

*Challenges in Top-Down, Bottom-Up and
Computational Approaches in Synthetic Biology*

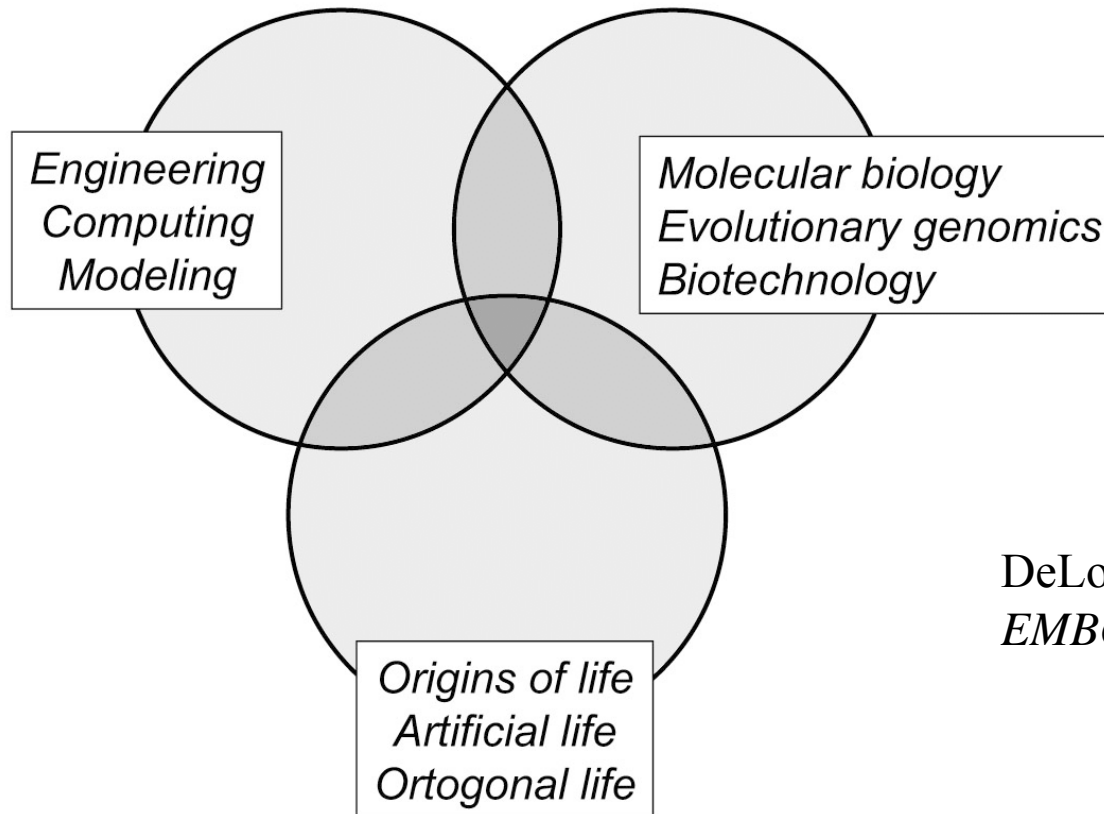
Nottingham, 19th March 2010

***Synthetic (constructive) biology:
From vesicles self-reproduction to
semi-synthetic minimal cells***

*Pasquale Stano, Pier Luigi Luisi
Biology Department, University of RomaTre, ROME*

stano@uniroma3.it

Three pillars of synthetic biology



DeLorenzo, Danchin,
EMBO Reports 2008

Thanks to **synthetic (constructive) biology**
we can study biological systems
that do not exist in nature

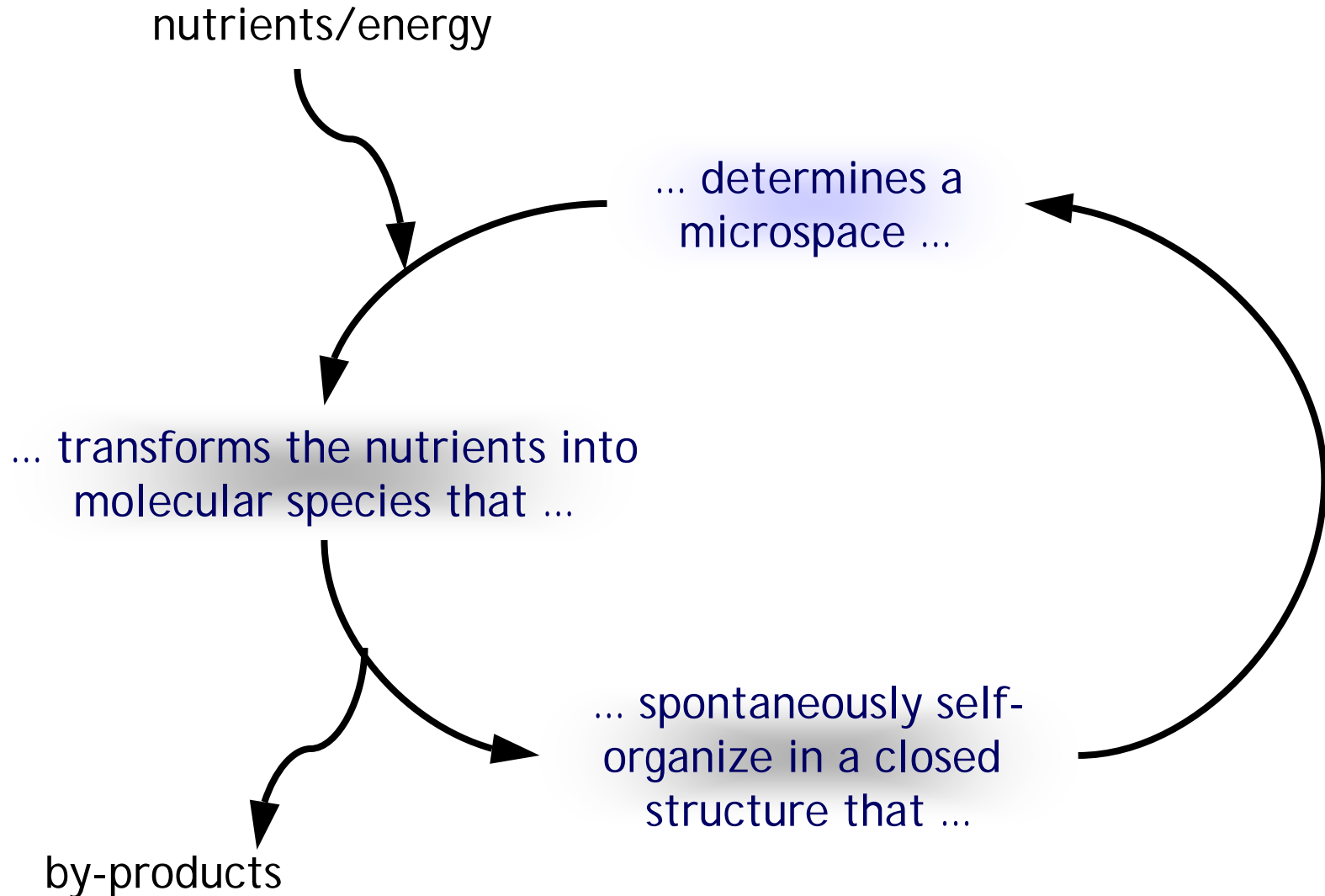
e.g. synthetic cells, primitive cell models

Towards the construction of minimal living cells

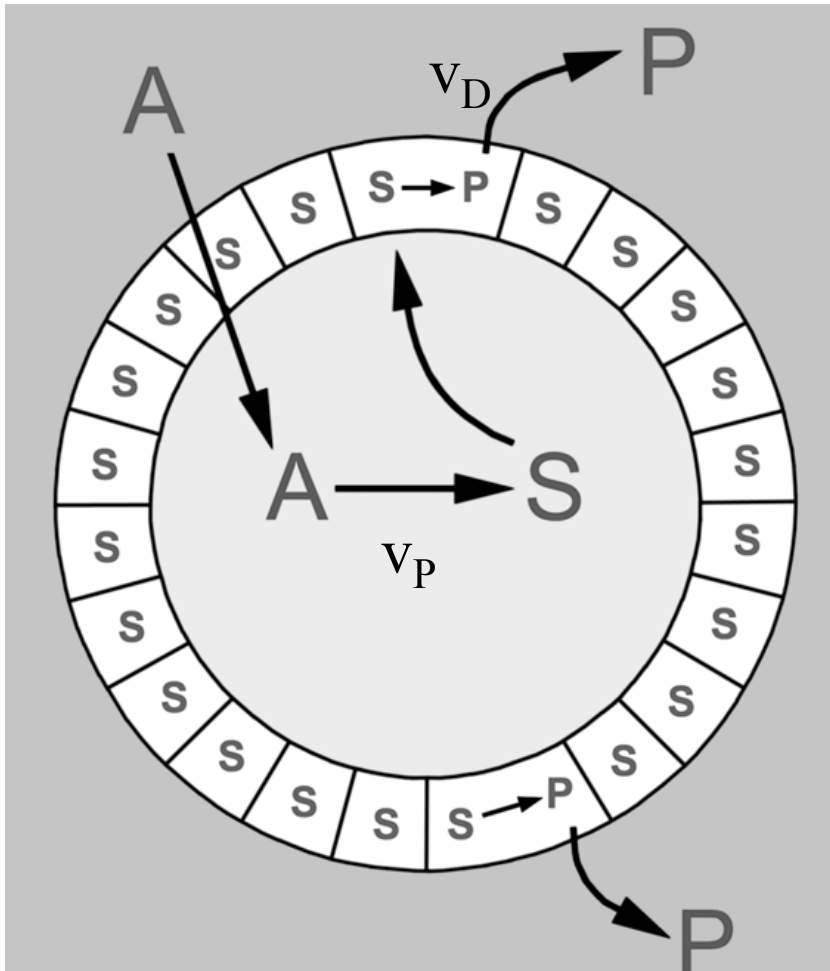
- Autopoiesis and the logic of life
- Vesicles (liposomes) as cellular models
- Semi-synthetic minimal cells

A cell is...

...a chemical system confined by chemical boundary, that contains a reaction network, which is able to self-maintenance and self-generation



AUTOPOIESIS (self-production)



- self-bounded
- self-maintenance (self-identity)
- "cognition"

The **organizational pattern** remains constant. The **material components** that realize such pattern change.

Despite the chemical transformations of the parts, the whole is conserved!

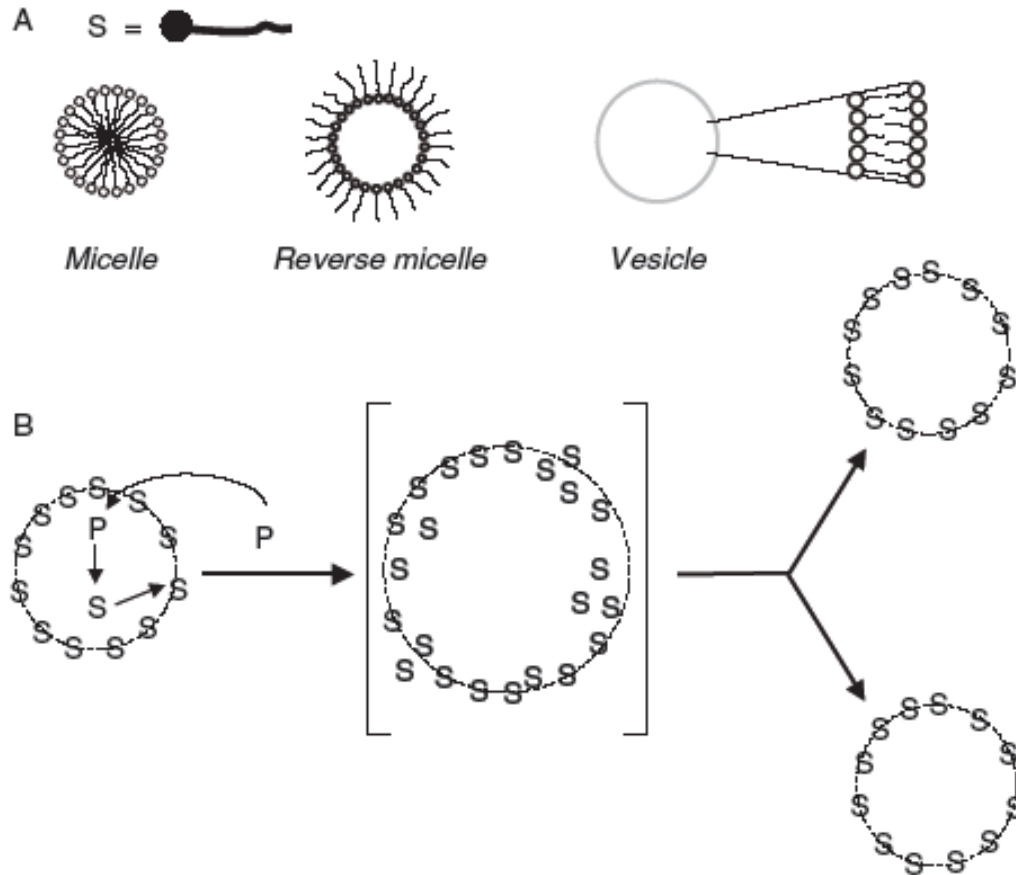
$v_P - v_D > 0$ growth, self-reproduction

$v_P - v_D = 0$ homeostasis

$v_P - v_D < 0$ death

Is it possible to make
simple autopoietic systems
in the laboratory?


