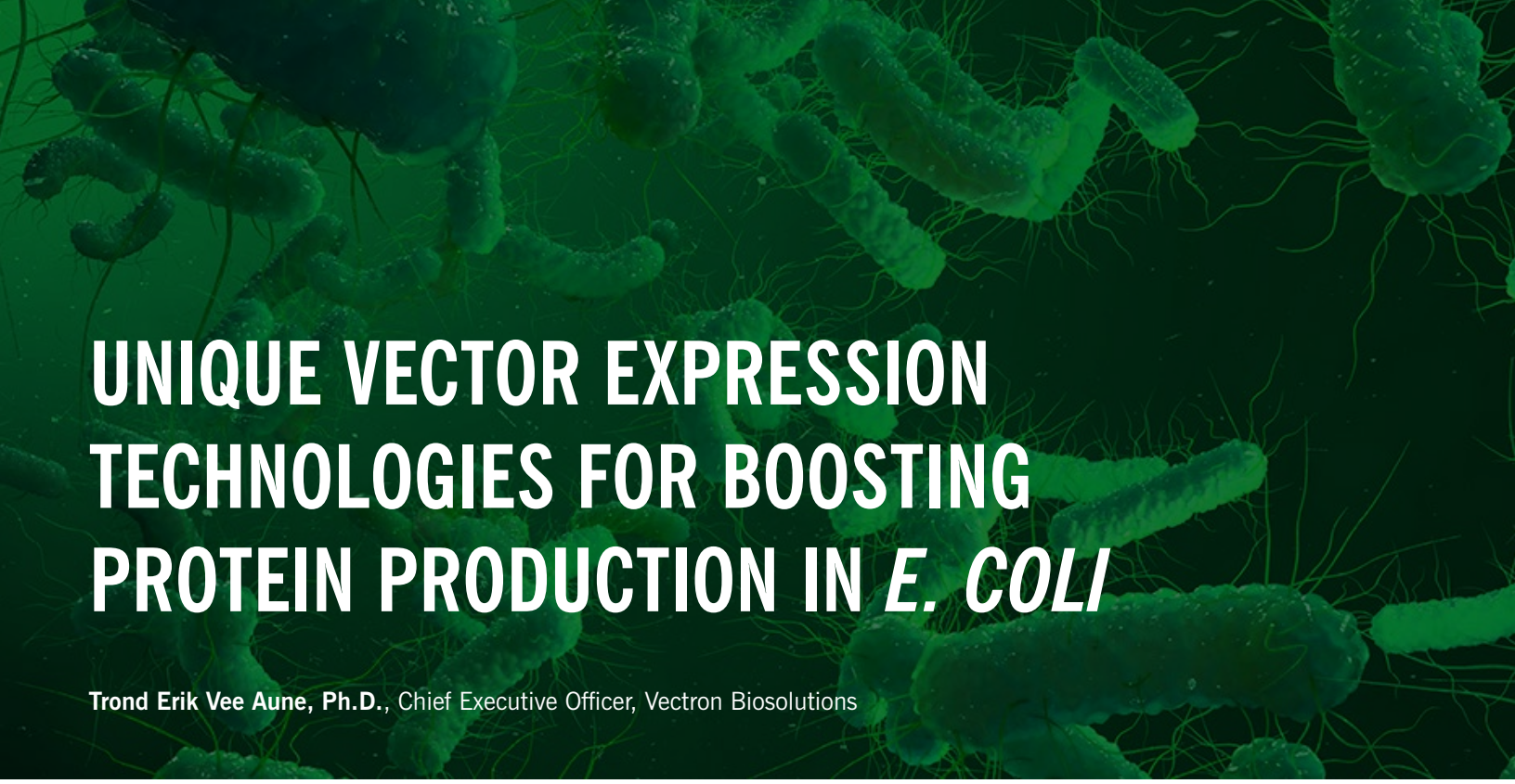

UNIQUE VECTOR EXPRESSION TECHNOLOGIES FOR BOOSTING PROTEIN PRODUCTION IN *E. COLI*

Trond Erik Vee Aune, Ph.D., Chief Executive Officer, Vectron Biosolutions

Despite decades of study, there remains much to be learned about controlling protein expression in bacteria, particularly with regard to enabling researchers and manufacturers to increase their success rates and yields and to reduce cost of goods for biotherapeutic and industrial proteins. Vectron Biosolutions has developed unique vector and vector evolution technologies that enable the development of expression systems designed for high-titer production of high-quality proteins, even for proteins that are otherwise difficult to produce. Here, Vectron's CEO Trond Erik Vee Aune, Ph.D., discusses the origins of the technology and the founding and evolution of Vectron, as well as the technology itself, its advantages, and what the future holds for the company with *Pharma's Almanac* Editor in Chief Director David Alvaro, Ph.D.

DAVID ALVARO (DA): To start, can you tell us about the research in Professor Svein Valla's laboratory and how that set the stage for the genesis of Vectron Biosolutions?

TROND ERIK VEE AUNE (TEVA): Professor Svein Valla's research group at the Department of Biotechnology at the University of Science and Technology (NTNU) in Trondheim, Norway, was investigating the basic mechanisms of how bacteria synthesize proteins. It is an area where it can be difficult to have any major breakthroughs, because extensive work has already been completed. However, there are still a few areas within the field where our understanding is incomplete. Svein focused on understanding some further nuances in bacterial transcription.

To do that, he developed his own expression vector systems as research tools. I don't know if Svein actually had any idea that they could have a commercial value; maybe he did, though – he was quite acute when it came to business. It turned out, though, that these expression vectors could drive protein production at industrial levels.

