Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in the life sciences. I'm your host, Steve Lewis, and I had the pleasure of welcoming Dr. Ramesh Jha to the show for today's episode. Ramesh is a technical staff member at Los Alamos National Laboratory in New Mexico, where he's worked for the past 13 years. He specializes in biocatalysis, computational biology, and he loves sharing about all the applications of his work to other areas of science. I could have easily spoken with Ramesh for probably a couple more hours. And I'm really excited to share with you the highlights of his work today. We jumped right in by discussing the focus of Ramesh's group at Los Alamos, and the implications of their work on a greener future for our planet.

Ramesh Jha, PhD 01:02
So the biome group at Los Alamos is focused towards working with microbes. And as I told you earlier, we are actually looking into kind of, you know, developing greener technologies for manufacturing, renewable chemicals, and fuels. The biome group also works around understanding the interaction between different microbes in the environment and how we can actually learn from them and work around them to address some of the key environmental problems and climate problems.

Steve Lewis 01:42
Interesting. So working alongside the existing environment, you're almost working within constraints. So, it seems like a bit of a design challenge as well, that you take on, on a regular basis.

Ramesh Jha, PhD 01:57
Exactly. One goal is to perform some kind of interesting bioconversion, which means that you start with kind of simpler molecules and convert into more interesting or useful or value-added chemicals and fuels. This process apparently needs a number of biocatalysts to actually convert the simpler molecule into a more complex molecule. And the big challenge there is that how exactly you engineer those biocatalysts. One thing which you actually rightly mentioned, is porting certain pathways, metabolic pathways, from one organism to the organism of interest and feed them the simpler renewable feedstock which they can be able to kind of convert to your value-added chemicals. But many times the pathway which consists of a number of enzymes, or biocatalysts, they are not very efficient. And there is, there you have a big challenge. How do you optimize those biocatalysts or enzymes to be able to actually perform the bioconversion at industrial scale?

Steve Lewis 03:10
So, this is a phenomenal preview, I think of the molecular biology aspects of our interview that I'm definitely going to ask you about. Let's stay at a high level for the start of the conversation. I am curious, how did you get to Los Alamos? And how did you find out that this area was of interest to you?

Ramesh Jha, PhD 03:32
This is a very interesting question, actually. So, in my grad school, I joined a lab where we performed computational protein design and tested them in the laboratory. And what we actually saw, I mean, during the course of five years, what I could do was basically design dozens of these proteins, express them in microbes, purify them, and then basically test them for their function. So, when I was about to graduate, I did see a very interesting requirement here at Los Alamos National Laboratory, where we

wanted to work at the interface of computational protein design and experimental validation. And that was actually towards designing new enzymes which can perform interesting bioconversion for biomanufacturing or bioremediation. So, definitely, it was the right time and a right place for me to actually come and explore other areas of computational protein design and have some application-based products being produced from my work.

Steve Lewis 04:38
That is a perfect segue probably to talking into some of the molecular biology aspects. Would you mind sharing, since you mentioned libraries and you mentioned variants, at a high level do you mind sharing some of the molecular biology tools that you utilize?

