Transcript

16th November 2023, 14.00

The seminar commenced with Laura Daniela Martinenghi[1] officially starting the session and expressing gratitude to all attendees. She introduced Christoph Gradmann[2] and Jørgen Leisner[3] as her research's principal investigators (PIs), mentioned herself, and highlighted the presence of Nora Ottens[4], their colleague responsible for note-taking. During this introduction, an online participant interjected, pointing out difficulties in hearing Laura Daniela Martinenghi unless she was near the microphone. Despite these audio challenges, caused partly by the seminar being held in an old room without installed microphones, Laura Martinenghi assured everyone they would try to speak loudly and clearly. Christoph [Gradmann] then remarked on the necessity of better hearing for recording purposes, to which Laura Martinenghi responded that the session was being recorded on Zoom, which Christoph [Gradmann] found satisfactory.

Laura Daniela Martinenghi: So, we're starting with ... Christoph is going to present our work.

Christoph Gradmann: Yes, I'm very happy to do that in the presence of two of my dear colleagues, Laura and Jørgen. My name is Christoph Gradmann, and I'm a professor of history of medicine at the University of Oslo[5]. And one of the things I did over many years was working on the history of antibiotics (1–3). And you can say that the history of antibiotics has gone from great promise to great tragedy (4–6). And at one point, I think Jørgen [Leisner] and I started discussing we should work on that. We shouldn't just take that for granted and lament the end of antibiotics. But we should make this the subject of historical inquiry. And that's how the project called the DryAP came about[6]. And I think we had a not-so-successful attempt to have it funded with a funder whose name I won't mention. And ultimately, the Research Council of Norway gave us the money[7].

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[2] Christoph Gradmann - Professor - Department of Community Medicine and Global Health – University of Oslo. https://www.med.uio.no/helsam/english/people/aca/ulrichcg/index.html.

[3] Jorgen Leisner - Associate Professor - Department of Veterinary and Animal Sciences - University of Copenhagen. https://www.linkedin.com/in/jorgen-leisner-53b033b/.

[4] Nora Ottens - Secretary of the Department of Veterinary and Animal Science- University of Copenhagen.

[5] For more information: https://www.med.uio.no/helsam/english/about/organization/departments/community-medicine/index.html.

[6] https://www.med.uio.no/helsam/english/research/projects/antibiotic-resistance-big-pharma/

[7] For more information about the Research Council of Norway: https://www.forskningsradet.no/en/.

What we're studying in this project is the phenomenon of the empty pipeline, which is often referred to when people talk about the problem of antibiotic resistance and what to do about it. And then they just say the pipeline is empty - we have to restart it[8]. And our question is, so how did that come about? Because in the first couple of decades after the Second World War, there would be lots of antibiotics (7,8). And it became obvious to us that while the public started to lament about the lack of antibiotics in the 1990s, the decline that Jørgen [Leisner] and I thought we should look at actually somehow started in the 1970s.

We'll mention just that one example with industry, I think, losing faith in classical screening programs [in the 1970s] (9). And then many other things happened, and at the end of the day, in the early 2000s, big companies left antibiotics and anti-infective medicines in large numbers (10,11).

And in our project, we're looking at very, very different dimensions of this process. For instance, at a certain company, Bayer, which used to be really big in anti-infective medicines. They also were the inventor of the last blockbuster, Cipro [ciprofloxacin], and then they said goodbye to everything. Why did that happen? (3)

And then we're looking at the empty pipeline as a policy window, for instance. So, there is a coincidence between the lack of interest in drug development and the rising interest or the window of opportunity for politics on AMR in the 1990s[9].

And many other approaches. We're looking at the inhabitants of the pipeline because, very often, the pipeline is represented through lighthouse figures; we have Domagk [Gerhard][10] or whoever they are. But actually, the pipeline has hundreds of people in it, and it has a very interesting gender structure. The pipeline that I studied for many years at Bayer had male figureheads, but it had female staff. So, we're looking at that also.

And we're looking at the fate of strain collections[11], which are very important in drug development, and today are not the same thing after gene technology arrived, and so on.
So, all in all, we have five projects, four PhDs, and one postdoc, it's distributed to five European countries[12], and the whole project is run from Oslo. It wasn't so

[8] For more information: https://www.emptypipeline.org.
[9] Polices and regulations from the European Commission in the AMR pipeline https://health.ec.europa.eu/antimicrobial-resistance/eu-action-antimicrobial-resistance_en.
[10] Gerhard Domagk, discovered sulfonamidochrysoidine (Prontosil; KL730). He received the 1939 Nobel Prize in Physiology or Medicine. https://www.nobelprize.org/prizes/medicine/1939/domagk/biographical/.
[11] Cultural strain collections, refers to microbial strain collections.
[12] Clusters or groups inside this research group: https://www.emptypipeline.org/pipeline-clusters.

easy starting that during the pandemic[13], but now we are in a phase where we are deep into collecting data, and one way of collecting the data is doing such witness seminars where we bring together people who are involved in stories that relate to the interest of our project.

And I think, that's probably where I hand over to Laura [Martinenghi] because this is a story that is very much studied in Copenhagen.

Laura Daniela Martinenghi: Thank you, Christoph. As Christoph [Gradmann] said, there are five groups in this research. We have Oslo, which is mainly working on pharma and research on Bayer, as Christoph said. We have Dublin, that is doing technology and new business related to the pipeline. Then we have Spain, which works with gender relations inside of the pipeline. It's very interesting work. And then we have France, which does policies specifically. And we have Copenhagen, that we do a little bit more on wet lab, and we research on targets in drug development. On the historical part, we have historical cases; the main one is Plectasin.

And this is the group[14]. There are two pictures, because there were two meetings held in Oslo last year (2022) and this year (2023). We have a website where you can read about us, Empty Pipeline. We are founded by the Research Council of Norway. Christoph [Gradmann] is our PI, and today, he is going to be our discussant. That means at the end of the day, he will come with some remarks and some conclusions.

Jørgen [Leisner] is my direct supervisor. He is the interviewer today; he's been working on the questions I'm going to ask today. Belma [Skender][15] couldn't be here today; she's a postdoc, she's in Oslo, and she's sick, unfortunately, but she's also part of this.

I [Laura Martinenghi] will be doing the questions, and Nora [Ottens] will be taking the minutes. We're coming now up to the witnesses, which is all of you.

So today, we have divided this witness seminar into three blocks. On the first block, there are some questions. The idea is that it's not an interview; it's just trying to remember what happened back then, how you felt as a researcher, what your perspectives were, and a little bit about the background.

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[13] Covid-19 short for "Coronavirus Disease 2019", is a highly contagious respiratory illness caused by the novel coronavirus SARS-CoV-2. It was first identified in Wuhan, China, in late 2019 and has since spread globally, leading to a worldwide pandemic. https://www.bbc.com/news/world-asia-china-51466362.
[14] Showing Power Point presentation.
[15] Belma Skender - Postdoctoral Fellow - Department of Community Medicine and Global Health- Oslo University. https://www.med.uio.no/helsam/english/people/aca/belmask/index.html.

So, first of all, a short presentation of yourself so that Nora can make some notes. Very short, but who are you, and what you're doing now? What are you ... (interruption).

(Note: Before the discussion, Christoph Gradmann made a quick technical remark, suggesting that participants identify themselves by name for easier transcription. Laura acknowledged and agreed with the suggestion).

Block 1: Discovery phase

Laura Daniela Martinenghi: Right. So, we start in the corner... down there[16].

Per H. Mygind: Yes, so I'm Per H. Mygind, as you can see on my little sign here[17]. I worked at Novozymes[18] in the early 2000s, and since then, I moved to Novo Nordisk[19]. Now, I'm in a smaller company called Ascendis Pharma[20] in Hellerup[21].

Laura Daniela Martinenghi: Okay. Perfect.

Hans Henrik Kristensen: Yes, hello, my name is Hans Henrik[22], and I joined Novo Nordisk; it was back in the day in '98, I guess. Two years later, they separated, and a demerger was formed, and Novozymes was formed, where we landed. And I stayed there, we stayed there, some of us for quite some years, and then I moved into oncology in various companies, and now I'm in a vaccine company that also has one oncology program. And like all of you, I'm now a vaccine expert.

Laura Daniela Martinenghi: Of a vaccination... Right. Thank you.

Olivier Taboureau: I'm Olivier Taboureou[23]. I came to Novozymes in 2001 as a postdoc and stayed there for three years, I think. And then, I moved back to university at the Center for Biological Research with Céline Grenac. I stayed there for six years, and I came back to France, taking the opportunity of a professorship in bioinformatics and molecular modeling, things that I was doing at Novozymes. And voilà[24].

Laura Daniela Martinenghi: And voilà.

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16 Laura's comment refers to a physical location within the seminar room, pointing out one participant.
17 Per H. Mygind - Director of Clinical Biomarkers & Immunogenicity at Ascendis Pharma https://www.linkedin.com/in/per-holse-mygind-221bab2/.
18 Novozymes (now part of Novonesis) is a Danish biotech company specializing in the development and production of enzymes and microorganisms for a wide range of industries. https://www.novozymes.com.
19 Novo Nordisk is a global pharmaceutical company headquartered in Denmark. https://www.novonordisk.dk.
20 Ascendis Pharma is a biopharmaceutical company headquartered in Denmark and with operations in the United States. The company specializes in developing and commercializing innovative therapies for rare diseases. https://ascendispharma.com.
21 Area in the northern part of Copenhagen.
22 Hans Henrik Christensen - Medical director, head of regulatory affairs, medical affairs, and scientific communication at AJ Vaccines. https://www.linkedin.com/in/hans-henrik-kristensen-31a319.
23 Olivier Taboureou - Professor of bioinformatics and biostatistics at Université Paris Cité. https://www.linkedin.com/in/olivier-taboureau-2b04574/.
24 "Voila" is a French word that is often used in English to express success or completion.

Eefjan Breukink: Okay, Eefjan Breukink[25] here. I actually was not involved in the original discovery of Plectasin because I'm not affiliated with Novozymes or Novo Nordisk. But I am working on the target, lipid-II [26], and I am also now working on Plectasin to finalize its mode of action, and we hope to publish it soon. And hi, Hans-Georg[27]. Just... I'm not sure if he... Oh, he doesn't hear me.

Laura Daniela Martinenghi: No, I don't know if he hears. But...

Eefjan Breukink So, I go back to also with Hans-Georg [Sahl] on the lipid-II story. So, I'm now actually focusing on trying to find a novel antibiotics that work on … at the level of lipid-II in Utrecht[28].

Laura Daniela Martinenghi: Perfect.

Leonardo De Maria: So my name is Leonardo De Maria[29]. I worked at Novozymes from 2002 to 2014, so 12 years. And those 12 years, I overlapped with all those that were at Novozymes at the time. Among the things I was working on were also antimicrobial peptides that work. Then, in 2014 I went to Novo Nordisk, and I spent four years at Novo Nordisk, 2014-2018. And from 2018 I have been at AstraZeneca[30] in Gothenburg. And I'm not doing vaccines, but...

Kirk M. Schnorr: Good and I'm Kirk Schnorr[31], and I joined Novo Nordisk enzyme business in 1997. And I'm still there. We're now called Novozymes. We split in 2000, as Hans Henrik said. I have worked the whole time finding new enzymes for different industries and at an increasing pace, fast, fast, very fast, so developing new molecular biology tools. Some of the tools I developed could also find other secreted proteins than enzymes, and Plectasin was one of these. I went across to the other building and handed the printout, which I think I had just found, to Dora and Hans Henrik to have a look. And that was my role, more or less.

Laura Daniela Martinenghi: Right.

Per H. Mygind: The rest is history.

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25 Eefjan Breukink - Professor in Membrane Biochemistry & Biophysics. Utrecht University. https://www.linkedin.com/in/eefjan-breukink-89b3b0/.

26 Lipid II is a lipid-linked peptidoglycan precursor, which means it plays a critical role in the formation and maintenance of the cell wall structure in bacteria (23).

27 Refers to Hans-Georg Sahl - Emeritus Professor at the University of Bonn.

28 University of Utrecht, Netherlands. https://www.uu.nl/en

29 Leonardo De Maria - Principal Scientist at AstraZeneca in the Advanced Drug Delivery department of Pharmaceutical Sciences. https://www.linkedin.com/in/leonardod3/.

30 AstraZeneca is a global, science-led biopharmaceutical business. https://www.astrazeneca.com.

31 Kirk M. Schnorr - Senior Scientist at Novozymes. https://www.linkedin.com/in/kirk-schnorr-319b6a/.

Laura Daniela Martinenghi: The rest is history.

Dorotea Raventos: Yes, I'm Dora Raventos[32]. I'm a molecular biologist. I have been in Novozymes for 22 years, and the first, I think 10, was with antimicrobial peptides, and the last 12, I think, are also working in the enzyme business. I'm in the same department as Kirk [Schnorr], so no more antimicrobial peptides. Unfortunately (*laughs*).

Laura Daniela Martinenghi: Unfortunately (*sarcastic tone*). And Hans Sahl, you have to speak loud.

Hans-Georg Sahl: Oh, okay. My name is Hans-Georg Sahl. I was at the University of Bonn[33], or I should say I'm retired by now. And I met Hans Henrik in the EU project PANAD in 2000-2003. I don't know if you remember it, Hans Henrik. It was a new project on antimicrobial peptides. And then, after Plectasin was discovered, I think Hans Henrik was asking about the mode of action, and since we had all these platforms for the mode of action analysis, particularly in the cell wall, and particularly the lipid-II, which in those days was not a target, actually. I was surprised that it turned out to be a target, to some extent, at least. But, that was my contribution, basically, to analyze the mode of action, and we did the first paper, Science paper in 2010, I think (12,13).

Laura Daniela Martinenghi: Before we start, we would like to hear a little bit about the context of how the group was established. How did you get into Novozymes, and from Novo Nordisk to Novozymes? I just want to have the background of the group before I ask the rest of the questions.

Hans Henrik Kristensen: Question to me?

Laura Daniela Martinenghi: Yes.

