Steve Lewis 00:10
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 we continue our exploration of groundbreaking research and technology with Dr. Gudrun Stengel. Gudrun has worked in genomics for over 10 years, with a background in DNA sequencing, proteomics, transcriptomics and more. In 2021, she co-founded Alida Biosciences, a company focused on next generation epigenetic analysis. I hope you enjoy our fascinating conversation. We begin by diving right into Alida Biosciences, what they do, the products that they offer, and what Gudrun is most excited about in her work.

Gudrun Stengel, PhD 00:58
Alida is a startup company located in San Diego, we started out four years ago and are developing new tools for the analysis of RNA. So our mission as a company is to unlock the function of RNA modifications in biology and to use this knowledge towards new applications in diagnostics, new therapies, and also synthetic biology. I was the inventor of the core technology, which is a proximity barcoding platform for reading multiple RNA modifications simultaneously. And it's very exciting to see this technology grow, and we are, in fact, very close to having our first official launch.

Steve Lewis 01:46
That's fantastic. Tell me more about proximity barcoding and the technology you've developed.

Gudrun Stengel, PhD 01:52
So, RNA modifications are very difficult to detect. So when you do regular next gen sequencing, they are silent and don't give a signal. So proximity bar coding is the concept of using a molecular recognition element, for example a protein binder, that is tagged with a barcode, and to recognize the modification and then transfer the barcode that IDs the modification to the target RNA, and then you can read out the target RNA with a barcode by next gen sequencing and infer what modification was present.

Steve Lewis 02:31
What was the inspiration for your concept?

Gudrun Stengel, PhD 02:36
I've been working for 15 years in the genomics industry in San Diego, and my core expertise is really sequencing technologies. So I've lived through next gen sequencing becoming a standard tool in biotechnology, and for me, like reading primary sequence of our DNA was at some point, really a staple. I don't think there are too many innovations to be made. In principle, reading primary DNA sequence is a solved problem. But what isn't solved is really reading epigenetic markers, and those markers are really important for understanding how the cell uses the genetic code, and they are applied dynamically in response to environmental triggers. And the RNA, or the function of the RNA modifications is probably the least understood today because the field has been so hampered by the lack of good detection methods. And for me, as a company founder, I was really looking for what are the blank spots in the genome market, like or genomics market, it was really like understanding epigenetics and building the best-in-class tools for it.

Steve Lewis 03:58
So in a way, developing a tool set for identification of the mechanisms of action around post transcriptional modifications?

Gudrun Stengel, PhD 04:07
Perhaps I should explain what RNA modifications are. So there are about 170 unknowns today, and 50 of them alone occur in humans. So they are naturally occurring chemical modifications that are installed by rider proteins, they are recognized by readers, and some of them are even removed dynamically by eraser enzymes. So they are very important for the survival of the cell. If you knock them out, like mammals aren't viable. And they have complex functions. So for DNA methylation, it's quite straightforward what it does, it turns the transcription of genes on or off, and that information is inherited. But for RNA modifications, the function really depends on the RNA target. So in regulatory RNA, it they have a role in stability and proper folding, and in messenger RNA, they're involved really in the protein production by regulating, splicing, translation efficiency, and error rates, but also trafficking within the cell and formation of stress granules. This has implications for disease and our understanding of disease, because it is known that they can be deregulated in in many common diseases like cancer, neurodegenerative diseases like Alzheimer's and Parkinson's, even cardiometabolic disease like diabetes, you find abnormal patterns of RNA modifications. So there's really opportunity for new biomarkers, but also new drug targets and identifying new pathways for drug targets, and that's why we are so interested in giving researchers tools to detect these RNA modifications.

Steve Lewis 06:07
So in the central dogma you're really looking at before the protein is developed and then, ultimately, how can you perhaps modify some of the protein production, how the what protein is produced, or how it's translated, by targeting the mRNA itself?

Gudrun Stengel, PhD 06:27
Yeah.

Steve Lewis 06:28
Talking a little bit about the overall market and business before we dive into the more molecular techniques perspective of the conversation, tell me a bit about your inspiration, aside from the gap in the market for what Alida Biosciences focuses on, and where you think, you can scale to?

Gudrun Stengel, PhD 06:50
Yeah, for me, it was important. So I'm a first-time company founder, it was important to really enter a blue ocean market opportunity where there's no need to displace competitors, because that's a much harder game. So we are not the only company that cares about RNA modifications, but I think there's a great opportunity because scientists always get excited when they can read out the new multi or like level of the multiomic layers. The pharma industry is very thirsty for new drug targets, and the first drugs are already in clinical trial that target the m6A writer enzymes. So there is, there are signs that there are real opportunities here to develop new drugs to address the major diseases.

