Summary, conclusions and outlook
Although biosensor technology has evolved tremendously, it has not reached yet its full potential. Although a variety of new devices have been reported, the amount of biosensor devices that actually made the transition from benchtop proof-of-concept to in vivo applications remains small. Nowadays, only a handful of sensor types are available for continuous biomonitoring of glucose in a clinically relevant environment. All biosensors designed for in vivo applications are electrochemical, mostly amperometric and enzyme-based. Current state-of-the art Continuous Glucose Monitoring (CGM) devices still heavily relies on this type of biosensors.

Despite the relative success of this type of biosensors in glucose biomonitoring, there are several aspects that hamper the daily use of these sensors by diabetic patients. Within this thesis I have identified, studied and hopefully contributed to a better understanding of some of those factors. Additionally I have attempted to apply these factors in the development and characterization of novel biosensors not only for CGM, but for in vivo biomonitoring in general.

In the first chapter (Chapter 1) it is explained why, there is still a need for a better CGM, despite decades of development in glucose monitoring tools in general, and biosensors in particular. Although various proof-of-concept biosensors, with multiple biorecognition elements coupled to a large array of transducers has been described, in vivo biomonitoring with biosensors is still confined to electrochemical (amperometric) enzyme-based biosensors. Therefore the fundamentals of electrochemical biosensors mechanism, in particular enzyme-based ones is presented. It is concluded that a better understanding of the mechanisms underlying amperometric enzyme-based biosensors may be the key to improve the use of CGM in diabetic patients. Additionally it may enable the emergence of biosensors for continuous monitoring of other key biomarkers.

Then, in chapters 2 and 3, (Chapters 2 and 3) a series of experiments aimed at a better understanding of basic amperometric enzyme-based biosensors electrochemistry is described. Chapter 2 is focused in understanding how permselective membranes (a major breakthrough in biosensor technology) enable improved selectivity of amperometric electrochemical biosensors. The surface of microelectrodes coated with various permselective membrane configurations was evaluated electrochemically and by SEM. All membranes were very effective in reducing electrochemical interference, but they also significantly reduced sensitivity towards target analyte. This effect was amplified in the cases of membrane combinations. Surface evaluation by SEM allowed the identification of an “inner polymerization” phenomena that pointed to a close relationship between (reductions) in surface availability and membrane selectivity.

In the next chapter (Chapter 3) we investigated how surface availability, modulated by the choice of membrane assembly, influenced the performance of amperometric enzyme-based biosensors. We found that biosensors based on permselective membranes with higher electrode active surface were more sensitive, without significant changes in their affinity for glucose. By using a model for kinetics of enzymes immobilized onto electrode
surfaces we found that $I_{\text{Max}}$ and LRS, but not $K_{\text{M}}$ nor LR were dependent on biosensor surface availability. These data provide a better understanding of the relationship between enzyme kinetics and biosensors performance and the role played by surface availability. The knowledge acquired in Chapter 2 and 3 was used to develop and characterize the biosensors described in the following chapters.

In Chapter 4 we describe the development and characterization (in vitro and in vivo) of a novel biosensor device, for subcutaneous CGM in freely moving animal models. We evaluated in vitro, the performance of several designs, based on needle-type Pt based microelectrodes. We found that the use of a microdialysis membrane on biosensor assembly improved the intrinsically low LR of such biosensors. The most suitable biosensor design (PtIr/Nafion/GOx/PE) was then coupled to a wireless prototype, using a 2 channel potentiostat with a self-referencing system (Sensor and Background). The CGM wireless biosensor device was then implanted subcutaneously in freely moving rats. Its in vivo performance of the sensor was evaluated by submitting the animal to pharmacological challenges known to modify blood glucose levels. The described CGM was able to detect significant changes in subcutaneous glucose following intravenous administration of a glucose and insulin. We found a strong correlation in changes between blood glucose and the subcutaneous glucose levels monitored by the CGM regardless of the algorithm used to convert oxidation currents into subcutaneous glucose levels. Nevertheless, the use of multiple point blood calibration showed a higher correlation between blood and subcutaneous glucose levels. Although biofouling, due to foreign body response, had a significant deleterious impact in biosensor performance, the wireless CGM was able to accurately monitor glucose for 5 consecutive days. This prototype iMBD may pave the way towards a minimally invasive portable CGM.

