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BEHIND THE MASK of Dr. Keir Neuman Oral History

October 13, 2020

Barr: Today is October 13, 2020, and I have the opportunity to talk to Dr. Keir Neuman. Dr. Neuman is a senior investigator at the laboratory of single molecule biophysics at the National Heart, Lung, and Blood Institute (NHLBI), so we look forward to hearing about your research. Thank you so much for being with us today.

Neuman: You're very welcome. Thank you for inviting me.

Barr: So Remdesivir has become a household word during the COVID-19 pandemic. Can you discuss in lay terms how your research that is looking at the single molecule measurement of COVID-19 replication inhibition relates to the science behind how remdesivir and other therapeutics like it works?

Neuman: Absolutely. So, the remdesivir is a so-called inhibitor of viral replication. This is the key process that takes place during COVID infection, which is that the virus has to replicate. It's an RNA virus so it uses its RNA template to generate more RNA. The molecule that does that is this RNA-dependent RNA polymerase, so think of it as a copying machine. It copies the RNA. And the remdesivir and other similar viral inhibitors look like a molecule of RNA that's gonna be added. But they block the replication process and so that process then causes the replication to stop or the replication process to stop and can inhibit the sort of viral life cycle, if you will. So, what our research is involved in—so that you know these studies were found—what could you use to stop the viral replication from taking place? So, these compounds were found. Many of them have similar features, as they all look a little bit like a sort of RNA molecule. You're adding RNA molecules to a chain and they all look a little bit like RNA so the polymerase will accept them.

They'll incorporate into the growing RNA and then they'll cause it to either pause or cease replication altogether. That prevents the process from carrying on. What our research is involved in is understanding sort of at the molecular level, what are the details of that process? So what we do is we study this process one molecule at a time and we study the replication of a single RNA by a single polymerase or RNA-dependent polymerase. The notion is that by watching that process of inhibition in real time and looking at the sort of mechanical aspects of this or the physical aspect of the polymerase as it's moving along, we can understand what are the key determinants that actually lead to effective inhibition versus ineffective inhibition. There's a lot of these analogs, only some of them work, and by studying this in sort of molecular detail and teasing out the details of this process—how fast does it go? How long does it incorporate? Does it move backwards? Does it stall? When does it stall after it incorporates?

By understanding these processes in detail, the hope is that we can understand what really leads to inhibition of this enzyme that's effective clinically versus similar analogues that don't have the same clinical effects. We could then hope, as it's sort of a feedback loop, that understanding this process in sort of molecular detail, we could then make predictions or help people who are trying to tweak these drugs and make them more effective.

What are the real critical aspects of this? Then we could screen that at this molecular scale. What we're doing is we're really getting a microscopic view of an individual enzyme as it's moving, as it's replicating the RNA. Then the hope is to watch it in real time as it incorporates one of these inhibitors. Then ask what's happening in real time and not just seeing an end product where, oh, it's not working, but we can actually watch that process when it's incorporated. Does it slow down? Does it back up? Does it speed up? And these things are very difficult to tease out of a standard assay. A standard, you know, test tube, where you have billions and billions of these molecules working together; here we're watching one at a time and sometimes there's information that's encoded in those so-called dynamics, so if you're watching something moving back and forth something happens very quickly that you would miss if you're averaging over a test tube's worth of molecules. Whereas by watching individuals, we can tease out this whole pathway what's happening, and the hope is that we'll be able to determine what's the critical element of these inhibitors that lead to successful inhibition of replication versus not.

Barr: So, like you were seeing that you're looking at certain things like—how does it move? The length of time? But what are some of the other criteria that you're looking at—how these enzymes respond?

Neuman: What we're looking for is, what the model seems to be, that they produce either pausing or they produce termination [of replication]. So, it's either and so those are the endpoints that people have established. But, what's clear, is that there are lots of different inhibitors that will leave the same endpoints. So, the questions really amount to what distinguishes the ones that are effective versus the ones that are ineffective.

