# The mastermind research method: cracking the code

Mr Rector Magnificus, dear colleagues, dear friends, family, very valued listeners,

If I asked you if you would like to grow old, many of you would say: “Yes, but healthily”. Being healthy or feeling healthy is very important, and maintaining a good health or recovering it is worth a lot. We can certainly contribute to that by living healthily, and therefore have a primary responsibility in reducing our chances of becoming ill. But, there is no guarantee. Everyone can get sick. Some of those diseases can pass on their own. Another part will have to be treated with a drug, for a short period if the disease can actually be resolved, or constantly to at least suppress the symptoms of the disease. An important category of disorders are the brain disorders. Examples are: epilepsy, depression, trauma, stroke, brain tumors, Parkinson's disease and Alzheimer's disease. I do not have to tell you, I think, that the consequences of brain disorders can be huge. With many brain disorders, the functioning of the brain and thereby the skills of a person changes. In certain cases, this may lead to changes in feeling, behavior, memory, and even personality or character. It therefore has a major impact, not only on the person with the brain disorder, but also on the loved ones and on the social environment. Unfortunately, most brain disorders cannot be treated adequately. So there is still a lot to do. The brain has always fascinated me. This intriguing body manages all our functions. It also makes us the way we are. It determines our personality.
Both the awe for the normal functioning of the brain and the often shocking consequences of many brain disorders led me to do dedicated brain research with the aim of helping improve brain therapies. In this inaugural lecture I will try to convince your-of the need to pay much more attention in the research and development of brain drugs to the time dependence and interdependence of the many body processes that determine the effect of drugs.

## The key players in the effect of a drug on the brain

After administering a drug, a drug must first be able to reach its workplace. Then, it can start there and have an influence on body processes, which together ultimately determine the effect of the drug. In the body, drugs can move between blood and tissues quite easily through a process called diffusion. That works like a little dye in a glass of water: the entire water slowly colors until the concentration of the dye is the same everywhere. However, the brain is protected; the blood vessels of the brain form the blood-brain barrier. Only small substances and drugs can cross the blood-brain barrier through diffusion. Depending on the drug properties, that process goes faster or slower. Other drugs must be actively transported through the blood-brain barrier (hereafter: BBB) if they are to get into the brain. There are also many body components that drugs can bind to. For example, drugs in the blood can be bound to large blood proteins. If drugs are bound to those large blood proteins, they can no longer pass the BBB. So it is important to know what those unbounded drug concentrations are. We have to hold on to that. Once in the brain, there is a complex interplay of diffusion, transport through fluid flows, and active transport, which together determine whether a drug can arrive at the right workplace, at the right time, with the right concentration. But it can

also end up in an unintended place, which can cause side effects. Furthermore, a drug can also bind to brain components in the brain. In that case it is therefore not available for binding to the workplace, and it cannot work. Again, the unbound drug concentrations are also important here. You may already have lost count, but the most important thing to remember now, is that unbound drug concentrations are determined by many body processes that take place simultaneously and are interdependent. Let's compare it to a sports team. For a match, the players must be at a certain place, the sports hall, at the time of the match, and there must be enough players to be able to play the match. And players who hang out at the bar in the canteen, and who are therefore not in the sports hall, do not participate in the competition.

