ESF Synthetic Biology
ECSB II – Design, programming and optimization of biological systems

San Feliu de Guixols, 29 March – 03 April 2009

Vesicles as cellular models: from self-reproduction to semi-synthetic minimal cells

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SYNTHCELLS
Approaches to the Bioengineering of Synthetic Minimal Cells

HFSP

cost
SYSTEMS CHEMISTRY

Research within Luisi's group

- **vesicles self-reproduction, competition and selection**
- **semi-synthetic minimal cells**
- **"never born" proteins and RNAs**
- **small peptide catalysis (peptide bond formation)**

WHY MINIMAL CELLS?

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

UNDERSTANDING LIFE AT THE CELLULAR LEVEL

HOW DOES A CELL WORK?

A cell is defined by a physical boundary which allows the assimilation of nutrients/energy from the outside; the cell is able to self-maintainance and self-generation owing to the activity of the cell within its boundary.

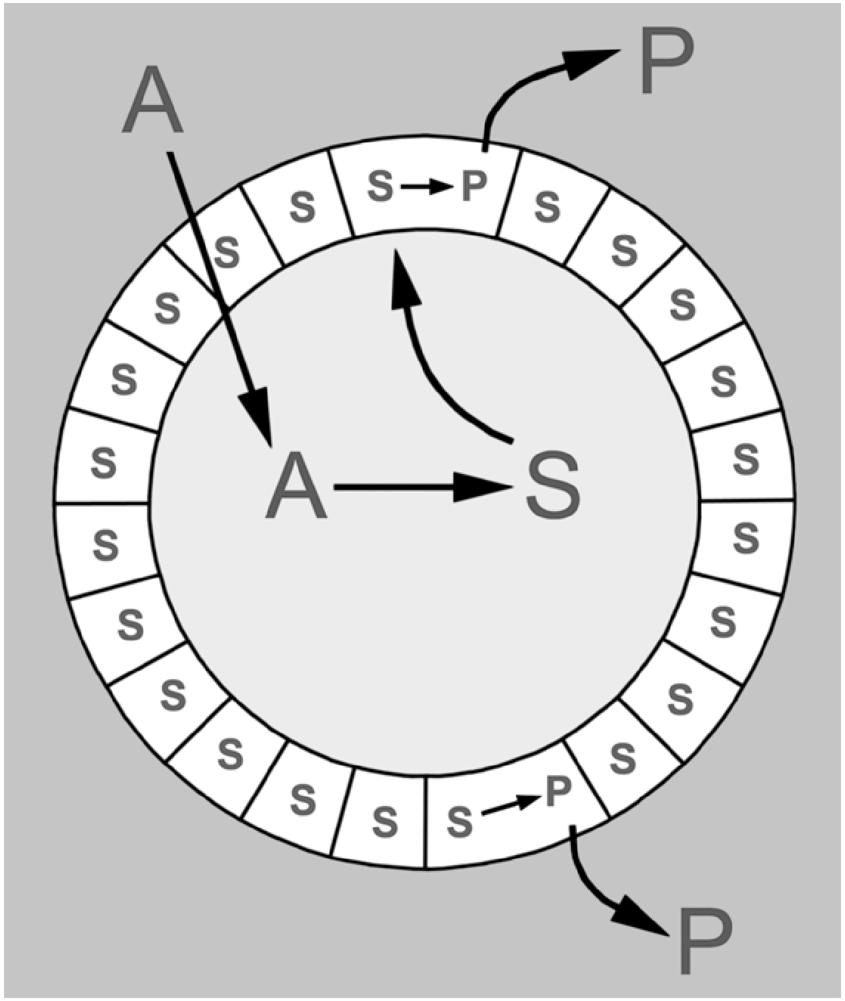
life "here and now"

autopoietic systems

"an autopoietic system is able to self-generate owing to a reaction network taking place within its own boundary"

Varela, et al. (1974).

AUTOPOIESIS stems from the Greek *auto* = self; *poiesis* = produce



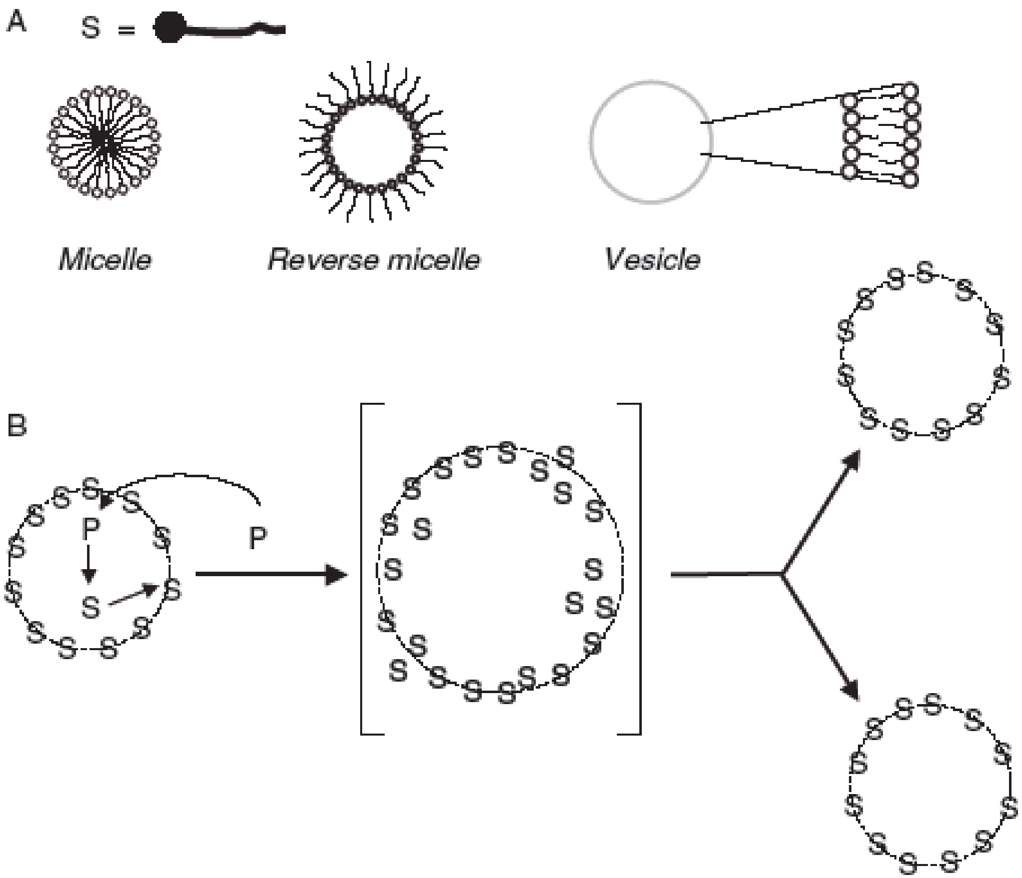
- self-bounded
- self-maintenance (self-identity)
- "cognition"

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

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

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

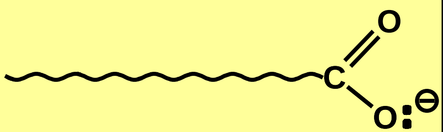
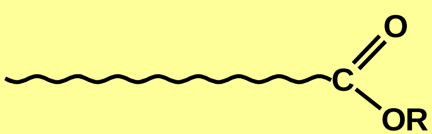
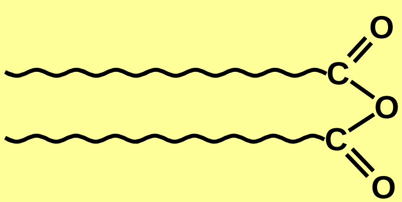
The **chemical implementation** of autopoiesis started about 18 years ago with a concept paper *[Luisi & Varela, OLEB (1990)]* and later developped experimentally in several ways.

