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 Steve Lewis, and I'm flying solo in the host chair for today's episode, and I'm thrilled to be joined by our wonderful guest, Dr. Elizabeth Bell. Izzy is a postdoctoral researcher in bioengineering at the National Renewable Energy Laboratories (NREL) in Golden, Colorado. She specializes in enzyme engineering and biocatalysis and works at the cutting edge of sustainability technologies that bring us closer to a green future. We hope you enjoy our conversation. We begin by asking Izzy about her first foray into science as an undergraduate and how her education and experiences brought her to her current job with NREL.

Izzy Bell, PhD 00:57

I started out my science career in my undergraduate degree, where I studied at the University of Cambridge. And when I was there, I did a mixture of courses, some in biology and some in environmental science as well. And from there, I kind of decided that what I wanted to do in a PhD was something that was very applied and something that was going to help the environment. That's what kind of spurred me to look for projects in that area. And then from then I ended up doing a PhD with Prof. Anthony Green at the University of Manchester, which is also in the UK. And my main project was engineering plastic-degrading enzymes, which are also known as PETases. So, they're the kind of enzymes that can break down polyethylene terephthalate (PET), which is the kind of plastic that's in plastic bottles. And from there, in the UK, the way that your PhD is examined is you have an examiner, and the examiner knows Dr. Gregg Beckham very well, he works at NREL, and I kind of made the connection through that. And yeah, that's how I ended up at NREL. So that's been pretty good. And now I work on other types of plastic-degrading enzymes, such as enzymes that can break down nylon and ones that can break down polyurethanes.

Steve Lewis 02:16

Amazing. A lot of cutting-edge research comes out of NREL, specifically related to energy. And of course, with a lot of the polymer resins being fossil fuel–derived for polypropylene in particular, that's the plastic resin that you see in PCR plates. Very nicely, thermal stable as a polymer. And I'm really intrigued because one of the things that I think overlaps this area is sustainability and recycling. And so knowing that you worked on really that seminal paper, I would say of thermal stable enzymes for PET degradation is really just incredible. And I think our listeners would love to hear a bit about how you went from a focus in environmental science to specifically that area.

Izzy Bell, PhD 03:16

So basically, I really enjoyed working in a lab. And I wanted to be on my feet all day doing things. And that was a kind of area where I could carry on doing biochemistry, but doing a project that had a real-world application. I tried projects when I was in my undergrad, it was more basic science. And it just it doesn't excite me as much as doing something that I can like see in the real world would have an application for real people. So, that's kind of why I was really interested in that. And yeah, I think the reason it was PETases and plastic degradation, PET or polyethylene terephthalate, is basically one of the easiest plastics that we can imagine can be degraded by enzymes. And that's just because there are lots of esterases around already in nature. So there's quite a good reason to go after that as a first try if we can make this process viable.

Steve Lewis 04:06

There are so many questions I have I guess about your origin in terms of how you got to this space. Were you just, you know, looking around at a plastic bottle, saw it degrading, and were like, "I bet I can find out the esterase in nature that can break that down." What was your inspiration?

Izzy Bell, PhD 04:26

So, for me, it was yeah, from my like undergraduate degree. These are the kind of like sustainability issues that I had learned about. And then when I moved into a PhD project it was at the time that I started doing the project there was a couple of years after IsPETase had just been discovered. So that's an PETase enzyme that's from an organism called *Ideonella sakaiensis*, and it was basically discovered by a Japanese group in a waste recycling plant for plastic bottles. And there's been a lot of hype around the enzyme, but nobody had actually made it commercially viable yet. There were a number of problems with the enzyme. And yeah, it kind of raised a number of questions about how can we actually make these types of enzymes better? What would a better enzyme look like? And basically, the lab that I joined for my PhD, their specialty is directed evolution. So the question then became, can we take this enzyme that's caused a lot of hype, lots of people are excited about it, can we use these techniques that are quite well known for engineering enzymes for like pharmaceutical applications and can we instead apply it to a sustainability application? And that was sort of the springboard from which my project came out of.

