Anton Simeonov

Behind the Mask

December 14, 2022

Barr: Good afternoon. Today is December 14, 2022. My name is Gabrielle Barr, and I'm the archivist at the Office of NIH History and Stetten Museum. Today I have the pleasure of speaking with Dr. Anton Simeonov. Dr. Simeonov is the Scientific Director of the National Center for Advancing Translational Sciences (NCATS). Today he's going to be speaking about his COVID-19 research and experiences. Thank you very much for being with me.

Simeonov: Thank you, likewise. Happy to be here.

Barr: To begin, what was your role in the creation of a COVID-19 OpenData Portal, and how has it evolved since it was launched in 2020?

Simeonov: This is a great question. A lot of my comments today will revolve around this general theme of data sharing and dissemination because it's really part of our mission here at NCATS—to disseminate findings in this translational space. Even outside of peer reviewed publications in many cases, and with COVID-19 that is especially true. Sharing before publishing is pretty important, because it really allows you to beat the clock, for lack of a better term. We actually decided to do the OpenData portal not just because of the mission, but because of tradition and experience with rapid dissemination of data. It can many times take a year before a peer review publication is completed in some cases. Sometimes you go through several journals, etc. We've actually built multiple databases to share by getting data on small molecules, starting from publishing gigantic data sets in NCBI's [National Center for Biotechnology Information] PubChem database, from around 2005-2006 all the way to present day. We've published well over half a billion data points today. Those data have been used by many people out there to build predictive models and to run their own analyses and so on. Doing the OpenData Portal took a village. Multiple people dropped what they were doing, and we were able to get it stood up in literally days. It's really a result of institutional experience, expertise in coding, and also culture—wanting to make data available. Not just their own data, but obviously agglomerating data from elsewhere. The portal has evolved. We've been putting our own data into it, obviously, and collating and collecting data from all sorts of additional sources out there. We added data on variants and on convalescent plasma more recently. And actually, with late summer developments, we're adding monkeypox data and resources. We're hoping that it will evolve with time into a resource not just for the immediate SARS-CoV-2 efforts, but also a resource for other related efforts.

Barr: You mentioned the Small Molecule Antiviral Compound Collection. Can you speak a little bit more about the creation of that resource and how it's being used to discover broad spectrum antiviral drug molecules for SARS-CoV-2 and other pathogens, some that haven't even emerged yet?

Simeonov: Again, I'm sort of repeating my response. It's really a result of what we've been doing over many years around here. Early on, literally 10 to 12 years ago, we realized that a lot of these efforts are associated with pathogens that are resistant to existing treatments, rare diseases for which there is practically no business case to be made for developing a new drug, like treatment resistant cancers or you name it. Repurposing existing drugs to treat the new condition is actually a very important and potentially viable path to therapy because the molecule already exists, and what if you find a new use for it? It's already being manufactured, there is a lot of data around it, and so on. How do you actually go about discovering new uses of existing drugs? First of all, you have to figure out what the existing drugs are. That is actually not obvious. There is no one single database that tells you what they are. You may say, "Doesn't the FDA know that?" Well, there are many drugs that FDA does not regulate, but are still in existence in their beautiful molecules, impacting humanity. Back around 2009 or 2010, our informaticians and others embarked on this journey to actually figure out how many drugs are out there. Are they on patent or are they still in commerce? Have they been sunsetted? Are they drug candidates versus approved molecules? We were going around the world, pouring over many databases.

As a result, the so-called NCATS Pharmaceutical Collection was created in two forms. One is in a virtual form as a platform that actually tells you what these are. You can call that an informatics effort. What about a drug that is only approved in Japan and not by the FDA? You want to have that one in your toolbox. What about an old, abandoned drug that is way off patent, but is still a good molecule? You want to know about it as well. A lot of these are not within the FDA "directory," if you will. The other part of this is building a physical collection. How do you actually obtain these molecules? Not all of them are readily available. We had to synthesize some of them in-house. We had to procure them through various mechanisms. They're really not available as an off-the-shelf catalog item. It took several years to build that collection. Some of the molecules are extremely precious and expensive, so you cannot obtain too much of a particular molecule. That was published about 11 or 12 years ago in *Science Translational Medicine*. That collection has been updated a couple of times in the interim. As a result, we develop institutional knowledge and expertise and experience building these custom collections. There have been offshoots of this collection of, for example, oncology related molecules—not just approved drugs, but molecules that are candidate molecules in clinical investigations. Following that sort of reasoning, we also built the antiviral collection to not just tackle COVID-19, but to anticipate other similar public health emergencies. We also had a lot of experience with Zika virus from about half a decade ago. I can go into more details on that later on, but that's really what prompted us to build this collection. It's been serving us well.

