KEYWORDS: COVID-19, *Coronaviridae*, infectious diseases, genome sequencing, future antiviral treatments

Photos Courtesy of Dr. Robert G. Hammond

OAKWOOD GRAD Pursues Research Dream

BY ALEX AAMODT

When Dr. Robert G. Hammond began to study them, coronaviruses were just another obscure pathogen on the outskirts of the collective consciousness. Yes, the SARS outbreak of 2003, which killed some 700 people and sickened thousands more, was still a vivid memory for some—especially within the countries in Asia that were hardest hit—but the average person likely couldn't identify what a coronavirus even was. Another coronavirus disease, MERS, emerged as a deadly threat in the Middle East during 2012, again killing hundreds, but did not make the jump to a truly global crisis.

It was 2013 when Hammond started his doctoral studies in chemistry at the University of Alabama at Birmingham (UAB). He would be working in a new lab, established by Dr. Margaret Johnson, who had also just joined the institution as an associate professor and researcher. Some of Johnson's previous work had included

studying the minute structure of the original SARS virus, and the new lab would continue working to better understand such viruses. The implications of such research at the molecular level are profound, for it can be the foundation for developing groundbreaking antiviral drugs.

Hammond was intrigued by the significance of the viruses, but expected the work to remain in an obscure scientific niche.

Dr. Robert G. Hammond

"I [was] just resigned to the fact that my work would probably never be understood or relevant to people," Hammond told me recently. "No one knew what it was."

Of course, in just a few short years, all that would change.

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The COVID-19 pandemic, caused by the SARS-CoV-2 virus, that has uprooted life in every corner of the globe during 2019 and 2020, seemed like a shock out of the blue, but it really shouldn't have been a surprise. The journalist David Quannmen, writing in *The New Yorker* not long into the pandemic, recounted meeting with Dr. Ali S. Khan in 2006. Khan was then in charge of combating emerging diseases for the CDC in the United States, and while there are many fascinating and terrifying diseases caused by pathogens, Khan had a quick answer for what he thought was the most interesting: SARS.

"Because it was so contagious, and so lethal. And we were very lucky to stop it," Khan said.[1]

A quick refresher, if one doesn't remember the details of the original SARS: In late 2002 and early 2003, a cluster of pneumonia patients—whose conditions couldn't be traced to any standard diseases—began to concern health officials in Southern China. Eventually a new-to-humans virus, SARS-CoV, would be identified as the cause. It quickly became concerning, as evidence emerged that it spread from person to person with rapidity. A large number of eventual cases were traced to one man sick with the virus who stayed in a hotel in Hong Kong—a superspreader. Other hotel guests on his hallway became sick, and spread the new disease to several countries. Eventually it was contained, with less than 10,000 known infections, through a concerted effort to isolate patients and trace their contacts. Still, many health authorities, including Khan, thought that avoiding a global disaster had been extremely lucky; all the elements of a pandemic had been there.

The coronavirus family, *Coronaviridae*, has many different members, and can infect everything from plants to mammals to birds. Seven are now known to infect humans. Ascending the coronavirus family tree, there is one of the four genera that has been relevant to the most dangerous human infections: betacoronaviruses. Ascending even further, betacoronaviruses are separated into four lineages, labeled A, B, C, and D. SARS and COVID-19 come from lineage B, MERS from C (two from lineage A are among the many viruses that cause the common cold).

All of the complicated taxonomy is to say, there are many coronaviruses, a few of which infect humans, and a few of which cause serious diseases when they do so. A connecting thread is that most, if not all, likely originate in bats. For reasons that scientists are still exploring, bats are excellent hosts for viruses; they seem able to carry many of them without being negatively affected. There are other betacoronaviruses found in bats that haven't infected humans but are similar to those that cause the dangerous diseases, and studying them can be useful in finding weaknesses that might apply across the whole of the betacoronavirus genus. Perhaps there is a common thread that could neutralize all of the dangerous ones in one fell swoop.

