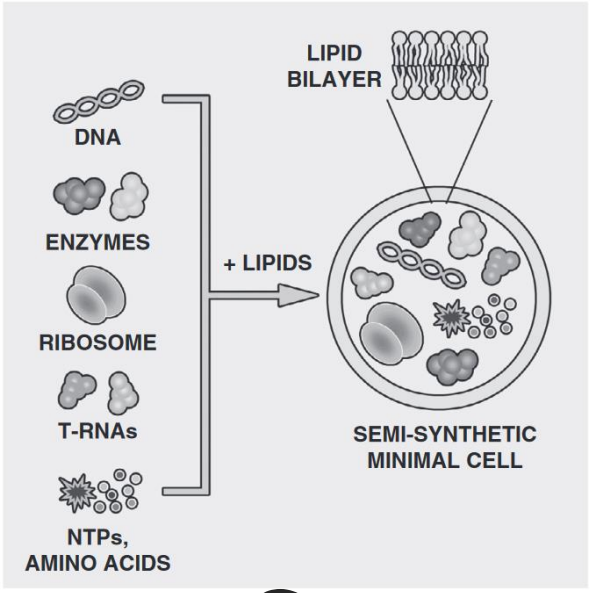
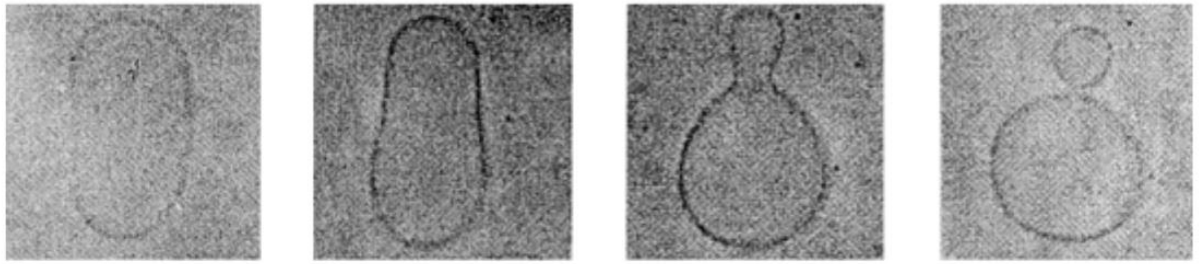




Steps towards the development of a minimal cell

SUSANNE REINHARDT, ANNA BAPTIST

18.11.2019

S. SVETINA, B. ZEKS, SHAPE BEHAVIOR OF LIPID VESICLES AS THE BASIS OF SOME CELLULAR PROCESSES, THE ANATOMICAL RECORD, 2002

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

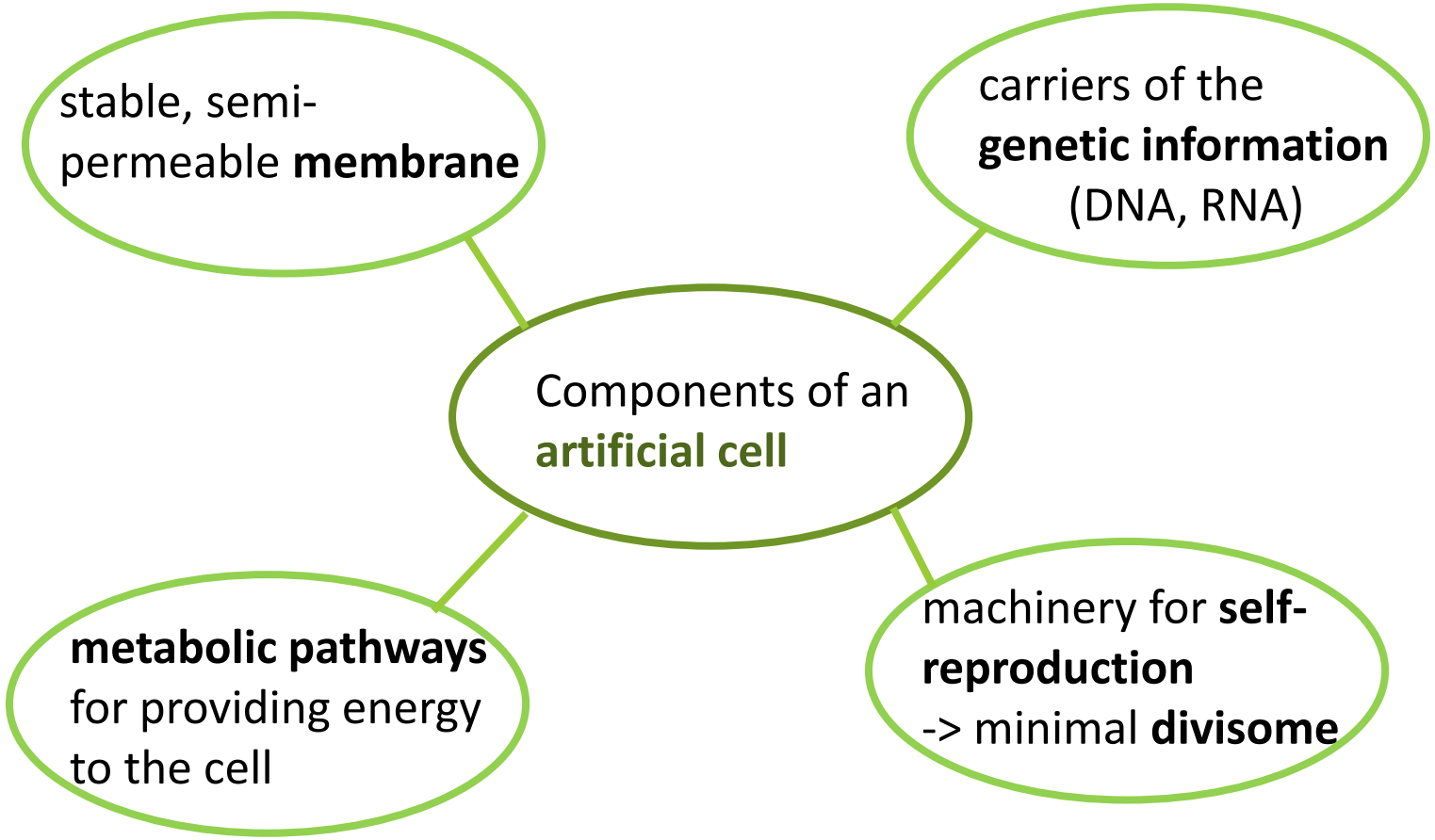
1 Motivation: Why an artificial cell?

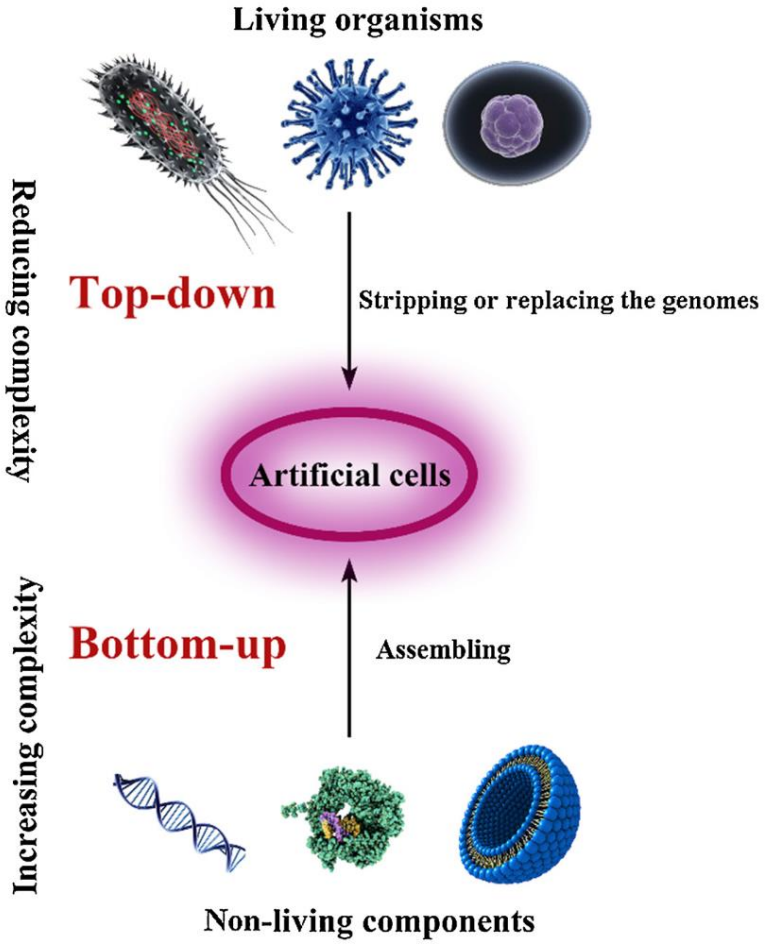
Artificial cell: (synthetic) entity that mimics functions of a biological cell

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

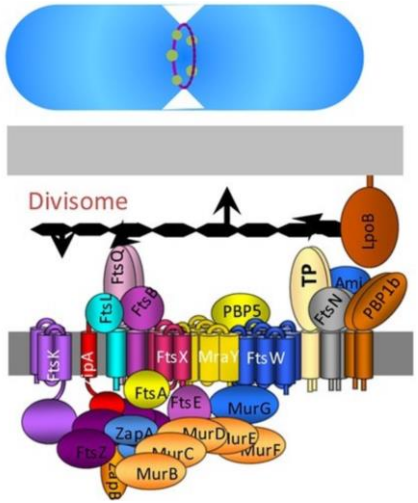
1 Motivation: Building an artificial cell





