Steps towards the development of a minimal cell

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18.11.2019
Outline

1. Motivation
2. Triggered Gene Expression in Liposomes
3. Different approaches for the assembly of a minimal division machinery
4. Summary and outlook
„What I cannot create, I do not understand“

Richard Feynman
Motivation: Why an artificial cell?

Artificial cell: (synthetic) entity that mimics functions of a biological cell

- new insights about the **design principles** and the **origin of life**
- more easily controlled and more robust than natural cells
  -> addition of **new functions**
  -> **applications** e.g. in medicine
Motivation: Building an artificial cell

Components of an artificial cell:

- **Stable, semi-permeable membrane**
- **Carriers of the genetic information** (DNA, RNA)
- **Metabolic pathways** for providing energy to the cell
- **Machinery for self-reproduction** -> minimal divisome

**Top-down**
- Stripping or replacing the genomes

**Bottom-up**
- Assembling

Non-living components

Living organisms

Reducing complexity

Increasing complexity
Some important definitions

**Divisome**: complete macromolecular machinery able to effect division in the living cell includes proteins and also membranes that take part in the division.

**Phase Transition Temperature** $T_m$ of a lipid membrane:

- **Gel Phase**: Lipids rather immobile
- **Liquid Phase**: Lipids diffuse freely in the membrane

Dependence of $T_m$ on:
- lipid chain length
- saturation
Triggered Gene Expression in Fed-Vesicle Microreactors with a Multifunctional Membrane

- **Solution A:** Enzymes & Cofactors
- **Solution B:** Feeding Solution (Nutrients & tRNA)

**PUREExpress:** commercial kit for minimal gene expression machinery
Triggered Gene Expression in Fed-Vesicle Microreactors with a Multifunctional Membrane

A: Enzymes, Cofactors

B: Nutrients, tRNA

DNA Template

bilayer membrane

Z. NOURIAN ET AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012

PICTURE: HTTPS://UPLOAD.WIKIMEDIA.ORG/WIKIPEDIA/COMMONS/0/0F/LIPOSOMES%2A.PNG
Liposome Preparation
Basic Principle: Lipid Film Swelling

Dissolved lipids

Solvent evaporation

Stacked lipid bilayers

Buffer solution:

Osmotically driven flow into the bilayer stacks

Lipid film swelling

Vesicle formation

H. Stein et al.: "Production of isolated giant unilamellar vesicles under high salt concentrations", Frontiers in Physiology, 2017
Liposome Preparation: Protein-synthesizing liposome microreactors

Formation of stacked lipid bilayers by solvent evaporation

- Even Surface → Glass Beads
- Increased active surface area
- Increased yield in liposomes

Z. NOURIAN ET AL.: "TRIGGERED GENE EXPRESSION IN FED-VEICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
Liposome Preparation: Protein-synthesizing liposome microreactors

B Lipid Film Swelling at $T > T_m$

Rehydration Liquid

PURE Solution A: Enzymes & Cofactors

DNA Template coding for autofluorescent protein (green)
Liposome Preparation: Protein-synthesizing liposome microreactors

C Immobilize Vesicles on Coverslip

D Exchange Enzyme Solution with Feeding Solution

E Incubation at 37°C
Membrane Permeability: A Requirement for Gene Expression in Liposomes

A: Enzymes, Cofactors

B: Nutrients, tRNA

Product: emGFP

DNA Template

Bilayer membrane
Membrane Permeability: Test of different Lipid Compositions

Biotin-PEG-lipid + TRITC-lipid

- **DMPC**
  - DM: 14 C
- **DMPG**
  - DP: 16 C
- **DPPC**
  - DPPG: 1 double bond
- **DOPC**
  - DO: 18 C

*Z. NOURIAN ET AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
### Membrane Permeability: Test of different Lipid Compositions

