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STELLINGEN

BEHORENDE BIJ HET PROEFSCHRIFT

Sustainable membrane biosynthesis for synthetic minimal cells

door Marten Exterkate

1. Implementation of membrane protein insertion and folding systems will be essential for *in vitro* transcription/translation-based membrane expansion in a synthetic cell.
2. Engineering a synthetic cellular membrane module in its minimal form is a challenging and complex task. To maintain simplicity, implementation of alternative (non-biological) approaches should be explored.
3. For the development of a synthetic minimal cell based on *in vitro* transcription/translation of the cellular constituents, it is necessary to first develop functional sub-modules based on purified proteins that can be combined and transformed into a genome encoded system.
4. The promiscuity of cardiolipin synthases towards a multitude of phospholipid polar head groups allows for tunable and diverse lipid biosynthesis by means of a single enzyme, and offers a simple alternative for the synthesis of complex synthetic cellular membranes.
5. The anionic lipid-dependent activity of the translocon shows that not only the presence of, but also the ratio between certain lipid species is essential for a functional synthetic cellular membrane.
6. Although DOPC is not a membrane constituent in *Escherichia coli*, it is often used *in vitro* to stabilize liposomes due to its bilayer-forming propensity and thus should be considered as a constituent of a synthetic cellular membrane.
7. The moment you read DOPE on a hoodie as dioleoyl-phosphatidylethanolamine, you are fully integrated into the lipid field.
8. Doing a PhD is so much more than committing to ambitious research. You learn all the ins and outs of managing a four (mostly five) year project, which is not only beneficial for any future career, but also a great experience for many others aspects of life.
9. The spirit of research: We're on a road to nowhere, Come on inside. Takin' that ride to nowhere. We'll take that ride. (Talking Heads; Road to Nowhere)