Steve Lewis 00:09
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis and today I have the pleasure of welcoming Dr. Sarah Barry to the show to learn about her amazing research. Sarah is a Reader in Chemical Biology at King's College London, where she researches bacteria, the molecules that they produce, and the chemical pathways that shape those processes. We hope you enjoy our conversation.

We begin by asking Sarah about her background in a broad range of scientific disciplines, and how they inform her current work.

Sarah Barry, PhD 00:50
I started as an organic chemist, actually, that's my background, is in organic chemistry. From PhD to postdoc, I kind of transitioned into a more biology-focused career. So, I started doing more microbiology and molecular microbiology, and then ultimately, in my first independent position, that became chemical biology. So, I guess we would define chemical biology as using chemistry to solve or investigate a biological problem, in the broadest sense.

Steve Lewis 01:22
Do you view it as distinct from biochemistry?

Sarah Barry, PhD 01:25
Yes. So I guess I come at every problem from the perspective of, what's the chemistry going on? What's the chemical transformation? And I think that's good. I think it's actually complementary to a biochemical approach. And I guess throughout my career, I've managed to incorporate that more biochemical view. I have found it really useful to go to biochemistry conferences and see how biochemical colleagues approach different problems, I guess from a more, often from a more protein-centric or macromolecular perspective. And I would say many of my organic chemistry colleagues would be focusing on, well, what's the enzyme doing? Like what chemical transformation is being performed? What bonds are being broken and made in the course of that reaction? It's really useful to have those complementary approaches to a problem.

Steve Lewis 02:14
Absolutely. The multidisciplinary, interdisciplinary approach has been a theme throughout this season. And it's really interesting, too, that you transitioned from chemistry into biology, but you have a bespoke approach, as you mentioned. What was your “aha” moment for how you made that transition from O-chem to the bio space?

Sarah Barry, PhD 02:39
So I have, I guess, I have a little bit of a leg up, in that I did a science degree. So, a more general science degree where I'd done some biology classes and a little bit of physics and some chemistry—so I'm a chemistry major. And then when it came to doing my PhD, I ended up in organic chemistry. And I guess my moment was, I'd seen a lot of natural product total synthesis talks, I was all Ooh, there's a lot of cool chemistry here. But at the same time, a lot of these are focusing on synthesizing natural products in many steps. And that led me to think about, well, how does nature make these molecules? So these are all natural products, right? They're all natural molecules that are being isolated from bacteria, or sponges, or whatever it is. I guess I started to look into that a little bit more and, as it happened, my PhD supervisor had a colleague in another university who was investigating natural product biosynthesis. And this person gave a talk in our university, and I was like, oh, right, okay, this is how bacteria put these things together. And it's all enzyme-based, of course, and they're doing transformations that are almost impossible using traditional organic synthesis. So in some of the catalysis that goes on and promoted by these enzymes, we either use heavy metals, or transition metals, to

carry out those reactions in organic synthesis, or it's something we just can't do with the same kind of selectivity. And I find that fascinating. And so ultimately I went on to do a postdoc in that field, which really completely changed my perspective.

Steve Lewis 04:20
And with the transition metals that you were mentioning, can you expand on that a little bit? Are you referring to cofactors for those transformations, or am I totally off?

Sarah Barry, PhD 04:25
Yeah, so I guess if I'm, so yeah, two things. So, I guess in organic synthesis, we'd use things like palladium, for example, as a common transition metal that we would use to do carbon-carbon bond formation reactions, right? That's a big area of organic chemistry and C-H bond activation chemistry. And if you take that into, say, natural product biosynthesis, then you've got things like cytochrome P450 enzymes that use a, they also use the transition metal at their center, but it's iron. And they use a heme cofactor. But they're capable of activating really inert C-H bonds to do really selective transformations that will give you stereoselective products in a way that we would really struggle to do with any kind of iron chemistry in organic synthesis. So these kinds of reactions are really powerful, and it's kind of what leads to people being interested in these kinds of enzymes to do biocatalysis.