The positive results of chapter 5 encouraged us to take a “leap of faith” and to try to bridge the gap between benchtop technology and “applicable” biosensor applications.

Sterility is a prerequisite for biomedical devices in order to be used routinely both in clinical environment and at home. Therefore, in Chapter 5 we investigated the effect of several sterilization methods on biosensor performance, both acutely and up to one month after sterilization. Although the various sterilization methods had distinguishable effects on biosensor performance, all treatments caused a significant decrease in several key biosensor performance parameters. However, and despite such strong effects, some of the tested sterilization methods (EtOx, $H_2O_2$ (alone and combined with y-radiation) may allow a proper sterilization without impairing the ability the biosensor to selectively monitor glucose.

In the first chapter it is mentioned that the ability of the human body to regulate its own blood glucose levels is intrinsically related to the normal function of the endocrine system. In a diabetes patients, the ability of these mechanisms to regulate blood glucose levels is either severely impaired or, in extreme cases, absent. Therefore, diabetes patients rely on frequent glucose monitoring to maintain blood glucose levels within its “normal” range. Although glucose regulation is immediately associated to the endocrine system, new evidence points to an involvement of the CNS in glucose homeostasis. There is growing interest in the
Putative ability of the brain to control blood glucose availability. Moreover, it is thought that abnormalities in brain energy metabolism may be associated with early diabetes stages.

Therefore in Chapter 6 I describe the development and characterization of a multiplex biosensor device (MBD) for continuous and simultaneous *in vivo* biomonitoring of key biomarkers in brain energy metabolism. First we developed and characterized amperometric enzyme-based biosensors for *in vivo* biomonitoring of lactate and pyruvate. After we assembled a multiplex biosensor device comprising the most suitable pyruvate and lactate biosensors, along with the glucose biosensor (described in Chapter 3) and a background sensor. *In vivo* performance of the MBD was evaluated by submitting an anesthetized animal to pharmacological challenges known to induce, significant changes in blood glucose levels, as described in Chapter 4.

The prototype MBD was able to simultaneously and accurately monitor independently and simultaneously basal brain levels of all the target biomarkers (glucose, lactate pyruvate). Additionally, it was able to monitor, continuously, simultaneously and in real time, differential changes in glucose and lactate in response to the pharmacological challenges. Although the functionality of the pyruvate biosensors incorporated in the MBD was assessed after explanation, no significant changes in brain pyruvate were found. Nevertheless, the described MBD has proven to be a valuable tool to better understand the energetics of the brain and clarify its role on diabetes onset.

Despite the success of amperometric biosensors in brain monitoring, better spatial resolution remains a goal in the development of new tools for experimental neuroscience. In Chapter 7 we try to move towards the miniaturization of needle type amperometric enzyme based biosensors. Tungsten (W) is the strongest metal and microelectrodes based on tungsten might be downscaled to even a few nanometer in diameter. However, in order to use W microelectrodes as a basis for amperometric enzyme-based biosensors, its surfaces need to be coated with a highly electroactive metal, such a gold (Au). Therefore we have developed and characterized (*in vitro* and *in vivo*) biosensors based on W-Au needle type microelectrodes. We characterized the electrochemical profile of W-Au microelectrodes (bare and coated with permselective membranes) in presence of both target analytes and its putative electrochemical interfering compounds. This characterization allowed us to identify the most suitable potential to ensure continuous monitoring of H₂O₂ with high sensitivity and selectivity. These microelectrodes were then used to build glucose biosensors, whose performance was evaluated *in vitro* and *in vivo*. Amperometric enzyme-based W-Au based glucose biosensors were able to monitor, with high degree of sensitivity and selectivity, changes in glucose both *in vitro* and *in vivo*, in the brain of anesthetized rats.
8.1- Outlook

Although amperometric enzyme-based biosensors are already employed in in vivo biomonitoring, either in experimental physiology, or as diagnostic tool (in the case of the CGM devices) it is fair to assume that they haven’t reached yet its true potential. There are far too few “real” applications of biosensors, when compared to the abundant proof-of-principle devices described in literature. For biosensors to be regarded as reliable bioanalytical tools, capable of providing data that can decisively impact either preclinical research and/or disease management in clinical settings, there is still a long way to go.

