Amy Harris: I'm standing here in the middle of the room. We're gonna get started. Everybody, welcome to the Science Cafe tonight. It's great to see everybody. I'm glad you're all here. I've been around talking with folks, and there are many, many new attendees. We're delighted to have a lot of new folks here tonight. It's always great to see friends who have been here many times before. My name's Amy Harris. I'm director of the Museum of Natural History. We've been offering the Science Cafe series for 15 years. We're really proud of it.

I hope you enjoy tonight's program. Tonight's program is about the future of MRNA vaccines. Usually what I do at this point is tell you about future events at the museum. We actually take a little bit of a break from public events over the summer. What I can tell you is that the museum just expanded its hours for the summer. We don't have a lot of public events over the summer, but we're open 7 days a week from 10:00 a.m. to 5:00 p.m. We are air conditioned. It's really not that hard to park there. We look forward to seeing you at the museum over the summer.

I'd like to thank Connor O'Neill for making this space available to us over these 15 years. Please join me in giving them a round of applause. I also wanna thank last month's sponsors, who are with us tonight. They weren't here in April, but Lee Green and Michelle Eyecult 00:01:40 are good friends of the museum, and longtime donors. They underwrote last month's Cafe. We wanna thank them now that they're here to join us. Thank you so much. Tonight's sponsor is Sigma Sci, the Scientific Research Society in University of Michigan chapter. I'd like to invite Cynthia Costello up. No, I didn't get it right. You'll introduce yourself.

Cynthia Marcelo: Marcelo.

Amy Harris: Oh, Marcelo. Sorry. Cynthia Marcelo is here from Sigma Sci, and she's going to tell you a little bit about it. Then we'll get on with the program.

Cynthia Marcelo: Thanks. Oh, I have a microphone. Okay. Thank you.

Amy Harris: Please speak into it, so *[crosstalk 00:02:24]*.

Cynthia Marcelo: Okay. Gotcha. Thank you so much. My name is Cynthia Marcelo. I am the secretary of Sigma Sci. Sigma Sci helps sponsor the

Science Cafes. We've been doing it for a couple of years. I figured this is a great opportunity for me to tell you a little bit about Sigma Sci. No, we are not a sorority. We actually are the world's largest multidisciplinary honor society for scientists and engineers. It was formed in 1886 when Phi Beta Kappa refused to let scientists into their group. I guess we weren't good enough.

Our chapter here in Michigan was formed in 1903. We have over 1,000 members all over the university. The mission of our society, and it's a national society, in fact, we have international members also, is to enhance research, foster integrity in science and engineering, and promote the understanding of science. In this later mission that is promoting the public understanding of science we support activities like the Science Cafe.

We also judge and award prizes to the Southeast Michigan Science Fair, and support a whole bunch of very young, and very special, and very bright students. We give cash awards to the science math teachers, who help stimulate STEM cell education here in the Ann Arbor area. Okay. Thank you so much for letting me explain to you who we are.

Kira Berman: Thank you so much to Sigma Sci for their ongoing support of the Science Cafe series. My name is Kira Berman. I coordinate the Science Cafe, and help put together the topics. I see a lot of new faces. I'll go over our format so everybody knows what to expect. In the beginning, we will have some presentations from our two speakers, whom I'm about to introduce. Then we will take a short break. There are discussion questions, information, and readings at your table to help you have a good conversation, and the speakers will circulate around the room.

Then we'll come back together for a group discussion, and Q and A session from about 7:00 to 7:30. Then at 7:30 we'll end, but you're welcome to remain in other parts of Connor's, but we will relinquish the room that they have so generously donated. We do pause before the last question is asked to fill out the little blue evaluations on your tables. Those help me get ideas for future Science Cafe topics. Please let me pick your brain, fill out an evaluation at the end. With that, I will introduce our speakers in the order they are speaking.

I'm really, really excited about this topic, and really excited about these two speakers, Nils Walter, who's standing right over there, is currently the Francis S. Collins Collegiate Professor of Chemistry, Biophysics, and Biological Chemistry. That's a lot, but there's more. He founded and currently directs the Unique Single Molecule Analysis in Realtime, abbreviated SMART Center as well as he co-founded, and currently co-directs the Center for RNA Biomedicine at Michigan, so very on topic.

Nils earned the equivalent of his bachelors and Masters degrees from the Technical University of Darmstadt, and did his doctorate while studying molecular invitro evolution of DNA, and RNA using fluorescence techniques with Nobel laureate Manfred Eigen at the Max Planck Institute for Biophysical Chemistry. For his post-doctoral studies he turned to RNA enzymes at the University of Vermont. His research interests focus on non-coding RNA through the lens of single molecule techniques.

Based on his work, he has received so many prestigious awards that I could be here all night listing them. From the Otto Hahn medal for outstanding researchers of the Max Planck Society in 1995 all the way to the Students' Choice Faculty Mentor award of the Cellular and Molecular Biology Graduate Program in 2020, unless there's a more recent one that I forgot.

Nils Walter: *[Faded voice 00:07:23]*.

Kira Berman: Okay. Please welcome Nils Walter. Our second speaker will be Rachel Niederer. Did I say it correctly? Excellent. Who is an assistant professor in the Department of Biological Chemistry, a faculty scholar of the Center for RNA Biomedicine, and member of the University of Michigan Rogel Cancer Center. She received BS degrees in biochemistry, and cell biology in genetics from the University of Maryland, College Park in 2009.

