# The Novozymes Prize

*Professor*
*Gunnar von Heijne*

2018

# Nomination of Gunnar von Heijne

The 2018 Novozymes Prize is being awarded to Gunnar von Heijne for his scientific breakthroughs in studies of membrane proteins, especially his pioneering work on developing methods that can predict signal peptides and how these are cleaved from proteins and predict the topology of membrane proteins.

Gunnar von Heijne studied biochemistry at the Royal Institute of Technology in Stockholm, Sweden and received his PhD in theoretical physics there in 1980. Following a two-year postdoctoral fellowship at the Department of Microbiology and Immunology of the University of Michigan, he was recruited as Assistant Professor in Theoretical Molecular Biology at the Royal Institute of Technology and then later promoted to Associate Professor. In 1991, he was recruited to the Karolinska Institutet as group leader at the Centre for Structural Biochemistry. Since 1994, he has been Professor of Theoretical Chemistry at Stockholm University, where he established and directed the Stockholm Bioinformatics Center and later the renowned Center for Biomembrane Research.

The work of Gunnar von Heijne deals primarily with membrane protein biogenesis and structure, and his work has been instrumental for current understanding of the mechanisms of protein translocation and membrane protein folding. His most significant contributions include: 1) identifying signal peptide sequences and cleavage sites, 2) the positive-inside rule, 3) the existence of dual topology proteins and 4) methods for studying how proteins are inserted into membranes.

Translocation and targeting proteins to specific locations is key for the proper functioning of all living cells. Many cellular proteins target the extracellular environment, where they can serve as hydrolytic enzymes or hormones, or the cytoplasmic membrane, where they serve as transporters or receptors. It has long been known that a signal peptide that is cleaved when the protein has been appropriately targeted determines this targeting of proteins. Gunnar von Heijne, together with Henrik Nielsen and Søren Brunak of the Technical University of Denmark, developed algorithms to successfully predict the signal sequences in proteins, including the site at which they are processed by signal peptidases. The resulting prediction tools, SignalP, are now standard in the field, and the latest version (SignalP 4.0) published in 2011 already has more than 2350 citations. From bioinformatics analysis of signal sequences, Gunnar von Heijne showed that these are largely conserved in organisms from all kingdoms of life, which has been supported by a wealth of experimental data and is now textbook knowledge. Among the findings is the -1, -3 rule, which specifies that the -1 and -3 positions just before the signal peptide cleavage site harbour amino acids with



a small side chain such as alanine and glycine, whereas bulky amino acyl side chains such as those present in aromatic amino acids are excluded. Gunnar von Heijne has published seven articles on predicting signal sequences and cleavage sites, and these articles alone have more than 23,000 citations.

With the advent of gene (and later genome) sequencing, it became apparent that plasma membrane proteins are composed of multiple transmembrane alpha-helices, separated by cytoplasmic and external loops. Gunnar von Heijne noted early on that the cytoplasmic loops have an excess of positive charges compared with the external loops. This bias is particularly manifested by the presence of abundant lysine and arginine residues, and this is now known as the positive-inside rule. This rule has been used for more than two decades to obtain an initial model of the topology of a membrane protein, and it is considered a landmark study in structural membrane biology. Later, Gunnar von Heijne showed that, in addition to the key position of lysine and arginine, integral membrane proteins use the positional information of other amino acids, such as aromatic residues and proline, to insert and maintain the proteins in the membrane, another property that appears to be conserved in species from all kingdoms of life.

Most proteins embedded in biological membranes have vectorial functions: that is, it is essential that these proteins have unique orientation in the lipid bilayer. One of the best understood signals for ensuring this is the positive-inside rule. Analysis has, however, shown that many membrane proteins contain homologous domains with opposite membrane orientation. The presence of such proteins led Gunnar von Heijne and colleagues to search for membrane proteins with a weak or no bias for positive-inside residues. They found that individual subunits of the multidrug transporter EmrE have such dual topology properties, and very recently he showed that these different subunits attain their final orientation independently of dimerization, suggesting that topological inversion of membrane proteins is unlikely to happen *in vivo*. This has broad implications for membrane protein evolution.

Gunnar von Heijne has also used his extensive competence on membrane proteins to develop a clever method that enables the determinants of membrane topology to be studied. Taking advantage of the positive-inside rule and the insertion of specific glycosylation sites, he developed a simple assay that enables researchers to determine whether a certain protein sequence is embedded in the membrane. This is now widely used, and it has allowed any potential peptide sequence – natural or synthetic – to be tested for its suitability for being embedded into the membrane.