Nowadays, biosensors for in vivo biomonitoring still require miniaturization. Not only to increase its spatial resolution but also to enable better patient compliance. Biosensor miniaturization may be achieved by using new, more resistant materials in microelectrode manufacturing. However, as size does matter in terms of biosensor performance, the continuous downscale of these devices, will come with a price. As biosensors will get increasingly smaller, they will also become less sensitive. To overcome this size dependent limitation, surface modification will be necessary. The use of carbon nanotubes and metal-based nanoparticle, alone or in combination with conductive polymers or even graphene, may allow an adequate surface to area ratio in increasingly small, thus less invasive, biosensors.

However, miniaturization is not the only challenge faced by the biosensor community towards widespread application of biosensors in in vivo biomonitoring. As, at least in the next few decades, biosensors for in vivo biomonitoring will be most likely, invasive, better understanding of the FBR mechanism is fundamental. A deeper insight on FBR may lead to the necessary breakthrough in material sciences, enabling more favorable interactions of the biosensors with living tissues, with the obvious benefits for in vivo biomonitoring. Only then, biosensors can finally unleash its true potential as bioanalytical tools for in vivo biomonitoring.
Nederlandse Samenvatting

“Real-time biomonitoring” van de bloedsuikerspiegel in diabetespatiënten is een technologische uitdaging waarvoor nog geen optimale oplossing bestaat. Hoewel er reeds biosensoren in een klinische setting worden toegepast, staan lage selectiviteit en/of gevoeligheid, afstoting door het lichaam en een korte levensduur toepassing op populatieniveau in de weg. Het huidige onderzoek heeft zich daarom gericht op 1) het meer inzicht verkrijgen in de biochemische mechanismen die de eigenschappen van biosensors bepalen en 2) de ontwikkeling en optimalisatie van een elektrochemische sensor die online glucose, lactaat en pyruvaat kan meten in levend weefsel.

Het eerste hoofdstuk bespreekt de huidige stand van zaken in het onderzoeksveld. Aan bod komen de voor- en nadelen van bestaande klinische methoden om glucose te meten, met een focus op amperometrische biosensors op basis van enzymen. Hoofdstuk twee en drie gaan dieper in op de eigenschappen van verschillende ion-uitwisselende membranen, welke de selectiviteit van de biosensor versterken. In deze hoofdstukken blijkt dat een nafion membraan in combinatie met PPD de meest optimale in vitro biosensor eigenschappen bezit. Deze bevinding wordt preklinisch relevant in hoofdstuk vier, waar de gevoeligheid voor glucose en levensduur van de sensor wordt getest in vivo. Door koppeling aan een draadloze transponder is de sensor in staat om de bloedsuikerspiegel tenminste vijf dagen accuraat te meten, waardoor de sensor potentie heeft om te worden gebruikt in een klinische setting. Vereiste hiervoor is dat de sensor gesteriliseerd kan worden. Daarom wordt in hoofdstuk vijf het effect van verschillende sterilisatiemethoden onderzocht, waaruit blijkt dat - ondanks een reductie in gevoeligheid - de sensor ook na sterilisatie geschikt zou zijn om glucose te meten in vivo.