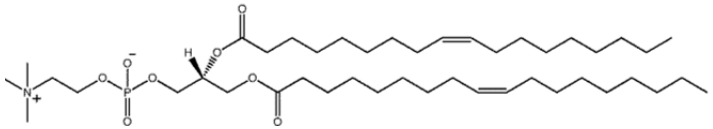
1 Some important definitions

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



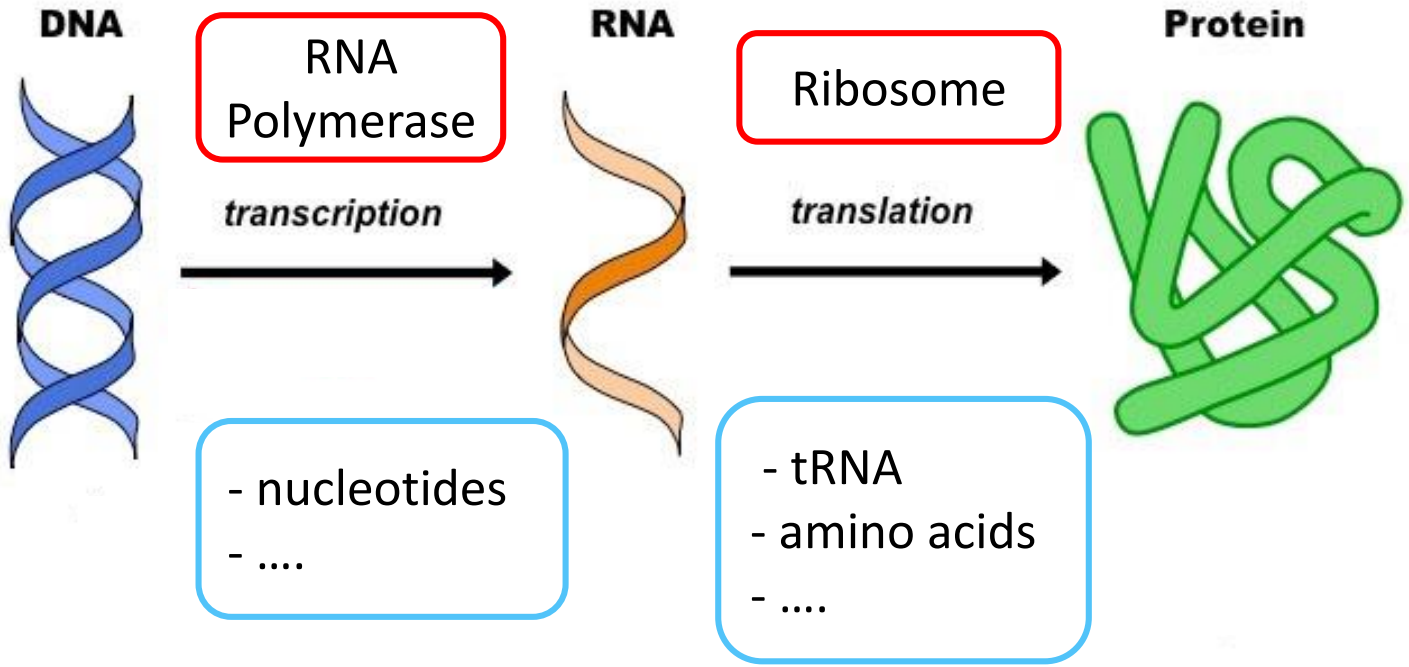
Phase Transition Temperature T_m of a lipid membrane:

Dependence of T_m on:
- lipid chain length
- saturation



PICTURE: HTTPS://EN.WIKIPEDIA.ORG/WIKI/DIVISOME
PHASE TRANSITION TEMPERATURE: CISSÉ: HTTP://GUAVA.PHYSICS.UIUC.EDU/~NIGEL/COURSES/569/ESSAYS_FALL2006/FILES/CISSE.PDF

2 Triggered Gene Expression in Fed-Vesicle Microreactors with a Multifunctional Membrane



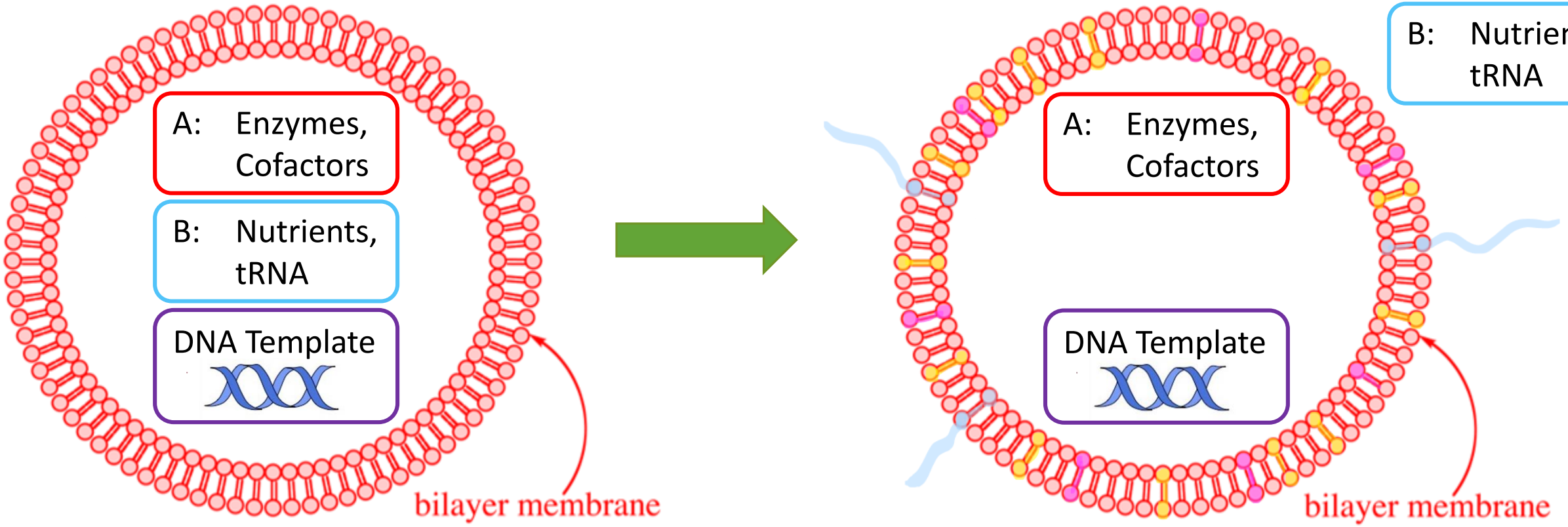
Solution A:
Enzymes & Cofactors

Solution B:
Feeding Solution
(Nutrients & tRNA)

PURExpress — commercial kit for minimal gene expression machinery

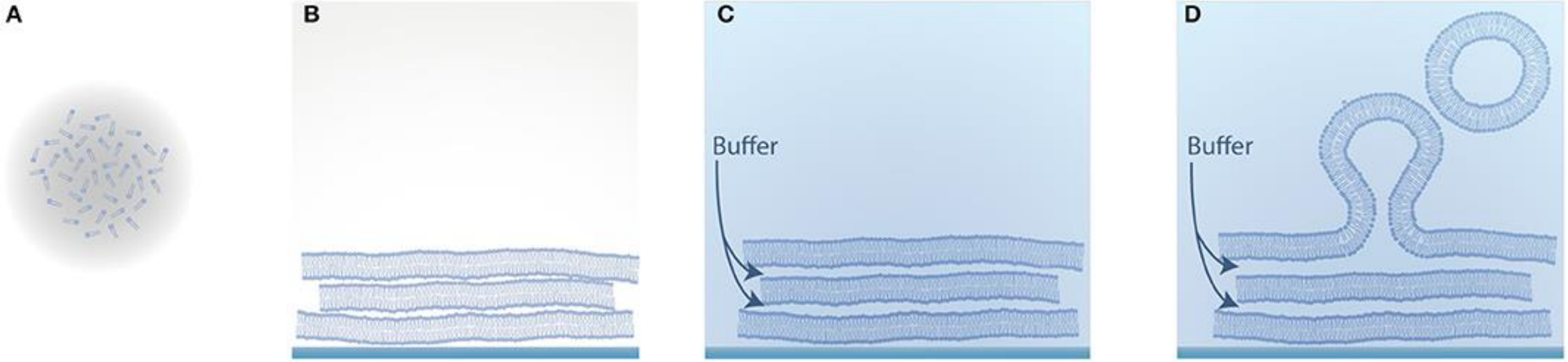
PICTURE: HTTPS://UPLOAD.WIKIMEDIA.ORG/WIKIPEDIA/COMMONS/0/0F/LIPOSOME3%2A.PNG
PUREEXPRESS: NEW ENGLAND BIOLABS: HTTPS://WWW.NEB.COM/PRODUCTS/E6800-PUREXPRESS-INVITRO-PROTEIN-SYNTHESIS-KIT#PRODUCT%20INFORMATION