<table>
<thead>
<tr>
<th>Lipid Composition</th>
<th>$T_m$</th>
<th>Swelling Temperature</th>
<th>$T_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DM</strong>: 14 C</td>
<td>$T_{m,DM} \approx 23^\circ C$</td>
<td>$T_{s,DM} \approx 30^\circ C$</td>
<td></td>
</tr>
<tr>
<td><strong>DP</strong>: 16 C</td>
<td>$T_{m,DP} \approx 41^\circ C$</td>
<td>$T_{s,DP} \approx 45^\circ C$</td>
<td></td>
</tr>
<tr>
<td><strong>DO</strong>: 18 C + double bond</td>
<td>$T_{m,DO} \approx -20^\circ C$</td>
<td>$T_{s,DO} \approx 30^\circ C$</td>
<td></td>
</tr>
</tbody>
</table>

**Swelling Temperature**

- **DM**: $T_{s,DM} \approx 30^\circ C$
- **DP**: $T_{s,DP} \approx 45^\circ C$
- **DO**: $T_{s,DO} \approx 30^\circ C$
Membrane Permeability:
Test of different Lipid Compositions

DP (16 C)  NO gene expression!
DM (14 C)  Gene expression!
DO (18 C) double bond  Permeable for nutrients & tRNA!

Damaged PURE system?  Non-Permeable?
Membrane Permeability: Test of different Lipid Compositions

Swelling Temperature $T_S = 45^\circ C$:

- Damage on PURE System
- **BUT:** Gene Expression is still possible
- Gene Expression!
- No Gene Expression!

Z. NOURIAN ET AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
Membrane Permeability: Test of different Lipid Compositions

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<th>Gene Expression</th>
<th>Permeability</th>
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20 Z. NOURIAN ET. AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
Membrane Permeability: Test of different Lipid Compositions

DP (16 C)

DM (14 C)

DO (18 C) double bond

$T < T_{m,DP} = 41^\circ C$

Gene expression!

$T > T_{m,DM} = 23^\circ C$

$T > T_{m,DO} = -20^\circ C$

NO gene expression!

$T = 37^\circ C$

Z. NOURIAN ET. AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
Membrane Permeability: A Challenge for large Molecules

\[ \text{tRNA: too large for Passive Diffusion through the membrane} \]
Membrane Permeability: A Challenge for polar & charged Molecules

Polar / Charged components of the Feeding Solution:

- tRNA
- Nucleotides
- (Some) amino acids

Membrane Permeability: Unknown Mechanisms

The *mechanisms* enabling the observed *semipermeability* remain *unknown*!
Membrane Permeability: Nourian’s Suggestion for tRNA Permeation

1. Osmotic Pressure
   - Membrane Defects: Transient membrane rupture and resealing
   - Permeation pathway

2. tRNA-bilayer interaction: Adsorption on membrane
   - Electrostatic: phosphate group (tRNA) & lipid headgroup
   - Hydrophobic: exposed nucleobases (tRNA) & lipid tail
   - Stronger interaction in liquid state (lipid packing less dense)
   - Increase of local concentration of tRNA on the membrane
Stochastic nature of Gene Expression in Liposomes

Heterogeneity in intensity / expression levels between individual vesicles!

- Liposome formation: Random partitioning of solution A molecules between the vesicles
- Efficacy of matter exchange with feeding solution: Surface / Volume ratio

Confined protein synthesis (in liposomes) ↔ batch reactor experiment

Z. NOURIAN ET AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
Assembly of a minimal divisome

next goal: implement compartment division of a minimal cell

need: elementary molecular machinery that supports the division of a cell model

several different approaches towards a minimal divisome conceivable
Different strategies

**Strategy 1: Lipid biosynthesis route**
- Excess membrane lipids or change of lipid composition
- Change of the physical parameters of the lipid bilayer
  - Cell shape deformation
  - Scission into smaller progeny cells

**Strategy 2: Membrane-deforming protein route**
- Membrane deformation at midcell with the help of certain proteins
  - Division into two halves
Strategy 1: Lipid biosynthesis route

