART About Protein Therapies

Imagine the world's most complex game of dominoes. Now shrink it down to fit inside a cell, with each domino representing a chemical reaction. A single missing or misshapen domino could be disastrous. The scientists of IBBR's SMART therapeutics program are working to engineer "smart" assemblies of proteins that dynamically respond to their environments and alter particular chemical reactions, with the ultimate goal of combating disease.

IBBR's first proof-of-principle SMART therapeutics project is to design an enzyme that selectively destroys the cancer-promoting RAS protein. This project is a collaboration among IBBR Director **Thomas Fuerst**, IBBR Fellow Emeritus **Philip Bryan**, and IBBR Fellows **Eric Toth** and **John Orban**.

Mutations in one of the three *RAS* genes are involved in roughly a third of all human cancers, often resulting in a RAS protein stuck in the active state, which leads to tumor growth. Current therapeutic interventions against RAS-driven cancers have had limited clinical effectiveness. To date, the SMART therapeutics team has created a protein machine that selectively destroys active RAS in a test tube and in cell model systems. Their next step will be to determine how well their machine controls RAS signaling in cancer cell models. The team plans to apply the principles learned in the RAS study to additional therapeutic targets.



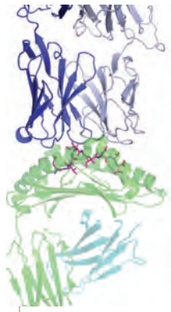
T cell researchers at IBBR (left to right: Brian Pierce, John Orban, and Roy Mariuzza)

Target Recognition

The primary component of each T cell receptor complex is the *T cell receptor* (TCR). TCRs bind small pieces of foreign proteins—peptides—displayed by infected cells, cancer cells, or immune cells that alert T cells to an infection. When TCRs inappropriately recognize the body's own proteins, autoimmune disorders can result. A deeper understanding of how TCRs bind and recognize their targets will help researchers manipulate their function.

To that end, IBBR Fellow **Brian Pierce** and his group have developed a web server called TCRmodel that creates high-resolution models of TCR structures from user data. TCRmodel has generated over 2,000 TCR structures since its release in Summer 2018. Pierce's group is now working to extend the tool's abilities to include modeling exactly how individual TCRs recognize and bind their targets.

In 2019, the team released a related tool called TCR3d, a searchable and continuously updated database that contains all known TCR structures. It allows researchers throughout the world to look for patterns that shed light on the structure and function of TCRs of therapeutic and disease relevance.



One of the TCR complexes in the TCR3d database

Information Relay

IBBR Fellows **Roy Mariuzza** and **John Orban** focus on the signaling event that occurs after a TCR binds a foreign peptide—the signaling that initiates T cell activity.

Recently, they used molecular dynamics simulations from Pierce's team combined with nuclear magnetic resonance (NMR) spectroscopy to explore that event. They learned that the binding of a TCR to a foreign peptide, which happens at one end of its structure, causes a ripple of changes along its entire length, in a process called allostery. The changes at the opposite end of the TCR, where it interacts with additional components of the TCR complex, propagate the signal inside the cell through a chain reaction that prepares the cell for its mission.

Together, these IBBR labs are enhancing our understanding of T cell immunity and opening the door to new T cell- and TCR-based therapies for cancers, autoimmune disorders, and infectious diseases.

Molecular Package Delivery

Our bodies and cells contain elaborate "postal systems" and, like mail-order prescriptions, drugs must be delivered to the right place within the body to be effective. IBBR Fellow **Silvia Muro** studies nanoscale carrier structures developed for the controlled and targeted delivery of therapeutics.

Nanocarriers have the potential to deliver therapeutics to precise targets across cellular layers of tissues or organs, such as the blood-brain barrier or the gastrointestinal lining of the gut. Muro also uses these powerful research tools to better understand cellular processes that regulate molecular transportation and how these processes are altered in disease.

In a collaboration with industry partner Genisphere LLC, Muro's group recently used an antibody specific to an abundant lung protein to "address" a nanocarrier made of DNA to the lungs. The antibody increased lung targeting over 400-fold, an unprecedented specificity for a DNA-based nanocarrier.

