

# Cascade Dynamics Observed in Self-division of Giant Vesicle Containing Amplified DNA

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# Missing Link in History of Life

3.5 billion years

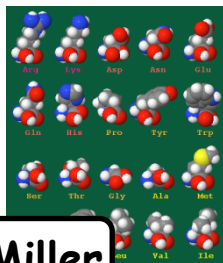
present



Inorganic Material

Amino Acid

Organic Molecule



S.L. Miller

Coacervate

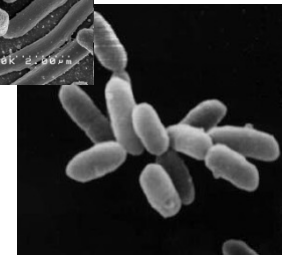


A. Oparin

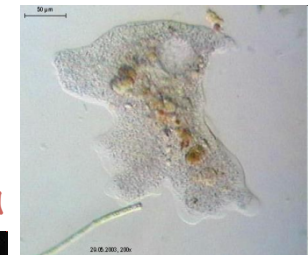
Prokaryote  
Bacteria



Archaea



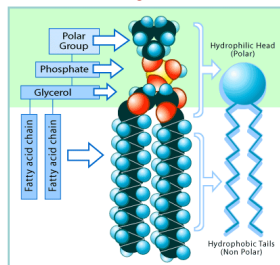
