Jason Anthony: Welcome to Proto, a podcast that explores the frontiers of medicine. I'm Jason Anthony.

Luke Chao: And I'm Luke Chao. Today we're talking about an innovation in basic research transforming the way we develop new treatments. The 2017 Nobel prize in chemistry was awarded to three scientists for their contributions to electron cryo microscopy, cryo-EM. This method allows us to directly see the molecules of life in breathtaking detail. We use this tool in my own lab to understand how molecules work and malfunction in disease.

Jason Anthony: And I'll visit one of these instruments located in New York City and explore how powerful this technology has become over the past few years.

Bridget Carragh...: If this tiny little three millimeter grid was Southern California, one of the proteins we want to look at is one person in there, and there's a lot of people in Southern California.

Jason Anthony: Coming up on this episode of the Proto Podcast, brought to you by Massachusetts General Hospital.

My co-host today is Luke Chao, molecular biologist at Massachusetts General Hospital. Welcome to the Proto Podcast, Luke.

Luke Chao: Thanks.

Jason Anthony: So can you tell our listeners what exactly does a molecular biologist do?

Luke Chao: Molecular biology studies the basic rules of life. All things that make us sophisticated organisms are governed by molecular interactions happening at the atomic level. Molecular biologists try to understand some of the fundamental rules and an important aspect of this is understanding the shape of molecules.

Jason Anthony: Now, this plays into this discovery, this Nobel discovery that was announced in early October, the news about cryo electron microscopy said that it could help us see proteins better. So that's important, sort of seeing those shapes is important to work as a molecular biologist?

Luke Chao: Yeah, proteins are really versatile molecules. Central to their abilities to do a variety of functions is their really amazing shape, and cryo-EM gives us a chance to get a glimpse of these shapes in very native-like contexts.

Jason Anthony: Can you give us some examples of proteins in the body that we might be familiar with?

Luke Chao: Hemoglobin is a really critical protein that transports oxygen in your blood from your lungs into your tissues. Antibodies are really important molecules that respond to foreign pathogens.

Jason Anthony: But there are a lot of kinds of proteins as well, sort of beyond those that we might understand is that right? How many proteins, different kinds of proteins are there in the body? Do we know the answer to that?

Luke Chao: That's actually a difficult question to answer. There are about 30,000 genes that code for proteins, but there are a lot of modifications of the proteins themselves that customize them for different roles.

Jason Anthony: So there was a lot of excitement about cryo electron microscopy, this ability to look at proteins and see what they look like. Why is it important to see them? Why is it important to understand their shapes?

Luke Chao: Shapes are central to how they work and how they function, and they're also really important if we want to actually modify their function, or block their function in the case of many drugs.

Jason Anthony: We've known the shapes of some proteins for a while, as I've understand it, sort of mapping proteins goes back, I think, gosh, to the fifties. Has cryo-EM always been the standard for understanding what proteins look like and if not, how do we start understanding what the shapes of proteins were?

Luke Chao: Well, the real champion in this field that's generally referred to as structural biology is this technique called x-ray crystallography. And x-ray crystallography has determined the structures of the vast majority of proteins up until this point. This technique uses a beam of very intense x-rays and blasts crystals of proteins.

Jason Anthony: So presumably the laboratories would need to synthesize a lot of a protein and then somehow turn it into a crystal and then sort of shoot it through with x-rays. Is that about right?

Luke Chao: Yeah. One of the advantages of cryo-EM is the fact that you don't have to grow a crystal.

Jason Anthony: And how does that help? How is that an advance over this crystal method?

Luke Chao: In some cases, when you actually grow the crystal, you might lock out certain confirmations or positions of the molecule. And cryo-EM, because it doesn't require a crystal, allows you to capture some of those potentially.

Jason Anthony: So you can see the protein, maybe in different shapes or in more shapes, more of its stages of motion than you might with a crystal.

Luke Chao: Proteins are really dynamic molecules. They're constantly moving around to do their role, to work. And what's exciting about cryo-EM is trying to get more of those snapshots, more pictures of how proteins while they work.

Jason Anthony: So we'll talk about practical applications for this in a minute, maybe what some therapeutic applications are, but I should say here that you're an investigator at Massachusetts General Hospital. Is it unusual for a hospital, which sort of sees patients coming, walking through the doors with maladies, is it unusual for them to have a department of molecular biology?

Luke Chao: Really sharp institutions recognize the importance of basic science. Many of our major medical breakthroughs have their origins in fundamental research.

Jason Anthony: So there's a department there that looks at these sort of very basic, very structural interactions happening in the body with the hopes that eventually they will lead to new treatments and new ways to understand disease.

Luke Chao: All of these problems that we're studying in the department are fundamental problems that in many cases go awry in cases of disease. So understanding how they work normally is really the first and essential step in order to ever treating them.

Jason Anthony: Well, thank you, Luke. Coming up, I'll take a tour of a cryo-EM lab and meet two researchers doing pioneering work with this powerful tool.

Luke Chao: You're listening to the Proto Podcast, a production of Massachusetts General Hospital.