The beauty of this system as a therapeutic platform lies in its flexibility. The nanocarriers can be engineered to carry small drug molecules or large protein therapeutics, and different antibodies can target them to different sites throughout the body. ■

Alexander Andrianov: *IBBR Professor*
Philip Bryan: *Founder, Potomac Affinity Proteins, LLC*
Thomas Fuerst: *Professor, UMCP Department of Cell Biology & Molecular Genetics*
Yuxing Li: *Associate Professor, UMB Department of Microbiology & Immunology*
Roy Mariuzza: *Professor, UMCP Department of Cell Biology & Molecular Genetics*
Silvia Muro: *IBBR Associate Professor*
Daniel Nelson: *Associate Professor, UMCP Department of Veterinary Medicine*
Gilad Ofek: *Assistant Professor, UMCP Department of Cell Biology & Molecular Genetics*
John Orban: *Professor, UMCP Department of Chemistry & Biochemistry*
Brian Pierce: *Assistant Professor, UMCP Department of Cell Biology & Molecular Genetics*
Eric Toth: *IBBR Assistant Professor*

TOP PHOTO BY JOHN T. CONSOLI, TCR IMAGE BY BRIAN PIERCE

One for All: A GLOBAL ANTIBODY

Mass spectrometry researchers Michael Pettit and John Schiel

PHOTO BY JOHN T. CONSOLI, NISTmAb IMAGES BY TRAVIS GALLAGHER, ASPIRIN STRUCTURE BY BENJAH-BMM27/WIKIPEDIA

Part of the mission of the National Institute of Standards and Technology (NIST) is to develop standards and reference materials that underpin advances in bioscience and biotechnology, contributing to human health and the US economy.

In 2016, NIST introduced one of the world's most intricate reference materials—a monoclonal antibody (mAb) dubbed ***NISTmAb****.*

MONOCLONAL ANTIBODIES and other protein therapeutics are much more complex than small molecule drugs. The long chains of amino acids comprising proteins fold into complicated three-dimensional structures and can be decorated with complex patterns of sugars. Tiny changes to the amino acids can change the overall structure in ways that affect function. Determining the identity and quality of biopharmaceuticals requires techniques that can measure and describe this *higher order structure*. And these analytical techniques require protocols to harmonize and routinize the measurements across laboratories—a job for NIST.

The NISTmAb is a representative of the mAb drug class and was manufactured by MedImmune, now AstraZeneca. NIST provided foundational measurements to characterize the mAb, certified the material, and made it available as a non-proprietary tool to spur innovations in protein therapeutics by industrial, federal, and academic scientists.

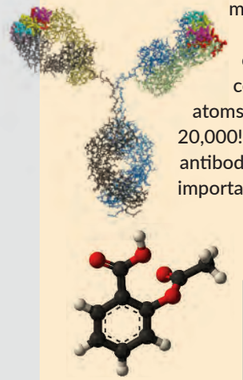
What is a mAb?

"mAb" stands for monoclonal antibody. Antibodies are immune system proteins that bind foreign particles. There are billions of different antibodies, each with an affinity for a particular foreign molecule. Monoclonal antibodies are identical copies of each other, sharing the same specificity.

Antibodies can tag infectious agents or cancer cells for destruction and can modulate the immune system to treat autoimmune diseases. Current mAb drugs are used to treat various cancers, as well as autoimmune disorders like multiple sclerosis, rheumatoid arthritis, and psoriasis, and hold great promise against infectious agents—in fact, the new Ebola drugs are mAbs!

mAb drugs constitute a $150B industry. Seven of the top ten drugs by sales in 2018 were mAbs or mAb-based drugs.

Each mAb is a large, complex molecule. For comparison, aspirin has 20 atoms, but an antibody has over 20,000! Accurate measurement of antibodies is challenging but no less important than for simpler drugs.



Top: A structural representation of the highly-complex NISTmAb. Bottom: The comparatively simple structure of aspirin (not to scale).

NISTmAb interlaboratory study leaders (from left to right: Robert Brinson, Jeffrey Hudgens, Trina Mouchahoir, and John Schiel)

NIST is committed to promoting US innovation and industrial competitiveness by advancing measurement science, going beyond publishing new methods and technologies by testing and validating them. That validation encourages use of the new technology and generates communal results, further proving and increasing the technology's value.

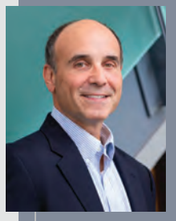
The work of NIST Biomolecular Measurement Division scientists located at IBBR supports the biopharmaceutical industry's development of safe, effective, and affordable drugs. The fastest-growing class of drugs are protein biologics and, as part of the NIST Biomanufacturing Initiative, IBBR researchers are developing new and better ways to characterize these complex biomolecules and establish benchmarks that industry and regulators can use to help inform decisions during the drug approval process.