So what we can see directly is the sort of intimate details of that process, for example, if you look at the COVID virus, which is sort of a clever virus and it not only does it have an RNA dependent polymerase, but it has a sort of a proofreading mechanism, so it mis-incorporates as it's trying to make a very faithful copy of its RNA and, if it incorporates the wrong sort, it doesn't make a proper base pair RNA against RNA; then it can actually back up, undo that and move forwards. That prevents some of these from being incorporated. So, for example, we could ask which ones are proofread, which ones are really coming down to the sort of critical timing and critical process of how it is incorporated, and then what happens to the enzyme during that incorporation process. That could help us understand which ones are successfully incorporated, which ones are removed, and importantly also, what is the precise process which leads to the actual clinical effects. So, for example, we can measure a change in rate, we

can measure how long it pauses, we can measure what's the efficiency, and the other thing we can measure is how long does it pause. We can measure the efficiency.

The other thing we can measure very accurately every time it incorporates—does it pause and then keep going, does it pause and then go backwards, does it pause and then fall off. By understanding that process we can measure sort of the series of different outcomes or different effects at very high resolution. Then what we can do—we've done it with other inhibitors for topoisomerases, which for example are chemotherapeutic targets; you know that the basis of this proposal was some of our work on topoisomerases. Here the traditional assay is you add it to a cell and you ask how does a cell die, so you can collect an IC50. Since there was some confusion over what's the actual mechanism by which it's working, and so by watching this process take place in real time we could then sort of measure these sort of biophysical characteristics that we could measure how fast it's going, how long it's inhibited, and what not, and find an absolute perfect correlation between one of the things we could measure, the sort of single molecule assay and the IC50, and so with that we could establish the molecular determinants of inhibition.

So, the hope is with these inhibitors we could do something similar and ask at the level of a single enzyme what it is that leads to successful inhibition versus not and is it really simply a chain termination. But some chain terminators work better than others and there are some sort of general properties we can tease out of this process by watching them individually and looking at these where some could be very small scale and also rapid dynamics that you just absolutely miss in these sort of ensemble assays that are traditionally used. So that's the sort of one aspect of the research.

On top of that, just by understanding this process in much finer detail we may be able to also probe other aspects of inhibition that get past these sort of chain terminator or pause inducing additions, we could potentially probe other aspects of the polymerase and inhibit it in other ways.

Barr: Interesting. So, what are some of the techniques and tools that you are using in your work?

Neuman: The main tool we use is something we call magnetic tweezers. The way this works is we take a single molecule of RNA in a test tube—that's well understood—and we could make some fraction so that the viral genome is about 30,000 RNA monomers in a long chain. We can make some fraction of that, say half of it, or, if we really had to, we could make the whole thing. What we do is take normally… what you would do in a standard biochemical assay to make a whole test tube full of these, thousands and millions of these, and you would let the enzyme work on those. Then you would ask how and what it did. You would analyze that through various sorts of standard biochemical techniques.

Instead what we do is we have ways of attaching basically a little handle-like, molecular handles, on each end of the RNA, we put it onto a slide, a microscope slide, one end of which is attached to the slide and the other end is attached to a tiny micron-sized magnetic particle. We can now watch the motion of that magnetic particle with nanometer scale precision. The nanometer scale is one step. If you think about walking along this RNA molecule, each step is about, let's say a third of a nanometer or half a nanometer, and so we can watch the motion of that bead to almost a single nucleotide position. So now what we can do is introduce the polymerase as it is converting this long piece of RNA single-stranded RNA as it copies it. It changes its elastic properties, so, if you take a spring and change it—I'm pulling on a spring and a constant force and I change the elastic property—the spring will move.

So, what we can see is by pulling on this RNA as it's being replicated, then we'll see a decrease in the extension of the RNA. That tells us exactly how many nucleotides have been incorporated, how fast it's going, and in the case if the polymerase walks backwards, we can see the length of the RNA get longer again. We can also measure when the enzyme stops working and what the kinetics are: How quickly when it stops working; how quickly does the enzyme fall off; and this sort of thing.