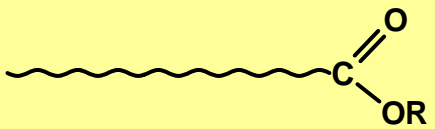
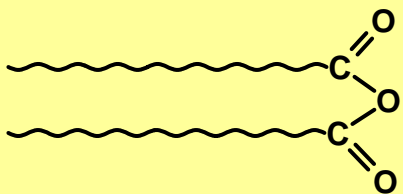

The **chemical implementation** of autopoiesis started about 18 years ago with a concept paper [*Luisi & Varela, OLEB (1990)*] and later developed experimentally in several ways.



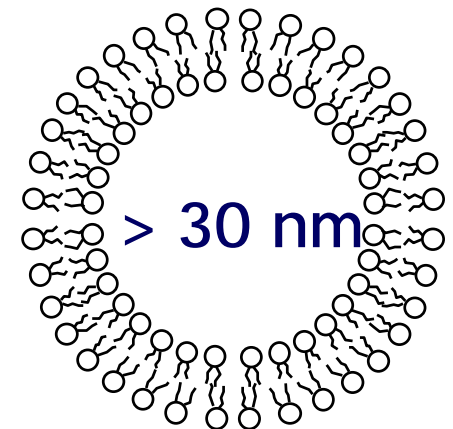
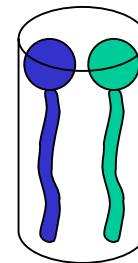
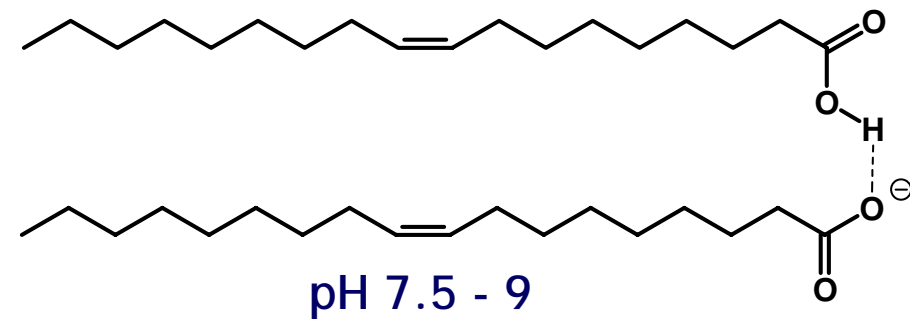
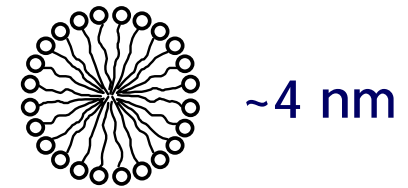
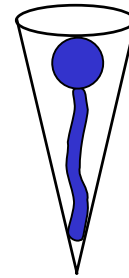
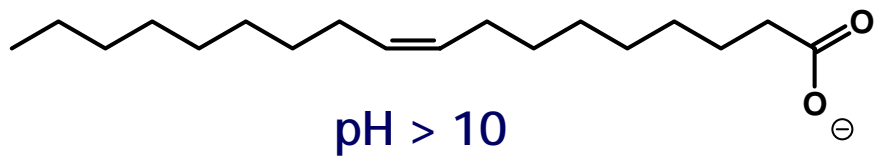
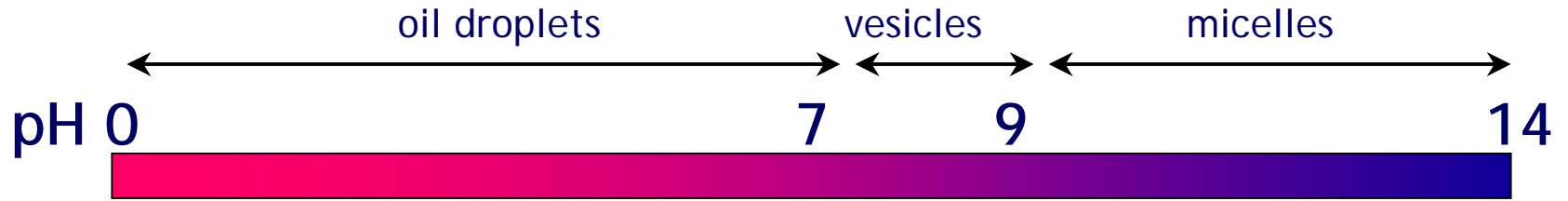
ETH 1990-2003
P.L.Luisi
P.Walde

The **self-reproduction of vesicles** is a pre-requisite for studying more complex **core-and-shell reproduction**

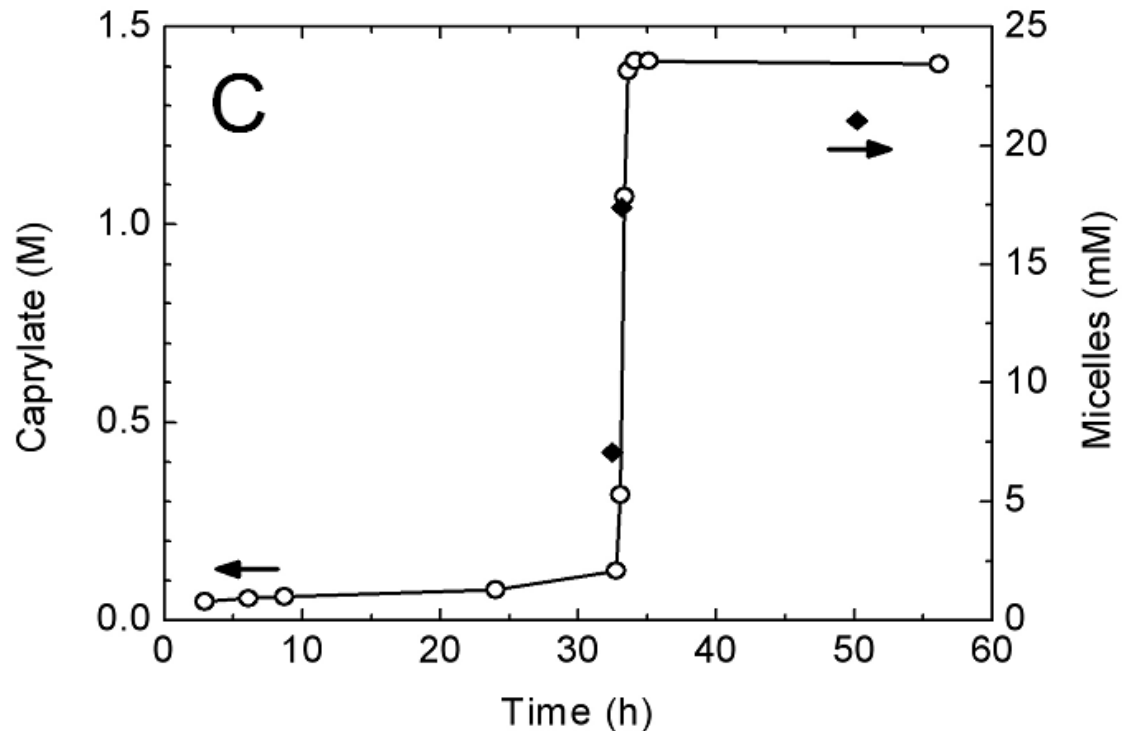
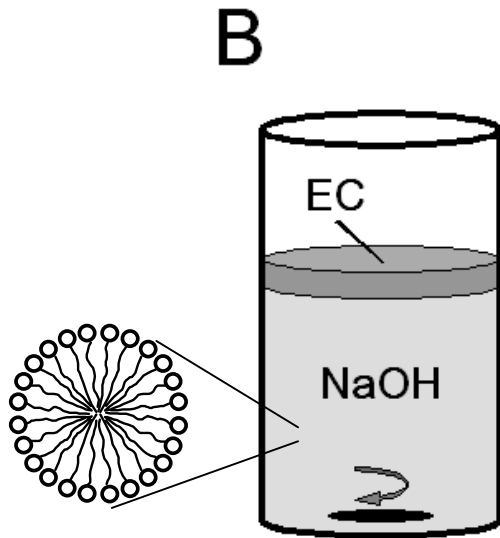
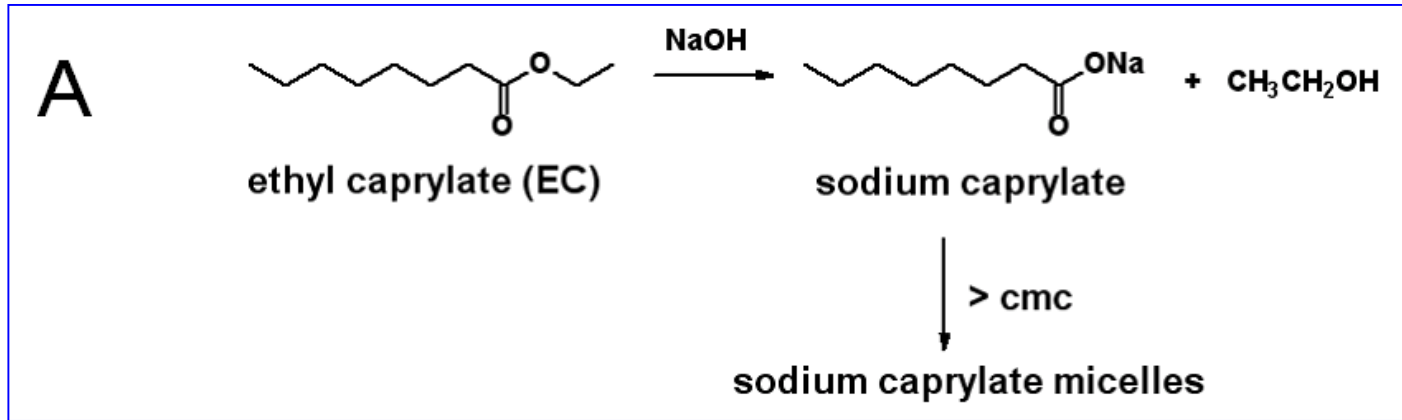
Self-reproduction of supramolecular structures - List of precursors

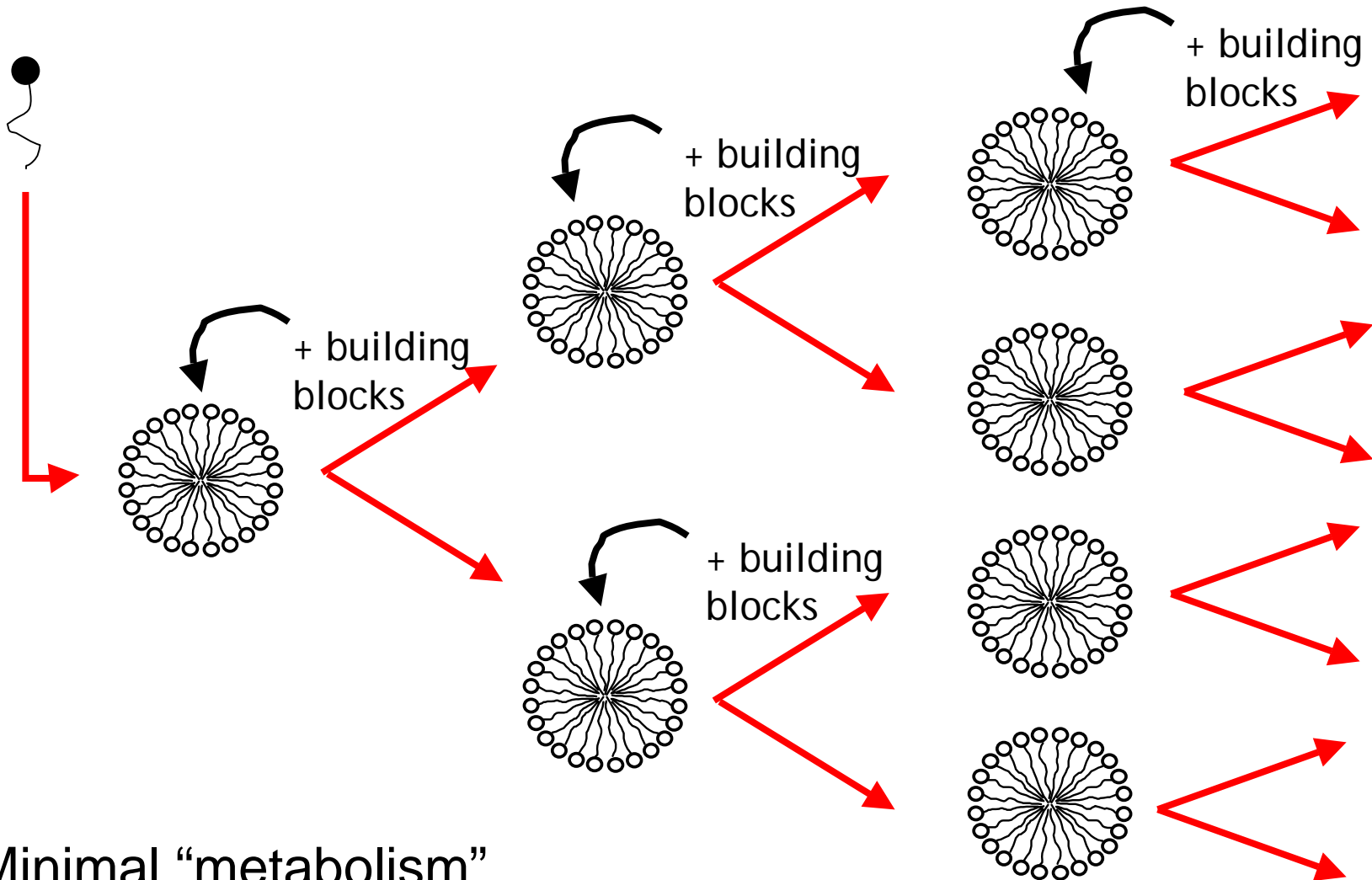
	In aqueous phase		In apolar phase
	High pH (micelles)	Intermediate pH (vesicles)	Reverse micelles
		Bloechliger et al., 1998 Lonchin et al., 1999 Berclaz et al., 2001a,b Cheng & Luisi, 2003 Rasi et al., 2003a,b Stano et al., 2006	
	Bachmann et al., 1992	(Schimdli et al., 1991) Luisi et al., 1993 Bachmann et al., 1994	Bachmann et al., 1990,1991
		Luisi et al., 1993,1994 Walde et al., 1994a,b Wick et al., 1995 Oberholzer et al., 1995 Morigaki et al., 1997 Zepik et al., 2001 Berclaz et al., 2001	
	Bachmann et al., 1991		Bachmann et al., 1991