**Approach 1a:**
Incorporation of excess lipids into the membrane

- change $\frac{A}{V}$ or $\Delta A_0$

lipid uptake from micelles in the environment or internal synthesis of lipids from precursors

- vesicle tubulation into long thread-like shapes
  separation due to gentle shearing
Strategy 1: Lipid biosynthesis route

Approach 1b:
Modification of the lipid composition

Example:
membrane composed of lipids in different phases (with different order)
shape transformation due to minimization of line tension at boundary
fission of a bud at the phase separation line upon moderate heating from 30°C to 35°C
Strategy 2: Membrane-deforming proteins

**Approach 2a:**
Use of membrane proteins involved in cell division in natural cells

**Example:** bacterial division machinery of E. coli
- multiprotein machinery
- Z-ring, comprising FtsZ, FtsA and ZipA, as the central element of the division machinery

J. LUTKENHAUS, S. PICHOFF, S. DU, BACTERIAL CYTOKINESIS: FROM Z RING TO DIVISOME, PMC, 2014
Strategy 2: Membrane-deforming proteins

Essential steps of cellular division:
- Z-ring formation
- Force generation -> distortion of the lipid membrane
- Progressive constriction of the Z-ring
- Completed division

FtsZ (together with FtsA and/or ZipA) provides a highly attractive route towards the fission of an artificial cell!

Z-ring constriction by bending force of FtsZ-filaments

Constriction of a membrane due to the contraction of the Z-ring

Upper image: M. OSAWA, D. ANDERSON, H. ERICKSON, CURVED FTSZ PROTOFILAMENTS GENERATE BENDING FORCES ON LIPOSOME MEMBRANES, THE EMBO JOURNAL, 2009;
Lower image: M. EXTERKATE, A. DRIESSEN, SYNTHETIC MINIMAL CELL: SELF-REPRODUCTION OF THE BOUNDARY LAYER, ACS OMEGA, 2019
Strategy 2: Membrane-deforming proteins

**Approach 2b:**
Use of non-canonical membrane remodeling proteins

**Example:** BAR-domain of a protein
- positively charged residues on its concave surface interact strongly with lipid headgroups
- "scaffold" for membrane curvature

BAR-domain of a protein binding to the membrane surface and bending the membrane

J. ZIMMERBERG, M. KOZLOV, HOW PROTEINS PRODUCE CELLULAR MEMBRANE CURVATURE, NATURE REVIEWS MOLECULAR CELL BIOLOGY, 2006
First successful attempt of triggered gene expression in liposomes via an outside feeding solution.

Two promising strategies for the implementation of a minimal divisome: lipid biosynthesis route and membrane deforming proteins route.
Outlook

Remaining challenges:
- increase in complexity when combining all elements of an artificial cell
- effective communication with the environment
- implementation of movement of the artificial cell
- construction of artificial cell networks
- ...

Applications / Benefits:
- engineered organisms to produce fuels or pharmaceuticals
- applications in biomedicine: e.g. imaging, drug delivery
Main sources

H. Stein et al.: *Production of Isolated Giant Unilamellar Vesicles under High Salt Concentrations*, Frontiers in Physiology, 2017
Y. Caspi et al.: *Divided we stand: splitting synthetic cells for their proliferation*, Syst Synth Biol, 2014
M. Exterkate et al.: *Synthetic Minimal Cell: Self-Reproduction of the Boundary Layer*, ACS Omega, 2019
C. Xu et al.: *Artificial cells: from basic science to applications*, Materials Today, 2016
A. Martos et al.: *Towards a bottom-up reconstitution of bacterial cell division*, Trends in Cell Biology, 2012
Thank you for your attention!
Backup Slides

[Image: HTTPS://UPLOAD.WIKIMEDIA.ORG/WIKIPEDIA/COMMONS/0/0F/LIPOSOME3%2A.PNG]