## The importance of correct measurements – to measure is to know

After studying Biophysical Chemistry in Groningen, I conducted my PhD research here in Leiden, under the supervision of: Prof. Meindert Danhof, Prof. Douwe Breimer, and Dr. Bert de Boer. My goal was to investigate whether the microdialysis technique could be used to measure unbound drug concentrations in the brain, in order to determine the BBB transport of the drug. The microdialysis technique uses a very small tube. We can let a liquid flow run through this tube, to the end and back again. At the end of the tube is a membrane with small holes. As a result, only small substances from the environment can enter the tube. When small substances enter, the liquid flow will entrain the substances. This liquid stream is successively collected in fractions. The dust concentration is then measured in each of these fractions. So that can be the drug. In that case we obtain information about the time course of the unbound drug concentration at a certain place in the brain. The insertion of that small microdialysis tube into the brains of small laboratory animals requires very sophisticated microsurgical procedures. Small cannulas must also be placed in the blood vessels. The test animal must then have a recovery period. During the experiment, a drug can be administered into the blood via one cannula. Blood samples can be taken via the other cannula. The microdialysis samples are also collected at the same time. During this experiment the test animal can move, eat, drink and sleep freely. With this experiment we obtain a lot of related information from one and the same test animal, and we therefore need fewer test animals. I hear you thinking: but if you insert such a tube into the brain, doesn’t that result in damage? And then you may be measuring something that you do not mean to measure. Very well! That was precisely the question that I wanted to answer with my PhD research: is the microdialysis technique suitable for measuring BBB transport or is BBB transport fed by inserting the tube? Before that I did a number of investigations and the microdialysis technique came through brilliantly. Part of this research was conducted in collaboration with Dr. Alfred Schinkel, Dr. Jan Wijnholds and Prof. Piet Borst; the top researchers from the Netherlands Cancer Institute. They had genetically modified mice, with which we could do research on active BBB transport systems.

*A step back in time*

In the 80s and 90s of the last century, microdialysis was actually only used to measure the body's own substances that can ensure signal transmission between brain cells. These are the so-called neurotransmitters. Drugs can change these neurotransmitter concentrations and

thereby influence the functioning of the brain. In many studies, neurotransmitter concentrations were measured before and after drug administration. However, such effects were not associated with the unbound drug concentrations in the brain. If you measure changes in neurotransmitter concentrations for a particular drug, you do not know which unbound drug concentration in the brain caused this. So you have thus not gained insight into the mechanisms of action of the drug. For example, if there is no effect, you do not know if this is because the drug cannot get into the brain or if the drug does not work. Specifically that is what we wanted to know and so we started measuring. The quantitative measurement of drug concentrations in the brain with the microdialysis technique was revolutionary, yet it was not frequently used. Many thought it was too complex. After all, you could always use the same analysis to measure neurotransmitters. To quantitatively measure drug concentrations in blood and microdialysate, you had to develop new analyzes for each drug. Many researchers therefore preferred not to be that complicated. In addition, there were also many researchers who were unable to properly estimate the quantitative measurement of unbound drug concentrations.

With Prof. Margareta Hammarlund-Udenaes from the University of Uppsala in Sweden I have had many discussions about the BBB transport of drugs. My microdialysis results provided new insights into the mechanisms of BBB transport, and old paradigms could be thrown overboard. For example, previously no explicit distinction was made between the speed and extent of BBB transport. In other words: apples and pears were compared.

I may not have to explain why speed of BBB transport is important. You can imagine that we want a drug that works quickly against a migraine attack, so it can get into the brain promptly. However, that speed for combating a chronic brain disorder, such as schizophrenia, is much less relevant.

The extent of BBB transport is an equilibrium ratio. Namely, the ratio between the unbound drug concentrations in the brain and those in the blood. If only diffusion takes place, and the unbound drug concentration in the blood is kept constant, then the unbound drug concentrations become the same everywhere. Then this ratio will be equal to 1. So, everywhere unconstrained concentrations, whether that takes a short or a long time due to fast or slow transport over the BBB. But for drugs that are pumped out of the brain by active transporters, that ratio will therefore be smaller than 1. So you have to take this into account if you want to achieve the right brain concentrations.

*Another short flash back*

At the end of the last century, Margareta and I were at a convention in Tenerife, the "Monitoring Molecules in Neuroscience". Erik and Sjoerd were with us. Sjoerd still in the package. Margareta and I stood there in a corner, by our posters with our results about the BBB transport of a number of drugs, measured with microdialysis. There was actually no attention for it.
That was the moment when we decided that this had to change. For example, we started organizing the international symposia on "quantitative microdialysis in drug research and development". We have thus formed a community of quantitative microdialysis scientists. This is still active. Next year, in June 2020, the 9th Symposium will again take place in Berlin.