Hoofdstuk zes beschrijft de ontwikkeling een multiplex biosensor die simultaan glucose, lactaat en pyruvaat meet. Omdat de hersenen een belangrijke rol spelen in de regulatie van de bloedsuikerspiegel wordt door middel van deze multiplex sensor het verband tussen perifere en centraal metabole processen bestudeerd. Een interessante bevinding in dit hoofdstuk is dat de pyruvaatconcentratie in de hersenen constant blijft, ongeacht sterke fluctuaties in de glucosespiegel. In het hoofdstuk zeven wordt de ontwikkeling van een mini-biosensor op basis van een goud-gecoate tungstenelektrode beschreven. In vitro en in vivo experimenten laten zien dat ook deze elektrode accuraat glucose kan meten, wat potentie biedt tot minder invasieve biomonitoring in diabetespatiënten.
ΔE_p - Difference in peak potential
AA- Ascorbic Acid
ANLS- Astrocyte-neuron lactate shuttle
ANOVA- Analysis of variance
BG- background sensor
BSA- Bovine serum albumin
CGM- Continuous glucose monitoring
CGMS- Continuous glucose monitoring system
CH- clorohexidine
CV- cyclic voltammetry
DA- Dopamine
DOPAC- 3-4-dihydroxyphenylacetic acid
DNA- Deoxyribonucleic acid
E.C. - Enzyme commission number
ECF- extracellular fluid
EtOx- Ethylene oxide.
FAD - Flavin adenine dinucleotide
FBR- Foreign body response
FDA- U.S. Food and Drug Administration GA- Glutaraldehyde
GABA- γ-aminobutiric acid
GOx- Glucose oxidase
GluOx- Glutamate oxidase
HBA1c - Glycated hemoglobin
HLA- Human Leukocyte Antigen
HPLC – High-performance liquid chromatography.
I_p – Current on the peak Potential
ID- Inner diameter
IDDM – Insuline-dependent Diabetes Mellitus
ISF- Interstitial fluid
IG- Interstitial glucose
IPA- Isopropyl alcohol
i.v.- Intravenous
J_max - Maximum movement of solutes (from Fick’s law)
kDa- KiloDalton
K_m- Michaelis-Menten constant
appK_m- Apparent Michaelis-Menten constant
LBL- layer-by-layer
LC-MS – Liquid chromatography-mass spectrometry
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOx</td>
<td>Lactate oxidase</td>
</tr>
<tr>
<td>LR</td>
<td>Linear Range</td>
</tr>
<tr>
<td>LRS</td>
<td>Linear range sensitivity</td>
</tr>
<tr>
<td>MBD</td>
<td>Microbiosensor device</td>
</tr>
<tr>
<td>iMBD</td>
<td>Implantable microbiosensor device</td>
</tr>
<tr>
<td>MPBC</td>
<td>Multiple point blood calibration</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>nA</td>
<td>Nanoampere ($10^{-9}$ A)</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non Insulin-dependent Diabetes Mellitus</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>OPPy</td>
<td>Overoxidized polypyrrole</td>
</tr>
<tr>
<td>OD</td>
<td>Outer diameter</td>
</tr>
<tr>
<td>pA</td>
<td>PicoAmpere ($10^{-12}$ A)</td>
</tr>
<tr>
<td>PAN</td>
<td>Polyacrylonitrile</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PE</td>
<td>Polyether sulfone</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene Glycol</td>
</tr>
<tr>
<td>PET</td>
<td>Positron-emission tomography</td>
</tr>
<tr>
<td>mPFC</td>
<td>Medial prefrontal cortex</td>
</tr>
<tr>
<td>PG</td>
<td>Plasma Glucose</td>
</tr>
<tr>
<td>POx</td>
<td>Pyruvate oxidase</td>
</tr>
<tr>
<td>PPD</td>
<td>Poly(phenylenediamine)</td>
</tr>
<tr>
<td>PmPD</td>
<td>Poly(m-phenylenediamine)</td>
</tr>
<tr>
<td>PoPD</td>
<td>Poly(o-phenylenediamine)</td>
</tr>
<tr>
<td>PreC</td>
<td>Pre calibration</td>
</tr>
<tr>
<td>PostC or PC</td>
<td>Post calibration</td>
</tr>
<tr>
<td>RC</td>
<td>Regenerated Cellulose (when applied to membranes)</td>
</tr>
<tr>
<td>RC</td>
<td>Rejection Coefficient (when applied to biosensor performance parameters)</td>
</tr>
<tr>
<td>SAL</td>
<td>Sterilization assurance level</td>
</tr>
<tr>
<td>SAM</td>
<td>Self-assembled monolayer</td>
</tr>
<tr>
<td>SC</td>
<td>Selectivity Coefficient</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy (when applied to imaging)</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean (when applied to statistics)</td>
</tr>
<tr>
<td>$SI_{\text{Max}}$</td>
<td>Surface independent maximum current</td>
</tr>
</tbody>
</table>
SI app K_M – Surface independent Michaelis-Menten constant
SMBG- Self-monitoring of blood glucose
SPBC – Single point blood calibration
T1DM- Type I Diabetes Mellitus
T2DM- Type II Diabetes Mellitus
UA- Uric Acid
UV- ultraviolet
V_{Max} - Maximum reaction rate
W.H.O- World Health Organization
“Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained.”

*Marie Curie*

“Never, never, never give up!”