"Everyone's poster included the NISTmAb."

—KYLE ANDERSON, NIST RESEARCH CHEMIST, REGARDING A RECENT BIOPHARMACEUTICAL SCIENCE CONFERENCE

NISTmAb has been quickly adopted and is on pace to become one of NIST's top-selling reference materials. Close to half of NISTmAb sales to date have been to biopharmaceutical companies, with another third going to manufacturers of the sophisticated instruments used to analyze protein therapeutics; many academic scientists also see value in using this industry-relevant material in their work.

IBBR researcher Kyle Anderson explains the value of this broad dissemination: "No one wants to publicly share details of their proprietary materials, but we can all compare methods that are tested with the NISTmAb."



John Marino, Leader, NIST Biomolecular Structure and Function Group

IBBR Associate Director John Marino adds, "The NISTmAb really 'raises all boats' and is a great example of how US federal science can build bridges to solve common industry measurement problems through open-access technology development."

NISTmAb in Action

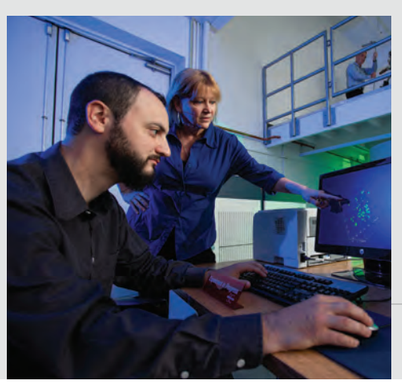
Prior to the release of NISTmAb, IBBR Fellow **John Schiel** coordinated its initial characterization by crowdsourcing data from groups around the world, work documented in a comprehensive three-volume book series. Now, NIST researchers are taking a deeper dive into its characterization. They are designing and coordinating larger studies and wider collaborations to further develop new methods for reliable, accurate, precise, and robust biomolecular measurements. Three recent global interlaboratory studies provide fantastic examples of the power of the NISTmAb to bring the biopharmaceutical research community together and enable innovations that will accelerate the discovery, development, and approval of important new drugs.

2D-NMR Study

Nuclear magnetic resonance (NMR) spectroscopy can be thought of as MRI for biomolecules. Two-dimensional NMR provides a "fingerprint" of the atomic-level structure of a biomolecule that can be used to compare batches of a drug or to compare drugs under development to current, approved products.

In order to test the accuracy and precision of 2D-NMR measurements to characterize mAb drugs, IBBR Fellow **Robert Brinson** coordinated a study in which 26 industry, academic, and government labs around the world received a fragment of the NISTmAb, along with a detailed protocol.

Despite being collected in various settings and using instruments of different ages and magnetic strengths, the data showed high levels of agreement among measurements. The NIST NMR team concluded that 2D-NMR is incredibly reliable and can be used for assessing therapeutics in various settings and applications, including for regulatory decision-making.



NMR researchers Luke Arbogast and Amanda Altieri

PHOTOS BY JOHN T. CONSOLI

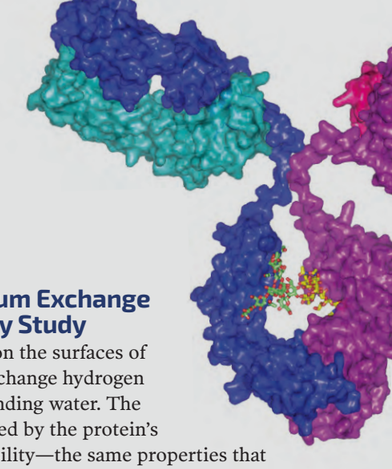
Mass Spectrometry MAM Study

Currently, dozens of measurements are made to characterize the features—or *attributes*—of a biopharmaceutical. IBBR Fellow **John Schiel** and IBBR researcher **Trina Mouchahoir** are working to develop a single platform capable of collecting data on multiple attributes simultaneously.

Schiel and Mouchahoir are members of the MAM Consortium, a group of scientists from more than 70 organizations working to develop and implement the multi-attribute method (MAM), a relatively new application of established liquid chromatography-mass spectrometry (LC-MS) techniques.