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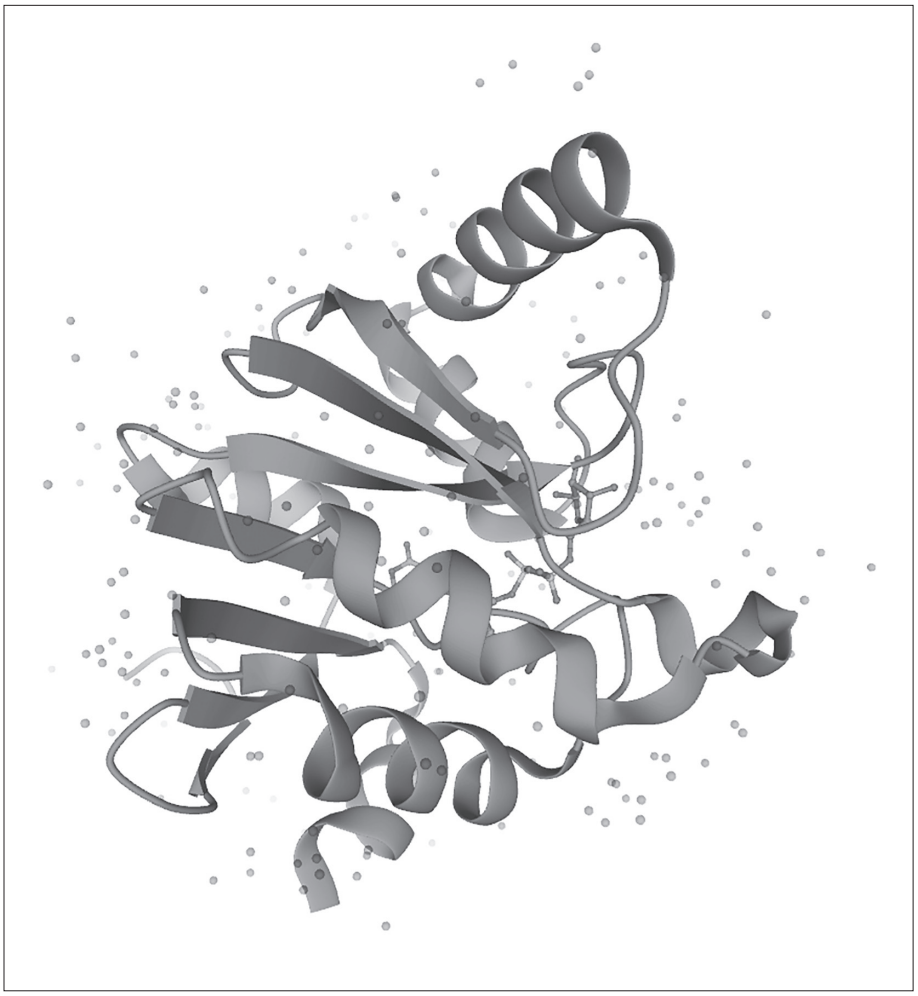

Image courtesy of the RCSB Protein Data Bank (rcsb.org)
PDB ID: 6MEA
Hammond, R. G., Schormann, N., McPherson, R. L., Leung, A. K. L., Deivanayagam, C. C. S., Johnson, M. A. (2019) Crystal structure of a Tylonycteris bat coronavirus HKU4 macrodomain in complex with adenosine diphosphate ribose (ADP-ribose) doi: 10.2210/pdb6MEA/pdb

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Robert Hammond grew up in the Dallas-Fort Worth area of Texas. He attended Forest Lake Academy in Florida for part of high school, and then went on to complete a degree in chemistry at Oakwood University, graduating in 2008. He was interested in doing an MD-PhD program, wherein he could engage with scientific research alongside medical practice, but had trouble finding the best path forward.