*Winston Churchill.*

*But also my Dad, my Mom, my sister and my wife. And all of those who kept me going...*

I am not aware how many years, in average, a student takes to finish his/her Ph.D. But I am pretty sure that I took much longer than average. Therefore, allow me to have a “larger than average” thank you note. Over the years, there were many people that helped me carrying out this task. I hope (but I am sure I will) I won’t forget anybody.

First of all, I would like to thank my supervisors, Ben Westerink and Thomas Cremers. Thank you for allowing me to pursue my dream of actively work on the fascinating field of biosensors. Thank you for hosting me. First, at the now defunct Biomonitoring and Sensoring department for my MsC, and after for inviting me to do a Ph.D. in this amazing topic. Thank you for allowing me to fail and to try again. Only to fail again, and again, until something somehow meaningful was finally found.

Ben, I thank you, above all, for all the time you spent on me. Time is the most precious gift anybody can get. I feel grateful and honored to have you as my supervisor. I cherish, now more than ever, all the time we spent together. Mostly discussing biosensors and its deviant behavior, but also, in these late years, my thesis. Thank you for guiding me, with your own unique way, from the point that my thesis was no more than a “Big Table”, to a proper book.

Thomas, I thank you for more than I can honestly express. I feel grateful to be able to work with you. On my thesis, but also far beyond, for the past 10 years. Thank you for pushing my limits, beyond what I thought it was expected from me. Thank you for the trust. For the talks. For the travels. For the opportunity to try to push, even so slightly, the boundaries of what biosensors could do. Thank you for feeding me, time after time, with increasingly challenging and ambitious ideas. For stimulating my drive and ambition, and to motivate me to keep improving myself. For the harsher words, when they had to be said. It was hard, very hard at times. But as you well say, it only makes my skin “thicker”. I wish you good luck in whatever challenges you chose to face. I truly hope to be somehow involved in whatever you choose to do.

I also would like to thank Prof Susan Lunte, Prof Menno Prins and Prof Anton Scheurink, for taking the time to critically read and assess my thesis. Your comments helped me to significantly improve my thesis.
I also need to thank Martin de Vries for his help, in many aspects. Although you were absent during the final stages, your mark is still quite visible. I am sure I wasn’t able to finish it, if you wouldn’t have been so patient with me while this “plane” was taking off. I finally managed to land it. And for that I thank you!

It all started in the now extinct Biomonitoring and Sensoring department of the University of Groningen, first as a MsC student, and later as a PhD student. In that sense, i have to thank my fellow Ph.D. students at the Biomonitoring and Sensoring department, Jelle and Wahono. Thank you Jelle (and Marjoleine) for your kindness and help in the start of this journey. And thank you Wahono, for your friendship throughout these years. I still believe you will finally understand how strong you (really) are. Please do not forget to call me when that happens.

From the Biomonitoring and Sensoring, I was “transferred” to the Pharmaceutical Analysis department. It didn’t start there, but I certainly finished there. Therefore, I need to thank Prof. Sabeth Verpoorte, leader of the group, for integrating me and allowing me to finalize my project within the department. But I need to thank Patty as well, for all the help. You have no idea how important you were. Without your help and trust, I would have done much less. Perhaps not enough. I would also like to thank JP. For all the coffees and talks, that helped me ease my mind. I hope the future will bring you some peace! And a lasting friendship.

I would also like to thank all the Ph.D. students I met in these last few years, wandering around the department, sometimes almost like a ghost. I hope I won’t forget any. Thank you Hanan, for all the insightful talks. And for sharing lots of great ideas and concepts. Even though I could not even properly grasp some of them. You will be fine, trust me on that! I am looking forward to your promotion. I also would like to thank Pim. For waiting for me to finishing, my (long) experiments. But also for the discussions we had over a cup of coffee. Thank you Maureen, for the nice vibe in the lab, and for the discussions in between experiments. Thank you Nadiah. If I managed to finish my PhD, so can you! Keep your faith and do not give up! I would also like to thank you Gert and Macieij. I wish you guys all the luck in your adventure. Thank you Sergio and Margarita. And thank you Klaus. We should definitely have dinner more often.

