University of Nottingham

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Semi-synthetic Minimal Cells

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SYNTHCELLS

Approaches to the Bioengineering of Synthetic Minimal Cells

HFSP

cost

SYSTEMS CHEMISTRY

Research within Pier Luigi Luisi group

- **vesicles self-reproduction, competition and selection**
- **the semi-synthetic minimal cell**
- **the “never born” proteins and RNAs**
- **the “fragment condensation” approach**

Minimal cells in origins of life scenario

Modern cells

Conceptually a top-down approach. We aim to construct a minimal living cell, that *does not represent an ancient cell*, but it has a minimal set of functions.

When we use extant enzymes and nucleic acids to assemble a synthetic cell, we are building a *semi-synthetic minimal cell*. It may have relevance to understand life and for biotechnologies.

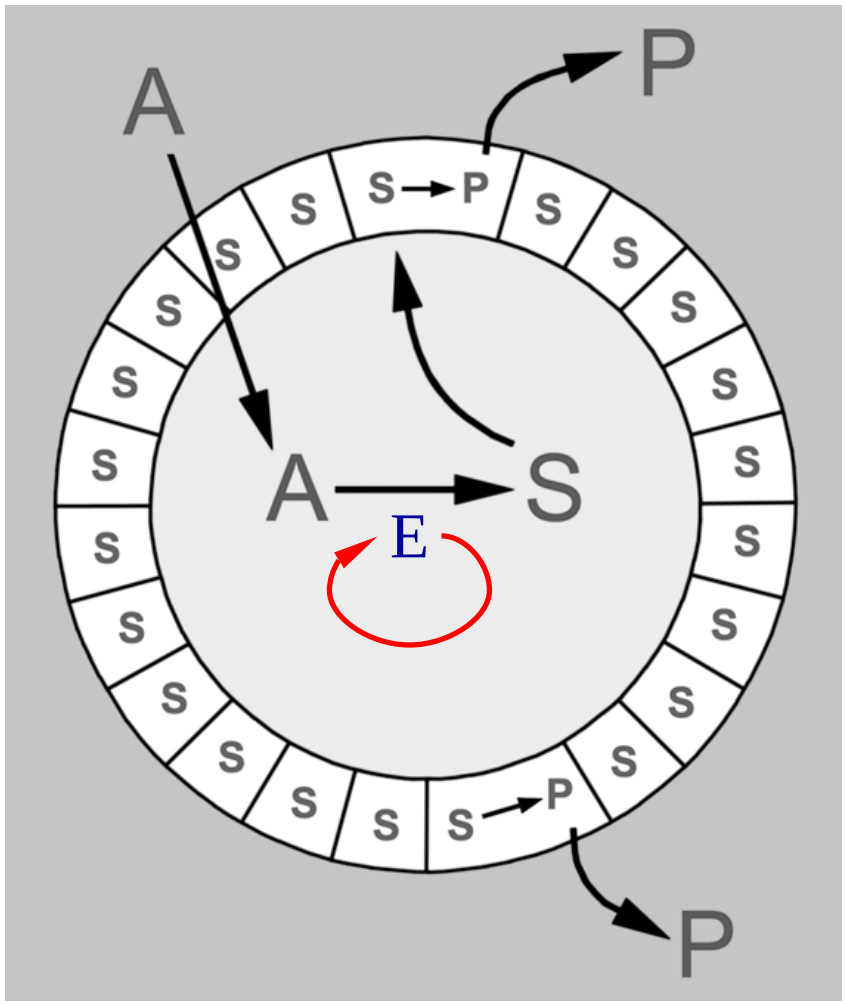
Minimal Cell

This approach is typical of "prebiotic chemistry".
Problems:
- historical reconstruction?
- *contingency* in origins of life scenarios

A completely synthetic minimal cell can in principle be made starting from synthetic components. This attempt is currently carried out by S. Rasmussen and coworkers: the *Los Alamos Bug*.

Simple molecules

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

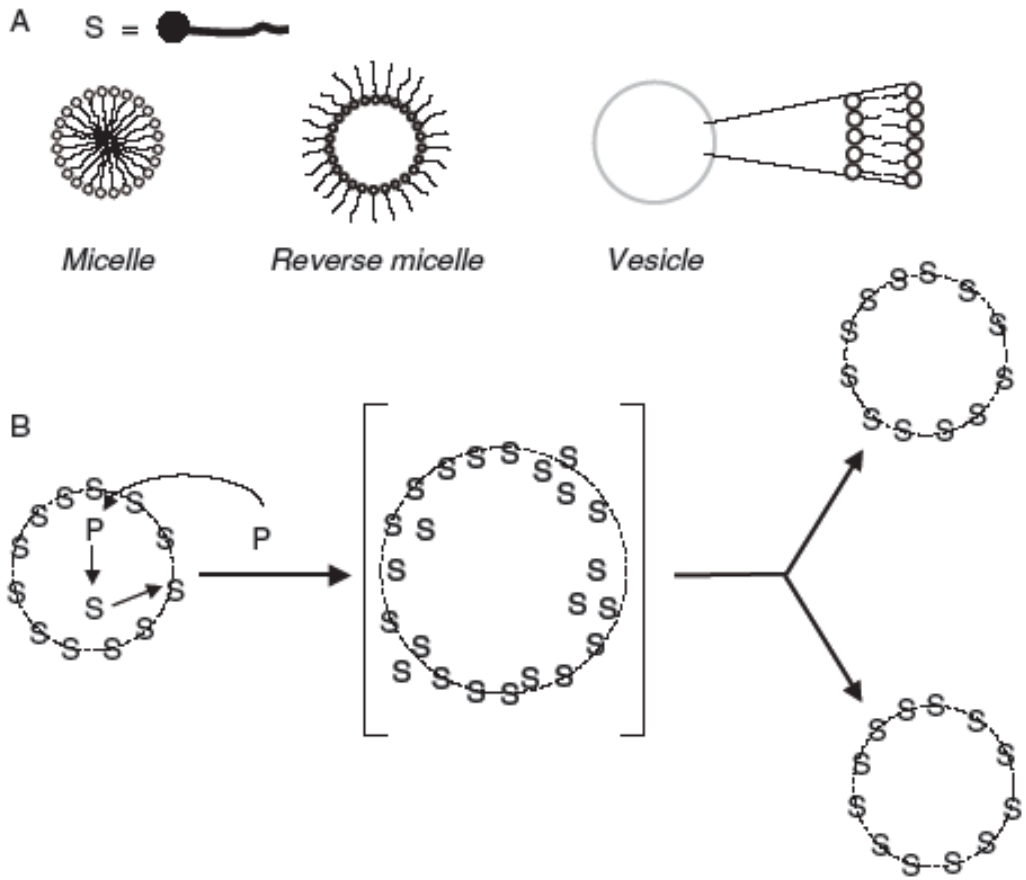


- self-bounded
- self-maintainance (self-identity)

