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Immunoreceptor MerTK: A journey from the membrane into the nucleus of human dendritic cells

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Epilogue

With the emergence of quantitative biophysical tools reaching single molecule sensitivity, we are now better than ever capable of observing single biomolecules and characterize their behaviour in the context of living cells. The field of nano-immunology has particularly benefitted from these technical advances to reach a more thorough understanding of the molecular mechanism of action of many key immunoreceptors, those serve in the conventional immunological approach to classify and describe different immune cells. The availability of such techniques is thus causing a shift from the conventional descriptive approach with a main focus on how cells function within the immune system or the entire organism to a more quantitative, fundamental and molecular way of researching. As a consequence, new insights are being obtained and a whole range of new questions can now be addressed. In Part I of this thesis I've made use of some of these novel imaging techniques reaching unprecedented spatial and temporal resolution to address various biological questions, all of them with a direct implication in immunology. Moreover, I also showed an example where tools from the microengineering field have been transferred to biology to study the interactions between different proteins at the cell surface. In part II, I have exploited various types of optical microscopy, combined with biochemical approaches, to obtain a first quantitative insight on the subcellular distribution and location of receptor tyrosine kinase MerTK. Whereas this receptor is implicated in a diverse number of cellular functions and its misregulation can lead to auto-immunity and cancer, there is still very little known about the molecular working principles. Our work uncovered new facets that were completely unexpected for this receptor, including its clear nuclear localization that appears an exclusive trait for immune cells and the possibly that MerTK participates in gene regulation during the transition of monocytes to fully differentiated dendritic cells.

As a whole, my PhD research is a clear demonstration of how the right combination of novel microscopy techniques, quantitative approaches and conventional biochemical tools are together providing new insights with unparalleled levels of detail into cellular processes within a broader biological context. On the other hand, my work also advocates for the future development of functional assays with similar molecular sensitivity in order to simultaneously visualize and understand the activity of biomolecules within their biological context even more thoroughly. As a biologist with a very strong interest in fundamental molecular research, I hope that my work serves as a driving force for physicists and engineers to make further progress in the development of such crucial tools.

In an era in which direct visualization of single, functioning biomolecules becomes more and more feasible, and image processing requires very elaborate quantitative analysis, it is easy to lose perspective. Biomolecules do not function without context, and a single imaging technique, even if reaching the highest resolution and level of detail, cannot uncover all the layers of biological complexity alone. And most importantly, things are never exactly how we hypothesize them to be. It is thus of vital importance to create situations in which we can get surprised and allow ourselves to see the unexpected in our research. As a biologist in this multidisciplinary landscape I therefore feel compelled to be the one trying to think and work from the cell's perspective. This confronts me with the challenge but also the excitement to find the best combination of tools and approaches to answer multidimensional questions biology confronts me with. To zoom in as much as possible for fundamental understanding and molecular detail, but also to allow myself to zoom out and letting the cutting-edge techniques aside every once in a while to remind myself of the undeniably complex living system I am trying to tame. Bear with me, and you might even find yourself wondering what a membrane receptor is doing in the nucleus!!

Acknowledgements

As I am about to finish this manuscript, it is inevitable to look back at the past six years with both joy and sadness. Joy for everything that I've learned and experienced, the people I've met and the person that I've become. Sadness because this academic life is so strongly connected to saying goodbye all the time, and that it's my turn to move on now. It is thanks to all of you that I am who I am writing this today.

Let's start out at the beginning by turning to Maria, my supervisor. Six years ago she took on a Dutch girl working in Immunology, within a group of biophysic guys pretty heavy on the physics side. But she saw something in me, gave me a chance in her group and offered me a position as a PhD student once my master internship was over. Maria, I am truly grateful for the way you have valued my scientific opinion from the very start, how you have always given me the chance to pursue things that I felt were the right path and the way you took me seriously even already as a master student. I honestly think this was the best thing anyone could have done for me at that moment. I have greatly enjoyed our discussions over the years in which neither of us was afraid to skip the politeness and strongly advocate for what we thought was important. Now that I'm moving on, I realize that a good part of my experience in ICFO has also been made by how open you always were with us. Involving us in project proposals, updating us on ICFO business, sharing your personal experiences as a woman in science, telling us stories about Niek and Steven. We have discussed several standard styles of leadership, but I think yours is a really unique mix and I hope many more students will be lucky enough to enjoy this like I did!