Jason Anthony: Perhaps the most central and most challenging task in medicine is to understand what happens at a molecular level inside your body. Every second, billions of complex transactions are ticking away to keep you alive. Cryo-EM is a tool to help us see the molecules involved in those interactions, some of them for the first time. To see cryo-EM at work, we visited the New York Structural Biology Center in Manhattan, a place where research institutions in the area can pool their resources and work with this kind of cutting edge technology. My guide is Bridget Carragher, a director of the Simons Electron Microscopy Lab at the center.

Bridget Carragh...: This is what we call our control room and behind these four doors are the big microscopes. So people don't go in the room with the microscopes, because people are source of noise and heat and vibration. And those microscopes have to be exquisitely well-controlled and free of all of those aspects. We're trying to look at atoms after all. So any vibration is going to blow those atoms.

Jason Anthony: Now, even though these big microscopes are incredibly delicate, what do you say we open a door and look at one of them? Will we destroy anyone's research if we do that?

Bridget Carragh...: Let's go and ask one of the scientists if we can do that. Hey guys, could we open a door for this? We won't mess any of that? They just went to have a quick look.

Speaker 4: Sure.

Bridget Carragh...: Okay. And we try not to open the doors too often, but they're in the changeover time. So they're allowing us to do that. So here's one of the big guys as you see, it's huge.

Jason Anthony: This is 10 feet tall, easily.

Bridget Carragh...: 14 I think.

Jason Anthony: Holy moly, just a sleek structure, black and silver. And now, so at the top of this structure, the electrons are being shot down on the sample. Is that correct?

Bridget Carragh...: Exactly. There's an electron gun that produces the electrons. Those are focused with electromagnets and they go through the tiny little thin sample, which is somewhere about in the middle of the structure. And there are more electromagnets. And then these fancy new direct detector cameras are at the bottom, collecting the magnified images.

Jason Anthony: Next, we went to the room where samples themselves are prepared. The researchers must first isolate many copies of the protein that they want to look at, but even a million protein molecules don't take up too much space.

Bridget Carragh...: The sample is incredibly small, and it's hard to explain in physical terms, because it's so small, but let me show you the support thing we put them on. So this little copper mesh, which is three millimeters across-

Jason Anthony: So just for reference, I would say that's about the size of an O on a computer keyboard.

Bridget Carragh...: Now, one of those molecules in that sample, relative to this little O on the keyboard, as you're talking about, is like a person in the whole of Southern California.

Jason Anthony: And then the sample is frozen, the cryo part of cryo-EM. The same machine that spreads the sample on the mesh also contains a small bowl of chemicals to do the job.

Bridget Carragh...: So it's liquid nitrogen, is the bubbling staff. And many people have seen liquid nitrogen, you can make ice cream with it at parties. And in the middle is a different fluid called liquid ethane. And that's what snaps these samples into a frozen state, very, very rapidly.

Jason Anthony: This was the innovation behind cryo-EM, that tiny complex molecules could be frozen in an instant. They would preserve their shape surrounded by an ultra thin layer of water molecules that wouldn't interfere with the electrons that these microscopes use to see very small objects. After the tour, Bridget and I were joined by Clint Potter, another director of the Simons Electron Microscopy Center.

So cryo-EM has been around for a while, but in recent years, something has happened so that you're able to see these pictures in much, much sharper focus.

Clint Potter: It's really this new detector technology that's been a major component of this-

Bridget Carragh...: And by detector technology, Clint means camera.

Clint Potter: And the idea, before we could really only take still pictures and the new cameras allow us, they give better image quality, everything, but they also allow us to take very fast movies. And when that electron beam hits the sample, there's distortions being caused over time, and by being able to take fast movies, we can correct for these distortions and get much better quality images.

Jason Anthony: Wow.

Bridget Carragh...: And that was one of the things that led to this recent revolution five years ago. But prior to that was 20 hard years of work by lots of different people doing other aspects that were also very important. The camera gets the credit because it was the last thing to be added that made it all possible. But plenty of people did lots of hard work before that, on other aspects.

Clint Potter: Yeah. Just coming up with the algorithms of how do you come up with these three-dimensional structures from these two dimensional particles where you don't know the orientation of the particle.

Bridget Carragh...: Very noisy pictures of those particles, too.

Jason Anthony: So have there been any sort of landmark discoveries with cryo-EM, is there any work that's going on with cryo-EM that really sort of put the technology on the map?

Bridget Carragh...: One of the first structures that was solved with cryo-EM at the beginning of this revolution was the TRPV1 channel by Yifan Cheng in University of California, San Francisco. And this was very important. Ion channel has to with how you sense pain and heat and cold, and it had never been solved by crystallography. So it was an important structure. And that was at the very early beginnings of this, people were really impressed and taken it back and said, oh, wow, this is a technique worth looking at. But since then, the spliceosome, that's another incredibly complex molecular machine that very sloppy and it moves a lot and that's been solved by many groups and-

Clint Potter: Many other membrane proteins. And what's happened is a lot of these x-ray crystallographers are now using cryo-EM techniques and they had all these great samples in their freezers and they couldn't get crystals out of it. And now they're able to use the cryo-EM technique and are getting structures.

