Sustainable membrane biosynthesis for synthetic minimal cells

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APPENDIX

Beknopte samenvatting

Acknowledgements

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BEKNOPTE SAMENVATTING

Een van de hoofddoelen geformuleerd in de nationale wetenschapsagenda (NWA), is de creatie van een synthetische minimale cel vanuit individuele, niet-levende componenten. In de biologie kun je deze uitdaging vergelijken met de race om de eerste man op de maan. Diverse internationale consortia proberen momenteel zo'n synthetische cel te bouwen, waaronder het Nederlandse initiatief BaSyC (ondersteund door een NWO zwaartekracht programma). Het werk gepresenteerd in dit proefschrift heeft als pilot gediend voor BaSyC. Hierbij was het specifieke doel een systeem te ontwikkelen dat de groei van de omringende laag van zo'n synthetische cel (het membraan) kan realiseren. In de natuur bestaan zulke celmembranen uit verschillende fosfolipiden die zich spontaan oriënteren in een bi-laag structuur, die als scheidingswand fungeert tussen de binnen- en buitenkant van de cel. Hier beschrijven we de constructie van een bio-synthetische route, die twee essentiële fosfolipiden voor het leven produceert. Door een combinatie van gezuiverde anabole en katabole enzymen, afkomstig uit verschillende soorten bacteriën, kon een cascade-achtige bio-synthetische route worden samengesteld, die fosfolipiden produceert vanuit simpele wateroplosbare bouwstenen. De synthese van de fosfolipiden heeft geresulteerd in de groei van een al bestaand membraan, dat model staat voor de scheidingswand van een synthetische minimale cel. Dit groeiende membraan functioneert niet alleen als barrière, maar kan daarnaast ook de activiteit van in het membraan gelegen enzymen ondersteunen, waaronder een systeem dat verantwoordelijk is voor de translocatie van eiwitten over het membraan. Alles tezamen is deze substantiële productie van fosfolipiden, met daaraan gekoppelde membraangroei, alsmede de functionele integratie met eiwittransport een eerste, belangrijke stap richting de constructie van een synthetische minimale cel.

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7 years ago, I was following the master course Organelle and Membrane biogenesis. I still remember the lectures from a certain professor Driessen regarding membrane transport and other membrane-related events. I was fascinated by this particular topic and decided to do a master internship in the group of Molecular Microbiology. During this internship, I discovered that I really enjoyed doing research and I got aware of the possibility to do a PhD. So, after my masters I approached Arnold with the idea in mind to extend on my earlier research. Instead, he introduced me into a new topic: the synthetic minimal cell. I immediately liked the concept of engineering something from the ground up, as this allows you to really control and regulate all conditions. Moreover, I thought it was cool that we would participate in a joined effort to engineer a basic form of life from nothing. As I would be working with membranes, this was the perfect topic for me, but there was just one little detail. There was no position available. No problem according to Arnold as there was this great opportunity for us to get some funding. So I started to write a research proposal, which turned out to be the beginning of my PhD. Since that moment time literally flew by, in which I did not only gain experience regarding membranes, synthetic cells and research in general, but also developed as a person. Overall, I truly enjoyed my time as PhD-student, in- and outside of the lab, in which I met a lot of new people and made new friends. Altogether, a great experience that will proof to be useful in all kinds of aspects of life. However, like most of us that do a PhD, I also experienced difficult periods full with setbacks in which I really learned a lot about myself. It is especially these struggling moments of the PhD that can feel lonely, in which the support of family, friends, and colleagues is essential. Therefore the next section is dedicated to all the people that supported, inspired and encouraged me throughout my entire PhD.