Eukaryote



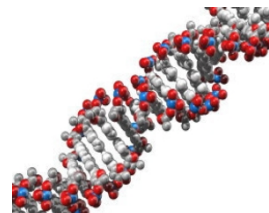
Ancient Earth



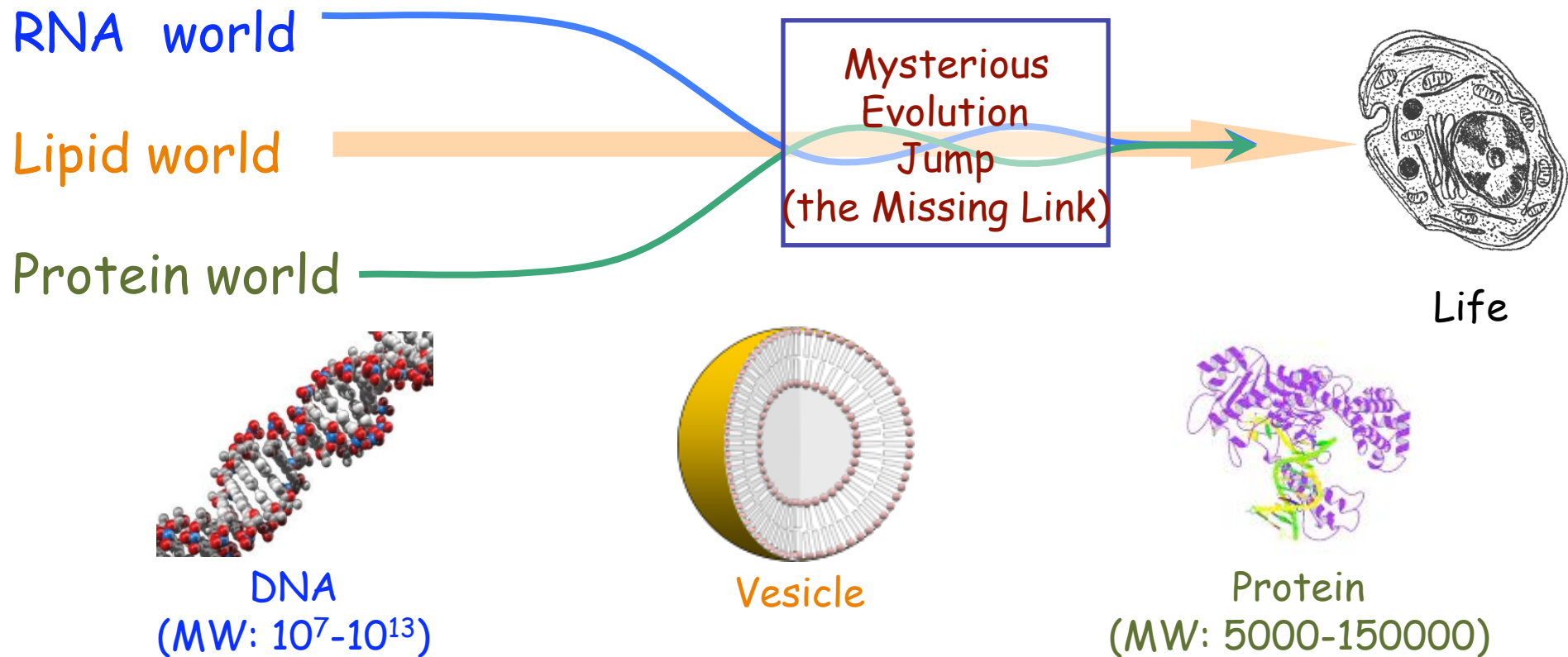
Lipid



RNA / DNA



# Search for the Missing Link

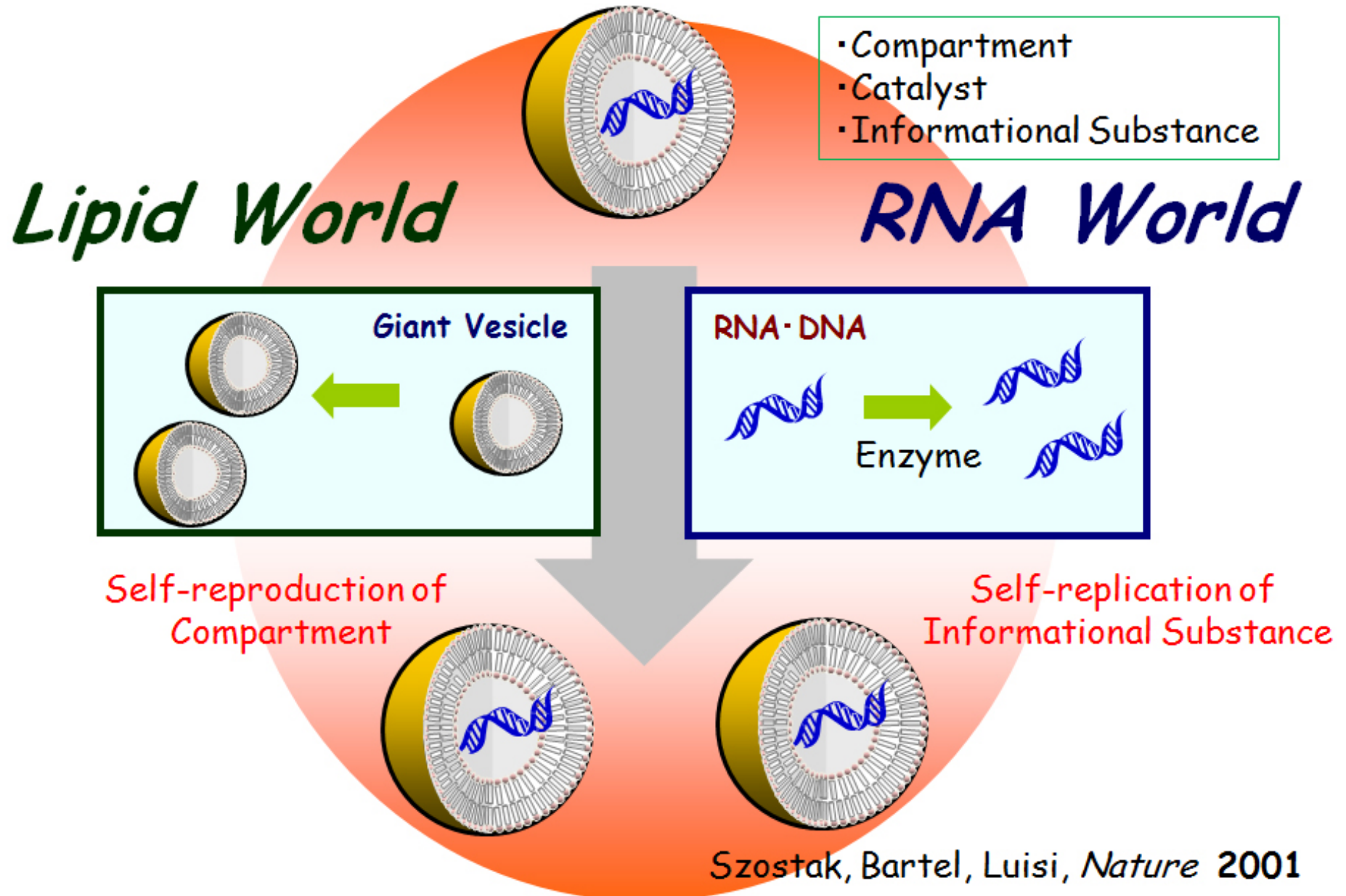


## Constructive Approach to Origin of Life

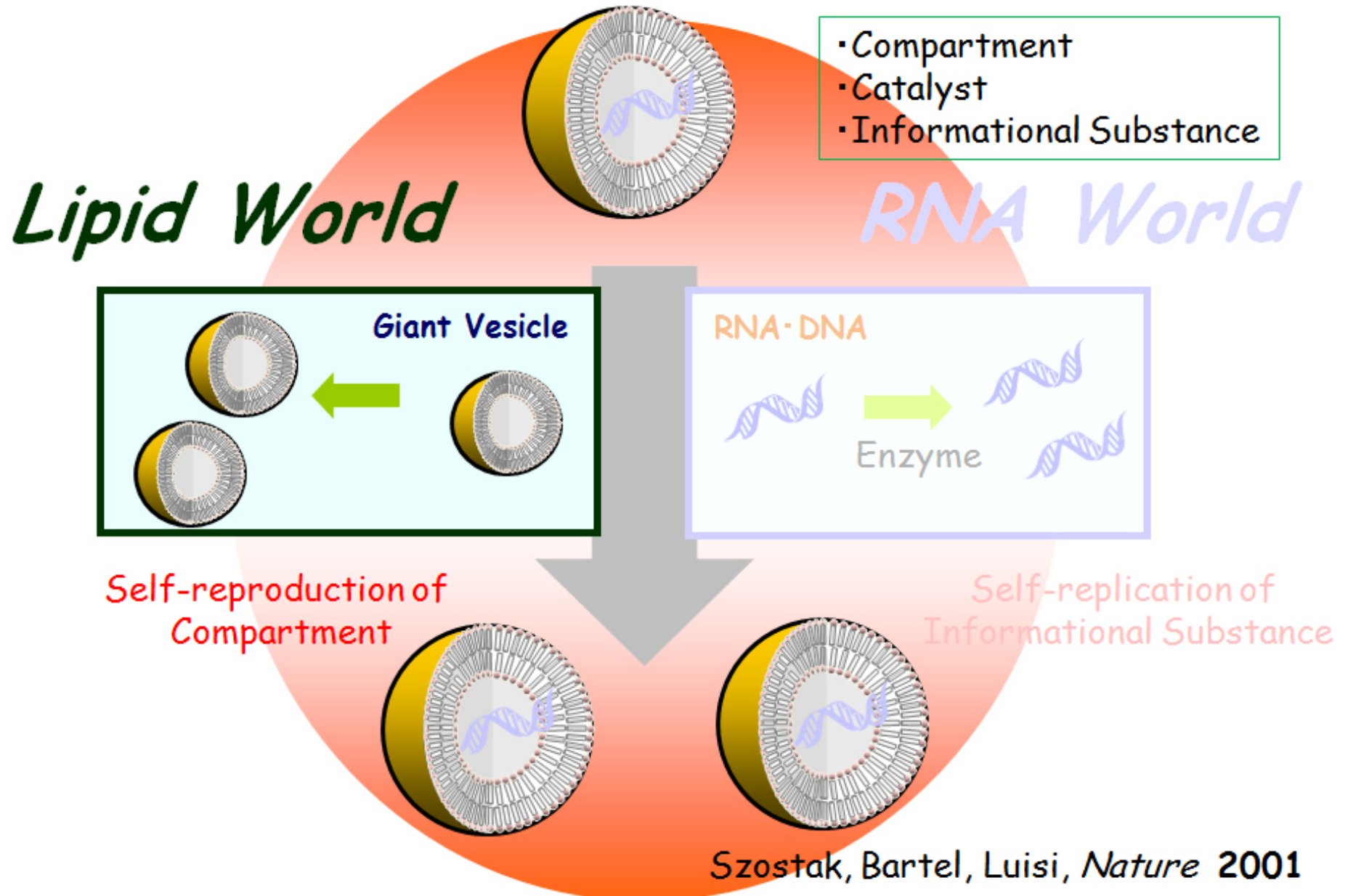
- Prebiotic : Construct a "Minimal Cell" using prebiotic materials
- Semi-synthetic : Reconstruct a "Minimal Cell" using purified biomaterials obtained from a living cell