So, what we're doing is really taking a single piece of RNA and, by monitoring the position of the bead attached to the end of the RNA, we can monitor the conversion of single-stranded RNA into double-stranded RNA. That's exactly what this process does. And the really critical thing is we can do this in real time, so I can measure this hundreds of times per second. I'm watching a single enzyme as it's walking along and polymerizing this RNA, making a new RNA strand. I'm watching that in real time. So, all of, you know, the back and forth motions or the stalling or even, you know, it does differently. It will incorporate different nucleotides at different rates which I can see in real time.

Then as we introduce these inhibitors, as I slow it down, how does it stop? Does it move backwards? The other thing we can then do is with this instrument, we've combined it. This is actually all the work of my colleague, Dr. [Yeonee] Seol, who's really the pioneer of this project. I'm the head of the lab but she's the one who actually thought this through and built all the instrumentation. What she's also done is added to this instrument the ability to track fluorescent individual particles. What we can do is by combining the two, I can ask how does this mechanical change take place? Then, if I put a fluorescent dye on the polymerase, I can ask a question, when does the polymerase leave? When does it stop? When does it leave? That tells me what is this mechanical activity or what is this enzymatic activity replicating the RNA?

Then when it stops, I can ask questions after it's stopped, how long does it remain bound because I can watch it through this fluorescent signal when the enzyme leaves the fluorescence and departs. So, by combining these two what we call single molecule techniques. So, I'm manipulating a single molecule of RNA. I can engineer it such as a single RNA polymerase or RNA-dependent RNA polymerase. We'll work on that single RNA and I can watch that single RNA polymerase by attaching a fluorophore or a dye molecule to it, and then, using microscopy techniques, to watch that directly.

Barr: So, with these two—that's quite a process about when the enzyme is there and correlate those two. Have you changed your approach at all or refined it since you began this particular project?

Neuman: No. This is a technique that we really established. I worked on it as a postdoc and then we've sort of refined this technique since coming to the NIH. This particular technique is really the focus of my lab and what we're doing now is our first foray into viral replication at all. What we're doing is sort of adapting this technique we've used primarily for things like helicase work or topoisomerases work and now we're adapting it to study the replication by the RNA dependent RNA polymerase. So right now, it's really a matter of just building up, the making the actual RNA substrate, obtaining the proteins, and with the hardware in place, it's really a matter now of sort of adapting hardware and some of these approaches to make these measurements.

Barr: Have there been challenges in adapting your approach and your hardware to a very different type of project? It sounds so different than what you've done in the past.

Neuman: The hardware itself is fairly robust and, as long as we can attach this magnetic bead, we can make the measurements. What we really struggle with is making this RNA substrate for the time being. It's a matter of we have to not only make this long RNA substrate, but we have to engineer what we call handles. So, we need little bits of molecular Velcro to stick it to the surface on one side and stick it to the bead on the other. We've traditionally worked with DNA where you have a little bit more control over things, and so working with RNA is just a little bit more challenging. We're working through that process of very precisely adding Velcro A at one end and Velcro B on the other end, and then stick it between the slide and the bead. That's really where we are right now and that's an ongoing process. I think once that's established everything else will fall into place and the measurements, too. Dr. Seol has really perfected the art of this measurement technique. So, once we have the substrate in place and then we have an enzyme that's active, I think we'll be off to the races.

Barr: That's great. Has anything surprised you so far in doing this research?

Neuman: What surprised me is obviously we just jumped into this and now we're reading the global literature and the pace of the field is remarkable. It's incredible how quickly people pivoted, and all the incredible research being done, not just for the NIH but around the world. We have a sort of a unique angle on this, so I think we'll be able to make some headway. It's really astonishing how much and how quickly the research is progressing. It's really satisfying to see that we come together as a community, as a research community, and push very quickly. I think it'll be interesting to look back in a year or two and realize just how quickly we did make progress on this sort of horrible disease from multiple aspects.