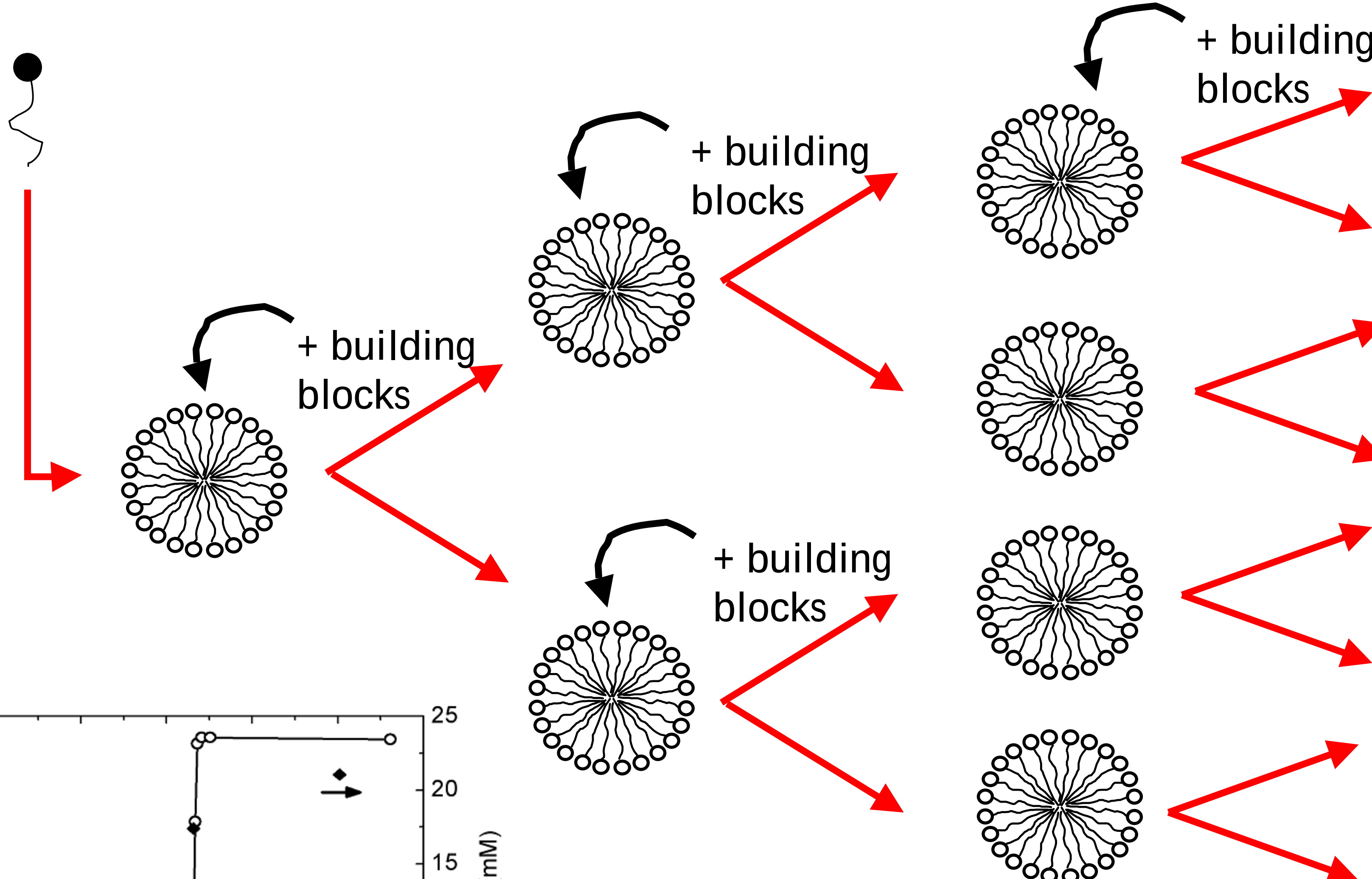


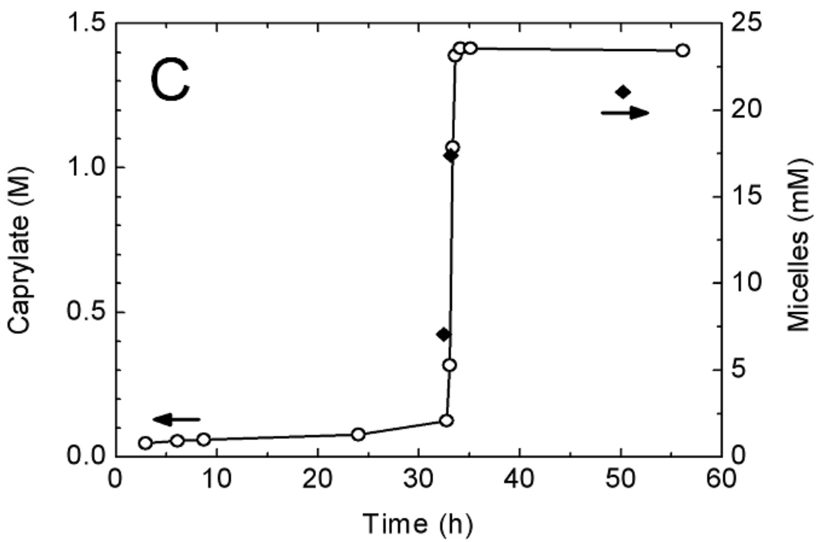
ETH 1990-2003
P.L.Luisi
P.Walde

The **self-reproduction of vesicles** is a pre-requisite for studies of 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



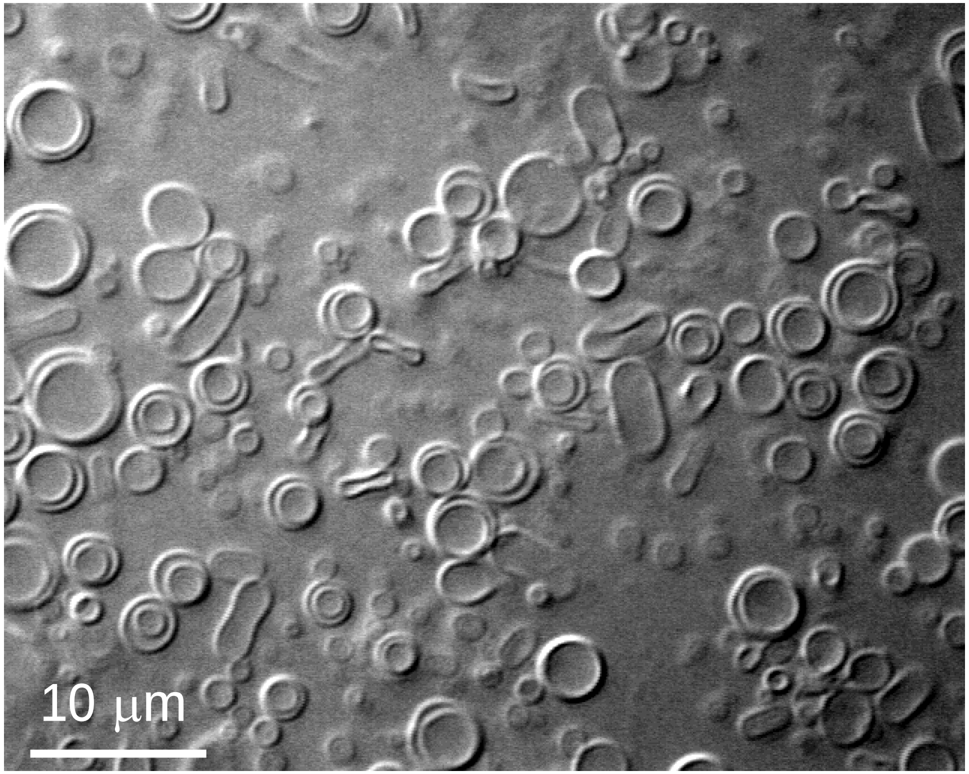


- autopoietic
- autocatalytic

Bachmann, Luisi & Lang, Nature 1992

Fatty acid vesicles

$CH_3(CH_2)_nCOOH$

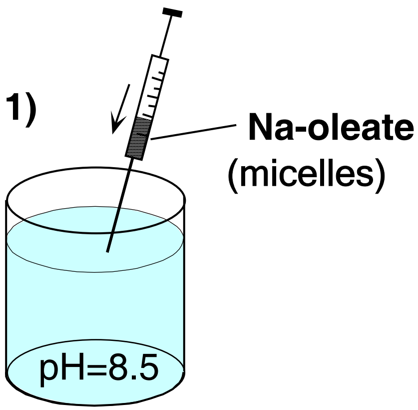


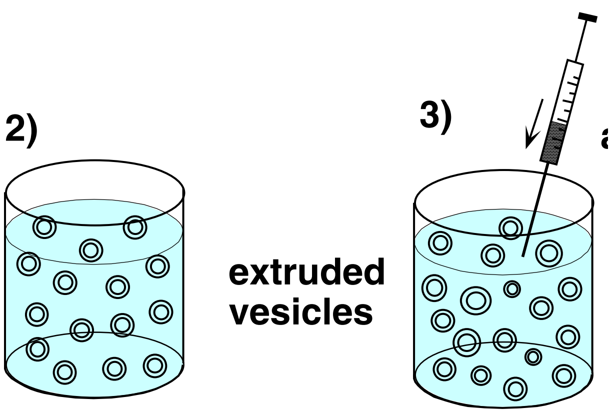
giant oleate vesicles

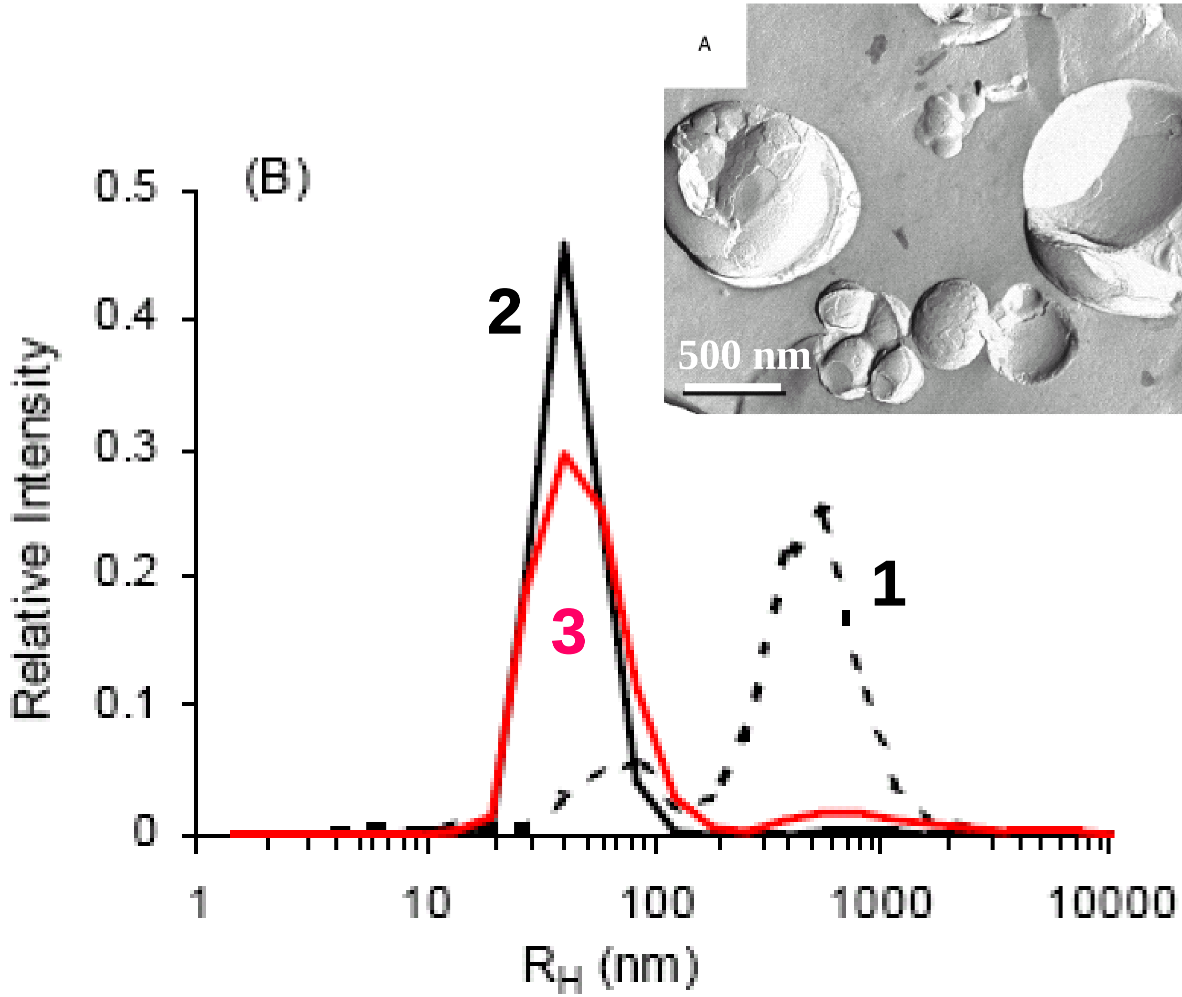
It has been suggested that fatty acid vesicles may have played an important role in the origin of life.

