Steve Lewis 00:10
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its training applications in life sciences. I'm Steve Lewis, and today I'm joined by Dr. Hanno Hermann and Dr. Thanh Tu Hellmich-Duong. Together, they form the leadership team of Applyo Jena, a fascinating company specializing in reagents, lyophilization and freeze-dried molecular biology solutions. Hanno and Thanh Tu boast more than 40 combined years of experience in the biotechnology space and it was a pleasure to get to learn from them today. I hope you enjoy our conversation as much as I did. Here's Hanno to start things off.

Hanno Hermann, PhD 00:54
Maybe first, I introduce myself a little bit. So I came from plant breeding and genetics, but finally, I finished my PhD in biochemistry and molecular biology, and that was also the base of my professional life in the biotech industry. So as sales force, as product manager in development, and last, not least, in the production, also in leadership positions. Approximately 2010, I came to a big diagnostic company here in Jena, and there, my way of life crossed the way of Thanh Tu, and since that time, we are working together.

Thanh Tu Hellmich-Duong, PhD 01:39
Yeah, I'm Thanh Tu. My background is, I'm a physicist with some specialization in biophysics. I worked for a pharmaceutical company and developed point of care diagnostic systems for industrial customers for a couple of years, and then after that, I worked for company which produces diagnostics products.

Steve Lewis 02:04
So Applyo Jena develops and produces freeze dried reagents for in vitro diagnostics, life science research and pharmaceuticals. Hanno, why don't you break down what that means to the audience?

Hanno Hermann, PhD 02:17
So, we are company with a mission to stabilize sensitive reagents under any circumstances, especially under high temperature environment. Normally, you have to use freezers for transport and storage of these reagents to keep them in function. And we found solutions to store and transport these reagents at ambient temperatures up to 60 degrees in, for some time, to keep them stable. And this is a crucial thing for the diagnostic industry, especially for point of care diagnostics.

Steve Lewis 03:01
Applyo' s core product are these lyo-beads. They're lyophilized master mixes, essentially. Thanh Tu, why don't you tell me about how you came to that idea and why there was a need for making it?

Thanh Tu Hellmich-Duong, PhD 03:01
In my last job, I developed a point of care system together with many other colleagues and also Hanno. In that time, we worked together for almost seven years in a very small office and developed an HIV cartridge for field application for detection of HIV.

Hanno Hermann, PhD 03:36
It was one of the first point of care diagnostic systems which was developed at that time. It was very innovative, and so we were lucky to be part of this. And so one important task for us was to make the reagents stable, just for in storage and application under the circumstances in Africa or other hot countries. And that was a challenge at that time, and we solved it by finding the innovation to make lyo-beads so small portions of master mixes designed for one reaction with all reagents inside as ready-to-use and to make sure that the diagnostic assay is finished in less than one hour. And this was really a very important starting point for us in the later development of Applyo Jena.

Steve Lewis 04:39
Freeze drying, I think, came probably to public knowledge as a technology around the time that astronaut ice cream came out. And I want to kind of highlight the technology, if I can paint a picture, these beads are freeze dried beads of liquid, very similar to, if you've ever felt astronaut ice cream, they're just much smaller. And it sounds like the idea Hanno, as you describe, it is there may not be a cold chain that can get from one area to the next without needing refrigeration, for example, or freezing of reagents to be able to enable a lab in an area that might be very hot, for example. So it sounds like you came up with this idea as part of, “Can we get these reagents ready to utilize in the field from point A to point B without any refrigeration at all.” Is that correct?

Hanno Hermann, PhD 05:46
Yes, that was a basic challenge and idea. And we were happy we found the ideas and the innovations together to make this possible.

Steve Lewis 05:54
So inside these lyo-beads that a Applyo Jena makes you have components of molecular biology reactions. You may have enzymes or master mixes, and then they are freeze dried. Tell me a bit about the resuspension process when these products arrive at their point of use.

Hanno Hermann, PhD 06:17
The resuspension process with our lyo-beads is very fast, so they are redissolved within a second. If I add an aqueous nucleic acid solution or whatever, so it is redissolved in this time. And reason for this is that the surface, or the inner surface, of the beads are very large, so there's naturally contact with the water or aqueous solution, and in within a second, the enzymes are rehydrated and can start to make their job. So it's like an immediate start of the reaction after the redissolving process within a second.

Steve Lewis 07:04
And when these reconstitutions happen, when they're going from that freeze dried format into liquid format, is the enzymatic confirmation is, it's exactly the same, right?