Barr: Will you share how you took part in an effort to determine synergistic and antagonistic drug combinations against SARS-CoV-2 using modeling and matrix screening? What were some of the challenges that you and others on your team encountered?

Simeonov: Yeah, this is a very important topic for us. We've been doing drug repurposing and combination screening for well over a decade. We've actually developed very efficient, very novel ways to test combinations of two molecules. It may seem very trivial, but it's actually extremely difficult when you start thinking about the number of possible combinations—and the expenses that go with potentially testing drug A and drug B in custom ratios and actually analyzing that data rapidly. We developed what's called acoustic dispense technology to literally spot picoliter volumes in the solutions of these drugs and build these matrices of drug A and drug B variable doses using [what was] very novel equipment back in the day, around 2011 and 2012, and do it on a

massive scale. What combination of drug A, the standard of care, and the entire pharmacopeia of say, 3,000 drugs, are synergistic and so on? That was done in the past, and we've been doing this for a decade now–over 100 disease projects, probably more. A lot has been published.

The work on COVID-19 is, again, a natural utilization of that paradigm and that platform. The other part is the collaboration with the UNC [University of North Carolina] team of Alex Tropsha and others. We've had many collaborations with that group over the past decade or so on predictive toxicology, on flagging potential interferences during processes of drug discovery. We have a long history of joint publications with that group, and it was only natural to partner with them to first predict certain top rank combinations, and then plug them into our platform to rapidly test those. Of course, finding antagonistic interactions is also important because that is often overlooked. Everybody's looking for the gold nugget and what synergizes. But the opposite is also important. You really want to inform the clinicians and practitioners what pairs are actually counter-indicated and what it is you should not be prescribing together for whatever reason. Again, in the same vein, we disseminated those results as rapidly as possible to allow others to immediately embark on downstream studies. You'll hear this over and over again—we don't pretend to own everything. It's not an A-to-Z operation. We try to put stuff out there in the public domain to catalyze the community. In that sense, we're sort of very selfless. If I can open a sort of bracket here—at NCATS, we don't have tenure and tenure track, because with translational research and translational science, it's all about team effort and multidisciplinary approaches. With us not having the pressures of tenure—where someone who's on tenure track has to develop their own program and really focus on one line of investigation based on the traditional requirements or demands of tenure committees—it can be, and has been, very liberating for us, allowing us to better respond to public health emergencies like this one.

Barr: Will you speak about your contribution to an NCATS-UNC collaborative study that sought to identify allosteric binders of ACE2 without enzyme inhibitory activity and discover small molecules which allosterically bind ACE2 and are also active against SARS-CoV-2 in cell-based assays?

Simeonov: I'm going to brag here, but it was a very novel approach where we utilized a still somewhat new biophysical technique—the microscale thermophoresis and related approach is to run an unbiased screen to find small molecules that bind ACE2 regardless of where they bind. For ACE2 [angiotensin-converting enzyme 2], which is a protein that also has enzymatic activity, traditionally in drug discovery you look for modulators for small molecule binders—candidate drugs to bind to the catalytic pocket and inhibit the catalytic activity of the enzyme. As such, you run tests based on the enzymatic reaction. That's the natural progression of such studies. Here, we wanted to find binders of ACE2 that would essentially block the docking interaction of SARS-CoV-2 with the spike protein. They would not block the catalytic activity of ACE2, because that's part of its normal function throughout the body. How do you go about doing that if you have only the enzymatic assay? You're stuck. You will only be finding enzymatic inhibitors. With the biophysical approach, you find binders in this unbiased fashion, and then exclude those that are interfering with the catalytic activity. You'll find the so-called allosteric binders and so on. It hasn’t really been done too many times before because the techniques are simply lacking. They're not able to interrogate too many molecules with microscale thermophoresis. In my group, actually, we've been running all sorts of pilot tests, improving the technology, and working with the original inventor for the past five or six years to perfect it and get it to the present stage, where it can be deployed on a more

massive scale. Again, with the UNC group, they help with some of the analyses and predictions and essentially help guide the study. It's really an exciting angle that we try to explore.

Barr: It's still very much in the preliminary phase—there's no medications done with this technology on the market?

Simeonov: Not really. We just recently published the initial findings. As is always the case with these projects, the bar is relatively high. You need something that is extremely potent. You need something that is extremely safe and easy to administer. Honestly, given the state of COVID-19 worldwide, it has to be cheap and easy to distribute, because the vaccines are making a difference. Other drugs are making a difference. The bar is actually shifting as to what the next therapeutic should be as far as overall profile.