Fatty acids have isolated from carbonaceous chondrite meteorites (Murchison), and it has been suggested that they can be formed (with an iron-based catalyst) from CO and H_2

Self-reproduction of oleate vesicles

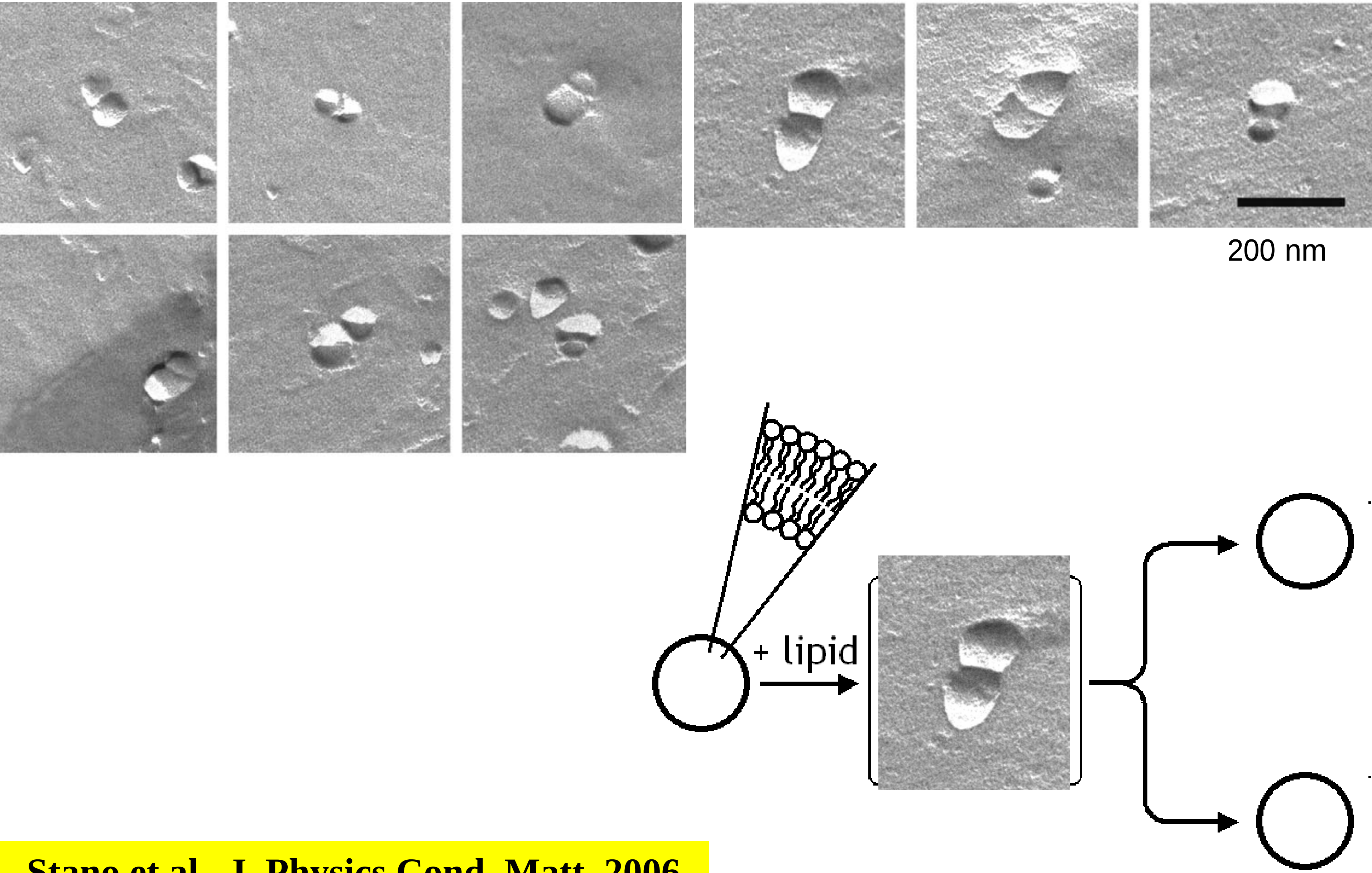






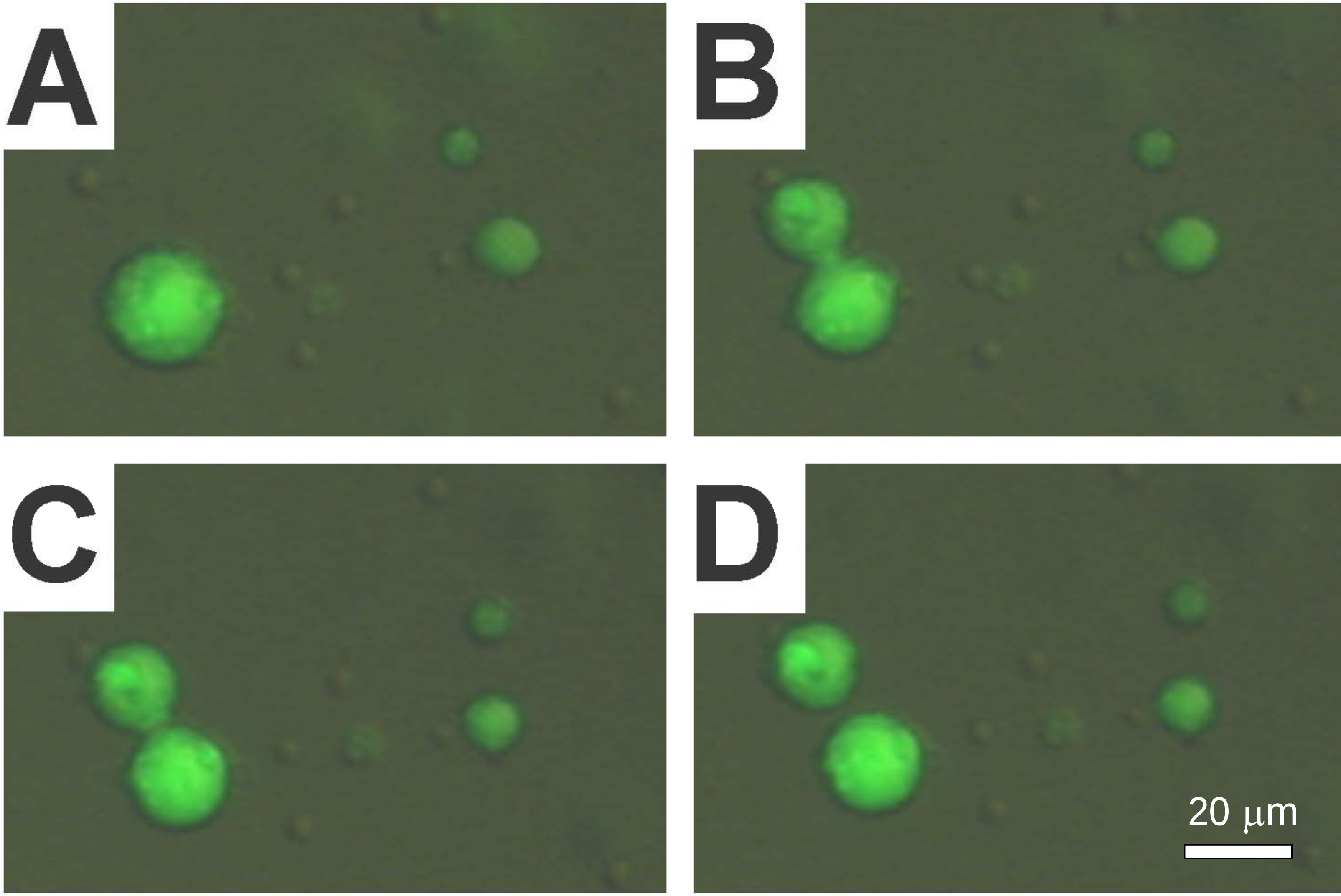
Bloechliger et al. 1998
Lonchin et al. 1999
Berlcaz et al. 2001a, 2001b
Rasi et al., 2003,2004