I feel like I've had 2 different lives in the group of Maria. When I arrived at ICFO in 2012, I didn't have the easiest of times in my personal life. But somehow something beautiful grew from it. Together with Thomas, Carlo, Mathieu, Bruno & Martha, Juan, and later on Alberto & Noslen, Ksenia and Iza & Lukasz. Thomas and Carlo, the two of you have been a huge support for me during those first months, and I think it's fair to say that without this I would have never made it from master into PhD student. Thanks to the both of you for giving me the confidence to move on and having my back like 2 older brothers, not only then but ever since. Together with the rest of the gang we made 2012 into a crazy party year full of sun, happiness and new beginnings. I think we all got a bit more serious since then, proven by the 4 weddings, 3 babies and 2 PI positions that followed. I have had to say goodbye to almost all of you already, but I still look back at those times with great joy!! You were the first to make being part of an international group of scientists feel like my home and the place where I belong, wherever we might all be now in the physical sense.

But as much as saying goodbye is hard, it is also an opportunity to get close to new people. My second life in the group has been colored by Pamina, Sarah, Felix, Maria Sanz, Enric and just for the last few months by Nico. I really loved working alongside all of you, even as much so that I dragged my newborn baby on several occasions into the coffee room during my maternity leave just to spend time together. I am greatly missing you lot already, even lengthy group meetings! Felix, I admire your ability to be encouraging and critical at the same time. I am sure that will make you a great PhD supervisor. I really appreciate how much you've been thinking along on my project ever since I first met you in CRG. Pamina, I think our friendship was sealed even before you officially started to work in ICFO. I love how we can always talk about anything together: happy or sad, energizing or frustrating, big stories or just a little smiley. A tight hug from you is one of the best things a person can get to feel better after having a rough time. I hope that one day we will settle down not so far from each other and be able to regularly share as much coffee as we can possibly drink in a day! Sarah, as you said already yourself, our timing has not been the best. I got to know you while I was pregnant, and once I came back to work after maternity leave we both knew that my time in ICFO was almost over. However, in this short period you have become one of the people that I will miss the most! I can't stop making up excuses why you should quit ICFO and come work in Paris (it's still possible you know! ;)). I'll especially miss our talent for not taking ourselves too seriously when we talk, while knowing at the same time that you will always have my back and the one of Aimé when need be. I wish you all the best for your PhD, you can always ask me for anything, and hopefully we will have better timing in the future!

In those six years in ICFO I have met and interacted with many other great and talented people. Guys from Melike's group: it was a true pleasure to share joint group meetings, the coffee room, Christmas lunch and paper celebrations with you! Jason, thanks for being so patient with me when I was figuring out how to do STORM in the nucleus! Maury, thanks for spending those last couple of weeks before the birth of our babies together in the dark and dedicating your time to my project! I can't imagine how ridiculous we looked walking the corridors together with 2 huge bellies while being fully focused on work. Laurent, thanks for all the great time that we spent together and keeping the French spirit alive for me during those long months that Mathieu was away! I know that you don't like Paris but never say never ;) Merche, thanks for being the only person that I felt completely confident with speaking in Spanish! I'm not sure how you actually managed to do that since this doesn't come easily to me at all (ask Laurent ;)). And thanks to all the others not mentioned by name. All together you have made ICFO feel like my home away from home and allowed me to grow from a little master student into a proud soon to be Doctora in Biophysical Cell Immunology (is that a thing?!).

I feel fortunate to not only have spent time in ICFO, but also have interacted with many other researchers in Barcelona. One of my usual spots was Hospital Clinic, more specifically in the group of my co-supervisor Daniel Benitez. Dani and his 3 girls Gina, Raquel and Carol have been keeping immunology alive for me while being in a physics institute. Thanks to all of you for helping me out with my DCs, accepting my crazy schedule and welcoming me into the group as a foreigner. I am very happy to have experienced the local scientific community by working in your lab and attending some of the meetings you organized, and not only live in the international bubble of ICFO! Gina, I enjoyed sharing part of the PhD life with you, and I think it's great that you are now continuing your journey in Nijmegen! I was also able to spend some time in CRG, in the group of Vivek Malhotra. From the scientific point of view this has been a real torture. I have done the same little experiment so many times over without any serious success for almost 2 years. But Nathalie and Amy have not only made this bearable, but even made me look forward to my occasional days there! Thanks girls for taking me in without any hesitation and treating me like I was one of you! I greatly enjoyed our lunches on the roof terrace and forgetting about yet another failed experiment by having some drinks together after work (better not go by car!).