Steve Lewis 05:46

It sounds like one of the things that you were inspired by was there wasn't a commercially viable process. And even in a lot of ways today, there's not really a commercially viable process for making this at scale and being able to apply it so that different polymers can be broken down. What do you see as your greatest challenge today working at the cutting edge of this area?

Izzy Bell, PhD 06:12

I'd say, for PET, we're sort of on the way to getting that industrial process. There are enzymes out there, we've now got ways to engineer them and that's really great. But the biggest challenge at the moment is what about all of these other types of plastic that you could possibly degrade with enzymes. So, you've got things like nylon that contains amide bonds, those are the bonds that you would have to break to depolymerize it. There are lots of amidases out there in nature, can those enzymes break down nylon? And actually, it's not as easy as the story for the PET. So, for nylon for polyurethanes, those well-characterized enzymes just don't exist. So, it's really kind of right back to the beginning of where the PETase field was about 10 or 20 years ago, where we're still going out trying to find those enzymes, find enzymes with good characteristics. But the promising thing now is that we've got the PETase as a good case of like how to find an enzyme, how to engineer it, how to make it work better on plastic. So the hope is that that process of creating an industrially viable recycling strategy for these other plastics is going to be speeded up a bit, because it's been a long time since PETases were discovered to actually creating that commercially viable process.

Steve Lewis 07:29

I'm curious, from your perspective what is next? Are polyethylene terephthalate, you mentioned that there were other polymers, where do you think, the next opportunity is related to polymer degradation?

Izzy Bell, PhD 07:46

It's multiple things, but you have to kind of take into account where enzymes fit into this process. So, we're not saying that enzymatic recycling is the best way of recycling and that's how you should recycle plastics. But what enzymes could be really good at is because enzymes are so specific, that means that they could potentially degrade certain plastics from a big mixture. And at the moment, a lot of plastic waste that goes to recycling centers is a big mixture of waste. And you have to sort it out to be able to put it into lots of different processes. And obviously, that's quite time intensive and it takes a lot of money. But the sort of next big thing where you could see enzymes being deployed is that if you can get enzymes that can degrade different plastics, then you could maybe give them this mixed plastic waste, and they could selectively degrade the different plastics down and then you wouldn't need to do the sorting. So, that would be a really interesting next application. And another thing is in textiles. So, textiles contain cotton and PET. You can't recycle textiles very well at the moment using chemical methods because they break down the cotton as well. And actually, we'd quite like to get the cotton back as well. We'd like to recycle the cotton. So maybe that's another application of enzymes where you can use the enzymes to break down the PET, and then you can recover the cotton as well.

Steve Lewis 09:05

That is just fascinating. It's a very topical problem that takes a lot of really creative ideas to tackle. In your particular case, directed evolution it sounds like, was the next logical step after doing a bit of research related to the naturally occurring enzyme. I'm curious how did you select the specific esterase and then how did you decide maybe the organism or vector that you wanted to use to ultimately target your directed evolution toward?

Izzy Bell, PhD 09:42

Basically we did look at a number of different esterases, and the reason that we selected the esterase from IsPETase is because it's the only known the *Ideonella sakaiensis* organism that it comes from is the only known organism that's been shown to use PET as a carbon source. So, the idea is that this organism has actually evolved to break down polyethylene terephthalate. And that's kind of different from a lot of the other esterases. We'd say that they're more promiscuous PETases. So, that means that like they've evolved for a different substrate. And then it just so happens that they can also break down PET. So, that's what kind of sets apart the IsPETase. And we kind of thought that maybe that would mean that it had an unusual catalytic machinery, maybe it had some interesting properties that might make it better than some of those promiscuous cutinases, and one of the main problems with this enzyme is that it's really not very thermal stable. And if you want to be doing plastic deconstruction at scale, you want to be running reactions at higher temperatures, so sort of 60 to 70 degrees. And the IsPETase basically stops working about 40 degrees. So, it was another interesting question of what happens if you thermostabilize these types of enzymes. The cutinases only work really well at high temperatures and the activity at lower temperatures is almost completely gone. Whereas the IsPETase has got okay activity at low temperature, but it doesn't work at higher temperatures. So, it's an interesting scientific question about whether we can make this enzyme more thermal stable. And then what does that look like for plastic degradation? And then, yeah, why directed evolution? Directed evolution is like really good at optimizing multiple parameters at the same time. So, it means that you can look at thermostability, you can look at activity, you can look at different types of substrates and all of these selection pressures can be done within the same experiment. For directed evolution, you need to be able to do genetic manipulation, transform with plasmids. So we actually use model organisms to do that, we use *E. coli* and that's because all of the genetic tools to do that kind of plasmid transformation, and manipulate the DNA is very well characterized in *E. coli*. And luckily, the IsPETase enzyme expresses really well in E. coli, which isn't the same for all enzymes. It's just really handy if you can get your enzyme to express in *E. coli* because it makes the whole workflow a lot easier.