Gunnar von Heijne's production of scientific articles is truly impressive. The quality and high relevance of his work is clearly demonstrated by the very high number of citations for his articles. The fact that many of his articles are highly cited even many years after their publication is an excellent indicator of the fundamental, long-term impact of his research and its original and independent nature. Besides his impressive scientific production, Gunnar von Heijne plays an active role in popular-science writing and is actively engaged in communicating science to society in general. He has received numerous awards and honours and is a member of several academies, including the Royal Swedish Academy of Sciences, through which he has served over several years on the Nobel Committee for Chemistry, both as Chair and currently as Secretary.

In conclusion, the work of Gunnar von Heijne can be characterized as highly original, often surprising, elegant, thought-provoking and imaginative. By combining theoretical and bioinformatics work and systematic biochemical testing and validation, he has developed a series of concepts that are now textbook literature. His work represents outstanding research contributions that have benefited the development of innovative biotechnological solutions. Gunnar von Heijne is therefore an undoubted and worthy recipient of the 2018 Novozymes Prize.

# How life *(un)folds*

By Morten Busch

Life seems to be ruled by chance: where we are born, what we become, who we meet, how we die. Novozymes Prize winner Gunnar von Heijne has devoted his life to bringing order into the chaos life constantly seems to create. He has become an expert in predicting how life unfolds – and how it folds. He is a pioneer in developing biological prediction methods.

Proteins are the building blocks of all life. Most proteins fold correctly – but when they misfold, diseases can evolve. Understanding how proteins fold and are transported to the right destination inside the cells is crucial to understanding life. Particularly important is how the proteins are embedded in the cell membranes, where they serve as ion channels, pumps and receptors – ensuring that the cells get the right signals from the outside and are maintained sustainably on the inside.

"About half the drugs we use that you can buy in a pharmacy actually target membrane proteins. Of the total number of proteins, maybe 30% are membrane proteins, but more than 50% of the drugs target the membrane proteins, so they are disproportionately important."

## A cryptophysicist

Gunnar von Heijne's very theoretical approach in often highly experimentally oriented biology has created great revolutions, but why this career path? Chance, he claims. His interest in science arose very early. He grew up in the 1960s – the heyday of science and technology with a space race, computers starting to be developed and much happening in science. Most people were excited about science and technology.

"I remember getting a couple of Christmas books; one was a popular science picture book about mathematics, and I still remember a picture of a French mathematician lying on his back in his office on a couch and looking at the ceiling and thinking deep thoughts about mathematics. That must have made a great impression on me. I must have thought that's how to spend my life."

He also got another Christmas book about rockets and space, but the lying-on-the-back image triggered his interest in science. This interest got his chemistry teacher to give von Heijne a stipend in high school to attend the Berzelius Days, an annual event of the Swedish Chemical Society, but spectacular and colourful experiments did not excite him.

"About half the drugs we use that you can buy in a pharmacy actually target membrane proteins. Of the total number of proteins, maybe 30% are membrane proteins, but more than 50% of the drugs target the membrane proteins, so they are disproportionately important."

"The only thing I remember was a booklet about quantum chemistry, really theoretical chemistry, that I don't think I had been exposed to in high school, and I thought it was really neat that you could calculate things about molecules. I felt attracted to the theoretical aspects from the very beginning, but I had a better chemistry teacher. So, I was kind of a cryptophysicist."

## Totally crazy

Although theoretical physics fascinated von Heijne, the inspiring teacher got him to choose the more practical chemistry studies at the Royal Institute of Technology in Stockholm. But when he had to choose a topic for his MSc project and asked his teacher Stig Ljunggren for advice, fate determined that this chemist also had a physicist hidden inside:

"I've done this microwave spectroscopy all my life, but we measured it, and you can only do this on very simple molecules, and we measured all the molecules you can do it on. So, this has no future. So don't come to me to do your master project. But instead, the guys who really know the game are the theoretical physicists, so get in touch with the physics department and see if they have anything that you can do."

However, the theoretical physicists had no great interest in chemistry. Fortunately, a young professor with a certain interest in "chemistry or biology or something" had just been hired. Clas Blomberg later also became von Heijne's PhD supervisor. However, Blomberg would not tell von Heijne which project to choose.

"He said: 'You have to find your own project and your own problems to work on.' Which is totally crazy: how can you just set a PhD student loose like that? But that is how it was in that department, and for me it was great, because we were a couple of PhD students in that group, and we were all shopping around for projects, reading papers and talking: 'Maybe we can calculate this, maybe we can make a model of that.'"