Hans Henrik Kristensen: I don't remember (*joyful tone*). My personal story was, and it's not that interesting, but I graduated, I got my PhD., and then I applied for a scholarship in the US, and I applied for jobs in Denmark at Novo Nordisk. Within the same week, I got offered a job at Novo Nordisk to do phage display and biopanning. And I got two grants if I can say that, to go to the US. One from the Science Foundation[34] and one from Carlsberg[35]. And I chose, of course, the

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[32] Dorotea Raventos - Science Manager at Novozymes. https://www.linkedin.com/in/dora-raventos-21557248/.

[33] University of Bonn .https://www.uni-bonn.de/en/university.

[34] The National Science Foundation (NSF) is an independent agency of the United States federal government that supports fundamental research and education. https://www.nsf.gov.

one from Carlsberg. So, we went to the US. I stayed there for two years to work on TB[36]. In the US. And I still kept in contact with the guy who actually tried to hire me at Novo Nordisk, Torben[37]. And he was at another US company in a collaboration called Maxygen[38]. So we met and kept in contact.

And then they got the first EU project, I think, funded by the EU and included a Dutch group from Waageningen, a German group, and possibly a couple of other groups. And that German guy on antimicrobial peptides. The purpose was for us to see if we could produce them for washing powder and industrial applications like in the rest of the project, which maybe had other applications in mind. But coming from the medical school at Stanford, I wanted to do pharmaceuticals. So that's the interest that I had.

So quite quickly, in my little EU project, and I was the sole one as far as I remember, internally at Novo Nordisk, it was about production, recombinant production of small peptides that were very difficult, of course, to produce in microorganisms. It might since have been solved to some degree. And then the combinatorial aspect of it, that we at Novo, in some division at Novo, had tools where you could manipulate the genes, make many variants, and then select the ones that were better or had a different spectrum of activity. Maybe they weren't inhibited by salts, as can be an issue with some of these. So that was all of my interest. And I did a lot of speculation around that and some experiments. And then, it is a bit blurry, I have to say, but again, my focus was on the medical side.

Did you join Novozymes or Novo Nordisk? [question to Dora Raventos]

Dorotea Raventos: Novozymes.

Hans Henrik Kristensen: OK. So then I probably met Hans-Georg [Sahl], as he mentioned. We were talking about a common new EU project, completely separate from the other one, and of course, with a pharmaceutical focus. And you have to correct me, Hans-Georg [Sahl], if it's not entirely correct. And then we got the funding of quite a large program with several partners involved.

Hans-Georg Sahl: Yes, we discussed going for a new project at the '99 Gordon Conference[39] on Antimicrobial Peptides in Italy. And then I was sitting back and writing the proposal that we got granted in PANAD project, as it was called.

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35 The Carlsberg Foundation supports visionary and innovative basic scientific research. https://www.carlsbergfondet.dk/en.
36 TB: Tuberculosis.
37 Torben Vedel Borchert- Scientific Director at Novo Nordisk. https://www.linkedin.com/in/torbenborchert/.
38 https://www.maxygen.com.
39 1999 Gordon Research Conference. https://www.osti.gov/biblio/1088857.

Hans Henrik Kristensen: Yes. So it all started at, probably in a bar at a luxury hotel in northern Italy, at this Gordon Conference in '99. So, I really enjoyed it, and it was a great introduction for me so we kept in contact. The project was funded, and suddenly, we had positions open that we could hire for this EU project. I was coordinator of the EU project PANAD and still have the application, reports etc. Novozymes had two postdoc positions which were filled with Per Mygind and Dora Raventos we were 6 partners from Denmark, Norway, Sweden, Israel, Italy and Germany.

Per H. Mygind: Short term, as I remember.

Hans Henrik Kristensen: Yes, yes.

Per H. Mygind: Repeatedly short term.

Hans Henrik Kristensen: No, that's up to you. So that's how it started. So, with Dora and Per on board, we took off on a longer journey. So maybe that's enough.

Laura Daniela Martinenghi: That's a very good introduction.

Per H. Mygind: What was it you didn't remember? (*joyfully provocative tone*).

Hans Henrik Kristensen: Don't get me started (*laugh*).

Dorotea Raventos: Well, he doesn't remember, he doesn't remember.

Laura Daniela Martinenghi: So Per, how did you...When you started, what was your role, and how were you starting in the group? You say it was for the short term, and it was after longer?

Per H. Mygind: Yes, so I'm not sure how long you've been with Torben [Torben Vedel Borchert] and in protein design when we were hired in, maybe a year or two. It was very shortly after the split of Novo Nordisk and Novozymes. That I joined. And I stayed there until 2009, I think. Gradually moving this antimicrobial business that Novozymes wanted to try out at that point.

And I think we had a lot of room to do this. But Novozymes had, I guess, the aspiration also to do what you were saying, drug development in maybe a faster, more intelligent way than the normal pharmaceutical thing and enzyme business.

But I started there, so that was with early discovery and research. I was happy that Kirk [Schnorr] came with molecules; it was not the only one.

Leonardo De Maria: That's a funny story that you should tell.

Laura Daniela Martinenghi: Right. But, Kirk, they mentioned you, so how did you end up in this group? You were part of another group.

Kirk M. Schnorr: So, I would say I was associated with the EU projects. I belonged in, I think, at the time it was called Fungal Screening. And there I was responsible for enzymes for many industries. So, I was primarily cloning enzymes, but the tools I developed[40] would be used to find all secreted proteins, essentially (14). And I'm a curious kind of chap, so when I see something interesting that, okay, Hans Henrik has this project, and this is something that is highly related to an insect defensin and that came up on the homology list. I think I'll just walk that over to one of you, and let's see what Hans Henrik has to say about that. And I think later, it was a couple of months later, you sent me an email, okay, we've tried to express it in yeast, and it was banging out.

I don't know if you did that, Dora [Raventos], or if it was another. But that was, one was a discovery of the molecule, and the other was it could be expressed easily in our normal industrial host, which at that time was *Aspergillus* oryzae[41], but also yeast. So, I think those two things together, that it was active, and I don't know when you tested the activity of it, shortly after expressing it, I would imagine. And then it could be expressed in a good quantity. I was joking, you could use it for floor polish, right, because it could produce it to such a high level.

Leonardo De Maria: It is fair to say that Kirk [Schnorr] invented that technology that could essentially, you know, fetch all the secreted proteins that an organism could produce, right, because that was kind of the interesting part of Novozymes, right when you produce this in big quantities, it's important that they are out of the cells because the first step in the big production tanks is filtering out cells from the broth. And that already, if you have things that are secreted, this is important.

So, Kirk [Schnorr] invented that, it's a technology that was used pretty actively until genome sequencing came about, right, and that's a different thing.

And I also remember that Plectasin had a very interesting structure because it had a signal peptide, yes, and it had the active peptide[42] at the end, but there was, in the middle, there was a pro-peptide[43], right, and that combination of signal peptide and

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[40] Task transposon-assisted signal trapping.
[41] *Aspergillus oryzae* known as kōji mold is a filamentous fungus (a mold) used for expression of peptides (24).
[42] The active peptide is the segment that binds to other molecules (such as substrates, inhibitors, or receptors) and carries out the protein's primary function (25).
[43] A propeptide is a segment of a protein that is present in the precursor form of the protein but is usually removed during post-translational processing to yield the mature, active form of the protein (25).

pro-peptide has been used to express other things, and it's quite powerful, so you can get things expressed.

Per H. Mygind: I remember it was also quite early days for bioinformatics, right, so there were not that many hits; I would assume if Kirk [Schnorr] took the same sequence now and made the same search as he did at that point in time, I'm sure the earth would have been exploding with sequences like this.

At that point in time, it was unique, but suddenly it was hitting something. I recall it was very, you know...

Leonardo De Maria: And again, this was our first paper, right? It was the first fungal defensin that was characterized because Kirk [Schnorr] found that it had similarities to other defensins, but they were not fungi. So that was very...

Per H. Mygind: You said similarity to an insect defensin, I don't know what ever happened to that one, but that was the catch, that was the hinch.

Kirk M. Schnorr: Yes, it was a dragonfly defensin[44]; it was a hit in the database; what happened to the dragonfly, who knows, because it couldn't be expressed properly, or yes, prominently, or...No, I don't know if anyone...We've expressed some defensins from insects later because I had projects later on mealworms and some other insects, carpet beetles, and such, so they didn't express as well; it was still the fungal ones, expressed in fungi that are a good combination, I think.

Hans-Georg Sahl: There was later also a peptide called Eurocin (15), I remember.

Leonardo De Maria: Yes.

Hans-Georg Sahl: It came also from this approach.

Leonardo De Maria: Yes, I mean; I clearly remember after the first hit, Per [Mygind], Dora [Raventos] and me really blasting the Novozymes databases and discovering new potential molecules, really on a, probably every couple of weeks there was something coming, because we knew already that, what was the structure, right, so a signal peptide, this pro-peptide, and it had a very clear, you know, di basic, you know, positive charges, where the protease will cleave, so we, and there were these fingerprints, and then once we found these things, we said, ah, there's yet another one that could be...

[44] Dragonfly defensin is a type of antimicrobial peptide found in dragonflies, known for its ability to target bacteria, fungi, and viruses (26).

Kirk M. Schnorr: And the scaffolds[45], concern among defensins, also across mammals.

Leonardo De Maria: Yes, the, also, these molecules, they are very small, right, but they are, they have a three-dimensional structure, and computational chemistry. And the interesting thing is they are so small that they cannot have what bigger proteins have, which is hydrophobic residues inside, so they compensate that by having three disulfide bridges, right, and those also were kind of a fingerprint. They were rich in cysteines that then were linked together and made this super stable structure, you know, you could boil Plectasin, and it survived. One way of purifying Plectasin was boiling, right? Everything else went, and Plectasin stayed.

Eefjan Breukink: Just out of interest, how high is the expression in yeast, in milligrams per liter?

Dorotea Raventos: I have no idea. I don't remember.

Hans Henrik Kristensen: In yeast? No. I don't remember either.

Dorotea Raventos: But I think there are papers now, are there not? China, I think, is expressing it in yeast (16).

Laura Daniela Martinenghi: In yeast and other microorganisms.

Hans Henrik Kristensen: Yes, maybe we'll get into that later, but expressing it in our...Novozymes, expression strains, and *Aspergillus oryzae* were really the key because it was massive amounts. I don't know if we are allowed to say how much, but...

Dorotea Raventos: But it was not milligrams.

Hans Henrik Kristensen: No, no, and it was orders of magnitude higher than it has ever been made in various yeast systems. Much higher than E. coli and […]. And again, industrial enzymes that have to substitute chemicals have to be dirt cheap. So that's sort of what had been developed throughout many years.

Leonardo De Maria: It's very important, you know, these antimicrobial peptides can kill the expression host very easily because that's what they do, but since they were from fungal origin, they were somehow... and this is a gram-positive mostly, right? Plectasin. So, it was not harmful to itself. So that's why you could get loads of these very lethal molecules to gram-positive bacteria that didn't do anything to

[45] In molecular biology and biochemistry, "scaffolds" refer to structural frameworks within molecules, particularly proteins and peptides, that give them a specific three-dimensional shape and functionality.

the fungi. So, you could express. Because sometimes when you are making...when you are using *E. coli* to express everything, you know, everything cannot be expressed in *E. coli* because it kills *E. coli*. So...

Eefjan Breukink: We do express it in *E. coli*.

Leonardo De Maria: But in inclusion bodies, so it stays inside. Not folded. Yes.

Eefjan Breukink: Not folded, yes. Correctly folded. In the cytosol.

Laura Daniela Martinenghi: But now Eefjan, because you are not part of the discovery team, but you are part of the present projects.

Eefjan Breukink: Yes, I'm now working on the mode of action.

Laura Daniela Martinenghi: Yes, do you want to introduce us a little bit about it?

Eefjan Breukink: Oh yes, so...At a certain point in time...So, I'm working on the lipid-II story, the lipid-II system itself. And I think if you have one good target, then that's lipid-II for antibiotics. Because it's essential and unique, of course, but it's also not a protein. And in my view, that makes it extra good. So, in principle, I'm interested in any kind of target that...any kind of molecule that interacts with lipid-II. And the Plectasin mode of action was, let's say...I wouldn't say it solved, but because it was studied in micellar systems. And we work with more with...biological, more relevant bilayer systems and intact cells. And so, with the coliform NMR department, we have now, in the past 7-8 years, we have looked into the interaction of Plectasin with lipid-II in bilayer systems.

And, well, we discovered one that...I've been telling that to Leonardo [De Maria]. It's cation-dependent. So, it's a bind calcium with high affinity, which is logical if you look at the structure. It aggregates upon interaction with lipid-II. It actually also can form pores, but only in certain membrane systems. So, I wouldn't say that we solved the structure of the complex because that's even more difficult. But we've come quite far. And, like I said, we hope to publish it soon in a nice journal. And, well, I can talk more about it, but that comes into more detail.

Laura Daniela Martinenghi: There …we have now an update on Plectasin…

Eefjan Breukink: So, yes, and besides that, of course, we're also working on trying to improve Plectasin. And I don't think you can improve Plectasin much if you just use normal amino acids. So, we're looking into ways to chemically synthesize it and correctly fold it and then improve it from there. Using chemically modeled, modified amino acids.

Hans Henrik Kristensen: I'm sure Dora [Raventos] would disagree. She can do anything.

Dorotea Raventos: What do you need to improve? Wonderful molecule.

Eefjan Breukink: I'm not saying it's not wonderful, but it can be even more wonderful, in my opinion.

Per H. Mygind: Yes, so I guess these variants that we screened out were never, you know, distributed outside. I'm just curious whether some of these have been, you know, evaluated for target affinity or if it's something else that we screened out. If it's some other part of the mechanism that we improved.

Hans Henrik Kristensen: I don't think they went out for and said NZ2114[46] didn't go out for anything that animal studies and in vitro MIC[47] tests, as far as I remember. But I don't... maybe...Yes, we made a lot of mutations, but if we sent and said NZ2114 off to Hans-Georg [Sahl] to confirm the mode of action, I don't remember, because the mode of action work was done on the wild type, which preceded any variant by some years.

Dorotea Raventos: But we had a paper now, a couple of years ago, with one of the variants. It was a PhD student, remember Per [Mygind]? But I don't remember which variant was that. I don't think it was 2114; it was another variant.

