University of Groningen

Sustainable membrane biosynthesis for synthetic minimal cells

Exterkate, Marten

DOI:
[10.33612/diss.98704569](https://doi.org/10.33612/diss.98704569)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Exterkate, M. (2019). *Sustainable membrane biosynthesis for synthetic minimal cells*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen. <https://doi.org/10.33612/diss.98704569>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Sustainable membrane biosynthesis for synthetic minimal cells

Marten Exterkate

The research described in this thesis was carried out in the Department of Molecular Microbiology, part of the Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, The Netherlands.

It was financially supported and funded by the "BaSyC – Building a Synthetic Cell" Gravitation grant [024.003.019] of the Netherlands Ministry of Education, Culture and Science (OCW), and the Netherlands Organization for Scientific Research (NWO) graduate programme: Synthetic Biology for Advanced Metabolic Engineering (022.004.006).

Author:	Marten Exterkate
Cover:	Anneke Rijnberk
Lay-out:	Ilse Modder, www.ilsemodder.nl
Printed by:	Gildeprint – Enschede, www.gildeprint.nl
ISBN:	978-94-6323-834-2
ISBN (<i>electronic version</i>):	978-94-034-2119-3

© M. Exterkate, the Netherlands, 2019.

All rights reserved. No part of this thesis may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording or any information storage or retrieval system, without prior permission of the author.

rijksuniversiteit
 groningen

Sustainable membrane biosynthesis for synthetic minimal cells

Proefschrift

ter verkrijging van de graad van doctor aan de
Rijksuniversiteit Groningen
op gezag van de
rector magnificus prof. dr. C. Wijmenga
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

vrijdag 25 oktober 2019 om 14:30 uur

door

Marten Exterkate

geboren op 9 juli 1990
te Utrecht

Promotores

Prof. dr. A.J.M. Driessen

Prof. dr. M. Heinemann

Beoordelingscommissie

Prof. dr. G. Maglia

Prof. dr. B. Poolman

Prof. dr. W. Huck

TABLE OF CONTENTS

Scope of the Thesis	9
Chapter 1	13
Synthetic Minimal Cell: Self-Reproduction of the Boundary Layer	
Chapter 2	39
Growing Membranes in Vitro by Continuous Phospholipid Biosynthesis from Free Fatty Acids	
Chapter 3	69
Cardiolipin Biosynthesis by the Promiscuous CIsA in a Synthetic Cellular Membrane	
Chapter 4	93
Two Distinct Anionic Phospholipid-Dependent Events Involved in SecA-Mediated Protein Translocation	
Chapter 5	123
Continuous Expansion of a Synthetic Minimal Cellular Membrane.	
Chapter 6	139
Summary	140
Samenvatting	148
Appendix	157
Beknopte Samenvatting	158
Acknowledgements	160
About the Author	166
List of Publications	168

Scope of the thesis

SCOPE OF THE THESIS

Over the past decades there has been an increasing interest in the construction of a synthetic minimal cell from lifeless, individual components. Only once we can faithfully construct a synthetic cell that is capable of reproducing, we will be able to understand the molecular basis of life and how the individual processes together are able to form a living entity. A key aspect of cellular reproduction is expansion of its surrounding boundary layer. This thesis focuses on the *in vitro* generation of such a growing surrounding layer: a phospholipid membrane, that not only functions as a barrier, but also has the ability to act as a matrix that supports other membrane related processes (e.g. membrane protein activity).

Chapter 1 provides a general introduction into the self-reproduction of synthetic cells/ compartments, thereby mainly focusing on the latest developments with respect to membrane growth and division. Although self-reproduction based on a variety of boundary layer building blocks is described, mainly phospholipid-based compartmentalization is discussed. Growth of such compartments is driven by phospholipid biosynthesis, based on the synthesis route in the model organism *Escherichia coli* that has been studied in great detail. Current developments in synthetic compartment division are also mainly based on *E. coli* cell division, but, as this is a challenging task, alternative mechanisms derived from archaea are discussed as well.

Chapter 2 forms the fundament of this thesis as it describes the engineering of a growing synthetic compartment based on a newly designed and developed phospholipid biosynthesis pathway, employing purified (membrane) proteins. This pathway supports the quantitative production of the phospholipid species PE and PG starting from simple building blocks. The construction of the *in vitro* phospholipid biosynthesis pathway is discussed, and includes a detailed description of its versatility, efficiency, and further possibilities to tune its membrane composition.

Chapter 3 continues on the work described in chapter 2, and adds cardiolipin as one of the products of the developed *in vitro* phospholipid biosynthesis pathway, further diversifying the pathway. Multiple cardiolipin synthesizing enzymes are characterized. They turn out to belong to a class of enzymes that are highly promiscuous, which make these enzymes interesting targets for the simple integration of other phospholipid species in a synthetic minimal cell.

Chapter 4 describes the specific anionic lipid-dependent activity of the Sec-translocase, responsible for protein secretion in bacterial cells, which serves as example of a membrane protein complex that can be functionally integrated in a synthetic minimal cell. The

dependence of the translocation activity on the anionic phospholipid concentration revealed two critical lipid-dependent steps that could be faithfully reconstituted in a growing membrane system. Since this chapter covers the coupling of protein translocation with membrane growth, it provides a first step toward a functional synthetic minimal cellular compartment.

Chapter 5 summarizes the major achievements with regard to membrane expansion of synthetic compartments, after which a perspective is provided on how to progress the field. This chapter mainly focusses on the development/design of a continuously expanding system and discusses the requirements for its success with an outlook to the future.

Chapter 6 summarizes all the topics described in the above chapters.