In summary, the microdialysis technique was revolutionary in that it can provide information about the time course of unbound drug concentrations in the brain, with which you can determine the BBB transport of drugs. This brought us a big step forward, and a great deal of mechanistic insight could be gained into the processes that determine the BBB transport of drugs. The processes that subsequently determine the distribution of drugs within the brain, including the workplace, could also be further mapped.

I have to admit that for quantitative measurement of drug concentrations in the brain you have to do a lot. To be honest, I sometimes got tired of it myself. But then you have something! I will make that clear to you later.

In addition, Margareta and I started the annual course on "BBB transport in drug development: principles, measurement methods, and influence of diseases". This course is full of important new insights and without exception extremely high. This October in Uppsala the course is celebrating its 10th anniversary.

## Unmet need for better brain drugs

As stated earlier, most brain disorders cannot currently be adequately treated. In addition, we are dealing with a growing number of people with brain diseases. Let us take dementia, public illness no. 1, as an example. There are currently more than a quarter of a million people with dementia in the Netherlands. Despite the fact that we know more about how we can help prevent these diseases through a healthy lifestyle, this number will increase sharply in the coming years due to the increasing number of elderly people. So there is a great need for good drugs for dementia, but also for many other brain disorders.

## Problems in the development of brain drugs

The development of drugs for brain disorders is one of the most challenging tasks for the pharmaceutical industry. Therefore:

1. You need to know what is different or what has changed in the brain of a patient with the brain disorder - so you need to have insight into the disease
2. You need to know what the workplaces are where brain processes can be influenced in such a way that they can run more normal again.
3. Do you have to make the drug that can cause that influence at that workplace, and
4. Must this drug have the right concentration at the right time, in the right workplace, in order to have the right influence?

This means that for the development of good drugs for brain disorders, there must be sufficient knowledge and understanding on all of these four points. It is crucial to sufficiently understand the time dependence and interdependence of all processes that play a role in this. We do not have that enough understanding. We can state that the development of brain drugs is problematic.

Naturally you may ask yourself: “Why does it go wrong so often? ". A very good question! I'm going to try to answer that.

First of all, we have insufficient insight into the processes that play a role in the development and further development of many brain disorders. Underlying genetic, physiological, neurochemical, neurodegenerative and inflammatory processes often play a role simultaneously. The contribution of each of these disease processes can furthermore vary greatly between patients. So it's complex.

In addition, the diagnosis of, for example, Parkinson's disease and Alzheimer's disease can only be made at a relatively late stage of the disease. Often so much has changed in the brain that the chance of a cure for the disease is zero. At best, the further development of the disease can be delayed, or only symptoms of the disease can be suppressed. It is therefore important to investigate what is going wrong at an early stage. Then we can probably still really intervene.

Then the question: what should we actually do? If we have already found a process that has been derailed in a brain disease, how can we prevent or eliminate this derailment. What should we intervene on? And also, when so many processes play a role at the same time, how big is the chance that we will be successful by tackling only one process?

And how do we know if the drug ends up at the right time, in the right place, with the right concentration? Is the drug getting too little, too much, or exactly enough? Is the drug coming too slow, too fast or exactly on time?
For this we therefore need that knowledge about the unbound drug concentrations in the blood and in the brain, with which we can determine the speed and extent of BBB transport of the drug. But we also need to have knowledge of the distribution of the drug in the brain, through diffusion, fluid flows, active transport and the binding of the drug to brain tissue components.
Far too little attention has been and continues to be paid to the impact of all these factors and their interdependence in the pharmaceutical industry.