2 Triggered Gene Expression in Fed-Vesicle Microreactors with a Multifunctional Membrane



Liposome Preparation
Basic Principle: Lipid Film Swelling

2



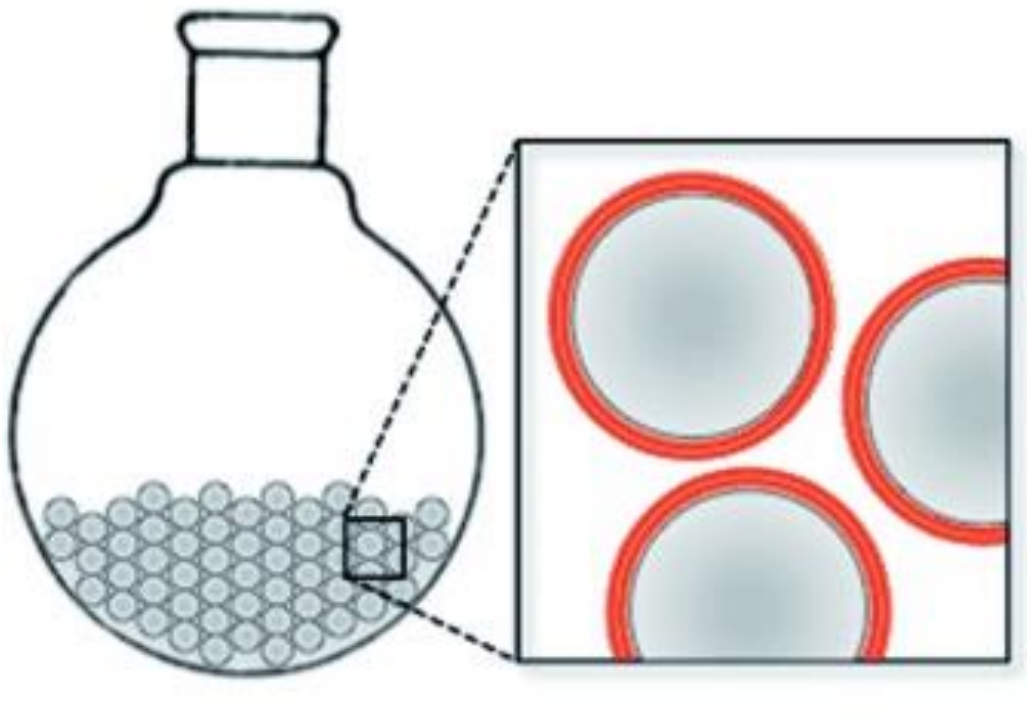
Dissolved lipids

Solvent evaporation
➡ Stacked lipid bilayers

Buffer solution:
Osmotically driven flow into the bilayer stacks

Lipid film swelling
➡ Vesicle formation

2 Liposome Preparation: Protein-synthesizing liposome microreactors



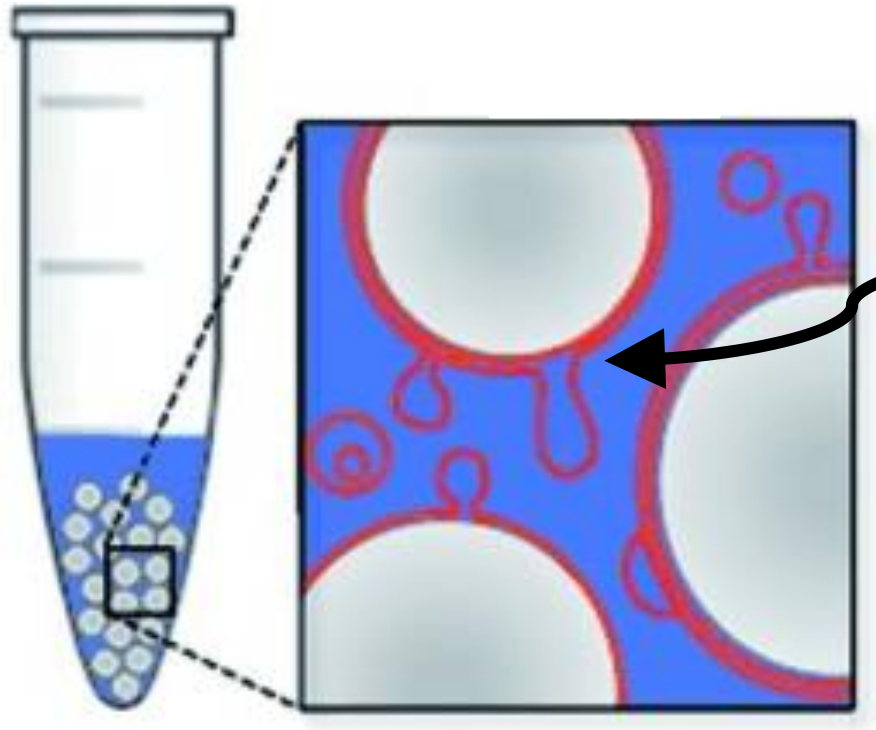
A Formation of stacked lipid bilayers by solvent evaporation

Even Surface → Glass Beads

↳ Increased active surface area

↳ Increased yield in liposomes

2 Liposome Preparation: Protein-synthesizing liposome microreactors

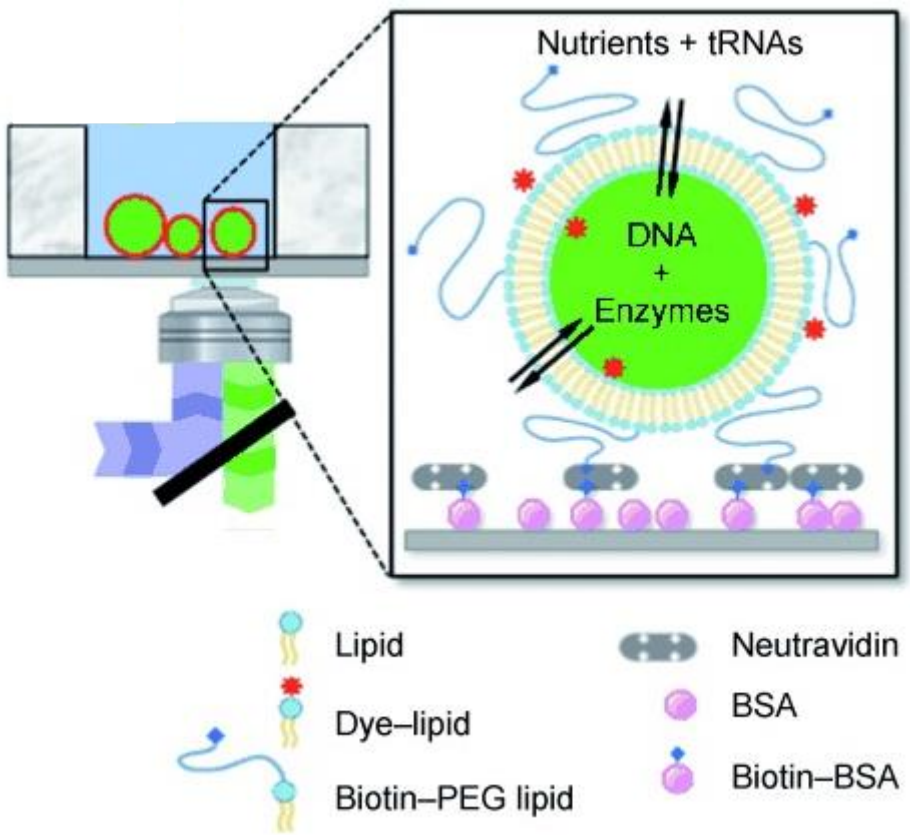


B Lipid Film Swelling at $T > T_m$

Rehydration Liquid

- PURE Solution A: Enzymes & Cofactors
- DNA Template coding for autofluorescent protein (green)

2 Liposome Preparation: Protein-synthesizing liposome microreactors

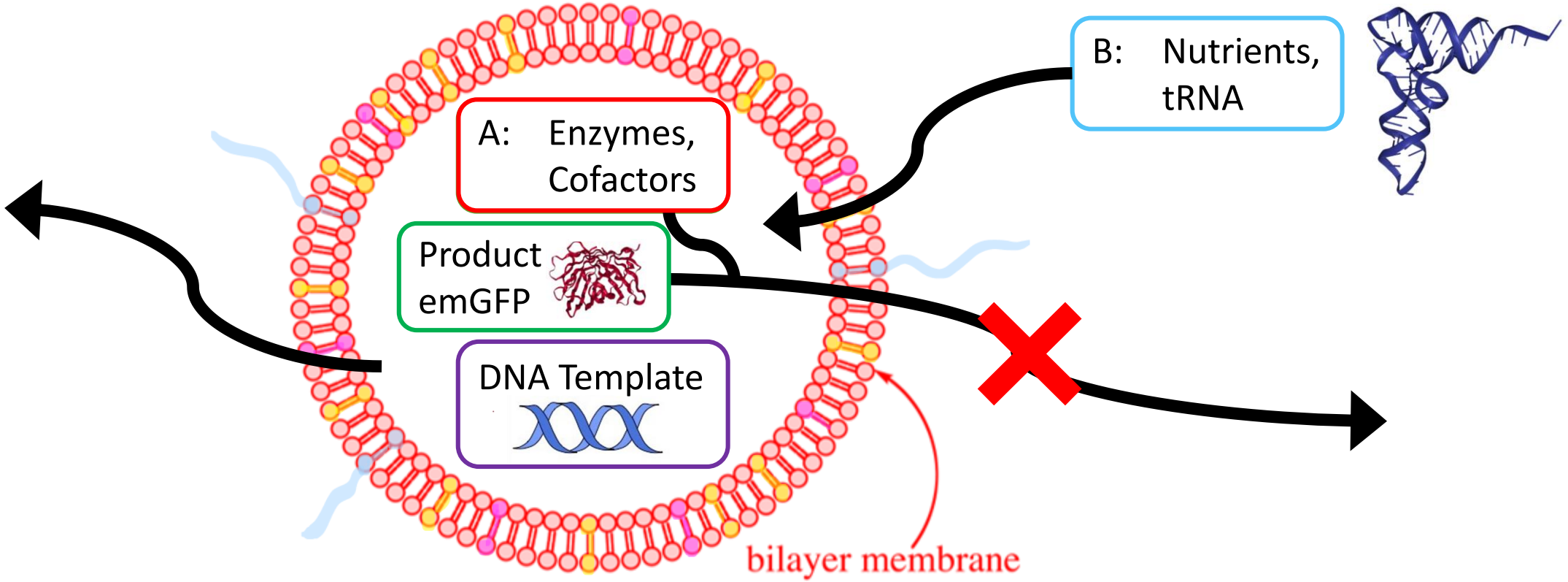


C Immobilize Vesicles on Coverslip

D Exchange Enzyme Solution with Feeding Solution

E Incubation at 37°C

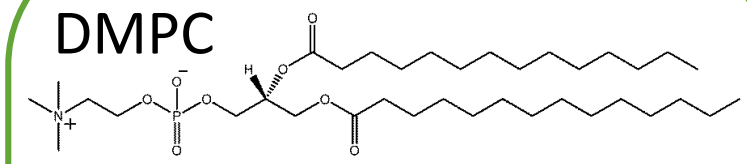
2 Membrane Permeability: A Requirement for Gene Expression in Liposomes



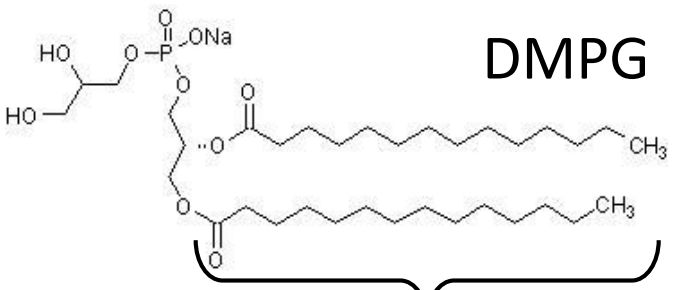
2 Membrane Permeability: Test of different Lipid Compositions