Steve Lewis 05:33
That's really a great context. What was the biosynthetic pathway? Do you remember?

Sarah Barry, PhD 05:38
The biosynthetic pathway that kind of got me into this whole thing was prodigiosin, a complex natural product that has this macrocyclic ring transformation, which is absolutely impossible

using organic chemistry, like no doubt impossible, because it involves the activation of two very inactive chemical functional groups. And it does it in a very selective way. The molecular biology is so important for investigating natural products—biosynthesis, and also enzymology. And the change, I would say, in the last 20 years has been phenomenal. It's completely transformed the field. And I guess the primary reason for that, or at least the initial reason, has been the ability to sequence genomes. And the reason for that is because some of the genomes for the bacteria that produce many important natural products are relatively large. And the biosynthetic pathways themselves within bacteria are often clusters of genes. So it's quite nice as if you identify one biosynthetic gene, you can often look upstream and downstream of that gene and find the other genes that are in the cluster. And people have designed bioinformatic tools to be able to identify these clusters, using the sequencing data we have, because we have a decent understanding of how these pathways are organized in genomes, or how the genes for the pathways are organized. And so the more data that's come through, through sequencing, the more we learn, the more we can identify these pathways. Then you can start knocking out genes to look for production, link production to genetics. You can find the biosynthetic gene clusters within the genomes, and then go looking for production of a natural product, which is the thing we couldn't necessarily previously do. And it has enabled us to learn huge amounts about the physiology and biochemistry of these pathways and how they're regulated. For example, certain pathways being turned on by the absence or presence of another metabolite. So iron-chelating compounds are one example. So, lots of bacteria produce iron-chelating compounds known as siderophores, into the media if you restrict iron from the media. So there's a regulator that senses the presence or absence of iron, and then turns on the pathway. And this is really fascinating stuff, right? Because this is really complex, and biochemistry turned on by the absence or presence of a fairly simple metabolite or a fairly simple nutrient.

Steve Lewis 08:12
There's a lot of directions that we can go from there, but I'm wondering if we can talk a little bit about your process. Do you start with an end product in mind? Or do you look at a product and you're like, how the heck did that get made? And then you start working backwards?

Sarah Barry, PhD 08:29
Yeah, so you can do both and we have done both. For one molecule that we worked on, we very much started with, we knew of its existence. So it had been actually isolated in the ‘60s. And as a lot of natural products, a lot of natural products work at that time, and many of them had been isolated, reported, and that was kind of a, you know, reported antimicrobial activity or whatever activity it might have. And now with the availability of genome sequencing, we can take a natural product that might have been isolated in the ‘60s and go, okay, well, the genome of that organism has now been sequenced; can we identify the genes that might be responsible for that? And the process is, you've got a few major families of natural products. So nonribosomal peptide synthetases, or polyketide synthetases, or terpenes, which is one that a lot of people know, that kind of cross the bacterial and plant families. And there are core genes involved in those pathways that you can identify fairly easily. And then the question is, can you link it to a specific natural product? So you can link it in a couple of ways. One is via the kinds of modifications of, say, a peptide or a terpene scaffold that you might expect to involve particular types of biochemical transformations—so say, for example, oxidation or a specific reduction. And you might say, oh, well, I know that that enzyme isn't capable of doing that, so I will try and find that kind of enzyme in a particular pathway. And the other is, we hope because we have a really good understanding of many of the enzymes that catalyze the formation of the, I guess the core scaffold—something that allows me to identify something clearly as a polyketide, or clearly as a terpene, or clearly as a

nonribosomal peptide—then I can look at those core genes, or that core gene, and the encoded protein, and say, I know the size of polyketide and type of polyketide that's going to make; I know the size of peptide that that's going to make. And that's actually quite predictable, with exceptions. And that's extraordinarily powerful.

Steve Lewis 10:40
What application areas would this approach benefit? And is the benefit speed to production? Cost to production?