Eefjan Breukink: Well, we have been working with NZ because it's supposed to be better active against the *Staphylococcus aureus*. But we can't really determine why. So, it has the same affinities for most of the lipid-II variants that we can make.

Hans Henrik Kristensen: But it has a charge increase, so wild-type Plectasin is not very positively charged.

Eefjan Breukink: Yes, I know, but we looked into, does it bind better or not, but … it is not detectable, better binding to bacterial cells. So, it's still a puzzle.

Hans Henrik Kristensen: But I guess we consider maybe a local higher concentration at the negatively charged membrane, since it was more cationic. That would have brought it closer or a higher concentration to the target lipid-II. But I think that's outside of my comfort zone.

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[46] NZ2114 was developed as an optimized version of Plectasin with enhanced antimicrobial properties.
[47] Minimal Inhibitory Concentration.

Laura Daniela Martinenghi: Coming back, how did you get into the group? I haven't heard from Oliver; how did you get invited into the group?

Olivier Taboureau: Yes, Olivier speaking. So, actually, I came, as I said, for a postdoc position, and it was more on the protein design. I'm a computational chemist at DTU[48] and during my PhD, I was working more on small molecules and trying to use some machine learning methods to optimize the activity of the small molecules to a protein target, whatever it is. And when I was discussing with Torben [Torben Vedel Borchert], at this time, and Alan Svendsen[49]. They said, OK, maybe we could apply this technology protein, try to optimize the stability and the activity, make some mutations, and use some computational tools.

And so, when I arrived here, it was essentially working on this border, from Novozymes, and it was not working so well. And then, I mean, and Cedric came with this project and said, OK, maybe you could help us to try your tools on peptides and see if you could optimize the activity by making some mutations. And we started with another enzyme, which was Novospirin[50], because we have some activity on it as well, and we said, OK, we have a set of Novospirin analogs with some mutations, and could you tell us if we can still improve the activity or the stability?

And actually, yes, it was working quite well. And I think Dora did some tests, and there was some mutation that was designed by the tool, and it was quite good, and then they said, well if you can do it with this peptide, try it with the Plectasin. And that's how I arrived at this project, trying to propose some mutations and some of them, I'm not telling which ones, I don't know if we have a lot of notes, but some of them were also quite interesting because we were still interested in activity, but also stability. And so, we proposed some mutations that would help with that. And it was working well, and that's how I arrived at the project.

I think it was one good thing I really liked in Novozymes and working in Torben's group [Torben Vedel Borchert], that you have a project to work on, but then you have some time also to work on something else. And it gave us some freedom. OK, you are working on other Plectasin things, specific enzymes, do your job on that, and if you have time, then you are welcome to work with the other colleagues from Novozymes, and so that's how we could work together, because we had this freedom also to be complementary on the specific projects, and not being from the same department. I mean Kirk was in another department, where they specifically

[48] Technical University of Denmark. https://www.dtu.dk.
[49] https://www.linkedin.com/in/allan-svendsen-8341a311/.
[50] Novospirin G (10) is a synthetic alpha-helical antimicrobial peptide designed in an effort to develop alternative treatments against *Chlamydia trachomatis* and other bacteria associated with bacterial vaginosis (27).

worked on bacterial peptides, and I was not, Leonardo as well, but still, we could use some time, our time, for something else.

Per H. Mygind: With your big computers and big screens, right?

Olivier Taboureau: That I don't remember. And it was really an excellent work, and I really appreciate it.

Block 2: Challenges in the Plectasin story

Laura Daniela Martinenghi: The last, Leonardo, how did you get in contact with the group?

Leonardo De Maria: Okay, so I actually applied to Novozymes twice. Once I applied for the postdoc position you applied for, Olivier.

Olivier Taboureau: I'm sorry for that.

Leonardo De Maria: I didn't get it. But then there was a permanent position the year after, and I applied to that, and I got that. I'm sorry for that [referring to Olivier]. And that was 2002. And when I arrived at Novozymes, I arrived to do computational support for enzyme engineering. I remember at my interview, I already met Dora [Raventos] and we started speaking Spanish. I was born and grew up in Colombia, and then she's Catalan, but she speaks Castellano as well.

Dorotea Raventos: With a strong accent.

Leonardo De Maria: So, and as Olivier [Taboureau] says, there was always space to work on other things on the project. So, I was allocated to enzyme engineering projects for different industries. So, they were there in 2002. We were all part of this enzyme engineering unit that was headed by Torben Borchert [Torben Vedel Borchert], that Hans Henrik [Kristensen] mentioned before. We were all in this very primitive setup. It was some prefabs, like the C2 building, remember? So, it was a prefab. And we were all together, also physically together. So, it was the enzyme engineering people doing wet lab, the small computational team, and then the team doing antimicrobial peptides. And there was also a small team doing expression, optimizing hosts for expression in fungi. We were all there, right? That was the building.

So, there was also a lot of talk and all that. So that's how I got it. It was 2002, and the group already existed. And you could see, at some point, you guys were not anymore on temporary money, but you became kind of permanent team, right?

Dorotea Raventos: It took some time.

Per H. Mygind: Yes.

Leonardo De Maria: And then hire more people. You hired Dorthe Sandvang[51], right? [question to HH Kristensen] To do the microbiology. So, they built a class three…

[51] Microbiologist at Novozymes 2000-2005. https://www.linkedin.com/in/dorthe-sandvang-78708a41/.

Dorotea Raventos: No, class two lab to try on.[52]

Leonardo De Maria: Class two lab to try on. So, it was really going...We all had the impression that it was there to stay, right? There were always some kind of questions, you know, periodic. But the team became established and was growing. So, it was...Yes.

Laura Daniela Martinenghi: Hans-Georg, can you hear me?

Hans-Georg Sahl [via zoom]: Yes.

Laura Daniela Martinenghi: Okay, now it's good. It's a mic, it's different.

Hans-Georg Sahl: Yes, I can hear you better also.

Leonardo De Maria: We hear you loud and clear, all of us.

Laura Daniela Martinenghi: When you came across Plectasin, what was your thought? I mean, you're an expert in peptides as well. What were your thoughts about Plectasin or a new peptide, a fungal peptide?

Hans-Georg Sahl: We were very positive. In those days, we were very positive about antimicrobial peptides and their potential. So, when Hans Henrik gave us Plectasin for mode of action studies, we were very enthusiastic about it. And particularly since it turned out to be a specific antibiotic in the sense that it would really have a specific target. Because the general view of antimicrobial peptides was that they would rather unspecifically destroy bacterial membranes, and that was obviously not true here. So, I think Hans Henrik, it was about right after your Nature paper(12) that you came to us with a peptide for mode of action studies, right?

Hans Henrik Kristensen: Mm-hmm (*consenting*).

Hans-Georg Sahl: Yes. And it was actually, these studies went very quickly because we had set up most of these assays. At first, we were surprised in a way, but we also had some evidence from a human peptide, the beta-defensin-3[53], that it would do similar things in those ways. So, over time, we got used to the idea that there are defensins out there with very specific activities. And, yes, I don't remember that much, I think, but Hans Henrik [Kristensen], you gave it also to

[52] "Class 2" and "Class 3" labs are terms used to describe classifications of laboratories used for work with genetically modified microorganisms (GMOs).

[53] The human beta-defensin-3 is a broad-spectrum antimicrobial peptide with multiple functions including exerting host defense responses against bacteria, and fungi by binding to and permeabilizing the microbial membrane (28).

Arny Bayer[54], I think, and he did the endocarditis model studies (17), and he was very enthusiastic about it. He said this was the best cohort he'd ever had in this model.

Hans Henrik Kristensen: Yes. That's true. I think I do remember, Hans-Georg, that, of course, you're biased regarding lipid-II because you worked with antibiotics for many years. But initially, you had a hunch that it might also bind, as you say, lipid-II or something like that, to be specific, given its characteristics, and it wasn't very cationic and amphipathic. But initially, you came back and said that actually, Tanja Schneider[55] couldn't make it bind lipid-II[56], so you were a bit disappointed, and maybe you had to look for a different target. And then you had a, or she had a, an eureka moment, whatever it's called, moment and thought that the binding, of course, is stoichiometric. Maybe I'm remembering wrong, but when the right amounts of Plectasin and lipid-II were mixed, then it actually did bind. Something like that.

Hans-Georg Sahl: Yes, it had a 2-in-1 stoichiometry[57]. Right? I must say that I don't remember. We had simply too many of these peptides later on. And they all had all sorts of stoichiometries. And yes, the most recent thing was Daptomycin, which has a stoichiometry of 15-to-1, something like that. So, these lipid-II binders can vary a lot, but I think it was, was it 1-to-1 or 2-to-1, the stoichiometry of these peptides? Because I remember that there was also one of these peptides. Was it Eurocin (15) or was it this peptide from another fungus that had a 2-to-1 stoichiometry?

Hans Henrik Kristensen: Yes, maybe that was part of it. And I don't remember, but as you got the amounts adjusted, it worked out quite fine.

Hans-Georg Sahl: Yes, yes.

Laura Daniela Martinenghi: Great.
But moving forward to challenges on this project, which is the main topic of my research, I should say. We make different questions, but specifically, what were the initial expectations for Plectasin? What was the idea, and how did that expectation develop over time? And yes, how did that evolve in the process?

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[54] Investigator, The Lundquist Institute. Professor of Medicine, David Geffen School of Medicine at UCLA. https://lundquist.org/arnold-bayer-md.
[55] Tanja Schneider was first author of a Plectasin mode of action paper (13).
[56] Hans-Georg Sahl comments here: actually, I do not remember that we had any difficulties about showing Lipid II binding .. and we never were disappointed. these studies went rather straight forward, and there was only discussion about the binding stoichiometry which turned out to be 1:11.
[57] The term "2 in 1 stoichiometry" here refers to a scenario where two moles of one reactant combine with one mole of another reactant.

Hans Henrik Kristensen: Yes, maybe, I can give you the thought. But in, maybe in a normal pharmaceutical company, you would have sort of an indication you want to address. You might have a target group, a specific, it's not indication, such as non-small cell lung cancer or something like that, and then you're working towards that.

We, by chance, were given a gene and a molecule from Kirk [Schnorr], and then we started to explore. So, we had no end goal; we were just seeing, you know, would it be active against fungi, gram-positive, gram-negative? Of course, eventually, we became anti-infective discovery so we had the anti-infective goal, but we were just seeing where did it lead us with that project. It's sort of my impression. And the spectrum initially was streptococci, and one of our proponents from Denmark, Niels Frimodt-Møller[58], from the Statens Serum Institut[59]. He was extremely excited about, oh, I can kill all *Streptococcus* spp.! And tried. They tested all the serotypes, and the MICs were very low, it was an important indication. But then, when you went out into congresses, or investors, or other companies, *Streptococcus* spp, a very narrow gram-positive [in terms of target spectrum], was not that interesting. So at least it has to, it had to include other gram-positives. *Enterococcus* spp. were among the spectrum, but of course, *Staphylococcus* spp., and ideally, multidrug-resistant *Staphylococcus* spp. And the activity there was maybe in the 2 to 8 to 16 μg/ml range, MIC-wise, as far as I remember, so not very impressive.

But initially, that wasn't a concern; then we were just exploring, you know, the potential. Oh, it does kill bacteria! You can add salt, it still kills bacteria, which is a big thing for some of them. We had, again, this: when we would do one animal model of infection, it worked exquisitely. It had a half-life that you could work with; it was stable in serum, and it didn't get degraded as the others. So, it gradually built up and became a molecule with antibiotic potential.

Albeit not the right spectrum, and that's another project in some sense we were into, but especially Dora [Raventos] and her team, engineered (the molecule) , also Per [Mygind]. Yes, yes, engineered the activity to include *Staphylococcus* spp. as well. We did acquire and test a lot of clinically relevant strains that we got from that.

Yes, so I think what sort of made it take off internally was, and we're all very proud of that, is the Nature paper, which is a key part of the journey for the molecule.

And I don't know if I should talk about that, but it sort of came about in some sort of a strange way because we wanted to publish something on Plectasin because we

[58] Niels Frimodt-Møller - Professor, Senior Consultant, MD. Department of Clinical Microbiology, Section 9301. Rigshospitalet. Denmark. https://www.linkedin.com/in/niels-frimodt-møller-67413655/.
[59] Statens Serum Institut prevent and combat infectious diseases and congenital illnesses through research, monitoring, diagnostics, and advice. https://www.ssi.dk.

were very academic, all of us, and, you know, but we were busy, and we didn't have, I don't know where we were targeting.

Per H. Mygind: I remember it was Michael [Zasloff][60] from the US who actually suggested this for a high-ranking paper. We were writing on it, thinking, well, we need to publish it, but we weren't thinking that it was going to be a high-ranking journal. And I think it was Michael [Zasloff] who actually said that, yes, being American.

Leonardo De Maria: But also, I will add that publication of papers was not a priority at Novozymes, right? So, our priority was to support the project, progress, and generate intellectual property rights, which were patents. That's what you should do. And if you had time, right, or if it suited the purpose, it was published.

Now, you know, now I work for AstraZeneca, and the story is completely different. I have in my KPIs[61], publishing papers on this and this impact, and all that. So, it's a different story.

Hans Henrik Kristensen: Yes, but again, our situation was a bit different, because we were, we considered us a biotech, a startup company within a larger company. So, we had, as we heard, extreme academic freedom. We were an academic group, most of us, but in a bigger company with all the support and all the support functions in bioinformatics and screening.

And it was clear early on that Novozymes could not and were not allowed to do clinical development. So, we had to attract partners prior to phase one of the campaign, which is quite important because that's not where there is any value in an antibiotic project. I guess that's true. I would claim that after phase I, where you have shown in vitro and in animal models that it works, it's safe. So that's when, okay, now we believe in it. Of course, you can see signals further along, but that's really where you get, I would claim a significant value increase. And we were not allowed to go there, so we had to attract partners in the preclinical phase to these programs to do alliances or partner them all.

So, we did a lot of business development, we did a lot of going to congresses, also in the northern part of Italy, in order to do that. So, we, getting back to your point, Leonardo [De Maria], so we actually have published quite a number of papers with the people here. So, it was part of it because if we weren't visible, then why would they take us seriously?