"Synthesizing Life" : J. W. Szostak, D. P. Bartel, P. L. Luisi, *Nature* 2001

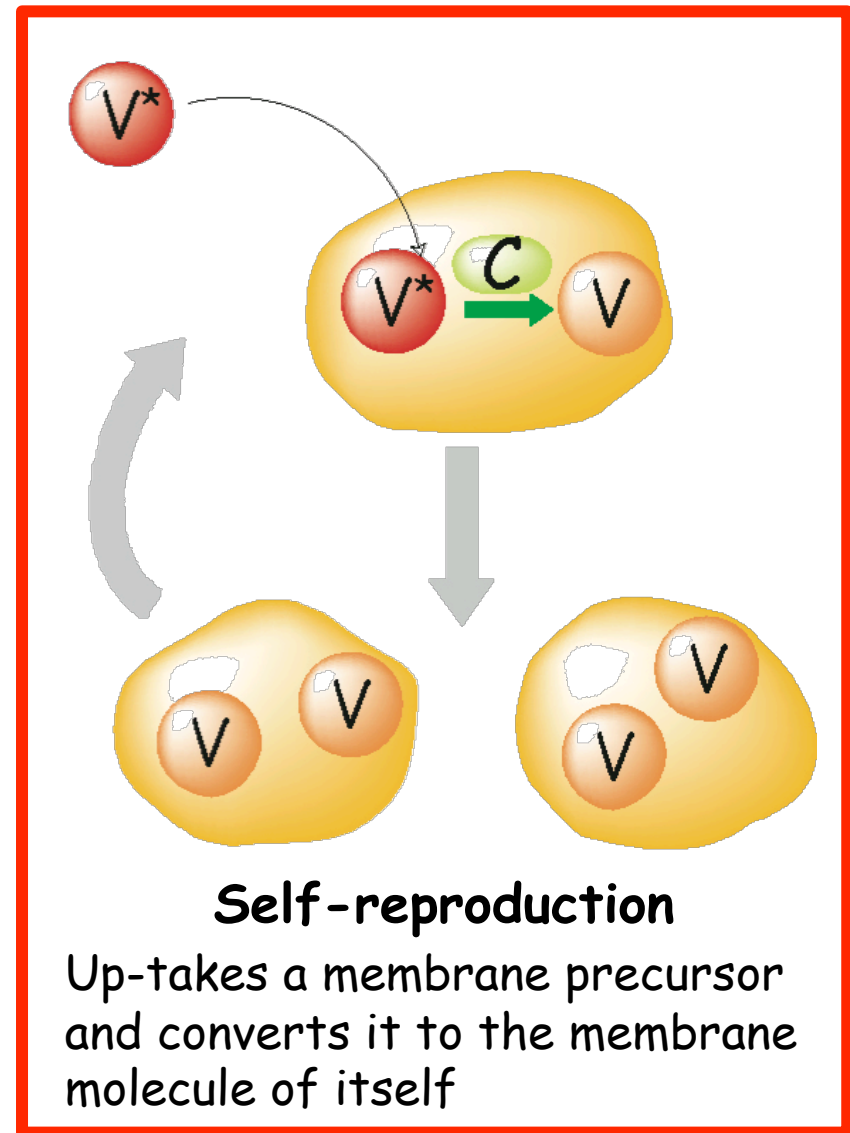
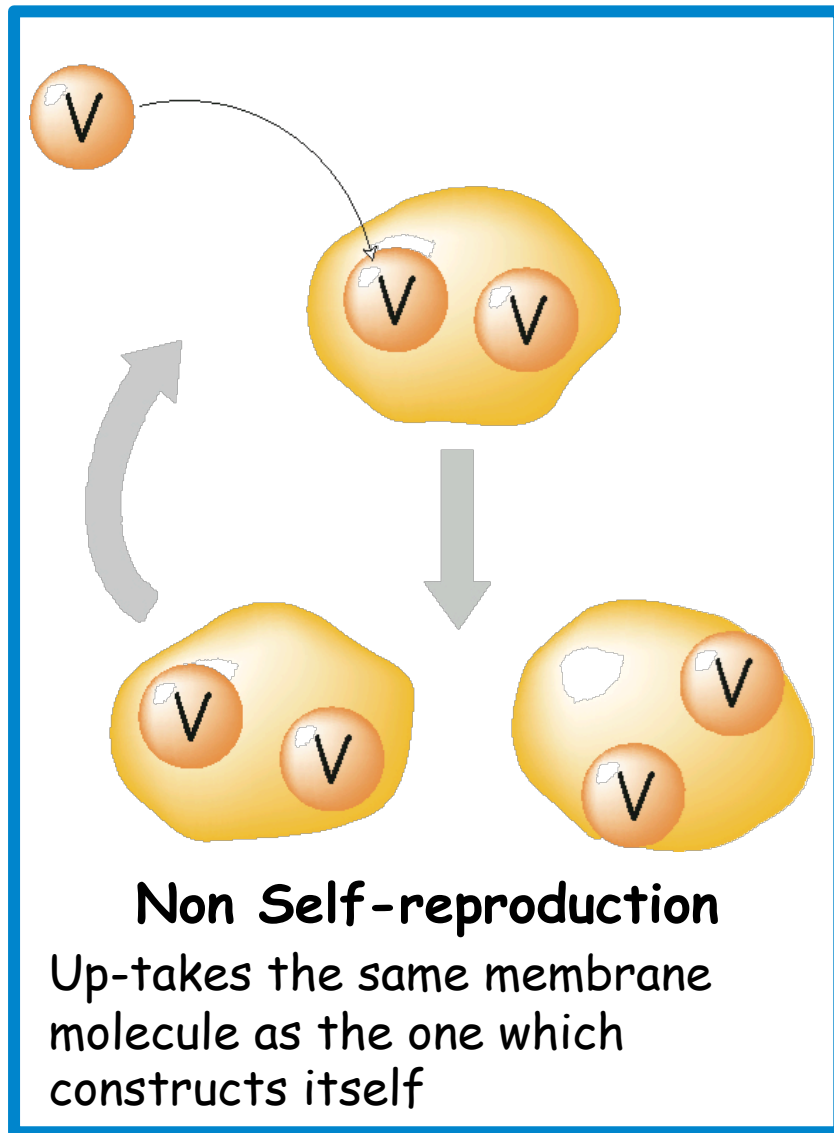
# Giant Vesicle-based Artificial Cell