Jason Anthony: Are there any diseases say that cryo-EM is helping to treat?

Bridget Carragh...: Well it's early days yet and drugs take 20 years to develop sometimes and cryo-EM's been doing this for five years, so it's a little bit early, but one beautiful example of this is the cystic fibrosis protein. One of the proteins called CFTR that is involved in cystic fibrosis and Jue Chen at Rockefeller University has solved several of these now using cryo-EM. And that's been a holy grail for cystic fibrosis because of you need to target these proteins with drugs and you need to understand what they look like, what they're doing before you can do that. So that's one of many, many, many, many examples, but one that's a local one and Jue uses our facilities as well as their own facilities in Rockefeller, and also Janelia Farm. But they've been collecting data with these facilities so we're kind of jazzed about the fact that this is a huge breakthrough for the community.

Clint Potter: And we have our project here understanding the structure of the insulin receptor, which is important not only for diabetes, but also is associated with Alzheimer's as well as some cancers. So understanding what this insulin receptor is very important, and that's being done right here as well.

Bridget Carragh...: And that's actually being done by scientists from Merck, so Merck Pharmaceuticals has a scientist here looking at the insulin receptor. So that's a very direct connection now back to the drug discovery community.

Jason Anthony: But so this cryo-EM, is happening not just in academic centers, but in drug development companies and private industry as well. It's sort of a revolution.

Clint Potter: Within the past really year or two, there have been big interests by pharmaceutical companies. They're buying their own microscopes now trying to learn the methods. These are x-ray crystallography groups in the pharmaceutical companies learning to do cryo-EM.

Bridget Carragh...: And there is a company that also does cryo-EM on a fee for service basis for pharmaceutical companies. Full disclosure, we're the founders of that company. And it's based in San Diego.

Jason Anthony: Well, Bridget And Clint, thank you so much for your time today. This has been exceptionally helpful and we really appreciate your time.

Clint Potter: Great. Thank you.

Bridget Carragh...: You're welcome.

Jason Anthony: I'm joined once more by my co-host, Luke Chao of the Department of Molecular Biology at Massachusetts General Hospital. Luke, that was a fascinating visit for me just to look at these physically imposing machines that could see these tiny proteins at a molecular resolution. I'd love to talk about how you use cryo electron microscopy. You have this technology at your disposal now. So how do you decide what proteins you'd like to map?

Luke Chao: In my group we're really excited about the molecular machines that shape cells. So cells are actually filled with various different kinds of compartments. And there are molecular machines that rearrange these compartments. For example, the mitochondria, which is known as the powerhouse of the cell, that organelle, one of these compartments, constantly divides and fuses with one another. And we're really excited about trying to understand those processes.

Jason Anthony: So you'd look at the proteins associated in that process to sort of see how they move and how they work together?

Luke Chao: Yeah, these proteins are critical for their function and they're mutated in a lot of diseases and cancers and neurodegenerative conditions. And we'd like to understand how they work so that we could discover better treatment.

Jason Anthony: So once the cryo-EM technology creates a map of the protein, how do you use that information in your lab?

Luke Chao: In many cases, the structures are really just the first step in understanding how the protein works. We're really excited about trying to put together the processes. This is a activity that's called reconstitution. It's basically building structures and events that happen in cells from purified components.

Jason Anthony: So you'd get these individual proteins and then you'd isolate them and watch them sort of at work together is that sort of the idea?

Luke Chao: In many cases, the proteins work together as teams. So to understand how they function, one has to put together many copies of them in the right environment, in the right context, to see if they can regenerate some of the activities they might be doing in a cell.

Jason Anthony: One of the things that we heard Bridget and Clint talk about were membrane proteins and how important those were. What's a membrane protein?

Luke Chao: So membranes are actually the boundary between compartments in a cell and in many cases there are really critical proteins that lie on this boundary that help transport things across the boundary. In our case, we're excited about proteins that are on this boundary and might actually reshape this boundary, redefine where it might lie.

Jason Anthony: And you hear there's a lot of excitement from pharmaceutical companies and sort of people working in this translation research to decode membrane proteins particularly, why would that be?

Luke Chao: Membrane proteins are really the target for a variety of drugs. And since they lie on the outside of the cell, they make a very accessible target for treating diseases.

Jason Anthony: So are there any clinical applications that might result from the work that you guys are doing, looking at these proteins in the lab?

Luke Chao: These machines that are involved in determining cell shape, they're often, as I alluded to earlier, mutated in a variety of neurodegenerative conditions and cancers. We're excited that some of these basic discoveries may lead to treatments down the road.

Jason Anthony: Luke Chao, thank you so much for joining us today. This has been incredibly informative and listeners, thank you again for tuning into the Proto Podcast.

Luke Chao: Today's podcast was produced by Emily Silber, Bradley Klein and Sarah Alger.

Jason Anthony: Thanks also to our technical director, Adam Keller, you can find the Proto Podcast on iTunes and Stitcher. Please subscribe. You can also follow Proto on Facebook and Twitter. See you next time.