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[60] Michael A. Zasloff is an American medical researcher, and entrepreneur. Zasloff is primarily known for his work on eukaryotic antimicrobial peptides, especially the Magainins (see footnote 101). https://www.linkedin.com/in/michael-zasloff-83b26b13/.

[61] KPIs: Key Performance Indicators.

Leonardo De Maria: But you are right, it was more kind of this biotech mindset, we know, show your research, this is what we are doing.

Per H. Mygind: So, I think we were also able to wait with this publication, so we actually had a lot, a substantial finding and research that went into this publication. We were not pushed to publish once you see something.

Leonardo De Maria: I will add that this was so uncommon, this is again Leonardo, that when the paper was published, our head of research at Novozymes, Per Falholt[62], invited everyone to the canteen to drink a glass of champagne and celebrate the publication of the paper. So, it was something important.

Hans Henrik Kristensen: And the joke back there was that the publication we got, or the increasing share price, I don't know, but the publicity we got was much larger with the Nature paper than with the demerger of Novozymes and Novo Nordisk, in which we all lost because we were the orphaned child. Sorry guys.

But maybe it's just a final since it's our moment, so we want to shine a bit.

Dorotea Raventos: But it was when we saw it was working *in vivo*, in the animal models, that then we said, OK, this is really good.

Per H. Mygind: That's serious.

Dorotea Raventos: It is, because before I remember, Per [Mygind] was like, yes, I'm writing, and I was like, Per, just write something. And it was the *in vivo* studies that would say, whoa, now this is good.

Hans Henrik Kristensen: No, that's exactly where - and, of course, we did have this - we did collaborate with what we would say are key opinion leaders. Professor Sahl [Hans-Georg Sahl] online [referring to the participation online in this seminar) is one of them, but we also had two other Mr. antimicrobial peptides, so Bob Lehrer[63] and Michael Zasloff, and they were both on our advisory team together with Niels Frimodt-Møller and at least Michael Zasloff was in Copenhagen at that point, I believe. Something around that, and he was highly enthusiastic about it, as was Bob Lehrer. And Per said, this is it, go for a high-ranking journal. We go Nature, and we sort of, and then we figured, why not? And then I tasked, probably I wasn't very specific, but I tasked Michael Zasloff to start writing on the paper as a consultant, and I tasked Bob Lehrer to write on the paper.

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[62] Per Falholt was the executive director responsible for research and development of Novozymes. Today he is Chief Scientific Officer at 21st.BIO. https://www.linkedin.com/in/per-falholt-2236402/.

[63] Robert Lehrer - Professor of Medicine, Emeritus, UCLA - Innate immunity - Phagocytosis - Antimicrobial peptides and mechanisms. https://www.linkedin.com/in/robert-lehrer-b28bb81b/.

Per H. Mygind: At the same time?

Hans Henrik Kristensen: At the same time. So, it wasn't very smart, but they were so interested that a couple of days later, sitting at home, I got their two versions of a manuscript for Nature, and I just said: how are these old elephants [taking it]? But somehow, I managed to take some from each, and it's a beautiful paper, and not because of us only, but the way these two, the insights they had, and the way they write about the divergence of fungus and insects, or insects and mollusks, 500 million years ago, and it was all in a higher context. And, yes, we couldn't publish the paper, and now I shut up because the *in vivo* data weren't good enough. So Niels Frimodt [Møller] had a sort of standard peritonitis model[64], where he is seeing a reduction in bacteria and CFUs in the peritoneal fluid, as far as I remember. And it's sort of a surrogate model, and it's not really a systemic infection, so Nature immediately said, you have to do survival studies in mice. And, you know, being who we were, I just called up Niels [Frimodt-Møller] and said, okay, go ahead, do that.

And it almost got me fired because, you know, Novozymes, of course had a highly ethical standard, and they had veterinary people, and you don't do survival studies. It's not ethical, and little did I know. So, yes, I was sort of hanging on at some point, talking to Torben, but he let me stay on. Probably not too much came out [within Novozymes], but the survival study was approved internally because the mice were euthanized, as you would do before they succumb to the infection. And then Nature took the paper, and, yes.

Laura Daniela Martinenghi: Personally, in each department, which challenges were in this Plectasin project? Each department is different, from bioinformatics and computer modeling to microbiology. What were the main challenges for Plectasin -that you can recall?

Leonardo De Maria: I recall one, I can tell. So, the structure was solved by one of our colleagues at Novo Nordisk, who later became my boss when I went to Novo Nordisk, Sven Ludvigsen[65]. And I remember when I got the first set of coordinates from Sven, I looked at it and the connectivity of the disulphide sites, so the way he connected the cysteins was not what it was in all the other defensins. And there were two options. Either this was new, or it was wrong. So then I didn't know Sven at the time, and probably I wouldn't have done it because he took very much pride in his work, but I ran simulations on both alternatives of the connectivity,

[64] A peritonitis model refers to a controlled experimental setup, often using laboratory animals, designed to replicate the pathophysiological conditions of peritonitis, an inflammation of the peritoneum. This model is crucial for studying the disease's progression, and underlying mechanisms, and evaluating potential therapeutic interventions (29).
[65] Sven Ludvigsen was the Project Director and later Vice President of Novo Nordisk. https://www.linkedin.com/in/svend-ludvigsen/.

and the one he was suggesting, the protein broke on the computer, it went all over the place. Meanwhile, with the one that was aligned with all the other defensins, the protein stayed as it should.

So, then I went to Sven and said, Sven, would you mind reassigning your connectivities? And he did, and I think that was one of the challenges that was there, and then that was how it was solved. It's minor stuff, but it could have been an odd thing if it was completely different than the others and not really right.

Per H. Mygind: I would say, and that's most of the credit of Dora [Ravetos], I think that the screening, setting up the screening to improve the pharmaceutical properties of Plectasin, by making colonies and overlaying that with something with a strain that was interesting from a clinical point of view. Setting that up was, I don't know if it was a challenge, but it was a lot of work.

Dorotea Raventos: One of them.

Laura Daniela Martinenghi: Any more challenges? Toxicity, for instance?

Per H. Mygind: Not with this molecule, I would say. It was very well-behaved

Kirk M. Schnorr: This is Kirk. I'm not qualified to comment on what I'm about to say, but someone is, I'm sure, and that was something about GMP-approved compounds in an industrial enzyme-producing company that didn't have much experience in that and also producing the molecule in *Aspergillus*, which has a glycosylation[66] and heterologous glycosylation[67].

Per H. Mygind: It's not approved as a GMP[68] strain, right?

Kirk M. Schnorr: No.

Per H. Mygind: It's not being used, so if you were to introduce that at the same time, it would be a challenge, I guess.

Kirk M. Schnorr: But I seem to remember that there were some issues or questions about using *Aspergillus* as a production host because that wasn't common practice.

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[66] Glycosylation is a biochemical process where a carbohydrate is attached to a protein or lipid (30).
[67] Heterologous Glycosylation refers to the glycosylation of proteins in a system different from their native source. This is often done in the production of therapeutic proteins where a protein from one species is produced in a different host organism (30).
[68] A "GMP strain" typically refers to a microorganism or cell line used for the production of pharmaceuticals in compliance with Good Manufacturing Practice (GMP) guidelines.

Per H. Mygind: No. I guess it still isn't.

Kirk M. Schnorr: I'm not qualified to know.

Hans Henrik Kristensen: You're right, Kirk [Schnorr]. It was a challenge. It was one of many, and I don't know what we should start with. But of course, Novozymes is a company, a production company, and they managed to set up GMP systems for that when that was required. They managed to produce it in sort of a medium scale, maybe 2, 3, 4, 5 cubic meter scale, and actually make 3 to 4 kilos, I believe, of what was in NZ2114, the clinical variant. And maybe it's still sitting somewhere. So, it was all done, and with the approval, because we made a very nice agreement, our licensing agreement with Sanofi Aventis[69] at some point, to their standards. And they did a very thorough due diligence.

We had a large organization. The department had 20 people when it peaked, and I'm counting everyone in. So, veterinarians and protein chemists, of course, did the work here. Yes, and again, a lot of the things were done externally. So, we had large strain collections tested, also to validate the results, so it's not just MICs we generated under our own conditions, but the industry standard. The same goes for receptor interactions and receptor bindings. There's a lot of preclinical safety studies you need to do. A lot of animal studies were performed externally as well, most of them, and of course, efficacy studies, which could be done by, as Hans-Georg mentioned, Arny Bayer from UCLA[70]. He was in LA.

Hans-Georg Sahl: UCLA.

Hans Henrik Kristensen: Yes, and he was sort of the expert on endocarditis models in rabbits. So, we had many of these in vivo infection models tested with the best people, again, to stamp or validate the data externally and again for external partners. Yes, PK [pharmacokinetics] and PD [pharmacodynamics] papers and studies were done with the experts. Now, his name slips me, but in, yes, somewhere in the U.S. That was all part of the business model.
Was that a challenge? No.

Laura Daniela Martinenghi: So, you mentioned Sanofi. What happened? What is the official [version]?

Hans Henrik Kristensen: Yes.

Laura Daniela Martinenghi: Why did they drop that?

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[69] Sanofi Aventis is a global biopharmaceutical company focused on healthcare solutions. https://www.sanofi.com.
[70] UCLA: University of California. https://www.ucla.edu.

Hans Henrik Kristensen: Yes, so probably for two reasons. Again, we were dating them, and we had business development people.

Laura Daniela Martinenghi: Dating?

Hans Henrik Kristensen: Yes, we were pharma-dating them for quite some time, and they became quite interested. And again, before that, we got project management support in the team, so all of our studies were meticulously cataloged and arranged. But eventually, they got interested. They did due diligence for a couple of days, but they came to Bagsværd in Copenhagen and stayed on site, and they were extremely impressed by everything.

Again, not just because of us … but …

Laura Daniela Martinenghi: The results?

Hans Henrik Kristensen: … but with the professional project management people that had come in. So, it was really – it became organized to a whole new level compared to when, of course, when we started. We produced the material for them. They wanted to validate key studies, I guess, and sort of, as I remember, they had a new CEO coming in, and he was not very interested. I think he cut like one-third of all the projects that they had running, and this project was one of them.

And there was a specific safety signal, and I guess that's what I can say, that we observed, but in my opinion, being very pro-Plectasin was not something that you wouldn't evaluate further. But it becomes important later on in the faith of Plectasin because it had that, and that was sort of the only negative thing we had ever seen about this wonder molecule. So, I think it was a strategic change and then a concern, and Sanofi has – and maybe Hans-Georg [Sahl] or you, if you remember – I don't know if it's Ketek[71], but they had an antibiotic on the market which gave kidney issues and actually resulted in people dying. So, they had something – they had a history with the safety of antibiotics, and it might not be Ketek, but something that could also have concerned them. So that's the reason why they gave it up. We got it back, and I don't know if you were still on board or you were –

Per H. Mygind: No, I left it.

Hans Henrik Kristensen: Then we discussed whether we could take it further ourselves, and it was decided not to do that. And it was because we had other molecules; we were looking at defensins, various types of human defensins for anti-inflammatory diseases. We had programs, and when I say we, it's in

[71] Ketek: the antibiotic Telithromycin.

Novozymes. And Kirk [Schnorr] had previously, some years earlier, found a very interesting peptide from a sandworm that we also worked on, and it had disulfide-rich elements. It had a very interesting spectrum for gram-negative bacteria, which sort of became commercially interesting at that point. So gram-positive, not so much. We have Daptomycin[72], and there is Linezolid[73], which was – or had been approved. So that became the focus, and then at some point, the patience of Novozymes ran out and said, now we are closing anti-infective discovery, and we are spinning out the program. And they spun out Arenicin[74] (18,19) and the beta-defensins, or whatever human defensins, but not Plectasin as far as I know. And the company became Adenium Biotech[75]. Yes. And we had one colleague, Søren [Neve] join that company. That's it.

Laura Daniela Martinenghi: Your research is the latest; I mean, it's the latest here in Europe, at least *(referring to Eefjan Breukink).*

Eefjan Breukink: I guess.

Laura Daniela Martinenghi: Yes, it is.

Which challenges did you face with Plectasin now? Because we are talking 20 years after, right? Or 15?

Eefjan Breukink: Yes, ¨so when we initially started this project, we thought, well, it should be quite simple to do. Well, now, eight years later, then we should say, well, it's not – it wasn't that simple. So, I remember that at a certain point in time, we were about to publish the Plectasin work we had at that point. But there was this issue with two sets of signals in the NMR[76] because of the probability related to the stoichiometry that we were investigating. And then, yes, so we waited for that. And then, well, actually, the stoichiometry that we get is one-to-one in terms of if you look at the phosphates and the interaction with Plectasin. So, and then there was always something coming along. And there was not a major issue, just all kinds of smaller issues that made it more interesting to look at that and include it in the paper. So, for instance, one of them is the calcium effect. The other one is the aggregation effect on the lipid tube. And then after we had connected to some

[72] Daptomycin: This is a cyclic lipopeptide antibiotic that is targeting Gram-positive bacteria.
[73] Linezolid: This antibiotic belongs to the oxazolidinone class and works by inhibiting bacterial protein synthesis. Linezolid is effective against a variety of Gram-positive bacteria.
[74] Arenicins are a group of antimicrobial peptides targeting Gram-negative bacteria. https://web.archive.org/web/20131029192604/http://adeniumbiotech.com/wp-content/uploads/2011/10/Arenicin-2010-HTS-final2_CAH.pdf.
[75] Adenium Biotech is a Danish biopharmaceutical company spun out from Novozymes A/S in 2011. https://www.crunchbase.com/organization/adenium-biotech-aps.
[76] Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique used in chemistry and biology to study the structure, dynamics, reaction state, and chemical environment of molecules.

– the group in Groningen who does high-speed AFM[77], and we also wanted to include that with Plectasin. Now, there were issues with affinities that we could not really explain with ITC[78], so we had to redo it with another method. Finally, it came to one paper and maybe another paper more specific on NZ, the number I always forget [NZ 2114].

But what's more challenging now? The biggest challenge we have is coming from the total chemical synthesis of the peptide. That's – even though it has been described in the literature that you have to treat the disulfide bonds specifically, so you can first make the other one and then the other and the other. If we try to repeat that in our hands, it doesn't really work that nicely. At least not – we don't get enough peptides to start playing around with. So, that's the challenge that we face now.