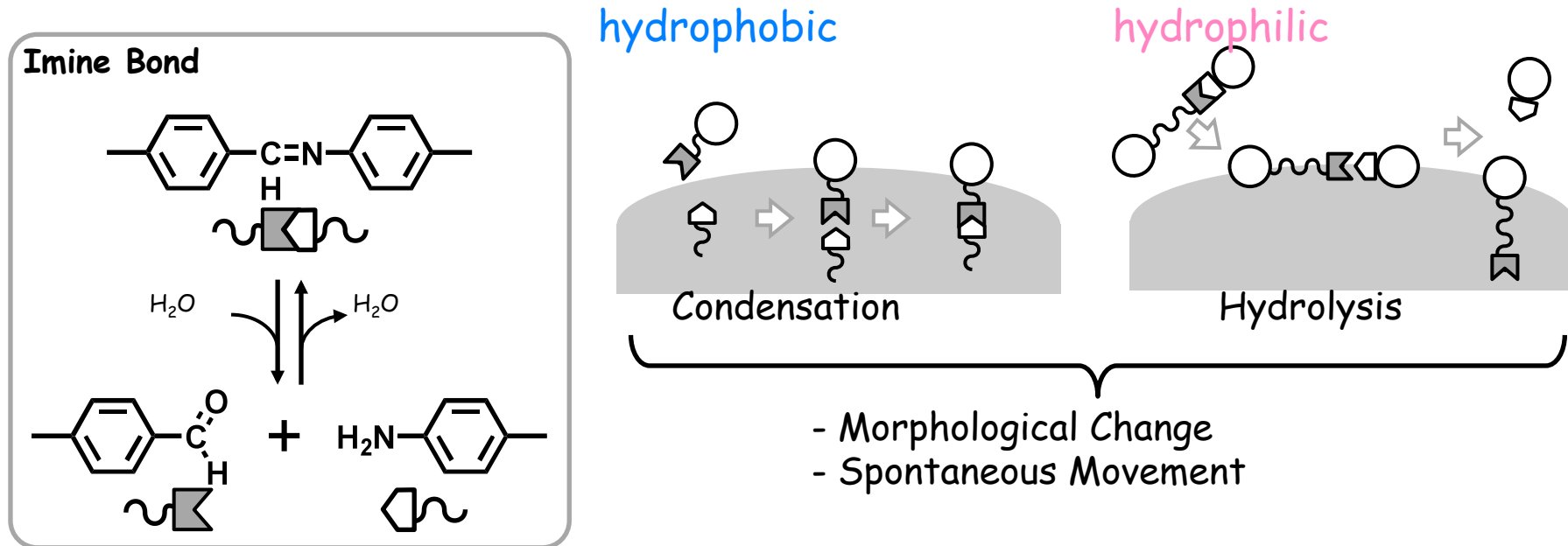
# Giant Vesicle-based Artificial Cell



# Self-reproduction and Non Self-reproduction

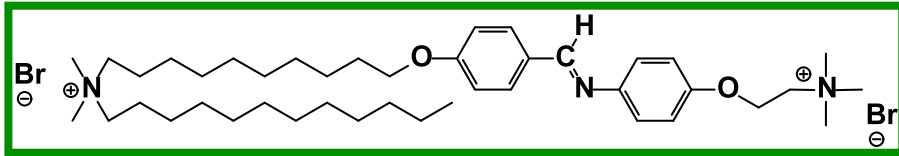


# Membrane Molecule with Imine Bond



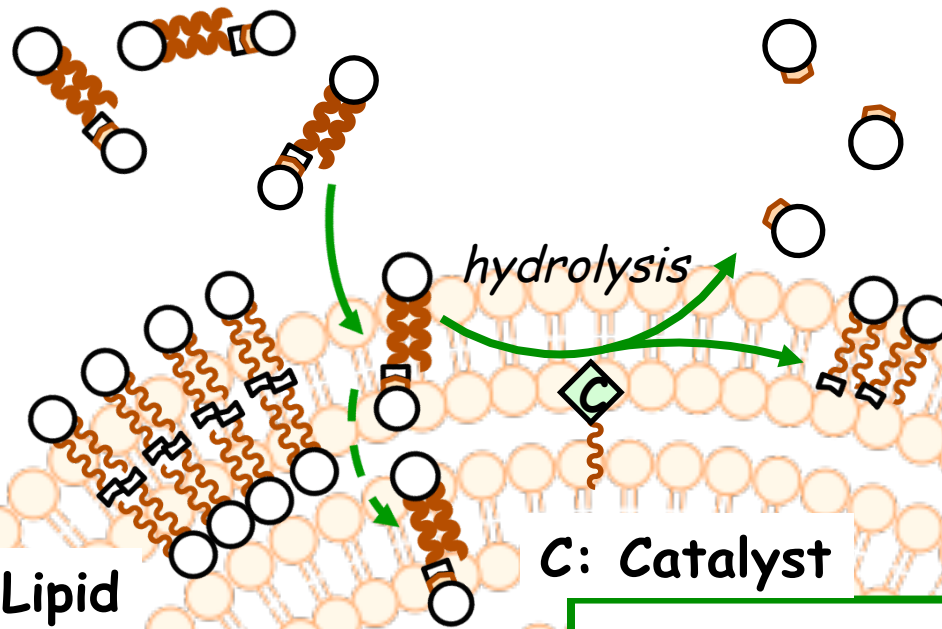
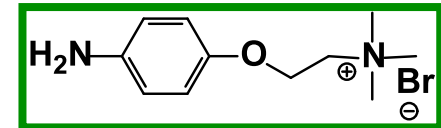
- Bond formation in **hydrophobic** environment
- Hydrolysis in **hydrophilic** environment

# Robust Self-reproducing Giant Vesicles



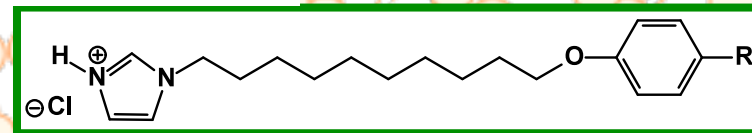
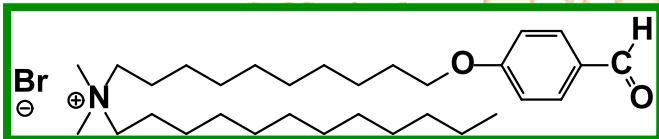
**V\*:** Bolaamphiphile

**E: Electrolyte**



**V: Lipid**

**C: Catalyst**

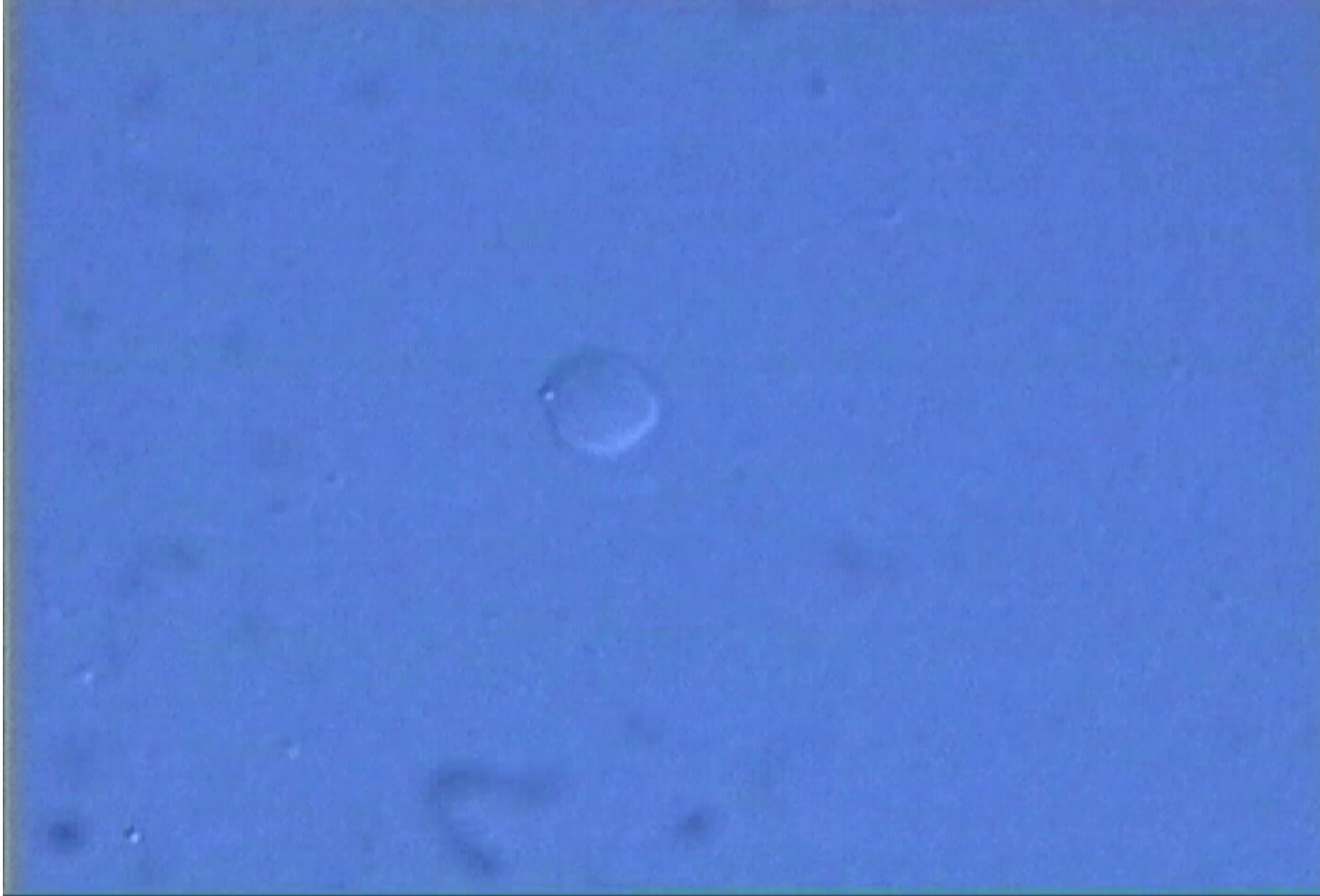


**Giant Multi Lamellar Vesicle**

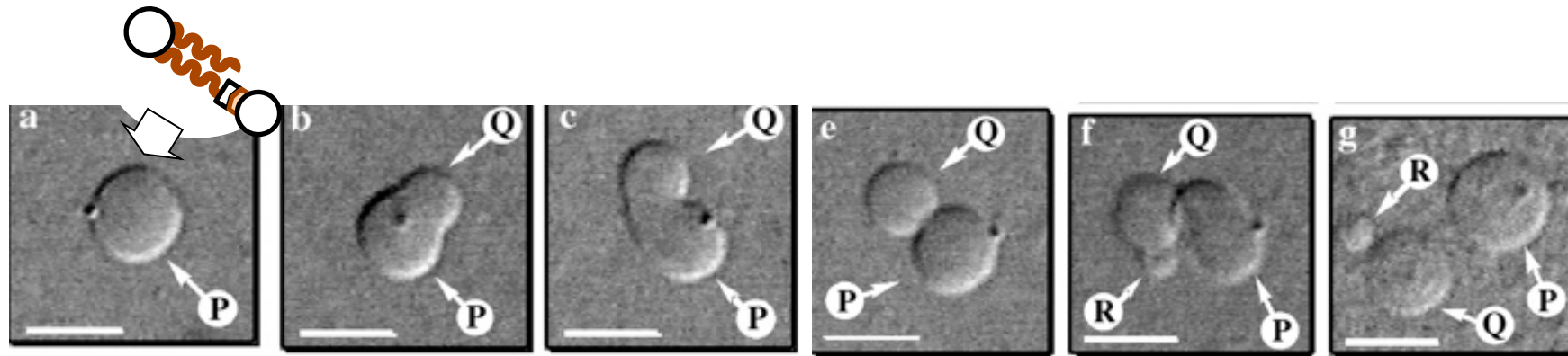
K. Takakura, *et al.*, *Langmuir* 20, 3832 (2004)



# Movie of Self-reproduction of Giant Vesicles



# Dynamics of Robust Self-reproducing Giant Vesicle



Plausible mechanism of quasi-equal division

Prof. T. Umeda (Kobe Univ.)