Steve Lewis 12:10

I'd like to tug on that thread a little bit. Do you mind explaining how you do the plasmid creation, and then the transformation and maybe what tools you use along the way?

Izzy Bell, PhD 12:25

Yeah, so for directed evolution, the first step that you need to do is create a library of mutants. And the way that we do this is by creating something called a site-saturation mutagenesis library. So, that means that you pick an amino acid in your protein of interest that you think is going to be important for function. And then what you do is you create some primers that are going to sit on that site where you want to mutate in the DNA. And at that site, we use what's called an NNK codon. It's a way of representing a codon that when you order it includes every amino acid. You then use that mutagenic primer in a PCR reaction called an overlap extension PCR. The first reaction is you create two fragments. So, the first fragment is going to have the start of the DNA that's encoding your gene, it will then have the mutated position that you want as that NNK codon, and then it has a little bit of an overlap. And then the second fragment that you make is the rest of the gene. And then in a second PCR, you basically stick those two fragments together. So, then you've got a whole-length gene at one position where you've got every amino acid encoded. That is what we would call a library at that position. The next process that you have to do is you need to clone that into a plasmid so that you can then express it in *E. coli*. There are a number of different plasmids that we use, one of the ones that I quite like is an E8K plasmid, and that's arabinose inducible, which makes downstream workflow quite nice. So, you would clone this mixed pool of genes that's got all of those amino acids at that one position into this plasmid. And then you have what we'd call a library of plasmids. This sort of mixed library of plasmids, you would transform into *E. coli* and then you can plate them out on just a normal agar plate. And what that means is that every colony that grows on that plate will have one plasmid in it, and that one plasmid will have one amino acid at that certain position that you're looking at. You can then pick those individual colonies into a 96-well plate. And then in theory, what you've got is now a plate of *E. coli* cells where in each well, you've got a different gene that's got, well, it's the same gene, but it's got a different amino acid at that particular position that you're looking at. And that would be your library plate which you can then grow up and express the proteins to try and test them.

Steve Lewis 14:53

I have two questions coming from that cloning process. Which are your most common and maybe even favorite?

Izzy Bell, PhD 15:01

Yeah, so a lot of my work, we just do restriction and ligation cloning. So we make it so that the genes have an NdeI and a XhoI restriction enzyme site at either end, and then the vector has the NdeI and XhoI, as well. So, then you will do a restriction digestion of the vector, and you do a restriction digestion of the gene fragment with those mutations in and then you ligate them together with T4 DNA ligase. Part of the reason for that is a cost thing. So, restriction digests are fairly cheap compared to some of the other methods. But we're now sort of moving towards things like Golden Gate cloning, because the mixes for that have come down a lot in price. So, it makes it a lot more viable to do at scale.

Steve Lewis 15:48

Yep. Now, my second question coming off of the process description was how do you screen?