Laura Daniela Martinenghi: Being part of this big pharma company, did you feel like Plectasin has gotten a fair chance? Being part of Novozymes, this group, this academic group inside of Novozymes, do you think that Plectasin itself had a fair chance back then?

Hans Henrik Kristensen: … maybe we haven't heard from you [referring to Dora Raventos].

Per H. Mygind: Can I say something?

Laura Daniela Martinenghi: Yes.

Per H. Mygind: I think Plectasin had great promises. I think the real issue is the business model for biotech. That's the reason why I'm progressing into a clinical phase. Because it needs to be financed in a different way than what you would normally do for a commercial drug candidate, in my opinion, because of the market potential it will have when it finally gets approved.

Laura Daniela Martinenghi: What do you think about that?

Eefjan Breukink: Isn't every antibiotic development now decided by money or not?

Per H. Mygind: And that's why it's not there.

[77] Atomic Force Microscopy.
[78] Isothermal Titration Calorimetry.

Hans Henrik Kristensen: But maybe going a bit in theory because I want to hear from you. But I think from a Novozymes perspective, taking a step back, we got a lot of freedom. But it is inside a production company.

But the products can be from the inception of the idea to being on the market, making a difference, earning money. It is maybe, we say, 15 years, maybe that's speeding it up. But the horizon is very different. And, of course, that's the main business. So, we felt often that we were de-prioritized much less because of the glow that the partner had and the interest in the project. So, it was easy to convince Kirk, Leonardo, and Olivier to work with us. But they had a natural interest. The same goes for purification, larger scale, and strain generation from other departments.

Laura Daniela Martinenghi: Jørgen?

Jørgen Leisner: I just have a question afterward.

Hans Henrik Kristensen: But we did feel deprioritized to some degree. So that was inhibiting. And I think to try and counteract that; we had an idea when we peaked with this 20 people department. That we would have all core competencies. So, we tested, we had library generation and screening, and we had purification on a small scale. Large-scale would be somewhere else. We had our own veterinarians; we had our own strain collections. So we could do all the initial work internally, being independent. And I think the only drawback to that setup was that we would be very sensitive if someone would leave us. If […] said, no, I'm going somewhere else. Then, we had a hole that we would need to replace. But people didn't leave in those days. So, I think it worked out as we had anticipated. And it allowed us to get things done.

And then, when we needed large-scale production, we would go to that department. But over time, it became more organized.

Leonardo De Maria: I'll add a comment before your question. I think one thing that you said, Hans Henrik, is really spot on, which is the time scale to get a product to market. And if you are into this pharma space, the times are longer. And what we could argue now, in hindsight, is that it was a slow process to build a team. Also, to build special labs and all that. And once they were ready to essentially fill the pipeline, Plectasin was one molecule. But at the time, we had found a dozen more. And there was space to really do research on many, many more. Remember that microorganisms are fighting each other with these chemical weapons. And that's what we were looking at. We were looking at enzymes, but there were other things there. But you could argue in hindsight, and I'm not in Novozymes anymore, so I can criticize more openly. Maybe it was a lack of patience. Maybe it was, okay, now we have this group. We have invested so much

time and energy in building it. Okay, we give you five years, ten years. Do some business development. It started with an eureka moment. Oh, this might be something interesting. But now you can do it backward. Where is the market? Where are the things that will bring money? You can prioritize high-reward projects first. So anyway, these things, you guys were ready to do that. And then, all of a sudden, it's closed. And that was also, in the big perspective, Novozymes started to do other things. Novozymes' [whim] was, we are good at fermenting, so let's go into pharma. Novozymes had also some sort of, you know, the hyaluronic acid experience, which was also a lot into this pharma space, but ended up in nothing, right? With a lot of investment. So, I think it was kind of a moment when Novozymes wanted to try things, and all of a sudden, it became a bit too difficult, too long-term, and said, oops, let's focus [on] where we are.

Per H. Mygind: Human serum albumin.

Leonardo De Maria: Human serum albumin. Novozymes purchased a company doing human serum albumin for excipients. So, it was really an interesting moment, right? Because we have these core competencies as a company. I think that was the thought of Novozymes. What else can we do that is not industrial, and all these things came about? But they are different. They have different complications. And whatever has to do with humans takes a longer time, right? And it has a higher risk.

Laura Daniela Martinenghi: So, Olivier?

Olivier Taboureau: It was exactly what you were mentioning. The feeling I have is the same. It was a protein engineering company. And so, they want to have something. The impression I have is that they want something in return rapidly. And even if we have some freedom to work on different projects. In the project, it was six months a year max. To have better enzymes, better stability, or better activity. And then we go for something else. And as Leonardo said, it's not working like that when we are talking about human health and finding drugs. It's not working for six months a year.

Per H. Mygind: So the whole machine was not set up to support that.

Olivier Taboureau: And I think they were not ready for waiting [referring to Sanofi] as much at Novozymes. Because of all the different products that we were developing in protein engineering, it was six months to a year. Max two years, but not more.

Leonardo De Maria: But also remember all those products. I mean, Novozymes is business to business, right? So, no one knows about Novozymes, but everyone has used Novozymes products. Either directly because you wash your clothes or

you wash your dishes, right? But also, indirectly, because you wear clothes that are processed with enzymes that Novozymes make. And you eat bread that is processed with enzymes that Novozymes makes, and so on and so forth, right? So, in business-to-business, you need a partner to develop products, right? And this antimicrobial peptide initiative started without a partner. And all of a sudden, after a lot of work, because what Hans Henrik Kristensen describes very succinctly, it's a lot of PR work going out and saying, look, look, look, a big partner comes, Sanofi, says, we are interested. I still remember the Sanofi people super smartly dressed, going to the 2C what, at the time, became a fancy building. They were, you know, in two days looking at these things that you prepared, right? All the printouts. And they said yes. Then, this big partner, after some time, says no. Right. So, then I think that also probably put a, because you, in business to business, you need a partner to co-develop your products.

Laura Daniela Martinenghi: Jørgen?

Hans-Georg Sahl: Maybe I can add.

Laura Daniela Martinenghi: Yes.

Hans-Georg Sahl: Is that okay, Laura?

Laura Daniela Martinenghi: Yes, okay.

Hans-Georg Sahl: I think that you, like they say, did get a fair chance. I cannot comment on what happened to the Novozymes. But when it left your hand, and it was at Sanofi, I had interactions with Sanofi in those days. And I knew one of those people who were concerned with it, Peter Hermann[79]. Do you remember him?

Hans Henrik Kristensen: Yes.

Hans-Georg Sahl: He talked about Plectasin a couple of times since he knew that he had been working on it and so on. My feeling was that it did not get a fair chance there because of the general skepticism about cationic antimicrobial peptides in the industry. I remember 15 years back, we did get Mersacidin (20) from Sanofi[80]. In those days, they were still hooks, I think. And they gave us Mersacidin and said, here is a peptide. Check what it does. If it is one of those disrupting peptides, forget about it. They had a fairly negative attitude towards that. And that was basically because everybody believed these peptides do cationic.

[79] Peter Hermann is identified as a sales manager at Sanofi.

[80] Mersacidin was from Hoechst which was a German chemical corporation founded in 1863. It became part of I.G. Farben in 1925 but was separated after World War II in 1951. In 1999, the company merged with Rhone-Poulenc and is now a part of Sanofi. https://www.sanofi.de/de.

They are, first of all, nephrotoxic, ototoxic, and so on. That is something that happens very often with these things. And, of course, it is unspecific. They don't have specific targets. And we could show them that Mersacidin didn't do that. But later on, with Plectasin, it was a similar thing. It had a specific target. But still, when it came to the higher-ups in the company, they would remember that general skepticism. My feeling was, talking to Peter Hermann, that that played a role. He was never specific about it. But my feeling was it played a role.

Hans Henrik Kristensen: Interesting.

Laura Daniela Martinenghi: Jørgen?

Jørgen Leisner: I just wondered if the issue of resistance played any role. I don't know how easily a target could be resistant. But I remember at that time, there was this guy Graham Bell[81] in Canada who raised the issue of resistance. So, I am just curious, did that play any role? Or it was not important?

Per H. Mygind: Can I comment on that? Because we actually did a study trying to challenge the chance of getting a resistant strain. And it turned out to be very hard to get a strain that was resistant to Plectasin. Far harder than many other antibiotics. It was taking a big bottle, you can say, of microorganisms and adding Plectasin in that and screening for whether there were any survivors there. It was a natural mutation all the time. And it was really, really, really hard. I think it was 1 in 10, in the 12 or something. So, we did do at least some initial assessment to see if it was too easy. And it could have been an issue.

Hans Henrik Kristensen: And we did overlap and also had the discussion internally if there would be any cross-resistance to our own antimicrobial types, defensins. And we also worked on all types of initial antimicrobial types like LL37[82], 39, or something like that. Some were human-derived. And, of course, the question would come up, is it really, given that antibiotic resistance always develops, is it really wise to use our own defensins in a more indiscriminate way? And certainly, that became an argument initially when we looked at industrial applications. You don't want to use your own defensins put it into washing powder or soap or toothpaste. So, it was considered, yes.

Per H. Mygind: I don't know if it was for a driver, though. I mean because there was this increasing antibiotic resistance. And, of course, it gave some…

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[81] Graham Bell, a professor in biology at McGill University (https://www.mcgill.ca/biology/graham-bell). The comment refers to a paper with Bell as a co-author: (39).

[82] LL-37 is a well-known antimicrobial peptide, which is a crucial component of the innate immune response in humans (31).

Hans Henrik Kristensen: I think it became an argument for Plectasin, for instance, that it is very specific. It has a completely different mode of action. It's not membrane-active. It targets something specifically and gives you [something], which we don't have. So, if resistance occurs, then it's not an issue for us in that sense. I think for the use of Plectasin, it is, but not for us in general, if it makes sense. So, it was discussed, and probably you being experts, Hans-Georg [Sahl]and Eefjan [Breunik] and you are aware of these discussions also.

Hans-Georg Sahl: Pardon me?

Laura Daniela Martinenghi: Discussion about the resistance of Plectasin or resistance of peptides or antibiotics. It could have been an issue for the development, but not in this case. Apparently, Plectasin doesn't have a higher rate for selection of resistant mutants.

Leonardo De Maria: I think that's the fundamental difference, right? So, this is not a small organic molecule that inhibits an enzyme in the bacteria. And the bacteria can engineer mutations that will still allow the enzyme to work but will circumvent this small molecule that you are putting there. Plectasin binds, as Professor Sahl is saying, to lipid-II, which is not an enzyme; it's part of the cell membrane. You cannot mutate that, right?

Per H. Mygind: But you still get vancomycin[83] resistance.

Leonardo De Maria: But how is that arising? They modify the lipid-II somehow?

Eejfjan Breunik: No, they modify lipid-II. Lipid-II is modifiable at the level of the pentapeptide. The high vancomycin resistance comes from a D-Ala-D-Lac conversion[84]. The C-terminal D-Ala is changed into a D-Lac. That gives vancomycin a factor of 1000-fold less affinity for lipid-II. That's a major issue. So, the Plectasin doesn't really bind?

Hans-Georg Sahl: The target binding site for Plectasin cannot be changed. This is a universal setup, basically. But what you can get is that vancomycin insensitivity phenotype called VISA[85], which is related to making the cell wall less negatively charged. But, this is not a high-level resistance mechanism. This is only a reduction of sensitivity. That's why these strains are called VISA. These things can happen,

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[83] Vancomycin belongs to a class of antibiotics known as glycopeptide antibiotics. This class is characterized by its glycopeptide molecular structure and is particularly effective against Gram-positive bacteria (32).

[84] Vancomycin acts by binding to the terminal d-Ala-d-Ala moiety of the bacterial peptidoglycan thus interfering with normal cell wall growth (33).

[85] VISA: Vancomycin-Intermediate *Staphylococcus aureus*.

and they would also apply to Plectasin. But a general high-level resistance mechanism would not be possible, I think.

Eejfjan Breunik So, what comes out of our study with Plectasin and lipid-II is that if Plectasin binds to lipid-II, we see high immobilization of the pentapeptide up to the level of the fourth amino acid. The fifth D-Ala is flexible. That implies that part of the interaction site is also concerning the peptide, at least up to the third amino acid. So, in our hands, if we look at higher order aggregation of Plectasin-lipid-II complexes, which helps for the affinity and also helps for the activity, then we do not see that if the lysine of the lipid-II is modified. So also implies that at that level the interaction may be important. And there is variability in the pentapeptide, starting from the third amino acid. So, the lysine or the DAP[86] or the amination[87] of the glutamate at the two positions. And there are some variations in affinity. So, no high-level resistance, but you can adapt a little bit to your sensitivity by changing the pentapeptide.

Hans-Georg Sahl: That's perfectly clear. We have published a nice reliable variability of lipid-II and the effect of antimicrobial pentapine. And that's certainly true.

Laura Daniela Martinenghi: Great.

Hans Henrik Kristensen: Maybe a comment if you just ask, but to the commercial model, again Hans Henrik. So, reflecting on what Leonardo said, it sort of didn't make sense, and also from Novozymes' perspective, because we did make a triple digit million-euro deal with, and that has been published, with Sanofi. But that would include royalties if approved; we would be the producer of the material, but we would make money then. So, there would have been a return on investment had it gone forward. And the risk, in some sense, was with Sanofi. So, they would see if they could go through phases I, II, and III and eventually get it on the market. But what I think hit us in some, help me, Dora, but once we had Plectasin out the door, it wasn't ours anymore. So, what then to focus on?

We had the Arenicin program, which we did, but maybe we weren't too focused. Again, it was Gram-negative. We had some antifungal peptides. Are we going into fungi? We had the human defensins, and then it became Crohn's and colitis and other types of inflammatory diseases where we had very little insight. So, in some sense, when you look at it, it wasn't a very professional start-up company. We were just lucky with Plectasin. It's sort of how I think.

[86] DAP refers to Diaminopimelic Acid, a derivative of the amino acid lysine.
[87] Amination is a chemical process that introduces an amine group into an organic molecule.