For her Ph.D. she worked with David Zappulla at Johns Hopkins where she examined the cellular response to short telomere induced senescence as well as structure function relationships within telomere's RNAs. Whew, that's a mouthful. She performed her post-doctoral work with Wendy Gilbert at Yale developing a high-throughput method to measure ribosome recruitment to thousands- to measure ribosome recruitment to thousands of RNAs in parallel. You get all the little RNAs all at once. Wow. Okay.

She's broadly interested in how RNA features effect biological processes. Currently her group uses a combination of high-throughput methods, and classical biochemistry to identify and characterize RNA regulatory features with the goal of engineering improved MRNA therapeutics. Please welcome Rachel Niederer. Without further ado, I'm gonna turn it over to Nils.

Nils Walter: Well, thank you, Kira, for the very gracious introduction. Thank you all for being here. We, as scientists, often forget to reach out to the public as much as we should. Clearly, there's a lot of miscommunications, a lot of confusion about scientific issues that prevail today. Thank you for coming, and listening to us make the case for RNA. Now as you can tell, we are going to talk about the future of NRNA vaccines. In order to understand the future, we have to look at the past as well. To me, as you heard, I got my Nobel prize—sorry.

I got my Ph.D. with Manfred Eigen back in Germany. I worked on invitro evolution, so understanding how evolution could have brought about life on earth, the origin of life. That was fascinating to me when I was an undergrad student. I read in a textbook for the first time about RNA enzymes. You probably know what enzymes are, and all you refer or think of them as protein enzymes that flowed into complex three-dimensional structures, and do everything in our body that makes the cell live.

However, there are also RNA enzymes. In fact, the ribosome that makes all proteins is such an RNA enzyme. We call it oftentimes the ribozyme for short. These RNA enzymes are really critical to bring about life as we know it. When I read about this, I was so fascinated that this also led to the idea that perhaps there was an RNA world where the chicken and egg problem. DNA storing information protein executing on that information could be solved by RNA being able to do both, store information in its sequence.

Also, in its complex three-dimensional structure that it can adopt be an enzyme that actually executes it. That then threw me into the field. I should say that all along over decades people like myself had the feeling that if RNA was so important for the origin of life, why is it not as important today. At that time, always MRNAs were thought to be the only thing that matters, but it turns out that there

are many, many other forms of RNA that count, and actually a majority of OM genome is not coding for proteins, but for these what we call non-coding RNA.

In other words, understanding the past, and the relevance of RNA, and the origin of life explains how it is so important still today. In order to give you that background, I wanted to go back a little bit in time. Where did it all start? You may have had heard of the primordial world. The idea is that it's a complex atmosphere with a lot of different molecules. It's more molecule *[unintelligible 00:12:07]* including methane, and hydrozincite, and water, and carbon dioxide, and so forth.

This is where life probably started in these with energy extended by these, by lightening, and so forth, and heat of the earth. The question is how do we get to the modern world? Where did it happen, and where did this all come together? I'm a chemist. When I put my Sherlock Holmes hat on, and think about this, how did we get there. Then I realize for one, that this is a question that has concerned humanity for a millennial. You think of stone hedge, and the place where they ask the gods with this question.

As a chemist, I also think that really there were other things that force complexity to emerge from simple molecules. Those are called chemical evolution, molecule self-organized. That what really leads to biological evolution as we now know it. There's a whole seminar I oftentimes get, and there's actually a YouTube video of one that I gave that you can listen to. In short, if you think about this atmosphere that I just described with all these inorganic small molecules.

An experiment not far from here at the University of Chicago done in 1953 by Stanley Miller and Harry Urey tested this hypothesis, whether more complex molecules could arise from these gas molecules from nitrogen, which is 78 percent of our atmosphere today, to carbon dioxide, which, as you know, can lead to climate change, to hydrozincite, which is actually toxic nowadays to us. Mitochondria, the energy producing entities of the cell actually get inhibited by hydrozincite.

Like you see normally associated with some German spy or so, they take a capsule, and then die. That's because of this molecule. This is, of course, a prejudice, or at least *[fading voice 00:14:14]*.

There are other molecules like ammonium is also pretty toxic. You wouldn't want to inhale them today. At the time, they were really important. This was proven by this experiment where Miller and Urey put these different molecules, including water into a flask under heat.

They boiled this flask, and the reflux where they condensed the water from the vapor back into liquid, and did this for days, weeks, and up to a month. Even after only two weeks of running this experiment, and adding some power in the form of lightening essentially sparks. It would create energy output inside the flask. They would find all sorts of what we now refer to as organic molecules, and molecules associated with life as we know it coming out of all these inorganic molecules.

If you look at this closely, then you'll see all the building blocks called amino acids of proteins found in these experiments. Nucleic basis, which are the basis for what's *[unintelligible 00:15:21]* bearing the DNA does, but also RNA are found here, and simple to organize from hydrozincite, ammonia, and other molecules. You can also build very easily sugars, which are essentially the backbone, or part of the backbone of nucleic acids of DNA and RNA. Finally, also lipids are found. Lipids that form a membrane around cells as we now know them.

In the 19th century, Hans Wiegel 00:15:49, a German fellow, thought of this as a primordial soup that the oceans were filled with these molecules that were created from the atmosphere, and then assembled it to more complex molecules. How can that be? Well, ultimately if you put in energy, what you will have is that complex molecules like a nuclear type, the actual building block of the nuclear base with the sugar and the phosphate in it as well assembles.