Sarah Barry, PhD 10:50
So, a few different areas. So discovery is one. So, natural products kind of fell out of favor with a lot of industry because it became difficult to find these compounds. And there were limits on the types of analytical tools available to detect things that were produced in very small quantities. And so what this approach allows you to do is A, confirm that a pathway is there that might be interesting, and, but B, allow you to manipulate that pathway, right? So, for example, using CRISPR. And linking the pathways to production is easier as well, because you can knock out the genes in an easier way. Discovery is one. Why do you want to discover these things? Well, because loads of them are responsible for the antibiotic activity, antifungal activity, anticancer activity—but antibiotic is the major one. The vast majority of our clinically used antibiotics are natural products. So if we're thinking about identifying new compounds to tackle, for example, antimicrobial resistance, these bacteria are a good place to start. Also, can we do things like heterologously express the pathways in other organisms to make them more productive? Can we manipulate the pathways to make different derivatives of these compounds that might ultimately become better drugs? So increase different, change the properties of these compounds, so that they are more effective drugs for evading resistance. So all of these things, I think, are a function of the incredible evolution of molecular biology techniques.

Steve Lewis 12:23
And let's talk about some of those tools and techniques. What do you do on a regular basis? Which of those tools do you use?

Sarah Barry, PhD 12:30
So sequencing is an obvious one, and we've done genome sequencing, which is just incredibly fast, and I was, oh, I was just super impressed by it. And I guess one of the things that's changed is, we used to PCR off genomic DNA. So we would isolate genomic DNA from whatever organism we are interested in, and PCR off to investigate a particular enzyme in a pathway, or so on. And we really don't do that anymore very much. We now order genes. So it's kind of, that's really changed, and the cost is probably about the same by the time you do a PCR. And by the time you isolate DNA, do a PCR, do the cloning…actually, just ordering the construct, already there, is probably about the same price.

Steve Lewis 13:23
It's rare that we get a guest who mentions *de novo* gene synthesis and associated clonal DNA and DNA fragments with it. Do you mind sharing what you all work with? Are they DNA fragments up to a certain base pair number? Is it clonal DNA inside some kind of vector that you love?

Sarah Barry, PhD 13:49

Yeah, so we've done both for relatively small, simple fragments, where the base pair composition is not difficult. So, where we don't have high GC regions or anything like that, it's very [ ] so, it's from *E. coli* or something, we might get a fragment and do the cloning ourselves. But we've done a lot of just—we have a favorite vector, and it's just this His-tagged vector for doing protein production. And we will just get our genes cloned into that for us. Sometimes these genes, particularly *Streptomyces*, will be one of the major classes of organism that we work with. And one of the problems with them is a very high GC content, which can cause problems with gene synthesis. And I think because of that process, then actually getting them cloned into a vector is often easier than getting the fragments themselves. But yeah, we've got colleagues who also will order fragments and then do the combining of the ligation of those fragments into their vector of interest.

Steve Lewis 14:50
That's what I was going to ask.

Sarah Barry, PhD 14:51
Yeah, yeah, for bigger—I think for bigger genes. Often ours are kind of in the region of 1,500 base pairs.

Steve Lewis 15:00
This is all really resonating with me. Do you all, for the processes that you just mentioned, I know that not all laboratories actually go and do the downstream purification themselves. What does that process look like for you?

Sarah Barry, PhD 15:17
No, we do it all in-house. It's a lot of work, yeah, and I feel for students sometimes, because I guess you do it once, and you work out what the optimal process is, for your particular protein. And then sometimes you've got to do it again and again, but I guess that's part of a PhD. You know, you get better at it, and you learn the tricks of the trade as you go through that. But I guess one of the problems when you're working with proteins like this, they're not always the most stable. And so you produce some, you use it in whatever assay you're doing, but you don't want to produce too much that you're storing it for months and months and you lose activity. So this is a little bit tricky, and we have a habit of producing some badly behaved proteins from *Streptomyces*, so my students are sometimes unhappy about that. So yeah, it's a balance, I think, between producing enough that you're not wasting your time doing protein purification all the time, but not producing so much that you end up throwing it away at the end of the day.