You know we're biophysicists and we don't typically get involved in sort of clinically related things, so that's exciting for us. I see that colleagues around the world are realizing this is an important thing to solve and it's an opportunity at the same time to do something really useful. So that's what's been remarkable is how quickly people pivoted and how fast the research is progressing. When you give people a really good problem to work on, it's important you see that motivation kick in.

Barr: That's my next question. Your background is in physics and applied math. How did you become interested in health and how do you think your background has helped you look at COVID-19 in many different ways than many of your colleagues?

Neuman: My trajectory from physics and math to biophysics really started fortunately as an undergraduate. I heard a very famous biophysicist, Steve Block, talk. He gave a seminar when I was an undergraduate at Berkeley and it changed my life. I walked in and I was gonna do physics and I walked out, and I wanted to do biophysics. In sort of thinking about it later, I actually ended up working in his lab and that was my introduction not just to biophysics but also to RNA polymerase. So actually, my graduate work was done on bacterial RNA polymerase, studying with very similar techniques using optical tweezers. That was really my entry point, working with Steve Block using optical tweezers to study RNA transcription.

Then I sort of moved on and studied these processes on topoisomerases. What we do really is we use two approaches—we take the tools of physics to build these single molecule instruments. We build them by ourselves. We build them from scratch. So, we use physical principles. We use magnetism obviously and optics to make these very high-resolution measurements, and then we take a very quantitative view. By watching individuals, we get these very complicated trajectories through space and time of what we're measuring but what that allows is very fine scale measurements of motion, a very fine scale rate of exactly what the enzyme is doing.

It turns out we can do very quantitative measurements on how fast does it go, on how long does it spend, what's the probability that it incorporates versus doesn't incorporate, and by measuring individuals we see not only these sort of coarse grain things but also we're following absolutely every motion these enzymes take.

To understand this, there's a lot of analysis that goes into this, a lot of sort of mathematical analysis in terms of how do you take these very noisy, messy traces that we can obtain and extract information from them. Our real goal is to make that quantitative and so what we're doing is taking tools, I would say of physics, which is both the tools and techniques of making very fine scale measurements, to make these measurements. If you think about it, this is thousands of times less than the width of a human hair and we're measuring this in real time in a solution at room temperature so the Brownian motion is moving everything around and we're able to extract these infinitesimal motions that are taking place in

real time and the physical basis of this is really extraordinary and we're building on the work of others obviously, but it's applying those physical principles, some engineering, in terms of actually building this, as I said, this is all built by hand.

We build these instruments ourselves; we code them ourselves; and then once we actually get the data as a matter of sort of taking the very messy, noisy data and extracting useful information from it. It's then very quantitative and so these are the tools and approaches that we take. What it gives us is a sort of unique view on biology in that we're really looking at sort of the mechanical aspects of biology. We're actually directly measuring the mechanical motion of this enzyme along the RNA. Again, it's a very physical view of what's happening, and I think we just bring in a different perspective.

These single molecule approaches were really enabled by the physics of being able to measure these, and any time you can watch a particular individual through time you learn things that are very different. So, for example, it could very well be that when you add these inhibitors it goes forward, it goes back, it goes forward. So, it's things like this where we see that sort of unique or rare behavior happening in only one of ten of these molecules. Anything that is really important and that turns out to be clinically relevant, and the other things that happen may not be all that relevant, and so again you know that by differentiating what's happening at sort of the individual level, that's what we've learned a lot about biology. That's been hard to get at through other terms, so it's just the invention of new tools gives us a new view on what's happening.

I think you know when anybody thinks about people, they think they know about the individuality of people, but enzymes are not quite like people. Think about the rich behavior of individuals rather than populations and that's really what we are measuring. We're measuring the individual and we all know that you can talk about the statistics of a population, but it's much more interesting to talk about a single person.

That interplay between those two things—that's sort of what we study—the individual enzyme doing its business. Then I would say, traditional biology is really looking at the statistics of what's happening and those two are very powerful.

Barr: How long do you think that your study will proceed?