As a result, Svein began working more specifically on bottlenecks in protein production and pursuing projects with industrial partners to work on specific topics of interest to them. It was a fairly vibrant research environment within a large group of researchers, including Masters, Ph.D., and postdoctoral students.

Eventually, Svein started looking for someone help him commercialize the technology. At the time, the life sciences sector in Norway was not very mature, and he wasn't able to find anyone with experience in getting a biotech startup off the ground. I was very interested in helping to advance the technology and was afraid that, if someone didn't step up, it would never be commercialized. I believed we had developed something really fantastic and felt strongly that I needed to make sure that it was actually applied by companies, and so Svein and I founded Vectron Biosolutions in 2008, and I took the position of CEO.

DA: How have the vision and mission of the company evolved from that beginning to today?

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TEVA: In a sense, our vision has come full circle. When we founded Vectron, we knew we had great technology, but understanding how that technology could apply to different customers and fit into different applications was more challenging. Initially, we didn't focus on any specific applications and didn't explore any real market segmentation. That came a few years later, when we decided to focus on the pharmaceutical industry. Today, we are once again broadening our offerings to customers outside of the pharmaceutical industry, but on a selective basis. Currently, 60% of our customers are in pharma and the remaining 40% include contract manufacturing organizations, industrial enzyme producers, and academia. Going forward, I expect the mix to remain similar.