A significant part of the work I present in this thesis was performed at Brains On-Line and at Brainlink, where I worked for the past 9 years. First, in the facilities at the also extinct, Antonius Deusinglaan, and later on the actual Brains On-Line facilities, at de Mudden. I couldn’t possibly finish this project without the precious help of the kind people that work or worked with me at BOL and BL.

Above all I would like to thank Gunnar Flik. Thank you for the freedom I could enjoy to work on my thesis, while performing my job as Scientist/Project Manager/Electrophysiologist, over these past years. I still can’t help smiling when I hear you talking with genuine interest in biosensors and its applications for \textit{in vivo} biomonitoring.

I also would like to thank Joost Folgering. Above all for your patience. Even in the very beginning, when it was not so easy to work with me (well, that may not have change so much after all:)). I will always remember that you always had (or “made time”) for me. Thank you
for having always an encouraging word (and there were so many!). I really enjoyed working with you, especially in this last couple of years. As I told you before…I am glad I was wrong on this one. That conference in Vienna was one to remember.

I would also like to thank Andy. Although you had no apparent direct input on my thesis, I learned a lot from you. And those lessons really helped through the final stages of this long process. I still intend to keep the promise of working with you again in the future.

I would like to thank Mariette and Ulrike, for “putting up with me” for quite some time, in our office downstairs. I know I am not the most talkative person on earth, but I surely talk much more these days. And for that I thank you.

All that I know in terms of animal experimentation, widely present in my thesis, is based on the extensive knowledge that existed on the biotechnical team of Biomonitoring and Sensoring department, later transmitted to Brains On-Line. I feel extremely grateful to be able to work and learn from all of you. I thank the ones from my beginning, Karola, Suzan and Harm. I have met you when I was still an MsC student, and you were there in the beginning of my Ph.D. Those were the certainly amazing years! I don’t think I can ever payback all that you have done for me. Inside and outside the lab. Thank you Suzan, for all the laughs, the limo tour, the way too drunk carnival in Zwolle, where I, obviously lost my wallet. Thank you Harm, for the trip across Portugal in my father’s filthy car. Wild day’s man! And thank you Karola, for too many things…Unfortunately, I cannot possibly neither summarize nor quantify how much you helped me. But I wouldn’t be here if it wasn’t for you and Ar. But I also need thank all the ones I met after. That witnessed and help on my Ph.D. struggle. And they are Annelie, Daphne, Lisanne and Tamara. It was really nice to work and learn with you.

All of the biosensors and biosensor devices of this thesis passed at some point through Brainlink. Either for manufacturing or just to discuss whether they were possible to be made. Therefore, I would like to thank Kirsten, for all the freedom I enjoyed while using Brainlink facilities and for the help on “making some sketches alive”. And the “ladies”. I may not say this enough, but I am truly thankful for all your help throughout these years. You are amazing! Thank you Silvia, Jeannette and Korrie.

At Brains On-Line, I met a couple of years back a clumsy gentle giant. After a while that clumsy giant showed up at the lab to start working with me. He turned out to be “the perfect partner in crime”. Thank you Taco. I think it is safe to say we make a good team. A team that involved at one point the sweet Antonella and later, once again Wahono. Thank you both.

I also need to thank my colleagues that although not directly involved in the thesis, made for many years, Brains On-Line a great place to work. The pleasant environment that you created at Brains On-Line, made it easier to go home and keep working long hours on my “hobby”. Thank you Robert, Corrie, Harold, Wim, Marius, Hedwig, Jietse, Chi, Mark, Kees, Ingrid, Jens, Lorenza and Julien.

And Elly. We started as co-workers and become very good friends. Thank you for being there, when things were not looking good. For your kindness and friendship. I wish you all
the luck in the future.

Throughout the time I took to finish my Ph.D. I met very interesting people in inside and outside the academic environment in the city I embraced as home. They left. I stayed. But I am thankful we met. Living in an international student house was, in a word, spectacular! I met so many interesting and diverse people. It was an eye-opening experience for sure… From that time I should thank so many. But above all I thank Laia, Steffano and Sintja. Crazy times hein? But also Marius, with whom I shared, first a kitchen, and after a whole house. You were so many times a precious confident. But mostly you were, and still are, a great guy! I hope we can keep seeing each other, every now and then. And Jon, thank you for being there when I needed a friend.