Self-reproduction of oleate vesicles



Stano et al., J. Physics Cond. Matt. 2006

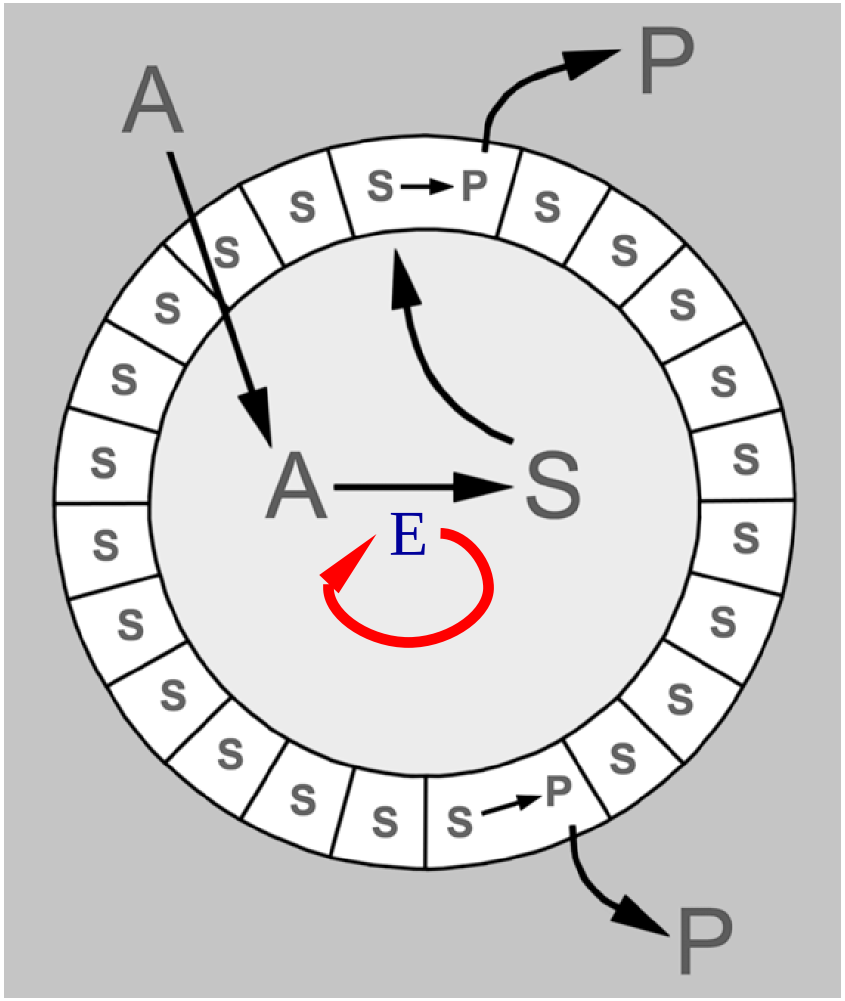
Self-reproduction of w/o droplets



Time interval: 3 s

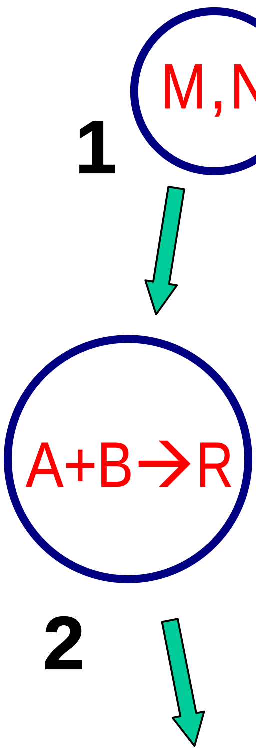
Fiordemondo and Stano, ChemBioChem 2007

CORE AND SHELL SELF-REPRODUCTION

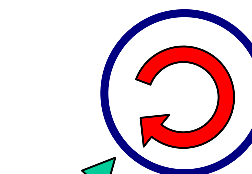


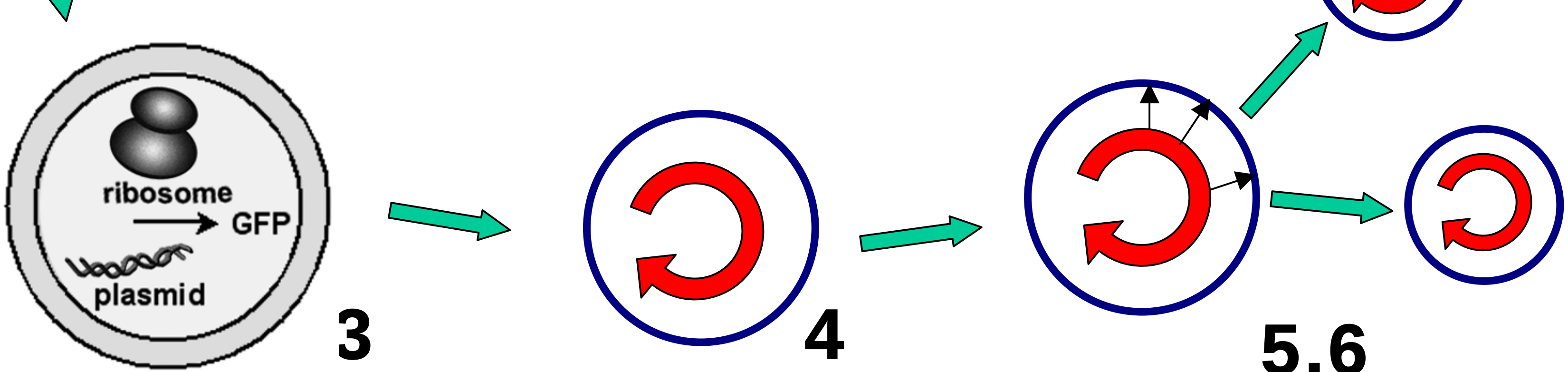
… moreover, core and shell reproduction should be functionally coupled…

A road map to the minimal cell



1. Compartments (vesicles)
2. Simple (bio)reactions in compartments
3. Protein expression
4. Self-replication of the components
5. Shell (membrane) reproduction
6. Core-and-shell reproduction
7. ... more?





Minimal cells in origins of life scenario

Modern cells

↓

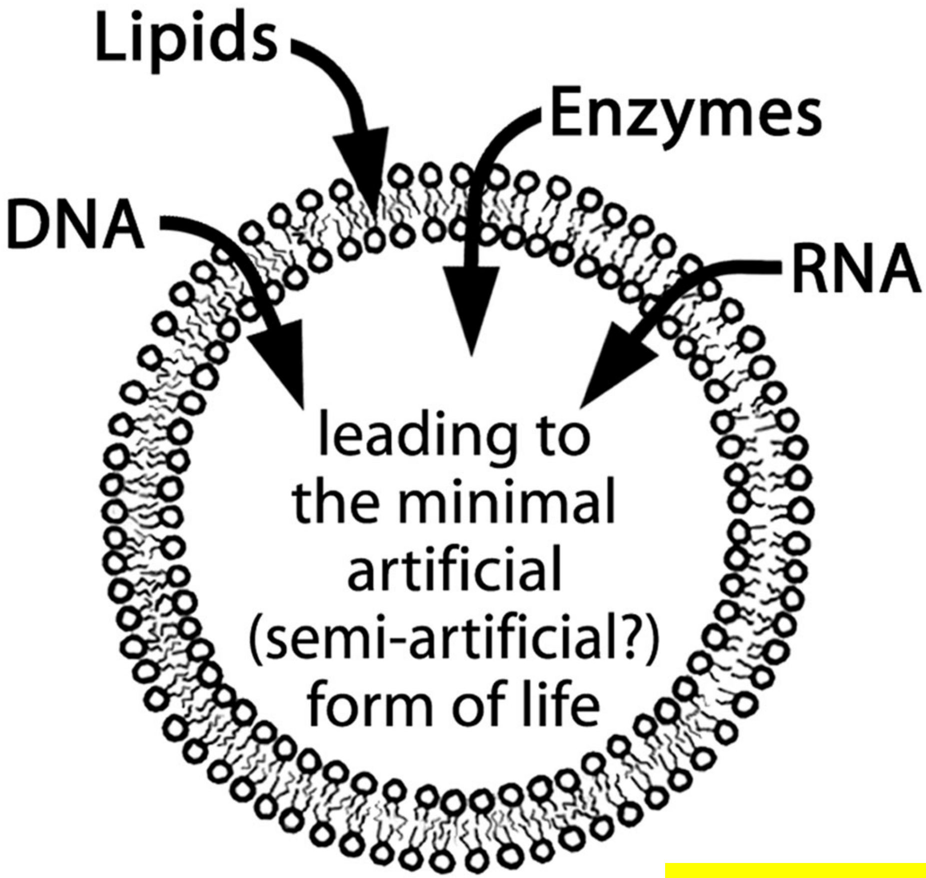
Minimal Cell

↑

Simple molecules

The notion of the Minimal Cell:

The **Minimal Cell** is a cell-like compartment containing the minimal and sufficient number of components (i.e., to perform minimal functions) in order to be "**alive**"



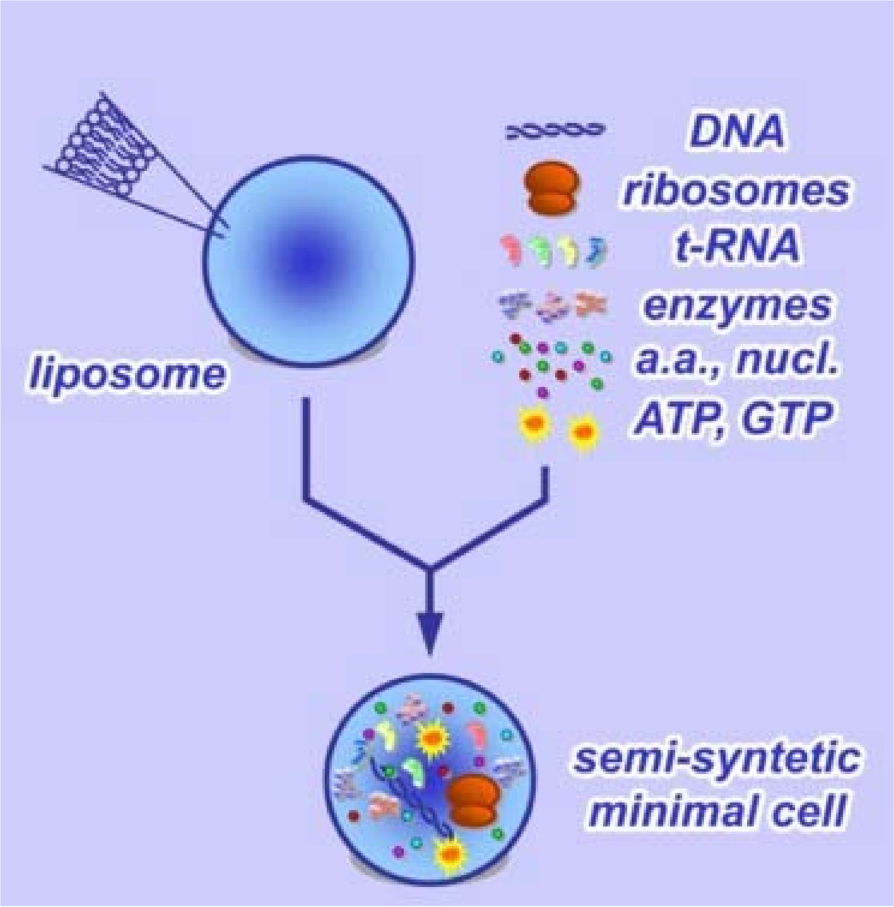
- self-maintenance (& self-bounding)
- self-reproduction
- possibility to evolve