"At the time, I didn't really understand what graduate school was like," Hammond said, remembering the difficult application process. He didn't feel that he had many mentors who could help navigate all of the complexity, and after not getting into the programs he targeted, looked for what to do next. "I had to figure things out and try a different method," he said.

Hammond settled on teaching. After doing several different jobs for a time, he taught science for two years at an intercity public high school in Texas and then at a suburban school. Still, the ambition to do graduate work remained, and when a scholarship for studying at UAB became available in 2013, Hammond took the opportunity.

Since the UAB lab where he would be working was brand new, there were challenges and work to do beyond what might be typical when starting a PhD.

"They had to renovate and make a new space for us," Hammond remembered. "It was interesting those first years, but I learned a lot, for sure, about how to start a lab." He also spent time developing protocols and standards for the operation of the lab.

Hammond worked to get several projects up and running. One that he spent a significant amount of time working on—an attempt to make a complex polymer—never functioned as planned. Around 2015, he started work on analyzing the genome of a bat coronavirus, catchily named HKU4, which is closely related to the MERS virus—it might be thought of as a sibling. So far, it has never been known to infect humans, but its genetics are very similar to the MERS virus that does.

Today, unlike the early days of working with infectious diseases, it's possible to do a multitude of research without actually handling an intact—and potentially dangerous—virus. If researchers are able to get a sample of a virus, whether from a human patient or from an animal, it's now a relatively simple task to sequence its genome, which can then be shared with researchers around the globe; everyone can look at the molecular details from afar.

HKU4 was actually found and sequenced before MERS made the leap to humans. In 2006, researchers

in Hong Kong published the results from 309 samples taken from different species of bats over a sixteen-month period.[2] HKU4 was one of six new coronaviruses they discovered, and was found in the lesser bamboo bat, a miniscule species with a body only an inch and a half long.

From his lab in Birmingham, Hammond looked at HKU4's genetic sequence to determine a promising research target.

A single virus particle, a virion, is a remarkably small yet efficient structure. "Viruses are perfect parasites," an author in the *Biophysical Journal* described[3]. They also exist in a strange area between the living and non-living. Without the mechanisms of an organism, they multiply only by hijacking cells, using the host's molecular machinery for their own purposes. The virion is just a bundle of genetic material that holds the instructions for replicating itself, surrounded by a shell of protein (enveloped viruses, which include coronaviruses, have an additional outer layer as well).

To be successful in replicating, a virion has to figure out how to enter a host cell, and then, once inside, copy itself. A simple idea, but understanding the details of the process at the tiny scale where it happens is difficult, and even today filled with many unknowns.