Thank you Lara and Luís. Lara, obrigado por teres vindo falar comigo na residência. Tenho a ideia que naquele universo paralelo em que isso não aconteceu, acabei o doutoramento mais depressa. Mas tenho a certeza que estes 10 anos não valeram tanto a pena. Luis, olhando para trás, acho que fica mesmo uma grande amizade. Grandes jogos! Grande golo pà! Grande golo mesmo… Longas noites. Longas tardes. Longos dias! Que tenhas sorte, e que nos vamos vendo. Obrigado.

The environment within the academic circle that intertwined the RUG and the UMCG that I found in Groningen was truly inspiring. I was fortunate enough to know some (not a lot) of people I can now call friends. And as it should be, most of the memories are from the time we spent outside our labs. Thank you Elena, Giulia, Cecilia and Ewa.

I also thank the friends I met at the Universidade do Algarve. It was nice to grow up together in Faro. It is nice to see where we all end up and what traces we leave behind, knowing that there is still a long road ahead of us... I hope we can still gather the “Antro” somewhere, from time to time, so we can all go back. When things were easy and dreams seemed so tangible…Thank you Jorge, Cátia, Cláudio, Pedro, André Lopes, André Santos, Marco e Eduardo. And Wilson and Rui, for putting up with a weird roommate for so many years. And for keeping the friendship alive. And later, through my lovely wife, Anna, Song and Kevin. But also Joana and Hilde. And Gusnaniar! You are truly an amazing person!

Although I left long time ago, I am fortunate enough to keep some dear old and new friends from my hometown. Sometimes, we have no more than time for a coffee in between something else. But those 30 minutes, every 6 months or so, are very important to remind me where I came from, and where I want to go. Thank you Nuno, for teaching me how to surf, but not how to SUP :). And thank you Daniel and Quim. For more than I think I deserve… E obrigado a ti também Guilherme. Pela tua amizade. Pelos concertos que vimos juntos em (literalmente) todo o lado. Acho que os universos paralelos nos quais partilhamos as salas de aula, são certamente melhores que os outros. Obrigado!

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Esta tese é mais que minha, é nossa.

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**Biography**

Carlos Cordeiro was born in Vila do Conde, Portugal in 1982. From 1990 until 2001 he was a competitive swimmer. He was the twice individual portuguese age groups national champion (1996 and 1998) and once 2nd division national champion by teams (1999). He started his undergraduate studies in 2000 at the Universidade do Algarve. He finished his licenciatura (MsC equivalent) in Biochemistry at the Universidade do Algarve (Faro, Portugal) in 2005. In 2006, he started an MsC in Biotechnology at the same University. In 2007 he was granted an Erasmus Mobility Scholarship and joined the Biomonitoring and Biosensoring group at the Faculty of Pharmacy of the Rijksuniversiteit Groningen to carry out his MsC research project. Later (in 2009) he received his MsC in Biotechnology at Universidade do Algarve (Faro, Portugal), with honors. In 2008 he started his Ph.D. at the Rijksuniversiteit Groningen in also in the department of Biomonitoring and Sensoring. Additionally, he started to work as Trial Manager and Scientist at Brains On-Line BV (Groningen, The Netherlands). Since 2011 he is also Trial Manager for in vivo electrophysiology studies at the same organization. In 2014 he joined the business development team at Brains On-Line. His main focus is the development of biosensors for in vivo applications. However, his work and scientific interests also comprise electrochemistry, neurochemistry and neuropharmacology, as well as surface chemistry and biotechnology, especially enzyme technology.

**Publications**


Cordeiro CA, Sias A, Koster T, Westerink, BHC, Cremers TIFH. In vivo real-time brain biomonitoring with enzyme-based microbiosensors based on gold coated tungsten (W-Au)

Patents

PT2895071 (T) — 2017-06-27
Rod Shaped Implantable Biosensor
Co-Inventors: Thomas IFH Cremers
Carlos Alberto de LBL Cordeiro

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2016- FLAG-ERA Joint Translational Call Grant-Graphene Flagship “Graphtivity” Project. Project Manager/Scientist at Brains On-Line, BV, in an European consortia involving also the Ruhr-Universität Bochum (Germany), KU Leuven (Belgium), The Italian Institute of Technology (Italy), Centre National de la Recherche Scientifique (France) and the International Centre of Biodynamics (Romania).