Steve Lewis 16:22
Are you in the market for a new PCR or gel electrophoresis instrument? If so, you should check out our Virtual 3D Lab. From the comfort of your own device and at your own pace, you can interact with our PCR and our gel electrophoresis instruments like never before. This immersive 3D tour lets you explore and experience what it's like to use these state-of-the-art instruments. To start your personal tour today, visit our website at www.thermofisher.com/molbiovirtuallab. That's forward slash mol bio virtual lab. And now back to the episode.

Steve Lewis 17:08
One of the common threads that I've noticed throughout my career is you can be an expert in an area of laboratory science, but when it does come to repeatably producing proteins, expressing them, purifying them, and then scaling that up, it almost in a way has its own domain of

expertise just for the expression and purification and scale-up aspects to it. Does that resonate with you at all?

Sarah Barry, PhD 17:43
Oh totally. I think one of the most challenging things of moving from chemistry to biochemistry was learning a different language in the lab and dealing…it's a very different approach. It takes, I don't know, a switch in your brain to move over into both an aqueous world and the small-scale world where you have to be very careful with temperature. Everything's done cold, everything has to be stored correctly—it's a very different way of working. But once you understand it and you learn it, I think combining the two skill sets is actually really beneficial because I think you can't do this—it's difficult to do this kind of work without a chemical understanding, at least, of the analytical techniques that are available to you, which allows you to really dig into what kind of reactions your enzymes are doing. And that requires, as I say, HPLC, mass spectrometry, NMR. But then you need the biochemical sensibility to be able to work with proteins and understand their inherent delicacy, and how to treat them to keep them working the way you want them to. I think molecular biology is just, inherently, can be quite complex and require a complex array of reagents and additives just to maintain the integrity of macromolecules. And that can be a learning curve for a lot of chemists, where we have, I guess, much more simplified solutions often, of very defined solutions that we think about and when we're going from molecule A to molecule B in a reaction.

Steve Lewis 19:25
Are macromolecules your primary area of work and interest, or do you ever work with smaller proteins and enzymes?

Sarah Barry, PhD 19:35
Enzymes are our focus, and we started working on lipids, which I guess you can think of as a small molecule, but actually, the way they behave is as macromolecules. They're like, they're crazy. And then we still do some synthesis. So, we still do organic synthesis, where often we are producing intermediates or products of standards for some of our reactions, or we're producing different versions of a substrate to be able to test the scope of our enzyme's activity. So, is it able to accept different kinds of substrates? Is it quite restrictive in its substrate scope? Understanding that's really interesting, because I think we often think of biochemical pathways as being quite, the enzymes in those pathways being quite selective, but actually sometimes they have broader substrate scopes than we think.

Steve Lewis 20:27
How do conformations play into your work? Do you take a molecule in its natural state and then you're like, oh, if I could just move this site over here... Is that a part of your work, or no?

Sarah Barry, PhD 20:43
Yes, we do site-directed mutagenesis, for sure, to modify active sites or investigate the role of a particular residue in catalysis, or modifying the shape of an active site, I guess, to see, does that change selectivity? So yeah, there's a variety of different ways of doing this. I mean, you can take it from single amino acids to multiple amino acids in terms of changing active sites. How successful you can be at that can depend on just how much we understand about a particular enzyme family, what residues are important for binding the substrate, what residues are important for catalysis itself. Perhaps sometimes we think we know more than we do. It's one of the most interesting areas of enzymology at the moment, I think, is predicting the chemistry of particular enzymes and understanding what determines substrate selectivity. And in some enzyme families, we have a good understanding of that, in

others, I think we are really unsure. And often, it can be quite subtle changes that result in distinct differences in both catalytic activity or substrate selectivity. It's a really interesting area. And I think that goes into directed evolution and so on.