Those can assemble on the surface of minerals here that catalyze a polymer formation that is aligning up with these building blocks that can then fuse together, and become a polymer of these monomers, and formed ultimately random strands of RNA. The idea is basically that all these building blocks are found in the primordial soup. Then they condense into what we now call ribonucleic acid, RNA, they can base there between a U, and a A, and a C, and a G. The only difference of DNA is it's a Urocell 00:16:51 instead of a Tiafinmine 00:16:53.

Then, of course, the disclaimers is important here, we don't know whether there may be another polymer preceded RNA, although there's good evidence to think that RNA is really the ideal molecule to bring about life. These can become living molecules because, again, as long as they're energy put in, you will have these random sequences. Adopt different structures and functions, and some of them may be able to align nucleotides ties that complement by the Watson Crick Base bearing, once again, A, U, C, G, and so forth along it.

It ultimately allow for the polymerization, the attachment of these nucleotides to one another to form a complementary strand of RNA that contains the same information as the first one just in reverse. You copy it a second time, you get the first one back. If you can separate these two strands, and then the red one here is actually a catalyst to catalyze, to favor this reaction, to accelerate the reaction, then you can imagine that you make both the catalyst, and the complement, the complement that makes the catalytic, and so forth. You do that many times over.

Now you have living molecules that start competing with one another for the resources of building blocks. Only the best ones that replicate the fastest will survive. You have to bring in evolution on the chemistry of these molecules. Now what is the driving force of this? Again, I give whole lectures on this. The idea is basically as long as you put in energy, then more complex materials will emerge inevitably, and probably not just on earth.

This is actually a cover for my Ph.D. thesis. The point of it is, and this is black and white, so it's a little more primitive at that time. The idea is basically if you have nucleic acid, you can replicate it so you can make more copies of it. Every so often an error will be made, which we call mutation. Now you've generated what my mentor, Manfred Eigen, called a quasispecies, which is essentially similar sequences, but not exactly identical because some of them carry an error, and are not exactly identical.

This just like a species of animals have slightly different phenotypes, look a little different, and have different functions from one another. This term quasispecies was actually termed by my mentor, Manfred Eigen, at the time. As long as there's energy input, you will inevitably get in this direction, and then eventually

have these molecules compete for one another through some sort of selection. The weaker ones, the ones that can't replicate well, will die out. They will degrade. They will decay, again, going back to dust.

Then the ones that are able to replicate more of themselves will survive. Then you get cycles of replication here, followed by mutation and selection that actually bring about more and more complex function in those RNA molecules. Again, all driven by this energy that can have a lot of different forms nowadays. It's A, T, P, and chemical forms of energy. Myself in the lab, for example, was playing a little bit of a chemist doing these experiments who can actually do these experiments in the test tube, and evolve a new function of an RNA or DNA molecules.

I should point out that this, of course, depiction of Einstein, who was a physicist, and a theoretical physicist at that, who would not have done these experiments. Be that as it may, you can then spin this thread further where if you do this often enough, then you can first make simple molecule that just replicates. Then becomes more complex, and it dots additional functionality to then replicate copies of it, and becomes more and more complex. This is really like the evolution of primates. Ultimately, leads to a formation of very complex molecules.

This is in ribosome RNAs is RNA molecule that the catalyst in the ribosome, as I mentioned earlier, all the way to the DNA, RNA protein board. Even, they teach this in my biochemistry class, evidence in our biochemistry of all these that RNA came first. Because RNA is being modified to become DNA in our cells. There are many reasons to think that as parts of these chemical processes are actually preserved in our enzymes nowadays.

Good evidence that this might've happened. Again, people have done these experiments, and can actually make an RNA molecule by in vitro evolution that replicates itself. Now this ultimately then led to the idea, or to the world that we live in today, that DNA makes RNA mixed protein. Of course, that's the central dogma of moleculer biology, but it's somewhat incomplete as we now know. Because, as I mentioned earlier, there's a lot of the DNA in our genome that is not coding for proteins.

It makes an MRNA, which is what codes for that protein, but only two percent of the whole genome in us actually makes MRNA molecules to make proteins. The rest, probably more than 90 percent of that, is actually transcribed at one time or another in different cells. Those of you who heard about epigenetics last year might remember that epigenetic code can basically silence certain genes, and RNAs are heavily involved in the silencing in different forms. Okay.

Ultimately, you can actually look at genomes of different organs, and soon you will see that the more complex the organism going from bacteria to yeast, which are single celled, to multicell of fungi, and others, there's more and more of the genome that's not coding for protein, and functioning on this non-coding level. There's good reason to believe that complexity of organisms, multicellular organisms, were enabled by the event of more and more functionality encoded in the genome of these non-coding RNAs.

To me, that's somewhat of a divine idea, and can be utilized nowadays, of course, to control gene expression. Rachel will talk to us about messenger RNA in more depth.

Rachel Niederer: Thanks so much, Nils. I also am a fan of all RNAs, non-coding and otherwise. What I'll be focusing on mostly today is MRNA, and really specifically I thought we'd hear a lot about MRNA in the context of vaccines. I think one of the important things for trying to really be able to appreciate the future, and all of the potential of MRNA as a therapeutic is really in taking as step back, and understanding why we have MRNA, and what it is doing in our cells in the first place. Because that's really central to understanding how we can repurpose it for our own uses in the future.

For me, I generally like to begin thinking about we're all composed of cells. This is the building block of life. If you're like me maybe are familiar with this really common cartoon of a cell, this is fairly unsatisfying as a description for the building block of life. Because this doesn't look anything like any organism at least that I'm familiar with. One of the first things that I was curious about when I first became interested in science is, how do we get from this to a whole person. This feels worlds apart.