A GMV has the following energy:

$$E = \frac{k}{2} \sum_{i=1}^m \frac{(A_i - aN_i)^2}{aN_i}$$

where

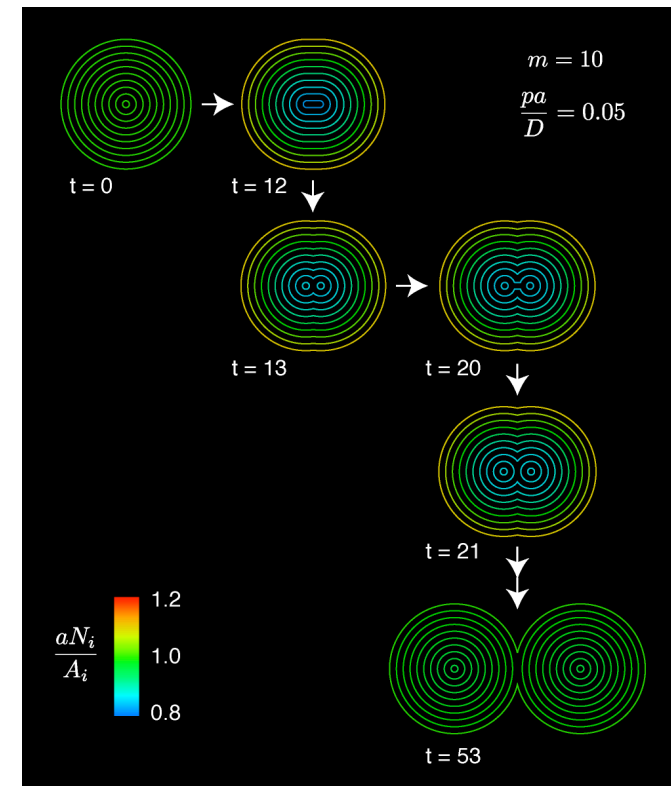
$m$ : number of lamellas

$A_i$ : area of the  $i$ -th lamella

$N_i$ : number of molecules in the  $i$ -th lamella

$k$ : elastic constant

$a$ : area per molecule



# Theoretical model of Self-reproducing Giant Vesicle

## Assumptions

1. A GMV has the following energy:

$$E = \frac{k}{2} \sum_{i=1}^m \frac{(A_i - aN_i)^2}{aN_i} \quad (1)$$

where

$m$ : number of lamellas

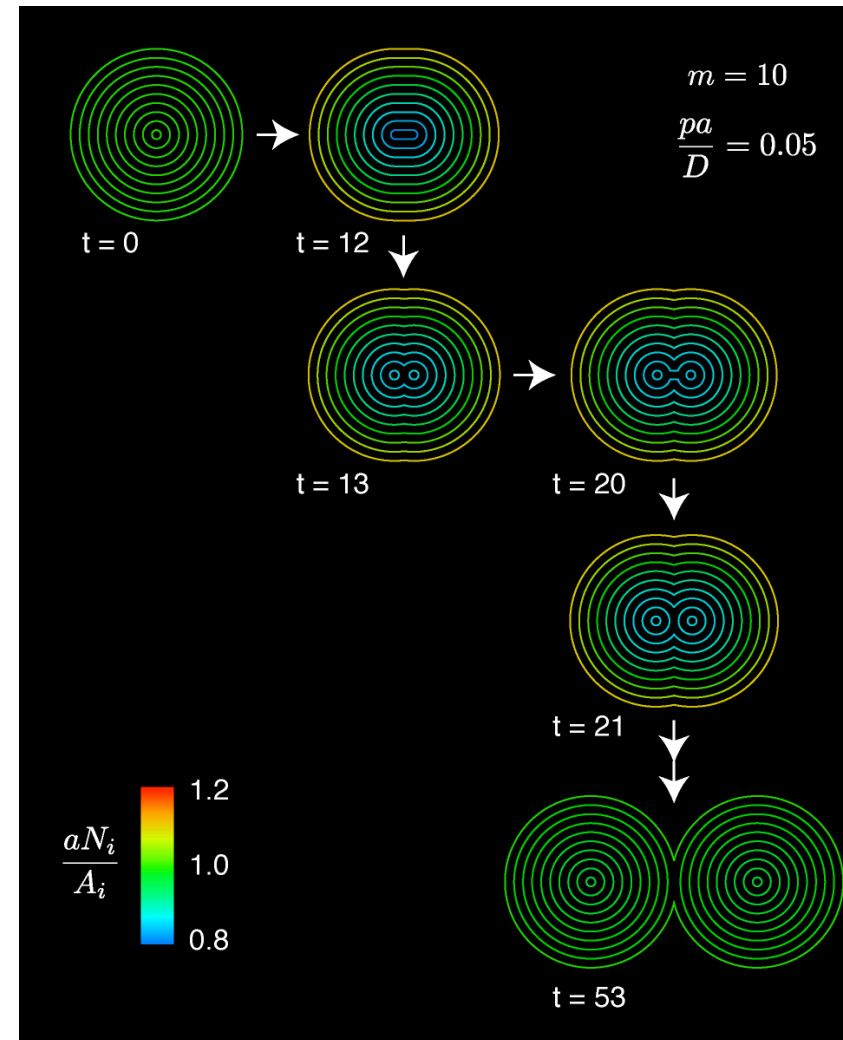