Hanno Hermann, PhD 07:20
Yeah, it's exactly the same, of course, also still the lyophilization status. So what we do in freeze-drying on lyophilization is that we keep the structure of the proteins as it is in the natural status, but we take the water away also the molecular bound water from the surface of the proteins. And instead of this, we add so-called excipients. These are special compounds, mostly carbohydrates, which are the

replacement of the water molecules. So this excipient keeps the structure, the 3D structure of the proteins, as it is normally. And in that way, we keep the structure all over the time. And so that's that stabilizes the function.

Steve Lewis 08:17
Absolutely. And one of the things that came to mind, as you were mentioning that is when traditional reagents are shipped, maybe in a tube, for example, glycerol is one component that might be used. Can you talk to me about what that process looks like?

Hanno Hermann, PhD 08:37
Yeah, glycerol is an anti-freezing, freezing agent, so it prevents freezing of a solution. Of course, this we don't need in freeze drying process, so we are working without any glycerol in our master mixes. And we don't need it really, because we keep the structure of the proteins by quite another way, by the stabilization of freeze drying and the excipients.

Thanh Tu Hellmich-Duong, PhD 09:03
So when we started dealing with lyophilization in 2010 it was very difficult to find enzymes in the market which did not had any glycerol. So the workaround, which we did in that time was to use very high, concentrated enzymes which contained still glycerol, most often 50%, that was too much. But with the suppliers together, we could reduce it to, for instance, 28% and when the concentration of the enzyme is high enough, then you can dilute it out in your lyo master mix, and this is then sufficiently to work. So the, in that time, we had the limit of approximately 0.8% of glycerol in the lyo mix that worked. But when you want to work with these freeze-dried reagents in the lab or during manufacturing, you have to maintain very, very low humidity environment of below 5 to 10% and had a very short time for processing.

Steve Lewis 10:20
You all were pioneers in this space. Were there other gaps that needed to be filled as you were looking toward expanding your technology?

Hanno Hermann, PhD 10:30
Yes, so we had a big challenge at the beginning, that we had to make small portions of a master mix. So just for one reaction, because in one so-called cartridge for the point of care product, there should be the reagent just exactly for one reaction. So we didn't need, of course, a large volume master mix and then make aliquotation. This is not possible in point of care. We needed precise portions of one PCR, RT-qPCR, or whatever assay reagents. And that was a challenge at that time, because we had a product which was counting virus numbers in blood of HIV patients just for control of therapy. And there was no chance to estimate it is 50 or 120 in a milliliter. So it was had to be precise, and that's why we developed a very precise method to make the beads. So our beads have a really an invariability of 2% or lower, and this is one of our features still in our products. The other challenge was to make a process, a production process, which is upgradable. Of course, we don't need a process for some thousandsf beads for industrial products. So we were able, and are now still able, to produce 100,000 and in near future, millions of beads in one batch. And that's a prerequisite for an industrial process. And another feature, which was important, if you have a very sensitive assay, and which don't need any

contamination, you need a very pure and you know how to say, process, and that's also true for our process. There is no contact, for instance, with liquid nitrogen, which is a dirty solution, so we use only pharma compatible materials within contact with our master mixes for therapeutics.

Steve Lewis 12:58
In the U.S., we have this brand of ice cream called Dippin Dots. Almost every baseball stadium, or maybe sports stadium sells this ice cream, where they're very similar to the lyophilized beads that you were just talking about scaling up. How much did, for commercial scale manufacturing, did you look at technologies that might have been making food products and perhaps apply them to the scientific processes?

Thanh Tu Hellmich-Duong, PhD 13:31
So when you start to develop a product, it's always wise to look at the state-of-the-art technology. Of course we looked at that and dripping droplets was also something we looked in and were astonished how it works. But this is not this technology applicable to lyophilization of reagents. When we started developing, producing beads we also use liquid nitrogen in the beginning for making beads. And where, when we looked at the details, we saw the beads are not homogeneously in size. You have several effects which happen when reagent mix hits the liquid nitrogen surface, you have an effect which is called inverse Leidenfrost effect. The water droplets, the reagent droplet is levitating on the on the vapor of the liquid nitrogen and is moving around due to this vapor. The cooling rate for the reagent is not very high. It cools down, then it starts to freeze, and then is submerged. But it takes some time, and just during this time, when you produce further reagents, they can merge together. And then you have a droplet which is double the size, or when it hits the nitrogen, it boils, and you get satellites so, and this is at the end, when you harvest the beads, the cryo-beads at the end of the process, you have to sieve them, because they are all different in size.

Steve Lewis 15:24
If I can take us maybe 10 years into the future, one of the breakthrough technologies that, I guess the FDA would have approved in the U.S. about it in the last 10 years is monoclonal antibody therapeutics, for example. Those are administered right now intravenously for the most part. And I'm curious, for monoclonal antibody therapeutics, is your technology something that could be applied and maybe enable a pill format?