Biotin-PEG-lipid + TRITC-lipid

DMPC



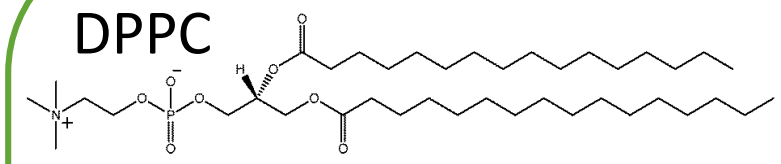
DMPG



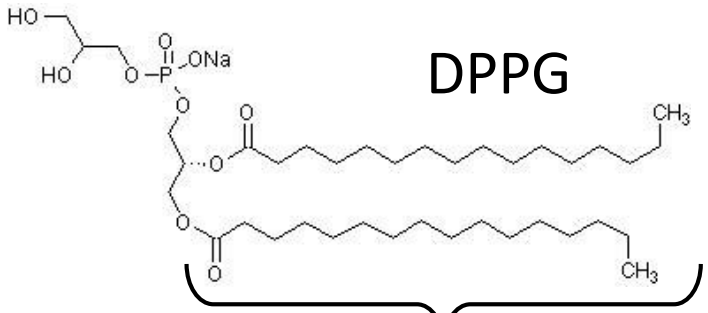
14 C

DM

DPPC



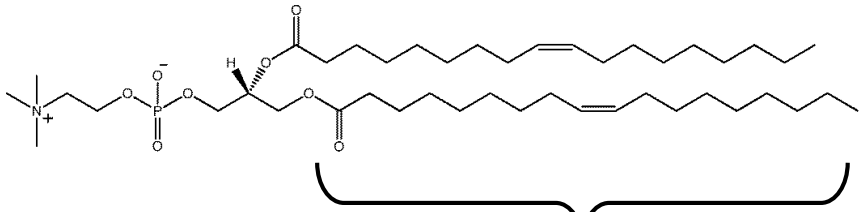
DPPG



16 C

DP

DOPC



18 C

1 double bond

DO

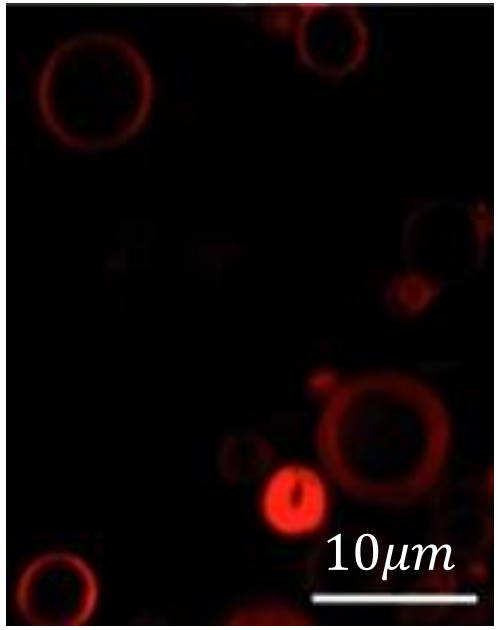
Z. NOURIAN ET.AL.: "TRIGGERED GENE EXPRESSION IN FED-VESICLE MICROREACTORS WITH A MULTIFUNCTIONAL MEMBRANE", ANGEWANDTE CHEMIE, 2012
PICTURES: HTTPS://WWW.ABCAM.CO.JP/12-DIPALMITOYL-SN-GLYCERO-3-PHOSPHORYLGLYCEROL-SODIUM-SALT-DPPG-PHOSPHORYLGLYCEROL-AB143953.HTML, HTTPS://WWW.ABCAM.CO.JP/12-DIMYRISTOYL-SN-GLYCERO-3-PHOSPHORYLGLYCEROL-SODIUM-SALT-DMPG-PHOSPHATIDYLGLYCEROL-AB143952.HTML, HTTPS://EN.WIKIPEDIA.ORG/WIKI/PHOSPHOLIPID

2 Membrane Permeability: Test of different Lipid Compositions

DM: 14 C	**DP**: 16 C	**DO**: 18 C + double bond
$T_{m,DM} \approx 23°C$	$T_{m,DP} \approx 41°C$	$T_{m,DO} \approx -20°C$
Swelling Temperature:	Swelling Temperature:	Swelling Temperature:
$T_{S,DM} \approx 30°C$	$T_{S,DP} \approx 45°C$	$T_{S,DO} \approx 30°C$

2 Membrane Permeability: Test of different Lipid Compositions

DP (16 C)

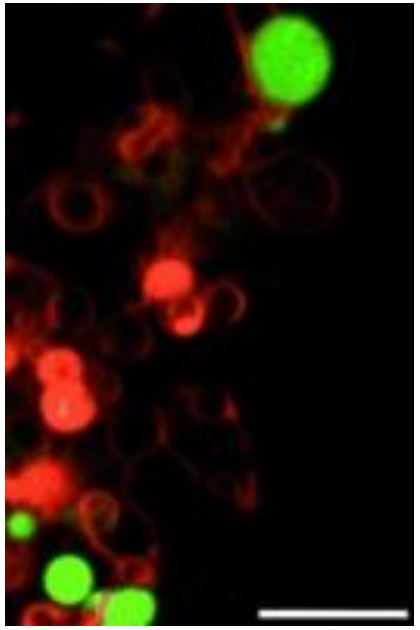


NO gene expression!

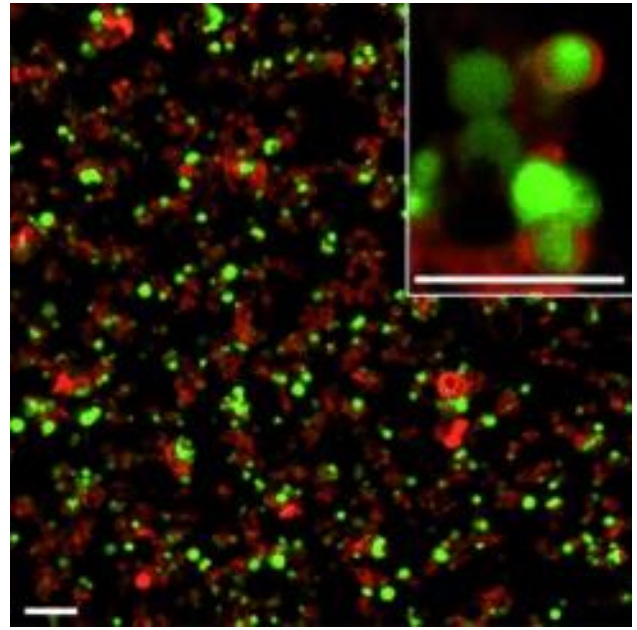
Damaged PURE system ?

Non-Permeable?

DM (14 C)



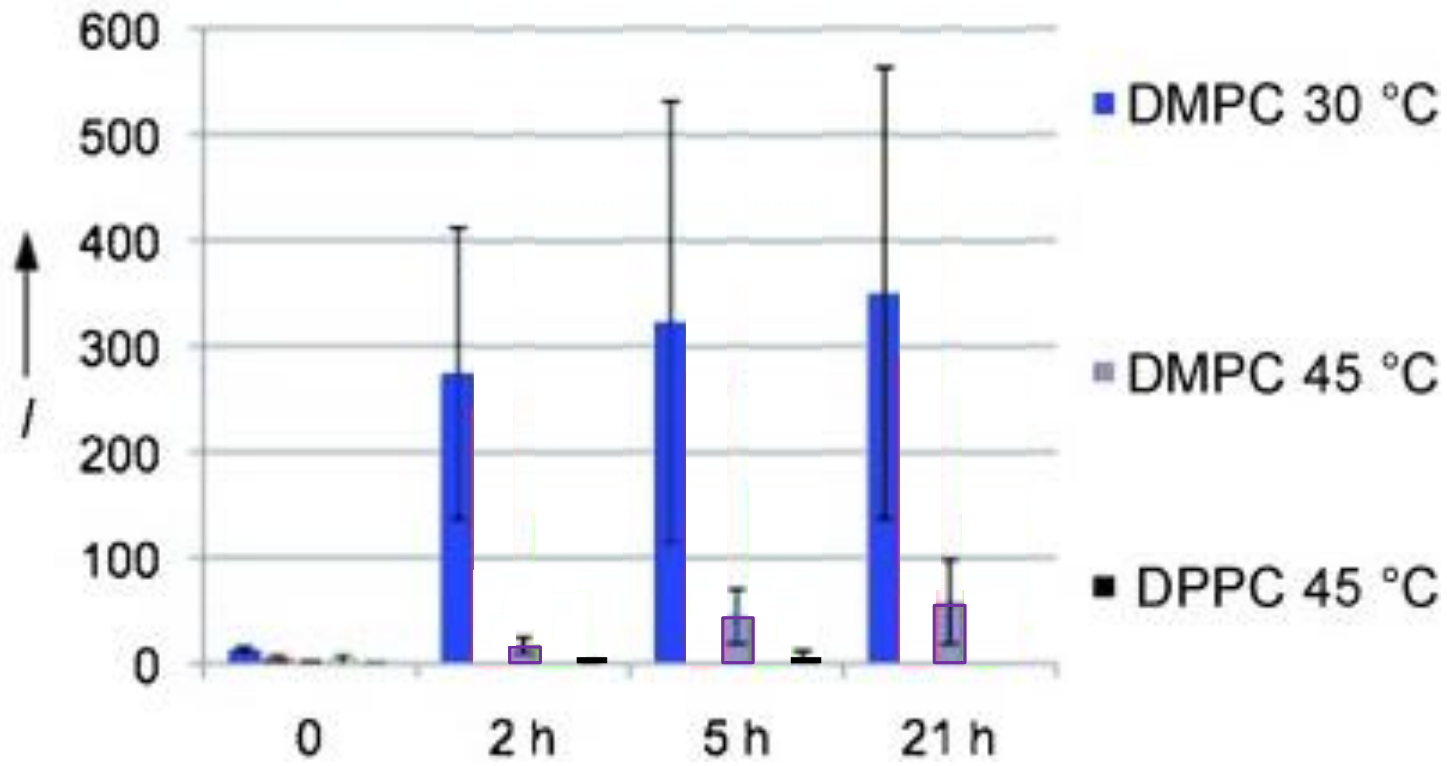
DO (18 C) double bond



Gene expression!

Permeable for nutrients & tRNA!