The semi-synthetic minimal cell

- minimal genome
- minimal metabolism
- minimal size
- (functional) models for early cells

Luisi, Ferri and Stano, Naturwissenschaften 2006

SEMI-SYNTHETIC MINIMAL CELLS



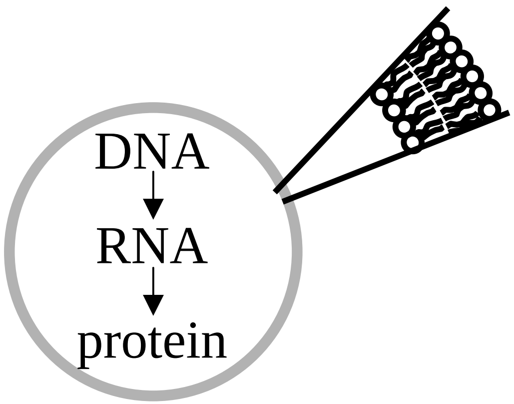
Operative point of view

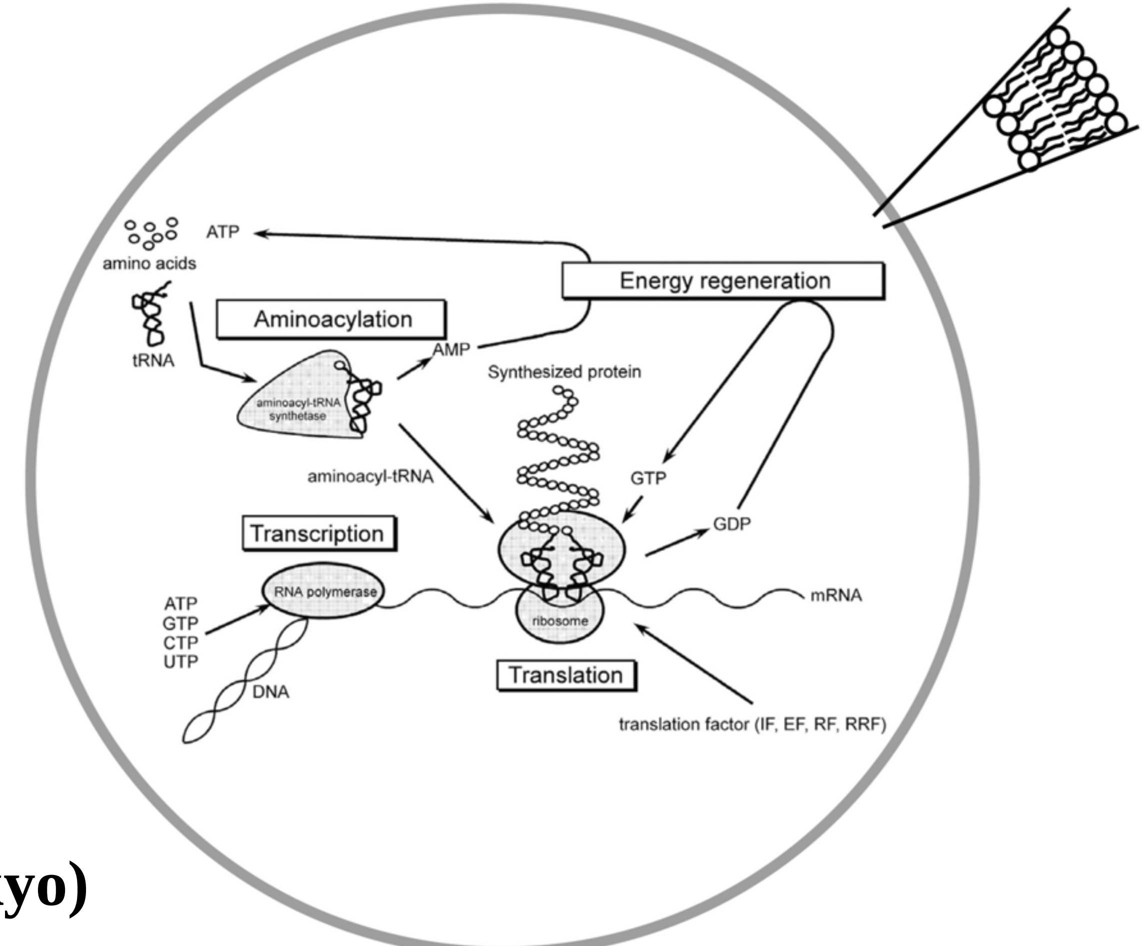
A combination of:

-cell-free/in vitro systems

- *liposome technology*

Protein biosynthesis as a paradigm of cellular metabolism





PURESYSTEM

(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

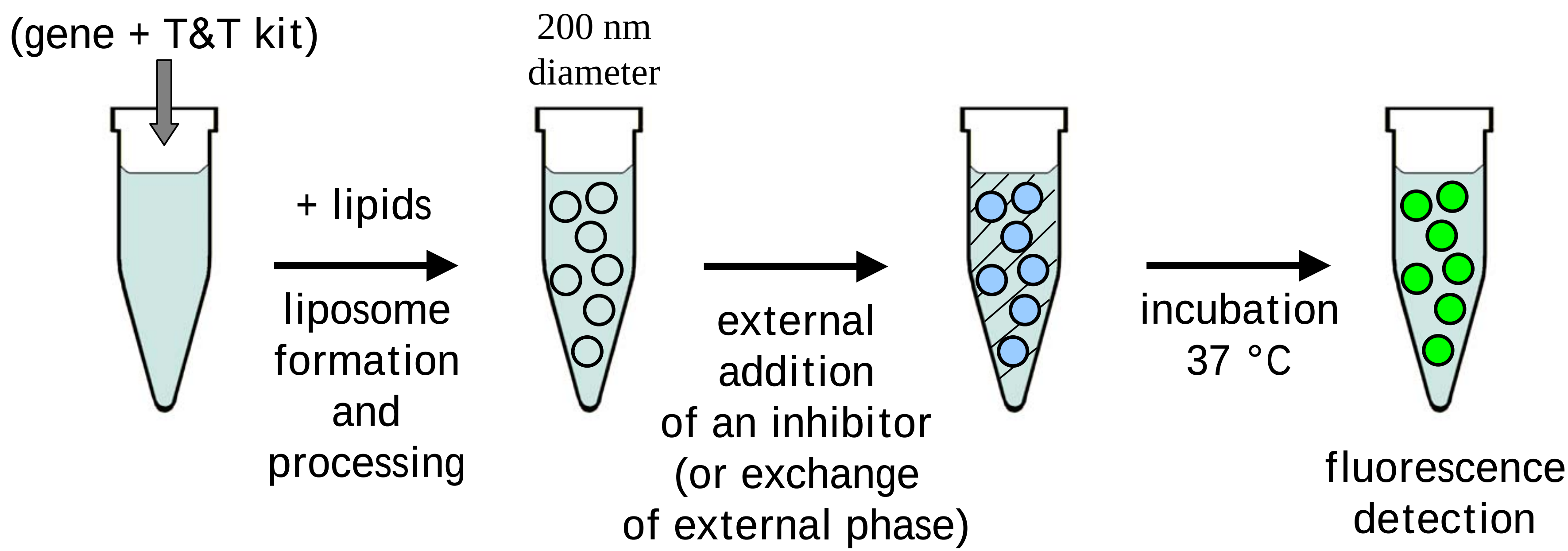
Shimizu et al. Nature 2001

The state of the art (March 2009)

Year	Authors	Results
1999	Oberholzer et al.	Poly(Phe) synthesis in vesicles (freeze-and-thaw/EDTA)
2001	Yu et al.	GFP expression in vesicles (dehydration-rehydration/RNase)
2002	Oberholzer and Luisi	EGFP expression in vesicles (injection method/EDTA)
2003	Nomura et al.	rsGFP expression in giant vesicles (GV) (natural swelling method/protease K)
2004	Ishikawa et al.	T7 RNA polymerase and GFP expression in vesicles (dehydration-rehydration/RNase)
2004	Noireaux and Libchaber	α-hemolysin and EGFP expression in GV (oil-to-water spin extraction)
2006	Sunami et al.	GFP expression in vesicles; PURESYSTEM; FACS select. (dehydration-rehydration/RNase)
2007	Murtas et al.	EGFP expression in vesicles; PURESYSTEM (hydration/RNase)
2008	Kita et al.	Qβ-replicase and β-galactosidase expression in VET400 vesicles; PURESYSTEM (hydration/Rnase)
2008	Kuruma et al.	Expression of two membrane proteins inside large vesicles; PURESYSTEM (hydration/RNase)
2009	Souza et al.	EGFP expression inside 200 nm vesicles, PURESYSTEM (extrusion or injection/RNase, protease, EDTA)

EXPRESSION OF GFP INSIDE SMALL VESICLES

THE MINIMAL SIZE OF CELLS?