Hanno Hermann, PhD 16:03
So, antibodies to stabilize by lyophilization is even not a problem, and also not in case of lyo-beads. And so far, the technology is usable for also stabilizing lyo-beads or other organic therapeutics. So but especially also another application could be the lyophilization of mRNAs. I think I have not to tell you what's importance of this type of molecules is especially as therapeutics for infectious diseases like Corona. You remember this story. And so far we think there it is a platform technology, and you can use it for any type of molecule, protein, antibody, organic compound, or even nucleic acids. And maybe one further idea will be the stabilization of phages by lyophilization technologies for the upcoming new antibiotic solutions, for instance, usage of phages.

Steve Lewis 17:29
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 on my desk, and I love how easily it connects to my phone. It's nice when I want a 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 18:13
You mentioned mRNA, and I'm curious how your technology may lead to new breakthroughs in that area of research, either for therapeutics or vaccines?

Hanno Hermann, PhD 18:31
Yeah, as you know, to stabilize mRNA for usage as a vaccine is not only the only challenge to stabilize the reagent, the reagent itself, you have also to packages before into special vesicles to keep it in function, even in the body of the patient. And of course, it's still a big challenge to freeze dry such vesicles. But nevertheless, the lyo-bead technology may help to make portions for one application, in a syringe, for instance. So the normal way now is you freeze dry some milliliters in a vial, in a glass vial, and then you redissolve it. And then you have to make aliquots from this, maybe for 5, 6 or 10 applications. And this is a source of mistakes. You have to concentrate that you have to make exactly five portions, for instance, from this volume and not more and not lesser. If you take beads, for instance, one bead for one therapy portion, then you can put this bead into a so-called dual-chamber syringe, and then you have a ready-to-use application tool to applicate one injection for one patient, and you can store it at room temperature. You can really solve it magically before the application, mix it, and inject it. And this, for this, you need a very good and precise portioning of the therapeutics, and this is possible with beads. Of course, a lot of things have to come together if this is, if this will be reality. But we think we are now at the beginning of a way, and I think it's, there is some need to bring different parts of the technology together, and then we will have the future, interesting new products for therapeutic application of freeze-dried biopharmaceuticals.

Steve Lewis 20:51
Moving into another molecular biology topic, maybe Thanh Tu, you can tell us a bit about RT-LAMP. Specifically on this show, we sometimes talk about isothermal amplification as it relates to PCR. And I'm curious why RT-LAMP technology is of interest to Applyo Jena?

Thanh Tu Hellmich-Duong, PhD 21:14
So definitely, for in molecular diagnostics, RT qPCR is a state of the art. And when you want to do molecular diagnostics in the field, you have challenges using real time cyclers in the field. And for this RT-LAMP is, of course, a very good technology as an isotherm amplification. This technology works at 65 degrees Celsius, which is at a constant temperature. And to this is a much easier specification for instrument in the field. At Applyo, we have developed an RT-LAMP mix which can be used in the field without any reader. It's a colorimetric LAMP assay which changes the color from red to green. And on the market, you find different RT-LAMP systems which change, for instance, the color from red, from

yellow to red. Those systems use this pH indicator for the color change, and we see that a pH indicator is not the best solution, because when you have buffered samples, this will interfere with the detection system, and we found a different colored change mechanism which does not have this bottleneck.

Steve Lewis 22:51
It's amazing to think that these colorimetric assays can be shipped at room temperature. I have worked in laboratory operations previously, and I just think about all the waste, and all of the energy and inputs and outputs that go into keeping reagents and assays cold and getting to the destination. Even, even still, with all those resources when it arrives at its at its location for use, there is still some question about whether or not it was, arrives in the quality that you would expect. So the surety around your technology, I just think is so fascinating when it comes to what freeze-drying enables, and it's such a unique area. In the next five years, one of the things that I am looking at is, we have all these instruments that are required, but this kind of convergence of isothermal amplification, your technology, and the idea of meeting use cases where they are. Do you see new form factors needing to be developed to enable use in maybe a more sustainable way?

Hanno Hermann, PhD 24:20
Even the freeze-drying is a sustainability factor. That maybe is astonishing on a first view, because it's an energy consumption process. We made exact calculations for this to be have a clearer view at the beginning, just for ourselves. And so we were surprised to find that making a certain kit - so if we have a model certain biochemical kit, and we produce it, then we have to store it at a refrigerator up to the selling point for a customer, and then we have to transport it on dry ice to any point in the Earth. And this is also energy consumption. And if you compare then as energy consumption of the traditional way and the way to make a freeze-dried product and to store it at room temperature and to transport it at ambient temperature, then you will find at the end that the energy consumption in case of freeze-dried product, is much lower.