2 Membrane Permeability: Test of different Lipid Compositions



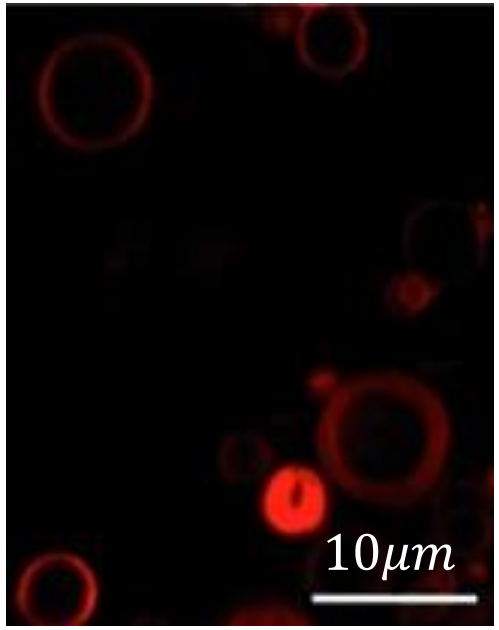
Swelling Temperature $T_S = 45°C$:

- Damage on PURE System
- **BUT:** Gene Expression is still possible

- Gene Expression!
- No Gene Expression!

2 Membrane Permeability: Test of different Lipid Compositions

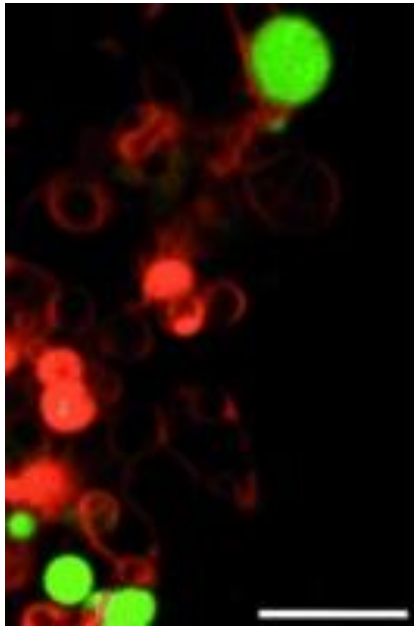
DP (16 C)



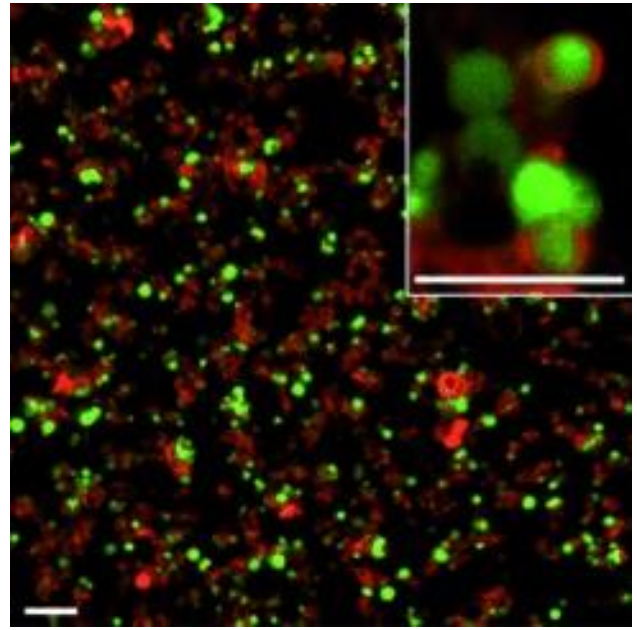
NO gene expression!

Non-Permeable!

DM (14 C)



DO (18 C) double bond



Gene expression!

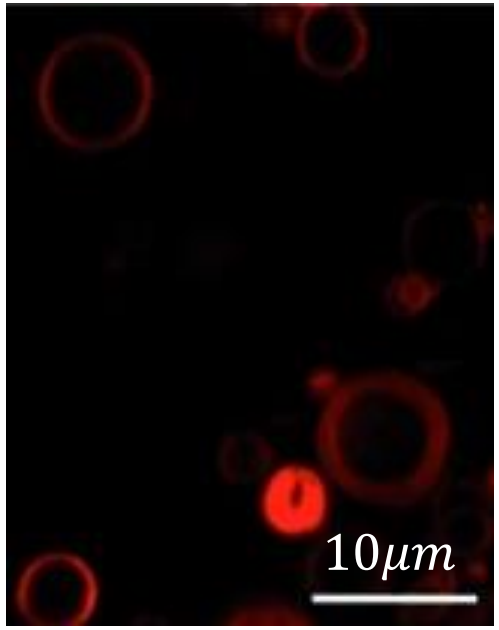
Permeable for nutrients & tRNA!

Membrane Permeability: Test of different Lipid Compositions

2

$T = 37°C$

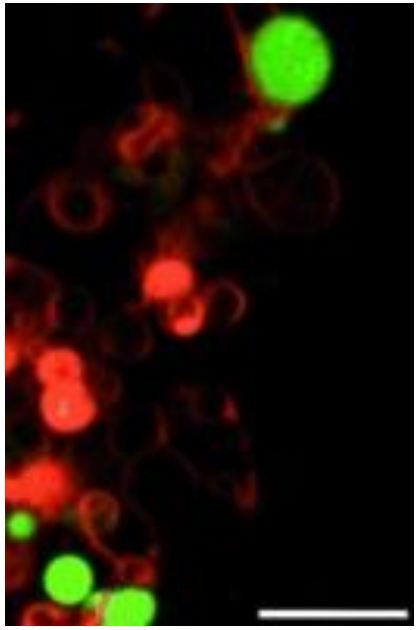
DP (16 C)



NO gene expression!

$T < T_{m,DP} = 41°C$

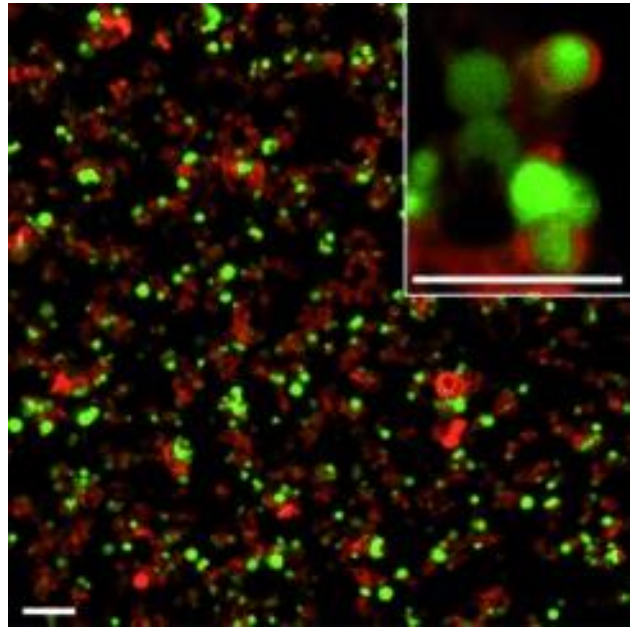
DM (14 C)



Gene expression!

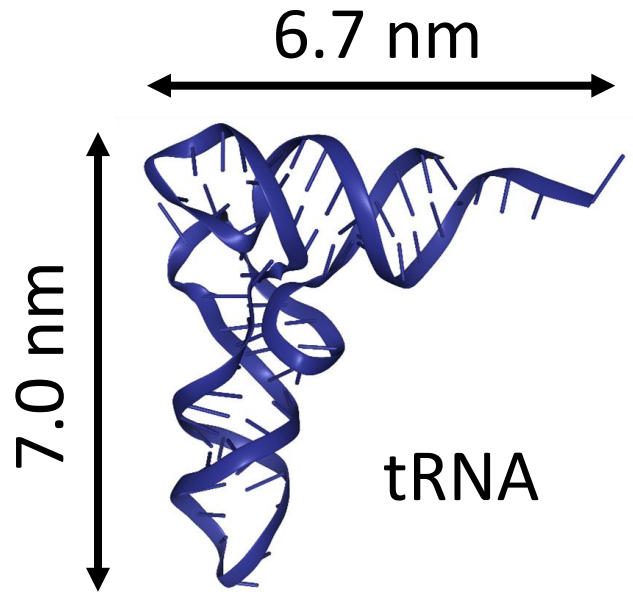
$T > T_{m,DM} = 23°C$

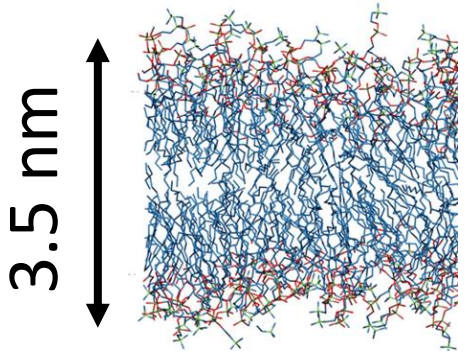
DO (18 C) double bond



$T > T_{m,DO} = -20°C$

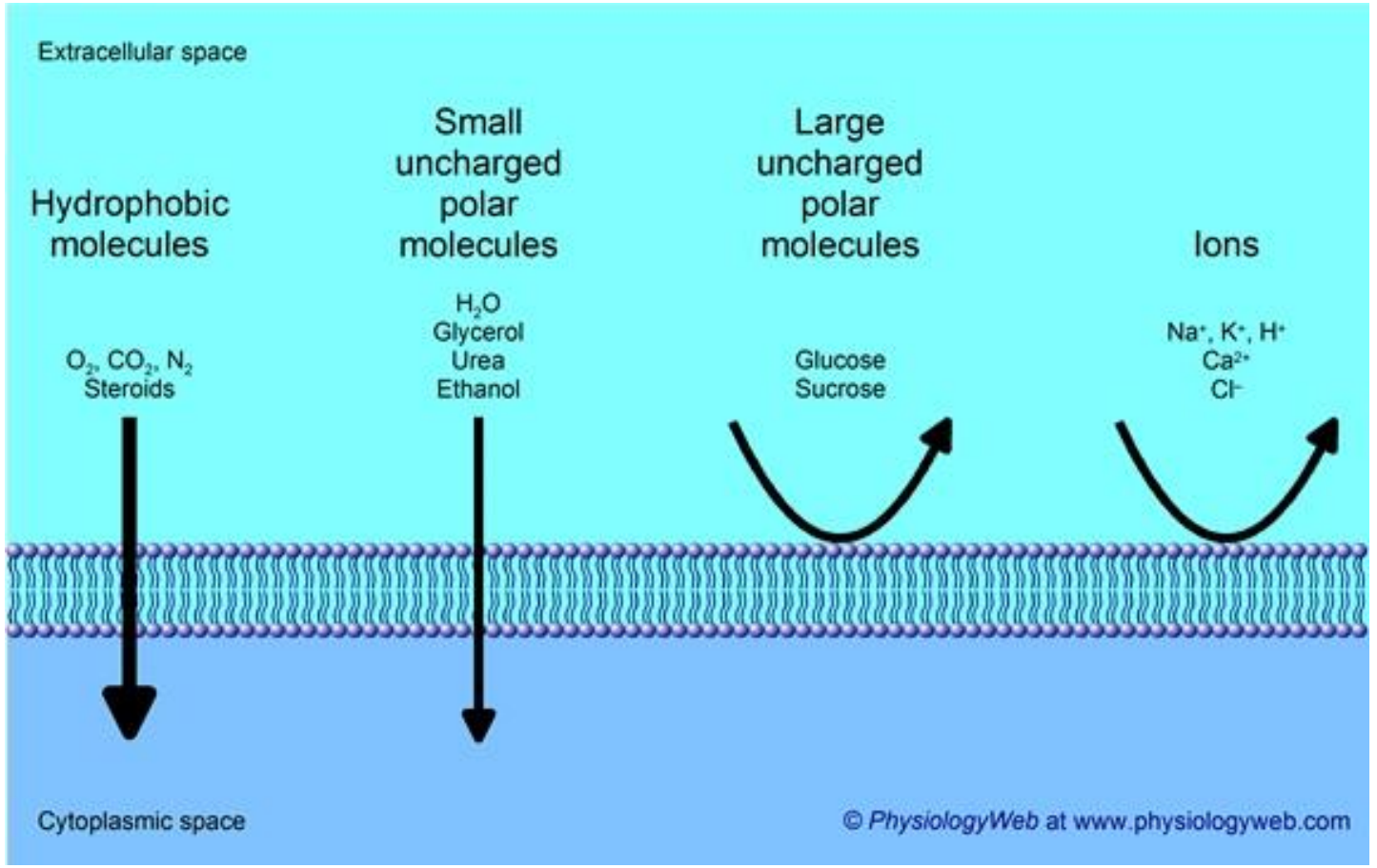
2 Membrane Permeability: A Challenge for large Molecules