Steve Lewis 22:07
That's actually very much in the scope for the mol bio conversation, so yeah, I'd love to know a little bit more about how you utilize site-directed mutagenesis or directed evolution as well.

Sarah Barry, PhD 22:19
So, sometimes we're doing it with a view to, we want to confirm that this particular amino acid is really important for catalysis. And so we will change it, do something like change it to alanine. That's the kind of the first initial step, change it to alanine. Does it kill the enzyme, or is it still active?

Steve Lewis 22:37 Why
alanine?

Sarah Barry, PhD 22:38
I think it's just because it's still got a chirality—so not glycine, because glycine has no chirality and it's more flexible. Alanine, you remove any functionality, but you've still got a methyl group there. So it's the, kind of, smallest amino acid that you can have. So yeah, we do that, and we said, well, does it knock out activity? Is the activity retained? And then we can at least make a determination of, is that amino acid involved in any catalytic activity, or does it have no effect on activity? Always you've got to be careful when you're producing, paying attention to producing the enzyme—is the enzyme still behaving in the same way? Does the protein behave in the same way? So during purification does it now start to look maybe a bit more insoluble? Have you compromised structural integrity? If you have, you can do things like look at the CD spectrum, perhaps, to see if the secondary structure is still conserved. So, you've got to be a little bit careful, right, because sometimes we inadvertently make changes that we think are relatively straightforward and they have big effects. And then, are we affecting substrate binding, or are we affecting catalysis? And after that, we'd look at the binding kinetics of the substrate. And then you can do bigger changes, right? You might do further mutations or more nuanced mutations, as I said, to change maybe the polarity of a particular residue or the size, going from a very large, like something like leucine, to something smaller, like valine or alanine. And that might just be about changing the space available in the binding pocket. So maybe you want to accommodate a larger substrate. And then you can get into more nuanced things, where you know there's a particular loop that's important for catalysis, but you don't know which residue is important. And so what you might do is something called an alanine scan, where you mutate every single residue in that loop to alanine, and you figure out which one is—maybe there's more than one—but you figure out which residues are in it. Yeah.

Steve Lewis 24:34
Sounds time-consuming.

Sarah Barry, PhD 24:36
I mean, there's lots of nice ways of doing this now. People have developed lots of clever ways of doing this in a more, I guess, combinatorial approach, which is really nice. We haven't done a lot of it yet, but there are really smart ways people have thought of doing some of this. And of course, you can do more and more *in silico*. So computational modeling allows you now, if

you've got the capacity, to model mutations and see what kind of impacts they might have before you go to the lab. And so because, as you say, yes, it can be very time-consuming, by the time you do the actual mutagenesis, even if you've got a combinatorial approach, you've still got to assay a bunch of colonies, look at the data, you can generate lots and lots of data doing this. And someone then has to analyze that and decide what's important and not important. And that can be quite time-consuming. So there's lots of challenges in this area.
There's huge promise, and there's huge potential in our ability to engineer proteins. I mean, I think this is another major change in molecular biology over my career—it has been our ability to much more easily engineer proteins. I mean, it's incredible. And it has changed people's outlook on how we utilize proteins, and biotechnology.

Steve Lewis 25:57
You mentioned at the beginning of the call, almost micro-environmental impact on the target products that you're mentioning. And that's a really unique, almost in a way, like application area design challenge. I'm curious, because oftentimes on this podcast we talk about intracellular, we talk about very controlled environments. But you actually brought this up at the beginning, so I was curious, can you tie that out to, do you look at enzymes and reactions in context almost, with that as part of your experimental design?