## Picked up by a Nobel laureate

The inspiration for what would become Gunnar von Heijne's lifelong interest came by serendipity. He had a very good French teacher in high school and because he liked French he took an evening class during his PhD studies and subscribed to a popular French science magazine: *La Recherche*.

"One day I happened to read a short article, a one-pager, about the signal hypothesis. There was a little cartoon, and it didn't make sense to me because it had some strange interactions between a protein chain and a membrane that I thought couldn't be right. So I looked up the original paper and read a bit more and realized that here is another thing that maybe we can make a simple theoretical model of."

The cartoon was from a paper that is now very famous and that later led to Günter Blobel winning a Nobel Prize. In the paper, Günter Blobel and Bernhard Dobberstein showed the first aspects of how proteins are secreted from a cell. As it turned out, von Heijne was the first to try to model this; Günter Blobel picked up on his paper and von Heijne was invited to present the model at a meeting in the United States.

"Everybody was there. This was when the field started, and the people there were all young scientists who drove this field for the next 20 years. I had never been to anything like it before. Everyone had 10–15 minutes to present and no time for introductions. So, it was super intense and super exciting and, of course, you know about the American way of creating excitement. So this really opened a completely new world for me."

## Big in pharma

The speculation at that time was that the secreted proteins all start with a short sequence, 20–25 amino acids at the beginning, that serves as an address label and thus tells the cell if, for example, the protein should be secreted from the cell. After the meeting in the United States, von Heijne tried to improve his models but could not do any experiments, so he felt stuck.

"But again, I was lucky. People were starting to sequence DNA then. I had the idea that I could collect some of these sequences and compare them and see whether they have anything in common that would define what a molecular address label looks like. So I did this: I collected maybe 20–30 of these sequences and compared them." What emerged was a canonical design of this little address label in the beginning of these proteins.

"If you count from the cleavage site, from the side where this peptide is cleaved from the protein, we called the amino acid right before the cleavage site the 'minus one' position, and the one two positions over the 'minus three' position. These two invariably turned out to be small amino acids."

Together with Henrik Nielsen and Søren Brunak at the Technical University of Denmark, von Heijne eventually developed more advanced prediction methods that not only predict cleavage sites but also identify entire segments as being signal peptides. Today all the big biotechnology and pharmaceutical companies have built these signal peptide prediction tools into their own sequence analysis pipelines. This knowledge is essential in producing protein-based medical products on a large scale using microorganisms.

## The positive-inside rule

The next step was natural for von Heijne, the theoretician. While looking at this very early part of the protein – the signal peptide – von Heijne would also look at the rest of the protein. Many proteins that have signal peptides are membrane proteins, meaning that they must have a signal peptide that targets them to the cell membrane.

"Instead of being completely secreted across the membrane, segments of the protein become inserted into the membrane. By just looking beyond the signal sequence and looking at other parts of the protein, I started to see that membrane proteins have segments of very non-polar, hydrophobic amino acids that eventually end up spanning the membrane."

The membrane-spanning segments fold up as helices inside the double-layered membrane. But between the helix segments, the proteins have stretches that form loops – either outside or inside the cell. This time, von Heijne's ability to predict led to more material for biochemistry textbooks.

"I think I originally looked at 10–15 proteins. But even this small number showed that the inside loops have a very different amino acid composition than the outside loops. In particular, what differs is that positively charged amino acids are much more abundant in the inside loops. So this became known as the positive-inside rule."

Gunnar von Heijne's positive-inside rule for the first time described which way a protein would face when it is inserted into the membrane: which parts would remain facing inside and which parts would be transported across the membrane to face the outside of the cell. Like the signal peptide predictions, this rule turned out to have lasting effects, since it enabled both science and industry to predict the structure of membrane proteins and thus be able to engineer new ones.

## Ad on a wall

Gunnar von Heijne's career then took a new and unexpected turn. In 1995, while his children were still young enough to go abroad for a semester, von Heijne and his wife spent a winter in Los Angeles visiting Bill Wickner's laboratory at UCLA.

"When I arrived with my laptop, he looked a little wary and suggested that I do something different for a change. He suggested I get my hands dirty in the lab. I decided to test the role of positively charged residues for membrane protein topology."

Importantly, the experiments showed that membrane proteins can be turned on their head in the membrane by relocating positively charged residues. More importantly, this experience moved von Heijne's research in a direction no one could have foreseen – even 6 months earlier. He became an experimentalist. The trip also brought about another piece of luck.