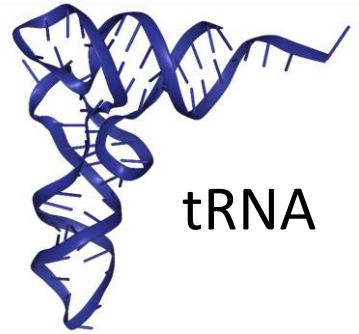


tRNA: **too large** for Passive Diffusion through the membrane

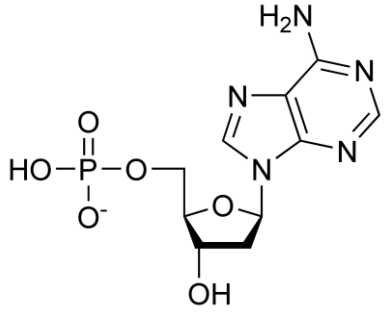
2 Membrane Permeability: A Challenge for polar & charged Molecules



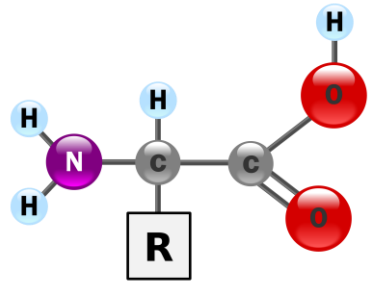
Polar / Charged components of the Feeding Solution:



tRNA



Nucleotides



(Some) amino acids

2 Membrane Permeability: Unknown Mechanisms

The **mechanisms** enabling the observed **semipermeability** remain **unkown**!

2 Membrane Permeability: Nourian‘s Suggestion for tRNA Permeation

1. Osmotic Pressure

- Membrane Defects: Transient membrane rupture and resealing

↳ Permeation pathway

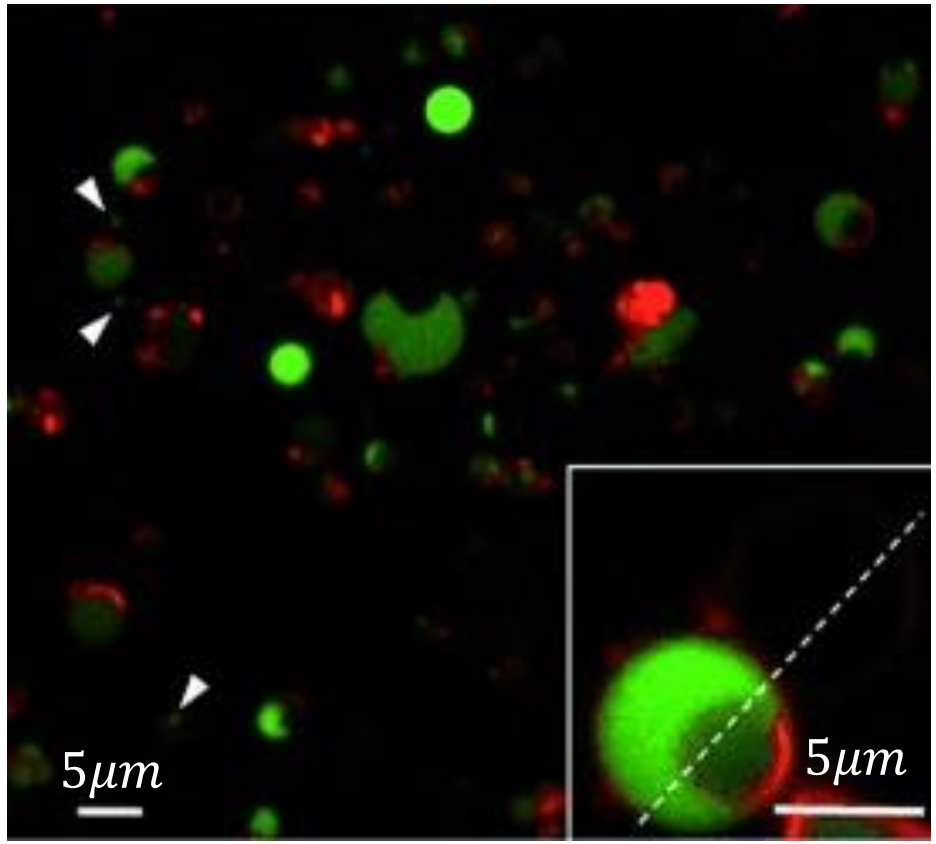
2. tRNA-bilayer interaction: Adsorption on membrane

- 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

2 Stochastic nature of Gene Expression in Liposomes



Heterogeneity in intensity / expression levels between individual vesicles!

Confined protein synthesis (in liposomes) ⟷ batch reactor experiment

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

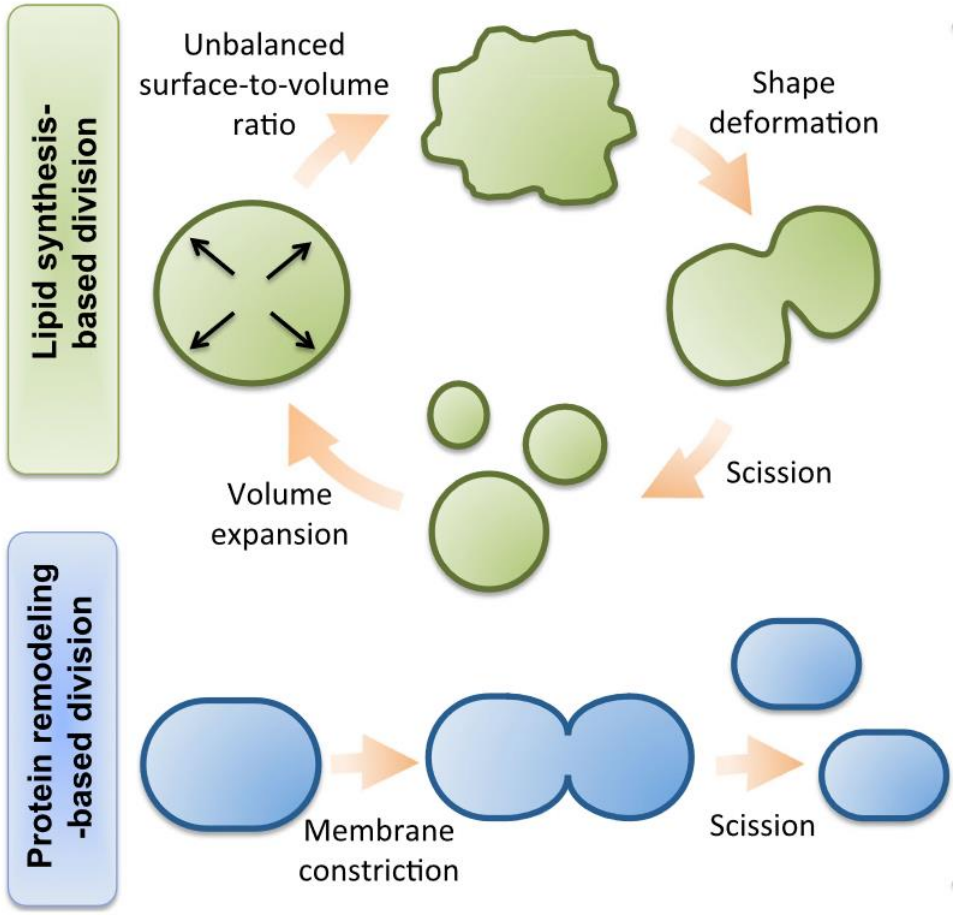
3 Assembly of a minimal divisome

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

3 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

3 Strategy 1: Lipid biosynthesis route

Approach 1a:

Incorporation of excess lipids into the membrane

➡ change $\frac{A}{V}$ or ΔA_0

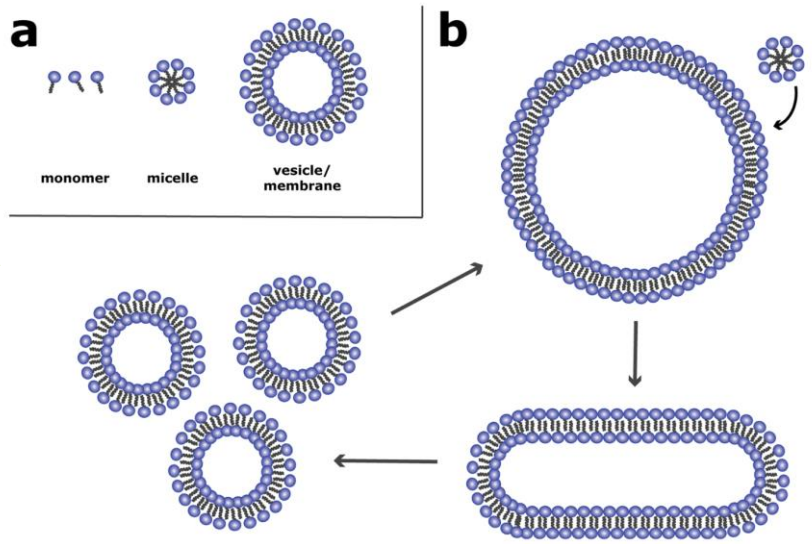
lipid uptake from micelles in the environment

or

internal synthesis of lipids from precursors

➡ vesicle tubulation into long thread-like shapes

separation due to gentle shearing

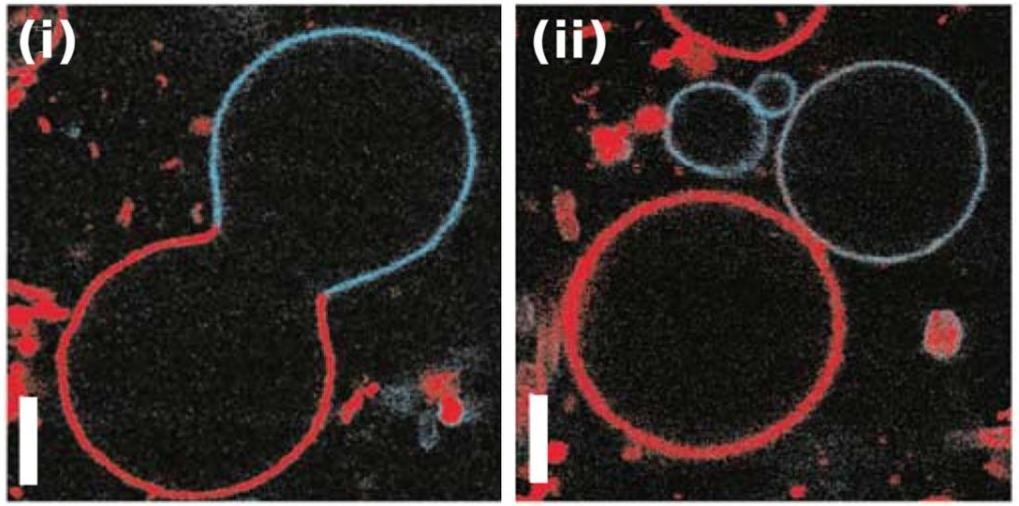


3 Strategy 1: Lipid biosynthesis route

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



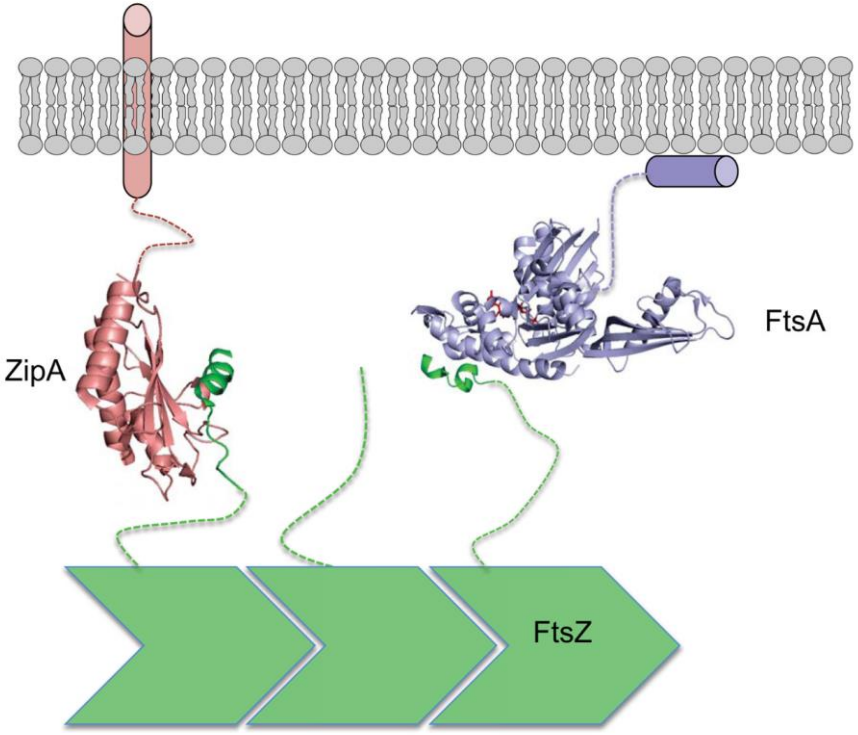
fission of a bud at the phase separation line upon moderate heating from 30°C to 35°C

3 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

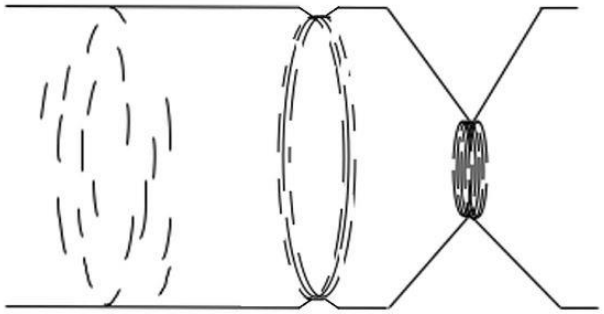


FtsZ filaments tethered to the membrane by FtsA and ZipA

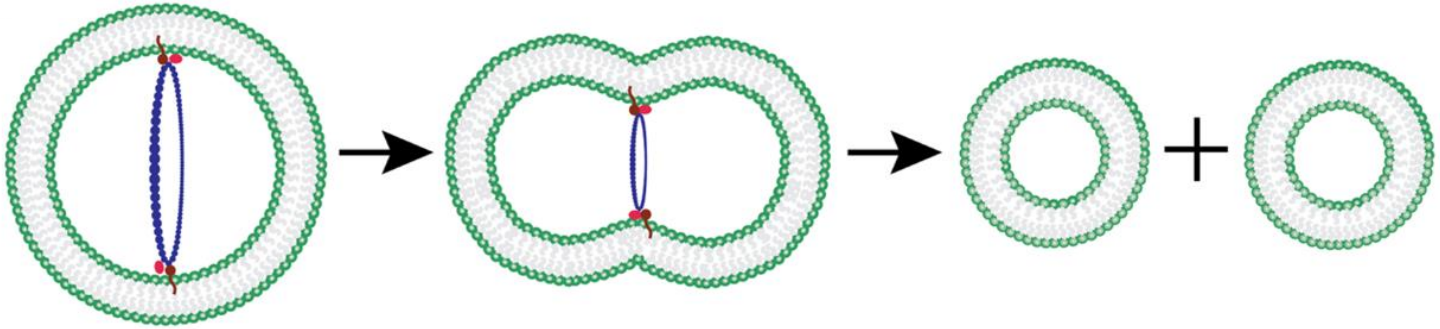
3 Strategy 2: Membrane-deforming proteins

Essential steps of cellular division:

- Z-ring formation
- force generation -> distortion of the lipid membrane
- progressive constriction of the Z-ring
- completed division



Z-ring constriction by bending force of FtsZ-filaments



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

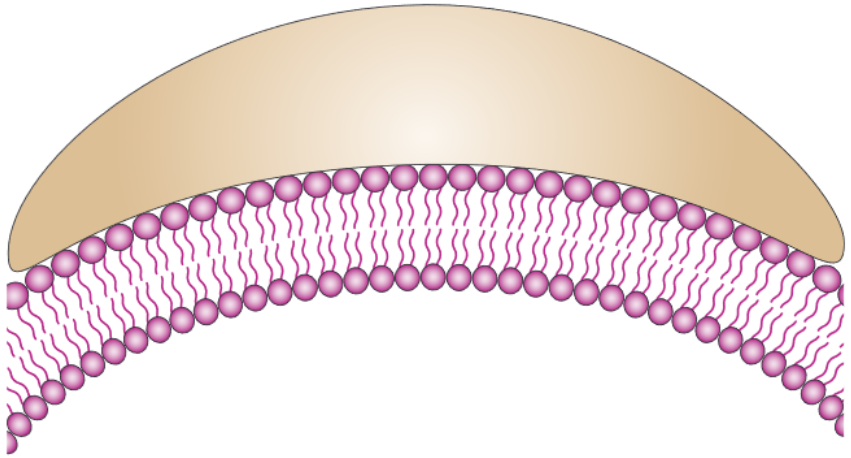
FtsZ (together with FtsA and/or ZipA) provides a highly attractive route towards the fission of an artificial cell!

3 Strategy 2: Membrane-deforming proteins

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

Example: BAR-domain of a protein

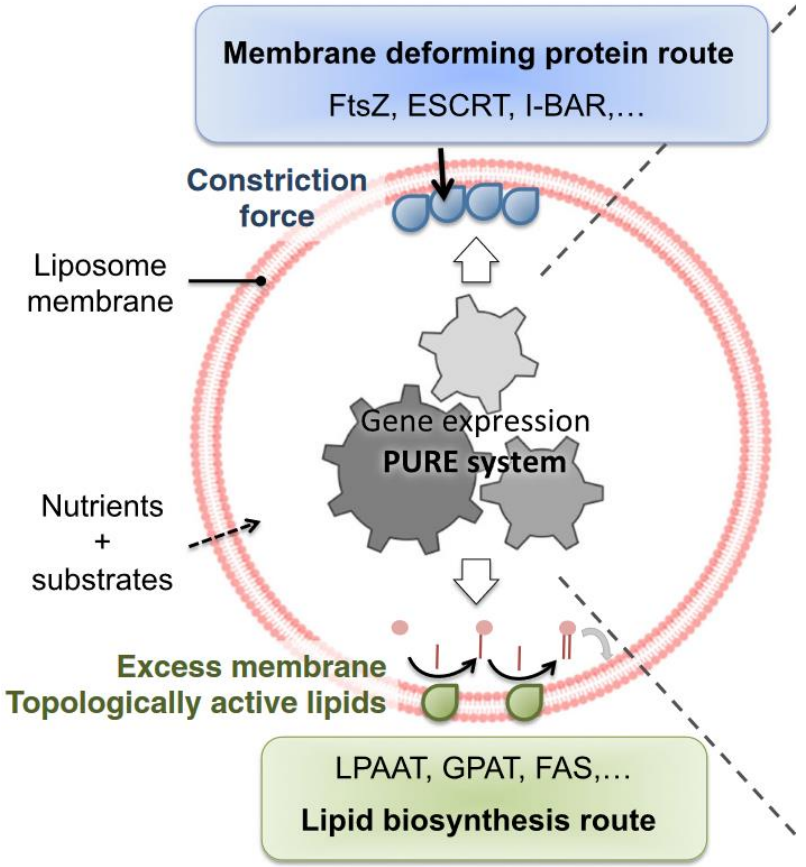
- positively charged residues on its concave surface interact strongly with lipid headgroups
- "scaffold" for membrane curvature