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

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

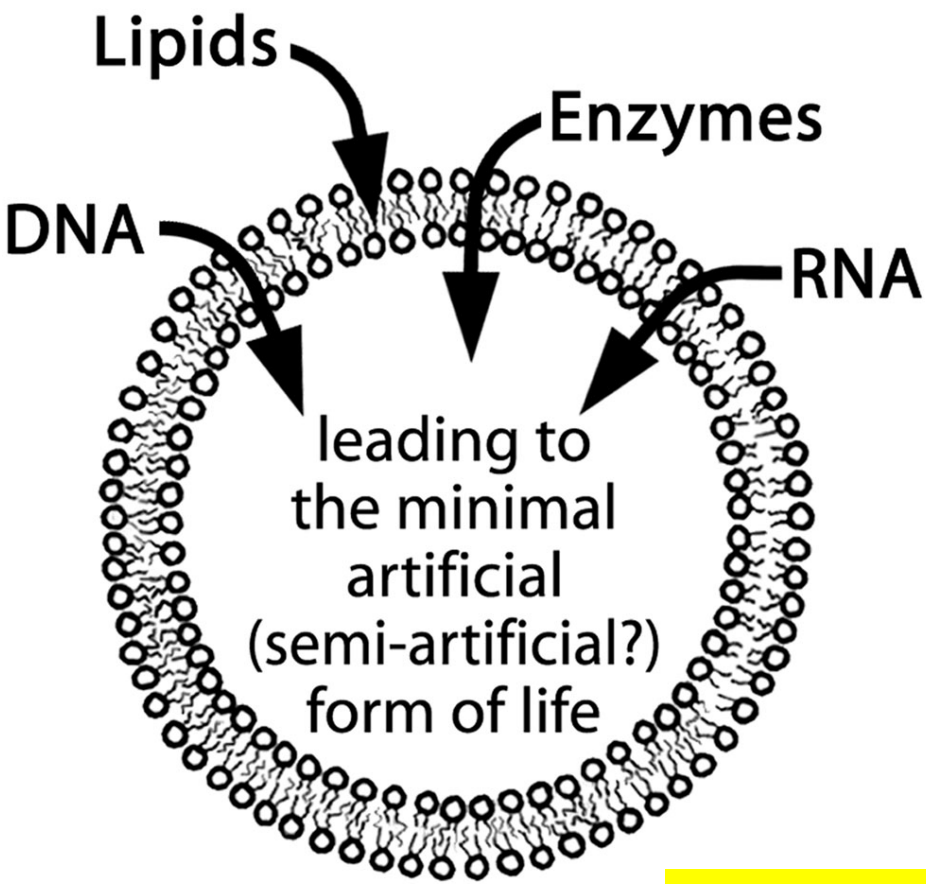
The **chemical implementation** of autopoiesis started about 18 years ago with a concept paper *[Luisi & Varela, OLEB (1990)]* and later developped experimentally in several ways.



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

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**"



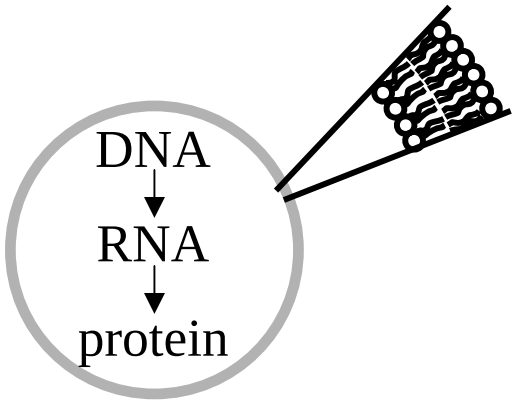
- self-maintenance (& self-bounding)
- self-reproduction
- possibility to evolve

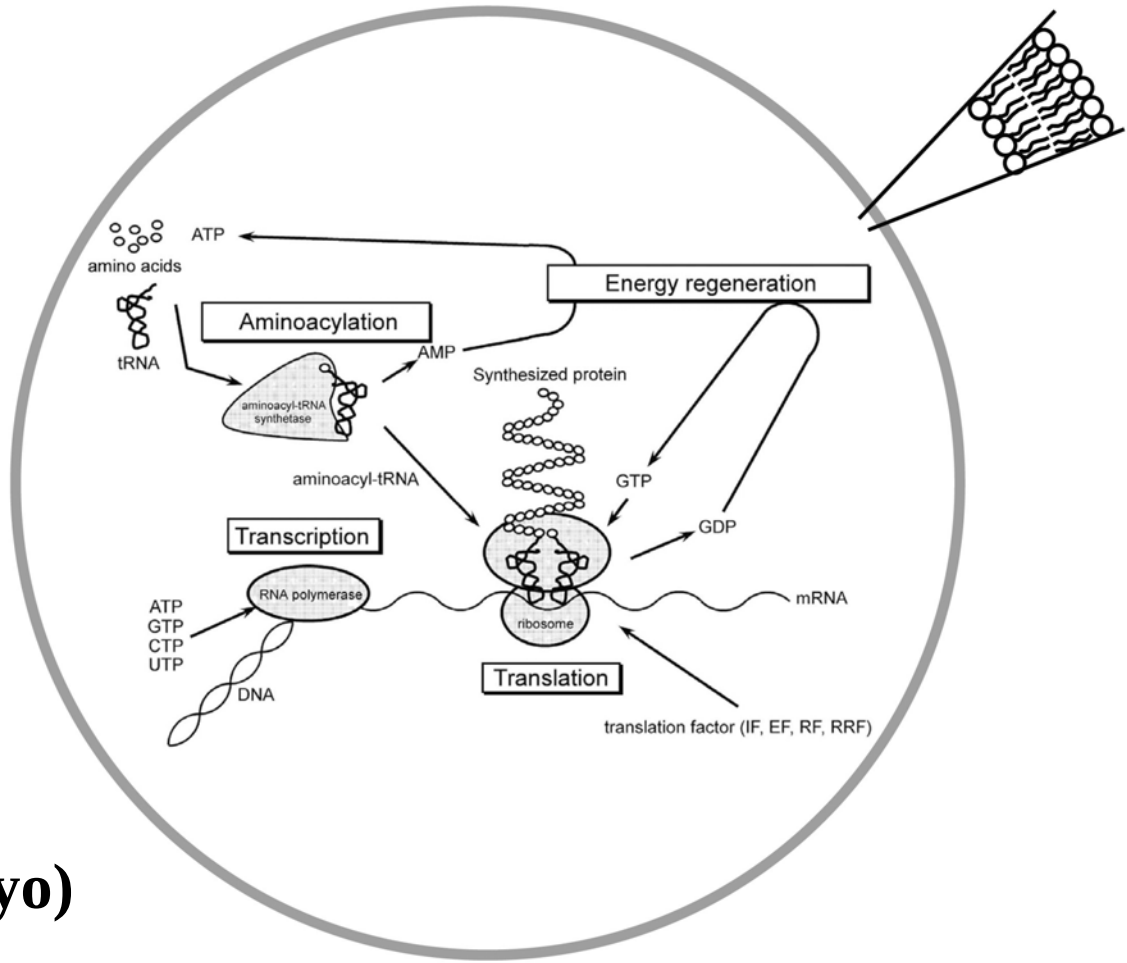
The semi-synthetic minimal cell

- minimal genome
- minimal metabolism
- minimal size
- models for early cells

Luisi, Ferri and Stano, Naturwissenschaften 2006

Protein biosynthesis as a paradigm of cellular metabolism





PURESYSTEM

(Takuya Ueda, Univ. Tokyo)