Steve Lewis 07:49
And some of the pathologies you mentioned, diabetes, Alzheimer's, Parkinson's as upstream considerations?

Gudrun Stengel, PhD 07:58
Absolutely, yeah.

Steve Lewis 08:01
Very fascinating. Tell me a little bit about how you move from genomics as your area of focus to this specific area, which is a little, I would say, intermediate, right between translation and transcription, and how, how do you make that transition to focus on almost the epigenetic targets as your area of focus?

Gudrun Stengel, PhD 08:31
So I have a PhD in biophysics and biochemistry, and I would say DNA and RNA has been the common theme in my academic and professional career. I think molecular recognition always has played a big role in these, in the, my early PhD days. Because I worked with biosensors, and the whole proximity bar coding idea is probably influenced by this experience. Because we have a protein engineering department, and we are evolving our own binders and also enzymes that are RNA modification-specific and yeah, recognize the modifications, and in case of the enzymes, also convert them. So this is the molecular biology background that inspired this idea, and having control over the binders means you have control of the accuracy of the method and can exceed what you could achieve with commercial antibodies, for example.

Steve Lewis 09:40
And you previously mentioned, was it m6A?

Gudrun Stengel, PhD 09:44
Yeah, so our current epiplex platform detects m6A in inosine, and we are expanding it to include to the pseudo uridine and other modifications long term. So and that will that cover the most prominent modifications in messenger RNA.

Steve Lewis 10:03
And tell me a bit about your life as a startup founder, compared to working in the industry, and how has that kind of shaped your perspective and philosophy at Alida?

Gudrun Stengel, PhD 10:16
Yeah, being a startup founder is very fun. You can imagine I wear many, many hats. Everybody talks about it in startup land. But I just thrive on diversity. I'd say, like diversity in the task and thinking and diversity of the people I interact with. In the early founding years, I spent much of my time in the lab doing experiments myself, which was super exciting, and knowing that your company depends on the experiments was very exciting. Back then, we were only four people. Now we're 20, and my role has shifted again, more into people management and fundraising, of course. But it is, it's a special

experience to see a scientific idea grow up and being translated into a product that enters the market and hopefully is used towards exciting exploration in biology. And also looking at signs, really, from many different angles, like considering the market, thinking about messaging and marketing is it's very stimulating. I like it.

Steve Lewis 11:40
Well, that's fantastic. And it sounds like you're making a lot of progress and not surprised to hear that the interest is in acceleration of timeline toward commercialization as well. What does that timeline look like in terms of you either going to market? Are you looking toward clinical transitions with your technology first? Or are you looking toward proof of concept, perhaps in like murine models? Tell me a bit about your roadmap?

Gudrun Stengel, PhD 12:12
Yeah, so our product has been in early access testing for the entirety of 2023. The first half of the year we offered our product as a service, and in the second half of the year, we have started really shipping a reagent kit. So again, I said it earlier, but our products are, it’s a reagent kit for an RNA library prep workflow and a software package that we are now offering through the DNA Nexus cloud platform. So we have done extensive beta testing and early access testing for the last half year, and we are all set to for full launch in January.

Steve Lewis 12:57
So you're looking from a platform approach, primarily?

Gudrun Stengel, PhD 13:02
Yeah, because this is a young research field, we think entering the discovery market is necessary. But of course, we already have ideas about how to address specific market segments later.

Steve Lewis 13:17
That's fantastic. It's a, it's a really great, I would say, uh, overall story about how, if you have a really great idea and you believe that you can get it to a point where you can make it a product, and along the way, get some intellectual property protection, it's a really just fantastic story about how innovation can really push a particular market toward new areas of discovery. That's really fascinating.

Steve Lewis 13:52
Imagine exploring innovative instruments and reagents in stunning 3D right from the comfort of your own space. Now you can with our Molecular Biology Virtual Lab. Whether you're doing PCR, electrophoresis, reverse transcription or more, there is something for you in the virtual lab. You can explore detailed 3D models of our instruments, learn about their functionality and discover how they can help elevate your research. You can also investigate the right enzymes or PCR plastics for your work. Ready to dive in? Simply visit thermofisher.com/molbiovirtuallab. It's a game changer for anyone looking to push the limits of their molecular biology research. Once again, that's thermofisher.com/molbiovirtuallab, all with no spaces or other symbols. And now back to our conversation.

Steve Lewis 14:52
So tell us a little bit about the molecular biology that you used in your labs? You mentioned you have a protein engineering group, for example.