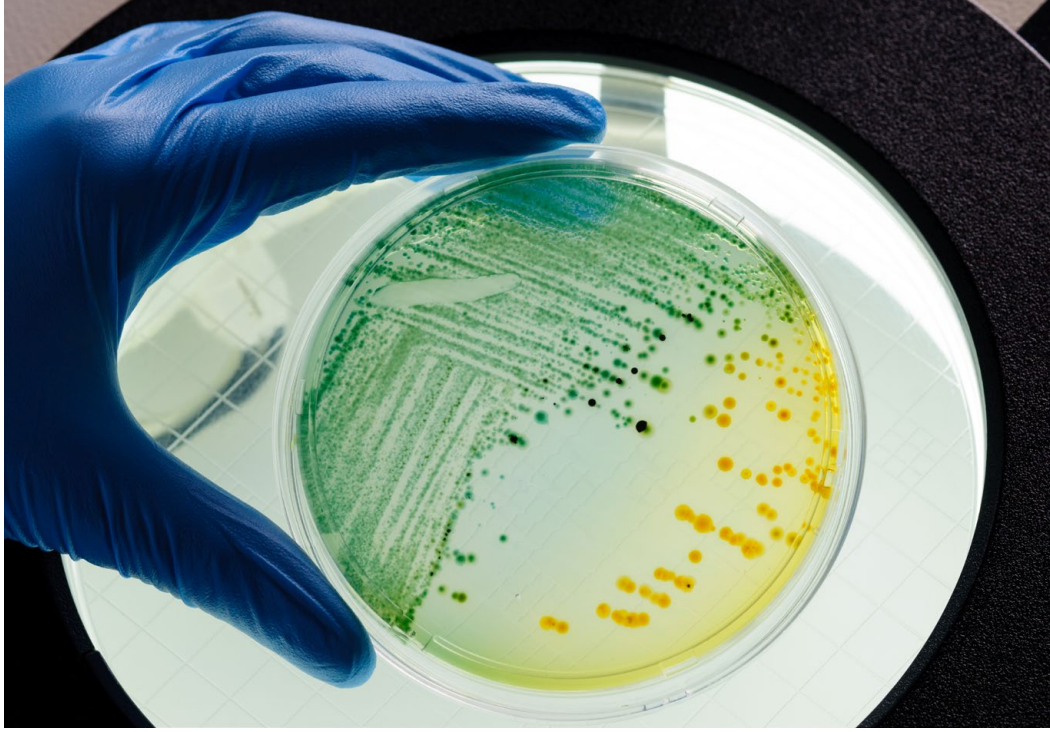
We have a technology for production of proteins, and it doesn't especially matter how the proteins will ultimately be used; the scientific challenges of creating a strain that produces a huge amount of a specific protein are the same. Of course, pharmaceutical proteins tend to have certain unique challenges, and industrial enzymes tend to involve different proteins. But for us, it doesn't really matter —we tackle every protein as a unique problem and provide a solution to that problem.

DA: Can you walk me through your foundational promoter / transcriptional regulator technology and some of the subsequent technologies you have developed?

TEVA: Svein studied gene expression, and he needed a model protein system. His choice was the *Pm* promoter, which was derived from *Pseudomonas putida*, where it regulates enzymes involved in the degradation of certain molecules.

Svein created expression vectors by placing the *xylS*/*Pm* promoter and its cognate transcriptional regulator into a naturally occurring RK2 plasmid that he had modified to minimize its size. This expression vector was completely novel and unlike the vectors used in industry at the time. Both the plasmid backbone and the promoter have features that are advantageous for producing proteins.

The RK2 plasmid can exist in many different Gram-negative species. To date, we have focused on *Escherichia coli*, but the system can transform other bacteria as well. We haven't yet really realized the commercial potential of this broad host range feature of our technology, largely because the pharmaceutical industry is conservative and not eager to try new bacterial species when it comes to protein production. It is a different story for the production of industrial enzymes, so we will be leveraging this property going forward.



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The *Pm* promoter is the key element of our vectors. It is a positively activated promoter, which means it requires an external inducer to bind to the transcription regulator to turn it on – rather than via a repressor mechanism, in which a repressor positioned on the promoter must be removed to activate the promoter. In addition, the inducer for the *Pm* promoter is benzoic acid, which readily and passively moves across bacterial membranes. Common bacterial inducers, such as isopropyl β-D-1-thiogalactopyranoside (IPTG), need to be actively taken up by the cell, which generally results in an all-or-nothing effect. If the inducer is taken up, expression is fully on, and if not, it is fully off – there is no means of regulating the level of expression. With our system, however, there is a linear relationship between inducer concentration and *Pm* promoter activity, which allows us to adjust the level of expression from the Pm promoter.

Controlling the expression level is crucial in maximizing yields of high-quality protein. Overexpression can lead to protein degradation and/or the formation of inclusion bodies by unfolded or misfolded proteins that in turn can cause stress responses within the cell, resulting in reduced growth rates and lower titers and protein yields. Being able to fine-tune the expression rate so that it is balanced with the protein folding rate allows all of the synthesized polypeptides to be folded correctly.

This VB Expression system was our first technology. Since then, we have added others, including VB Evolution and VB Secretion. VB Evolution is a directed evolution technology that mimics natural evolution. We create libraries of random expression vectors through mutagenization, then use an ultra-high throughput screening method to screen millions of expression vectors

to identify those that are the best for every specific protein. This approach goes back to our philosophy that every protein is unique and poses unique challenges. It really isn't possible to use just a few different expression vectors or strains to find the optimum solution in every case. Using VB Evolution, we essentially create tailor-made, bespoke expression vectors for each and every protein we work with by evolving a solution to every protein though an iterative process similar to what occurs in nature.