$A_i$ : area of the  $i$ -th lamella

$N_i$ : number of molecules in the  $i$ -th lamella

$k$ : elastic constant

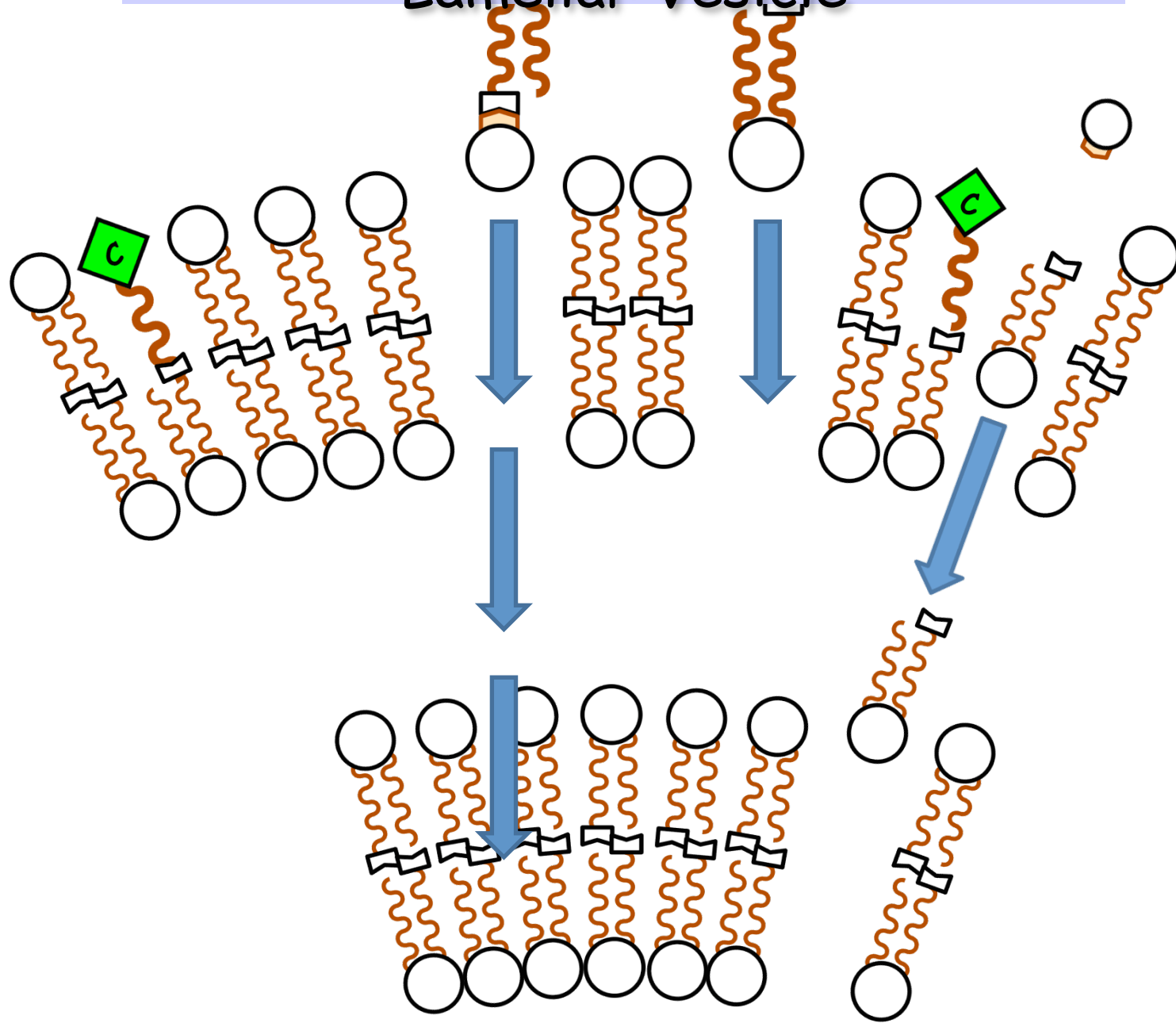
$a$ : area per molecule

2. Amphiphiles are added to the surface of the GMV at a constant rate ( $p$ ) per area, and diffuse into inner lamellas.
3. The distance between two adjacent lamellas is fixed when the shape of the GMV changes.
4. Energy minimization determines the direction of the shape and topology changes of the GMV.



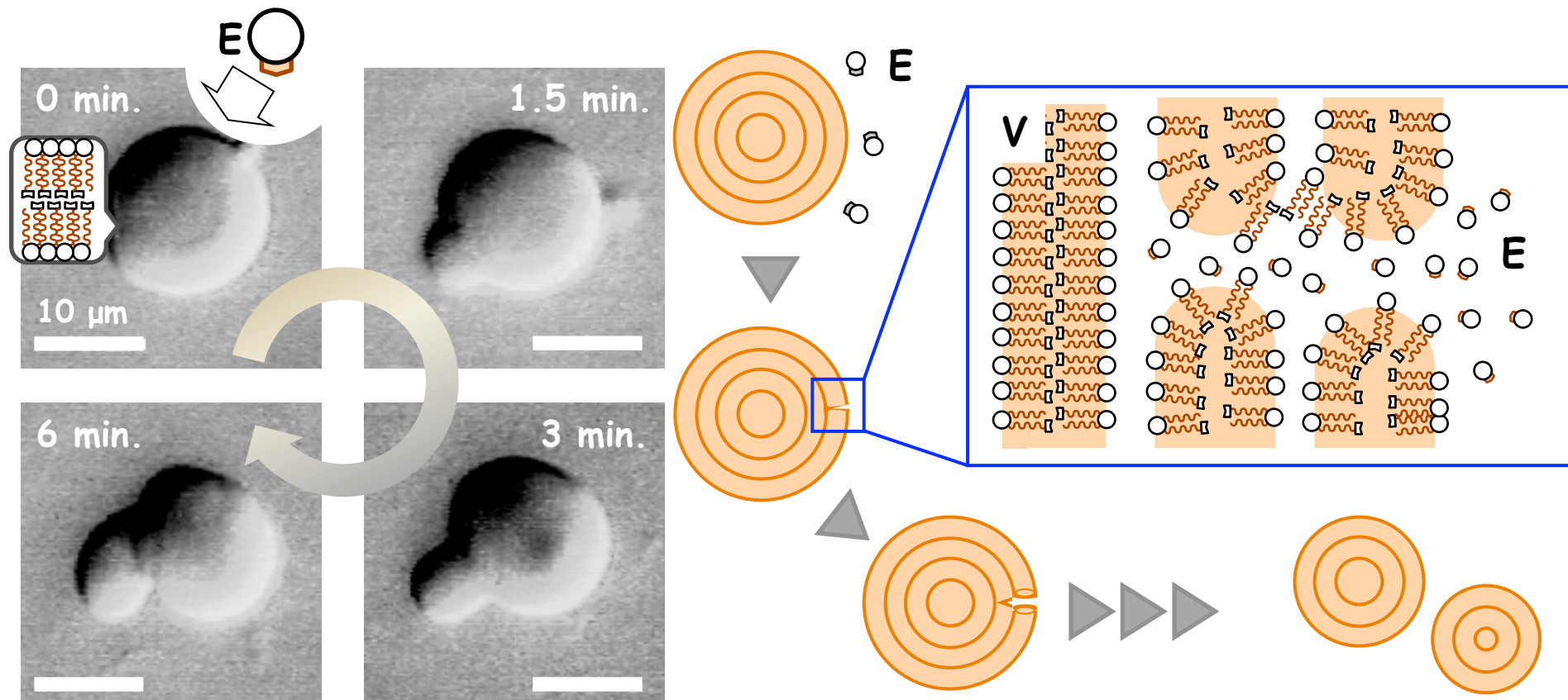
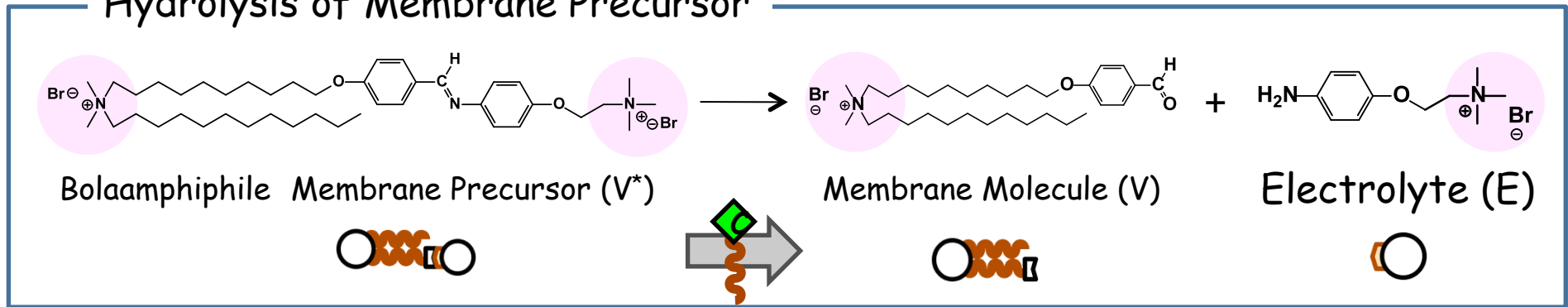
Movie