Gudrun Stengel, PhD 15:00
So, we make protein libraries of tens of thousands or millions of mutants. So naturally, we do a lot of cloning. We have a yeast display protein evolution platform, and we do a lot of like characterization of binding properties and also enzymatic properties. That's one part of molecular biology. But then, of course, our library prep workflow uses molecular biology enzymes. So our workflow includes like shearing of the RNA, phosphorylating its ends, ligating adapters, doing the proximity bar coding step, and then reverse transcription and PCR amplification. So you really use molecular biology components at each step.

Steve Lewis 15:59
Earlier, you mentioned some of your application areas, including synthetic biology. And I'm curious if you can share a bit about what you envision your technology and ultimately kit can be utilized for in that field?

Gudrun Stengel, PhD 16:12
Yeah, that's a really interesting question. When I mentioned synthetic biology, I was thinking mRNA vaccines. So not everybody knows that, but mRNA vaccines are heavily modified. They basically all naturally occurring U's in mRNA vaccines are replaced by a modification called one method 1-methyl-pseudouridine, and this modification was really a requirement for making the vaccines effective because they do two things. They increase the stability of the vaccine and they tone down the immune response. So if we understand better how RNA modifications change the usage of messenger RNA, we can start to copy this complexity in the design of mRNA vaccines. So we could, for example, start putting modifications only in specific regions of the mRNA. Of course this requires that methods for site specific labeling of mRNA co-evolved. But eventually this will come.

Steve Lewis 17:26
What other blue ocean opportunities do you see? Now that I think perhaps the discovery, as you mentioned, is being appreciated, what other areas do you think that it can perhaps open the door to so?

Gudrun Stengel, PhD 17:41
One application I personally find very interesting is monitoring the emergence of drug resistance during chemotherapy. There are a few papers that describe how glioblastoma patients during chemotherapy suddenly become resistant to the treatment, and this can be traced down to changes in the modification pattern of a few genes. That's certainly not the only root cause for the resistance, but it is something you could potentially use to monitor your treatment success and how it's going and to foresee upcoming resistance. Generally, RNA hasn't been used much in liquid biopsy because of stability problems of storing RNA after blood draw. But these problems are being addressed by different collection tubes. Also, people like to look now at other more stable RNA species that are encapsulated

in exosomes. So I think for the liquid biopsy market, there's more, there are more discoveries to be had by using more markers. I think there's a lot of opportunity. I mean, I'm a, I'm an optimist. I often, I always think that progress in science will eventually be used for the betterment of human health and humanity. So I think if there is, I believe currently, there is the sense that maybe all that DNA sequencing hasn't delivered enough actionable information. For me, it is just a sign that we still don't understand enough and we don't understand exactly how the genetic code is used and when it is reprogrammed in response to external triggers. That's why epigenetics is so important. And I think, yeah, studying really the complete multiome will eventually give more actionable information. And with the upcoming of AI, it will be more possible to digest the information and interpret it.

Steve Lewis 20:08
What does a customer persona look like for your startup company? And tell me what you're expecting your customers to utilize your products for? And let's say it’s their first day, it arrives at their lab and they're getting ready to do a experiment that they have planned?

Gudrun Stengel, PhD 20:28
We work a lot with customers who are very driven by a biological or disease relevant question. So there is a RNA modification community, and this community is often very focused on developing methods, but we work more with biology researchers that just really want to get the information in a very accessible format. They don't want to be experts at next gen sequencing. They want, don't want to be bioinformaticians. They want to get a workflow that is robust, produces reliable results, and then a user-friendly bioinformatics interface where you submit your sequencing data and queue them for analysis and get a summary report out that tells you between a test and a control condition, like, “How has the RNA modification, pattern changed?”

Steve Lewis 21:36
Tell me a bit about the analysis software?

Gudrun Stengel, PhD 21:39
It's an end-to-end pipeline. It takes FASTQ files as an input, then it performs established manipulation of sequencing data, you know, trimming of adapter sequences, alignment to a reference genome. But then it splits the data according to the proximity barcode we attach as the RNA modification ID, and we have written our own peak caller to identify the modified regions. So the raw data type looks similar to a ChIP-seq data set, for example, where you have read enrichments in the modified areas, and you pick that up as a peak in the enriched sample over a baseline solution control. And our peak caller, it uses a machine learning algorithm to identify these peaks. And the advantage of using a machine learning based peak caller is that we can train it with many input parameters. For example, it can learn that an inosine peak often has a different shape from an m6A peak, and it could take into account, in addition to read enrichment for example, sequence motifs under the peak or reverse transcription pattern. Like deletions or mutation hotspots that are often associated with a modification, either naturally or you can tweak, coming back to that reverse transcription reaction, you can tweak. We want to build large data sets and give each customer a database where they can store all their data and then perform meta-analysis by looking at all data you ever produced you can learn more, and this is something we want to enable in the future. We'll probably also go more towards disease specific applications. There's always

the discovery phase where you need to figure out what genes change, but then there's a targeted phase where you only want to look at these genes so you reduce the problem for a single sample, but then you can process more samples to get more information.