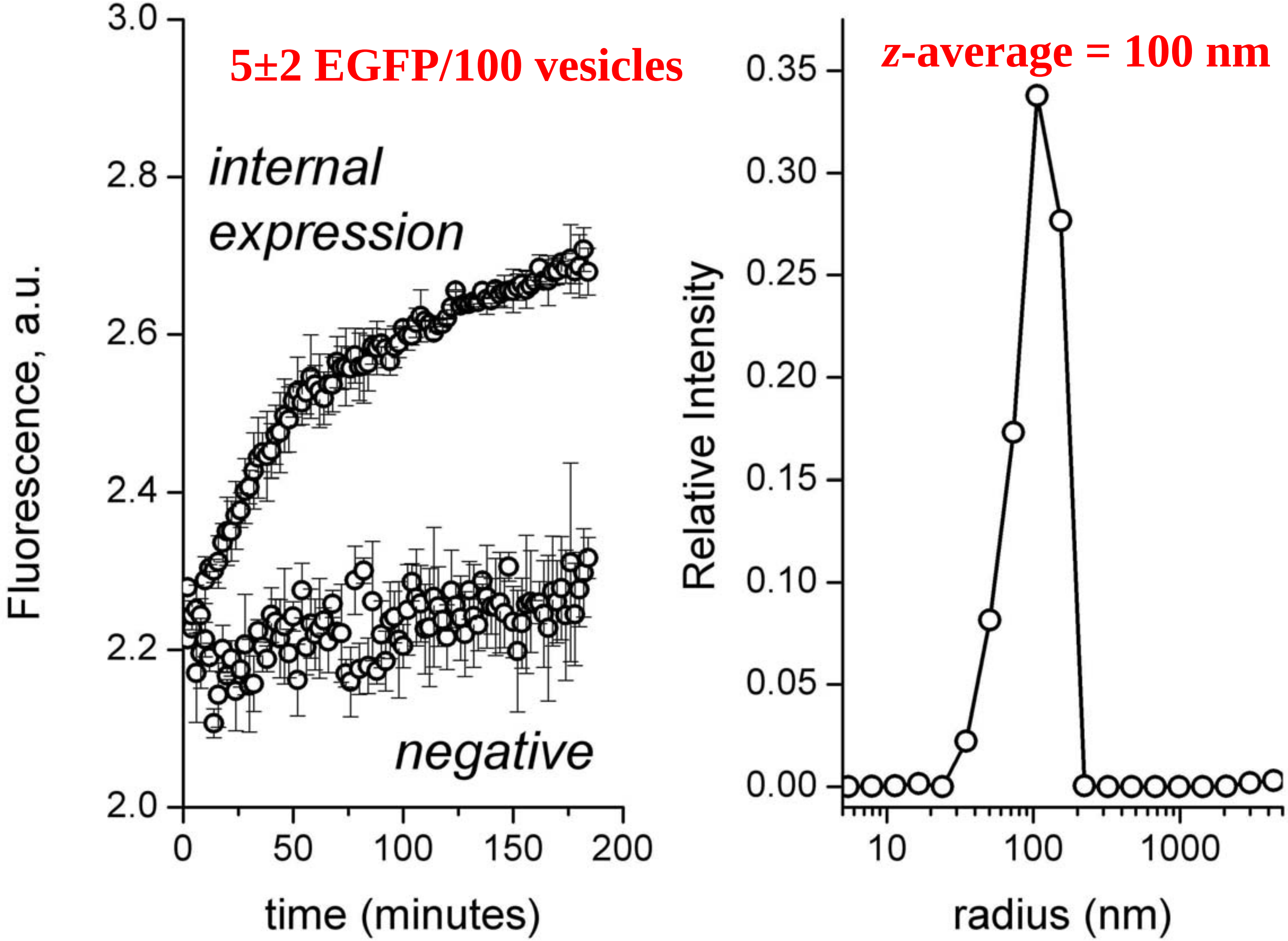
injection method

(or extrusion)

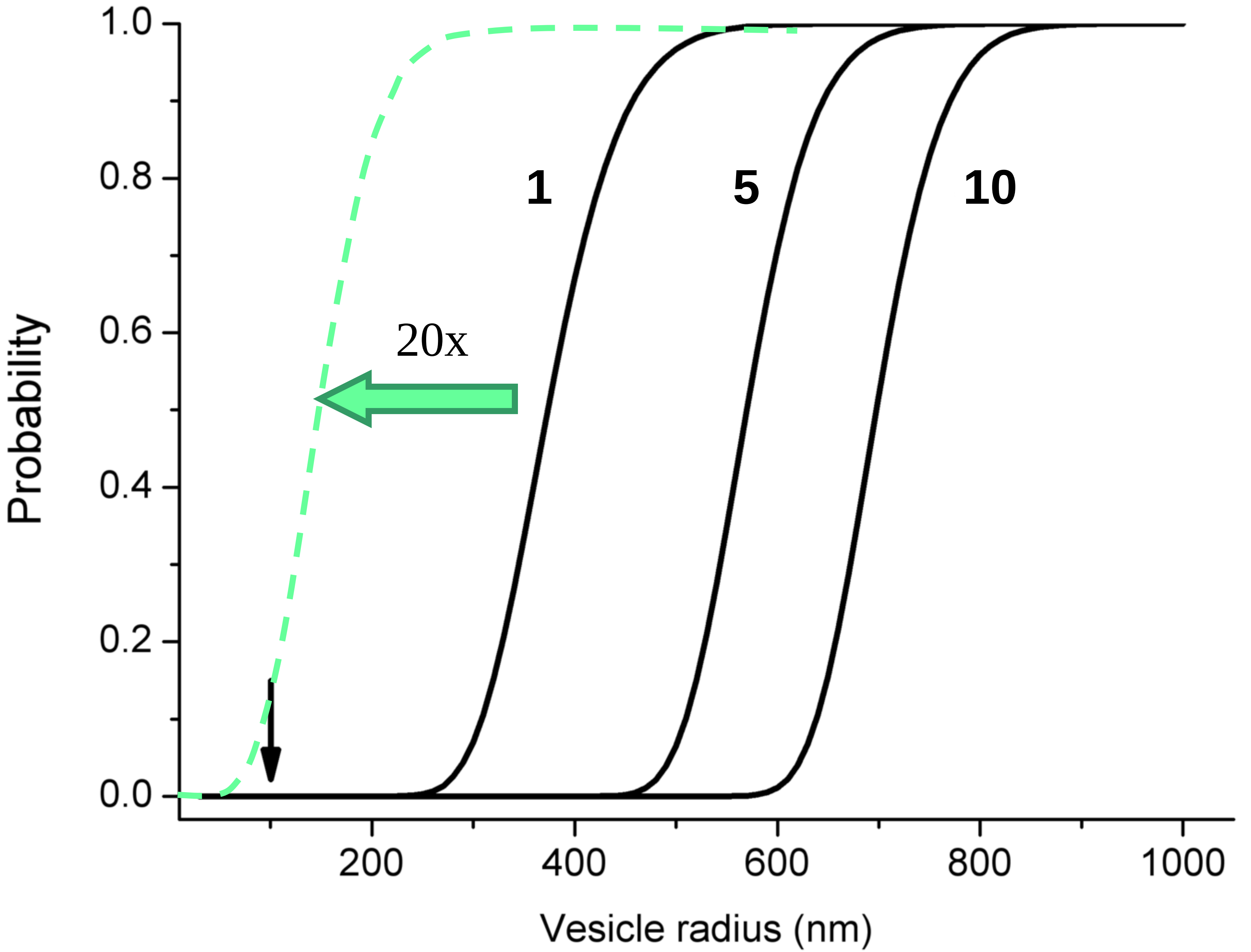
Injection method: small vesicles form spontaneously within the mixing time. Solutes are co-entrapped inside vesicles.

Souza, Stano and Luisi, 2009

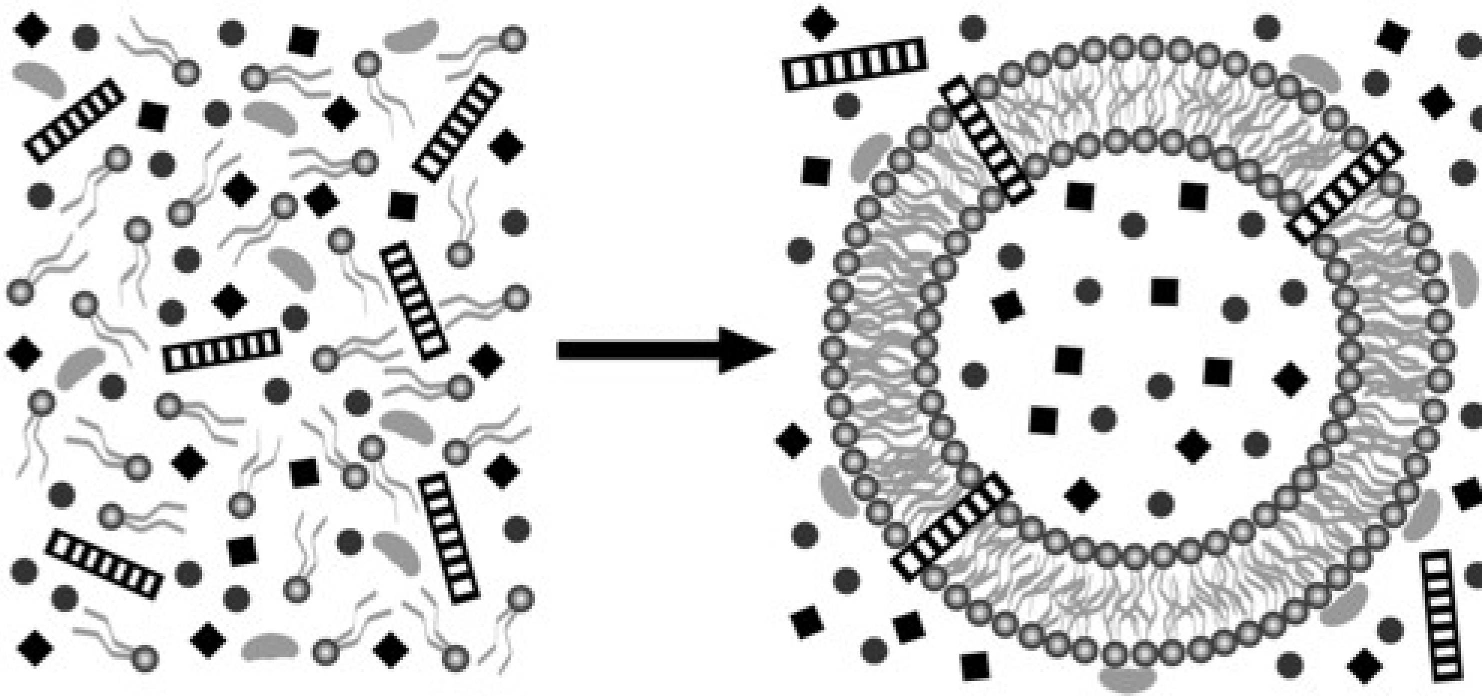
What is the minimal vesicle size compatible with internal protein biosynthesis?