Izzy Bell, PhD 15:54

Yes, so, I think we finished off where you picked your individual *E. coli* cells into a 96-well plate. So, you had a, you now had a plate with your library of plasmids arrayed across it, where you've got one gene now in each well, and that's encoding the protein, which is the PETase. So, then you would do a grow up. And that's exactly the same as how you would do a protein expression in a flask, it's just miniaturized in a 96-well plate. So, you take those *E. coli* cultures, and you'll do an overnight, so you'll grow them overnight just to let them get a bit more dense. You can then take a sample of all of those, and then you put them into fresh media, about 500 microliters of 2YT media and usually that's done using liquid-handling robots, because once you're getting up to sort of 20, 30 plates that's a lot of manual pipetting. So we tend to use either 96-well transfer pipettes, or that we have some robots that can do it as well. And then you let these cells grow again to a certain OD, you induce them for protein expression, and they start producing the proteins. Once again, you've left those overnight, you can spin them down and pellet the cells. Again, this is just the same as a normal flask protein preparation and pour off the supernatant and then you've got cell pellets. We then lyse those cell pellets. And that's going to release the PETase proteins into the solution. Actually, the PETase expresses so well that you can just use the lysate, so the crude lysate, to do your assays for activity. So, once you've lysed the cells, you can spin them down again to get rid of any cell debris, and then you again with a robot, you can take off the lysate and put that into some buffer. And then into each well, you put a PET plastic disc, and let that reaction go for as long as you want it to go. And you can incubate it at the temperature that you'd like to incubate it at. What then happens is the PETase acts on the plastic disc, and it releases soluble monomers. So, those are the components of the PET into the solution. And the monomers that you commonly get with PETases are terephthalic acid, MHET, which is just another monomer. And you also get ethylene glycol. What's nice about TPA, terephthalic acid, and MHET is that they are UV active. And that means we can basically put them into a HPLC plate. And then you can analyze these reactions by HPLC. And look for the terephthalic acid and the MHET. And then what you're looking for is variations in that. So, you're looking for any of those reactions where you've got more of those soluble PET degradation products than in a control reaction. And from that, that's where you'd say, "Oh, this one has a lot of MHET and TPA therefore it's a better PETase. Therefore, this is the PETase I'm going to look at further." And then you would go back and sequence that enzyme to find out what that mutation was at that position so that you can then characterize it more.

Steve Lewis 19:02

Steve Lewis 19:41

Izzy, I think you have a really interesting background in terms of how you got to really work at this cutting edge. What advice would you have for somebody looking to work in this area of sustainability?

Izzy Bell, PhD 20:01

Yeah, I think it's fully heart. You should be researching something that really interests you. Research can be really quite challenging. So, it's good to be doing something that you're really interested in so that you have that motivation to carry on. And what I would say is that, when I first started, there weren't so many examples of projects like this that were going on, but it's really taken off. And that means that there's lots of labs out there now that are interested in research in these areas, applying new techniques to these areas. I know in university courses, now, these kinds of topics are coming up. So, it's a lot more accessible now than maybe it was in the past. And it's just a case of doing a bit of research, finding the people that you're interested in reading the papers and contacting some of those people and seeing whether they've got positions available.

Steve Lewis 20:52

You highlighted a number of techniques earlier in the episode. And I'm curious what absolutely critical techniques must somebody know. It's pretty rare, at least in my conversing with our guests, that somebody can describe both the upstream and the downstream aspects of their process. And so, you know, I think that that is a really unique capability. What exposure does somebody need to have, either in school or extracurricularly in the lab, to be able to work in this space of directed evolution and manipulation or engineering of specific proteins for a purpose?

Izzy Bell, PhD 21:37

Like you say, it's a really sort of interdisciplinary sort of field, which means actually, you can come at it from lots of different angles. So, I would say, coming into it, I was a biologist. So, I knew a bit more about the genetic manipulation, what the plasmid does and how to transform into $E.\ coli$, that was my background. And then, because it's so interdisciplinary, whatever background you come in from, you're going to have to learn something. So, the

things that I had to learn were about the chemistry, about the polymer science, about how this fits in a process. But also, you find that people in working directed evolution, they might have started out with background in chemistry instead. So, they've got a really good handle of the sort of chemical transformations you can do with enzymes, and the sort of chemistry that's physically possible, but then they don't have the biological, the in-lab skills to do the directed evolution. So that would be the thing that they needed to learn. And then you've got the other type, a lot of people that do directed evolution come from a chemical engineering background. So, they've got a really good overview of the whole process and how it might fit together. And how will this look in a reactor? Whatever background you come from, you're going to have to learn something, but because it's quite, yeah, this interdisciplinary area, you tend to find that labs are quite happy to teach you that thing, because they know you're not going to come in with everything.