Clearly, there's a lot of fascinating science happening to get from here to here. At least part of the answer is that most of our cells don't really look like this. We actually have specialized cells that make up all the different functioning tissues, and organs in our bodies. These can look more physiological distinct from one another. The cells in our brain look very different from the cells that make up our skin, for example. Our muscle cells, and our intestine cells all have different forms, and completely different functions.

It's really necessary that we have this full complement of cells working as well as they can in order for us to develop happily and healthily. One of the things that I think is really fascinating about this is that all of these cells, in fact, are utilizing our genomes DNA as an instruction manual to tell them exactly what cell to become, how to form their different shapes and functions, and how to carry out their very important critical tasks. To me what became—so, one of the other obstacles to this is that actually this instruction manual is enormous.

If we actually typed out all of the letters in your genomes, it would turn into a book that is basically somewhere between the size of the Statute of Liberty and the Washington Monument. This is a tremendous amount of information. You might imagine that not all of the cells need the same amount of information. What's really fascinating to me is that even though these cells have completely different shapes, and completely different tasks that they all need to carry out, they are all working from the same enormous instruction manual.

This is a pretty tall order to ask all of ourselves. This is a real problem to sort through all of this information, and actually faithfully and appropriately execute the program that is necessary to grow and develop healthy cells. Here is where cells I think have devised a really clever solution to this to get from this enormous genome of information to this really wide variety of cell types. The answer is RNA. RNA is the solution to this problem for many reasons.

What cells do is they actually make smaller copies more or less, smaller temporary copies of specifically the instructions that are important for each of these individual cell types. Even though say the base code is the same in all of these cell types, they're only

making little instructional pamphlets for the parts that are relevant for their particular cell type. This has many advantages for the cell, and for us that we can take advantage of in the future.

The first advantage of this approach is that these instruction manuals, or the RNA themselves are temporary. This can be made in many copies. It's scalable. Also, these are copies that can be degraded if something wasn't copied quite right, or the cell can tell that it wasn't really up to snuff. They can degrade it, and it's fine. Nothing has gone wrong with this base code. The temporary nature of MRNA is really helpful in executing this wide variety of different programs.

As I mentioned, the scalability is also really important. If you need a lot of a particular item to execute your program, you can make many, many, many copies without having to have so many copies in your genome that your cell would then be burdened with having to maintain. They're also very portable. This book is enormous. If you need to transport it out, and recruit different machinery that's gonna make the different instructions, this is a very difficult thing to handle.

Whereas you can make an RNA copy that's smaller, easy to get in and out of the nucleus, which makes it feasible to execute these programs into processes. You might imagine that our neurons actually have this very interesting shape where some of these instruction manuals actually have to get very far into the *[distorted audio 00:29:07]*. This portability becomes really critical for their functioning overall. All of these features become things that we can use for therapeutics. These are all advantages in designing things that can help make us healthier.

The fact that they're temporary makes it so that we don't have to worry about such long lasting side effects. The fact that these can be scalable is something actually that my work is very interested in. How can we scale production of things from MRNAs? The fact that they're portable, we can deliver them. These are all taking advantage of properties that MRNA already exhibits that we can just use for our own purposes.

I've talked a lot about messenger MRNA, and how it is important in executing these programs. What do I mean by that? What is it actually doing? Messenger MRNA, as mentioned, is translated into

protein. If we think of the DNA as that big instruction manual that I mentioned, it's transcribed into these RNA templates, or these smaller pamphlets that you can scale up or down. You can make many copies, or very few. These are transcribed by the ribosome, or the original ribozyme into peptide that then folds into a functional protein.

It's these protein partners that execute most of the programs that we think of when we think of normal development. One of the great things about this is we can give our own instructions to the cell. This is a process that cells are already undertaking. They're already looking for RNAs. The ribosome is looking for targets to jump onto and translate. This is something we can use for our own purposes by providing our own MRNAs to cells, and directing them to do something that they're already doing, and they've been doing for millions of years.

The future from my perspective is really limitless. We can direct the cells to make therapeutic proteins, vaccine targets. We can introduce silencing that will turndown the scalability, the amount of an RNA for one protein or another. I know tonight we're primarily interested in vaccine target. I'll talk a little bit more about that. The MRNA vaccines, that I feel like we're all familiar with now, they're basically taking advantage of exactly this program that I've been describing.

Where we can provide our bodies with a vaccine that contains an MRNA in coding a specific protein, or a specific instruction pamphlet that the cells are then gonna take up and transcribe. In the case of COVID, it is targeting the spike protein, which is a known therapeutic target. It's very critical for viral replication. This gets translated, and then presented, and our bodies are able to mount an immune response.

Now I often get questions about how these MRNA vaccines compare with you can say flu vaccines, or more traditional vaccines that you might be familiar with. Just as a quick comparison, they really work I think fairly similarly from my perspective. Previous vaccine approaches say might inoculate you an inactive virus. That's more or less skipping ahead to this step. Our bodies see this inactive virus. It gets chopped up. Then we mount an immune response to some of these proteins. This is somewhat of a random process.

MRNA vaccines have a few advantages I think over this traditional process, although I wanna emphasize they're both very effective in utilizing similar responses from our immune systems. The first of which is using these inactivated viral systems tends to have a very long production time. You have to grow the virus. You have to be pretty good at guessing what viral variants there are gonna be. Another thing, as I mentioned, our bodies get this, and it's a little bit of anyone's guess as to which particular protein fragment you're body is gonna mount an immune response to.