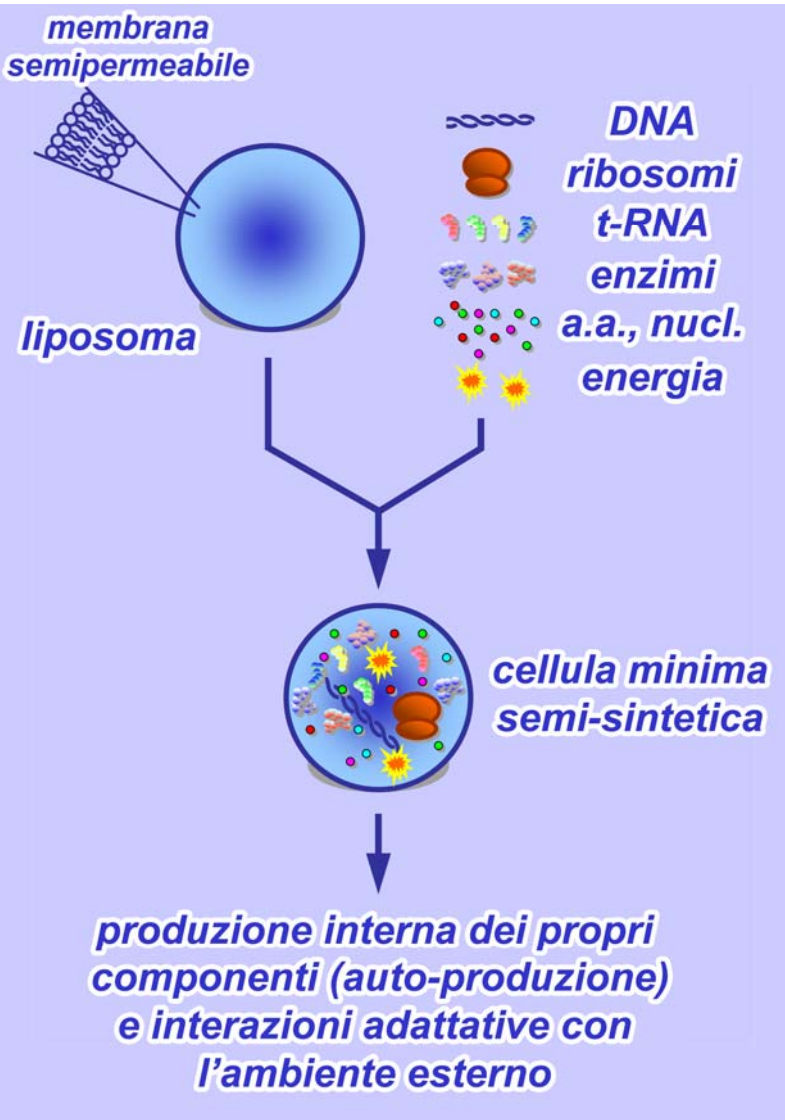
A molecular kit of 36 purified enzymes, ribosomes, t-RNAs, and low molecular weight compounds, which synthesize proteins starting from the corresponding DNA

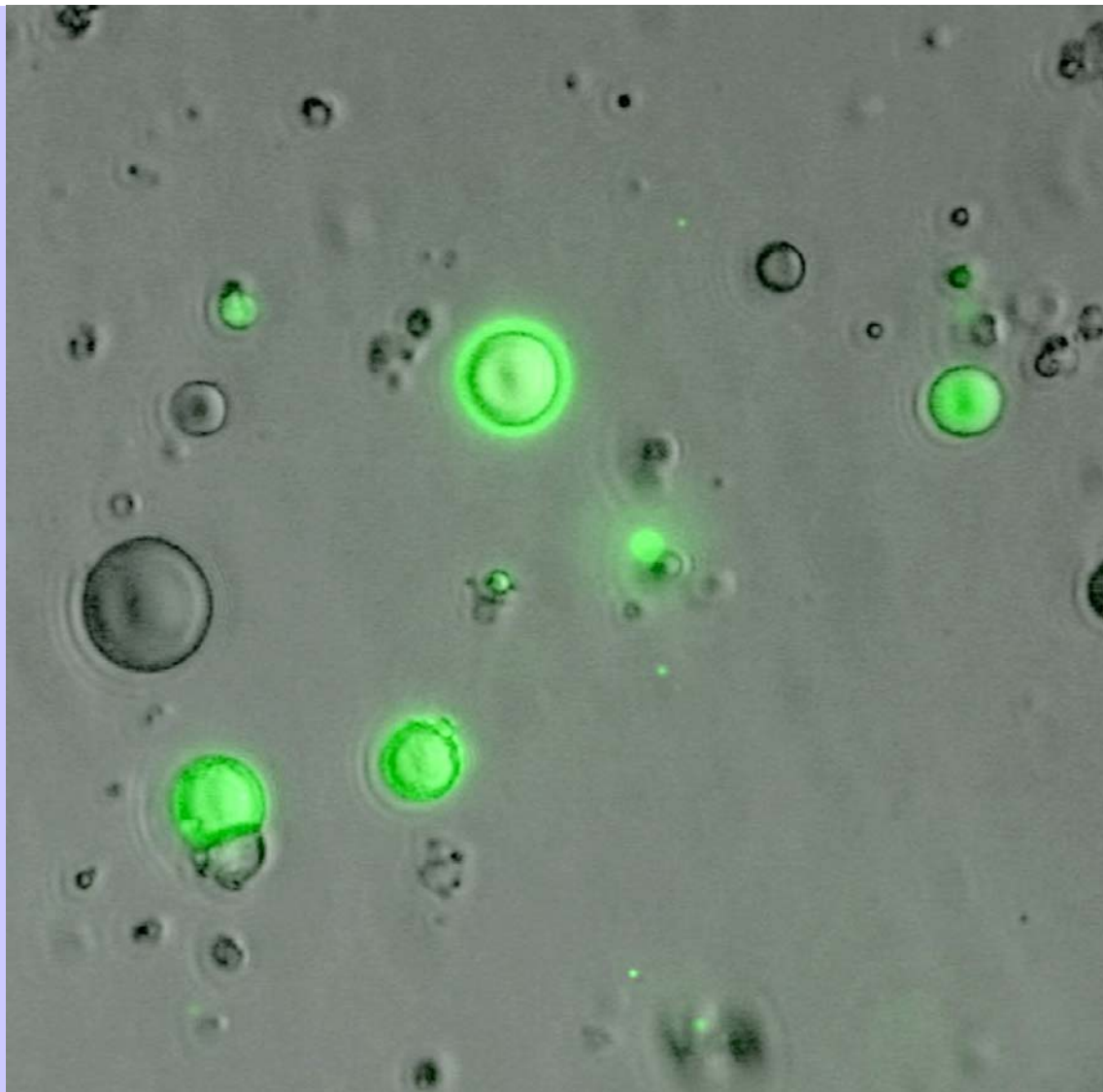
Shimizu et al. Nature 2001

The state of the art (March 2009)