"At a group meeting in Bill Wickner's laboratory, I glanced at the wall and saw a little ad from Henrik Garoff, who had just become a professor of molecular biology at the Karolinska Institutet in Stockholm. He was advertising for faculty to join his department. And I thought, maybe this is my chance."

## Inside out – outside in

Gunnar von Heijne sent a fax to Henrik Garoff asking whether there was any chance he could do some experiments in his department. The next day he got a fax: "When do you want to come?" Soon after that, von Heijne moved to the Karolinska Institutet and immediately started setting up a wet lab.

"That was one of the best pieces of luck I have had throughout my career. It's great to do theory and it's great to do prediction and you can find things by theoretical studies, but to actually see things work in the real world and to design an experiment and see the system behave as you thought it might behave. Or perhaps even more interesting, if it doesn't behave as you thought it would behave."

At Karolinska, von Heijne did many experiments to determine the fine details of the positive-inside rule and how the interplay between hydrophobic and charged regions affects the folding of membrane proteins. Some years later, he became a professor at Stockholm University and soon after that, he stumbled across a very interesting aspect of membrane proteins.

"Normally you would think that one part is always on the outside, while another is always inside, since the outside part has a different function than the inside part. But we stumbled across a class of membrane proteins that randomly inserts either way. How could, and why would, nature design a protein of that kind?"

Initially, von Heijne thought that dual typology proteins were an exception, but in a series of landmark publications, he and his colleagues showed that dual typology proteins are actually very common and especially among proteins that can transport small molecules back and forward across the membrane in and out of the cell. For example, cells use this transport mechanism to neutralize the effect of toxic compounds.

## The perfect system

Gunnar von Heijne's new mix of basic theory with experiments has turned out to be a potent one that has solved longstanding quandaries and invented new standards in the field. At a meeting at the University of Illinois Urbana-Champaign, von Heijne walked back to the hotel with his colleague Steve White.

"He had developed a new hydrophobicity scale for amino acids, where they were listed according to their tendency to stay in the membrane versus staying in the aqueous phase outside the membrane. What he needed was a way to measure inside the cell instead of in a test tube with purified proteins."

When White explained this, von Heijne realized that they had already developed the perfect experimental system to do this. This spawned long-term collaboration between them to construct a new biological hydrophobicity scale.

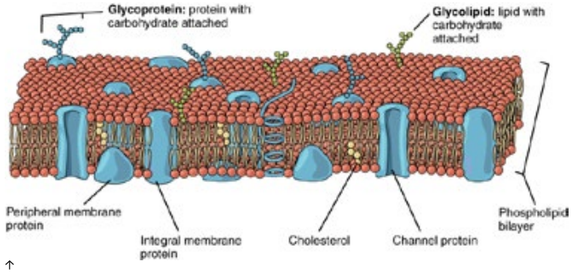
"This has given chemists invaluable insight into what it takes to make a segment of amino acids insert into a membrane – knowledge that is important in designing new enzymes for bioengineering and biomedical purposes."

However, once more – by chance – von Heijne changed his focus to start working on soluble proteins. He read a paper, this time by a group in Kyoto, Japan led by Koreaki Ito, describing short segments of a protein that have an uncanny ability to glue themselves into the tunnel of the protein production unit – the ribosome.

"So these peptides fit into little pockets in the tunnel, and they basically hold on to the tunnel walls and prevent further elongation of the protein chain. They arrest translation, leading to their name: arrest peptides. It was speculated that these arrest peptides might be pulled out of the ribosome by some external force and allow protein synthesis to continue."

Gunnar von Heijne's idea was to use these arrest peptides as force sensors: little devices that can be implanted in a protein that essentially measure forces acting on the protein chain at the precise time when the arrest happens. When the force of the growing proteins pulls sufficiently, the arrest peptides will dislodge.

"And this works. Now if we put a little arrest peptide right after the protein, when the protein gets into a location where it can start to fold, the folding process itself starts to pull on the arrest peptide. So now we have a force-measuring device we can engineer into any protein and look at the forces acting inside the cell on proteins as they are coming out of the ribosome."




↑ The membrane-spanning segments fold up as helices inside the double-layered membrane. But between the helix segments, the proteins have stretches that form loops – either outside or inside the cell. Gunnar von Heijne's ability to predict led to more material for biochemistry textbooks.



## No particular reason

Gunnar von Heijne's journey from theoretical physics to molecular cell biology has pushed the whole field forward towards more physical thinking and more rationally designed experiments that can get new essential information from a very complex system.