In LC-MS, components of a complex mixture are separated using an LC instrument, and then each component's mass is measured using an MS instrument. Researchers use the measured masses and signal intensities to identify and quantify each component. Attributes that can potentially be detected simultaneously using MAM LC-MS include sugar structures attached to a protein, chemical degradation of the amino acids that make up a protein, and impurities in the sample, to name a few.

Mouchahoir, Schiel, and their industry collaborator Richard Rogers of Seattle-based Juno Therapeutics Inc. recently coordinated an interlaboratory study in which each participant was asked to perform MAM analysis on three NISTmAb samples whose attributes had been deliberately altered. Participating laboratories reported each new or different attribute they found, and Mouchahoir and her colleagues compared results between labs to evaluate the reproducibility and capabilities of the MAM platform. Their findings provide important insights for improving the method across the industry.



Hydrogen-Deuterium Exchange Mass Spectrometry Study

Some molecular groups on the surfaces of proteins continuously exchange hydrogen (H) ions with the surrounding water. The rate of exchange is dictated by the protein's folding, binding, and stability—the same properties that determine protein function.

Exchange rates can be measured by immersing the protein in D_2O, which reacts like H_2O, and counting the number of exchanged deuterons (D). Since H weighs one atomic mass unit and D weighs two, deuterons are readily counted using mass spectrometry.

The entire measurement procedure, termed hydrogen-deuterium exchange mass spectrometry (HDX-MS), is widely used in biopharmaceutical research.

A recent interlaboratory study, coordinated by IBBR Fellow **Jeffrey Hudgens**, documented the reproducibility of HDX-MS measurements of the NISTmAb across fifteen labs and instruments, involving 89,800 measurements. The results will be valuable for establishing HDX-MS as a quality control measurement tool for biotherapeutics. ■

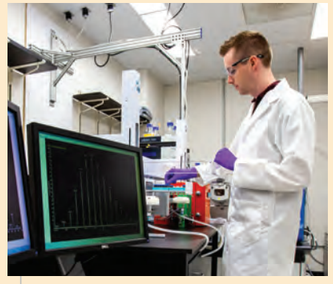
Kyle Anderson, *Research Chemist, NIST Bioprocess Measurements Group*
Robert Brinson, *Research Chemist, NIST Biomolecular Structure and Function Group*
Jeffrey Hudgens, *Research Chemist, NIST Bioprocess Measurements Group*
Trina Mouchahoir, *Research Chemist, NIST Bioanalytical Science Group*
John Schiel, *Research Chemist, NIST Bioanalytical Science Group*

Targeting Membrane Proteins Without Clogging the Mass Spectrometer

HDX-MS is frequently applied to drug R&D since it can detect changes in a protein upon binding to a drug. More than 50% of drug targets are proteins in cell membranes, and these proteins tend to behave most naturally in the presence of membrane components, namely lipids. Unfortunately, lipids pose a challenge for HDX-MS researchers since the greasy molecules "gunk up" the instrument.

Researchers can remove the lipids manually, but it's a very labor-intensive process, often causing measurement variation due to differences among samples and among lab protocols.

To address this challenge, IBBR researcher **Kyle Anderson** developed a way to automate lipid removal as part of the conventional HDX-MS process, thus improving measurement precision and facilitating the study of important membrane protein drug targets. This advance earned Anderson, and NIST, a big thank-you from a major instrument vendor. The vendor incorporated Anderson's method into an updated version of their product and credits him with solving a community need.



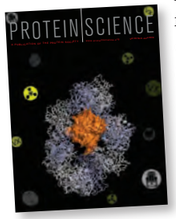
Kyle Anderson at the HDX-MS instrument

PHOTO BY JOHN T. CONSOLI, IMAGE BY TRAVIS GALLAGHER

Supporting Design of Drugs Against Nerve Agents

Nerve agents, such as sarin and VX gas, work by inhibiting an important enzyme of the nervous system called acetylcholinesterase (AChE), which is critical to the proper functioning of vital body systems. Treatment for exposure involves reactivating AChE, but current drugs vary in their efficacy.

Work of IBBR Associate Director **David Weber**, CBT researcher Kaylin Adipietro, and their collaborators was featured on the cover of the journal *Protein Science* in June 2019. The team developed and validated a simplified version of AChE that can be used to facilitate future structural biology studies aimed at a better understanding of the enzyme and the creation of new reactivating therapeutics.