Barr: That's so interesting.

Simeonov: Yeah. They call this sometimes the "better than the Beatles phenomenon." When something hits the market, whether it's blood pressure medication, diabetes medication, or for COVID-19, the next thing really has a tough time competing. It has to be better in some other way or else the clinician simply won't prescribe it.

Barr: That's something that I didn't really think of, but very true. Will you explain the importance of TMPRSS2 to the viral life cycle of SARS-CoV-2? How have you been involved in the efforts to find TMPRSS2 inhibitors through virtual screening as potential treatments for COVID-19?

Simeonov: Yeah, I mean, I'm not a virologist myself but it's well known now that TMPRSS2 is one of the key proteases. They essentially are part of this viral entry cycle because SARS-CoV-2 binds to ACE2 and then TMPRSS2 performs cleavage to cut sites, and that is sort of the productive step leading up to internalization of the virus. We didn't really discover that. We did not discover that at all. We actually learned about it through literature searches and knowing, I guess, about the virus in general and speaking to colleagues—because again, we're not virologists. What we were able to bring to the table from day one, in that regard, was experience—actually my group's and my own experience, when I didn't have a group—of screening proteases and knowing how to set up the test system to discover inhibitors of such proteases. It may seem trivial, but it is not.

Barr: Can you describe the process?

Simeonov: You have to think about the substrate, how to design the signal generation, and how to attach the right fluorophore, and potentially quencher, to the peptide that is being cleaved by the specific enzyme. There are a lot of biophysical and chemical factors you need to take into account. It is not a given that you can immediately perform a high throughput screen against the new protease. You have to essentially test different peptides and different sequences that flank the cleavage site to find the one that the enzyme will accept. Running an assay using the entire protein as a substrate is impossible—you cannot obtain so much protein. You have to synthesize a shorter version of the substrate that is only, let's say, five to 20 amino acids—something that is cheap and easy to produce. You typically attach a fluorescent moiety to one end, and a quencher on the other end. When you shine light on that sample, it does not fluoresce because the quencher absorbs the light

from the fluorescent binding. When the enzyme cuts that into pieces, the fluorophore is separated from the quencher. They're no longer part of the same molecule, and now the fluorescence is released. You get an increase in signal as the enzyme cuts and turns over. This is a very convenient technique to test literally millions of samples, where an inhibitor will slow down their rate of cutting so you can detect the inhibitor very easily. But in order to do that, you have to have the right substrate, you have to have the right signal, your background, and everything. It's not a trivial job.

Barr: Sounds really complicated. If anything goes wrong in the whole process, then it's messed up.

Simeonov: Well, it is, and you will end up with a lot of false positive molecules that potentially block the light or have physical properties that look like they're binding to TMPRSS2, but they're actually false positives. You have to create conditions for the assay to actually avoid such interferences or minimize their effect. That is where experience with prior projects comes into play. We've worked on cruzain, which is a major protease for Chagas disease, back in 2009, so we have that experience with tropical infection by a protozoan parasite. We actually ran a protease screen of the major protease for Zika virus a couple of years ago, again in my group, so we have all that institutional knowledge as to how to do it. We applied that with TMPRSS2 as well.

Barr: Can you comment on NCATS' work looking to see if the alkalization on the acidic compartments and host cells is an effective strategy to reduce viral infection, and that the lysosome is a viable target organelle for COVID-19 drug discovery?

Simeonov: Yes, so again, it was happenstantial almost. When COVID got here, we did a lot of literature searches and tried to educate ourselves rapidly. Chloroquine and hydroxychloroquine were drugs that were really being highlighted as potential treatments for COVID back in the early days. We knew from literature that they were acting by alkalizing, affecting the lysosomes. That was one of their mechanisms of action. But it was also known from the very early days of COVID that hydroxychloroquine will exhibit those dependent toxic effects. It would not be an ideal drug to use "on field," so we decided to look for new molecules different from hydroxychloroquine that would act by the same mechanism. If that mechanism was important, then finding other molecules acting by it that would potentially be safer and less toxic would be an important undertaking. That's what really drove our efforts. We use a specific dye that follows the lysosome license tracker, and we've set up an assay and then ran screens pretty rapidly—again, having the technologies to do that—and we found some new molecules. They were doing the same thing and essentially acted as a preliminary validation of the lysosome as a potential therapeutic target.

Barr: Will you discuss how and why niclosamide, which has been used for a variety of illnesses, can be applied to combating SARS-CoV-2?

