Propositions accompanying the thesis

Recycling to grow

Cofactor conservation for sustainable phospholipid biosynthesis in synthetic cells

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1. **W**ith a volume comparable to that of *E. coli*, giant-unilamellar vesicles of ~1-2 μm diameter are ideal compartments for a sustainable metabolism. – *This thesis, Chapter 1.*
2. **H**aving nice and supportive colleagues makes all the difference.
3. **A**rginine breakdown-mediated glycerol 3-phosphate formation in compartment is limited by phosphate depletion but can be avoided by co-reconstituting the glycerol 3-phosphate/phosphate antiporter GlpT. – *This thesis, Chapter 2-4.*
4. **T**he multi-chamber dynamic dialysis setup developed for bottom-up synthetic cells ensures continuous substrate feed and waste product wash from the external environment, thus promoting sustained metabolic activities in confinement. – *This thesis, Chapter 2.*
5. **I**ncreasing the number of reconstituted components has the cost of an enhanced stochastic distribution in large-unilamellar vesicles. – *This thesis, Chapter 1-2.*
6. **S**ynthetic cells with an internal volume of 0.05 fL would require several days to undergo a full growth cycle if they were to rely exclusively on arginine breakdown for ATP formation. – *This thesis, Chapter 4.*
7. **L**ong-chain fatty acids do not diffuse through dialysis filters as free monomers, making dynamic dialysis an unsuitable technique to feed these substrates in combination with the retention of lipid vesicles. – *This thesis, Chapter 3.*
8. **I**n reconstituted systems, poor protein preparation significantly limits reproducibility and experimental progress. – *This thesis, Chapter 5.*
9. **F**reeze-thaw-extrusion is not a suitable technique to encapsulate membrane-associated PlsB and PlsC into large-unilamellar vesicles. – *This thesis, Chapter 4.*
10. **E**ventually, the construction of an autonomous, life-like synthetic cell will reveal more mechanistic complexities than initially anticipated and raise further unforeseen questions.