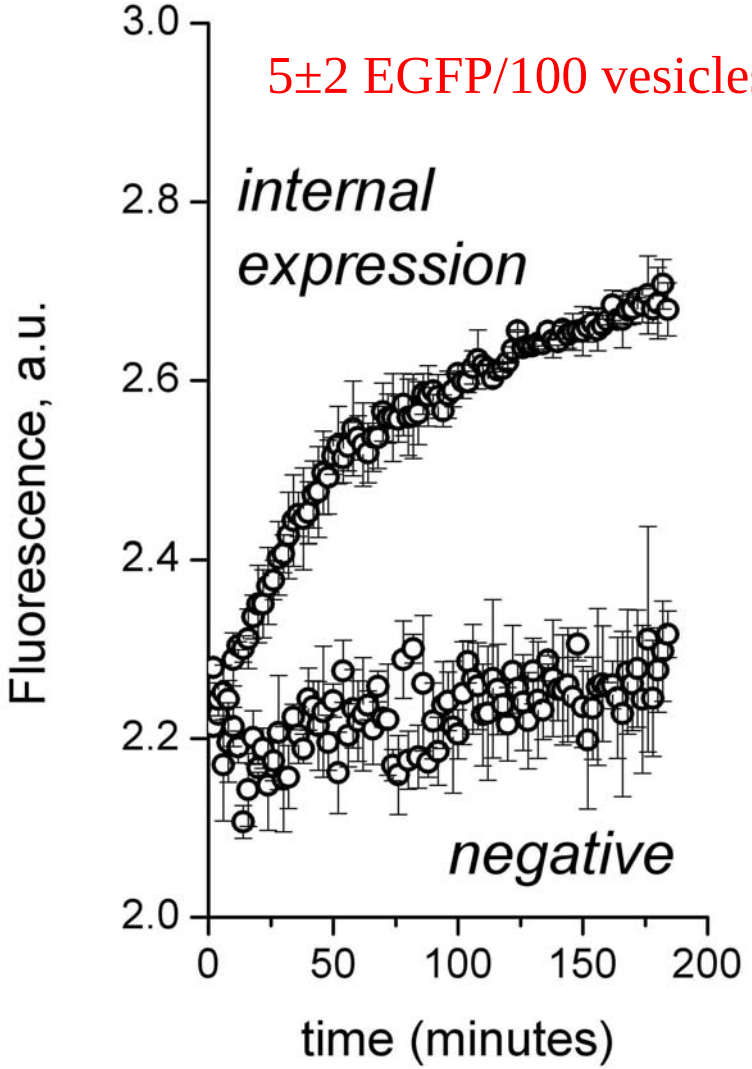
Year	Authors	Results
1999	Oberholzer et al.	Poly(Phe) synthesis in vesicles (freeze-and-thaw/EDTA)
2001	Yu et al.	GFP expression in vesicles (dehydration-rehydration/RNase)
2002	Oberholzer and Luisi	EGFP expression in vesicles (injection method/EDTA)
2003	Nomura et al.	rsGFP expression in giant vesicles (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 vesicles (0.5-1 um); PURESYSTEM (hydration/RNase)
2009	Souza et al.	EGFP expression inside 200 nm vesicles, PURESYSTEM (extrusion or injection/RNase, protease, EDTA)