Simeonov: This is an old drug that has been used for tapeworm and related helminthic diseases for many decades, actually—not just years, but decades. Interestingly, it's on the World Health Organization Model List of Essential Medicines. It is also an FDA [U.S. Food and Drug Administration] Category B drug, which means that it did not demonstrate risk to the fetus in animal reproduction studies, so it's potentially safe to give to women of childbearing age or pregnant women. We did run many drug repurposing screens in the past against other

parasitic targets. For example, it was found in drug repurposing screens of Zika virus. This was published some years ago in *Nature Medicine*, when Zika virus entered the headlines. We knew about niclosamide. Our medicinal chemists actually realized that it would not be an ideal drug to give outside the tapeworm application because it didn't have very good bioavailability and other kinetic properties. They started making analogs, making small changes to the molecule to improve of its properties. When COVID hit, it was sort of a "natural" continuation of this work to test all these molecules—that were created as part of the earlier projects for Zika and other projects—against SARS-CoV-2. That's really where we found some good activities—and again, we reported on those and, hopefully, others will take those to the next level.

Barr: Will you discuss your involvement in devising a strategy to develop SARS-CoV-2 neutralizing humanized nanobody constructs with sub-nanomolar affinities and nanomolar potencies, and how this strategy can be employed as a tool to mitigate the threat of emerging SARS-CoV-2 variants?

Simeonov: This actually comes from my own background before joining NIH. I actually did my postdoctoral work at the Scripps Institute in California working on antibodies. That involves protein engineering, phage display, a little bit of immunology, and directed evolution of proteins. NCATS historically has been focused primarily on small molecules over the years—for historical reasons, funding reasons, mandates, and so on. But more recently, with the opioid crisis, actually, I tried to introduce antibodies into the mix. Long story short, I actually had an army of one—one person working on antibodies, initially for purposes of targeting proteins related to pain, addiction, and overdose. As part of that effort, we had just developed a new library of small antibody fragments called nanobodies. Literally, right before COVID hit, we had that library established. With COVID, we decided to turn around on a dime and find nanobodies against the spike protein. That's really what resulted in this work. It was a great collaboration actually—with NIEHS [National Institute of Environmental Health Sciences], the sort of sister Institute within NIH with the cryo[genic] electron microscopy [cryo-EM] and cryo-EM facility, at NIEHS in North Carolina—to characterize the binding of these antibodies to the virus spike protein and understanding how the binding occurred and why the antibodies were neutralizing. We have not been able to test antibodies or develop them against all the variants. It would be a monumental effort. To be fair, others are reporting antibodies in this space as well, so we're trying to be mindful to the fact that there is actually plentiful effort within the biopharma space to develop such antibodies.

What nanobodies offer as far as advantages, is that they're much smaller and they're much easier to manufacture. This specific library that we developed is based on humanized nanobody. Originally, nanobodies came from camel, shark, and llama—those are three animal species that actually have these smaller antibodies that don't have light chains. This is a discovery from the late 1980s or early 1990s, actually, but the field has evolved significantly, and some versions of these smaller antibodies are non-immunogenic. They essentially have the backbone of human proteins, so when you inject them into human patients, they don't produce immune response. Our library was based on such antibodies. The hope is that if we discover an antibody that is actionable, it's going to be very easy to deploy in human testing because it will not be immunogenic. One side note that is important is the intellectual property associated with this discovery. We actually donated that to the World Health Organization as part of the White House effort to create a technology pool to enable developing countries to manufacture therapies for COVID-19 without having to pay the license fees. This was actually

highlighted back in the early spring in the White House press release. NIH donated several technologies and NCATS donated the nanobody technology as part of this effort.

Barr: As the Scientific Director of NCATS, can you mention a little bit about how NCATS' COVID priorities have shifted over the course of the pandemic? Also, briefly share some of the other COVID-19 research initiatives that you've been a part of or supported, both at NCATS and with any grantees? How do you accommodate non-COVID research, which was a big situation that you had to deal with?