Neuman: It really depends on what we find and I think it's a rich area so I think that we've discovered in sort of educating ourselves about this viral replication process that process in itself is a little disconcerting. Now I don't want to personify these enzymes, but the viral replication is quite clever and has a lot of tricks built into it to evade surveillance by the host cell to make very efficient use of his genome. So, you know part of our study is really focused on understanding the replication process and

inhibition. But there is another level of this which is understanding how the virus takes one long continuous RNA and makes several different transcripts that are made of the same RNA but yet have the same ends. So, there's a sort of discontinuous process which is fascinating.

This could turn into sort of a self-contained project of really understanding this viral replication from a more fundamental level. I would argue that that helps us in understanding and gives us other tools and other approaches perhaps to look for novel inhibitors. So, one aspect is that you know we talk about the polymerase, but the polymerase really doesn't act alone but acts as a whole complex, and so there's other accessory actors, including the helicase and this sort of proofreading enzyme, this exonuclease. So you have an understanding of the full complex and all of its sort of integrity is something else we could get a grasp on at the single molecule level, and possibly you could inhibit other aspects of this or even, if you could inhibit the interactions between these protein assemblies, that could also lead to inhibition. So, there's an aspect of this that is very focused. Can we understand inhibitors that exist currently and help in the process of improving them or, I think, recent work is sort of looking at repurposing other drugs and trying to know if we look at a collection of drugs, 20 or 30 different drugs, what are the ones, what's the relationship between clinical efficacy and what we see at the single molecule level?

But past that, I think there's an interesting question of just how does this work. There's a lot of mechanistic details that I think are fascinating. There's a possibility that would give us new therapeutic intervention avenues. So, I think it's always better to know more, to know your enemy. Right ?

Barr: That's very true. So, during the pandemic have you been spending the majority of your time on campus?

Neuman: No. As a senior investigator I spend most of my time directing the lab and writing proposals and writing papers. The lab people actually do the work because of the limitations on time and whatnot. I've decided to spend most of my time at home. I actually gave my office to a student in the lab so everyone in the lab now has an office to work in. They can take off their mask, be safe, and so I thought it was just most effective. I can still do most of what I need to do from home and the people are actually doing the actual work. They're in the lab; they're making the measurements; they're making the actual science. I'm giving them more time. So, I spend most of my time at home and then interact with the lab by a Zoom or Facetime or telephone calls and emails. So, I'm really stuck most of the time at home.

Barr: How's it been working from home? Has it been fairly seamless like communicating with your lab and doing things at home?

Neuman: I think when we first locked down, it took a little while to adjust. I tend to be a bit of an introvert, and so I thought being forced to interact with people, I had to sort of come out of

my shell and get better at initiating contact with people and really making sure the lab got together.

Now I think we sort of established a routine. So, we have our normal lab meeting and whatnot and then with individuals either telephone calls or private zoom meetings. I think things have reached, as very adaptive humans, a new normal. It's very strange and I miss a lot, being physicists and mathematicians and involved in hard science, of our work which is spending time thinking about things.

One of my favorite activities is the use of these massive white boards. We fight it out on the white board by taking two different colored markers and we write down our ideas and test different ideas. It's that interaction I really miss. It's hard to replicate that, so that's something I really miss. What's really nice about that is you get the whole lab involved, so you're standing at the board yelling at each other and having this intense debate. Other members of the lab come and jump in and pretty soon you got three or four people standing around thinking hard about what is the best approach, do we do this or that? Or how should we design our substrates, or what do we think is going to happen and how can we measure it? So those sorts of spontaneous interactions I really miss.

But I feel like we've gotten back to this new normal. I think people get what they need. The lab itself has been terrific about scheduling. We're under very strict occupancy rules. So, people feel really good about scheduling their time and their communication has actually gotten better in some sense because we're so tightly regulated. I severely miss everyone but even, if I were to come in, I still couldn't see people. And so, I decided that my time is better spent here and give the people who are actually at the bench in the lab making the measurements the opportunity of doing this.

Barr: So personally, have there been opportunities and challenges with the pandemic?