David Weber: *Professor, UMB Department of Biochemistry & Molecular Biology; Director, Center for Biomolecular Therapeutics (CBT)*

Exploring the Cancer-Fighting Promise of Galeterone

IBBR Fellow **Vincent Njar** is a medicinal chemist with an interest in small molecule anti-cancer agents. Galeterone, Njar's first lead clinical candidate, has shown promising activity against prostate cancer in Phase I and II clinical trials, and his team is working with other entities to continue to develop galeterone for this application. In addition, an upcoming Phase II clinical study for patients with metastatic pancreatic cancer will test galeterone alone and in combination with gemcitabine, an existing chemotherapeutic.

Vincent Njar

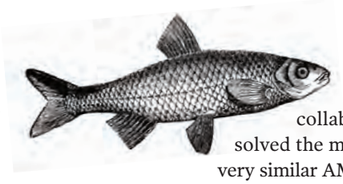
The team recently received funding from the NIH to support development of next-generation molecules similar to galeterone, and a related class of novel compounds is being explored for treatment of triple-negative breast cancer, prostate cancer, and dermatological conditions like eczema and chronic wounds.

Vincent Njar: *Professor, UMB Department of Pharmacology; Head, CBT Medicinal Chemistry Section*

Fish Antimicrobial Peptides May Hold Clues to Antibiotic Alternatives

Antimicrobial peptides (AMPs) are small immune system proteins found in all living things. AMPs from one organism bind to and disrupt the cellular membranes of competitor organisms. For example, many animals and plants produce protective, positively-charged AMPs that compromise the negatively-charged membranes of bacterial cells. Researchers are exploring these antibacterial AMPs as alternatives to traditional antibiotics.

IBBR Fellows **Ella Mihailescu** and **Vitalii Silin** and their collaborators recently solved the mystery of how two very similar AMPs have quite different toxicities. Two model AMPs from fish—piscidins 1 and 3—differ only slightly in structure, but p1 kills bacteria in minutes, while p3 requires hours.



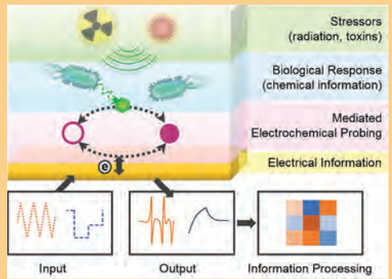
Both researchers are affiliated with the NIST Center for Neutron Research, a world-class, federal resource that provides neutron measurement capabilities for the US research community. Using a sophisticated method called neutron scattering to study the interactions of piscidins with membranes, Mihailescu and Silin learned that small changes in the number and position of positively charged amino acids called histidines can have profound effects on AMP biological activity.

Ella Mihailescu: *IBBR Assistant Professor; Research Associate, NIST Biomolecular Structure and Function Group*

Vitalii Silin: *IBBR Associate Professor; Research Associate, NIST Biomolecular Structure and Function Group*

Diagnosing Disease On-The-Go

IBBR Fellows **Gregory Payne** and **William Bentley** are taking an unconventional approach to developing a new biomedical diagnostic. Rather than figuring out a way to measure a well-studied chemical associated with a particular disease, these engineers are starting at the other end of the challenge.



Payne and Bentley first asked what measurements can be made robustly and quickly on a deployable device for use in a clinic, at home, or even on the battlefield. They settled on "redox" chemistry. Redox reactions—which cells use for a wide range of biological functions—involve the transfer of electrons, which allows "communication" between biological systems and an electronic device.

Now, with funding from the US Department of Defense, the team is collecting measurements from biological samples and looking for patterns that distinguish healthy and unhealthy states. They expect their work to inform basic biology questions as well as the development of their diagnostic sensors.

William Bentley: *Robert E. Fischell Distinguished Professor, UMCP Fischell Department of Bioengineering; Director, Robert E. Fischell Institute for Biomedical Devices*

Gregory Payne: *IBBR Professor*

VINCENT NJAR PHOTO: TOM JEMSKI AND MARK TESKE, UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

FISH BY WHITEMAY/ISTOCK, POPLAR TREE LEAVES BY GARY COLEMAN, MOSQUITO BY FLUBYDUST/ISTOCK

Insect Biology at IBBR

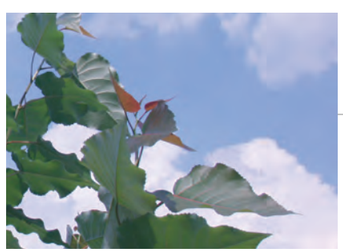
Researchers from around the world who study insect biology, including disease transmission, have a valuable resource in a unique facility housed at IBBR. The University of Maryland Insect Transformation Facility (ITF), directed by **Robert Harrell**, is an international authority for service and training in insect genetic modification technologies.