That can be good or bad. Many of these proteins, one of the things viruses are very good at is mutation to avoid an immune response. Some of these proteins has less selection on them as we just heard about from Nils. There's pressure. You can put evolutionary pressure on things to either evade immune system or not. Some viral proteins are under less pressure. If you happen to have mounted immune response to something that is gonna rapidly mutate, that's going to be less effective.

Whereas when we design MRNA vaccines, we can pick proteins that we know are absolutely critical, and very unlikely to change in the population of people. We have a lot more control about selecting targets that we know are not only gonna elicit good immune responses, but are also unlikely to evade immune responses very quickly. Another advantage I think is many of these traditional methods require inactivation from this infection state. You're starting from something that was infectious, and you have to do something to make it not so.

Whereas when we're starting with MRNA this was never infectious. It was never encoding an entire virus. This is a safety parameter that we don't necessarily need to worry about. We probably all become much more familiar with vaccine targets for COVID, but you can imagine that based on all of the properties that I've described, really this is a platform that can be applied to many different viruses, many different diseases that are very scalable, especially with the advent and explosion of our ability to sequence viral genomes, and human genomes.

We are really expanding our capability of designing good, safe targets for this technology. One other thing that we can do that I think is very cool, although as I said I really believe the sky is the

limit for this technology, is we can also repurpose this machinery that our cells are already using to design vaccines against something like cancer. For example, in many cancers because they're growing at this uncontrolled rate, you might be familiar with these many mutations that are likely to accumulate.

The result is that many cancers are actually expressing protein variants that are slightly different from what all of your healthy cells are expressing. What that means is if we can figure out what those slightly different variants are, these are called neoantigens, we could design an MRNA therapeutic that would specifically target them. This would be basically a vaccine that in effect is gonna look for cancer cells and target them. Because it's gonna view them as a foreign body.

Because the cancer cells are expressing these distinct protein profiles, this can be an effective strategy to clear the cancer, and get your own body's immune system to do that for you. This is just one example I think of many that is very exciting. I'm gonna hand it back over to Nils now to talk about this a little further.

Nils Walter: We are not just dreaming this up. I wanted to show some evidence that this is already working. This is from December of last year. One of the first announcements made by Portanda 00:36:11 where, of course, there are many MRNA vaccines were made, and Merck Pharma Company had a collaboration to investigate personalized MRNA cancer vaccines made the way that Rachel described. Sequencing the tumor, figuring out what sequences of RNA are in there, and giving rise to what proteins on the surface of these cancer cells.

Then making MRNAs that would make that protein and instruct the immune system to attack that cancer and reduce it. They had to stop the clinical trial because they couldn't have the placebo group not get the MRNA vaccine. Because it was so effective. This is just one example, a recent example. All of pharma industry now has major efforts in making MRNA and other RNA therapeutic modalities against all diseases. It's expected to be a many billion-dollar industry, and went way beyond vaccines against infectious diseases.

The critical thing to keep in mind is that RNA is a polymer that stores information. By designing an RNA based on information

that we can glean from a virus or a cancer cell by sequencing it, it's the only information we need to instruct how to make the next messenger RNA that becomes the vaccine. This can be done in record time by Moderna, for example, in 45 days they had a vaccine ready after the Chinese posted a sequence of the SARS-COV-2 virus.

The same can be true for cancer cells. Think about the problem of cancer is actually that the immune system does not do its normal job. Normally it would kill—it does kill 100 billion cells a day that have gone *[unintelligible 00:38:09]*. Here a cancer cell escapes it. Then gets the immune system to ignore it. That's how the tumor grows. You can immediately sequence the tumor, and make an MRNA that would help fight antigen, or make an antigen that ultimately reeducates the immune system to attack it.

If the cancer cell mutates and escapes, you sequence a new tumor, the metastasis, and you can do that over and over. Again, because of the speed of molecule biology of designing in the computer, and making it by standup procedures that, for example, the $750 million new investment by Pfizer into the Kalamazoo facility will make it large scale. You can actually do very, very fast responses. Much faster than vaccine making was at the beginning.

What I wanted to finally emphasize is that there's actually a lot of tradition for things related to this at the University of Michigan, right here in town. For the townies here something to be proud of I suppose. Martin Yuroburg 00:39:18, was an alumnus, got his Ph.D. actually in Rachel's department of biological chemistry a number of years back. Then later went on to help interpret the genetic code, and its function in protein synthesis. MRNAs how they function, how do they encode proteins that he co-discovered. Augie Orenbach 00:39:40] was an undergrad in my department.

His father actually a famous University of Michigan professor of physics, theoretical physics. He actually was the one in the 1980s who developed the way in which we now all make MRNAs by transcription using an enzyme called T-7 RNA polymerase, which comes from a bacterial phase. Something that affects bacteria. It makes this protein that can transcribe DNA into RNA for us in the test tube. That's exactly the process that to this day Pfizer and Moderna use now in kilogram scales.

Augie I don't think is getting any revenue out of this. He didn't patent this. We all can use it. This was developed in the 1980s. Finally, just this past December Melissa Moore, who is the CSO, or was the CSO up until that point of Moderna, and a good friend of mine actually she got an honorary doctor of science degree here at the university. This is from the ceremony where the *[unintelligible 00:40:42]*, whose name should not be spoken I guess, but gave her the certificate of the doctorate. Again, there's a lot of connection here at the University of Michigan.

Just to close that loop, in 2016 we found at the Center for RNA biomedicine that Kira mentioned at the beginning, my co-director Mats Youngman 00:41:03 and I, and the mission is to promote and develop process for collaboration on RNA across campus, and beyond, and mentor the next generation of RNA biomedical scientists. Maybe you have siblings, or kids that want to go, and embark in this new exciting science, and crossing over into medicine. This is something you can do here.