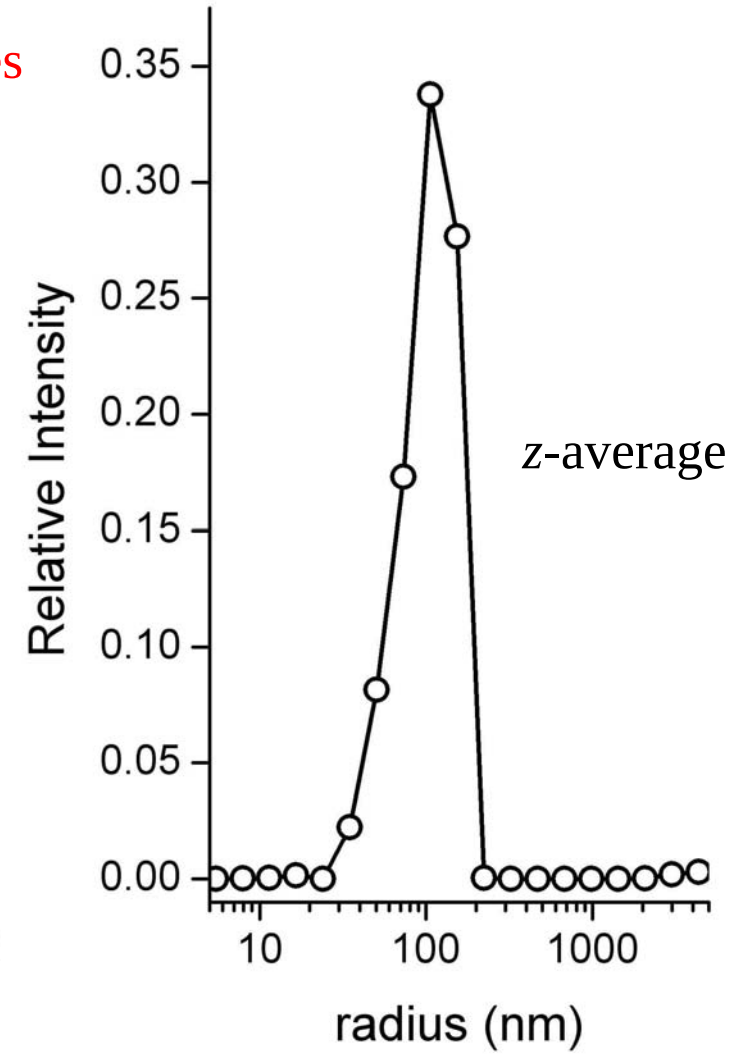
SEMI-SYNTHETIC MINIMAL CELLS





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

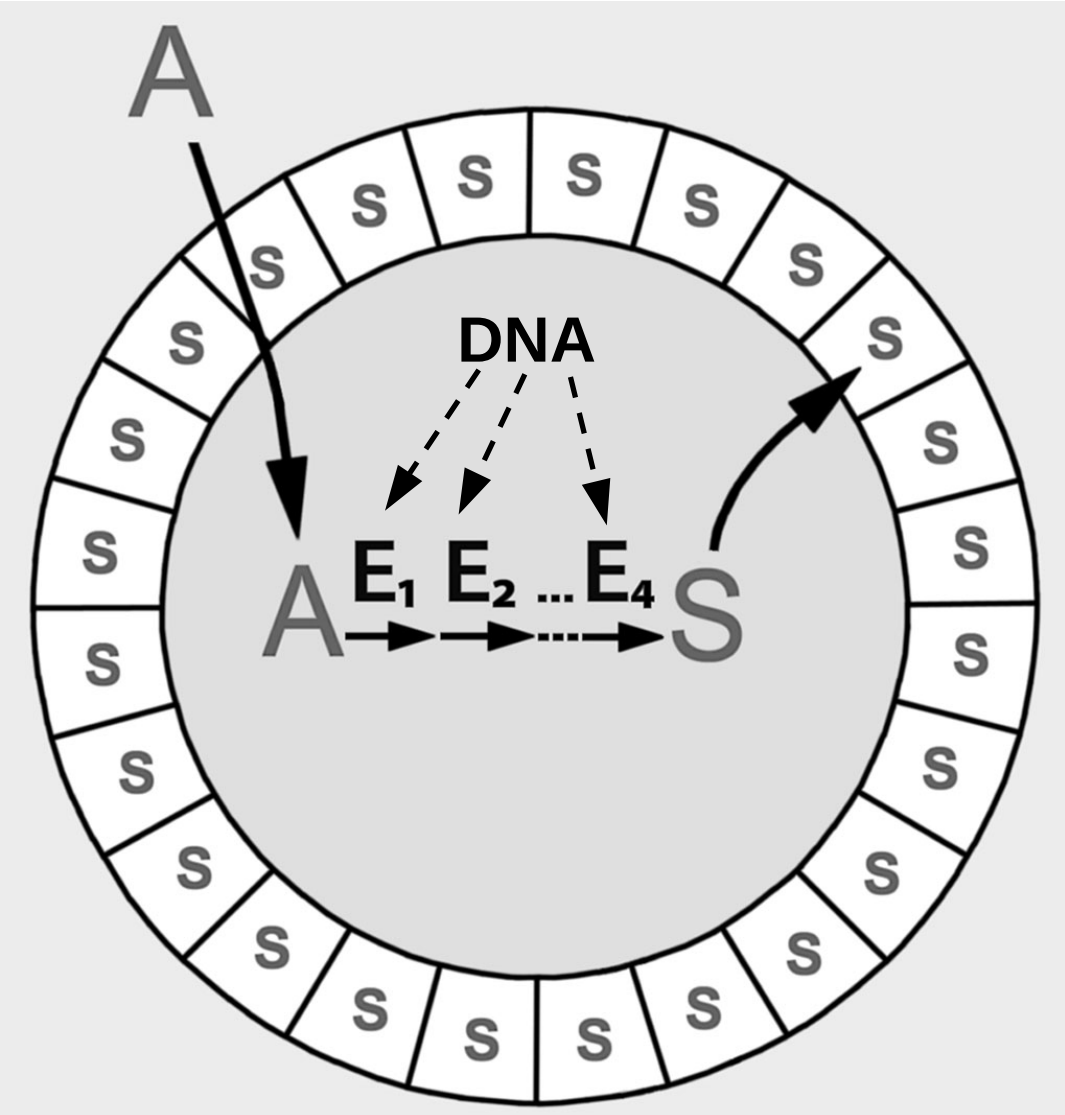




z-average = 100 nm

Souza, Stano and Luisi, in press

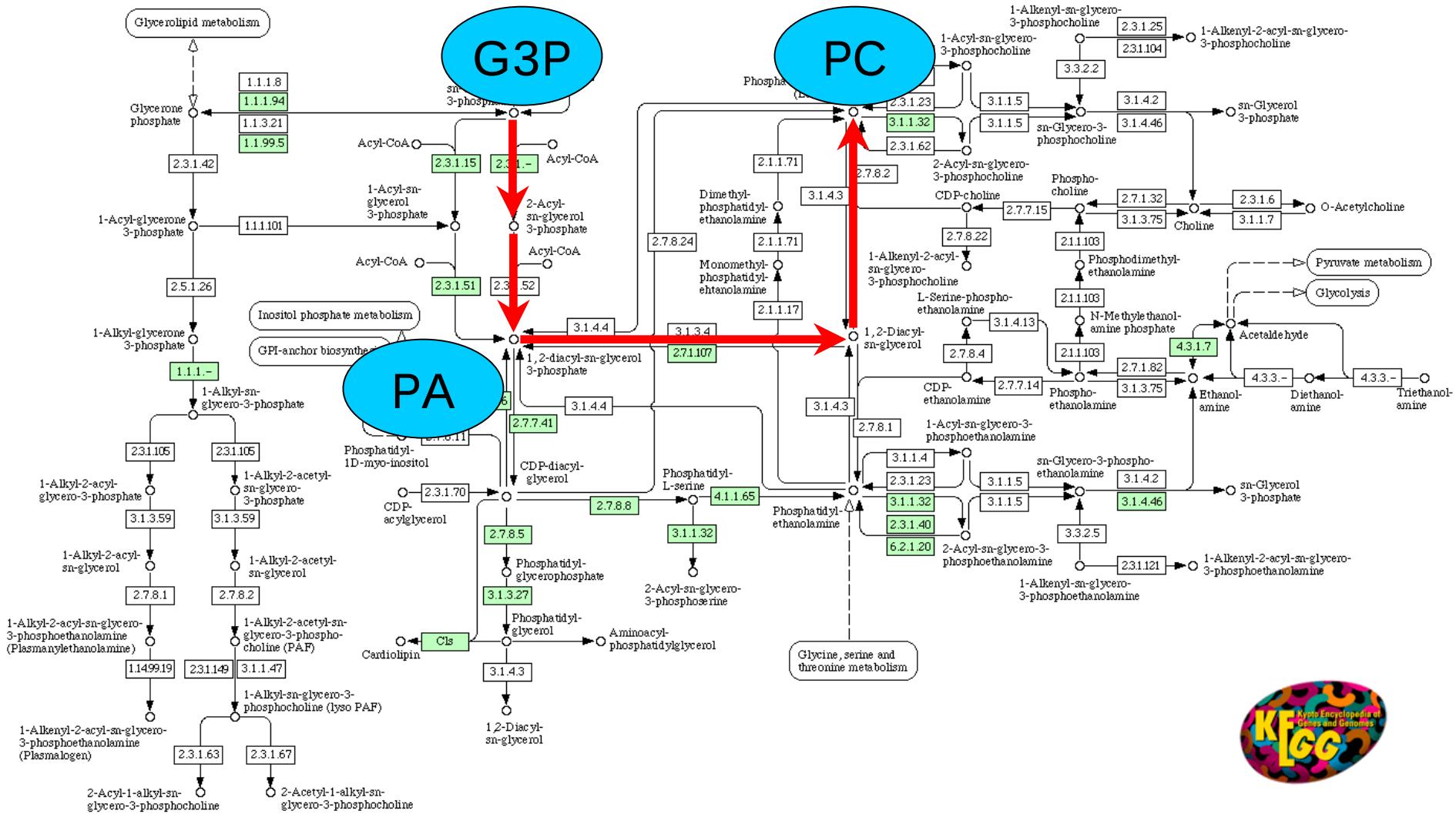
Minimal Cell: Synthesis of lipids from within



