Jonathan Shrimp, Ph.D., Research Scientist, Early Translation Branch, National Center for Advancing Translational Sciences (NCATS)

Behind the Mask
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Interviewed by Gabrielle Barr, Archivist, Office of NIH History and Stetten Museum, NIH

Barr: Good afternoon. Today is October 27, 2021. My name is Gabrielle Barr. I'm the Archivist at the Office of NIH History and Stetten Museum. Today I have the pleasure of speaking with Dr. Jonathan Shrimp. Dr. Shrimp is a research scientist in the Early Translation Branch at NCATS. Today, he's going to be speaking about some of his COVID-19 research experiences. Thank you very much for being with me.

Shrimp: Thank you, Gabrielle, for having me.

Barr: To begin with, you've done a lot of work on TMPRSS2 in COVID-19. What role does TMPRSS2, also called transmembrane protease serine 2, play in the virus's lifecycle? Why is it such an attractive target for therapeutics against COVID-19?

Shrimp: Within the virus life cycle, in order for the virus to actually replicate, it first has to enter a host cell. In this case, that's the first step in the whole lifecycle. That's the part that TMPRSS2 is relevant in. It's relevant in viral entry. One mechanism for SARS-CoV-2 entry occurs with a spike protein. That's a protein that's on the surface of the virus. It binds to a receptor on the host–cell surface, and that host–cell–surface receptor is ACE2 [angiotensin-converting enzyme 2], and then after that occurs, there has to be cutting at two sites on that spike protein. The cutting allows for a conformational change of that spike protein. Then that allows for a fusion to occur between the virus and the human host cell. TMPRSS2 has been shown to be capable to cleave at those cut sites. In addition to its relevance in SARS-CoV-2, it's been demonstrated to influence viral entry in other viruses, such as other coronaviruses.

Barr: So you've worked on that before in previous work?

Shrimp: No, I've never worked on any coronavirus work or anything, but just within the literature. Within the literature, it's been shown to be relevant in influenza entry into a host cell, as well as some other coronaviruses. For that reason, it's become an attractive therapeutic target, not just for COVID-19, but also for influenza.

Barr: When did you begin working on TMPRSS2 in regard to COVID-19? And can you talk a little bit about some of your initial work?

Shrimp: Yeah, sure. So I began working on it in the beginning of the pandemic around April [2020]. Around that time, in April, a paper had just been published. It's been widely cited since then, over 11,000 times. It showed that if you add a molecule known as camostat to cells, that it could reduce infection of the virus in lung cells. This molecule camostat—in the paper, it was described as being a TMPRSS2 inhibitor. But the thing is, really, from that publication there wasn't any direct evidence showing that camostat would actually inhibit TMPRSS2. That's really where the work that I did came into play because it would really address that question—does camostat really directly inhibit TMPRSS2?

Basically, I developed a biochemical assay. What that means is that I obtained purified TMPRSS2 protein from a commercial vendor. I also obtained a fluorogenic peptide substrate. The amino acid sequence of that peptide substrate matched a recognition sequence that's known for that enzyme. Basically, if TMPRSS2 was active and it was able to cleave that peptide, you would see an increase in fluorescence from the base substrate that was a fluorogenic peptide. Overall, we optimize this assay to be capable to be used in a fifteen thirty-six [1,536]–well plate [a flat plate with 1,536 wells used as small test tubes]. That's significant because then you could use this assay to test thousands of inhibitors while using just a little bit of reagent. It allows you to be able to test a lot while using only a little reagent. Then also within the assay, we were able to demonstrate inhibition of camostat directly on TMPRSS2, which was a question that we wanted to address at the time.

Barr: So you've continued this analysis. Can you talk a little bit about where you have gone since that initial paper? I know you that you have analyzed more than 6,000 compounds for testing.

Shrimp: Yeah. So originally, in that first publication, it was just to test a few molecules, in particular camostat and nafamostat, which are two inhibitors that went into clinical trials for a COVID-19 therapeutic. Since then, we screened over 6,000 compounds and tested the inhibition on TMPRSS2 of those. Then also, we made that data publicly available immediately on a website called Open Data Portal. All that data was made public.

Barr: How long did it take you to test these 6,000 compounds? I would imagine that takes quite a lot of time and organization.

Shrimp: Yeah, for sure. It only took a few weeks though because the compounds that we tested were already well organized into plates, so I was able to do the experiment pretty quickly. Within a few weeks, I was able to generate that data. We were able to post it on the website and make that publicly available.

Barr: Were you surprised by any of your findings, or did you find anything particularly interesting?

Shrimp: I think what was surprising at first to me was the low number of actual inhibitors that we discovered out of 6,000 compounds. There was a very low hit rate, but that just really indicated that the enzyme was very selective as to what it actually bound in the active site. That was surprising to me.

Barr: Have any of these compounds been tested in animal trials and in human trials yet?