Fatty acid micelles and vesicles



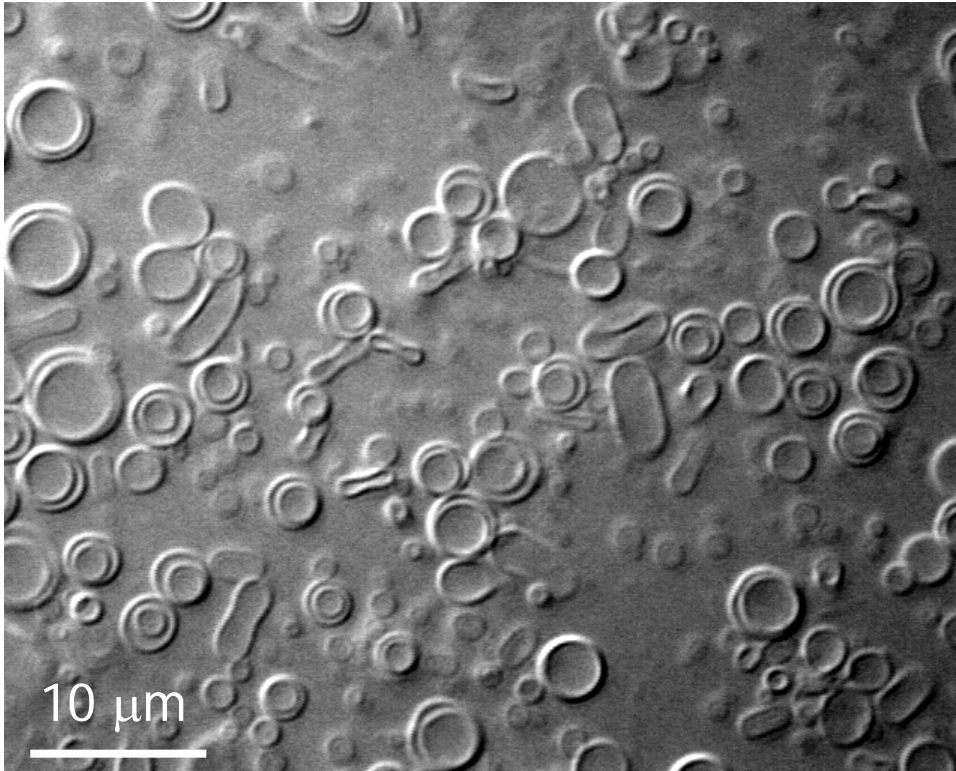
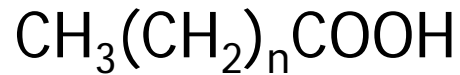
Autocatalytic self-reproduction of caprylate micelles





Minimal "metabolism"
1 reaction
↓
Self-reproduction!

Fatty acid vesicles

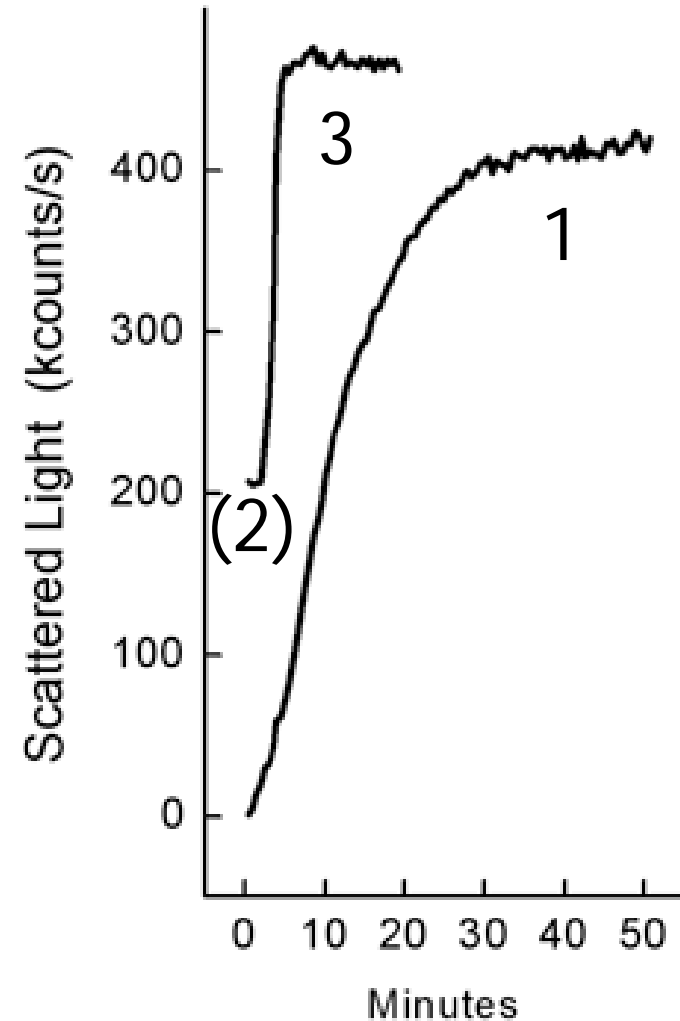
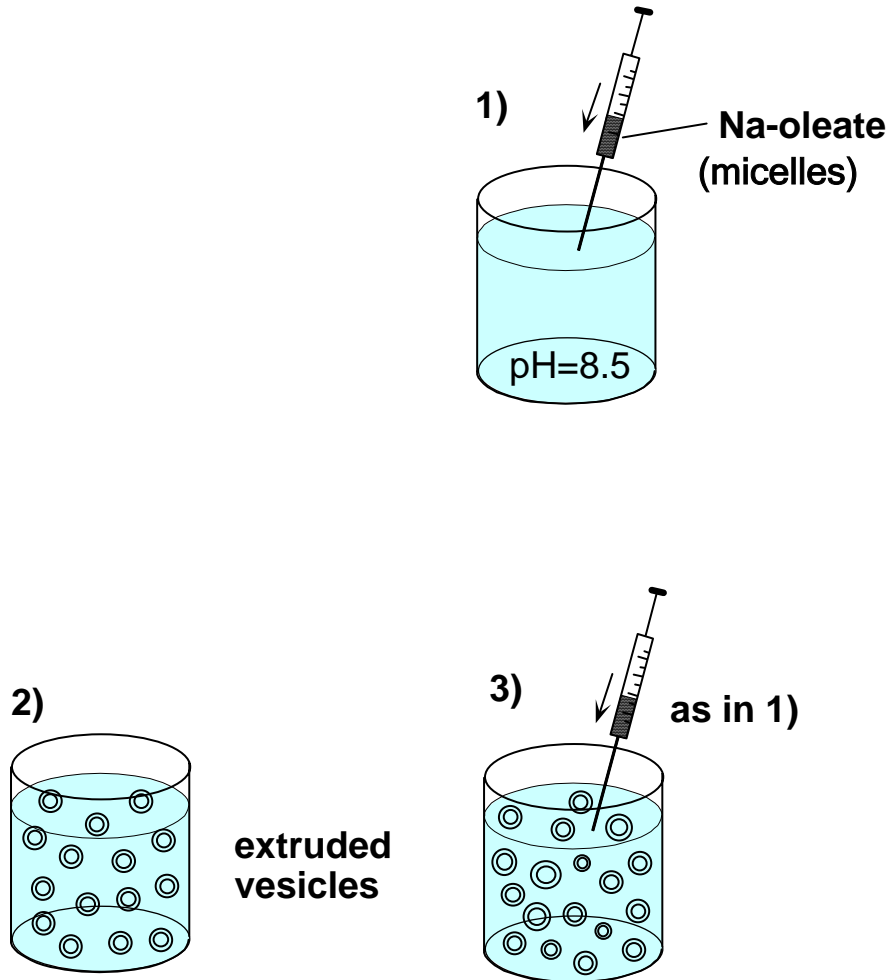


Fatty acids have been synthesized under simulated prebiotic conditions and have been isolated from carbonaceous chondrite meteorites (Murchison).

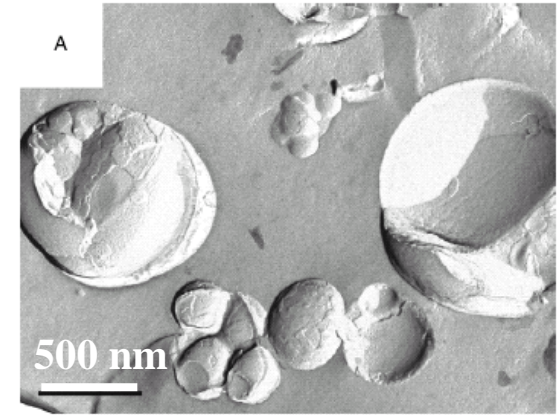
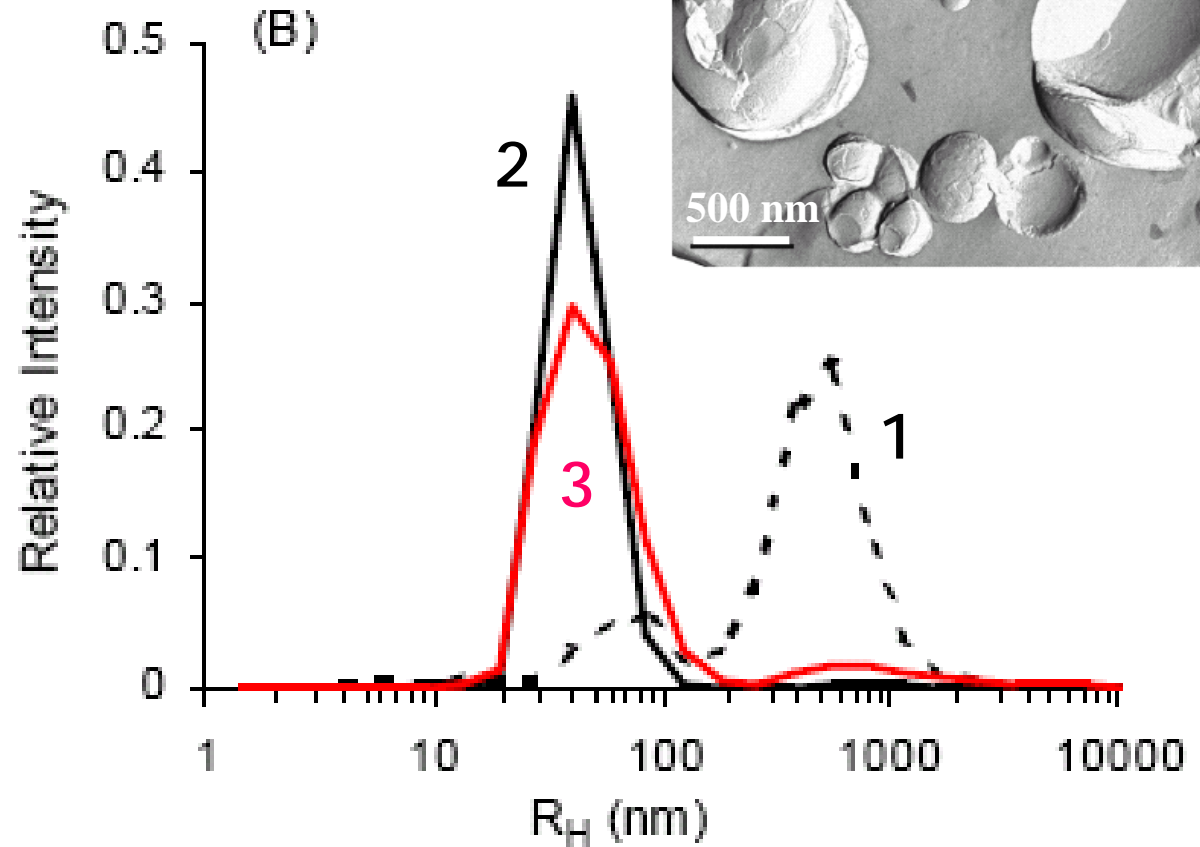
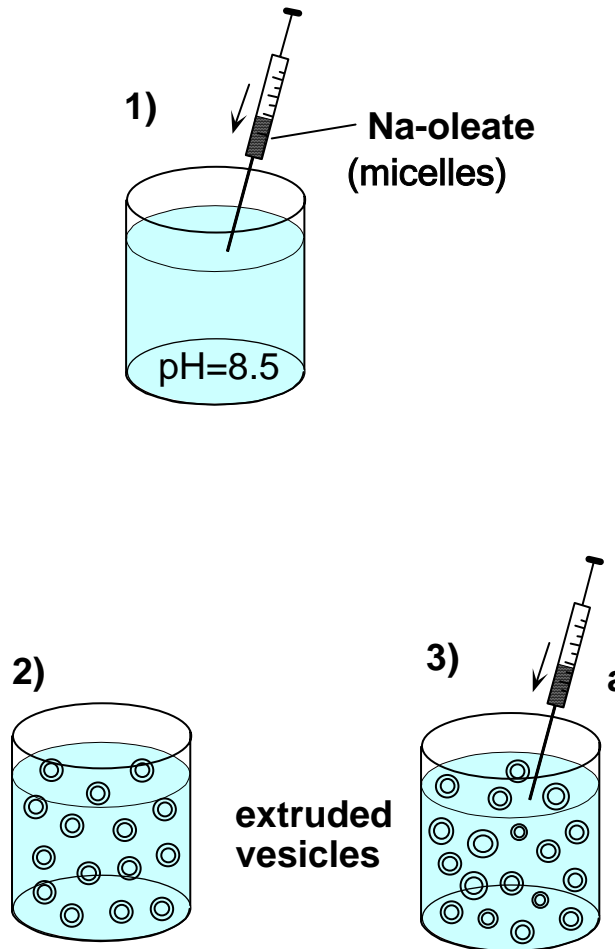
It has been suggested that such vesicles played an important role in the origin of life