Steve Lewis 22:52

When you create your team or you build the group that's going to work on these areas, is it equally as interdisciplinary? Do you get together and talk to those maybe bioprocess engineers and say, "Here's what I'm thinking, poke holes in it?"

Izzy Bell, PhD 23:08

Yeah. So, that's actually one of the really nice things about working at NREL. So, I'm the biochemist, my stuff is creating enzymes, optimizing enzymes, producing enzymes. I then work with a polymer scientist; she can make polymers for me. I can request things, she can tell me what the polymer looks like, how it looks after degradation, and she can give me lots of information on that. We then also have the option to work with sort of I was saying about looking at the economics and things like that. There are people here that do these techno economic assessments, so we can give them data from our enzymatic reactions and say, "Is this feasible?" And they will do the modeling and run it and go, "Actually, no, that's not a good idea. Maybe you could change this, maybe if you change this thing that will become more economical or use less greenhouse gases." And then once we've got that on a smaller scale, here at NREL we also have the capacity to scale up, we can do the 96-well plate reactions. We then have bio reactors as well and they go from as small as 15 mL up to two liters and beyond. So, we really have the option to pull in all that different expertise and work the whole way through the process.

Steve Lewis 24:17

I want to talk a little bit about scale-up, in general. Difficulty in scaling up of some of these processes, because as you mentioned earlier, it's not just making the enzyme work, but you know, you have to be able to envision it in perhaps a larger scale. Why don't you just tell us how you think about scale-up as you're going through your experimental design?

Izzy Bell, PhD 24:43

Yeah, so there's lots of things that you want to think about because when you're doing directed evolution one of the sort of mantras is you get what you screen for. So, you want to make sure that the conditions that you're screening under are as close to those industrial conditions as you can get within the kind of workflow of doing it in a 96-well plate. And the sort of things that you might need to think about for scale up are, “How am I going to express this enzyme at scale? Is it a really poor expressor? Is it really hard to purify? Are those things that we also need to improve?” So, that's one of the considerations. Another consideration is, “What substrate am I actually going to use? You can imagine using a cut-out PET disk for the 96-well plate assays, but actually, for a big reactor is a powder better? How is that enzyme going to act on a powder? Is it going to act differently on a powder to what it does on a PET disk?” Also, some people are looking at like foaming plastics increase the surface area, does that help with the degradation? And then the other things are kind of like, “What is the process going to look like at scale? How much enzyme am I going to need in that reactor? How expensive is that going to be? How much enzyme do I need in comparison to how much substrate is in, is that something that needs to be very optimized? Or actually, can we run a different enzyme–substrate loading and still get the same effect?” So, there's lots of different things to think about.

Steve Lewis 26:10

And after the downstream processing at the scale that you're working at, of course, the next step would be to how do we move to that that next level, whether it's 100 mL flasks, or all the way up, like you said to 2 L, microbial bioreactors. When you're talking about yields and titers inside of a bioreactor, what's a generally good expression level for the enzymes that you're working with?

Izzy Bell, PhD 26:40

At the moment, the bioreactors that we use, we add purified enzyme. So, the enzyme is grown in the bioreactor and then you'll purify it out, and then you will put it back in a new bioreactor with your PET plastic. And it kind of depends on how good your enzyme is about what sort of enzyme loading you need. So that can be really enzyme dependent.

Steve Lewis 26:59

You just highlighted kind of like the novelty of the process and also, I guess, my ignorance of it in a way. Putting it in a bioreactor, you have to, like you said, put the actual polymer in there, and you were highlighting earlier could be a powder, which I guess would be like mimicking a finely ground up like final product. Wow, there's so many aspects to this, you've really, really got my mind going. So, I mean, how do you decide like, how much polymer to put into a reactor?