# Intrusion of Membrane Precursor into Giant Multi-Lamellar Vesicle



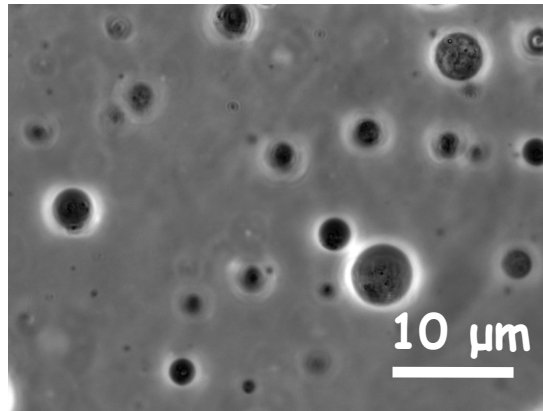
# Role of "E" in Self-division

## Hydrolysis of Membrane Precursor

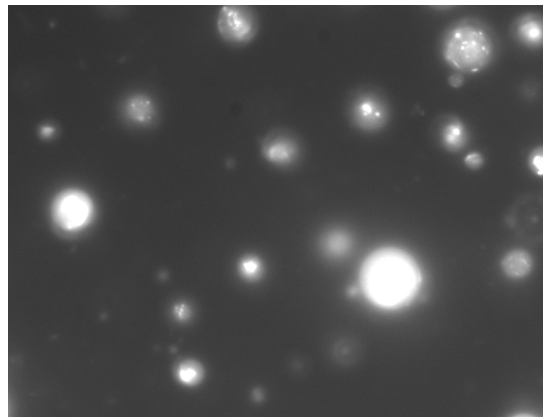
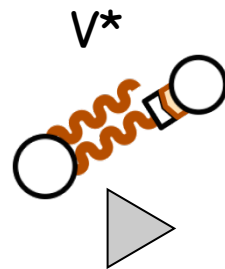
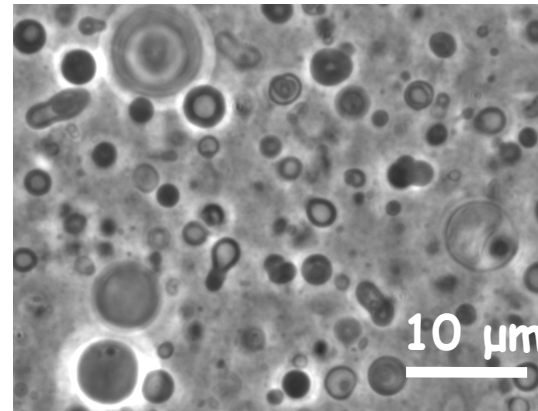


# A Large Number of GVs are Self-reproducing!

Initial Sample



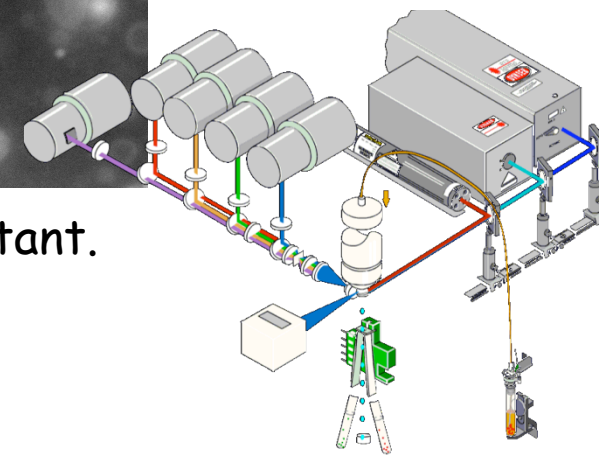
After self-reproduction



Mass-scale analysis of self-reproducing GVs is important.

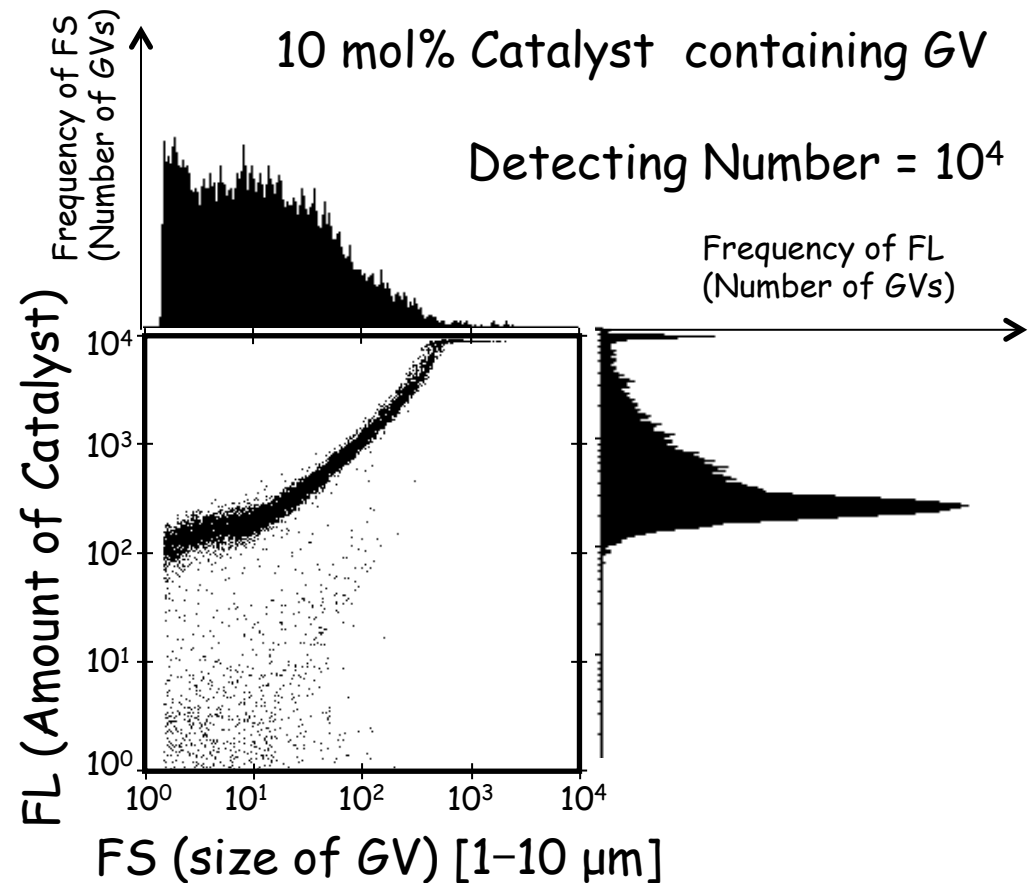
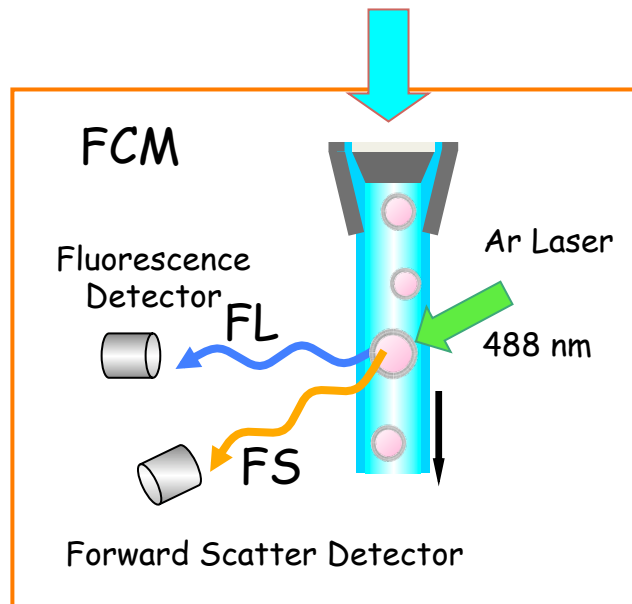
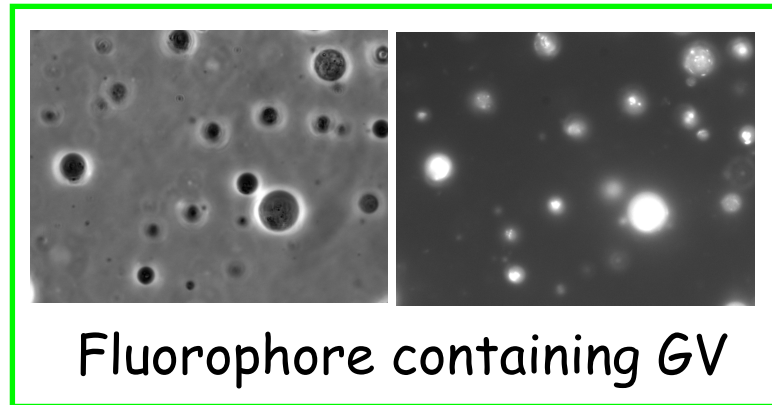