giant oleate vesicles

Self-reproduction of oleate vesicles: the matrix-effect

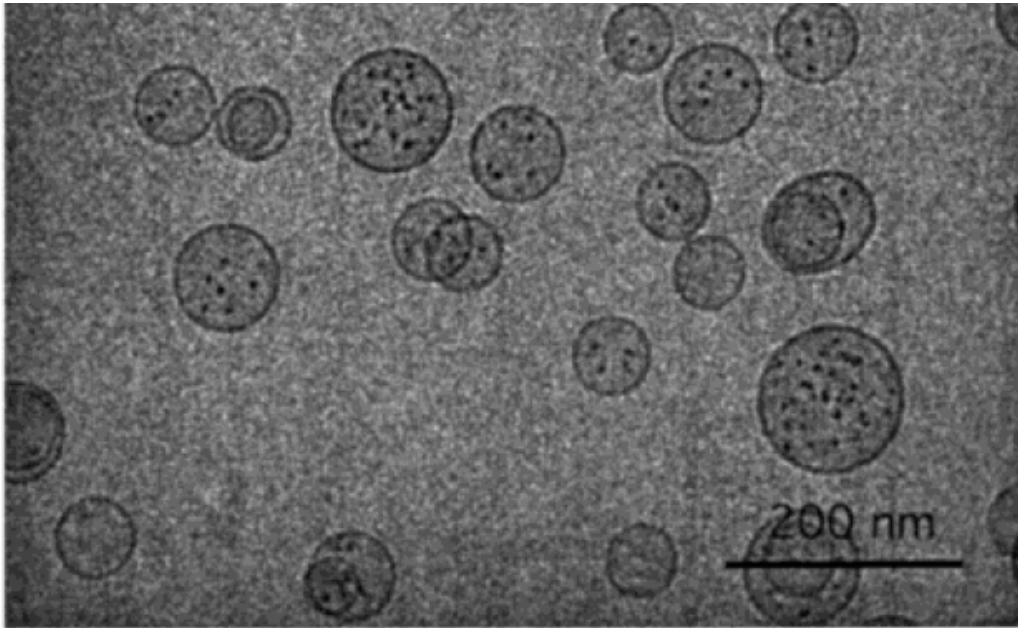


Self-reproduction of oleate vesicles: the matrix-effect



Bloechliger et al. 1998
Lonchin et al. 1999
Berlcaz et al. 2001a, 2001b
Rasi et al., 2003,2004

Following an entrapped probe



+ oleic
anhydride



+ oleate
micelles



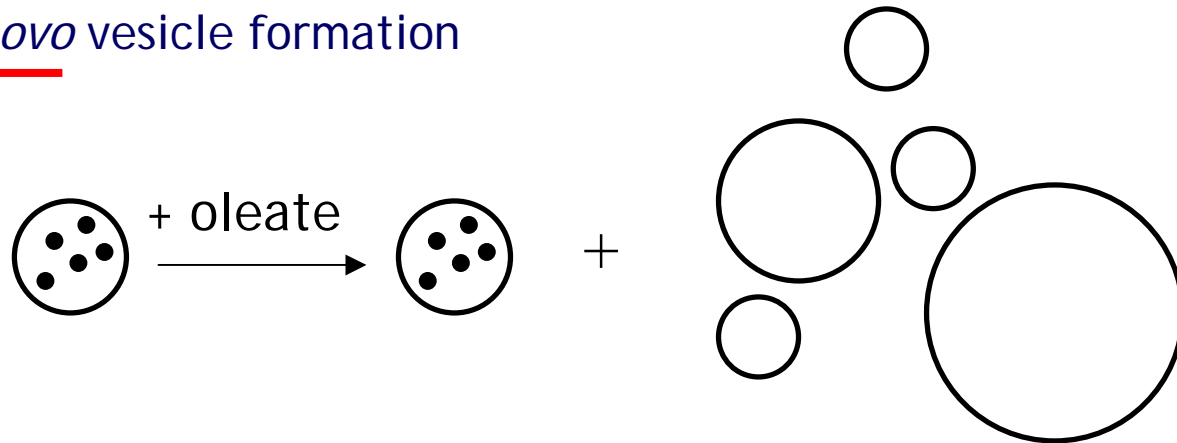
Ferritin

Direct counting of vesicles before and after the addition

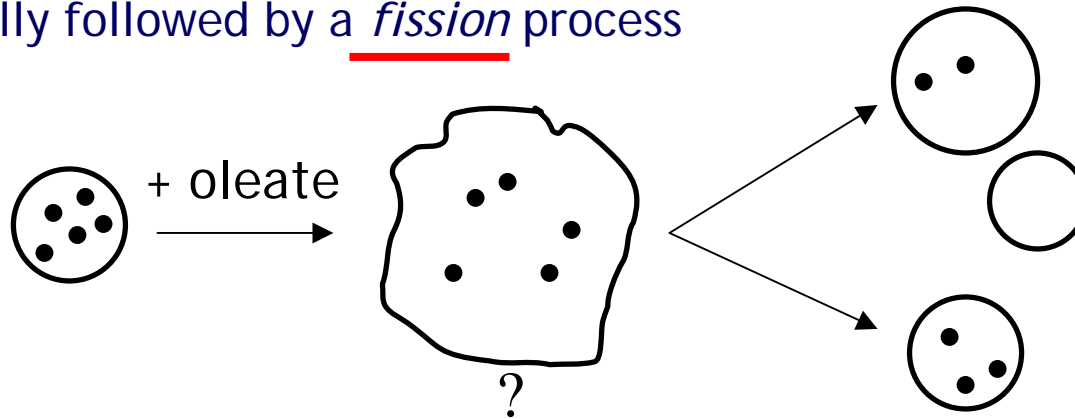
- **detection of small vesicles (cft. DLS analysis)**
- **larger and smaller filled-vesicles suggest growth & division**

Experiments with ferritin and calcein

(I) de novo vesicle formation



(II) growth of the preformed vesicles eventually followed by a fission process



Berclaz et al., 2001a,b

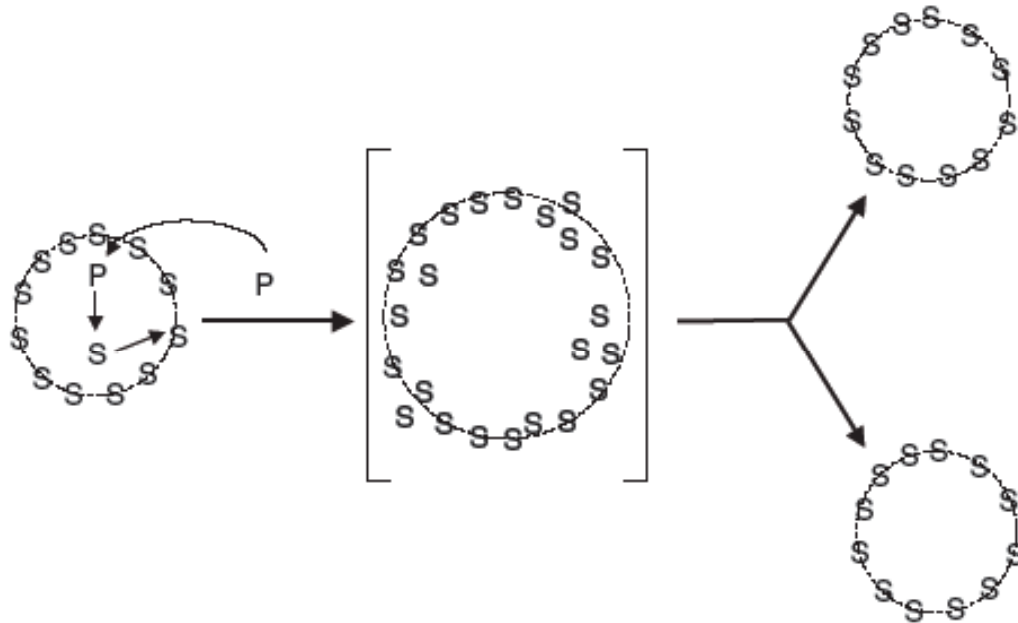
Calcein-release tests indicate only a minor leakage

Stano, Wehrli & Luisi, 2006
Rogerson et al., 2006

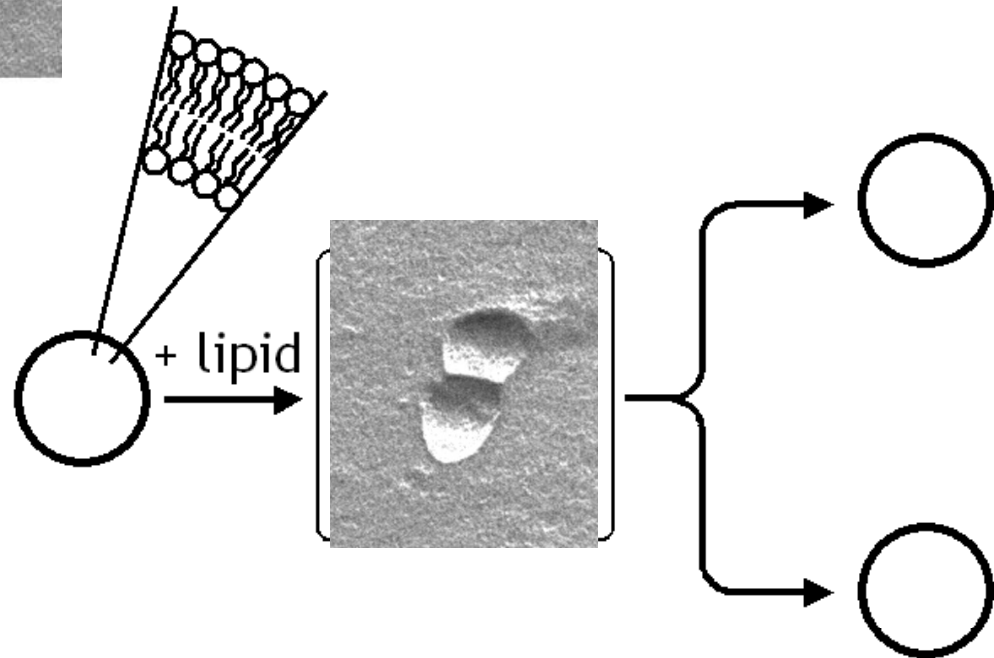
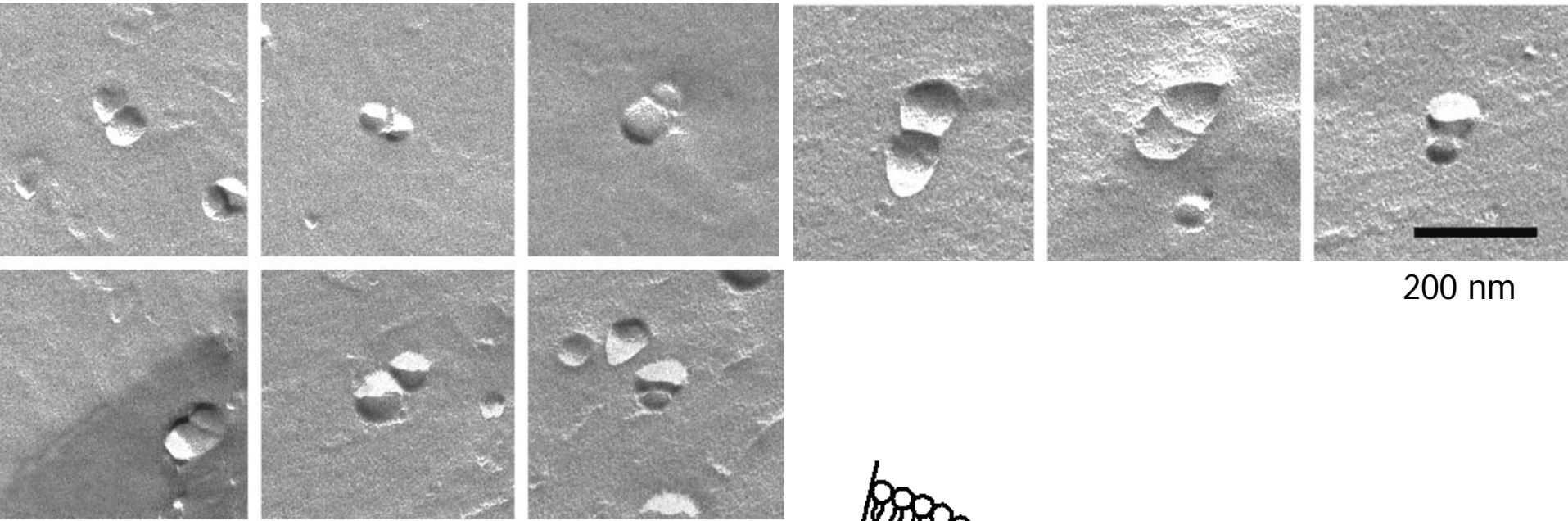
From observations to models