And what then is the binding of the drug to the workplace? This binding is usually only determined in a cell system, or in laboratory animals with a PET scan, while it ultimately depends on many body processes, which can be very different in humans - and certainly in patients – than in the cell system or laboratory animal.

Another problem is that we are not allowed to take measurements in people's brains.

*Just an interactive interlude: is any of you prepared to have a tiny microdialysis probe implanted in the brain for scientific research? And then it remains deafeningly silent. That says it all.*

So those crucial unbound drug concentrations cannot be measured in people's brains. There have been exceptions, for example with traumatic brain damage. The microdialysis technique provides information about the patient's condition rather than clinical signs by measuring the body's own fluids. And in those microdialysis fractions which were collected for that purpose, the drug concentrations could also be determined. For example of morphine. However, today it is forbidden in many countries to use the microdialysis samples to measure drug concentrations, because that would not be in the patient's immediate interest. But the tubes are already there…. I wonder how we can ever get further with this. Now I hear a few of you

think: can we measure things in people's brains with MRI and PET scans? You are right about that. With MRI and PET scans we can make a number of physiological processes visible. With PET scans, drug-workplace binding can also be determined. But unfortunately this information is insufficient to be able to determine the interdependence and time dependence of processes.

And then I think we have the biggest problem: for many years, researchers in the pharmaceutical industry and also in academic institutions have ignored the complexity of the brain. For example, many measurements were performed in cell systems and test animals that could not be compared directly with each other. In this way you cannot gain insight into the mutual coherence and time dependence of the body processes that you need to be able to make the transition to humans. Particular emphasis was placed on the speed of the measurements for a huge number of candidate drugs, the so-called high-throughput. In my eyes, a waste of time and all that money! And for academics; due to assessment criteria, they are under pressure to publish quickly and extensively. Then it is more beneficial to measure fragments and publish on each, instead of publishing one in-depth research into complex coherence and time dependence. As far as I'm concerned, something is wrong there. If we look at the efforts and the money invested in the development of drugs for the treatment of Alzheimer's disease. That has led to major disappointments so far. A number of pharmaceutical industries have therefore pulled the plug from drug development for Alzheimer's disease. That speaks volumes.

*It has become a valley of tears ... is there still hope?*

## What are important steps to a solution?

In my opinion, we need a very structured and extensive research approach, and therefore also a long breath. And with that we finally come to the title of my inaugural lecture: "**The mastermind research method for cracking the code"**. What do I mean by that? I am referring to the Mastermind group game. This game is known to many Dutch people. For those who don't know it a short explanation. It is a game between a codemaker and a code cracker. The codemaker makes a secret code and chooses five of the ten available colors for this and puts it in a certain order. The code cracker must be able to crack the code in a number of steps. The code cracker first places a random set of colors in a random order, so a complete guess. The codemaker gives feedback on the number of good colors and the number of good colors in the right position with white and black pins. To crack the code it is very important that the code cracker logically analyzes the feedback from the codemaker to test a next strategically chosen new combination of colors. It should be clear that the use of only one color will not cause the code to crack.

I use this game as an allegory for the way I think research should be conducted. Nature has the codes, and we, as researchers, have to crack the codes. Because processes in the body are coherent and time-dependent, we must therefore ultimately not study them separately, but as much as possible in conjunction. The codes of nature are of course much more complex than those of the Mastermind game. As researchers, we therefore have to use advanced mathematical models, which we of course design ourselves, but with which the computer can

do calculations that we ourselves could not, or certainly not, be able to do so quickly. The ultimate goal of such a mathematical model is that the result of calculations must match the measurement data we have obtained from research. If so, we cracked the code, as it were.

## An example of the mastermind research method

I would like to share all the research with you, but there is simply no time for that.
Let me try to explain here how we have developed our best model so far. This can predict well what the unbound drug concentrations are at various locations and at different times in the brains of laboratory animals and humans.