Dorotea Raventos: No, we were not lucky; we were clever. I'm never saying we were lucky.

Hans Henrik Kristensen: But I had some concerns with the Arenicin, and it was spun out into Adenium [Biotech]. It did reside there with funding for a number of years and didn't make it anywhere. So, yes, it was sort of that setup.

Per H. Mygind: There wasn't a clear business plan.

Hans Henrik Kristensen: No, and we were sort of slow. I sensed this; it's probably organized a bit differently. Right.

Per H. Mygind: So, no one noticed it as well.

Block 3: future perspectives of Plectasin and reflections

Laura Daniela Martinenghi: So, just coming back from the challenges, and it's a little bit of a reflection question. Knowing what you know now, 20 years after, what would you do differently in a business mode, in bioinformatics and microbiological discoveries? What would you do differently from back then? If you said anything different?

Per H. Mygind: I can't find anything.

Christoph Gradmann: Choose a different profession (*joyful tone*).

Laura Daniela Martinenghi: That's a big thing.

Leonardo De Maria: I think probably that's the only thing we will not do, because it was very interesting what we were doing. So, I still find it very interesting. And I don't work with them. I'm now in health care, so we are obsessed about humans. But in Novozymes, there was this window that you were looking at: the diversity of microbiology, the diversity of microorganisms in nature. And that was really very exciting, very interesting to see how microorganisms do the things they do. All the fungi that degrade wood, for example, they have an army of different types of enzymes that need to make sure that you get to the cellulose. I mean, all these things, those were super interesting things, at least for me, when I was there. That's one thing I will not do.

Christoph Gradmann: I didn't think so.

Laura Daniela Martinenghi: But in the process, do you think there were some steps that could have been done better, skipped or that you used too much time on, and maybe today it would have been easier? Or if you compare the research back then to today, with your experience and with the technologists today, how have things in the pipeline changed?

Olivier Taboureau: Olivier speaking, I mean, I think from bioinformatics parts, there is now a lot of progress in automation, in systematization.
I mean, you have a lot of advances now in bioinformatics that's also with the genomics technology where you can more easily get access to data, so find new peptides, modeling also in a systematic way. So, I think we could go faster, at least for some prediction or some explanation about interaction also with different targets that could facilitate now to do the same thing in a faster way from a bioinformatics perspective, I would say.

Hans-Georg Sahl: But I guess it's not really surprising in the sense that you did this work 15 years ago.

Olivier Taboureau: Yes, no, no, but that's...

Hans-Georg Sahl: And there's quite a transition in technologies in the meantime.

Laura Daniela Martinenghi: Would that have changed any results?

Olivier Taboureau: Probably not. I don't think so. But it would have been faster.

Laura Daniela Martinenghi: And what that would have meant for Plectasin if it was faster?

Olivier Taboureau: That they would stop the company sooner and...

Laura Daniela Martinenghi: I don't know, It's a self-reflection on Plectasin. What do you think?

Leonardo De Maria: It's very hard to...

Jørgen Leisner: Maybe I can come with my question after you.

Leonardo De Maria: It's very hard to ... Because technologies change ... I mean, when I arrived at Novozymes, it was 2002, right? And... There was signal trapping, which was what you developed, which allowed, for example, to focus on secreted enzymes. But still, there was a lot of painstaking work, too... If you find that a microorganism is doing an interesting enzyme, go back to the enzyme. There was still a lot of cutting the genome of the microorganism, cloning in *E. coli*, and all that. For probably ten years, I went to see my colleagues doing that. To... Okay, we have found this microorganism that makes an interesting cellulase. We will sequence the genome of the organism. And we get everything, right? In ten years. So, that was a complete change in how to look into this diversity. The same goes for automation; the same goes for cloning. And maybe what would have been different is that, instead of finding one Plectasin, here, we would have found 20. Plectasin or Plectasin lookalikes, right?

And I remember with Dora [Raventos], you know, you remember, we were drawing to get the MICs. At some point, we were just [doing] drawing things, right? And then you have this thing that could take the picture. And then we made an Excel program. And it was very artisanal, right?

Today, we will do it probably. We have the resources and the support. Because again, what Hans Henrik said, it was always not asking for a favor, but the resources were not the resources that the guys in enzyme optimization were using. They had the robots; they had all the support, right?

So, in hindsight, with the resources you have now, you could have found 20 different Plectasins.

Per H. Mygind: But then... They might already be there. And we probably wouldn't have had the time …

Leonardo De Maria: We wouldn't have time to devote so much careful study to the mode of action, for example, because you don't have time. If you have 20 molecules, then you just screen your way through.

So, it's a different mindset. So, we would do different things because technology has changed dramatically. What would that mean for Plectasin? Plectasin wouldn't be the only one. But maybe we will know less about all the others than all that we learn about Plectasin. It's just... Because if you have more things, you know less. Or maybe you have a machine learning model that learns what a Plectasin molecule looks like, and then you cannot even screen but ask the generative model to generate sequences that have antimicrobial...

Dorotea Raventos: Well, you have AlphaFold[88] now. That would help you.

Leonardo De Maria: Yes, that would help not to argue with the NMR scientists. But maybe not.

Hans Henrik Kristensen: Hans Henrik. I think that these reflections become what would you have done today with the tools you have available today. That's probably... Yes, that's one reflection.

I think from my point of view, we probably could have benefited from more structure... As I remember, in the early days, we were all over the place. At least I was. There were so many interesting molecules. We made all sorts of... We patented a lot of different concepts and things. We had a suicide expression system, which we thought was a great screening system. We had quenching domains that would counteract the antimicrobial activity. We were all over the place. None of us had pharmaceutical experience. None of us had any leadership experience. So, someone like us today stepping in and guiding and leading the department would have been great.

But we had fun. I think we had fun but wasted a lot of time. And as we have discussed, it was very opportunistic. It wasn't that we had defined this as our technology platform; this is how we're going to use this, this is the education we're

[88] AlphaFold, developed by DeepMind, is a groundbreaking artificial intelligence program that has made significant advancements in the field of protein folding. https://alphafold.ebi.ac.uk.

going for, and this is when we're getting there. It wasn't like that. Any company now would be like that. But again, that was the way it was set up back then.

And then the final thing, what I regret the most is, sort of, that I didn't let Plectasin go when it formally didn't go with Adenium Biotech[89]. Because I was one of the believers in the molecule, we had this 3-4 kilos GMP material standing that still could be used. But I didn't manage to raise any funding. Novo [Venture][90] had said "no" to Plectasin. They did start a company already with the other assets. So, I personally just gave up, and maybe another person would be more persistent.

I see that recently, and it's now many years after the hyaluronic business was probably closed, but that has found a new life outside of Novozymes with investor funding.

Kirk, would you like to say something?

Kirk M. Schnorr: Not sure, actually.

Dorotea Raventos: It's only the cosmetic.

Hans Henrik Kristensen: Yes, but I just thought as someone managed to take something out of the albumin half-life extension, I guess, did that go to Albumix [Albumedix][91] or something? It might still be … . In principle, we could have done the same. In principle, that would be a regret.

Eefjan Breukink: Is the 3 kilos still there?

Hans Henrik Kristensen: Ask Kirk [Schnorr]. I think it is.

Per H. Mygind: You can find it.

Eefjan Breukink: Can you spare a gram?

Kirk M. Schnorr: I have a new name to contact that might know where the four kilograms are.

Dorotea Raventos: five

Laura Daniela Martinenghi: five kilograms?

Per H. Mygind: I've heard five.

[89] Adenium Biotech dropped the Plectasin program, prioritizing Arenicin development.
[90] Novo Venture - the early venture arm of Novo A/S.
[91] Albumedix is a company focusing on best-in-class albumin-enabled solutions. https://albumedix.com.

Laura Daniela Martinenghi: Can I have a picture?
Jørgen?

Jørgen Leisner: If you look at bacteriocins[92] as antimicrobial peptides (21,22), at that time when you were discovering Plectasin, I think it was still an interest in using them for bio-preservation of food. Now, it appears that there was an interest in using them, maybe as a cell-killing agent. Do you have any thoughts about Plectasin compared to all the others? Does it stand out, or is it one of the many? And if so, how do you see the stakes for all these molecules from now on? Will any of them succeed?

Hans Henrik Kristensen: Hans Henrik again, Hans-Georg, and if you know the field better. But I think bacteriocins...

Hans-Georg Sahl: I did not get all of the questions.

Hans Henrik Kristensen: Please, Hans-Georg.

Eefjan Breukink: We need to repeat the question.

Laura Daniela Martinenghi: You have to speak a bit higher on the question.

Jørgen Leisner: Okay. Yes, so the question is, if you compare Plectasin with all the other antimicrobial peptides, including the one of bacterial origin, does Plectasin stand out? Can you tell us something about the potential success of the new compounds that are being are investigated now?

Hans-Georg Sahl: I didn't really get the question.

Laura Daniela Martinenghi: He's asking on … what is that makes Plectasin stand out in comparison with other bacterial peptides in the pipeline of peptides.

Hans-Georg Sahl: As antimicrobial drugs?

Laura Daniela Martinenghi: Yes.

Hans-Georg Sahl: As I was saying, there is this general skepticism around towards cationic amphiphiles[93] that most of the bacteriocins would be. And if they

[92] Bacteriocins are bacterially produced proteinaceous or peptidic toxins normally targeting related species or genera. Some of them, however, have a wider antibacterial spectrum (22, 34).
[93] Cationic amphiphiles are molecules that have a unique structure comprising of two distinct parts: a hydrophilic (water-attracting) head that is positively charged (cationic), and a hydrophobic (water-repelling) tail (35).

are not cationic, they tend to be calcium-dependent. So, it will become cationic. I mean, Daptomycin told us that it is possible to develop such compounds. But that's clear. It is doable, and it would have been doable with Plectasin as well. I'm very sure.

But of course, as long as there are easier alternatives, companies would go the easy way. And I think chemistry can still do a lot on beta-lactams and beta-lactamase inhibitors. And that is where the field is currently going. There are quite a few new antibiotics coming from that area. And that will save us for a while, and we will do the same.

So basically, the question is it economically feasible to go that way? There is a general problem with new antibiotics, even with those that will work coming from the beta-lactam and beta-lactamase inhibitors field. And this economic aspect is even more critical when it comes to entirely new concepts such as bacteriocins and peptides. But maybe there are other areas where they would be useful. I think bacteriocins, in my view, could be much more easily applied in animal health, for example.

Was that what you were asking?

Laura Daniela Martinenghi: Yes.

Eefjan Breukink: So, if I may add to that, I think the novel antibiotics are still out there. Myself, I have been involved, of course, in the Teixobactin[94] and the Clovibactin[95] work. So, these are molecules that work similarly to Plectasin, have similar effects, and are maybe even smaller. So, if they are better, I don't know. Plectasin is already very good.

I know that there are also people looking at vancomycin, and I know of vancomycin derivatives that outperform vancomycin itself and even other antibiotics. So, there is some development in there. Also, Bacitracin[96] is being looked at and can be modified, so it's even better. And also in alternative ways.

So yes, there are novel bacteriocin-derived peptides that may still make it.

Hans-Georg Sahl: But the focus in the industry is really on Gram-negative, and all the compounds that you mentioned are anti-Gram-positive.

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[94] Teixobactin is a peptid-like secondary metabolite targeting various Gram-positive bacteria that was discovered from a screen of hitherto uncultured bacteria (36).
[95] Clovibactin is an antibiotic that was isolated from an uncultured soil Gram-negative bacterium called *Eleftheria terrae ssp* (37).
[96] Bacitracin is a polypeptide antibiotic targeting various Gram-positive bacteria (38).

Eefjan Breukink: But still, staph [*Staphylococcus* spp.] is a problem.

Hans-Georg Sahl: Of course, you have to think two decades ahead when it comes to resistance. Currently, we have the issue it once was with the MRSA, and then ten years later, it's the Gram-negative that we are now in. There will be solutions, as I was saying, from the beta-lactam field and beta-lactamase inhibitors and these combinations. But what happens in another 10 years? And then the Gram-positives may be the bad boys again. But there is no strategic thinking in the pharmaceutical industry anymore. The business is really it has not as strategic in terms of development as it was in the 70s and 80s. These times are gone, and as long as the market is completely dominated by financial and economic issues, I think it's going to be difficult to develop entirely new compounds and new compound classes and really bring them on the market. Don't forget that there are companies out there, smaller companies who come out with entirely new things. They got the FDA approval[97], and the day they got the approval, they went bankrupt.

It's the economy that dictates basically the development also.

Eefjan Breukink: True. If I talk to companies, they always want to have at least almost pre-clinical trials before they are even interested in the level of toxicity studies, that in vivo efficacy models like what Hans Henrik [Kristensen] has talked about on the Plectasin level. For someone with a group that's focusing on a mode of action and trying to find novel ones, that's tough to get at that stage. You really need more financially heavy partners in that.

Per H. Mygind: You need another level of financing.

Eefjan Breukink: Maybe.

Per H. Mygind: At least that's what Novo [Nordisk] has introduced very recently. Before that, Novo Foundation[98] went into this field, but now Novo is actually also playing a role here. That's more to try and see if there's a path for financing these programs into clinical development. Something like the vaccines that we just experienced. Officially, countries buy in on vaccines. They don't even know whether they're going to need them. They pay them upfront. I guess you could see a similar kind of solution for antimicrobials.

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[97] The U.S. Food and Drug Administration (FDA).

[98] Novo Nordisk Fonden is a Danish commercial foundation that was established in 1989 through the merger of two previous foundations: Novos Fond and Nordisk Insulinlaboratorium. https://novonordiskfonden.dk.

Eefjan Breukink: I heard Dame Sally Davies[99] also mention that they wanted to install that. They have installed that already in England? Kind of a subscription to...

Per H. Mygind: Yes, so they're subscribing to a potential need in the future.

Hans Henrik Kristensen: For vaccines or antibiotics?

Eefjan Breukink: Antibiotics.

Laura Daniela Martinenghi: It's a subscription, so in a way, it's helping the companies.

Eefjan Breukink: They have basic funding, but if the need comes, then they have to produce.

Laura Daniela Martinenghi: Sort of that.

Laura Daniela Martinenghi: Hans-Georg [Sahl], how do you see the fate of peptides in the antimicrobial pipeline? In general.