Yutetsu Kuruma

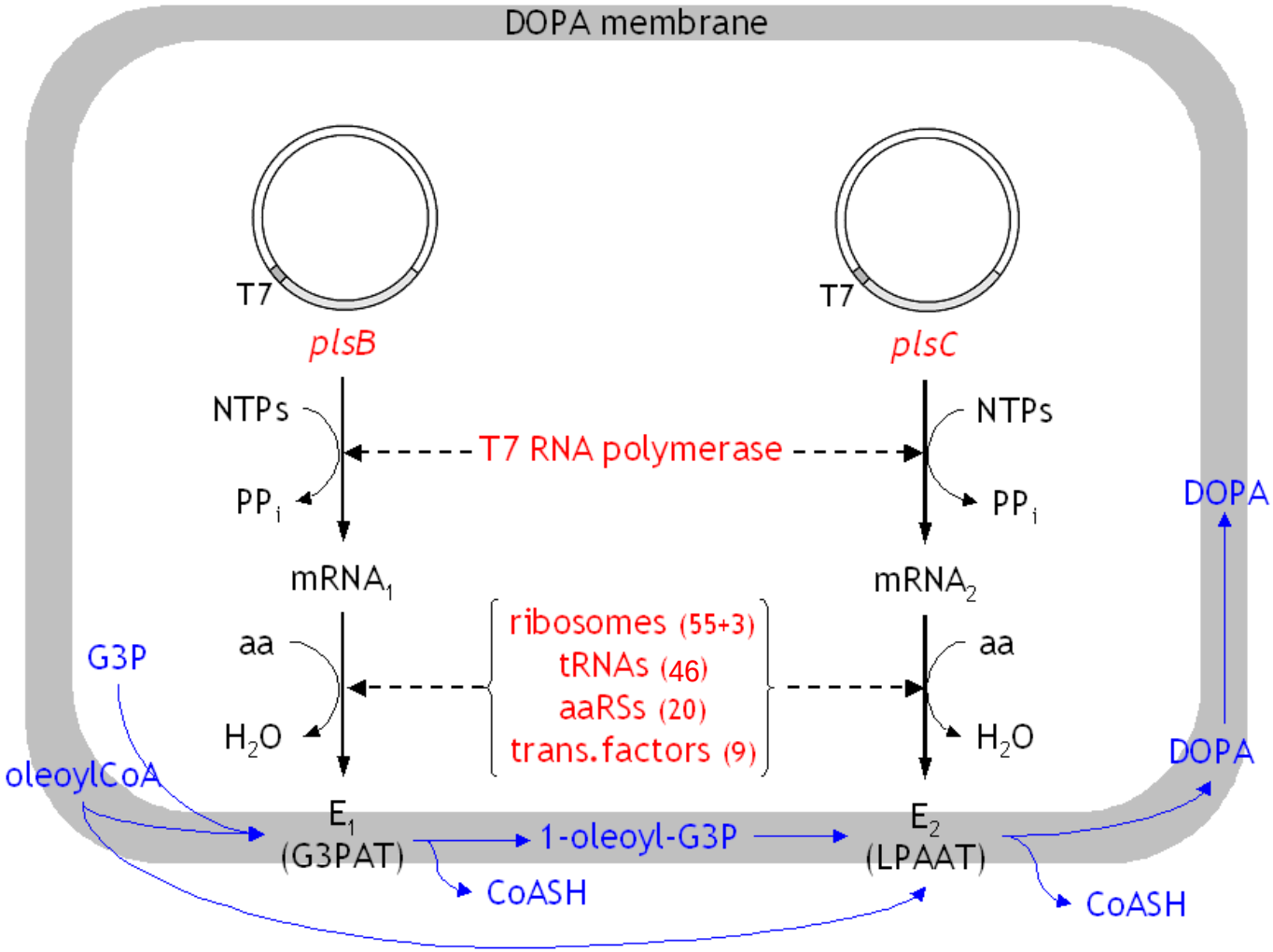
Enzymatic synthesis of the lipids from within and autopoietic growth.