Ramesh Jha, PhD 04:57
So, our overarching goal is actually to create enzymes, and there are lots of enzymes I guess in nature, but when you start to work with industries you definitely need to actually improve its function which could be stability, longer shelf life, faster catalytic efficiency. So, basically, in order to do those, there are lots of molecular biology techniques which we can actually use. Definitely there are ways to kind of create diversity in your gene, that gene, which encodes for the enzyme. The problem is that when you start to kind of improve or make changes in any enzyme, one big problem happens is that usually you need dozens of mutations to have a substantial improvement in the function. But then it can take a single mutation to completely ruin your enzyme. So, you need to create these libraries. And if you just think about, like, you know, an enzyme being encoded by amino acids, these amino acid chains actually give you an enzyme and each position can have up to 20 different possibilities, you'd see that by the time you touch five different positions, there is 20 to the power of five different possibilities, which is already kind of you know, hundreds of thousands to millions of variants. So, now you will be actually touching lots of these positions. Computational design help you choose not at each position, not all those 20 amino acids, but a very subset of those amino acids. So, there will be kind of two to three variations. So now, when you have two to three variations, at positions at each position, you can actually touch upon 20 different positions and make a combinatorial library. Which means that each position you will have all those two to three variations, which computational design predicted. So, now this library when you create, and we often use these PCR-based methods to actually introduce these variations at each spot. So, now you are actually looking at kind of, you know, millions to tens of millions of variants. But then the big question remains is that, you know, how do you actually test that. And that's where actually one of our key technology is to create a gene circuit. We'll be testing all the efficiency of all these enzymes in actually a whole microbial cell format. And those microbial cells will have a gene circuit consisting of a transcription factor, which can actually detect the product of the enzymatic activity. And when that product binds to the transcription factor, it actually expresses a reporter protein, which will be a fluorescent protein. So now, each cell based on the activity of enzyme inside is actually you know, producing certain level of product the product is actually activating the gene circuit and you are getting a code-related fluorescence response. When you have a microbial cell with certain fluorescence response, you can sort them based on the fluorescence using flow cytometry. Los Alamos has happens to be really, I mean, we have a huge resource in in the flow cytometry area. In fact, flow cytometry was actually invented at Los Alamos and we happen to have like, you know, really good resource in that area. So, our approach, where we start with creating mutations in an enzyme, and linking the activity of an enzyme with these gene circuits, what we also call as biosensor circuit,

which ultimately gives you a correlated fluorescence response. We are able to utilize that or couple it with flow cytometry to kind of sort good performer from the bad performer. So that is actually our approach.

Steve Lewis 09:14
There's a lot to unpack there, right? There's a lot of different areas within what you just mentioned that I'll call the entire biology stack. I'll try to go stepwise from an upstream to downstream perspective. You mentioned and alluded to adjusting essentially protein folding to improve perhaps performance at active sites within enzymes. So, that might relate to five or six different folds in an amino acid, it could be in alpha fold or beta sheets, or what have you. Where do you select a starting point from in your modeling?

Ramesh Jha, PhD 09:55
So basically, the starting point would be actually to look at the natural diversity, what is actually there, okay. But then the big question remains are those suitable for industrial applications or not? There are kind of possibilities where you are more interested in some kind of anthropogenic kind of, you know, reactions or event you talked about bioremediation, and we have an active interest in that area. Those are actually these, you know, forever chemicals or certain pesticides which linger in the environment for years. And what you would see is that since nature hasn't seen those anthropogenic molecules for long, they did not have actually time to really evolve certain amino biodegrading enzymes for some of those molecules. But then there could be a possibility that some nascent activity has started to kind of pick up in an environment. Let's say plastic polyethylene terephthalate, that has been probably, you know, used for decades and all and apparently, in nature, some of these organisms have started to kind of utilize break down a of polyethylene terephthalate and ingest the molecules and kind of use it as a carbon source. I'm talking about some of those enzymes which are still kind of very early in their evolution. They are definitely not very great in the activity, and also our industrial scale, you need enzymes, which has, you know, better thermal stability, longer shelf life, can actually perform function in kind of, you know, a harsh environment, like having organic solvents. We have, you know, an idea that what we want to achieve, then we go after what's actually in the nature, okay, and start looking into it. And then we'll pick some of these good candidates and perform some baseline kind of, you know, activity, what kind of you know, functions they have, and then basically via computational modeling, understand kind of, you know, we can do some kind of, you know, mechanistic evaluation that how certain activity can actually happen, and then basically choose the areas which can be actually mutated for certain gain of function. And the computational tools, like, you know, Rosetta is a tool which uses a certain energy function which consists of physics-based and knowledge-based potentials and then it uses Monte Carlo search to actually look at certain protein certain positions and perform variations in it. And based on the energy, it can actually select, or not select, some of those mutations. So, that actually guides you to these different variations, which can happen in an enzyme. Definitely, one really interesting area is now with the AlphaFold, basically, you can kind of go a little bit beyond and start to kind of, you know, scribble certain kinds of sequences. If you really want to have kind of, you know, new activity engineered in a completely new protein for which you probably don't know if that is anything existing in nature you can start with certain, you know, very simple sequence, which AlphaFold can help you understand what this structure will look at and then that is kind of, you know, a