Our VB Secretion technology was developed in Professor Kelly Hughes' laboratory at the University of Utah. It is based on truncated versions of bacterial flagella, an organelle in bacteria used for locomotion, that have been engineered to act as pores or channels linking the inside to the outside of the cell. Most importantly, these channels direct proteins produced by the cell to exit the cell into the surrounding media, which eliminates the need for cell lysis and recovery of proteins from the complex mixture that results. As a result, the proteins are in a cleaner bioprocess fluid, and downstream purification is much simpler, leading to time and cost savings.

We started with one specific set of expression vectors, and then added a method for generating novel, unique expression vectors by mimicking natural evolution and engineered secretion technologies that allow us to send the proteins out of the cell.

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Together, these solutions comprise the most sophisticated technology platform for the production of proteins in *E. coli*. As a host, *E. coli* has not really been neglected, but, when the focus in biopharma shifted to antibodies, *E. coli* simply couldn't do the job, and mammalian systems became predominant. I think there was also a general consensus that there wasn't much more to find out about *E. coli*, because it already worked really well with the expression systems developed back in the 1980s and 1990s.

Svein, however, took the technology a step further, and Vectron has gone beyond his initial work to make *E. coli* an even better production platform, not only for those proteins that could be produced in *E. coli* before, but now also proteins that previously would typically be produced in yeast because of the secretion possibilities.

DA: What advantages does the VB Evolution technology offer over other high-throughput screening approaches?

TEVA: When we only had the VB Expression technology, we created a lot of different expression vectors for every protein using variants of the promoter, signal peptides, untranslated regions, and other expression elements. However, we realized that the variants we were using had been isolated 10–20 years ago because they were effective for a specific protein, and there was no reason to think they would be best for the other proteins we were working on. Rather than use predefined elements, we decided to create completely new elements through an evolutionary approach that would be tailored to each specific protein. In essence, the VB Evolution technology was conceived through us questioning whether we were missing something because the mutation space is so large.

VB Evolution addresses that concern, because we are screening through millions of different candidates. It doesn't necessarily mean that the results will be better in the end – there might be only miniscule improvements for some proteins. But for many, we are confident it will. The ultra-high-throughput screening method is also extremely efficient and allows us to screen those millions of candidates in a couple of days. More rounds of mutagenization take longer, of course, so the full project timeline depends on the number of desired iterations. Generally, though, we expect VB Evolution to be much quicker. It is especially attractive for customers that have unexpected or unexplainable problems in clinical development and are anxious to get back on their planned development schedule.

With respect to other high-throughput screening technologies, I know of no other company doing something similar. The concept of evolving new expression vectors isn't necessarily revolutionary; I'm sure others have thought about that. What is innovative is our ultra-high-throughput screening method. Normally, you would have to develop a screening method for each protein, which could take months. Ours is a universal screen that works independent and agnostic of the target protein, because we are screening for titers, not activities. All that is required is some cloning to establish the client's gene inside our library of mutagenized vectors. It is a matter of only weeks from the time we have a client's gene until we generate an expression vector that produces very high amounts of the client's protein.

DA: What was the impetus for the acquisition of the VB Secretion technology?

TEVA: We want to continue to add technologies and strengthen our position within bacterial gene expression services; we can do that by creating something ourselves or finding it externally. As a very small company, what we can achieve ourselves at the moment is limited, but we are also always open to innovations that come across our path.

T3S Technology, the company founded on the basis of Kelly Hughes' research, was looking for a partner for collaboration. The secretion technology had originally been developed for *Salmonella*, which is not an attractive host for the production of proteins, especially pharmaceutical proteins. T3S learned that Vectron was good at *E. coli* genetics, so they contacted us and initiated a research collaboration with us with the intention of moving this secretion system from *Salmonella* to *E. coli* and using it in combination with our expression vectors. It is very complex and involves many changes to the *Salmonella* genome. Moving it to *E. coli* required more changes to allow control of the expression of certain genes.