The facility has a special focus on mosquitos, but ITF personnel undertake projects involving other insects as well. Recent efforts have supported:

- a molecular understanding of mosquito biting behavior, and identification of small-molecule drugs to suppress host-seeking and biting;
- a study of how mosquitos choose egg-laying locations based on water salinity, a project that could inform interventions to decrease mosquito reproduction; and
- an investigation into the relationship between the parasite that causes the tropical disease leishmaniasis and its sand fly host, through the first-ever CRISPR-Cas9 modification of this insect species.

Agricultural Biotechnology Center

Food safety, nutritional security, renewable energy, development of plant-based therapeutics—these and other challenges are the focus of IBBR's Agricultural Biotechnology Center (ABC), directed by IBBR Affiliate Fellow **Angus Murphy**. The ABC supports collaborative projects across IBBR and the University of Maryland, College Park, towards the discovery and application of science-based solutions to pressing challenges of 21st century agriculture.



Poplars offer rapid growth and quickly produce substantial plant biomass with high cellulose and low lignin contents. The high cellulose content provides the carbohydrates necessary to produce bioenergy. The low lignin content simplifies the extraction of carbohydrates from the biomass for conversion into liquid transportation biofuels.

IBBR Fellows **Edward Eisenstein** and **Shunyuan Xiao**, and IBBR Affiliate Fellows **Gary Coleman**, **Yiping Qi**, and **Vijay Tiwari**, are taking a multidisciplinary approach to enhance the value of poplar trees for bioenergy and bioproduct use. Their efforts include:

- evaluating and improving how plants absorb and use nutrients, with the goal of optimizing growth and minimizing fertilizer application to reduce runoff pollution;
- understanding and harnessing how trees respond to biological stresses, such as pathogens, and acclimate to abiotic stresses, such as drought and ultraviolet radiation; and
- developing methods to probe and control the mechanisms that plants use to balance growth and defense.

Gary Coleman: *Associate Professor, UMCP Department of Plant Science & Landscape Architecture (PSLA)*

Edward Eisenstein: *Associate Professor, UMCP Fischell Department of Bioengineering*

Angus Murphy: *Professor, PSLA*

Yiping Qi: *Assistant Professor, PSLA*

Vijay Tiwari: *Assistant Professor, PSLA*

Shunyuan Xiao: *Professor, PSLA*

IBBR's Translational Management Office

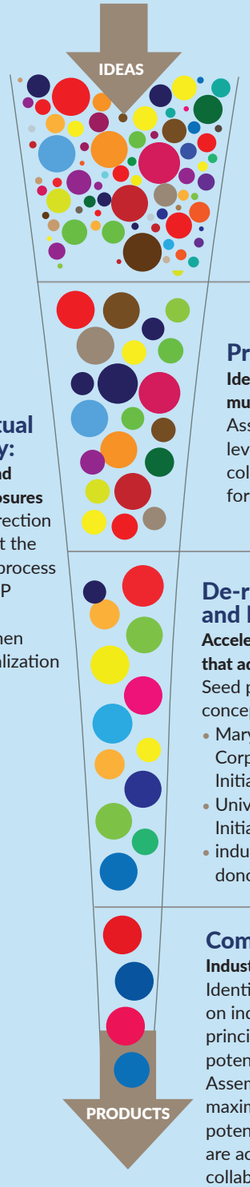
Accelerating Discoveries to the Marketplace by Bridging the Academic-Industrial Divide

What is translational research?
Applying findings from basic scientific research to create new therapies and diagnostics that answer unmet medical needs.

EXCITING DISCOVERIES ARE MADE IN ACADEMIC LABORATORIES EVERY DAY, BUT MANY NEVER ADVANCE TO PRACTICAL APPLICATION IN THE COMMERCIAL MARKETPLACE. To prove and showcase the commercial potential of an innovation requires access to alternative sources of translational funding and industry-experienced project managers that complement researchers' scientific knowledge. IBBR's Translational Management Office (TMO) partners with Institute researchers to bridge this "academic-industrial divide."