Steve Lewis 25:29
So far, we've spoken about a couple of molecular biology techniques in isothermal amplification and one of the things that comes to mind is how your technology could be applied to other areas of biotechnology and molecular biology. Things that come to mind, for example, are cloning, or even NGS, or maybe nanoparticle applications. Can you speak to any of those?

Hanno Hermann, PhD 25:56
Yeah, you are right. Because we have a platform technology with the process to make lyo-beads, we can applicate it also for other fields, than isothermal reactions or PCR. And you mentioned just one of this, we can do, of course, cloning kits inside the lyo-beads, but also preparation kits for next generation sequencing. And a very special point I will come to closer, is the lyophilization of nano- and micro-particles. We did this with some different kinds of particles, especially with magnetic particles, and this we did also in cooperation with colleagues from Thermo Fisher.

Thanh Tu Hellmich-Duong, PhD 26:48
Yeah, so when you want to purify or clean up nucleic acids, today, you don't use silica membranes anymore. So many of the industry is changing to magnetic particles, because it's much simpler. But

when you leave the slurry over the weekend or after certain days in the fridge and you want to homogenize this solution, it's really cumbersome. You need harsh mixing conditions or ultrasonics for many minutes, half an hour. And this is something, yeah, which you need to take into account in your daily work. Yes, and we were able to demonstrate, to show that we can freeze-dry single reaction beads which contain magnetic nanoparticles. And yeah, so when the user takes these beads, they can immediately start using things, these beads for purification of their samples immediately.

Hanno Hermann, PhD 27:57
The redissolving time is the same as in the other cases with it, with the lyo-beads. So they are redissolved in a second. So you have a well portioned number of magnetic beads, put your aqueous DNA or RNA solution on top of it, and it redissolves in a second. And then you can make maybe a magnetic separation or cleaning process with this material. And another positive effect is to freeze-dry magnetic beads, you have always special molecules on the surface of the magnetic beads. In some cases, also sensitive, maybe again like antibodies. And by the freeze-drying process, of course, you can protect this sensitive functional layer by freeze-drying and you can store it at room temperature up to the point of usage.

Thanh Tu Hellmich-Duong, PhD 28:58
You know the difference in freeze-drying of particle-based systems is that the particles tend to agglomerate or to sediment down, and this you have to translate into a production regime, where you have to maintain always a homogeneous solution, a slurry, and make a single reaction beads out of that. And this is some challenge we have mastered and can ship already to some customers. And if you look at other particles, systems like fluorescent polystyrene beads, so those systems are normally not for freezing, but we demonstrated that we can freeze it, freeze dry it, and resuspend it and maintain the functionality.

Steve Lewis 29:54
That's just incredible to think about all the, all the tech, technologies that this this platform can touch. I think even about sample preparation to chromatography as well with some of the areas you just discussed. As we move toward the end of our podcast today, one of the things I am very eager to hear about from you all is, what does a Applyo Jena look like in 10 years?

Hanno Hermann, PhD 30:25
That's a good question. Why, you would ask, what about five years? But nevertheless, we think the company will develop well further. So we are now nearly five years old, and we got always good development to become bigger, smarter and more successful. And we now hired a lot of nice people, which are very well educated and very well motivated. And this is the basis of our work, to work with these people. And we have a lot of new ideas for new products in the field of isothermal reactions, in the field of lyophilization of particles, and so on. And so we will also develop pharmaceutical applications in the near future. And so far, we think we are well prepared and have a lot of ideas to develop further. And what will be the situation exactly in 10 years, I don't know, but we will be, I think, on the market within new fields and with some more people in the company.

Thanh Tu Hellmich-Duong, PhD 31:53

It's very difficult to predict what comes in the future. Of course, we will develop further in the diagnostics market with new ideas, new product, and also hopefully enter the pharma market. But to be honest, the pharma market is a very long story. It gives you a lot of chances, but where chances are there is there are also many risks. And yeah, we need to deal smart with this in order to be successful, not only in pharma, but especially in diagnostics.

Steve Lewis 32:41
Well, here's to many more years of success. I'm very excited to see where you head. I thank you both very much for your time today. It's been a really fascinating conversation, and I look forward to seeing where Applyo Jena goes in the future. Thank you very much.

Hanno Hermann, PhD 32:58
I thank you too.

Thanh Tu Hellmich-Duong, PhD 33:00
And have a great day.

Steve Lewis 33:03
That was Dr. Hanno Hermann and Dr. Thanh Tu Hellmich-Duong, COO and CTO of Applyo Jena, in Jena, Germany. 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.