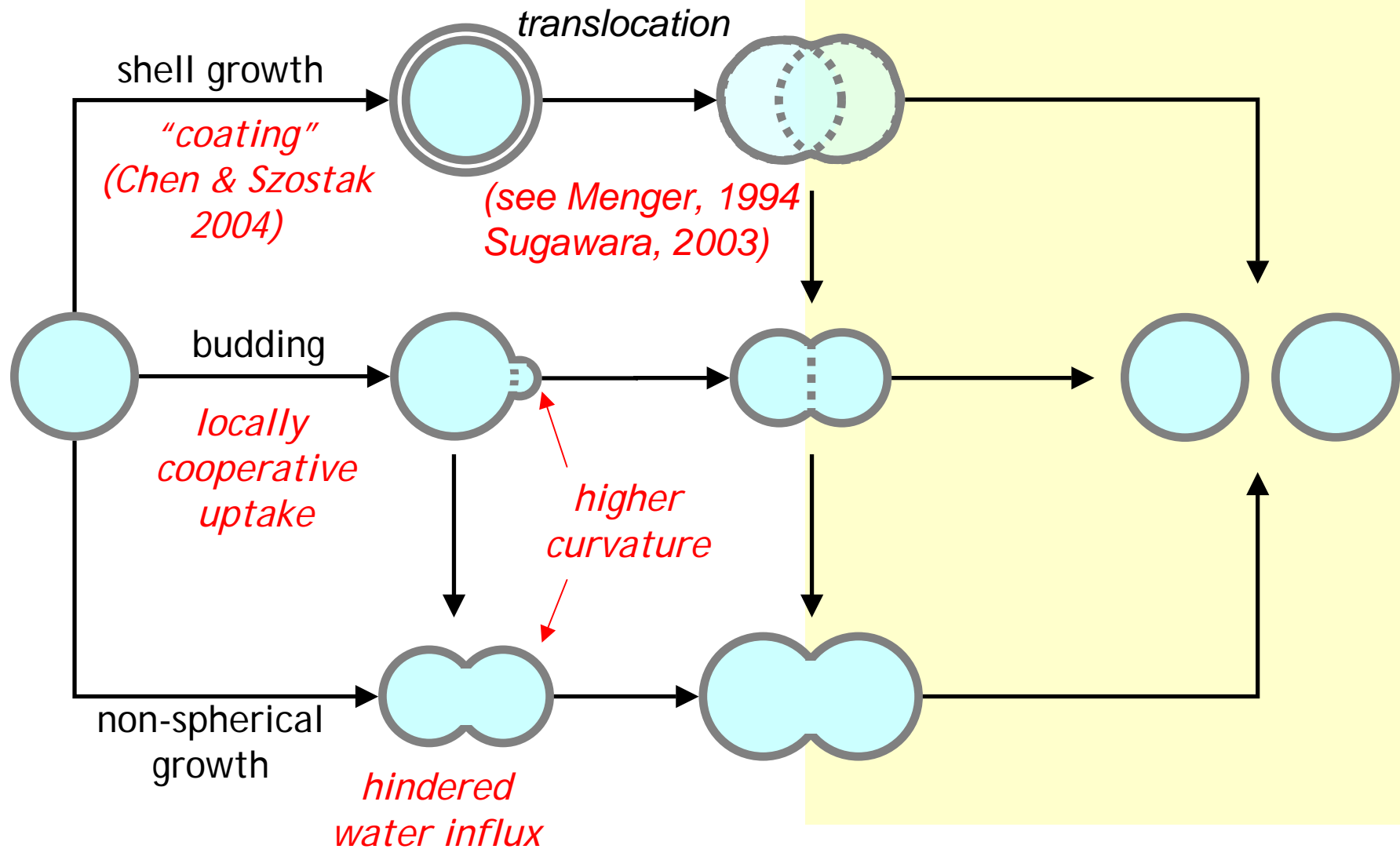
turbidimetry: faster reaction

- DLS: approximate constant size distribution
- cryo-TEM: smaller and larger filled vesicles
- fluorescence: only minor leakage during the transformation



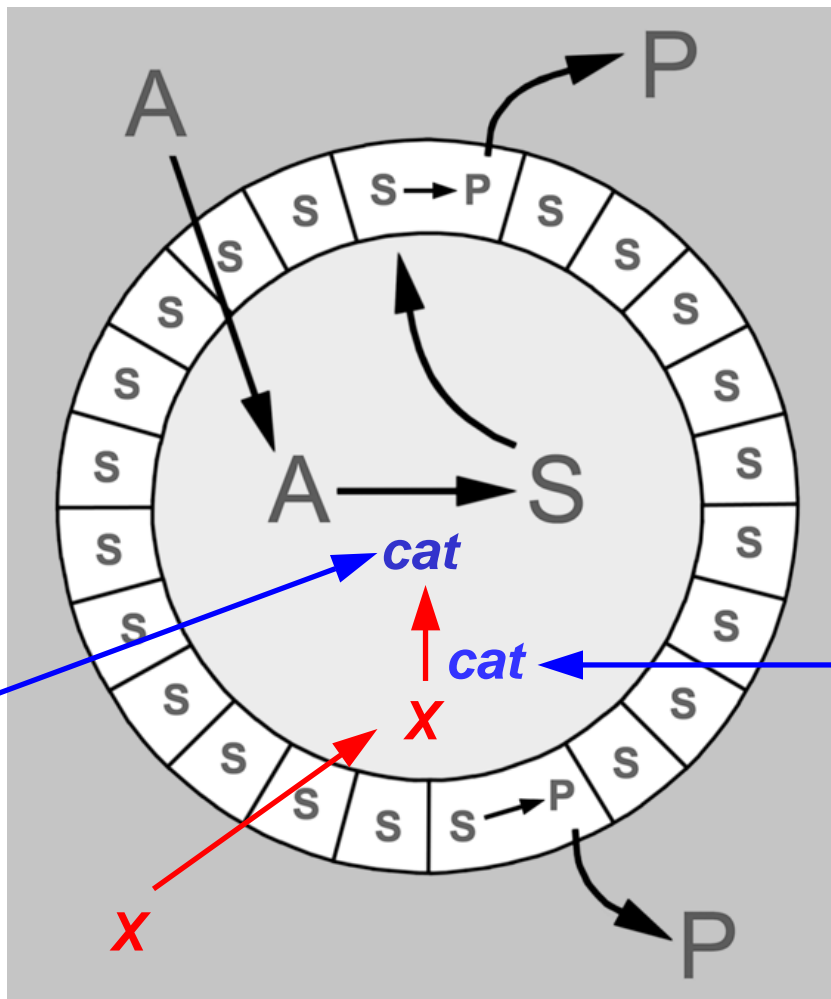
Self-reproduction of oleate vesicles





Can we build
self-reproducing
minimal cells?

What does it mean “minimal”?



*with the help
of a catalyst*

*with the help
of another
catalyst*

*It stops only with a
**COLLECTIVELY
AUTOCATALYTIC
system***

-----> and so on... ----->

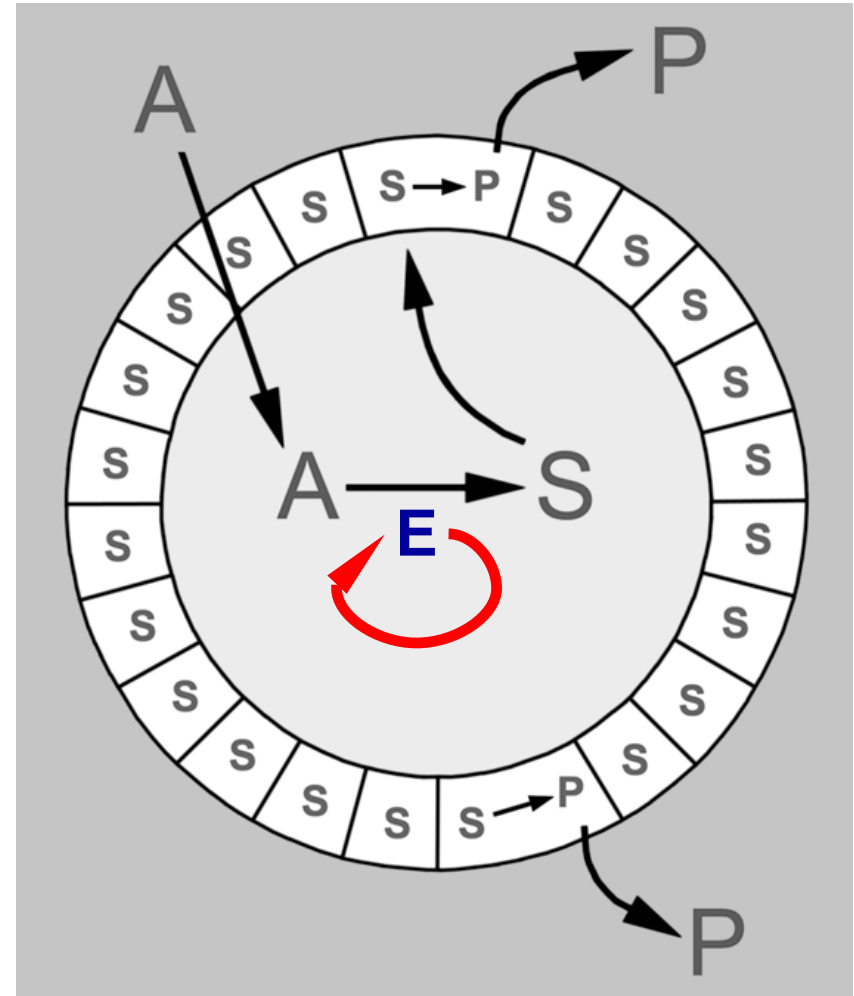
*which in turn
must be done
by another
catalyst*

CORE AND SHELL SELF-REPRODUCTION

This minimal collectively autocatalytic system is a...
minimal metabolism

Which can be:

- a protometabolism
- or related to cells as we know them, and to the **minimal genome**



What is the minimal complexity of “E”?

Modern cells

*semi-synthetic
minimal cells*

Minimal Cell

SIMPLICITY \neq PRIMITIVENESS

*primitive cells
models*

*artificial cells
synthetic cells*

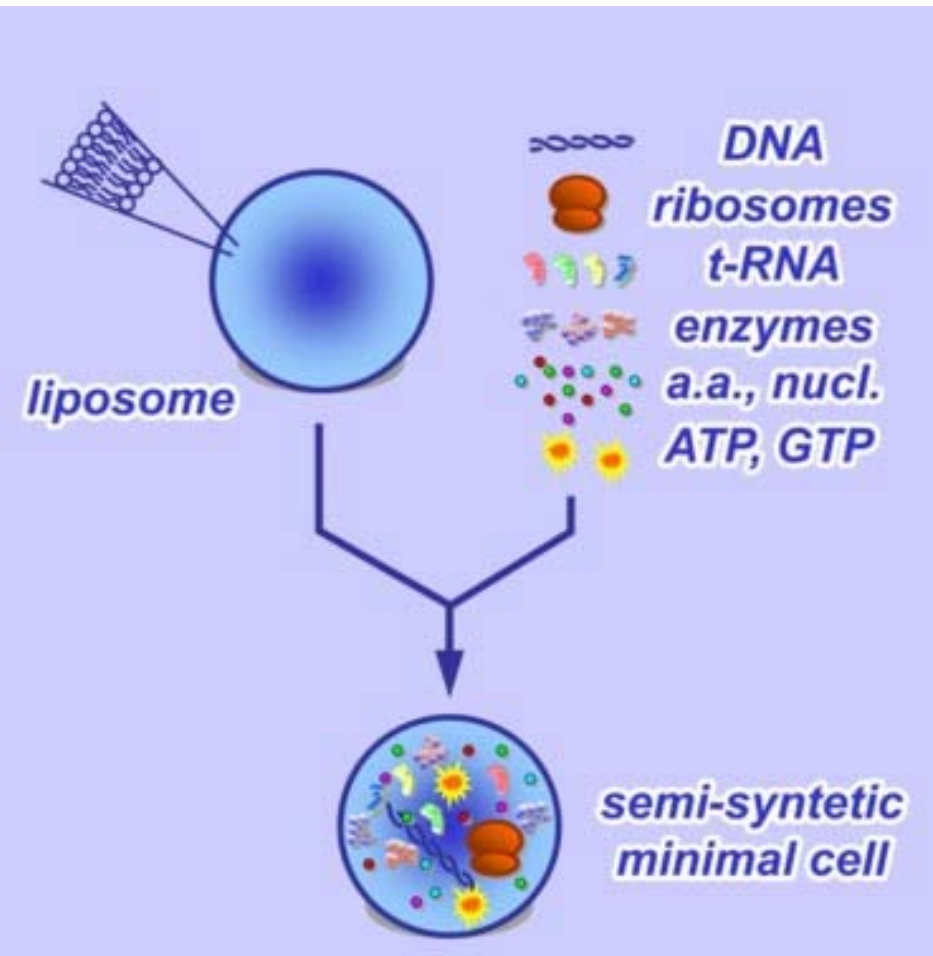
**prebiotically plausible
molecules**

**simple or complex
synthetic molecules**

(e.g., ribozymes, short peptides)