For that, I first take you to mathematical models that you all use: the navigation systems. Navigation systems can calculate how quickly and via which routes you can reach your destination. You can enter how you travel, and the system knows which roads and transport routes are available, at what speeds you can use them, and, depending on the time, to what extent the journey can be a little slower due to the traveling of fellow human beings, or is influenced by a road break, or you name it.

I come back to the sports team that has to play a match. Let's take the sports hall in Leiden as the venue for the competition. The game can only be played there if there are enough players in the sports hall to be able to do their work there during the game.
In drug language: the right concentration, the right workplace, and the right time. The navigation system can predict how quickly you and your teammates will arrive at the workplace, the sports hall.

If the next match is to be played with that team in the sports hall in The Hague, the team is the same, but the city is different. And even though cities have many similarities, such as having roads, canals, bridges, railways, buildings, etc., transportation is not the same because the maps of the cities are different. A navigation system has therefore collected extensive information about the city map, the average crowds at different locations in the city, at different times, in order to be able to make a good travel forecast.

We actually did the same for the brain and the drug. We have, as it were, developed a brain drug re-activation system. For this we first determined the map of the brains of laboratory animals, based on data from the literature. We then placed small microdialysis tubes in various places in the brains of laboratory animals in order to collect data about the “pressure” of the drug in various places in that brain map. We have brought all this information together in a mathematical model. So we have developed the brain drug navigation system for the test animal. We then replaced the map of the brain of the test animal with that of humans. And with that we also have the brain drug navigation system for humans. The microdialysis technique has played a crucial role in the development of this model, and it has taken around 12 years. The PhD research of the PhD students Joost Westerhout and Yumi Yamamoto was of great importance in this. And now we have something! Instead of using laboratory animals, it is sufficient to enter the properties of a drug, or candidate drug, into the model, and then determine how it ends up at different brain locations at different times. This is very useful for the pharmaceutical industry, for testing candidate drugs, but also for designing clinical trials in humans.

*Of course we don't stop there!*

We already have the basis for further development of this model ready. In addition to predicting the unbound drug concentration at different locations in the brain, we also want to incorporate the work of those drugs in different workplaces.

The insights into the interaction of drugs with their workplace come from Wilbert de Witte's recent PhD research, which we recently published in Nature Drug Discovery Reviews.
This research is also based on the coherence of different body processes. The starting point was the idea that a long interaction of a drug in the workplace, or a long residence time in the workplace, results in a long effect of the drug. Wilbert has demonstrated, among other things, that the residence time measured in a cell system does not guarantee a long residence time of the drug at the workplace in the body, because the residence time at the workplace in the body is also influenced by other body processes. Conversely, he showed that a high concentration of drug at the workplace in the body can ensure that interaction with the workplace can continue for a long time. In sports terms, if you have a very large team you can still play the game despite the departure of a number of players.
In short, understanding the coherence and time dependence of all body processes appears to be important again. This is therefore a huge step forward in being able to further predict the effects of drugs on the body.

In addition, through the recent PhD research by Willlem van den Brink, we have made further pioneering steps in better understanding not only the intended drug effects, but also effects that were not anticipated. Of course, we don't want to discover these surprises until the drug is tested in people, but before that time.
By measuring not only the drug in blood and microdialysis samples from laboratory animals, but also as many body-specific substances as possible, Willem determined the relationship between the drug concentrations and the drug effects on the body's own substances. For the measurement of those many bodily substances, use was made of the so-called metabolome platforms of Prof. Thomas Hankemeier. Willem has in this way indeed found drug effects that were unforeseeable. The research of Willem van den Brink is therefore an important step forward to a more complete understanding of how a drug works.

## Our research today and tomorrow

With my current team of PhD students: Esmée Vendel, Anthony Gebhart, Tian Qin, Mohammed Saleh, and post-docs Yeon-Jung Seo, Luc Bisschoff and Frédérique Kok, we will continue to fight for better therapies for brain diseases, and also for other diseases.