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

4 Summary

- 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



4 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:

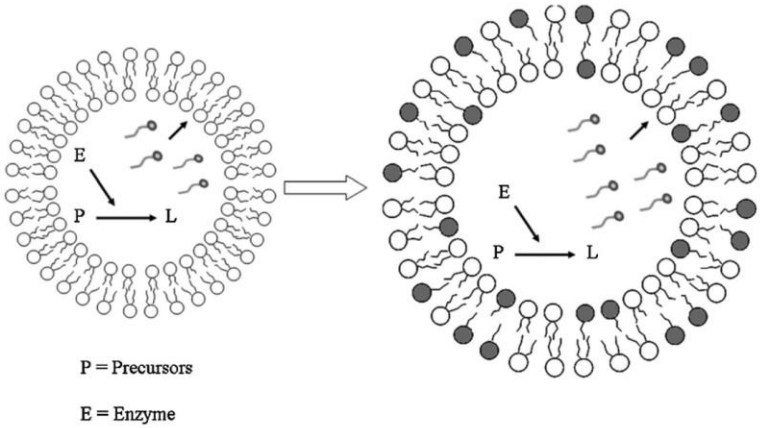
- engineered organisms to produce fuels or pharmaceuticals
- applications in biomedicine: e.g. imaging, drug delivery

Main sources

Z. Nourian et al.: *Triggered Gene Expression in Fed-Vesicle Microreactors with a Multifunctional Membrane*, Angewandte Chemie, 2012
H. Stein et al.: *Production of Isolated Giant Unilamellar Vesicles under High Salt Concentrations*, Frontiers in Physiology, 2017
Z. Nourian et al.: *Toward the assembly of a minimal divisome*, Syst Synth Biol, 2014
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



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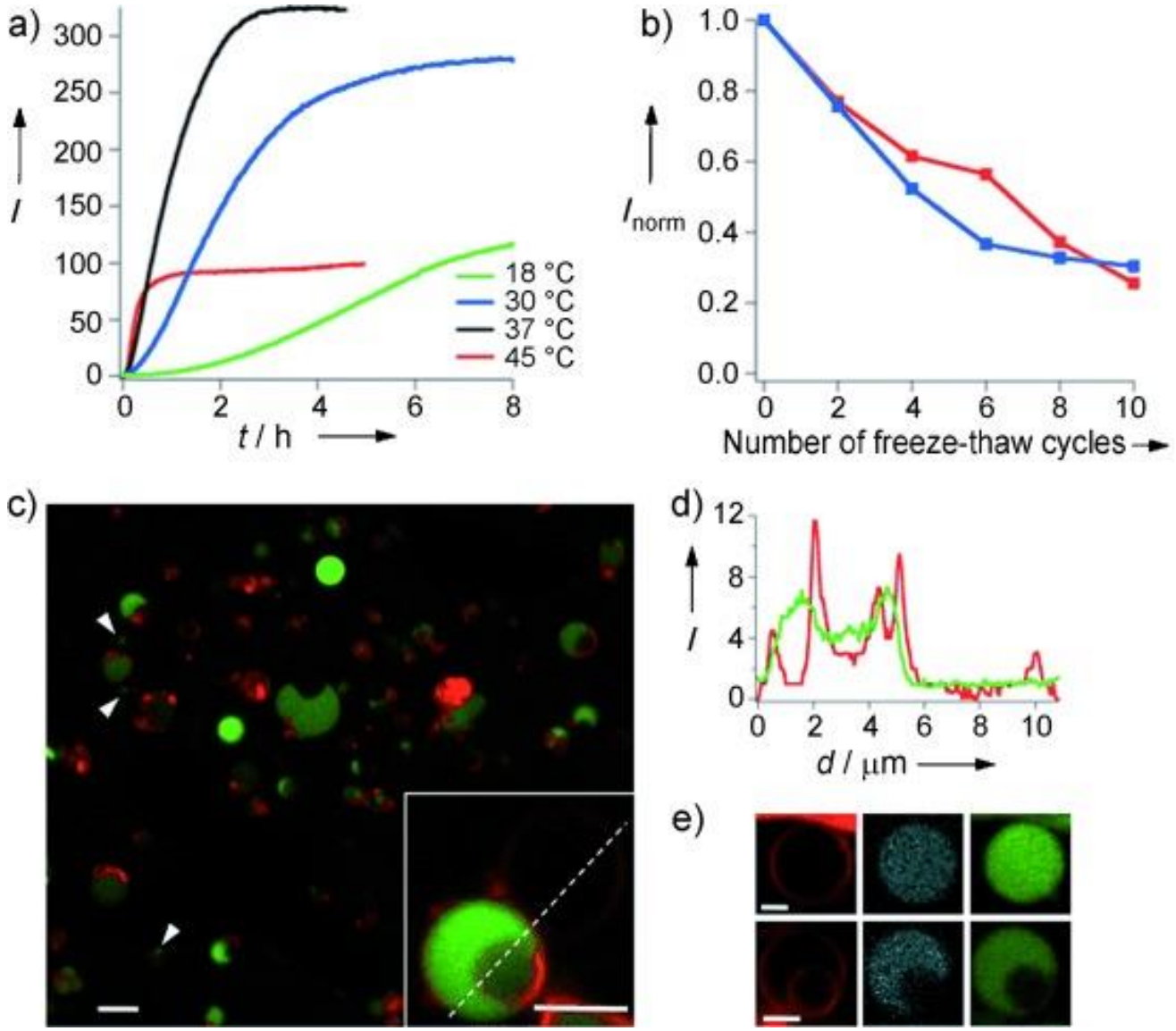
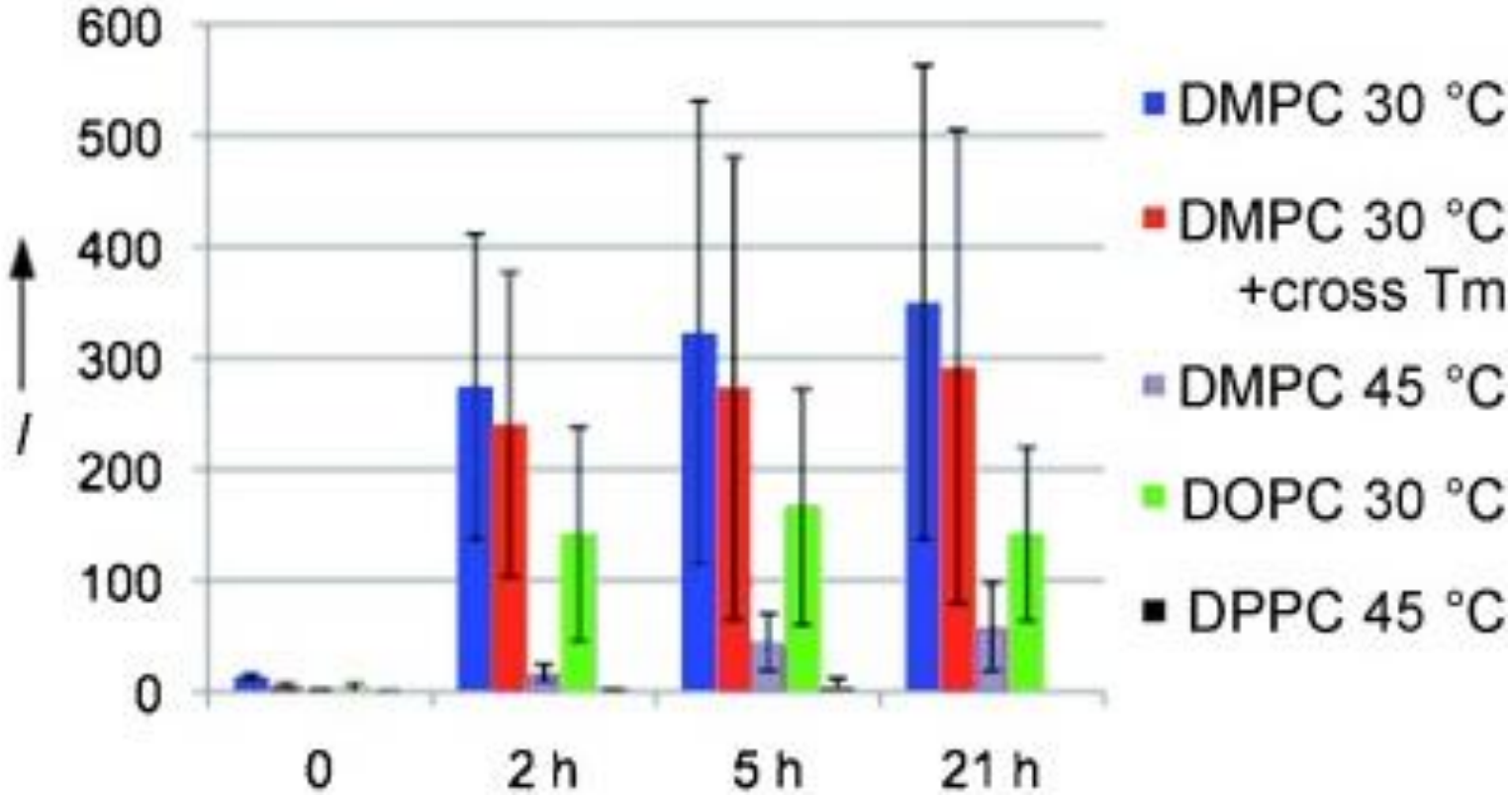

a)
300
250
200
150
100
50
0
I
0
2
4
6
8
t / h
18 °C
30 °C
37 °C
45 °C
b)
1.0
0.8
0.6
0.4
0.2
0.0
I norm
0
2
4
6
8
10
Number of freeze-thaw cycles
c)
d)
12
8
4
0
I
0
2
4
6
8
10
d / µm
e)



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