In 2018 we got a major award from the University of Michigan called the bioscience initiative. That allowed us to hire at the Center for RNA biomedicine five different faculty. One of them, and this was written in 2018, was to be on RNA drug targeting or as medicine. That became very clairvoyant in 2019, 2020 of the RNA virus SARS-COV-2 came to the fore. Then also we hired none other than Rachel. She's one of our star recruits as an assistant professor into the Center, and the department of biology and chemistry.

We have a certain phase wise increase. We wanna build out, and get sustainability. If anyone can cut $1 million check, they are welcome to do that today. Finally, we want to really bridge the divide between bench and bedside by finding new targets using a tool called bioinformatics, which is computation analysis. Basically, making MRNAs, but also other modalities that have different names here.

We can synthesize them in different ways through RNA manufacturing to target certain diseases with these RNA therapeutics including MRNAs. Then deliver them in a specific way, and you can see many, many different diseases can potentially be addressed with these RNA therapeutics. This is really the new

initiative that we are launching this year. If you are interested in reading a little bit more, we have this magazine.

I think one per table, RNA translated. It talks about what we call as MRNA therapeutics, M will be, hopefully the block M of the University of Michigan. Okay. With that, thank you very much for your attention.

Kira Berman: Wow. That's a lot. Thank you both of our speakers. That was amazing. I learned so much. We're gonna take a short break now. We'll come back just a little after 7:00 for our group conversation. Please take this opportunity to refill your glasses. Remember your servers, whose names are Chris and Maddie, and they can help you with that process.

Kira Berman: With that preamble, would anybody like to start us off with a question or comment? I see you.

Audience member 1: Thank you to the speakers. That was great. I wanna make a comment. I wanna encourage people to seek the amazing, wonderful book called, "The Code Breaker," by Walter Isaacson who talks about MRNA research crisper, the COVID vaccine development, the test development. It's an amazing book. It's beautiful in soft cover. You don't even need the hard cover book. It's just an amazing, beautiful book. Thanks.

Nils Walter: Just to add to that, Jennifer Dobb 00:44:39 is really a leader in the field who has bene in the field for many decades. I spent some time at her house when she was an assistant professor. I was a post-doc, and we ventured into a collaboration. I've seen her for many decades. She's a wonderful leader, a very strong leader who also thinks a lot about the ethics of using crisper. Really, somebody to admire.

Audience member 2: Long, long time ago, when I was in high school, I'd heard of messenger RNA. Then over the last, dare I say, half century I really hadn't heard much about messenger RNA. Meanwhile, I've heard lots of things about vaccines, polio, flu vaccine every year. It seems that in 2021 those things just started to come together. Was there something specific about COVID-19 which meant that they couldn't do things the way they had been doing them over the last half of century? They had to move this, what I'm assuming was a

new technology that had been going on in the background, but just had to burst on the scene at that point with COVID-19?

Nils Walter: Yeah. What's oftentimes forgotten is that, of course, a breakthrough now is decades of work that led to it. MRNA vaccines are no exception. In the 1990s a person, whose name I never quite get right—yeah, please.

Kira Berman: Carey *[unintelligible 00:46:33]*.

Nils Walter: Yeah, Carey *[unintelligible 00:46:34]* started working on this. In a very almost hardheaded way stuck with it for decades. Nobody wanted to believe her that MRNAs could be used to make proteins and cells, which he did. The challenges are that RNA can degrade, as you heard from Rachel. It's transitionary in the cell. It's there to be broken down after a certain period of time. You can switch to a new set of genes to be read out from the genome. The challenge was always to make it stable enough to survive so they can actually deliver it.

Then to actually get it taken up by ribosomes to be translated. There was decades long work on that. In 2000, a German company, CureVac, was founded. As the name implies, they wanted to use vaccines to cure, and use MRNA technology for that. If you followed that a little bit, they were actually late to the game. Because in the end, in 2021, they didn't include particular modification that everyone else knew to include. Their vaccine didn't work as well. They got sidelined.

2008 this German company, another German company, CureVac is German. Another German company, BioNTech, was founded around this idea. 2010 then Moderna. BioNTech later teamed up with Pfizer to make it larger scale. The story that Melissa Moore, for example, told us about Moderna is that they had just built the capacity by buying a warehouse, and equipment to make large scales of MRNA in 2018, not knowing what it was going to be used for.

They were just in the right time to then start up large production. They knew how to make it. They had read all the tricks that other people had come up with to make these MRNAs stable, and efficient, and all of that. Really, it takes a village to raise a child, in this case an MRNA vaccine. That was really true here. I should

also emphasize that, and you might've read about this, DAPA 00:48:55 for example, as well as NIAID under Tony Fauci actually funded Moderna for doing some of this work.

BioNTech and Pfizer had developed independently without federal funding, but federal funding played a major role also in getting this done. It was really a confluence of various different threads that developed over decades really. Again, I think it's important to keep in mind how long-term science works, and how there's often a discovery that nobody knows how to use that later becomes a breakthrough.

Rachel Niederer: Actually, I just wanted to add a personal antidote. 'Cause one of the things that—so, during my introduction it was mentioned that I did my post-doctoral work at Yale with Wendy Gilbert. I actually started my post-doctoral work with Melissa Moore, who Nils mentioned was the CSO of Moderna. Back in 2016 when I had only been a post-doc with her for a few months, she decided to close down her lab to leave to be the CSO of Moderna, which was obviously very sad to me at the time.