step by step, a bottom up approach where you can actually build some novel enzymes in that way. So, some of those areas of research are also being kind of, you know, looked into.

Steve Lewis 13:54
In talking about some of these molecular biology workflows, I'm curious if you could speak more to your approach overall, whether it's through library prep, or designing different downstream functional assays. What is your approach to workflow development?

Ramesh Jha, PhD 14:11
We actually always build a high throughput screening method to actually look at lots and lots of variants of enzymes which we have created based on our computational prediction. When you start to make changes in these proteins, you would be actually looking at millions of variants as soon as you touch kind of, you know, few positions to actually mutate, even after computational predictions. So now, when you have such a large libraries, you definitely want to actually test them all. And hence, we want to actually have a high throughput screening method. And one way to actually really tackle this combinatorial problem is to actually have certain you know biosensor design which can actually sense the product which is actually coming from the enzymatic activity and gives you some kind of you know signal. In our case, this biosensor gives you a fluorescence response. We carry these all I mean, the whole process is carried in a microbial cell. Each microbe actually has a unique sequence of enzyme being produced and based on the activity you have correlated fluorescence response. So, now in a single sitting you can actually look at up to 10 to the power of eight cells using flow cytometry. So, now, all you have to do is that, you know, based on the fluorescence, pick the best performing ones and those could be actually top 1%, top 2%. And you might have to, again, grow it, because I mean, even flow cytometry can actually give you some false positives, okay, because there could be a cheater, which just kind of, you know, get into your collected or, you know, sorted cell population. So you again grow, and you perform some of these, the similar kind of, you know, cycle of growth, giving it a substrate and seeing the fluorescence from the enzymatic activity, and then again, sorting that. So, basically, that's how you actually, you know, after multiple rounds, so, depending on what is the initial library diversity, you might have to introduce number of cycles to really converge to a few like, which could be a dozen of sequences, which are kind of definitely better than the others, okay? And then we go ahead, grow them, extract the plasmid, the plasmid was used to actually express certain variant of that enzyme, get them sequenced, and then we go ahead and actually produce those enzymes, and do some kind of, you know, next step analysis to see the gain of function in those enzymes. But then one very interesting area here is that, when you have large libraries, and you are capable of sorting them based on their activity just by looking at the fluorescence, you also end up actually getting lots of data. You can you know, at each step, you can go ahead and have different performers kind of sorted out. And then you could do next generation sequencing to look at a whole population that how exactly it looks, and how the sequence enrichment is happening. And that actually gives you some, you know, opportunity to perform the data-assisted kind of protein engineering where you can actually learn from large amount of data, utilize that, and then in the next round of design-build-test-learn cycle for enzyme evolution, you can utilize that data for improved design, which ultimately helps you kind of, you know, help you achieve the better sequences in less time.

Steve Lewis 18:12
The engineering sphere, I would say, is moving toward application areas that can have tremendous benefit to human life, biodiversity, and the other challenges that are being tackled, if you will, these are real tangible benefits that society can have, from the progress in these research areas. How do you all think about that, from a commitment to your scientific endeavors perspective? I imagine is that always in the back of your mind that you're doing this for a greater good?