In collaboration with Dr. Vivi Rottschäfer of the Mathematical Institute of our faculty, Esmée Vendel is conducting her PhD research into a 3-dimensional mathematical brain model. We started by mathematically defining the smallest possible brain block, which has the most important properties of brain tissue. We can eventually connect these blocks as lego bricks to imitate the brain in length, height and width. The great thing about this approach is that we can give each block certain characteristics. In this way we can investigate the 3-dimensional coherence of drug transport and workplace binding. This is important for brain diseases that have an impact on a certain area of the brain, such as brain tumors and brain trauma.

In collaboration with Dr. Jeroen Elaiassais-Schaap, Mohammed Saleh is conducting his PhD research into further improving the aforementioned brain drug navigation system. This is because it must be made suitable for further expansion. But Mohammed is also investigating how we can make the brain navigation model disease specific. The focus is primarily on traumatic brain damage and Alzheimer's disease. In these diseases, the "map" of the brain has changed. And that can therefore have consequences for brain drug concentrations, and their interactions with the workplaces in the brain, which may have changed again due to the disease itself. For traumatic brain damage, we have already had good cooperation with Prof. Dick Tibboel and Naomi Ketharanathan from Erasmus Medical Centre and we are looking for opportunities to continue this.

In collaboration with, among others, Dr. Dymphy Huntjes of Janssen Pharmaceutica and Dr. Jeroen Elaiassais-Schaap, Anthony Gebhart is conducting his PhD research on the relationship between drug concentrations in the gut, the intestinal wall and the blood, in order to ultimately be able to predict which drug is best at what concentrations. fight colon cancer. It may be interesting for you to know that there are a number of important similarities between the BBB and the barrier of the intestinal wall.

Then, in collaboration with Dr. Jonathan Mochel of Iowa State university in the United States, among others, the postdoc Yeon-Jung Seo conducts research into Parkinson's disease. We are investigating how we can achieve better L-DOPA concentrations in the brain for better treatment for Parkinson's disease. For this we also use the brain navigation model, as well as a secret form of administration about which I am not allowed to say anything here.

In collaboration with Prof. Elga de Vries of the Amsterdam University Medical Center, Dr. Geert Jan Groeneveld from the Center for Human Drug Research, and Dr. Eric Wong from Union Chémique Belge , are conducting PhD research on the early detection of Alzheimer's disease. The postdocs Luc Bischoff and Frédérique Kok also work in this area.

Alzheimer's disease has become a spearhead in our team because, as mentioned, despite all investments, recent clinical trials have failed again. There is an enormous amount of information but there is no coherence in the body processes that determine the course of Alzheimer's disease. In order to find possible treatment or perhaps cure for Alzheimer's disease, we really have to do things differently. As advocated, it is time for an in-depth, systematic and coherent research approach. The mastermind research method squared, as it were.

Unfortunately we have a research culture in which the desire for hypes and fast and many publications rules. Research funding agencies also require rapid results. Such a setting leaves little room for in-depth, systematic and coherent research approaches. We need to join forces better. Because what is it really about? Is it about power and status, or do we really want to solve problems? I keep hoping for the latter

If we look at the type of information that we can get from Alzheimer's from people, we see that it is very limited. Many healthy people were included in cohort studies such as the Rotterdam Elderly Study, who are followed during their lifetime. Blood samples and sometimes also brain fluid samples from very low parts of the spinal cord are collected from

these persons, while questionnaires are kept that relate to the ability to think and to be able to communicate. Some of these people have already developed Alzheimer's disease. By looking now at the body's own substances in blood and brain fluid, and at the questionnaires, a possible link can be made retroactively between changes in the body's own substances in the blood and brain fluid and Alzheimer's disease. But since, of course, no samples are taken from the brain, it will remain unclear what actually happens in the brain. We will not obtain mechanistic insights in this way.

The question is also what Big Data could provide us with insights into Alzheimer's disease. There is a lot of data, and besides the question whether all that data is of good quality and mutually comparable, I do not think that with the Big data we will obtain mechanistic insights into Alzheimer's disease..