She gave a very compelling explanation. That she felt like MRNA therapeutics were coming into their own. The combination of this ability to sequence pathogens rapidly, and our ability to deliver MRNAs, and to target them was really at the time when it was we could make a really big impact globally at improving human health. I was sad because my position was ending. That was again back in 2016. I think it was clear to a lot of the people in the RNA community that really we had figured out many of these obstacles to really address problems.

I think people were already working on this with Zika, for example, and other viruses, but it wasn't making headlines necessarily. To me the unique feature of COVID was just that everybody was on the same page about trying to address the same problem, and address the problem quickly. Many of the advantages to MRNA vaccines over traditional ones the scalability, the ability to target things quickly became major assets that were on view for everyone. To people in the field we had seen this developing over many years. That's a shorter timeline. Anyway.

Nils Walter: There's one other dirty secret about MRNA vaccines, which is it's generally difficult to deliver RNA to cells. Because RNA is

negatively charged. If you think about what the lipids and the membrane do, they do not like anything that likes water. They don't let it pass. They don't let water pass. That's their function. RNA is really what we call water loving hydrophilic. It doesn't get across by itself. It just happens that these lipid nanoparticles that are now used had been developed also years before as a way to deliver RNA to cells.

It only works for certain things like the liver, or for vaccines that you put into the arm, and then macrophages white blood cells immune system cells come along. They are used to gobble things up that don't belong there. Then it so happens that these lipid nanoparticles release the MRNA, and it becomes fodder for the ribosome.

Audience member 3: This is a follow-up to the first comment that was a recommendation for a book. I have a question about a book. I'm very taken by the discussion of molecular evolution, the evolution of RNA, and so forth. Can you recommend a good book, monograph, article, whatever? Something maybe akin to Canfield's History of Oxygen, or whatever? Something for general audiences for molecular evolution?

Rachel Niederer: That's a you question.

Nils Walter: Okay. It's actually not that trivial. There are many people who worked on it. What I failed to make clear is that there were also with many researchers working on it, many different opinions about how this could've happened. Of course, there are no fossils. We might internally have different opinions about what actually was happening there. There's an older now American Scientific article about evolution written by Leslie Orgel some people say. You might want to look this up. This is O-R-G-E-L, Leslie Orgel.

He was one of the leaders of the field, and founders of the field I think I mentioned his name was on one of our slides. I would start there. My mentor tried to put together a book that actually makes the analogy between evolution at the molecular level, evolution and biology, evolution of societies. If you look at that, they see the same things happen just at different scales. If you think about how societies evolve, there are certain things that amplify, and social media actually is a good example for how they can amplify, and become major themes or organization principles in society.

Much of what we experience daily is actually similar to what happened probably in molecular evolution. Again, things to look up.

Audience member 4: I have a question. First, I just wanna say this is the first time we've been here, and it's been so fun talking to people at our table. You talked about lipids. Well, we have here someone who worked for 30 years at Pfizer, and was on that team that figured out how to manufacture on a large scale, so that we could have—

Nils Walter: Did I say anything wrong then?

Audience member 4: - that I just feel such a privilege to have been able to hear from him. My question really is about the new MRNA vaccine, and the Keytruda. I'm not being so deep into science. My understanding was Keytruda was paired with another drug that was highly effective. I guess my question is, how much more effective, or is it at the same level now that you're pairing on the MRNA vaccine with Keytruda?

Nils Walter: They claim it's 44 percent more active. Now I'm not a physician, not running clinical trials myself. I don't know how to evaluate that exactly. I think that anything that can help in cancer is something that pharma industry is very interested in bringing to the market. Because there's potentially a lot of money to be made. Now this is for melanoma. Melanoma is not as big a problem as some other cancers.

I think there's also the idea that showing it for one cancer brings a lot of interest, and potentially money to making other MRNA, anti-cancer MRNA. Of course, also commercial interest to push this. My understanding is that it was significantly effective in supporting this other drug.

Rachel Niederer: I can check. It's obviously too really dependent on how unique the mutational signature of the cancers are. I think it's a very exciting area that I think has a lot of up-side. We're still at the early stages of being able to tell how well we can sequence. I don't know by signatures that are targetable. I think this is a really good proof of principle that I'm hoping that 44 percent is the floor, and we get better at identifying these new antigens.

Then these cocktail approaches to all of the proteins that are mis-expressed, or have different sequence signatures that we can then target. We're really at the early stages. It's a combination of needing to be able to sequence individual patients, and more of them, and then design MRNAs to target, and figuring out which ones work best.

Nils Walter: Yeah. There's one other thing of the packaging. You can package multiple different MRNA sequences in the same lipid nanoparticle, or at least the same cocktail. With that, you can say, "Okay. I don't know which of these two strains of an infectious agent, a virus will be the one that breaks through. Let's put both MRNAs in there." The same is true for anti-cancer drugs. You can use multiple of these neoantigens, newly developed antigens that are unique to this tumor for this particular person. Then give a personalized medicine that actually has multiple different MRNAs that cover all the bases.

Audience member 5: Okay. You answered the one question the gentleman asked over there. An amazing book I read two years ago is by Nick Lane. It's called "The Vital Question." His whole team has done a lot of breakthrough work at looking at the origins of life. He spends most of his time thinking about it. In fact, he's written a book on oxygen. I don't know if everybody else has been using ChatGPT, but it's a recent fascination of mine. It's a trick to use it to get good answers.