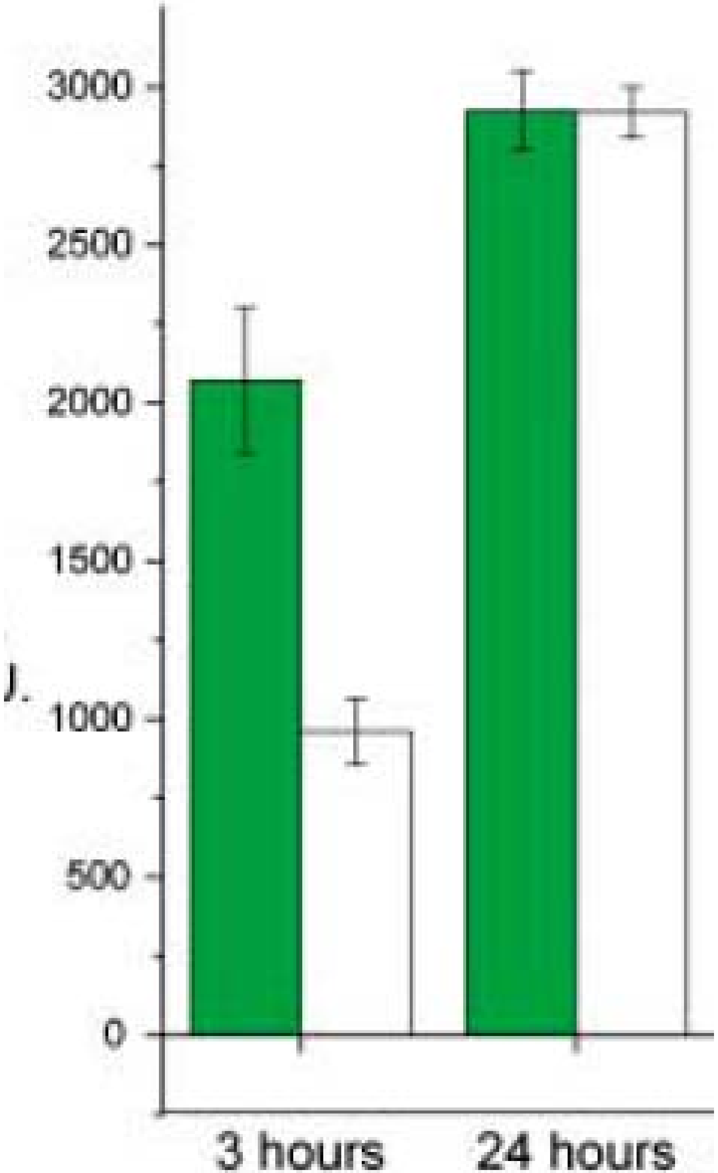


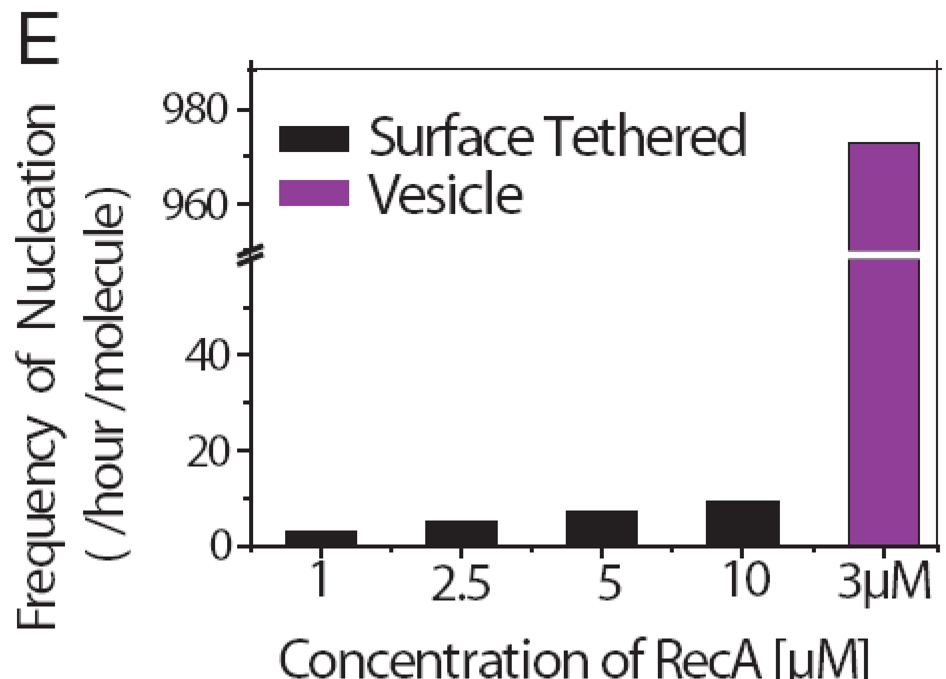
Souza, Stano and Luisi, 2009



Souza, Stano and Luisi, 2009



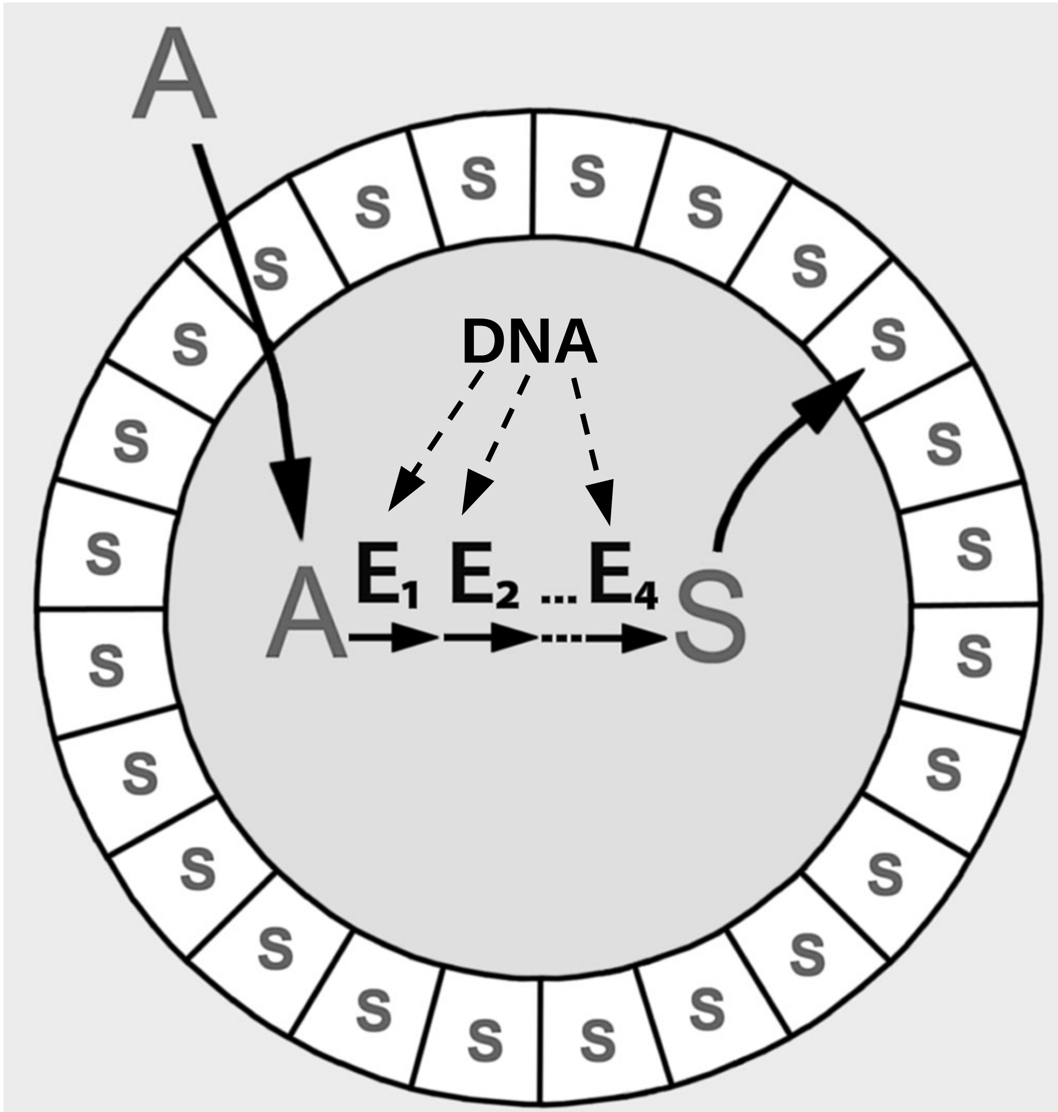




Cisse et al. PNAS 2007

Nomura et al. ChemBiochem 2003

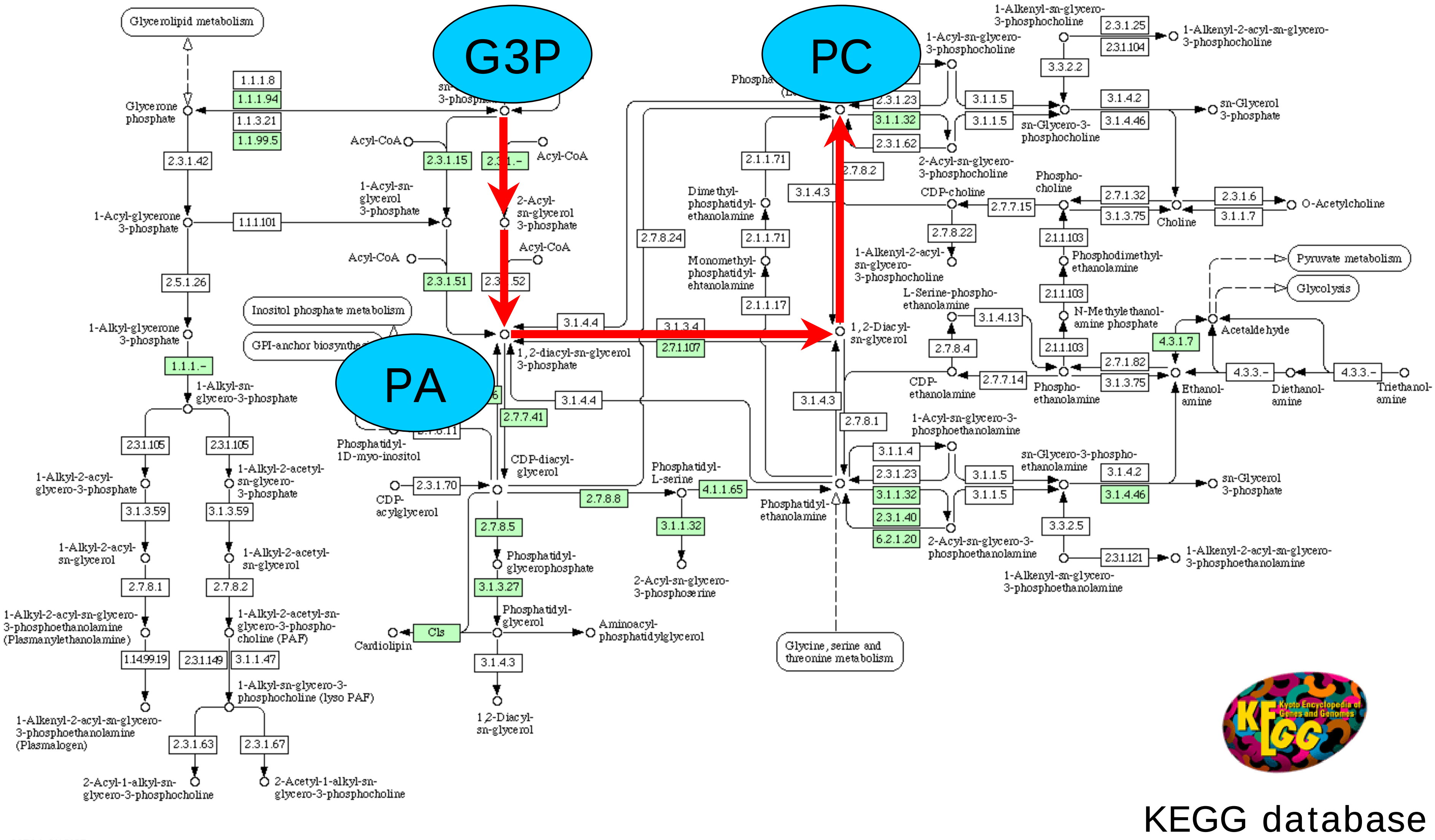
Minimal Cell: Synthesis of lipids from within



Yutetsu
Kuruma

Enzymatic synthesis of the lipids from within and autopoietic growth.

Glycerophospholipids metabolism