First of all, I would like to thank my promotor **prof. Dr. Arnold J.M. Driessen**. Beste Arnold, we kennen elkaar inmiddels al vele jaren. Hierin ben je eerst mijn docent geweest, daarna mijn mentor (gedurende de masters), en tot slot mijn werkgever. Ik wil je graag bedanken voor al het advies dat je me hebt gegeven gedurende al die jaren en voor de goede begeleiding van mijn projecten. Je hebt me veel vrijheid gegeven en er was altijd ruimte voor eigen inbreng, wat erg belangrijk is geweest voor mijn eigen ontwikkeling. Door de ontspannen sfeer tijdens onze meetings voelde ik me altijd vrij om alles te zeggen en ik heb dan ook genoten van onze, soms intense, discussies over bepaalde resultaten of vervolgexperimenten. Daarnaast heb ik onder jouw begeleiding flinke stappen gemaakt op het gebied van wetenschappelijk schrijven. Ik ben van origine nog al lang van stof, maar die wolligheid is inmiddels flink afgenomen, al wil het nog niet altijd lukken. Echter, er komen nog wat manuscripten aan, en ik hoop dan ook nog een tijdje prettig met je samen te werken en te schrijven.

Furthermore, I would also like to thank **Prof. Dr. Dirk-Jan Scheffers** en **Dr. Juke Lolkema**

for their valuable input during the Tuesday morning work discussions. It always helps to have an extra couple of experienced brains thinking along. Juke, een extra bedankje voor jou vanwege je hulp met de alignments en de hydrophathy profielen waarbij jouw zelf geschreven computer programma goed van pas kwam. Dirk-Jan, naast het werk, was het ook een welkome afwisseling om de sport, en dan met name het wielrennen, van dat moment even door te nemen.

Then, a huge thanks to **Antonella Caforio**. Dear Antonella, I don't know what would have become of me and my PhD without your incredible help and supervision. Especially in the beginning, you basically taught me all the ins and outs on lipidomics, Mass-Spec and many other lab procedures, which was vital to get me on the road. Moreover, your office was always open for me and you were always willing to discuss (yet again) a new research strategy, or a hypothesis I came up with. But most of all, thank you for the many conversations and funny moments we shared together as friends. For me it really felt like we were a team and I am grateful for your overall support throughout the entire time we spend together.

My paranymphs **Riccardo Iacovelli** (or should I say Lacovelli) and **László Mózsik.** Móóózsik László my friend, as you were my office companion, you are probably the person I spend most time with throughout my entire PhD. Despite the fact that you can be slightly noisy once in a while :p , I can honestly say that I could not have wished for a better office mate. You allowed me to listen and sing along with all the music I was playing, and we could share all our joy and frustration together, without holding back. Thank you for the great time and stay positive like our possum! Riccardo Jonguh! Despite your excellent level in Dutch, I am writing this in English anyways. Where Laszlo was my companion in the office, you were my mate in the lab. After we became lab partners we directly bought a good stereo with Bluetooth, to make life more bearable during work. Being both passionate about music, we played our songs non-stop meanwhile pipetting and discussing life. However, not only inside the lab, but also outside we hang out a lot in which we visited concerts, watched football, chilled in the park, etc. I want to thank you for all this time we spend together in which you really became a close friend of mine and you always had a listening ear for the struggles I had during my PhD.

Obviously, my paranymphs weren't the only colleagues around. During my time in MolMic I met so many people of which sadly the majority has already left, which is exemplary for any research lab. I always find it sad that for a couple of years we spend so much time together, after which everyone goes their own direction and we hardly encounter. Despite this constant coming and going of people, I always felt at home in our department, in which we had a very open, tolerant and pleasant atmosphere, where I always felt comfortable to be myself. You are with so many and that is why I am not going to mention any names here,

but just thank you all for a wonderful time, and I hope that at least with some of you I will meet in the future again.

A special thanks goes to **Sabrina Koch**, whom I worked close together with on our shared chapter on anionic lipid-mediated translocation. Sabrina, I know it wasn't always easy for you to work with a slightly less organized person like yourself ;p , but I think we managed quite well. Speaking for myself, I think back with warm feelings to our little collaboration, in which in the end **Megha Patro** and **Cesar A. López** also played a crucial role. This chapter we worked on together was the most demanding and challenging one of my whole thesis, but we persisted and managed to publish it in the end, for which I am proud.