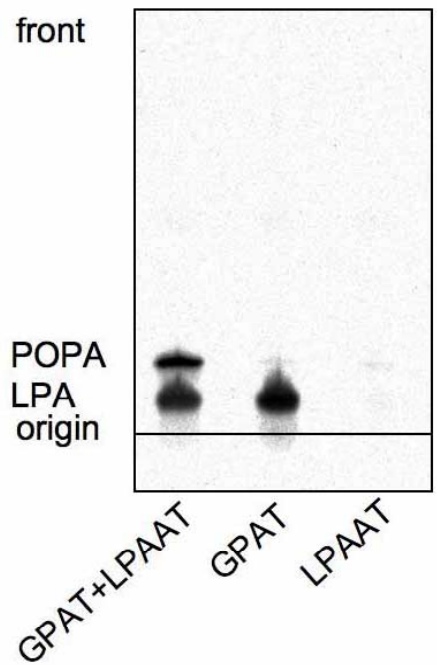
Glycerophospholipids metabolism



KEGG database

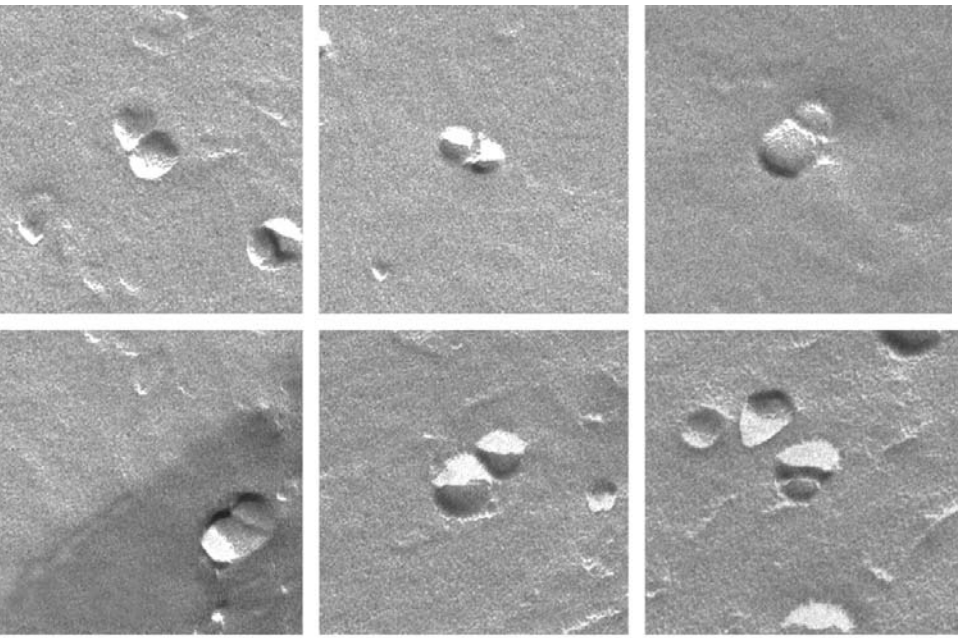
Enzymatic production of lipids

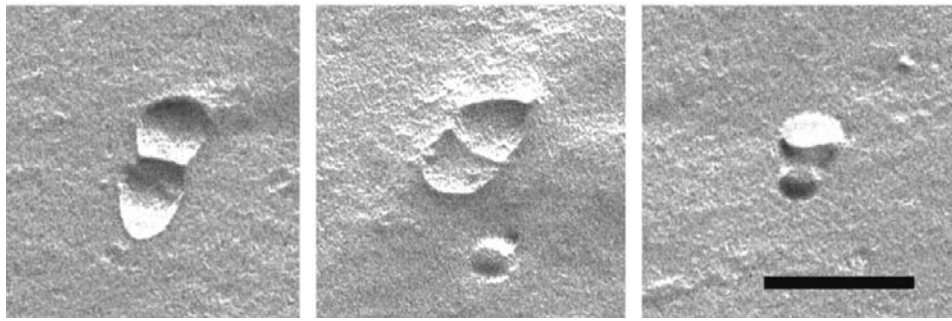




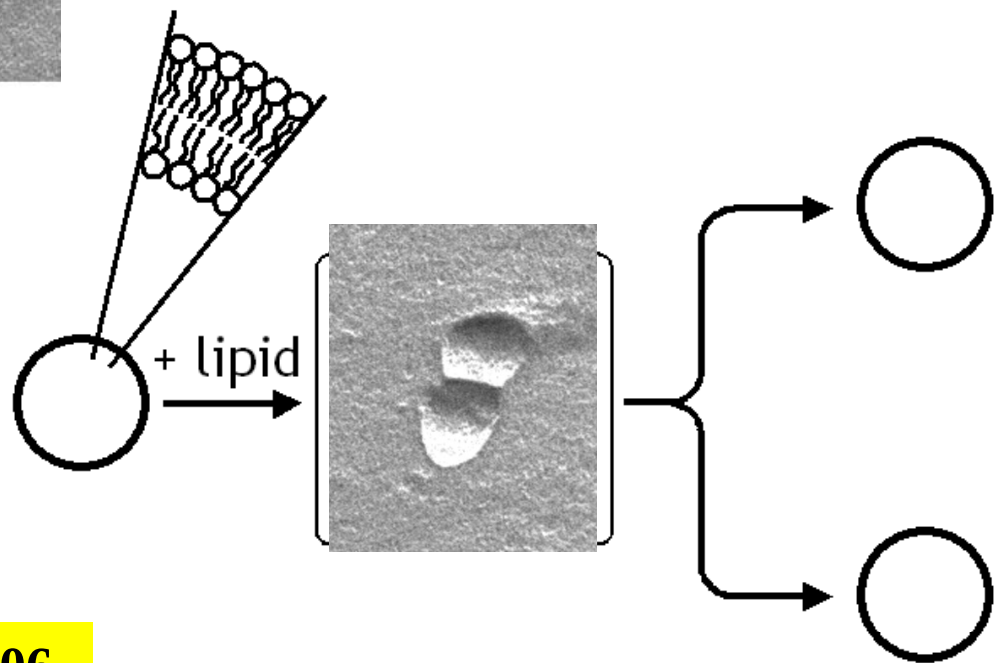
Kuruma, Stano et al., BBA 2008

Self-reproduction of oleate vesicles





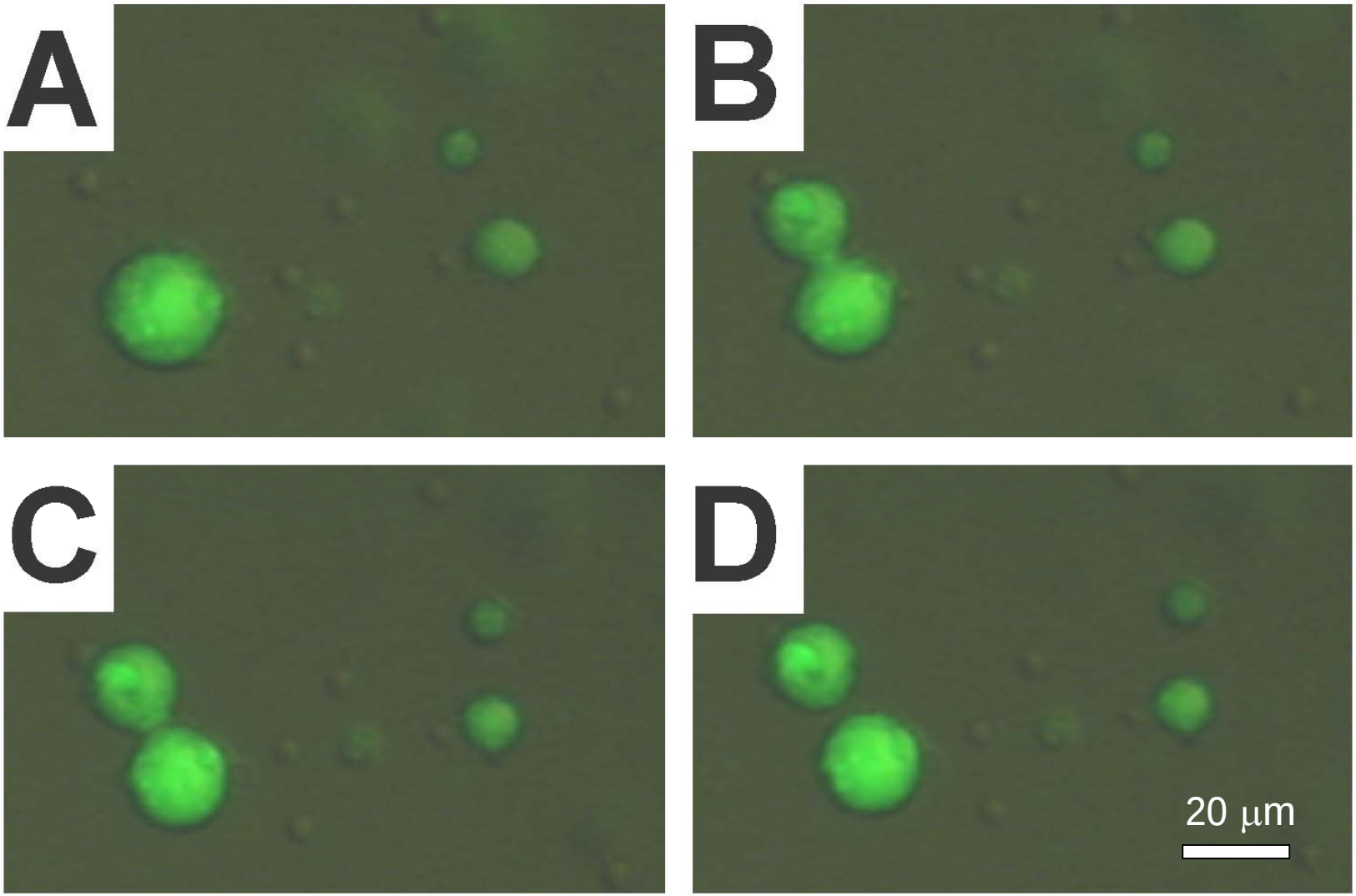
200 nm



See also: the “matrix effect”