(e.g., PNA)

SEMI-SYNTHETIC APPROACH



Conceptually:

**ELIMINATING COMPONENTS
SIMPLIFYING STRUCTURES**

Operatively:

**ASSEMBLING MOLECULES
INTO SYSTEMS**

Technologies involved:

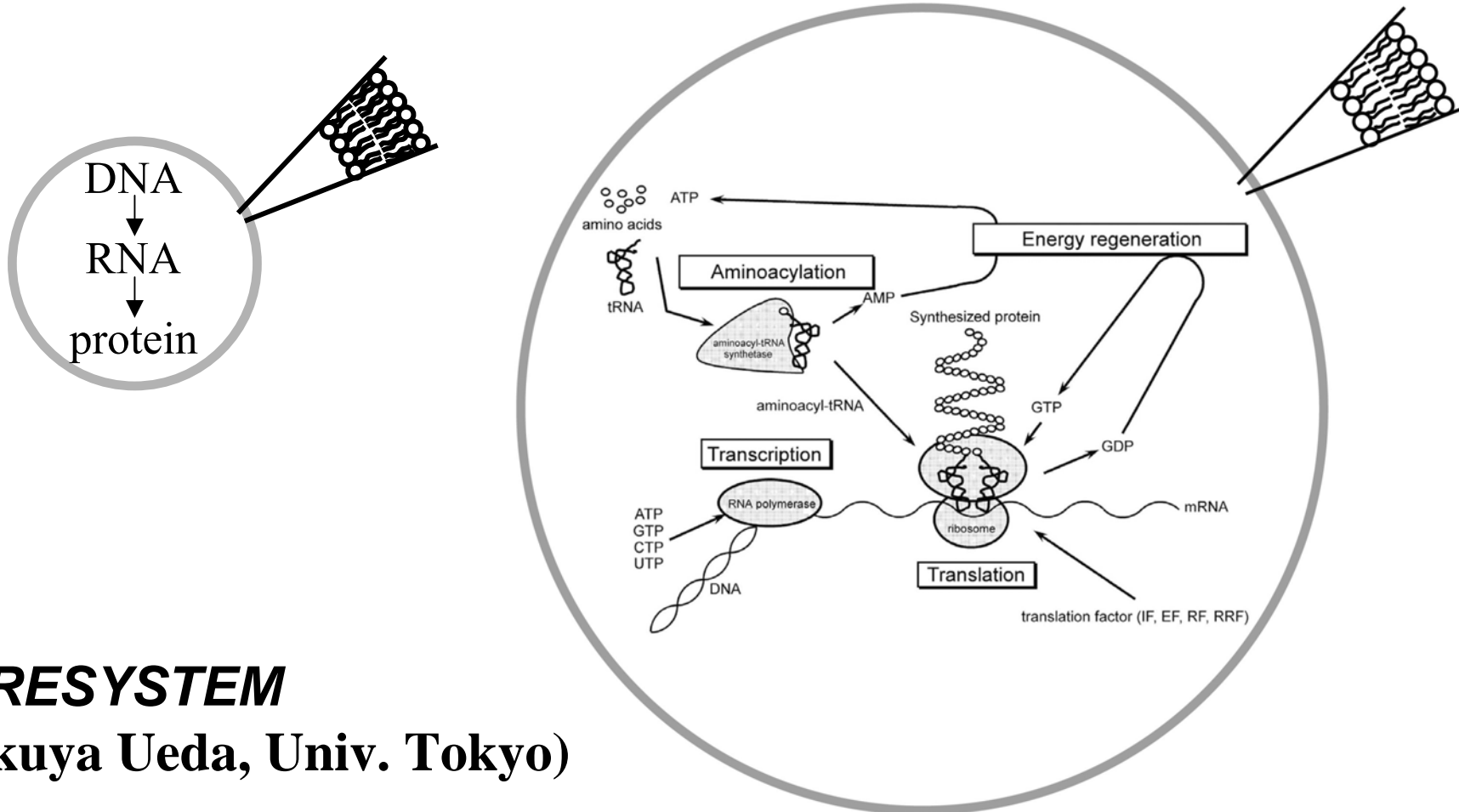
- **CELL-FREE SYSTEMS**
- **LIPOSOME TECHNOLOGY**
- ... **MICROFLUIDICS**
- ... **MODELING**

The state of the art (March 2010)

- a. **The expression of GFP as model protein inside liposomes can be considered now as standard result**
 - 1) **Protein synthesis in small vesicles is not trivial** ✳
(Luisi 2009)

- b. **There have been four main studies that imply major advancements:**
 - 1) **Two-step genetic cascade reaction (Yomo 2004)**
 - 2) **The alpha-hemolysin pore (Libchaber 2004)**
 - 3) **Self-encoded RNA replication (Yomo 2008)**
 - 4) **Synthesis of membrane enzymes (Luisi 2009)** ✳

Protein biosynthesis as a paradigm of cellular metabolism



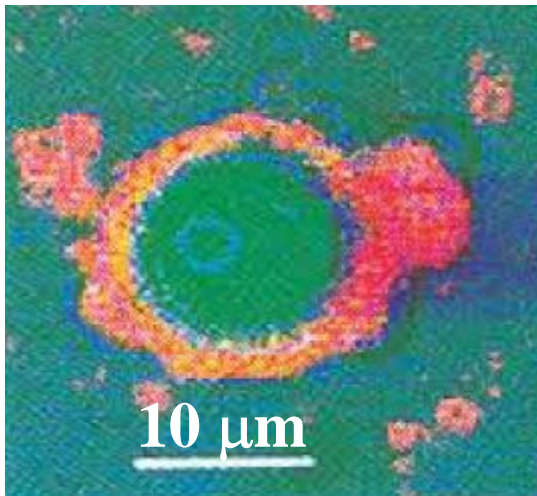
PURESYSYSTEM

(Takuya Ueda, Univ. Tokyo)

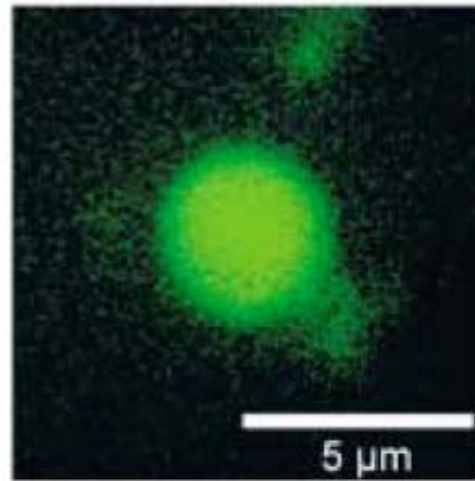
A molecular kit of 36 purified enzymes, ribosomes, t-RNAs, and low molecular weight compounds, which synthesize proteins starting from the corresponding DNA

Shimizu et al. Nature 2001

Protein synthesis inside large and giant vesicles



Yu et al. 2001



Nomura et al., 2003

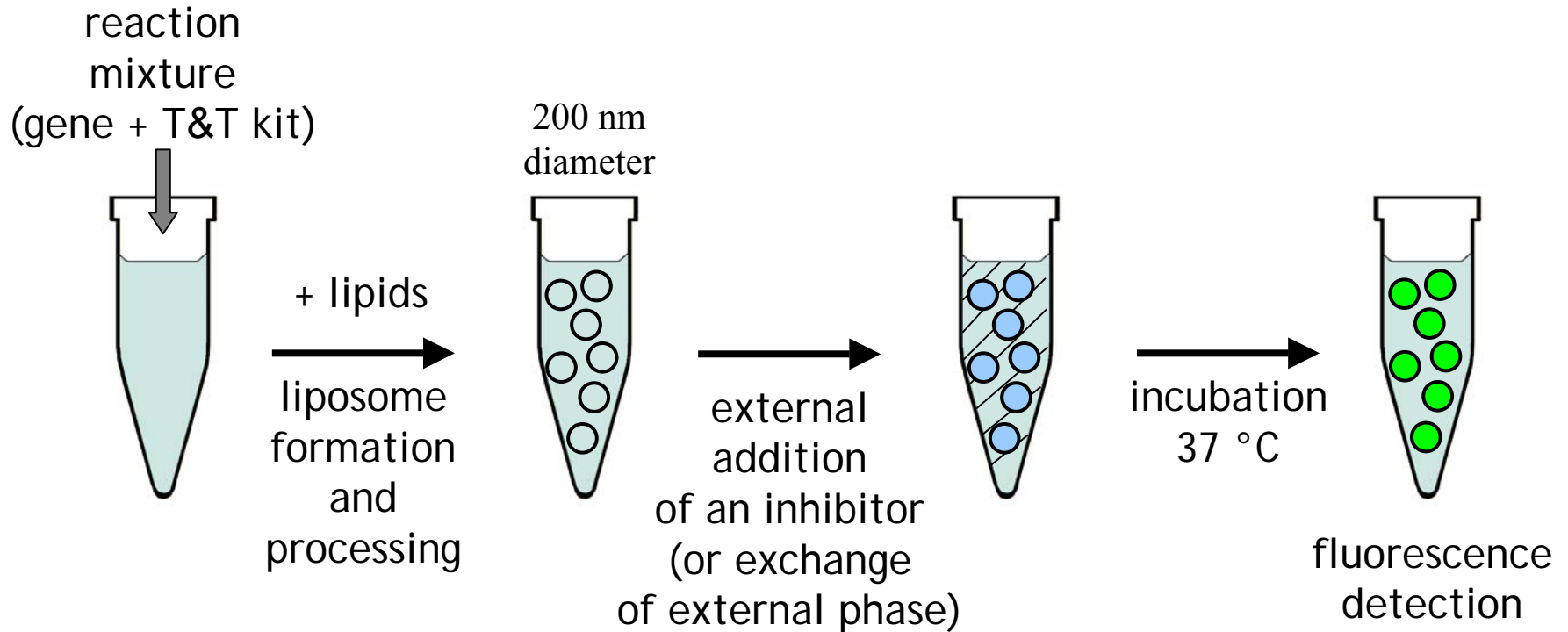


Noireaux and
Libchaber, 2004

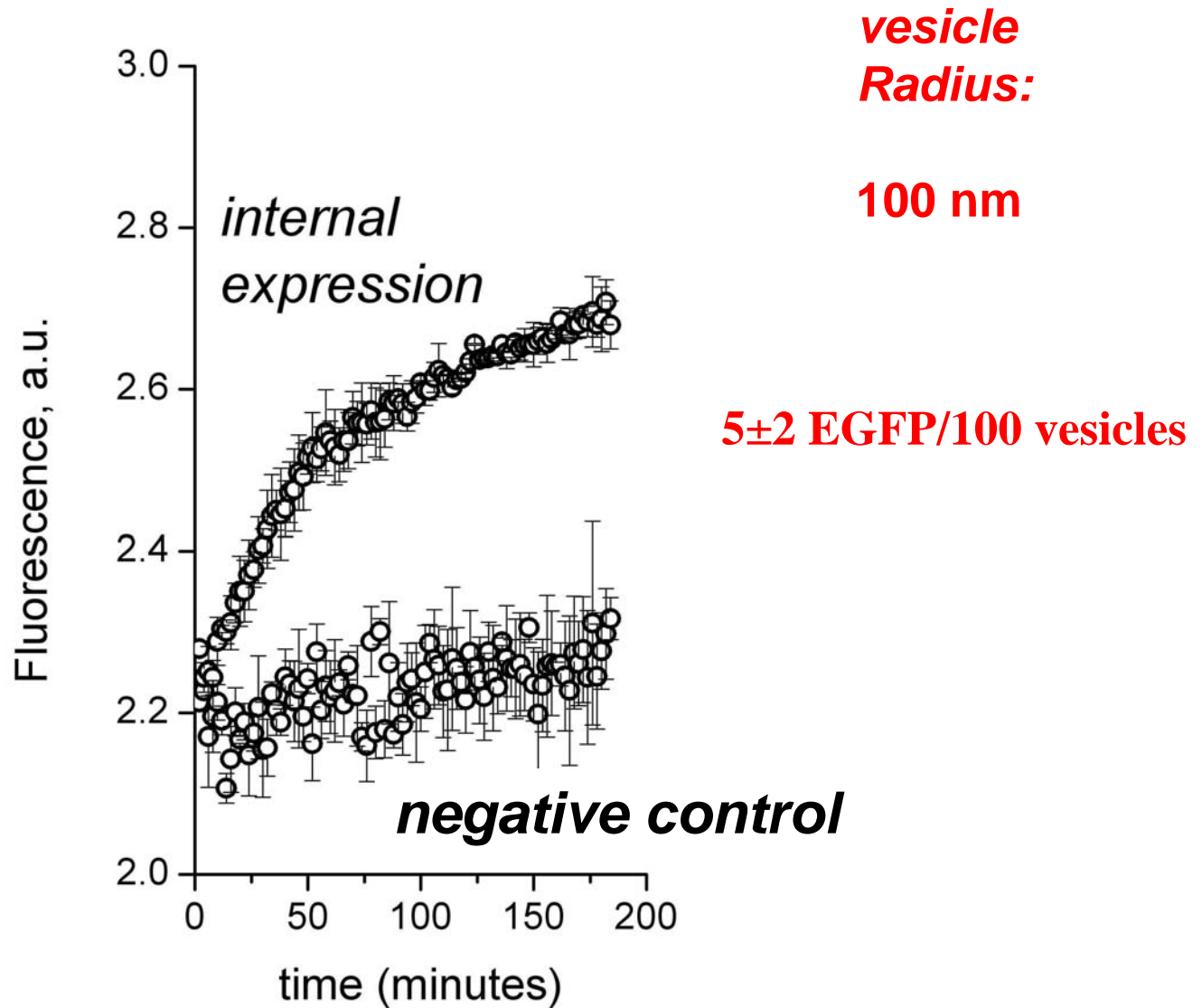
Recent reviews: Luisi, Ferri, Stano, Naturwissenschaften 2006
Chiarabelli, Stano, Luisi, Curr. Op. Biotech. 2009