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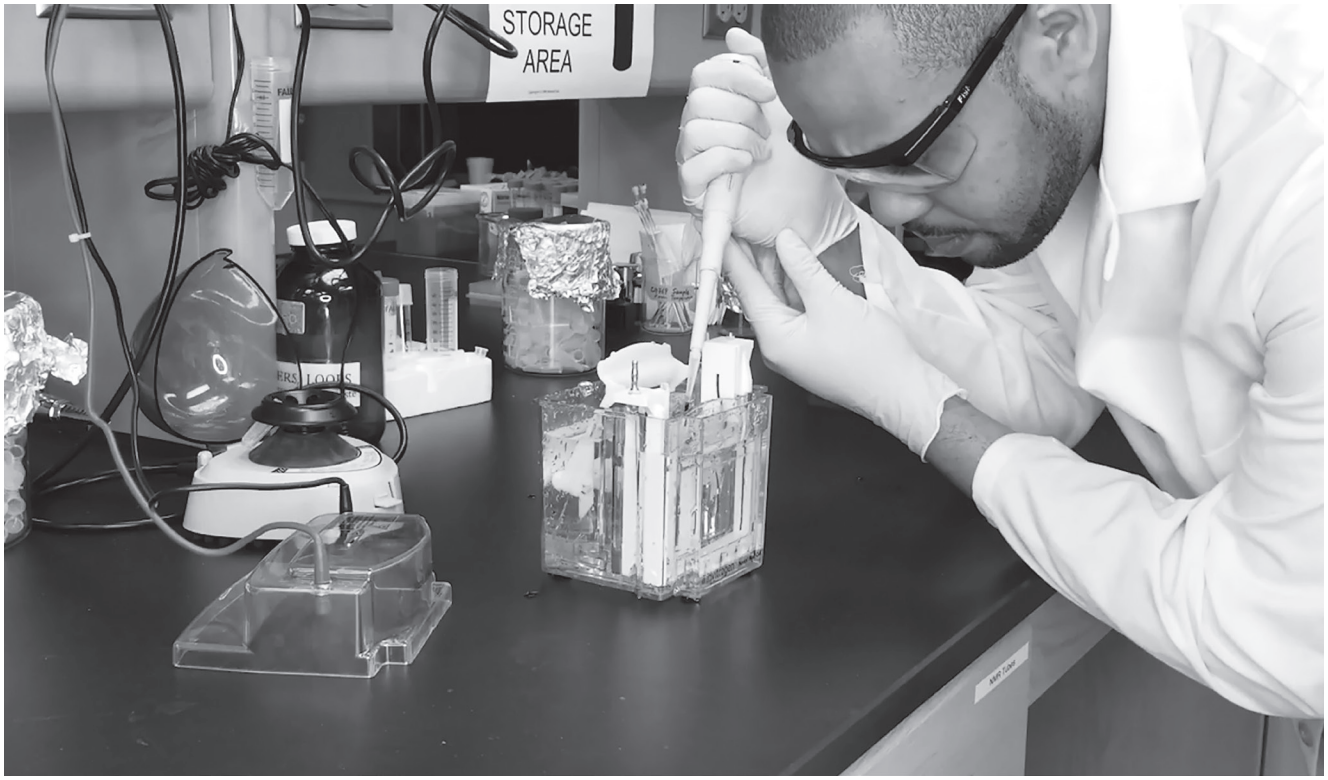
"I wanted to know the shape of some of the proteins that were made after the virus infects a cell," Hammond explained to me about the goal of his research. These proteins are of great importance because some are involved in perpetuating the replication process. Identifying the correct proteins and developing a drug that stops them—while also not damaging human cells—could lead to an effective therapy. Since many of the proteins are shared between different strains of virus, such a therapy might work across multiple examples.



Hammond settled on trying to study a section of a protein in HKU4 called a macrodomain.

"I tried to find a particular domain that had high physiological value," he said. With the potential section identified, he began working to create it in the lab so that he could then study it closely.

Creating a complicated protein is no easy task. First, Hammond had to clone the section he wanted to study, then implant it into E. coli bacteria. Next, he nurtured and fed the bacteria, so that it and the cloned protein would grow. Once properly developed, the protein then needed to be removed from the bacteria that had harbored it.

"It looks like this brown putty," Hammond said of the matured E. Coli and protein mixture. He concentrated it into a pellet, then chopped it up and added water to make a sludge-like material, which was bombarded with sound to break up all the bacteria cells into fragments. Only then could the fragments—the "guts" of the bacteria—could be filtered out, leaving only the desired protein, pure and unadulterated.

If it sounds like a complicated process, that's because it is. When he first started, it took Hammond around a month to advance through all the steps and get an individual sample. Later, with more practice, he cut the time to a week, though doing so sometimes required staying in the lab for eighteen to twenty hours straight to do multiple steps at once.

Originally, Hammond wanted to study the protein with a technique called nuclear magnetic resonance (NMR). NMR is related to the MRI machines familiar in hospitals, but shows the structure of molecules rather than tissues in the human body. It is a powerful tool that can give a three-dimensional look at the atomic level, but also is very sensitive and difficult to make work properly. In 2017, Hammond and his UAB collaborators used NMR to map the structure of a protein in another coronavirus, HKU9, and he wanted to use the same technique on HKU4.[4] Yet with time starting to run out and needing to finish his doctoral work, Hammond was still fighting with the NMR process and decided to move to the technique of X-ray crystallography (Hammond did eventually succeed with the NMR structure on a different HKU4 domain, which was published to the RCSB Protein Data Bank in 2018).