Sarah Barry, PhD 26:34
Yeah. So, I think microbes are totally fascinating. I guess there's historically been a bias that people assume that because an organism is multicellular, it's more complex. And I think genome sequencing has changed that, in a way, because it's allowed people to realize that actually, bacteria have huge genomes. And so I think because of that, they've got incredible ways of adapting to their environment, whether that's as a result of a lack of feedstocks—a lack of carbon or a lack of iron—and that then changes the internal biochemistry, what genes are switched on and switched off. And this is hugely important, for understanding almost everything. So I think disease is a good example. Microbiome, right? Even outside of disease. So understanding how our microbiome works, it's all bacteria, how do those bacteria interact with each other and how do they interact with the host? And that's all mostly chemical signals, right? Its small molecules being interpreted, and genes getting turned on and turned off, and other small molecules being produced, and that being a kind of whole signaling pathway in and of itself. It's so complicated. Then you've got the rhizosphere—you've got the soil where, which a lot of the bacteria that we work on, that's their kind of native environment. And that's a hugely hostile environment. In the lab, we try and replicate some of these environments—I mean, try in very difficult ways to think about if these processes are already turned on or off in specific circumstances, how do we replicate that? How do we understand what might influence a particular pathway being switched on? And sometimes it's relatively easy, but others are much more complicated and not always obvious, and partly it's because we don't always understand what a natural product is doing. Antibiotics, relatively easy to test. But you've got other molecules, which we really don't know what their function is in the ecological niche that that bacteria would normally live in. But it's a kind of fascinating chemical warfare that goes on, and chemical communication that goes on, between these organisms that controls gene expression and how they survive in the environment. Which is ultimately why we need to be able to manipulate these pathways, or heterologously express them, that kind of thing, because sometimes we just never find the right conditions to switch them on using media. And that's been the only way that we could do it.

Steve Lewis 29:18
There's a lot of great insights in that, and almost in a way, looking at the molecule but removing the context makes it really difficult. And I think what you were describing, the way I interpreted it,

is all of the feedback loops that are going on from these different cell types, whether it's plant, fungal, microbial, and in other ways, they all have their own feedback loops and their signal transduction pathways, there's transcription factors, but they're also interacting with one another while aiming for survival, right? For the limited number of resources.

Sarah Barry, PhD 30:00
Yeah. I mean, it's a network, right? It's a hugely complex network. And I think we focus on single cells, but there's a fascinating world of more complex chemical networks out there in these environments.

Steve Lewis 30:13
This has been a tremendous conversation, and I want to wrap by asking a few questions. What have been your keys to your success?

Sarah Barry, PhD 30:24
I suppose…okay, so a few things. One, I think, is a little bit of luck. And I think that's very important, being in the right place at the right time. I think taking opportunities when they present themselves. I think often, I see it in some of my own students that they have a plan. And while it's great to have a plan, sometimes you don't see different opportunities when they come your way. I would say yeah, taking…when opportunities come up that you didn't expect, think about it and sometimes it can lead you in a direction that you never expected. I did a science degree with the intention of doing biochemistry as a major, and then I switched to chemistry because I thought, people kept talking to me about all these cycles in biochemistry, but I didn't have enough chemical information to really understand them. And then the chemists were talking about what the structures of molecules were, and I thought, oh well, I understand that, that I get, that feels right to me. So I went into chemistry and then I ended up coming back in to biochemistry a few years later, because what I wanted to know, now I felt like I had the tools to understand the biochemistry in a way that made sense to me. So I still find some of those cycles intimidating, but now at least I can logically make sense of them, because I can say all right, well that transformation goes to that, it's catalyzed by that enzyme, I understand how that chemically works. But I think, had I been too rigid in my thinking, maybe I wouldn't have made that change. So I think it's being open to changing your mind, and moving into different, if it feels right, go a different direction, if that's the way you think things are going. And I think traveling has been really valuable. So, I come from a small country, I'm from Ireland originally. And science is one of those jobs that allows you to travel because there's a common language, if you like. That is something I think is really valuable, and allows you to experience different ways of working and to see different approaches to problems. And that's been, I think, really hugely valuable. I've seen completely different ways of approaching this kind of science and been exposed to different kinds of sciences, because of the ability to travel. And that's been great.

Steve Lewis 32:42
That was Dr. Sarah Barry, Reader in Chemical Biology at King's College London. 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.