Neuman: I would say mostly challenges just in terms of sorting through all of a sudden being cut off from being able to go to work and see everybody. That really sort of threw us all for a loop. I think everybody was sort of "thrown up in the air" for a long time; just the mass uncertainty of was it going to get better or was it going to get worse. What was going to happen? How long will this go on? So, I think that really sort of threw us for a loop for a while. I'd say that was a challenge.

About opportunities, because I have fewer interactions now in the lab and I've had to reach out to other people, for example, I think my sanity has been saved by a nightly yoga ritual which I've never done before. That's a way to connect with friends and to do something that also imposes some structure. That has been an opportunity. Also, it's nice that my daughters have been here as they have their home offices in virtual classes, but now we are sort of working and living together and I see a lot more of them. Whether or not they like that, I don't know. But there's a nice aspect of that as they get to see up close what I do all day.

In fact, my younger daughter has started helping with some figures. We're making figures for papers; she's now learning how to make illustrations and whatnot. She's very excited to learn the science and make the figures. She's a beautiful artist. So that's been really some upside. It again says power to adjust, so I feel like we've adjusted more or less. We are moving things forward as best we can despite these awful circumstances.

Barr: Was it hard for you in the Spring to manage your daughters' online schooling while working?

Neuman: They were pretty good about it. There's a little bit of challenge, and right now the big challenge is fighting over who's going to have the prime real estate. It's usually a negotiation— whose meeting is more important, who has to retreat to a bedroom versus using the nice living room space. That seems to be the most of it.

Actually, my daughters pointed out that, because they don't have to take the bus and because we don't have to drive to their various activities, they calculated that 40 hours a week were saved in transportation. That opens things up. I think the other thing that was a real savior was doing things that were a little bit more normal, like getting outside, biking and hiking. Then camping with my daughters was a lifesaver. I feel like doing something normal, like those things you would do before the pandemic, that were still very safe. Those were the sort of lifesavers, in terms of getting a sense of normalcy despite the craziness that was going on.

Barr: Do you have a favorite social media platform? I know virtual platforms are how we connect with others. Unfortunately, there are so many of them. Do you have a favorite?

Neuman: I've sort of gravitated toward Zoom. It's funny that on my various computers I have probably four or five different specific Zoom links. I have a yoga link. Also, my partner lives across the country and so we have a specific Zoom link for us. We can Zoom and spend part of the day each day on Zoom just working but seeing each other. Then I have the lab link and group meeting links. So, I think Zoom seems to be the one that I end up using the most.

Barr: It's very easy to use. It's very nice. Well, do you have anything else you want to share either as a scientist or as a person living through this pandemic?

Neuman: No. I think what's really striking and really remarkable is how quickly many of the communities have reacted and responded. How many disparate fields are coming into play and really pushing so hard on this. It's amazing what the scientific enterprise can do when

there's such a clear goal. It's also remarkable as, we'll see, you never know it's going to work. So, I think it's remarkable that people from so many different fields are focusing their attention and there's a synergy that comes from that.

Then there is an excitement that's gratifying. I think that's been something we'll look back and realize that this is a time when what's nice is it's organic, as no one was telling people what to do. There are funding opportunities, but I think people are doing this no matter and I think that there's something about that as a scientist. I've certainly found this true since I never expected as a biophysicist to ever be involved in clinical research; that was not something we ever anticipated. I think part of coming to NIH exposed me to this, my colleague, for example, Yves Pommier, and the NCI is working right at the interface of molecular biology and actual pharmacology and chemotherapeutics and getting exposed to that research and contributing in some small part.

It's really gratifying to be able to contribute. Remarkably, these single molecule tools that were so esoteric and thought to be studying only basic biochemistry mechanisms, and yet we have the opportunity. We've done this to some extent and other systems that understand these connections with medicine and that's been really gratifying. So, I think that highlights the power of the scientific community and that sort of organic, again, it's bottom up. We're driving this as scientists and seeing them come together in trying to solve these challenges and bring this huge intellectual force to bear on this problem.

Barr: That's really wonderful. Thank you very much for talking with me today. I wish you the best with your research.

Neuman: Thank you very much. It was nice talking with you.