Ramesh Jha, PhD 18:52
That is true, actually. I mean, and what is really kind of very interesting is that, you know, when, when we started, or when I started a group at Los Alamos, I started to identify some of these, you know, key problems, which, obviously, are very often highlighted in kind of, you know, news articles or by funding agencies and all. And, and those apparently, you'd see is like, you know, a very application-based problems. So, basically, can you solve “This?” So, can you have certain, you know, chemicals made from renewable sources, which can replace the one which is being produced from let's say, petroleum feedstock? And overuses of petroleum feedstock we all know that is kind of, you know, affecting resulting in these greenhouse gases and basically affecting the climate. That is one area. The second is the use of all these new kinds of, you know, chemicals, which we thought was like, you know, very interesting inventions in the last century. We have started to realize that they were meant to be kind of, you know, having long, you know, shelf life for a certain reason. But then now we have started to realize that they are actually kind of, you know, menace, I guess, basically it is affecting the environment. So, some of those problems we started to actually see, and we right away thought that, okay, we have these technologies, we know how to actually deal with these problems, why not, we go ahead and pick up some of those where we can directly contribute in terms of you know, addressing those problems. Basically picking some of those which has direct application. So, some areas, I guess, as you mentioned, polyethylene terephthalate, areas we have been trying to discover and improve the function of some of those natural enzymes, so that they can be used in an industrial setup.

Steve Lewis 20:57
We're excited to be in season three of Speaking of Mol Bio, and we know that we have you, our loyal listeners, to thank for the growing success of our podcast series. As a thank you, we're offering a free portable wireless speaker so you can listen to the podcast or your music anywhere. I have one at my desk, and I love how easily it connects to my phone. It's nice when I want to break from my headphones or want to share what I'm listening to with others. I hope you'll visit thermofisher.com/molbiopodcast to request yours today. Please note this item is only available in some regions and only while supplies last. Again, visit thermofisher.com/molbiopodcast to request yours. And now back to our interview.

Steve Lewis 21:46
One of the themes that I picked up on our conversation is the lines between chemistry and biology, with the intersection being biochemistry, and then life sciences and technology, with the intersection being biotechnology. It really sounds like those areas for you all, you can kind of be fluid between them in a way. And then simultaneously, you also mentioned this concept of we have chemical problems that we've created. However, they were designed perfectly for their different use cases. And now we have to come up with a biological solution in a way where, you know, we're aiming to do that, to ultimately

undo some of these you know, anthropogenic chemical, I'll say side effects that we have created. What do you think about the blending of those formerly bespoke disciplines getting more and more converged?

Ramesh Jha, PhD 22:49
So, I guess some of the environmental problems you're talking about definitely needs interdisciplinary approaches and a wide range of capabilities. So, basically understanding the chemistry let's say you want to do some kind of you know, degradation of certain anthropogenic molecule, understanding the chemistry becomes kind of very important. And basically, our group is actually, you know, great in utilizing biological materials to perform some kind of you know, this catalysis or the biodegradation. So, definitely we introduce the microbes, certain genes synthesis, basically the enzymes which are kind of, you know, encoded by those specific genes. So, finding an environmental problem, understanding the chemistry, and then using these kind of, you know, biological source materials, which could be enzymes or the whole microbial cell itself, to actually perform those function has been the key area. And I think that the environmental problem, or the research problem itself is so huge that blending some of those different areas would be kind of very important. And that is something again, kind of in our focus of our group and even at Los Alamos there are several, you know, funding programs which actually encourage you to bring capabilities from different areas and come together to actually solve or address some of those research problems.

Steve Lewis 24:34
Selfishly, this is an area that really interests me and I'm curious to hear your take. A couple of components of this conversation have danced into this new, or converged, area of multiple disciplines called biodesign. How often do you all have conversations in this kind of like, "This is a great idea, we think it would work". But then you get down the road in your conversation and you're like, "But you know what, like, we really actually need to think about the environmental constraints that we're working within." And this discipline of biodesign, considers itself, I'll say, like, that is where they kind of take their speculative design mindset and apply it to these problems. It's like, “Sure, you can engineer any plant you want, you can engineer to grow wherever it is, but if you don't even think about where the problem ultimately is, that doesn't necessarily help you, if you engineer a very productive plant that can't grow,” for example. I don't know that I communicated that very eloquently?