There was help from other scientists as well. Hammond met a researcher from Johns Hopkins who ended up having additional tests that would be helpful. Back in the lab, Hammond flash-froze some of his samples, put them on dry ice, and shipped them to Johns Hopkins—crossing his fingers that they would survive the trip. They did, and the tests delivered more useful information.

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At the end of 2018, Hammond graduated and moved on to the other part of his original plan: medical school. While at UAB, he published several papers, including one with work on another bat coronavirus, but, since graduating, he has been working to publish his most significant research, from the HKU4 virus. While always a significant topic, the start of the COVID-19 pandemic has changed the stakes for such research, with the whole world clamoring for scientific answers to the health emergency caused by the SARS-CoV-2 betacoronavirus. Suddenly, everyone knows the significance of what he was working on all those years.

When we first spoke, Hammond was still trying to publish his principal research, but in the summer of 2020, the full paper—coauthored with his collaborators at UAB and Johns Hopkins—was accepted for upcoming publication by the *PNAS*, the official journal of the National Academy of Sciences (known for being one of the most prestigious scientific journals in the world). The paper discusses both the structure of the macrodomain, and of three different mutations that Hammond created. The mutations are essentially small alterations to the protein, to see if it's possible to change how the virus replicates and thwarts the host's cellular defenses. And the

Identifying the correct proteins and developing a drug that stops them—while also not damaging human cells—could lead to an effective therapy.

paper concludes that yes, the changes did affect how the macrodomain functioned.

Hammond's work isn't a cure for betacoronaviruses, but it might contribute to a future antiviral treatment. As of July 2020, several of the many developers rushing to create a COVID-19 vaccine have announced promising early results, but there is still no guarantee that a safe and effective vaccine will ever be ready for widespread use. Hopefully, one will be, but even so, a future antiviral treatment could still be immensely important. There are many other betacoronaviruses out there, lurking in different bat species, with the potential to jump to humans. The odds are that in the coming years other ones will.

Now in his second year of medical school at Meharry Medical College in Nashville, Tennessee, Hammond is working to ready himself to do further science that leaves the sort of impact he wants to see in the world.

"I realized that in order for my research to be as effective as I wanted it to be, it needs to be as close to the community as possible," he said. The sum of his experience, from being a teacher to a lab scientist and soon a medical doctor, has left him wanting to develop his own lab in the future.

"I want to develop a research facility for people to enter the scientific process," Hammond said. "And I want different types of people other than [from] the selective process that I think our current system presents."

Hammond thinks there is a dearth of both minority and Adventist scientists who present their work in high-impact journals: a fact he is determined to change.

Endnotes

1. David Quammen, "Why Weren't We Ready for the Coronavirus?," *The New Yorker*, May 11, 2020.

2. Patrick C.y. Woo et al., "Molecular Diversity of Coronaviruses in Bats," *Virology* 351, no. 1 (May 2, 2006): 180–187, https://doi.org/10.1016/j.virol.2006.02.041.

3. Fredric S. Cohen, "How Viruses Invade Cells," *Biophysical Journal* 110, no. 5 (March 8, 2016): 1028–1032, https://doi.org/10.1016/j.bpj.2016.02.006.

4. Robert G. Hammond, Xuan Tan, and Margaret A. Johnson, "SARS-Unique Fold in The Rousettusbat Coronavirus HKU9," *Protein Science* 26, no. 9 (2017): 1726–1737, https://doi.org/10.1002/pro.3208.

ALEX AAMODT is a staff writer and the Roy Branson Investigative Reporter at *Spectrum*. He has degrees in English and Spanish from Walla Walla University and is based in Portland, Oregon.