"I think I was lucky because there weren't that many people coming from my kind of background, so there was very little competition. Nobody else was crazy enough to do these theoretical things. Everybody was just working at the bench. Molecular cell biology is such a rich field that being able to think about the more basic sort of fundamental issues is a good thing."

According to von Heijne, life is chaotic, and life events very often happen by chance, pushing you one way or another. Or you choose to go one way over another for no particular reason.

"Nevertheless, maybe from a deep psychological viewpoint, doing science is in a way trying to take control, trying to understand enough about the system that you can predict and control what's going to happen. I don't know if that's anywhere near true, but it's kind of an interesting dichotomy in life: being a scientist and also being a human being."

Although von Heijne has spent his whole career predicting, he had not predicted that he would receive the Novozymes Prize. However, he knew from his many years as a Chair and Secretary of the Nobel Committee for Chemistry that prize recipients are often surprised when receiving the good news.

"Typically, the news triggers one of three reactions. Some think it is a practical joke and take a while to be convinced. Then some think and may say that it was about time they got the prize. Finally, the members of the third and largest group are astonished and speechless that their colleagues have honoured them in this way. I belong to this last group and am deeply moved that researchers from outside my field have found me a worthy recipient of the Prize."

# Curriculum Vitae

## About Gunnar von Heijne

**1975**
MSc in Chemistry and Chemical Engineering, Royal Institute of Technology, Stockholm, Sweden

**1980**
PhD in Theoretical Physics, Royal Institute of Technology, Stockholm, Sweden

**1980–1981**
Postdoctoral fellow, University of Michigan, Ann Arbor

**1987**
Visiting Professor, Molecular Biology Institute, University of California, Los Angeles

**1989–1994**
Associate Professor, Karolinska Institutet, Stockholm, Sweden

**1994–**
Professor of Theoretical Chemistry, Stockholm University, Sweden

**2000–2006**
Director, Stockholm Bioinformatics Center, Stockholm University, Sweden

**2006–**
Director, Center for Biomembrane Research, Stockholm University, Sweden

**1998–2009 and 2014–**
Member, Nobel Committee for Chemistry

# The Novozymes Prize Committee

The Novozymes Prize is a European research award instituted by the Novo Nordisk Foundation. The Novozymes Prize is awarded in the name and with the funds of the Foundation. The purpose of the Prize is to raise awareness of basic and applied biotechnology research.

The Novozymes Prize is awarded to recognize outstanding research or technology contributions that benefit the development of biotechnological science for innovative solutions. It consists of a funding amount for the Prize recipient's research of DKK 2.5 million and a personal award of DKK 0.5 million. An additional element of the Prize is an international symposium within the Prize recipient's field of research. Prize recipients must have a current position at a public or non-profit research institution in a European country. They may previously have worked anywhere and may have any nationality.

The Novozymes Prize is awarded by a prize committee that selects the successful candidate based on scientific achievements after a confidential nomination and review process.

The members of the Novozymes Prize Committee are appointed by the Novo Nordisk Foundation Board of Directors. The 2017 Committee comprised the following six members:

- Jens Nielsen, professor, chair
- Søren Molin, professor
- Henrik Callesen, professor
- Liisa Viikari, professor emeritus
- Michael Broberg Palmgren, professor
- Birgitte Nauntofte, CEO, Novo Nordisk Foundation

The award event takes place in the spring at the Novo Nordisk Foundation Prize Celebration, at which the Novo Nordisk Prize is also awarded.

In addition, in celebration of the award, the recipient gives a lecture lasting about 1 hour at his or her workplace. Before the end of the year, the recipient and the Foundation arrange an international symposium within the scientific field of the Prize recipient.

Candidates for the Novozymes Prize can be nominated by the Prize Committee and former Prize recipients. In addition, a call for nominations is published in the spring, and candidates can be nominated based on this call.

The Committee meetings thoroughly discuss the nominated candidates with regard to their research contribution and impact, and a comprehensive bibliometric report is produced. A few candidates are then selected for thorough international peer review. Based on the international peer reviews, the Committee reaches a decision about the year's Prize recipient.






Previous recipients of

## The Novozymes Prize 2015–2017

| | |
|---|---|
| 2015 | Professor, Director Bernard Henrissat |
| 2016 | Professor Jens Nielsen |
| 2017 | Professor Emmanuelle Charpentier |
| | Professor Virginijus Siksnys |

Novo Nordisk Foundation
Tuborg Havnevej 19
DK-2900 Hellerup,
Denmark
Phone: +45 3527 6600

nnfond@novo.dk
www.novonordiskfoundation.com

novo
nordisk
fonden