Hans-Georg Sahl: As I was saying, when it comes to these antimicrobials, peptides, you have the issue that they have the reputation of being potentially toxic, nephrotoxic, specifically nephrotoxicity. I've never seen any data on Plectasin regarding that, Hans Henrik.

Hans Henrik Kristensen: No, it hasn't been made public.

Per H. Mygind: But?

Hans-Georg Sahl: Okay. So, …

Per H. Mygind: How is something going to kill you? We have in Novozymes a very easy answer.

Hans-Georg Sahl: So, this is what frightens people off; we go that way and start programs. And then there's also always the issue of proteolysis and immunogenicity. So, these are issues that need to be addressed in the first place. When it comes to applying these compounds in a systemic way, oral application is a different story. But I think the peptide people, Hans Henrik, you had a lot of considerations about these issues when you did the work ten years ago, didn't you?

[99] Professor Dame Sally Davies GCB DBE FRS FMedSci is the UK Special Envoy on antimicrobial resistance.

Laura Daniela Martinenghi: Regarding the problem of antibiotic resistance that is increasingly growing. In general, do you think that Plectasin or other peptides that have been shelved could have a second chance to be revived?

Eefjan Breukink: So, how long does the patent stay?

Laura Daniela Martinenghi: Isn't it finished?

Eefjan Breukink: So, it has to be another one.

Hans Henrik Kristensen: Or a different model. We were joking that why don't we contact Bill Gates[100] and then have him develop it so he doesn't have to earn any money. He has enough. So, he will pay for the... I have a bit of a socialistic instinct.

Per H. Mygind: I remember...

Hans Henrik Kristensen: It might be Per's idea. But we were considering something like that, yes.

Per H. Mygind: But they are not funding clinical research in that sense?

Hans Henrik Kristensen: No, they don't. It could be anyone who would just do it and make it and not have to make any money on it. But, of course, that's not the business model.

Laura Daniela Martinenghi: No.

Christoph Gradmann: That's not Bill Gates.

Per H. Mygind: No?

Hans-Georg Sahl: I don't know. I think the antimicrobial peptides field would really profit a lot from a success story. And there hasn't been a success story so far, which adds to the reputation in some ways. It started with the Magainin[101] failure, and then it was then Plectasin, which was a big hope, and it didn't come true. So...

My feeling was that Daptomycin, in a way, can be regarded as an antimicrobial, an antimicrobial peptide. Once it is bound with calcium, then it acts like an

[100] Refers to Gates Foundation. https://www.gatesfoundation.org.
[101] Magainins constitute a class of naturally occurring antimicrobial peptides found in the skin of certain frogs and toads.

antimicrobial peptide in the sense that it is attracted by the cell wall, accumulates in the cell wall, and then finds a target. So that could show the way how to develop such compounds. But as I was saying, is that economically feasible? And as long as it is not and it is risky, people wouldn't do it. That's my feeling when I look at the field.

Block 4: Bigger questions and concluding discussion

Laura Daniela Martinenghi: Do you have any questions? No? I have the last few questions.

Laura Daniela Martinenghi: A little bit on the field in general. But the first one is open; I mean, you can answer if you want. I know there are a lot of people working in pharma. Do you feel that big pharma business driving priorities can sometimes be in disagreement with healthcare's needs…?

Per H. Mygind: I mean, it's quite evident that antimicrobial resistance is a growing concern from society's point of view. But I'm speaking for my company as well. I, and we are in orphaned diseases. That's more attractive as a business model. Even ultra-orphan diseases are more attractive in oncology areas where you see maybe you have a clinical need, but it's also hitting very few people many times.

And I think that's … there is a disconnect between what the business is trying to drive and what the society needs. It may not be completely aligned.

Hans Henrik Kristensen: I guess another model, and I'm working at a company that is doing exactly the opposite. But if you would put your drug development into a public, it would be a state-owned entity. If the state would make your pharmaceuticals, then, of course, the cost might go down. That's one argument that is made. Of course, whether the state can be innovative enough and stuff like that is another one.

So, put antibiotics into an entity that is not driven as the pharmaceutical is driven. And currently I'm at AJ Vaccines[102], which used to be the Danish vaccine production. And that, of course, was never set out to make any money. So, it was the most futile entity among many in Denmark, I'm sure. You had a public entity selling to a public entity, and of course, that doesn't increase productivity.

Eefjan Breukink: I would add to that. I would add the same, let's say, an altruistic dream that the perfect company for making antibiotics will not be a company but just a government institution that profits from having more, … let's say you save on the clinical trials because you own the hospitals. Most costs go into the clinical trials.

So why? Because the patients are in the hospitals. The hospitals are paid by us. So why is this a costly business? The only thing that when I pitch this to others, I get

[102] AJ Vaccines is a global pharmaceutical company developing and manufacturing vaccines against infectious diseases. https://ajvaccines.com.

back, yes, this will be an institute that will have a bureaucracy from here to Tokyo. That you have to anticipate, that will happen. So that's difficult to set up.

Per H. Mygind: But that happens in the pharma, too.

Eefjan Breukink: So, in my perspective, you won't have to have a patent. The only thing that you need is to have it produced, and then you can give it out for free and maybe sell it. Within Europe, yes, I think that's a viable model.

Per H. Mygind: I think the idea of getting funding up front, I mean getting society to commit to develop. I'm not saying it needs to be government-funded by society. We can use the benefit of a commercial entity.

Eefjan Breukink: In my perspective we don't need the commercial. So, I think it's weird, the concept that you need medicine, that people need medicine, and that the only way the medicine is being produced is because you can earn money. That's very difficult for me to grasp. Yes, I know the business models, of course.

Per H. Mygind: But I think a lot of development in medicine wouldn't have happened if it wasn't because of the commercial interest.

Eefjan Breukink: Well, the first antibiotic that was really produced was penicillin. So, and then you know the British were more altruistic than the Americans, so they didn't file for a patent, but the Americans did.

Leonardo De Maria: But remember, the drive there was not to cure people. The drive there was to cure soldiers. And it was the Second World War. So, there was a strong push there.

Eefjan Breukink: So. they didn't want to earn money.

Leonardo De Maria: So … But you know, we as a society have essentially outsourced drug development to for-profit companies. And regarding these for-profit companies, probably our pension funds are investing in these companies as well. So, it's all intertwined. And again, the way things are priced these days is not your production cost plus 20%. The way things are priced these days is that 'I am going to calculate the benefit that what I am doing' gives you, and that is the price of the thing.

If I make a genetic medicine that will cure your hemophilia, that will cost the Danish state a lot of money from now till you die, and now you don't have that. A profit, for-profit company, how much is it going to cost as a medicine, right? Because now you have 30 years of not paying for coagulation factors that are more expensive than gold.

We live in this sort of paradigm, right, where profit drives things and profit also, you know, and maximizing profit is having the best possible guess about the benefit of what I am doing is going to bring you, right? And in the healthcare business, this is, I mean, how much do you price two more years of the life of your son that has leukemia, right? Or how much do you price that your son that has hemophilia will not have hemophilia anymore? I have a genetic, you know, medicine that will cure the hemophilia of your son.

Right, so this is where the paradigm will lead. So, in this sort of scheme, right, having one thing that you may need once in your life that you are unlucky enough that you get one nasty, nasty bacteria, right, and then you use it once, you are cured, and that's it, right? This is orthogonal to the paradigm that we are all living in, right.

So, of course, this needs a different way of thinking because it doesn't fit there at all. Right. It doesn't fit.

We all agree that if one of our family members gets a nasty bacterial infection and may die from that is because there is no antibiotic to cure my son or my daughter or my wife or my relatives, this is totally unfair.

Laura Daniela Martinenghi: But it's not only that bacterial infection, but a normal infection also makes you sick, you cannot work, you don't produce.

Leonardo De Maria: But you are not going to die, right? And we are, as a society, we are very emotional, right, and so it's also that antibiotics have not reached some point. The multi-persistent bacteria were on the news all the time. Now you don't hear about them so much. So, they are not on people's radar anymore. So, I don't want to bring bad luck, but maybe it takes an epidemic of nasty bacteria for this to start, and then again, you know.

Eefjan Breukink: It's a silent one.

Leonardo De Maria: Right? So, I don't know. Because with the vaccine, you know, it was a different story. It was super-fast, and everyone was trying to do it.

Hans Henrik Kristensen: Maybe going back to Eefjan's and Per's reflection on altruism. There are companies that like in programming, that I think one is called Open Source Fund[103], and I've met a guy called, an Indian guy, Jay Kumar or something, but the idea is to, again, non-profit, sort of copy the open source from

[103] http://www.opensourcepharma.net.

programming into pharmaceuticals to make them available for countries like India or Pakistan.

But I don't think they are that successful. And I know specifically that some of the issues that they faced...

BCG[104] is a very old vaccine. It was first used 102 years ago. There are a few producers, but many people could benefit from it, both for TB and for other reasons. So that's one of the things I know they have been looking at. And they talked to companies in European area, the answers, I think, about how to approve it in other indications, sort of from a non-profit perspective. And they ran into a lot of issues, most of them regulatory.

I guess it's not that easy, because whether you want not to make money from it, the regulatory authorities still require the same package, basically. And it's, you know, the problem with some products, it's not what we're discussing, is that the regulatory file is very old, so it's not living up to current standards, and they really cannot understand that I'm just getting another indication on the back of something that already has an indication or several indications. Why is that more difficult? It should be easy. No, it's not. And it requires ...

Per H. Mygind: It's a different standard.

Hans-Georg Sahl: I would agree with what Hans Henrik said, that regulators play a big role, but I think you can see that they are ... They don't want to be the bad guys that hold up the whole process. So, there has been a lot of rethinking among the regulators, and they've changed the rules. They have different ways now, and they create different ways to develop things, doing the clinical phase-3 studies, and so on.

No, but it's certainly right. I think in the future, we will see a mix of big pharma making money with some antibiotics that are well-established, and for innovation. Innovation doesn't come from big pharma. Innovation comes from small companies, and there must be more push and pull incentives and initiatives like GARDP[105] and many others, which are currently pushing the field forward in the sense that they would provide money to some extent to get ...

I mean, there has been this antibiotic, Zoliflodacin[106], but it wasn't in use when GARDP was responsible for developing further this compound that had been put

[104] The BCG vaccine, which stands for Bacillus Calmette-Guérin, is a vaccine primarily used for the prevention of tuberculosis (TB).
[105] GARDP stands for the Global Antibiotic Research and Development Partnership. https://gardp.org.
[106] https://gardp.org/positive-results-announced-in-largest-pivotal-phase-3-trial-of-a-first-in-class-oral-antibiotic-to-treat-uncomplicated-gonorrhoea/.

on the shelf by AstraZeneca years ago. It had already passed Phase 1, and they really came up with 250 million to develop this compound, which is now in Phase 3. It passed the FDA approval. We will see a mix of initiatives between really pharmaceutical, big pharma-driven money-making initiatives and this public money that has to go into it, particularly when you're going for reserve antibiotics where you would tell people that they have to put the things on the shelf for emergency cases. You would need public money that goes into it. You have to find somebody that develops and produces it and then put it away, and we will see these initiatives, I'm pretty sure. They are well on the way.

Laura Daniela Martinenghi: Right. Last question from me, and then I pass the word to Christoph [Gradmann] to do a discussion up.
Do you feel that sometimes interdisciplinary teams can lead to too many "cooks in the kitchen," causing a little bit of paralysis or diluted focus? What is your opinion?

Per H. Mygind: No, I think you need interdisciplinary groups. I mean, as long as there is some project management in some way or some plan as to where you're going, I think you need a mix of disciplines to move anything around.

Olivier Taboureau: I mean, you are cooking differently.

Per H. Mygind: Yes.

Olivier Taboureau: Sometimes it's a combination which makes a play in the simplicity. That's what I think.

Laura Daniela Martinenghi: Any thoughts?

Leonardo De Maria: I mean, for Plectasin you can see, we had all the complementary skill sets that were used on working on the same sort of problem or molecule. And this is typically a thing of medium-sized and large-sized industries, that you have colleagues from all walks of scientific life. You have your NMR expert next door, the crystallography expert here, and you can always, you know...

And yes, there are... So, I think interdisciplinarity is kind of intrinsic to this type of problem. The problems arise when there are similar cooks, right? So, when you have three different molecular biology teams working on similar things. So, I have heard that in the US, some of the PIs from the big groups put two postdocs on the same project and see which one gets the results first. That's where you have troubles. But if you have complementary skill sets and as Per said, that they are kind of loosely bound into the same task, this is actually very positive.

I can clearly remember my last postdoc position in academia was very interesting. We had European... And I'm speaking, you know, the late 90s, early 2000s, so European Union group research projects were smaller. Nowadays, they need to be gigantic; otherwise, you don't get funding, right? But still, you know, there was very little collaboration. You know, once the money came from Brussels, everyone went his own way, doing his own things, and sometimes you meet and discuss things, but if you didn't need to interact with the other, you didn't. While in the industry set up, in medium or large enterprises, everyone is there, and you have the expert next door, and you can go and ask, can you crystallize this, can you do a simulation of that, how to analyze this data, because I don't know. Do you have something that allows me to, from an image, get numbers and things like that?

So, this is actually a plus, not a minus, in my opinion.

Hans Henrik Kristensen: I think a general reflection would be, again, leadership is very important, that it's clear who's leading, and maybe in the Plectasin case, it became more complex at various levels because it was a project... No, but really not initially, but then we had professional project managers, we all remember Dorrit [Aaslyng][107].

Per H. Mygind: I stole Nico…

Dorotea Raventos: I think she's still in Novozymes.

Hans Henrik Kristensen: So, we had a project management team, project managers, they sort of had one idea where to go, we had our management, we had inside management, then at some point a bio-business was established at Novozymes. So, we had inside the business, bio-business, then bio-business wanted to determine because they were responsible for the finances for these projects; in that sense, I think it became complex and non-optimal, and I see it in my own line of work where I now am responsible for regulatory affairs department among other things, but who is driving the regulatory affairs strategy? It's not related to this, but is it the business, is it the regulatory department, is it QC, is it QA[108], and if there isn't clarity and everything, you know, the regulatory strategy is aligned to the business strategy, then of course it's sub-optimal. So, in that sense, yes, not too many cooks, that's fine; it's required, but when many parties are involved, there needs to be a clear direction, strategy, and execution.