Steve Lewis 24:13
Pulling on that thread a little bit more, what gaps do you see right now in the market for perhaps complementary exploration, or even in the same area?

Gudrun Stengel, PhD 24:25
Yeah, I think we want to help researchers to make this transition from RNA modification analysis being a very artisanal, experimental method that is labor intense and difficult to implement it as a routine analysis. I think a gap is still really getting single-base resolution of every modification. I mean, even we currently only focus on a handful of modifications, but if you could really read out all modifications of the transcriptome in all types of RNA species it would be amazing. But there isn't a method on the horizon right now that can do that.

Steve Lewis 25:14
How many years do you think it'll take before we are looking at that?

Gudrun Stengel, PhD 25:21
I think it could be 10 years. Nanopores are always an active area of research for both for RNA and also for protein sequencing. People really want to sequence these molecules directly, without going through any intermediates. And developing nanopores is just really, exceedingly difficult. I think it will still take a long time.

Steve Lewis 25:47
So I'm curious, as we kind of move into the final minutes of our recording here, what topics have we not spoken about yet, that you might want to make known to our listeners?

Gudrun Stengel, PhD 26:02
I just really enjoy raising awareness of RNA modifications, because if you mention epigenetics today, everybody will think about DNA methylation, and it's a different concept, because DNA methylation is inherited, whereas RNA modifications are really applied very dynamically to adjust to the environment. I think RNA, in general, is still a little bit of a black box. Only a very small fraction of the genome is coding. There's a lot of black matter people talk about. We researchers regularly discover new regulatory RNA that has, you know, discovered new functions. RNA is just a really rich, not fully understood matter, and we are seeing more and more interesting RNA, or applications of RNA therapies, as well where antisense oligos are used to, to block gene expression or to modulate splicing or to induce protein production via vaccines. I think it's just a really exciting area of research.

Steve Lewis 27:25
Very much so. And the enabling concepts of additional stabilization approaches for RNA and making sure that we have the opportunity to even explore what's going on in, inside of a cell at any given time

is really interesting. When we're talking about multiomics, what are the boundaries, in your opinion? How many different omics are there, and have we even identified all of them?

Gudrun Stengel, PhD 27:58
That is a great question. Let me count. So there's DNA, DNA methylation, histone modifications, RNA, RNA modifications, proteins, protein modifications, so at least seven. And I would say RNA binding proteins are also regulators, post transcriptional regulators of translation. So I would say at least eight.

Steve Lewis 28:24
Do you think that there are more coming?

Gudrun Stengel, PhD 28:28
I don't think so. I think we know fundamentally what types of molecules exist in a cell. We just need to understand how they interact with each other.

Steve Lewis 28:39
Gudrun, thank you so much for your time today. I always like to end our podcast with two questions of our guests, and my first question to you is, what have been the keys to your success so far?

Gudrun Stengel, PhD 28:55
I think persistence. So sometimes people ask me, like, “You've made so many great choices in life, how did you get there?” And I think you can't control all aspects of life, so it's a combination of your choices, but also some luck and the ability to overcome obstacles. And I think I'm a very goal driven person who can really work persistently towards a goal. And the other thing is I really enjoy what I'm doing, and that gives me the energy to show the persistence.

Steve Lewis 29:38
Persistence and passion. I think that's wonderful. And what would you say to maybe aspiring researchers who might be looking to follow in your footsteps? What advice would you give them?

Gudrun Stengel, PhD 29:52
Yeah, I would say, really follow your calling and be optimistic that all your hard work will be rewarded. And in the long term, people tend to look at relatively narrow windows of time for reward, but sometimes life might throw you curveball, and temporarily things don't look so bright, but you will get through it and look back and think, “Oh, it wasn't so bad overall.” So I think being optimistic about, that you may, your performance will pay off, but also optimistic about society in general. Doing science really gives you the power to work for the betterment of society. So it's a great passion to have, and I like to be an optimist and think that both society and science will bring about further improvements.

Steve Lewis 30:53
Well, best of luck to Alida Biosciences and anybody who may use your technology in the future. I'm very excited to see how it continues to develop and comes to market successfully.

Gudrun Stengel, PhD 31:08
Yeah, thanks so much for having me. It was a pleasure talking to you.

Steve Lewis 31:15
That was Dr Gudrun Stengel, CEO and co-founder of Alida Biosciences in San Diego, California. 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.