Ramesh Jha, PhD 25:48
Yeah, I see where you are actually going. I mean, probably that our ethical concerns about kind of genetically-modified organism plants, and basically using them in open areas. There are some of those concerns, I think. But what our approach has been, is to actually any, any genetically-modified organisms, which we produce to perform certain kinds of, you know, bioconversion, basically, those are still in very confined setup. So, you'll have a bioreactor basically, with some of those genetically-modified organisms and could be well off converting molecule A to B. But yeah, I mean, can we really kind of you know, have these designed, or engineered, microbes released in the environment to kind of you know, take care of some of those pollutants, which might be existing around? That has actually mean kind of, you know, some concern and some areas, which apparently, will be kind of, you know, sorted out. But I think there are kind of, you know, also some research going around like, you know, biocontainment. So, basically confined areas where actually in the bioreactor and all, you see that, we

will actually perform all our work in it. But then, as you suggested that, you know, could we actually build certain kind of, you know, self-contained organism, which means that it is able to perform that function, which it is, but then once I think it has eliminated some of those pollutants from the environment it will actually kind of, you know, get eliminated by a certain kind of, you know, kill switch or you know, certain kind of auxotrophy, which you have actually introduced into an organism. So, there are some research actually going around those areas, too. But currently, we might think that it is there is a kind of, you know, environmental concern. So, we want to actually create certain kinds of a feedstock, which you want to use for our microbes, and it has to kind of, you know, have certain component in that feedstock, you will definitely engineer that plant. But then when you actually grow them, you definitely grow them in a very confined setup and not necessarily, you would actually really use it in that environment abruptly.

Steve Lewis 28:19
So, as we are nearing the end of our conversation, one of the questions that I always like to ask our guest is, what do you think have been the keys to your success in your career?

Ramesh Jha, PhD 28:31
Well, persistence. Identifying good research problems and building good team to actually answer those problems. Work at the interface of multiple scientific fields or try to be very interdisciplinary. Identifying the good technology gap and try to fill it with some form of skill and capabilities is something which apparently, we always go after. And I think that has actually helped us kind of, you know, achieve some good success in our research.

Steve Lewis 29:10
What would your advice be to somebody who might be in undergraduate education right now looking to get into this field?

Ramesh Jha, PhD 29:20
Well, I think one of our focuses has been to kind of you know, generate interest in STEM in next generation. So definitely, I think what I would have to tell an undergrad who is actually looking into finding their long-term career in certain areas, that I would tell, I mean, these areas of research directly aligns with some of the key climate clean energy requirement. And obviously, we are capable of having this greener technology towards manufacturing the value-added chemicals or, you know, be able to come up with these sustainable fuels which can be actually used for automobiles or for aviation. And then I think, you know, there are these other areas of like, environmental cleanup, I mean, can we actually take care of some of these forever chemicals? Basically, those are really kind of, you know, very intriguing scientific problems. And using some of these biological agents, or you know, microbes and enzymes, we are capable of actually really addressing some of those problems. So definitely, it's a very exciting area, very right time to really get into those kinds of you know, areas just because we are seeing the effect off of some of those chemicals, or you know, the petroleum-based kind of, you know, feedstock that has been utilized over the years. We are actually seeing the effect of those. So coming up with some of these newer tools, newer areas of research, or building greener technology is kind of, you know, interesting, and a long-term kind of career for any newer research scientist.

Steve Lewis 31:22
That was Dr. Ramesh Jha, technical staff member at Los Alamos National Laboratory in Los Alamos, New Mexico. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Join us next time for more fascinating discussion about the wide world of molecular biology. Until then, cheers and good science.