Shrimp: Yeah. As I was mentioning a little bit, camostat and nafamostat are two very similar molecules. Those have gone into human clinical trials, and those went into trials last year as well as this year. Camostat has actually had results come out, but they have not showed any success. They've been used both in hospitalized patients as well as outpatients. Neither one of those trials have actually shown efficacy. Then [for] nafamostat there's still clinical trials ongoing and data on that should be coming out. Since my publication though, as well, there have been certain peptide-based inhibitors of TMPRSS2. Those have gone into mouse studies. I believe that they're probably being out-licensed to companies for further development.

Barr: That's exciting.

Shrimp: Yeah, but I don't really know details on that.

Barr: Can you talk a little bit about your approach for comparing structural models and analyzing binding sites, and how you went about using the NCATS extensive library of compounds for your studies?

Shrimp: Yeah, so for structural models, at the time of our assay development there were no crystal structures of this protein. The alternative to that was that our informatics team did develop a homology model of TMPRSS2. They did that by using an enzyme that's very similar to TMPRSS2, and then basing the structure of TMPRSS2 on that structure that is a similar protein. That was useful and we were able to use that for docking studies in order to see how a compound might bind to the protein. Then, for using the NCATS libraries, that's where the 6,000 compounds came from. There were libraries that were already set up in NCATS.

Barr: What were some of the challenges that you experienced with your studies with TMPRSS2?

Shrimp: Yeah, I think one of the biggest challenges for the assay setup was acquiring an active enzyme. So for this, I purchased several different constructs that were expressed in different expression systems and tested them for activity. Several of them, even though we purchased them from a company, they didn't have any activity. Fortunately, I was able to get one that was active. But even that was only fractionally active. It was very minor activity. Then I had to use a really high protein concentration in order to really see any activity. That was that was probably the biggest challenge to really developing the assay at the time.

Barr: What are your plans with this type of research going forward?

Shrimp: Since that one assay, we've looked to develop an orthogonal assay. It's an assay with a different detection technology. The utility for that would be if the first assay with one detection technology had identified something that was maybe a false positive, then that second orthogonal type of assay would be able to potentially identify it as a false positive, because it has a different detection technology. So that's one thing. And then just continuing to be able to profile new molecules that are synthesized. You can still use this assay and determine the potency of those molecules against TMPRSS2.

Barr: Interesting. Have you been involved in other COVID initiatives on campus, whether it be research or other types of initiatives like volunteering in some way?

Shrimp: The only other project that was relevant to COVID-19 research that I was involved in was a cell–based assay and it is with an NIH collaborator. For that there's a viral protein called ORF3a, and it's a pro-apoptotic protein. The thing that I was trying to do for that assay was, I would express this protein in cells and then add small molecules and see if I could reverse the pro-apoptotic phenotype. However with that assay, nothing really

showed that it could really reverse the pro-apoptotic phenotype, but that was another assay that I that I worked on, as well.

Barr: In addition to being a scientist, you're also a person who's been living through the pandemic. What have been some personal opportunities and challenges that COVID-19 has presented for you?

Shrimp: I think in the way of work, it's really opened up just a new experience for me for assay development, developing a biochemical assay, and just learning how to set that up from scratch from the very beginning. I've learned a lot just doing that, and then [I'm] also learning a lot just about this area of science in viral research and antiviral drug development.

Barr: Have you been in NIH for a long time or are you relatively new to the institution?

Shrimp: At NCATS, I've only been here a little bit over two and a half years.

Barr: Okay.

Shrimp: Yeah.

Barr: So that is relatively new in NIH years.

Shrimp: Yeah, yeah. So I did do a postdoc at the National Cancer Institute for four years. I guess overall, within NIH, it's been six and a half years.

Barr: Those are quite a lot of opportunities. Was it the first time you were a PI, with the enzyme assay project that you worked on?

Shrimp: Are you asking if this is the first time I was leading?

Barr: The lead, yeah.

Shrimp: It's not necessarily the first time I've ever been a lead on assay development, I guess, and really working on it. NCATS is a very supportive environment. I was able to get advice from other colleagues as well, which was really important for me developing the assay and getting this work done.

Barr: Yeah. Can you talk a little bit about people who you collaborated with or who assisted in some way with your COVID research?

Shrimp: The one project that was the ORF3a-related, that collaboration was with Michael Lenardo from NIAID [National Institute of Allergy and Infectious Diseases]. He had the initiative to start that project and really understand and study ORF3a. And then with TMPRSS2 and the assay development there, I had several colleagues really contribute. They're also listed on the publication.

Barr: That is really great. Well, is there anything else that you would like to share either about your COVID-19 research or experiences?

Shrimp: I gave you some opportunities, I guess, that I experienced through COVID-19. I think one of the challenges, just especially work-related, has been due to not having everybody working all at the same time. There's a heavy reliance on email and things like that. I guess the big challenge is just not being able to have the personal communication and [instead] having to rely on other types of communication in order to really interact with everybody at work.

Barr: I think you're not alone in that. I wish you continued success in all that you do, and of course, continued health. Thank you very much, once again, for sharing your research with us.

Shrimp: Thank you for doing this. I appreciate it, Gabrielle.

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