Moreover, I would like to highlight **Iris Verhoek** and **Niels Wolbert**, two master students that worked with me together on the cardiolipin project. You both were around in the final years of my PhD, in which I was mainly busy with writing. Therefore, it was a great help to me that in this busy time you both showed independency, and at the same time performed valuable research for this thesis. Thank you for all your effort and dedication and I wish you all the best for your own scientific path.

The next persons I would like to thank are **Alexej Kedrov** and **Jeanine de Keyzer**. Although, only Jeanine has been present in MolMic at the beginning of my PhD, you both have been an example for me. During my masters you both have guided and taught me the basic lab skills, while at the same time showing me how to perform research, thereby training me as a proper scientist. This has made a big impact on me and has helped me a lot throughout my entire scientific career, for which I am grateful.

Another crucial aspect of my PhD has been the LC-MS (also known as Lola). Although Lola most of the time provided me with excellent data, occasionally she wasn't willing to cooperate. However, in these moments of despair I could always rely on **Oleksandr Salo** and **Ton Schippers** (thermofisher scientific). Sasha, thank you for the numerous occasions in which you came to the rescue and fixed the machine. Ton, thank you for all the times even Sasha could not help us out and you were our last hope.

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Although the actual work (pipetting and writing) has all been performed at the university, a crucial aspect of a PhD is to not forget about life outside of the lab. For me this is best exemplified by the weekly band rehearsals. **Joost**, **Pepijn**, **Juul** en **Frank**, onze wekelijkse woensdagavond was altijd een perfecte onderbreking van de werkweek. Ik wil jullie bedanken voor alle mooie momenten die we daar hebben beleefd en alle mooie (en soms wat mindere) jams en liedjes die we daar samen hebben gespeeld en gecomponeerd.

Finally, I would like to thank my family. Lieve Anneke, Ric en Lieke, zoals opa zou zeggen: hij is eindelijk klaar met studeren! Ik denk dat jullie het wel weten, jullie beteken heel veel voor me. Niet alleen in het alledaagse leven, maar ook hier aan de universiteit zijn jullie belangrijk voor me geweest. Als ik advies nodig had tijdens mijn studie, en later ook tijdens mijn PhD, waren jullie altijd de eersten tot wie ik mij richtte. Omdat jullie me nou eenmaal door en door kennen weten jullie vaak goed hoe mij te helpen en kunnen jullie als de beste me het zetje in de goede richting geven. Maar bovenal, jullie hebben me altijd ondersteund, bij welke beslissing ik ook nam. Dat is erg belangrijk voor me geweest, waarbij jullie nooit druk op me hebben gelegd, maar me altijd vrij hebben gelaten om mijn eigen keuzes te maken. Bedankt dat jullie zijn er simpelweg altijd voor me zijn en jullie kunnen weer een belletje verwachten boven op de bergtop!

ABOUT THE AUTHOR

Marten Exterkate was born on July 9th in 1990 in the city of Utrecht, the Netherlands. After finishing his VWO (athenaeum) at the St. Bonifatiuscollege (Utrecht) in 2008, he started with the Bachelor Life, Science and Technology at the University of Groningen. Here, he followed the major Biomedical Sciences with an additional minor in Molecular Biology and Biotechnology. He was selected in 2011 for the research master Top-programme Biomolecular Sciences at the University of Groningen, where he successfully finished two research internships in the Departments of Molecular Microbiology and Molecular Systems Biology, thereby graduating his masters with *cum laude*. During the masters, he wrote a research proposal for a PhD position together with prof. dr. Arnold J.M. Driessen, that was granted within the framework of the NWO Graduate Programme: *Synthetic Biology for Advanced Metabolic Engineering*. The results of this PhD-research, performed at the Department of Molecular Microbiology at the University of Groningen under supervision of prof. dr. Arnold J.M. Driessen, are described in this thesis. Currently, Marten Exterkate is continuing his research in the same department, where he is active as a post-doctoral researcher within the NWO Gravity *BaSyC* consortium that aims to construct a synthetic minimal cell via bottom-up approaches.

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