When synthetic cells and ABC-transporters meet

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Cover design: Hendrik R. Sikkema, courtesy illustration synthetic cell to Bert Poolman.
Cover image: The construction of a synthetic cell, with OpuA represented in green and the enzymes arginine deiminase, ornithine transcarbamoylase, carbamate kinase, and the arginine/ornithine antiporter of the ADI pathway represented in orange
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I dedicate this dissertation to
Valentina, my family and my close friends
who provided me with the inspiration and energy to write it.

Enjoy the little things,
for one day you may look back and realize they were the big things.

Robert Brault

Contents

Scope of this thesis

This thesis covers multiple aspects of (synthetic) biochemistry. Starting from a global point of view and then going deeper into signalling on the single protein level. It provides insight into the state-of-the-art (synthetic) biochemistry and showcases the importance by zooming in on details and zooming out to see the broader context.

The first chapter provides a detailed analysis of important design principles of synthetic cells. The focus lies on the energy balance of the cell. We discuss several systems to (re)generate metabolic energy and give an estimation on how much energy in terms of ATP is needed to maintain a (synthtetic) cell.

In chapter 2 we use one of the systems that was presented in chapter 1 in the context of a cell-like environment. We have developed a system in liposomes that is able to use external arginine as a fuel to regenerate ATP on the inside of the vesicle. We show basic physicochemical homeostasis and use the ATP that is produced to fuel one of the key proteins in osmoregulation, the ABC transporter OpuA. In case of an osmotic upshift, OpuA is activated by ionic strength and is able to import the compatible solute glycine betaine against large concentration gradients, which is powered by ATP.

In chapter 3 we focus on this protein. With use of single particle cryo-electron microscopy we have obtained a number of structures of OpuA in multiple conformations that help in understanding the transport mechanism. We also show that OpuA is regulated by the second messenger cyclic-di-AMP, which acts as an emergency brake.

Chapters 4 and 5 focus on methodological advances towards single-molecule FRET studies on OpuA. First we demonstrate how we turned OpuA from a homodimeric into a heterodimeric protein complex (chapter 4). When homodimeric proteins are labeled with probes for *e.g.* smFRET or DEER spectroscopy, any mutation introduced in one protomer also arises in the subunit of the dimer. Transforming the protein into an apparent heterodimer, by tagging the two identical subunits differently, circumvents this problem.

Then, in chapter 5 we introduce an *in silico* approach to find new positions for labeling that can be used for smFRET or DEER spectroscopy. The approach uses two or more crystal structures as input and then systematically assesses all possible residue pairs and filters out positions with suitable accessiblility and spacing.

The final chapter (chapter 6) places the work presented in this thesis into perspective and provides a view on the possible future of the research.