KEGG database

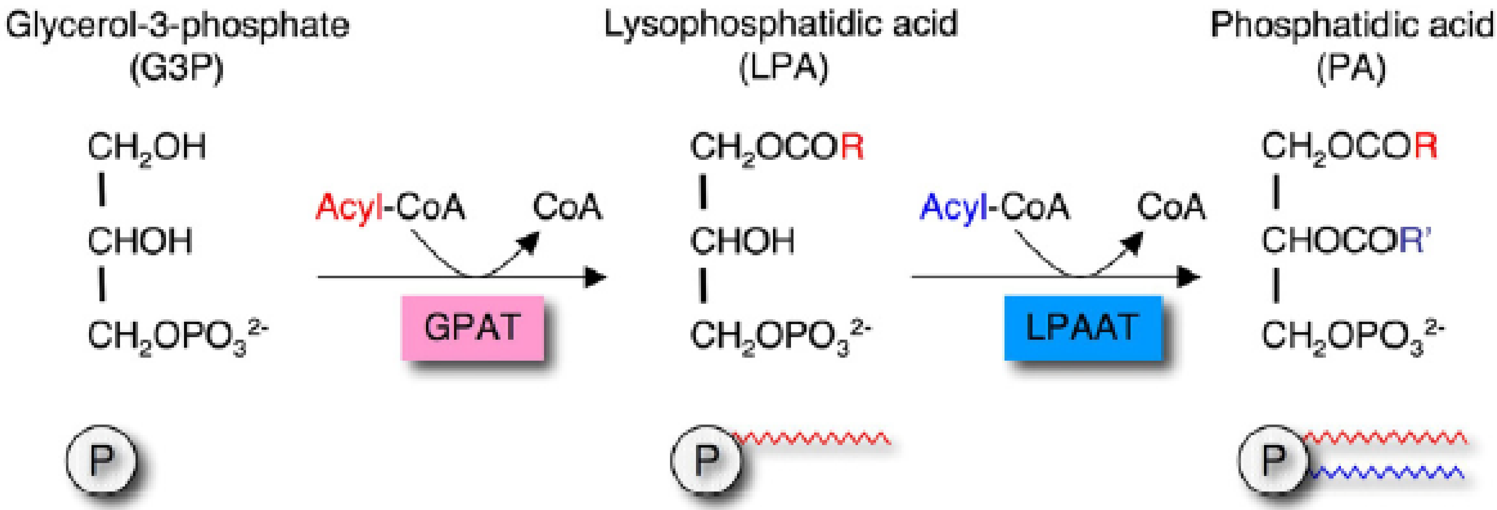
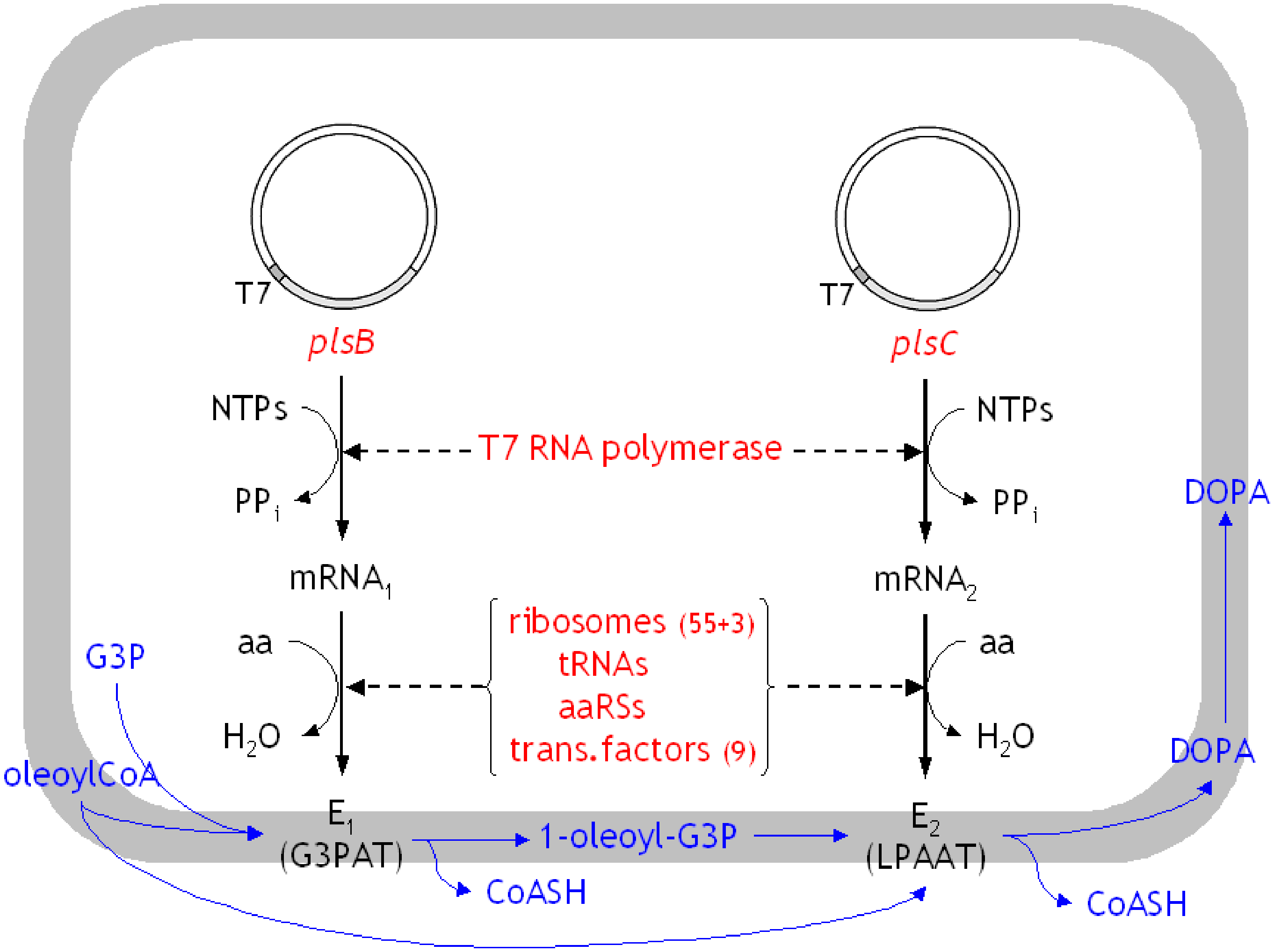


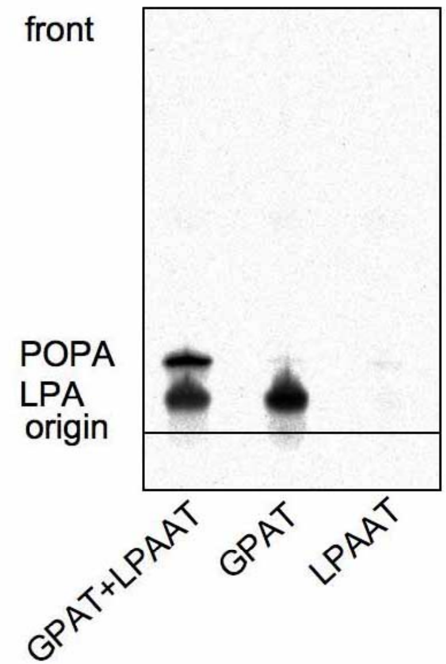
Table 1

Phospholipid compositions of liposome

Lipid composition (mol)	Conc. (mM)	Synth.	Encap.	Act.
POPC	200	++	+++	–
POPC/POPG (80:20)	200	+	+	+
Polar lipid extract (*E. coli*)	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	–	+++	–	–

Enzymatic production of lipids





Kuruma, Stano et al., BBA 2008