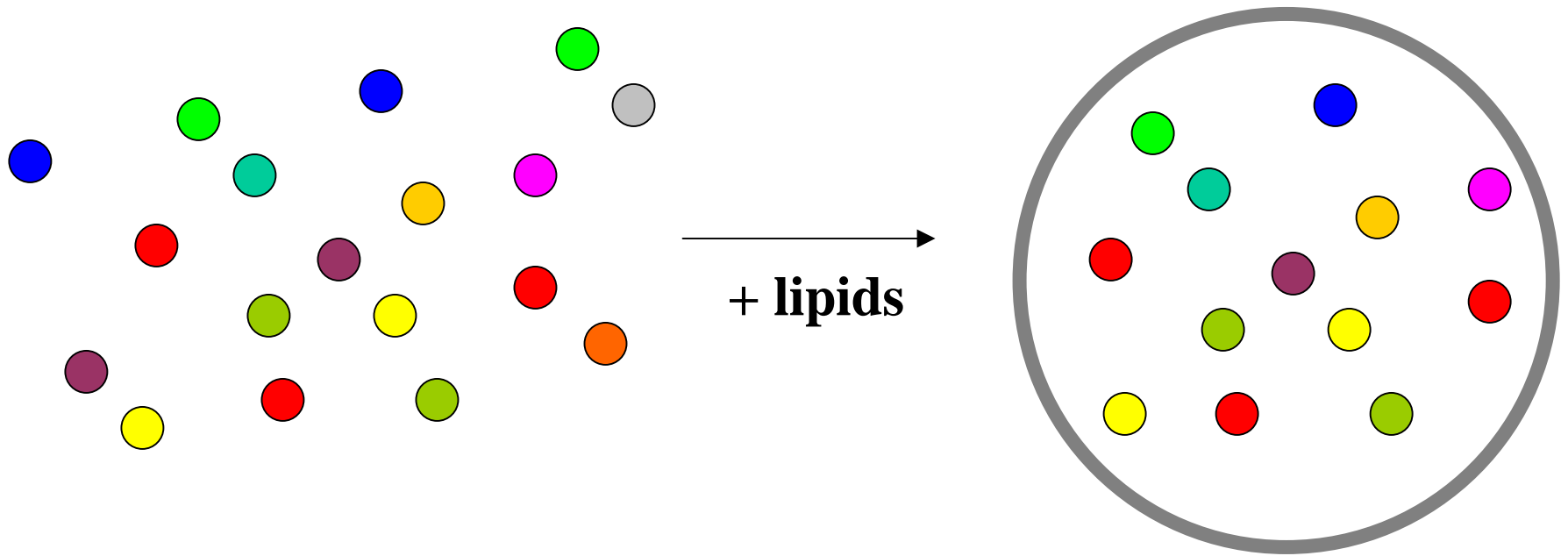
EXPRESSION OF GFP INSIDE SMALL VESICLES

THE MINIMAL SIZE OF CELLS?
synthetic biology is the tool for answering



EXPRESSION OF GFP INSIDE SMALL VESICLES

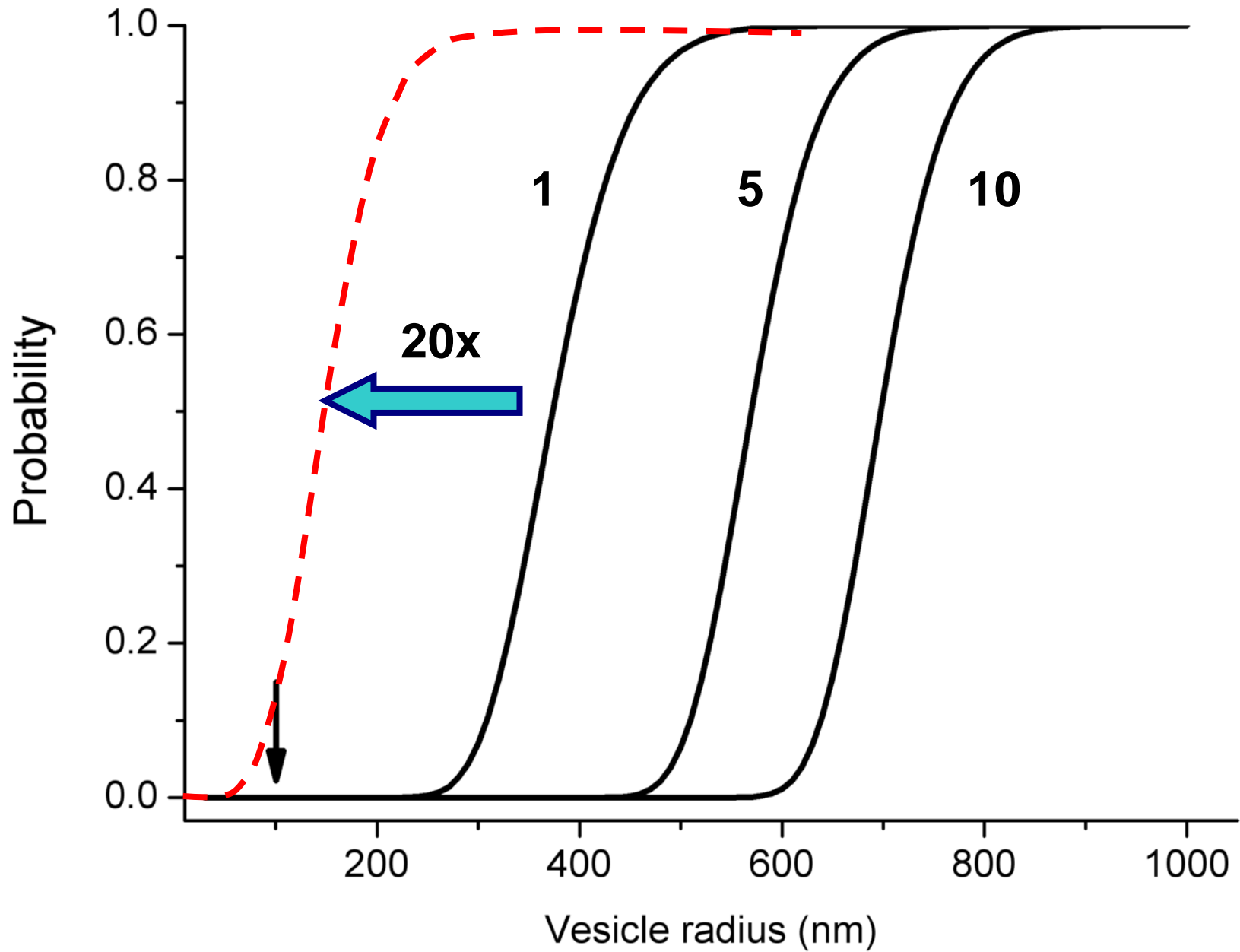


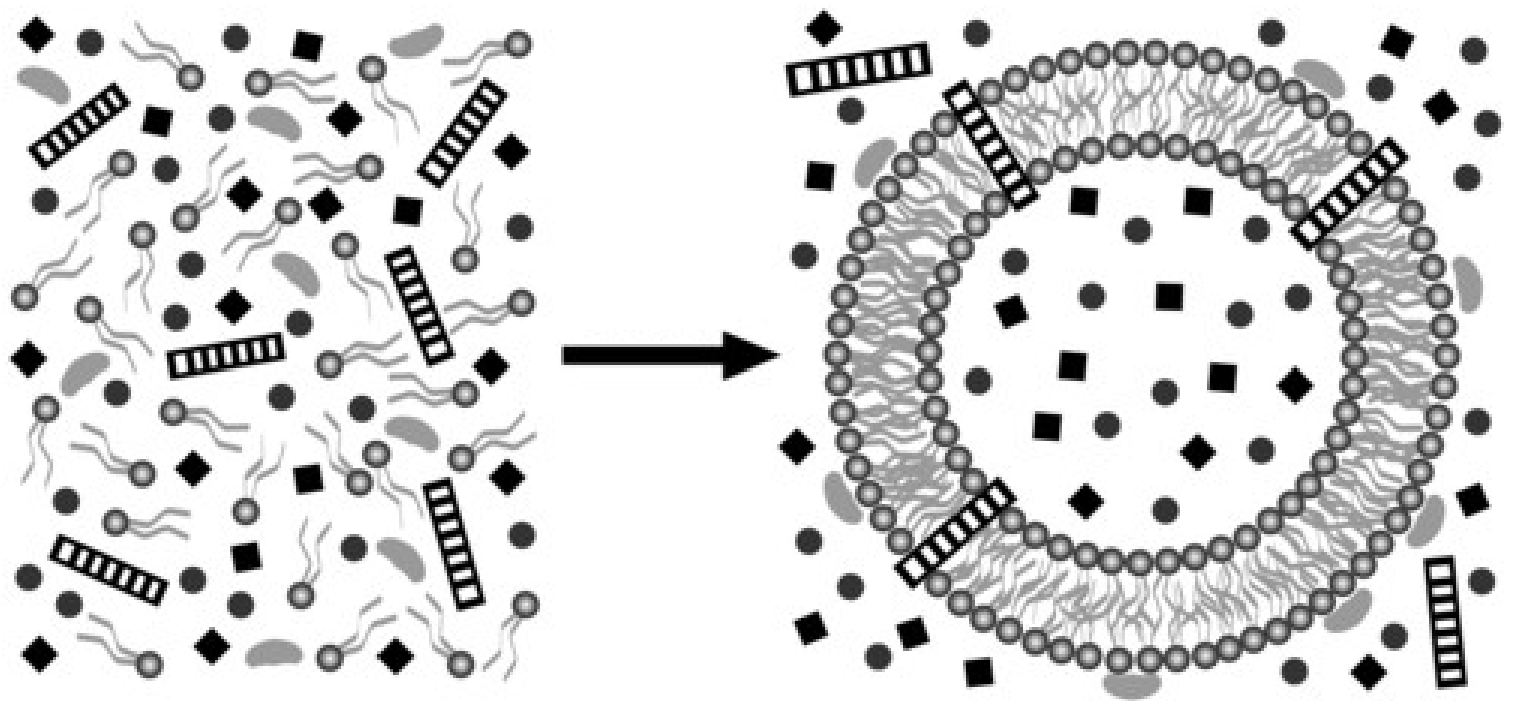


- A Poisson distribution is expected
- If the events are independent, the probability of co-entrapment is:

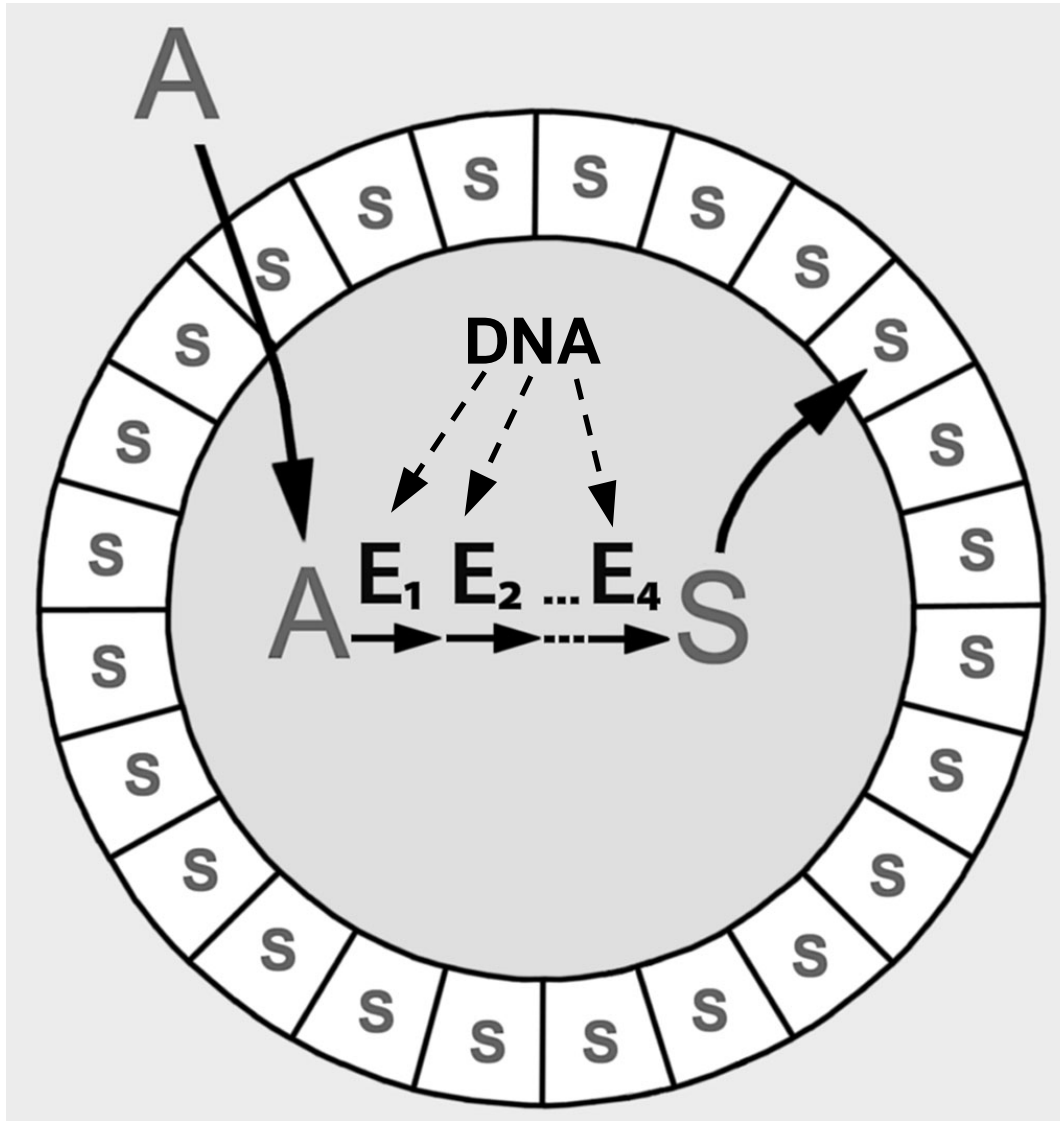
$$\wp_c(R) = \prod_{k=1}^N [1 - \exp(-C_k V(R))]$$

(probability of finding a vesicle with *at least* 1 copy of N species)





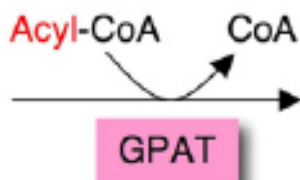
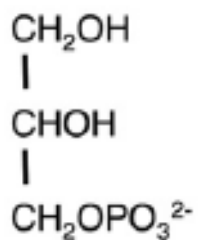
Minimal Cell: Synthesis of lipids from within



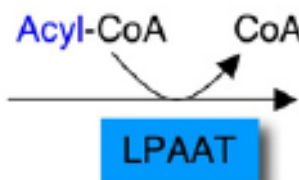
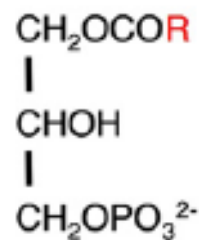
Yutetsu
Kuruma

Enzymatic synthesis of the lipids from within and autopoietic growth.

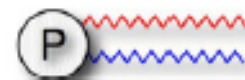
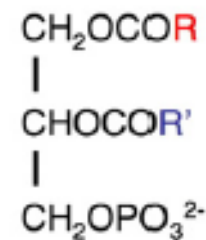
Glycerol-3-phosphate
(G3P)

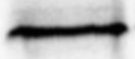




Lysophosphatidic acid
(LPA)




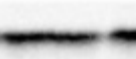

Phosphatidic acid
(PA)



protein	GPAT		
fraction	T	S	P
			

% solubility

9.7

protein	LPAAT		
fraction	T	S	P
			

% solubility

47.0

the enzyme must be synthesized

the synthesis must occur inside

the enzyme must be active

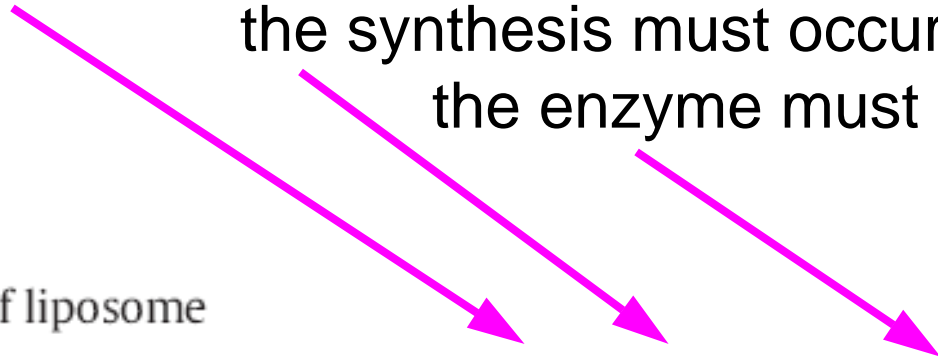
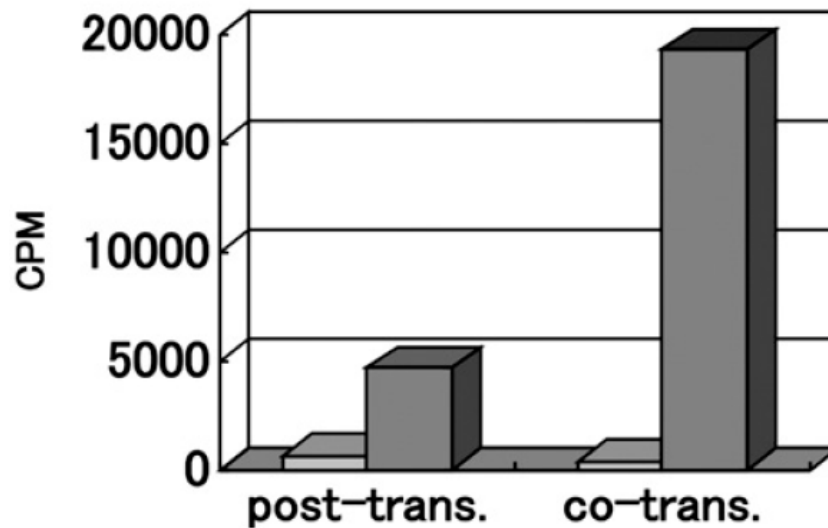


Table 1

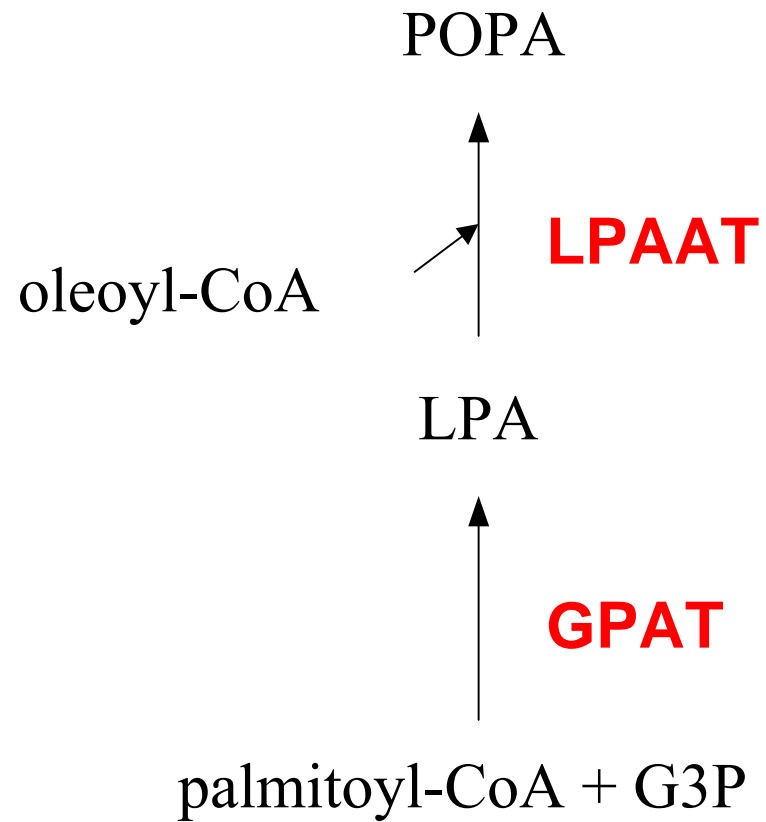
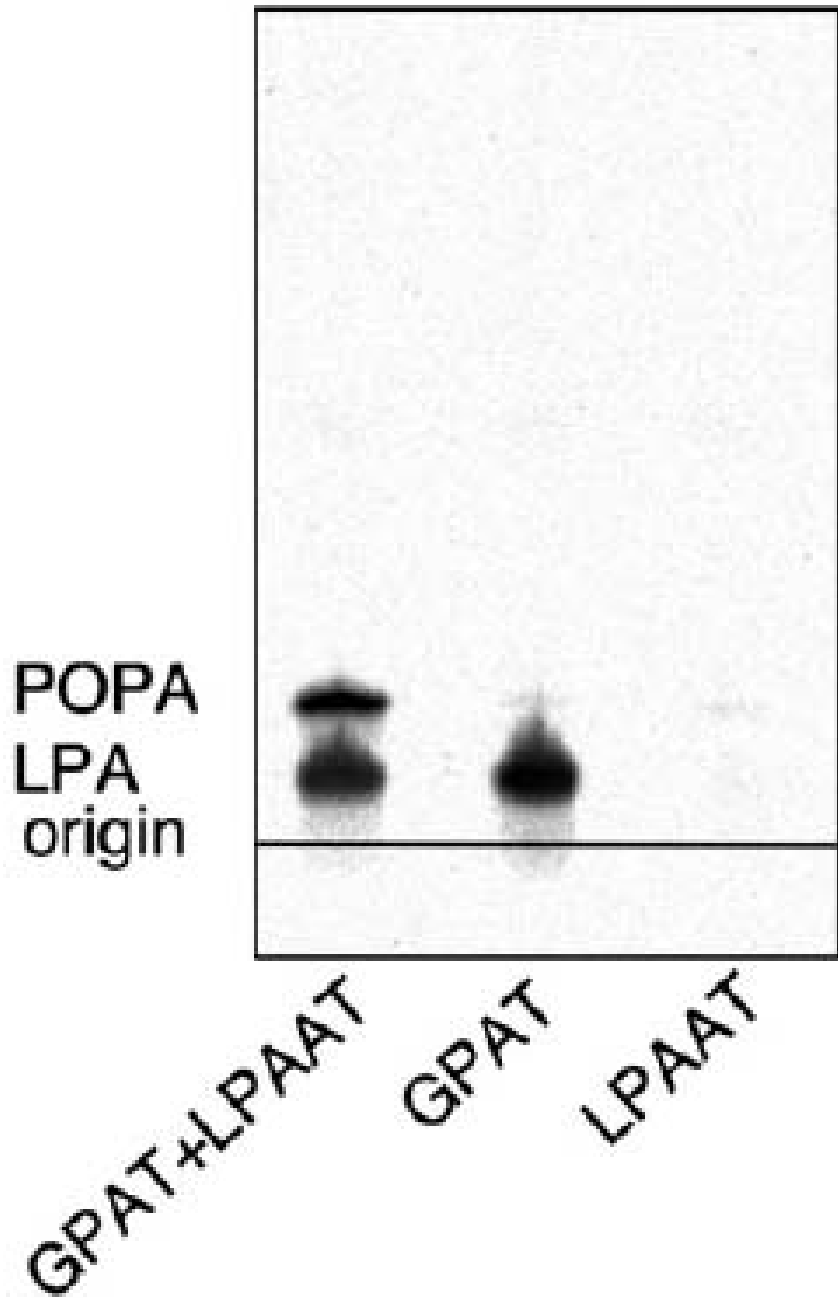
Phospholipid compositions of liposome

Lipid composition (mol)	Conc. (mM)	Synth.	Encap.	Act.
POPC	200	++	+++	-
POPC/POPG (80:20)	200	+	+	+
Polar lipid extract (<i>E. coli</i>)	1 mg/mL	±	-	+++
Polar lipid extract/POPC (50 ^a :50)	200	+	+	+
POPE/POPG/Brij35 (76.5:10.9:12.6)	50	+	-	Not tested
POPC/POPE/POPG/cardiolipin (50.8:35.6:11.5:2.1)	200	++	++	+++ ^b
No lipid	-	+++	-	-

lipids	no lipid		POPC		POPC/PG		Polar ext.		Polar ext. /POPC		POPE/PG /Brij35		POPC/PE /PG/CL	
RNaseA	-	+	-	+	-	+	-	+	-	+	-	+	-	+
GPAT														
% inside	0.4		11.5		10.8		2.1		11.8		0.8		8.9	



**GPAT synthesized
in the presence of lipids
is 4x more active**



Conclusions and future directions

- The synthesis of functional water-soluble proteins inside liposomes can be considered generally feasible. This paves the way to the construction of more complicated systems (gene regulation, sensing, multi-enzyme paths, etc..) that actually represent the “second wave” of synthetic cell work.
- Membrane proteins are more difficult to obtain, and this might limit the functionalization of liposome membranes.
- One of the goals remains the self-reproduction of minimal cells, by internal production of lipids (phospholipids, fatty acids)