In the fall of 2016 I also spent 4 months in Nijmegen, in the group of Alessandra Cambi. She was the person that got me into contact with Maria in the first place, and has kept an interest in my progress ever since. Thank you for keeping me connected to my scientific roots in Nijmegen and welcoming me into your group for those few months. Soon after arriving I came into your office with a green face, feeling like I was about to faint. You were actually one of the first people I told that I was pregnant and your reaction has very much helped me cope with the situation and not feel bad about the timing of things. Thanks as well to the rest of the group for helping me out and taking a few things off my plate! A special appreciation goes to Koen, who I got to know as a great example of the kind of junior researcher I hope to be one day. Ben, we already knew each other from various occasions in Barcelona and Nijmegen, and whatever we do it is always great fun! I really liked that you somehow became my missing link between Nijmegen and Barcelona, and how it always feels like it was just yesterday that we last saw each other, even after many many months. I know that Paris is not as relaxed as Barcelona for a quick visit, but I still hope we will keep bumping into each other every now and again!

The home front has been a part of this milestone as much as the people that I met along the way. Thank you all for cheering me on, coming to visit and being the roots from which I could continue growing. Thanks to my homies from Nijmegen: Lise, Iris, Jessie, Winnie and Annemiek. After studying together for 3 years we all followed our own path, and I'm extremely proud of the diverse group of women we've become. It has been very hard to

not have you guys around and enjoy our weekly dinner dates anymore, but I'm very happy to still have you in my life! Thanks to the two great girls that I got to know when I was still living in Utrecht: Gianna and Louise. I think it's just incredible that we are still friends after all this time and how both of you have supported me along the way. Your many visits, messages, pictures and memories have made it both easier and harder at the same time to be so far away. Thanks to my family, in particular Michal, Erika, Anja and Erik for your unconditional love and support. I have often wished that everything and everyone that is important to me could just be concentrated in the same country, but apparently this is not the case. Thank you for understanding and being in my corner all the same. A special word goes to my mother. When Michal and I were little, it was not easy for you to trust that we would be safe and to talk about how this made you feel. Now that I've become a mother myself, I see a lot better where these feelings came from and also realize how strongly they are connected to love. I really admire how much you have allowed yourself to evolve along the way. It must have been bittersweet to see me build a future somewhere else over the past six years, and I truly appreciate how selfless you have been throughout this adventure. I hope that I will have the same strength and grace when it's my turn to learn how to let go.

The original Greek muses were 9 goddesses, but I have 3 regular men instead. Erik, to the outside world our disciplines in science may seem nothing alike, but I know that I owe an enormous part of the researcher that I've become to your example. With a combination of logic, curiosity and a strong sense of what's your calling in this world you have shown me how to always remain in search of the truth. You taught me the power of clean argumentation, the ability to view things from an unconventional perspective and the value of being the odd one out. I feel very proud to be your daughter and I hope I will be able to pass these foundations to the next generation to come. Mathieu, our story started about 5 minutes after I first set foot into ICFO, even though you still believe that I didn't see you that day. Meeting you and becoming a little family together has been the single best thing that this Barcelona adventure has brought me. Your dedication to your work, your creative mind and your lawyer arguing are a true inspiration to me every day. You never cease to amaze me with your ability to learn new things and evolve along the way. Thank you for bringing out the best in me and believing in my potential for the both of us on the days that I was not able to see it myself. I couldn't wish for a better father and role model for our little patatje, and I am looking forward to see what the future has in store for us! Aimé, while I was trying to understand biology in the lab, a miraculous process was going on inside me. Knowing and feeling that I was going to become your mother soon gave me superpowers that nothing else ever could have, and I surely did my best work while you were cheering me on by pushing my ribs. I hope that dragging you across

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