TMO staff Yunus Abdul and Viqar Aslam

The TMO aims to:
1) facilitate the translation of new biomedical and other life science discoveries into commercial products,
2) help IBBR researchers obtain funding and form commercial partnerships, and
3) realize significantly greater value from discoveries for the University, its faculty, and partners.



Discovery
Assess and select commercially promising projects
Conduct market, competitive landscape, IP, business strategy, and scientific assessments to prioritize projects.

Intellectual Property:
Strategy and New Disclosures
Provide direction throughout the discovery process regarding IP strategy to strengthen commercialization prospects.

Project Management
Identify key components and create multidisciplinary teams
Assemble faculty-led project teams, leveraging UMB, UMCP, and external collaborators to achieve milestones for commercial application.

De-risking, Implementation, and Funding
Accelerate achievement of milestones that add value
Seed projects to generate proof-of-concept data. Funding sources include:
- Maryland Technology Development Corporation Maryland Innovation Initiative (TEDCO MII);
- University of Maryland Strategic Initiative: *MPowering the State*; and
- industry sponsors and philanthropic donors.

Commercialization
Industry outreach
Identify market opportunities based on industry inputs that help inform the principal investigator of the commercial potential and set relevant milestones. Assemble and align project teams to maximize translational impact. Notify potential partners as milestones are achieved. Negotiate and finalize collaborations.

TEDCO MII Funded IBBR Translational Projects

- Developing Novel Polyphosphazene Technology for Stabilizing Protein Therapeutics
  Alexander Andrianov, *IBBR Professor*
- Developing Next-Generation Multi-Specific Antibody Therapeutics for HIV-1
  Yuxing Li, *Associate Professor, UMB Department of Microbiology & Immunology*
- Developing Targeted Enzymes for Effective Treatment of Lysosomal Disorders
  Silvia Muro, *IBBR Associate Professor*
- Developing Drought-Tolerant Crop Cultivars with Improved Water-Use Efficiency
  Shunyuan Xiao, *Professor, UMCP Department of Plant Science & Landscape Architecture*

PHOTO BY JOHN T. CONSOLI

Green Initiatives at IBBR

"How do you eat an elephant? One bite at a time."

This oft-cited proverb applies to a formidable, but worthy, challenge that requires vision, creative thinking, and unwavering perseverance to achieve.

Mounting examples of the impacts of climate change are increasing the urgency with which organizations around the world, including IBBR, work to address the formidable challenge of dramatically reducing energy consumption and carbon emissions.

Research laboratories consume a tremendous amount of energy. IBBR's array of specialized instruments require a consistent environment while consuming power and generating heat around the clock. And, fume safety hoods require 5-10 times more ventilation of conditioned (heated/cooled) air than an office.

This all makes for a formidable challenge, but under the leadership of IBBR's Director of Facilities and Lab Services, Jim Johnson, we are meeting the challenge "one bite at a time."

2012
Facility-wide lighting upgrade: fewer and higher efficiency fluorescent lamps
savings: 70 homes

2013
New, more efficient chiller
savings: 13 homes

2015
New lab air safety monitors, used to optimize lab ventilation
savings: 18 homes

2017
Solar panel installations: parking lot canopy and roof arrays
savings: 22 homes

2018
Lighting upgrade to LEDs, including tunable lights for the greenhouse
savings: 51 homes

HVAC office control upgrade
savings: 3 homes

HVAC lab control upgrade, including fume hoods
savings: 155 homes

Chiller consolidation for entire facility
savings: 18 homes

According to the US Energy Information Administration, the average US home used 11,764 kWh of electricity in 2018.

Maintaining the momentum…

IBBR is dedicated to continuing this important work on the path to carbon neutrality by 2050, as outlined in University of Maryland President Wallace Loh's 2017 Climate Action Plan. We are exploring several ways forward:

- additional heating plant efficiencies
- steam turbine chiller to link chilling to our efficient heating capabilities
- energy production options aimed at independence from the power grid
- waste reduction strategies

Between April and September 2019, we used **21%** less gas and **26%** less electricity than during the same period in 2018.

HOUSE ICON BY ALEXANDER SKOWALSKY & LEAF BY YANTI FROM THE NOUN PROJECT, ELEPHANT BY LEONTURA/ISTOCK

UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE
& BIOTECHNOLOGY RESEARCH

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Exploring the structure and function of biomolecules

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