There are two options. Or, we accept that better diseases will not be available for diseases such as Alzheimer's. Or, after all, we will have to gain further insights through laboratory animal research in order to map the interdependence and time dependence of body processes in the course of Alzheimer's disease. In this way, clues can be found about what is derailed at an early stage of Alzheimer's disease. We have our plans ready for a systematic and in-depth investigation, and the protocol for this approach has been approved. The future will show whether this has been a wise choice.

## Word of thanks

First of all, I am grateful to the Executive Board and the Faculty of Mathematics and Natural Sciences for my appointment as a professor and for the trust placed in me.

I am also very grateful to my teachers Prof. Douwe Breimer, the Pater Familias of Leiden Pharmacology, and Prof. Meindert Danhof. You have let me develop as a pharmacologist, and have taught me to find my own way in the academic organization, and beyond. Furthermore, of course, many thanks to Prof. Margareta Hammarlund-Udenaes, for all the valuable discussions and also the countless activities that we have had the pleasure of organizing together.

I would also like to thank my former PhD students for the special collaborations I look back on with great pleasure: Dorien Groenendaal, Tamara van Steeg, Paulien Ravenstijn, Joost Westerhout, Jasper Stevens, Laura Kervezee, Yumi Yamamoto, Wilbert de Witte and Willem van den Brink. Thanks also for the very educational collaborations with the professors: Piet Hein van der Graaf, Thomas Hankemeier, Ad ijzerman, Joke Meijer, and the doctors Jan Freijer, Stina Syvänen, Gert Luurtsema, Pieter Gaillard, Rob Voskuyl, Hans Proost, Coen van Hasselt, Laura Heitman, Gerard van Westen, and Amy Harms. Mariska Langemeijer and Erica Tukker have been very important in earlier research. In more recent research, Dirk-Jan van den Berg and Robin Hartman contribute a great deal. Many thanks for that! Where would I be without all these contributions? Doing research together is a celebration and is also worth much more than doing research alone.

Thanks also to the many dear colleagues and students from the former Department of Pharmacology as well as the current “Systems Biomedine and Pharmacology” department of the L.A.C.D.R.

Collaborations with colleagues from the pharmaceutical industry, such as GlaxoSmithKline, Lilly, Pfizer, Astellas, Takeda, and Janssen, have also been very important and interesting. But of course I am also grateful to all other colleagues from the Top Institute Pharma projects, the PKPD modeling platform, the European consortia of EURIPIDES, DDMORE, and K4DD. Furthermore, of course, thanks to all the other colleagues in collaborations that I cannot name here all by name.

I would also like to mention the American Association of Pharmaceutical Scientists. In this environment I have been able to work together with many international colleagues on programs made possible for the exchange of knowledge between researchers, young and old, and from the pharmaceutical industry and academy. I have had the opportunity to develop leadership in a very positive setting and I am also very grateful for my distinction as a Fellow of this organization.

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We have a wonderful institute and have been able to cope with the enormous influx of students in recent years in education. I hope that the limits of our capacities are respected by the university system so that we can also cope with the future and maintain good quality in education and research. We must have the time to do that.

Then, of course, I want to thank my family and friends for all the good moments together! And there have been many and hopefully many more will come.

My parents, you are no longer there, but you have given me a start in life that I am still grateful for. Very idealistic, also a bit naive, but actually very wise. I could not have wished for a better basis. My sisters and brother, our bond is strong and very important to me. There is always unconditional support.

And of course last but not least Erik, Sjoerd and Casper. Sjoerd and Casper, now great sons, I am very happy and very proud of you!
And Erik, although you are not the easiest for me, you are the best! Despite my stubbornness, I have learned so much from you. Your support is worth a lot to me. We have experienced so many beautiful things with our boys, and I look forward to the rest of our lives together.

I have said