I realized fairly quickly that this technology can be extremely valuable, so we initiated discussions with T3S. The acquisition was completed in the summer of 2021, and we are in the process of finalizing that technology in *E. coli*. We have proof of concept but need

to increase the secretion rate to make sure that the technology is applicable to many different proteins.

DA: Is it your vision that most programs will integrate the three technologies?

TEVA: I believe that very soon VB Evolution will replace VB Expression to create expression vectors that produce huge amounts of high-quality protein inside of *E. coli* cells. Whether we will use the VB Secretion technology will depend on the specific proteins and how well secretion works for each individually, as we do expect the performance to vary. Part of that decision will be determined by the purification costs, whether they need refolding, and so on. For the majority of proteins, I think both technologies will be leveraged. But there will be cases where the secretion rate isn't high enough or customers are interested in inclusion bodies, and for those, cytoplasmic expression will be preferred.

DA: Are there certain types of proteins that benefit the most or projects that have greater potential to be enabled by Vectron's technologies?

TEVA: The fact that we have all of these different technologies and different elements and libraries of elements and can fine-tune and optimize so many aspects of the expression vectors gives us an advantage when it comes to expressing hard-to-produce proteins. We have earned a reputation for succeeding where others have failed with particularly challenging proteins. New pharmaceutical customers often come to us with more-or-less abandoned projects for which they have tried different systems before and failed. These difficult proteins include proteins that are prone to aggregation or may be toxic to the cells for some cryptic reason or are only produced in low titers.

Of course, our technology is also suitable for easy-to-produce proteins. We also have a good record with respect to manufacturing proteins in *E. coli* at titers of more than 60 grams per liter. That is not a titer I can guarantee for every protein, but it really demonstrates what we can achieve when everything works out, and increasing titers can really give customers a competitive advantage.

It's also worth noting that there is a general increase in interest in microbial fermentation, because working in bacterial systems is easier than mammalian cell culture, and results tend to be better for bacterial proteins and enzymes. Beyond that, our technology works well with any protein that can be produced in *E. coli*, whether that is antibody fragments or enzymes or hormones, and so on.

When a customer brings a protein, we listen to their experiences and the problems they have encountered when trying to produce it in *E. coli*. We then look at whether we have experience with any similar proteins to see what knowledge we might be able to leverage. We take a fairly broad approach, testing very different expression vectors with different properties to see what works. Once we get those initial results, we have a better understanding of the protein and can pursue more rational optimization from that point on – within the context of the VB Evolution technology, which is completely random beyond where the mutations are programmed.

DA: Ae there any other problems or bottlenecks analogous to secretion where there is potential for an engineered solution that would fit with Vectron's evolutionary approach?

TEVA: There are definitely technologies that we would like to add on, particularly on the level of the host cell itself. VB Expression and VB Evolution are plasmid-based, but changes to the cell environment can also dramatically affect protein quality, such as folding properties. We can envision an evolution of VB Evolution with mutagenization of the genome rather than the expression vector. That raises some regulatory issues, because you need to characterize the end product to a much larger extent, which may make such an approach less applicable in pharma. Generally speaking, though, improving the host cell needs to be done, and fortunately there are companies working in this area.

Better codon optimization is also an area of interest for us. We can use service providers to optimize the code and sequence of *E. coli*, but that doesn't always work, because there is often a lack of sufficient knowledge. I would also like a cleavage system for cleaving fusion partners. We occasionally use fusion partners to modulate expression that then need to be removed afterwards in a cost-efficient downstream process. There are few cleaving systems available that don't leave amino acid residues attached to the product, and it is important to maintain the integrity of the protein. There are also technologies on the downstream side – refolding, purification, etc. – that can be improved, because downstream costs are actually higher than those on the upstream side.

Additionally, there are certain proteins that cannot be produced efficiently in *E. coli* today, especially proteins that contain a lot of disulfide bonds because of the intracellular environment. Transporting them to the periplasmic space where the environment is more amenable for folding and disulfide bond formation is challenging.

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DA: Is there anything you can share about the Vectron team and how its makeup gives you a different perspective than other companies that aren't run by scientists?