Simeonov: I can start from the last part of your question. First of all, we're accustomed to turning on a dime. We actually have a pretty robust history of doing that. I mentioned Zika. I mentioned the opioid crisis. We've done work on Ebola when Ebola hit. We even did pretty interesting work around 12 years ago, when the British Petroleum (BP) Horizon oil spill happened in the Gulf of Mexico back in 2010, when oil started essentially poisoning the Gulf of Mexico. Back then, BP asked the EPA [U.S. Environmental Protection Agency] to essentially give a green light to the company to release into the Gulf of Mexico gigantic amounts of oil dispersants to help break up the oil slick and coverage into small droplets to essentially free up the fish and marine life. Unfortunately, EPA did not have data on animal safety of those dispersants. The company, BP, put EPA in the corner. They asked them, "Okay, we're about to release this gigantic number of dispersants to help. Are you okay with that?" EPA is a regulatory agency, so EPA had to render an opinion. Thankfully, we had this collaboration on predictive toxicology, where we actually were working with the EPA since 2008 or 2009 on running cell-based assays against chemicals to build predictive models of toxicity of chemicals. Long story short, over the Memorial Day holiday, our team—who decided not to take any vacation—stayed in the lab, ran estrogen receptor, androgen receptor, and related endocrine assays that we had in the lab against these candidate molecules. The clock was ticking on time—a span of probably hours or days, not a time span of months and years. Animal data could not be generated. Contractors could not be activated on such a timescale. We were the only game in town, and we delivered. We actually delivered data in five or six days from the initial phone call. People received FedEx shipments at their houses because it was the holiday, and the building was closed. Everybody was pitching in. EPA was able to basically say the dispersant was okay based on the very limited yet valid data, and essentially greenlit BP to work on containing the spill. It's extraordinary. It's not related to infectious disease, but it's another example of how we turn on a dime with public health emergencies. The team actually received a special award from the EPA based on those efforts.

It didn't really perturb us to have to do this for COVID. Of course, it was very resource intensive. Psychologically, people were affected just by the lab closures. I spent countless hours myself watching key fob entries, because we have to keep occupancy of certain labs to the bare minimum. We actually developed totally new work flows based on that, to not only be policing people to prevent overcrowding, but to also have maximum utilization of the lab space and to enable the work to continue. We were able to do those through extraordinary efforts from building support personnel to ID specialists to many others. It was pretty intense effort. A lot of things were learned with that. A lot of new collaborations were established.

Barr: Can you talk more about future directions?

Simeonov: We actually are part of the APP initiative, the Antiviral Program for Pandemics. This is a preparedness program funded by Congress for several years, where we're now working not just on SARS-CoV-2 type assays, discovery, and so on, but also other viruses and related parasites that could essentially result in the next pandemic. We actually have assembled dedicated teams to do that—essentially, to look at viral families and understudied viruses that have the potential to "explode" in the next pandemic. We're applying the knowledge that we essentially gathered from the COVID-19 response as far as platforms, predictive modeling, databases, and connections with others. We are beginning to execute on that. Part of the effort is also to improve the predictive models for these efforts. A lot of these drug candidates are tested in animal models that are not perfect. Can we improve the models to be better predictive of what happens in humans? This gets us to the topic of bioprinted tissues that are based on human cells. We have a 3D bioprinting lab that is actually making models of lung tissue, innervated and vascularized, that is infected with SARS-CoV-2. Can we get readouts on how SARS-CoV-2 infects the lung tissue? Again, we build on experiences from the past. For example, that same group has built a bioprinted human retina as a model of Zika virus infection of the human eye. We actually tested drug candidates on bioprinted retina, and we published that last year. We showed that it was a better model of testing the molecules than two-dimensional traditional cell cultures. A lot of platform building goes into this to prepare us for the next pandemic, whatever that one may be. That's what we're working on. We're also working on automating steps of the chemical synthesis for the small molecule drug candidates, because that skill remains a largely manual type of operation. When it's manual, it's not effective, it's not scalable, and so on. We're working on actually automating chemical synthesis and predicting chemical reactions that would produce a particular molecule. That is going on as well as part of this antiviral effort at NCATS.

Barr: In addition to being a scientist and an administrator, you have also been a person living through COVID the past two and a half years. Have there been any particular personal opportunities, challenges, or memories that stand out for you that the pandemic has presented?

Simeonov: There's not really a single [specific] thing to highlight. It's the intensity of the total effort that has been a challenge. You can call it the totality of circumstances, because as you detected, I wear multiple hats. How do you combine all this when you have to think about science—very specific science, the science of the day, the research results, the next steps—together almost within the same minute as personnel actions, managing the flow of people in the building, signage, and containing the virus in terms of how you operate within different labs? That's been the challenge, but it's also been very rewarding personally because you get to see the best come out of people, right? People actually united. They did not divide; they actually did unite. A lot of solidarity, a lot of help, and a lot of cross-learning happened. We actually onboarded probably 25% new people, extra individuals, during COVID. We have to work on how those people get integrated as well. These new team members are doing a great job. They're making a difference. It's been really invigorating—if I can end on that note.

Barr: Definitely. Thank you so much for all that you do, and I wish you the best of luck.

Simeonov: Thank you so much. It's been good talking to you.