Izzy Bell, PhD 27:39

Yeah. It's quite interesting. So again, this sort of, to work these kinds of things out, you can do them on a smaller scale. Try out some different enzyme loadings, try out some different substrate loadings. Look what's good but ultimately, you're always going to have to test them in that bioreactor to see whether the small scale translates to the big scale, and it doesn't always. But one of the sort of funny, considerations of bioreactors is, can it be mixed. If you put in too much PET powder, it gets too clumpy, and then it won't mix or if you put in strands, it gets wound up around the propeller. There are a lot of like practical considerations that sometimes it's not obvious that it's going to work until you've actually just tried it. Again, it's one of these things that is quite cutting edge about how to make these processes work. So, yeah, it's things that people are trying out. The purified protein that we use at the moment is in liquid form. So, it's quite nice just to add, so that makes that fairly easy. And then, one of the interesting things is that one of the ways that this could be like a viable process moving forwards that people are looking at is that you can actually express these proteins in fungi, which then excrete them. So, you could actually grow your fungi in a tank, let it excrete the protein, which would then move in across into your bioreactor. And you'd have this continuous process of producing protein and using it to break down plastic. That's kind of in the future, but it would be really interesting. Yeah, and for working out how much enzyme you need, it's really the case of you want the minimum amount of enzyme possible to achieve what you want to achieve. Part of that is to do with how fast that enzyme can actually break down the plastic. So, for example, with some of the enzymes we work with, it doesn't matter if you put in more enzyme, because there's just a certain rate that it can break down the plastic. Putting in more and more is not going to get you faster and faster depolymerization. So, I'd say as a general starting point, we usually start at sort of nought point one mg per mL. You'll find some enzymes you need more you will find some enzymes you can get away with a lot less but that would possibly be a good middle point to at least start off with to try and work out what the limiting points of your reaction are.

Steve Lewis 29:47

You're unlocking parts of my education that I have long forgotten for, uh Michaelis–Menten kinetics a long, long time ago for me. That is so incredibly fascinating. And I know that we are coming up on time, we always like to ask all of our guests what are, what do you think is the key to your success?

Izzy Bell, PhD 30:11

I think it's being able to communicate your science to lots of different people, especially in this field, where it's so interdisciplinary. Like everybody that I work with is very well educated around the subject matter, but the chemists might not always understand what goes on in the biological process. I might not always understand what's going on with the polymers. And so you need to be able to explain what you're doing and what the limitations are to these different types of scientists in a way that they can understand. And then also be open to learning those new types of science so that you can understand what they're

trying to tell you back. And it's only through that interaction that you can make progress in this field, I would say.

Steve Lewis 30:50

Being able to have that empathy and understanding for other areas of expertise and other areas of study. I think that particularly helps you in your area of research, because no one person can do it all. It's based on everything that you've said. Final question from my side is, what advice would you give to a young researcher that might be different than an established scientist or laboratory and who are trying to pivot into this area?

Izzy Bell, PhD 31:25

I would say that one of the beauties of this area is that it is all cutting edge, which means that you're never going to have the skills that you need to excel. Which in a funny way means that you can apply to any of these different areas or projects because no one else is going to have these skills. So you've just got to be able to show that you're willing to learn. I did my degree in sort of environmental science and biology; I didn't learn anything about plastics and polymer chemistry. And then when I went into my PhD, I was doing directed evolution that wasn't something I learned about in my degree. So, all of these techniques, because they're so cutting edge, it means that you don't actually have to have that perfect background. And a lot of the times what's actually more important is showing that you've got a willingness to learn and the passion behind the subject matter to like show that you're interested enough to be inspired to learn these different new areas.

Steve Lewis 32:18

That was Izzy Bell, postdoctoral researcher in bioengineering at the National Renewable Energy Laboratories in Golden, Colorado. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti, and Matthew Stock. Join us next time for more fascinating discussion about the amazing world of molecular biology. Until then, cheers and good science.