TEVA: I enjoy being around scientists, and I know most of our customers do as well. When it comes to describing our technology, it shines through that we are scientists and that we know what we are saying and what we are doing, which generates trust. That's definitely a positive.

We have quite a few grad students working with us and spend time supervising them in more basic research projects. That work, while it may not directly lead to sales revenues, creates a much better work environment, because it provides our scientists with an outlet beyond the continual performance

of gene expression studies and optimization for client projects. That ultimately helps in the longer term, both from an employee satisfaction perspective and with respect to the potential for making discoveries that can led to valuable innovations.

DA: Is there anything you would like to share about Vectron's investors?

TEVA: When we started in 2008, there weren't many investors in Norway that specialized in biotechnology. Most within biopharma were focused on drug development companies – finding the next blockbuster. It is much harder to calculate the risk of investing in a technology developer or service provider. Fortunately, we didn't need to raise money for a long time after first getting established. In addition, within the last five or six years or so, we have had some good exits in Norway, and investors have learned that it is possible to earn money in this sector.

That timing worked out, because we needed to raise money to acquire the VB Secretion technology. At that time, we also wanted to do more in the way of marketing and branding to drive more sales, so I was looking for an investor. It still wasn't easy, but I eventually found Dynamk Capital, which specializes in investing in the ecosystem of technology companies enabling biopharma manufacturing and has experience working with similar companies. The company leaders understand what we are doing, see the benefits and the value of a company like Vectron, and are aligned with us in terms of what can be achieved and where can we go.

Dynamk invested in Vectron in 2021 in connection with us acquiring the secretion technology from T3S. I wasn't used to having institutional investors, but Dynamk is a small team, and we have formed very close connections that I don't think would form with larger investors. It was a big decision for the shareholders in Vectron to let in a New York–based investor, especially considering the potential cultural differences and so on. Fortunately, I was able to convince them that this was the right decision for the company, and I think we are proving that it truly was.

I'm really satisfied with the collaboration going on, with my communication with Dynamk's team, and how they are helping out. They not only bring experience but a worldwide network of contacts to Vectron that significantly expands the smaller, more local network we have established. As a result, they are opening doors to new customers and new partnerships that would have taken us a long time to develop alone.

DA: What can you tell me about your larger, longer-term strategic goals and how you see Vectron evolving in the future?

TEVA: With our new investor, we now have some years ahead of us building the company – exploring and then realizing the full potential of our technologies. We will focus on building our capabilities in *E. coli* and cement Vectron Biosolutions as the expert in *E. coli* protein expression.

We also want to branch out beyond *E. coli*, leveraging our VB Expression and VB Evolution technologies in other bacteria and also examine whether we can engineer it to work in yeast. We are also thinking about bringing mammalian systems in-house, because so many types of larger proteins and antibodies are produced via mammalian cell culture and we aim to be a one-stop shop for protein production. Developing those capabilities ourselves will be too much, but we have connections and possible partnering opportunities.

Beyond that, we are presently just delivering production technologies. We are considering expanding beyond that to include delivering protein products as well, for tox testing, clinical trials, and so on. We may also build out our laboratory capabilities so we can offer more analytics and downstream services as well. There are many ways we can grow very naturally from where we are, and that is definitely what we are going to do. And I expect that the more we do, the more options we will have with respect to exit strategies. Right now, though, my job is just to continue building on what we have and making it better and better and more and more valuable. ■

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ABOUT THE AUTHOR

Trond Erik Vee Aune
CEO and Co-Founder of Vectron

Aune is co-founder of Vectron Biosolutions and has led the company as its chief executive officer since its foundation. Under his leadership, Vectron has been branded as a leading provider of cutting-edge technologies and services for microbial production of proteins. Aune has negotiated license deals with customers, suppliers, and vendors, a Series A investment, and the acquisition of innovative technologies from academia and industry. Aune holds a Ph.D. in bacterial gene expression. More importantly, Aune is the proud co-founder of two beautiful daughters who are a continuous source of happiness to him and his wife despite the long runway, unclear exit strategies, and obvious lack of profitability.

LinkedIn: www.linkedin.com/in/trond-erik-vee-aune-5a4b981