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

Self-reproduction of w/o droplets

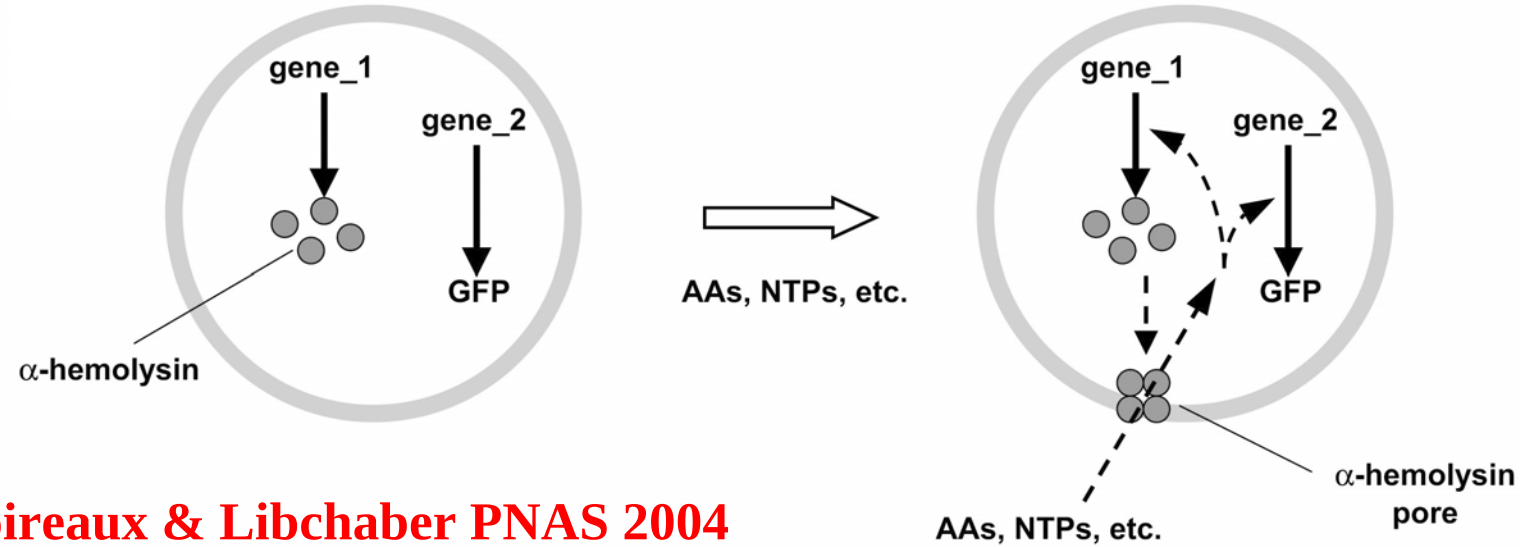


Time interval: 3 s

Fiordemondo and Stano, ChemBioChem 2007

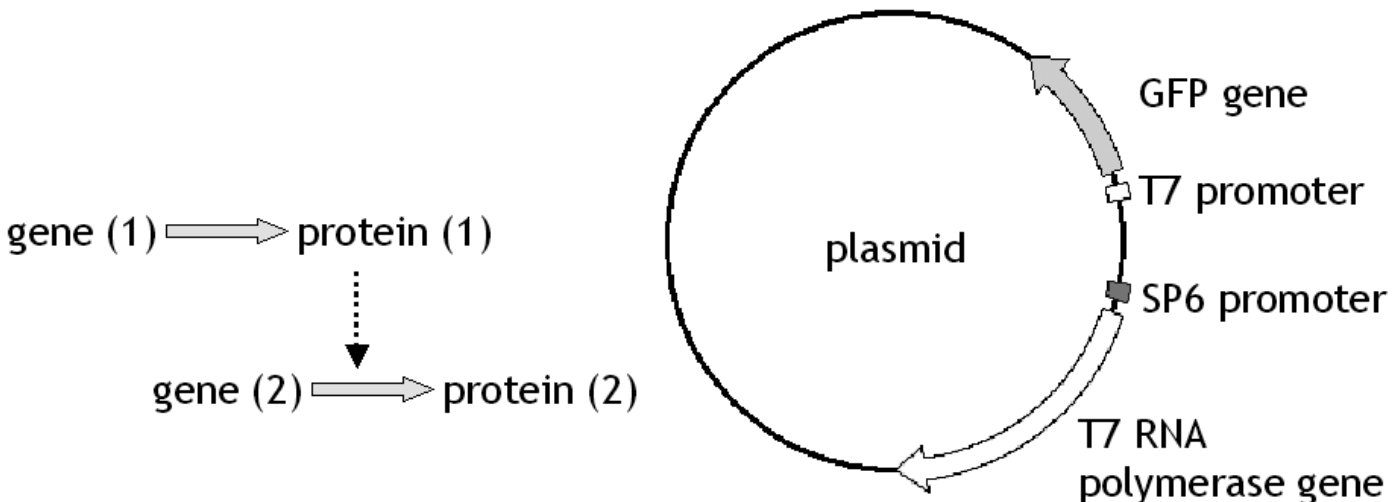
THREE IMPORTANT STUDIES HAVE TO BE MENTIONED

1



Noireaux & Libchaber PNAS 2004

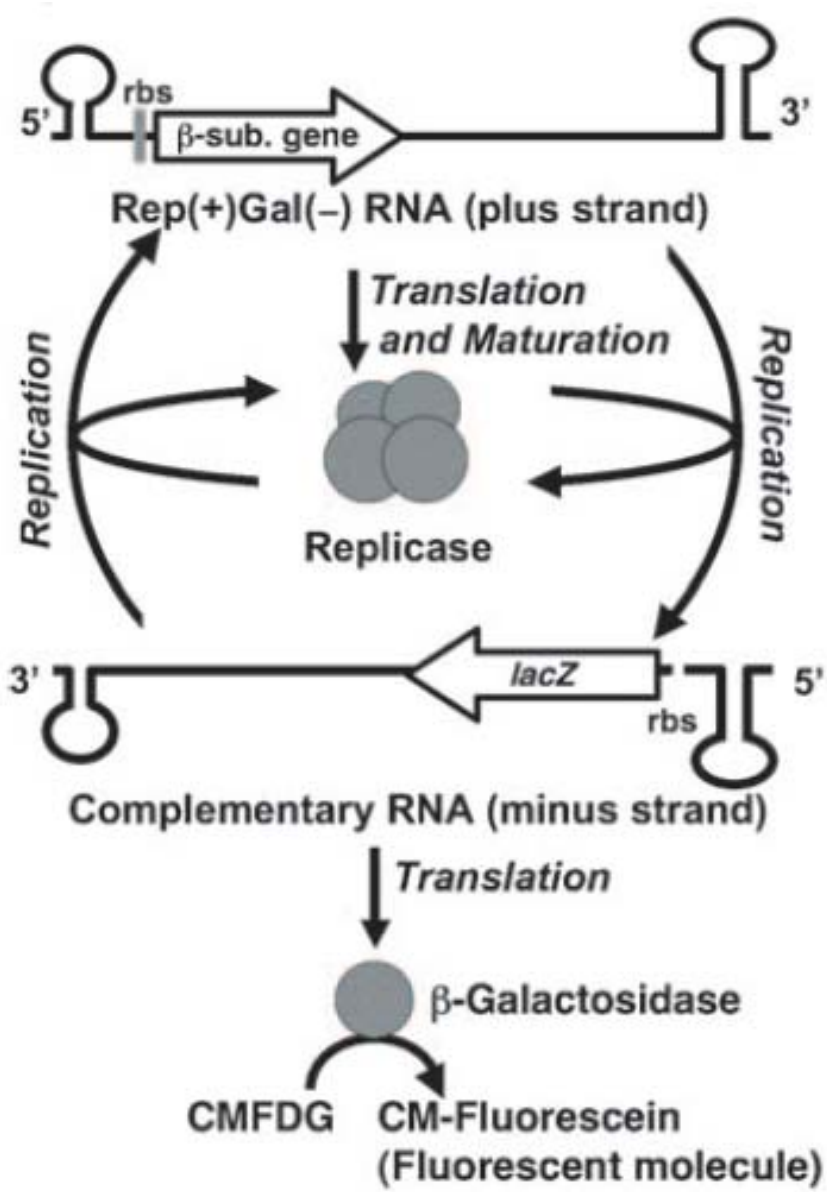
2



Ichikawa et al (Yomo) FEBS Lett. 2004

THREE IMPORTANT STUDIES HAVE TO BE MENTIONED

3



Kita et al (Yomo)
ChemBioChem 2008