Laura Daniela Martinenghi: Good ending to the question. Now I give you the word [to Christoph Gradmann].

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[107] Dorrit Aaslyng: project manager at Novozymes 2001-2013.
https://www.linkedin.com/in/dorritaaslyng/?originalSubdomain=dk.
[108] QC and QA stands for Quality Control and Quality Assessment.

Christoph Gradmann: I think, I have a fairly simple job. I had to hold back [during the seminar]; it's super interesting to listen to all the discussion, and generally speaking, I think 15 years of distance to the events are a good distance. There was a sense of reflection on events and people and structures [in the room] that you tend not to get when you talk to somebody who's coming straight from the lab, or the market. Then everybody is still fuming or doesn't want to do it anymore.

So, in that sense, this was very, very interesting. Coming to what I heard, I have a few comments to make, and they are comments of somebody who is not mastering any of the fields that you work in, … but who is able to follow and write histories about it. And some of my observations refer to commonalities, I think, in the history of drug development, and others are very specific, and then they connect to what a special company Novozymes was, and to the technicalities of the molecules.

First of all, there was a very strong sense that development started in something that was actually more like a university department in a company. And I think that's super common. I've studied drug development histories over the last century. There are different solutions from a company's perspective to that problem. Some companies do collaborate with universities, and they don't want to have too many eggheads in the company. And other companies have a big in-house development. But they always have this problem of the kind that the academic department moves on its own trajectory, and then somehow, they have to go and catch these guys, and see, can we make any products from what they do? I thought that was a very common feature.

In connection with that, I thought it was very visible from several people around the table that there was kind of a breakthrough/discovery moment or phase where you seem to have had the idea that you were starting something new, which was potentially a new class of antibiotics. Somebody said that 'suddenly we found it everywhere. It started by looking at defensive mechanisms of enzymes, and then this idea kind of rolled on, and suddenly they were all over the place'. And that's also, I think, something that is very typical for how innovation in the history of science works. Once you have a new idea, you can apply it to lots of things, and then it becomes pervasive. I thought there was a wonderful sense of that in the room.

And then the interesting thing is, and that's where it becomes very Novozymes-specific, is that the whole clinical development part was not at this table. In that sense, this created a very specific challenge of having to make, at one point, something that you thought works in a laboratory setting, and then you have to sell it to people who do clinical development. And, from the little I know, I think that's

probably more the normal situation these days. Speaking as a historian, 50 years ago it would have been a rare exception.

So, I think, in that sense, the drug industry has radically changed over half a century. Fifty years ago, that [process] would have happened within one company. And now it tends to be a question of different companies. You mostly described this as something that was a little bit peculiar about Novozymes, I would cautiously disagree. I think that's how it's going to be in the future. It's going to be less and less in-house. As a historian, I shouldn't speak about the future, but what I know is that in the past big companies, the big pioneers of antibiotics, Merck or so, or on the more chemical side, Bayer, would have huge in-house development [departments]. They would not collaborate with outside [incomprehensible; likely 'researchers'], actually. And they would do that in-house, but then they would typically stumble over the same problem.

So, then, they would need to make a business case [for a molecule]. This was a corporate business case in the 1950s and 60s, but it was the same problem that you had to look at and ask: they have a working molecule, but what's the competition? How much work will it take to remove all problems so that it becomes a pill that actually works at the end of the day? And how big is the market? Are we targeting a disappearing problem? Three essential questions.

I worked myself on one of the big companies in the 1980s and '90s, and it was typically at that stage [prior to clinical development] also inside the company where things get shelved. So, the deal with Sanofi not really working, I think, finds its parallel in many works that are being put on the shelf in big companies. I think, [it might happen] in a different way, but structurally, with exactly the same effect.

And looking at that I started to think about alternative histories. And there is a problem because if I look at the business side of drug development, which we haven't done that a lot [in this seminar], there's a lot of interest in big companies, in kind of getting these things closer and closer together. So, they're trying to avoid the waste of money in the scientific development, in the clinical stage, by bringing the business decision ever closer to what happens in the lab. The downside of that is that you shelve more and more molecules. I was hypothetically thinking, could there today be a successful business case for, let's say, an antibiotic drug, streptomycin, that was targeting a shrinking market, that was difficult to use because it's injected, you need a lot of personnel to do that, and that actually is not a very strong drug. It would not have been developed more recently. Soon after, there were other TB drugs [developed] that were much better. Probably, people would have developed just one. I worked myself on one of the big companies in the 1980s and '90s, and it was typically at that stage [prior to clinical development] also inside the company where things go off the rails.

Then [in the story of Plectasin] we had instances of contingent moments, what historians call the Cleopatra's nose effect (She was so beautiful, and then suddenly, world history took a different turn). And that's a CEO checking things out and all that. They were interested, and then suddenly, somebody came and threw out a large chunk of projects. 'Don't take it personally, but out it goes.' And that's not so rare. It goes like that, actually, in history.

Structurally, it relates to something that something I've worked on for the previous centuries, for the 19th century, and it's a typical problem, and some of you have been touching on it. So how do we translate something that works in the lab into an industrial system? And we studied that on the example of therapeutic vaccines. And all the problems that there were between the people who were producing that same tetanus vaccine and the people who had worked on tetanus in the laboratory.[109] And it's always a process that is very, very difficult. And I think we were at several points were touching on that transition, but you were all quite aware that you were on one side of this transition, so to speak.

There were a few comments in the other direction. So, when people were asking, is that bucket still around? You know, will we still do something with it? Yes. So that might be a thing [to do].

And then, yes, I had that business-is-moving insight. So, one way to make money in a drug company is to move the business decisions closer to the lab and involve market research and management more into drug development, but the challenge here is that it kills a lot of molecules that are already developed. And I think that's something that might be interesting [to study].

I have a second life as a historian of global health. So, the question of pricing that came up at the end [is something I am familiar with from that perspective]: It is done by people who were not around the table here, people from health systems research, and they have their metrical units, the QUALY's[110] and the DALY's[111] and so on, and they can put a number on, let's say, one more year [of life], and so on.

It's not as solid [as lab science]; I'm not suggesting these are very good numbers or even solid numbers; in that sense, I'm all with you. These are numbers that change over time, but still, the calculation is absolutely done for that [and the numbers are real, they have consequences].

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[109] Gradmann C. Locating Therapeutic Vaccines in Nineteenth-Century History. *Science in Context*. 2008;21(2):145-160.
[110] QUALY stands for Quality-Adjusted Life Year, which is a measure used in health economics and healthcare outcomes research to quantify the health-related quality of life for individuals or populations.
[111] DALY stands for Disability-Adjusted Life Year, and it is a measure used to quantify the overall burden of disease and injury in a population.

And my other comment is on what's been discussed on the question, how could we develop this further? Can we revive it, which very quickly descended into a discussion about whether it is a good idea to have this done in the economic profit model that it's currently in. I don't think it necessarily needs to be a discussion of, let's say, the socialist versus the capitalist model. I have been - that's my second anecdote - in a panel discussion with some people who are high up at CARB-X[112]. And then, one or two years before the pandemic, I asked the provocative question. So, let's assume you ever find anything, who's going to own it? And they said, obviously the company. And I said, wait a minute, it's all being paid by tax deductibles. This is all citizen money that you're using; why shouldn't it be owned by the citizens?

In that sense, it's interesting to take, to look not at socialism, but the vaccine market, which is a market where the big buyers have had the biggest say for decades. And strangely enough, it's been very innovative, and innovation didn't have a big problem being marketed. So, I think - more as a personal comment - probably, drug development for antibiotics can learn a lot from how the vaccine market lends stability to that market. But that is done through public health concerns. We need that vaccine, or we need that antibiotic, but we will not use it here and now.

The reasons why CARB-X doesn't like that are obvious. Because we will never be able to market antibiotics for prices like it was cancer medicine. It's not going to work. You don't get that [public money] without control of prices.

And that brings me to my little conclusion. What kind of story is it that we have heard? You call it an odyssey. I was playing around with the idea if it is a tragedy, but then [this is inaccurate]: it only becomes a tragedy in retrospect. The situation now is certainly one where there are things that are unfulfilled, and you all have the sense that this could have gone on, but it did not. But while it moved on, it wasn't a tragedy. The idea that something is a tragedy is only put together in retrospect. While it happens, it is driven forward by enthusiasm, and remember, all that happened at a point in time when antibiotics drug development, had been in a crisis for 20 years already.

Because when I look at the level of people taking marketing decisions about antibiotics, which is often what I do when I look at big companies, then they were having deep doubts about that type of medicine since the late 1970s, roughly. However, while the class of medicine is in a crisis, this is not something that we see a lot of in the laboratory work!

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[112] CARB-X Biopharmaceutical Accelerator is a global nonprofit partnership focused on supporting the development of new antibacterial products. https://carb-x.org.

So, thank you very much. It was very, very inspiring.

Laura Daniela Martinenghi: Thank you.

Jørgen Leisner: Yes, I just wondered if it was not a success story in one aspect, and that was that all the science involved gave a successful boost to the company profile showing they are very, very good at enzymes.

So, people can see you can also do this thing here. And you were talking about the Nature paper and the huge profile it gave you at that time. So, I wonder if it was not just about the company in that sense, regardless of the level of support or not. I don't know if you have a comment on that.

Hans Henrik Kristensen: From my perspective, yes. I think it was a success. And I look back with joy. I'm quite ambitious; we all are.

Per H. Mygind: So, I think the paper is a comfort. We were doing it to make antibiotics for humans, right? And if we couldn't get that, the paper...

Christoph Gradmann: It's a reflection of that very strong academic drive in the group. Actually, you see, return of investment, that's all very nice. We have a paper in Science!

Dorotea Raventos: And a Nature communication.

Leonardo De Maria: But I think for me what has always been puzzling, and I tried to tell it when I was at Novozymes is that drug development takes a lot of shots in order to succeed. This was our shot number one, right? And at Novozymes, we built the whole infrastructure that could have enabled us to have shot number two, shot number three, shot number four, shot number five, shot number 20. But we didn't.

We took shot number one, maybe number two, two and a half, maybe three molecules, and then we stopped after having hired 20 people, buildups, or whatever.

We were, as a company, we were ready to do [more]. And again, drug development is very difficult, but for antibiotic development I think it's the easiest possible drug development you can have. Because it's not like COPD[113], where I am working with chronic obstructive pulmonary disease, people can have their lungs ruined for

[113] Chronic obstructive pulmonary disease (COPD): This chronic inflammatory lung disease causes obstructed airflow from the lung.

so many different reasons. You need to find one molecular target, which is your best guess, invest years trying to modulate that target, and see if that works. Even the animal models for COPD are horrible. They don't work very well. You expose mice to cigarette smoke for months. And that's all you know, but this is not a good model anyway.

With antibiotics, you know the bacteria, you know the target nowadays, because you can sequence the bacteria, you can even know the mode of action because you can investigate it. You can in vitro take all the things and say, we are ready. The main concern is toxicity, but you can do cytotox already *in vitro*, then you can try your animal models. You can try the animal models with the right infection, which is the best possible model for a disease. So, in terms of drug development, it's the easiest one. I think safety is the big stopper. You put in humans, you have a bad readout, you stop.

Christoph Gradmann: The two big concerns I see are that tend to stop [development] are safety and market potential.

Leonardo De Maria: Yes, but market potential is something that we can, as a society agree. Because the market potential, of course, is, and again, vaccines are big volume, low price. In principle, every single child gets the polio vaccine. You know that. So, in a country, every child will get it. But in a country, you don't want every child to get an infection with a […] bacteria. The fewer, the better. And the less you use this molecule, the better because there is a risk of resistance. So, we can decide that there is money allocated to do the drug development, which is the easiest drug development of all the diseases.

And then, for the market potential, we just don't give it to companies that are for profit. There needs to be a balance.

Christoph Gradmann: I work a lot in global health. Currently, we have an interesting situation with regard to antibiotics. It's not that the market is not big. The antibiotics market is huge, but it's driven by generics. So, the way the structure is currently that it's very difficult to do innovation while it is very easy to do bulk. So, the market is such that there is growth but innovation is pretty much absent from it.

And of course, if antibiotics would be somehow a public property or the public would have a strong say, like they have in vaccines, that would, to some degree, remove that split, but it would also make antibiotics a public commodity and remove much of the need for innovation because most patients do very well with old antibiotics.

Leonardo De Maria: Yes. But it's... and how big is the problem? Again, you know, we don't hear any more in the news, right? But at some point, you know, we were having in the news cases of people that have to have an amputation because they had an infection, and the only way to cure the infection was, okay, your leg goes because otherwise, if this becomes systemic, we cannot cure it. So, you have one leg...

Christoph Gradmann: The problem is big. You can look at evidence from the, let's say, the very quickly rising problem of healthcare-related infections in low-income countries. It tells all that. The growing market produces big, big problems, but we're not currently doing anything about it.

Eefjan Breukink: It's old news.

Christoph Gradmann: Yes, that's old news. Absolutely. But that's for people like us. It doesn't mean that it's not important, but it hasn't really hit the public, I think. Actually, if you look at hospital infections or healthcare-related infections, people look at their ICU in a high-income country. They should look at hospitals and healthcare facilities in low-income countries.

Kirk M. Schnorr: I don't know how much more time we have, but I have one comment to the comment about whether Plectasin was good for Novozymes. And I will also say yes. Because you have to remember Novozymes split from Novo Nordisk in 2000. In 2000, what was Novozymes as a company? Then came the Nature publication. And then the institutional investors saw Novozymes ... Okay, they can also do this. They're not just an industrial enzyme producer. They've broken the code. They can produce with their technology. They can scale up. And those institutional investors are still looking at Novozymes.

We got on the map because of the publication because of the discovery of Plectasin, among some other things. But that was a major thing.

Christoph Gradmann: It's a proof of skill.

Kirk M. Schnorr: Yes.

Laura Daniela Martinenghi: Great.
I think we are good.
I would say thank you to everyone for ... also, online, thank you for being there, for coming, all of you.

End of seminar.

16th November 2023, 17.30.