I'm gonna treat you guys like ChatGPT. I hope you don't mind. I usually start all my questions by saying, "Answer this as if you were the expert in the subject." I try to get the negative answer. What I ask in this particular case is, if you were gonna make an argument for the concerns that people should have about the science, or the cons of MRNA vaccines, you mentioned one already. For instance, the delivery, and also sequencing the proper proteins on the exterior that you're gonna target. Because without getting the right ones—anyway. As the experts, how do you respond to the, acting as devil's advocate?

Nils Walter: You mean to take the other side? Present the *[distorted audio 00:59:48]* argument?

Audience member 5: No. Really, as you as a scientist. What do you see is the challenges? Not really play into the bad press, but.

Rachel Niederer: I know that you said that we already mentioned this. The major challenge right now is delivery. From our understanding of how translation works, I think that we can target a lot of—there's a lot of potential there. Really, the major obstacle right now is delivery. If all of the drugs that we're targeting end up only in macrophages or liver, that limits our ability to design effective therapeutics. Right now I would say if there are other obstacles beyond that one, they're masked by the fact that right now we're mostly limited to delivery to the liver.

That's a problem many people are working on. I have no doubt is gonna be solved soon. Then we will see, but right now, like I said, from a molecular biologist, biochemist perspective the other end of it seems like as soon as we can solve that, I feel like we can tackle many problems. It's really delivery from my perspective.

Nils Walter: Yeah. I hope we are a little higher in accuracy in ChatGPT I have to say. I think that there's another story I wanted to tell here which is, since the '70s and '80s so called antisynthetase were used to suppress genes that are undesired. Maybe the gene that gets made into a protein and ultimately, leads to cancer. In the '70s it was tried first. Then for decades developed. Companies were found around this. Then never quite took off. Okay. Because as it turns out now decades later that we didn't know how the machinery in a cell works, and couldn't utilize it to our benefit.

In 2006 the noble prize was given for small interfering RNAs, SRNAs which was another modality of RNA that can be used. Those are using specific machineries in the cell, so called RNA antisynthetase complex that ultimately regulates messenger RNAs. Since we now are using in this instant with the molecule that in principle looks like the former antisynthetase *[unintelligible 01:02:24]*, and SRNA that the cell itself also makes. Now it works much better.

This is just to show that what we are oftentimes limited by is the foundation, or the fundamental knowledge about how the cell works to utilize it properly. That's a strong argument for what in the U.S. is called basic science, which I think is a misnomer. In

German it's *[foreign language 01:02:52]* fundamental science because that's more proper to what it actually represents.

That's some of the questions I think on the table are around would you pay more taxes if more background can be understood, so that you can actually make more drugs. That's a question that affects all of us. The question that's important.

Kira Berman: Thank you so much. I need to get my microphone. I got it. It was muted. Thank you so much. We have time for maybe one or two more questions depending on whether you an answer them quickly. Before we do, I wanna remind you about those little blue evaluations on your table, and the little yellow pencils. We like to keep to amazing blue for these things. I saw two hands up. I have two questions. I'll have people ask them quickly. Hopefully, we'll get to both.

Audience member 6: Are there any situations in vivo, or in vitro that you can engineer an RNA molecule to become an enzyme to catalyze reactions?

Rachel Niederer: That's probably a better question for Nils. I think the answer is yes.

Nils Walter: I think engineering from scratch is difficult. We can't certainly because we don't know enough there's not enough fundamental science done yet. There's hope that AI will help there as well. There's the idea that proteins now can be designed with artificial intelligence software, and hopefully soon RNA. Then we can design RNAs of any function we like.

Audience member 7: Can you design something so that the messenger RNA can get through blood-brain barrier?

Rachel Niederer: I will take it. I think the answer will be yes, that's an area of very active research. MRNAs actually has many components to them. Part of the getting through blood-brain barrier is this delivery question. I think that's actually gonna be more of an issue of the lipid nanoparticle. One of the areas that I'm very interested in is, as I mentioned, these MRNA programs are read differently, or expressed differently in different cell types.

Even if we solve this delivery problem, if you're delivering an MRNA that the brain is like, "Well, this is only supposed to be on the liver. I don't care about it." It's less interesting. I actually think

that's an area of very active research, and it's one that I'm very interested in is designing RNAs that are expressed well in the tissues where we want them to be. Cells already do this very well. All we have to do is understand the code that they're following. Like I said, that's what I'm most interested in. Thank you for that question.

Kira Berman: Maybe one more quick one?

Audience member 8: May I?

Kira Berman: Yeah.

Audience member 8: Nils, in the beginning of your presentation you talked about the chemical evolution from the primordial soup into this. You mentioned that energy had to be added. Where does that energy come from?

Nils Walter: Yeah. We had this brief conversation earlier. There are many different forms from which the energy could have come. Certainly, discharges like lightening also can do something to build more complex molecules. A more likely scenario for the primordial world might be a hot under sea vent that also has heat for one, but also has minerals like sulfur, elementary sulfur that can serve as a building block to build energy. There's a pathway with which you can potentially create even ATP down the road from the energy that's stored in sulfur.

There's a mineral called pyrite, which is one iron, and two sulfur atoms together, which I collect. It's very beautiful. It's the fake gold. It's a fool's gold. That actually is a mineral that has this extra sulfur in it that could also be one of the mineral surfaces on which energy was available. Because sulfur can be oxidized. It creates energy that ultimately life could've used early on.

Audience member 8: Thank you.

Kira Berman: Thank you. One of the most wonderful things I love about working at a Natural History Museum is that it helps me to draw to connections between the very ancient past, and the future. Thank you for coming today. Please thank our speakers. Please join us in October for the next one.

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