FCM : Flow Cytometry



Flow Cytometer

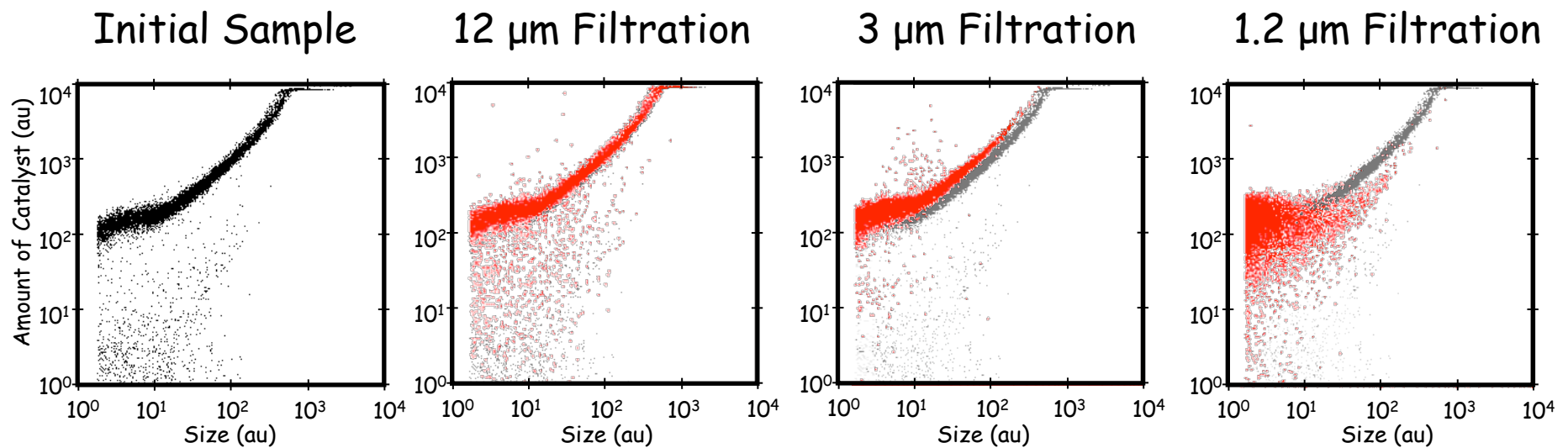
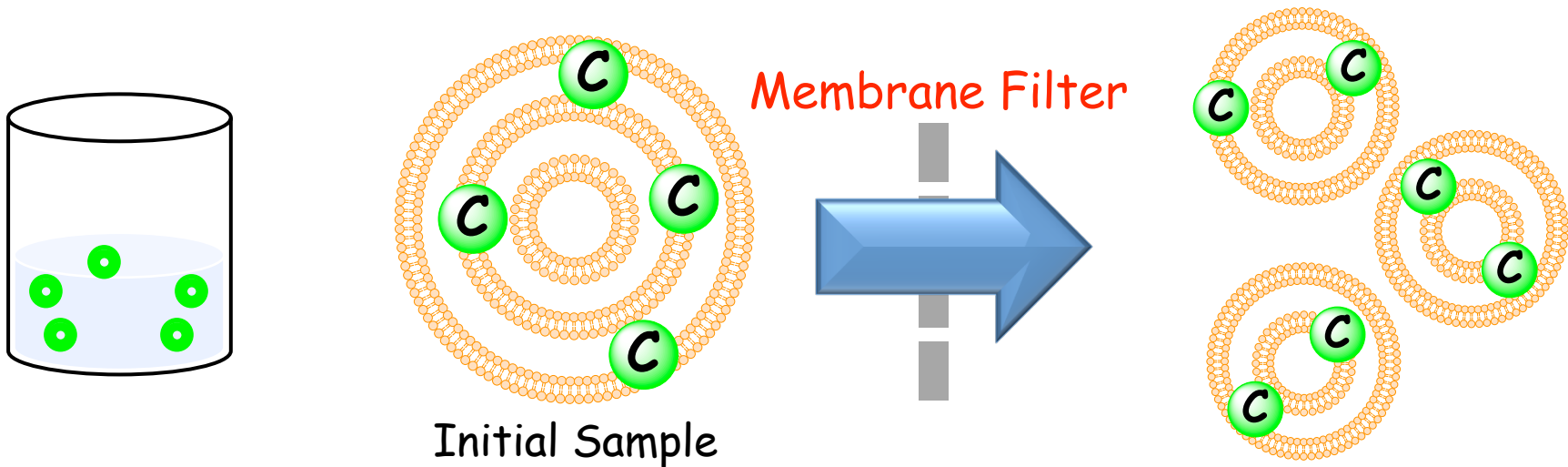
# FCM Analysis on Self-reproducing GVs



FS: Size of GV, FL: Florescence intensity of fluorophore per GV

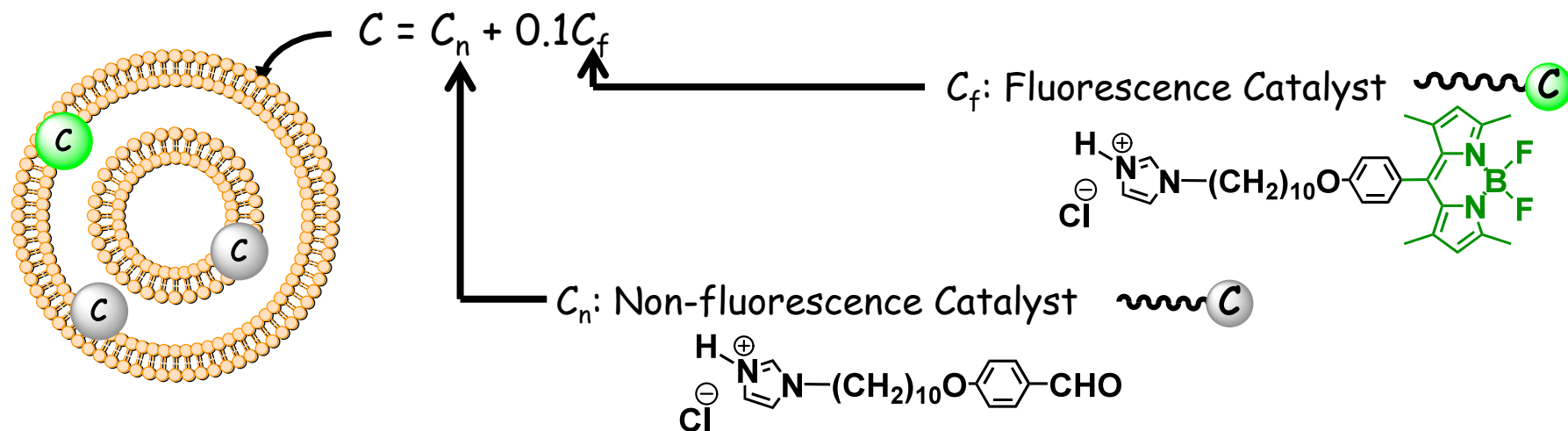


# Size-Calibration of GV<sub>s</sub> by Filtration

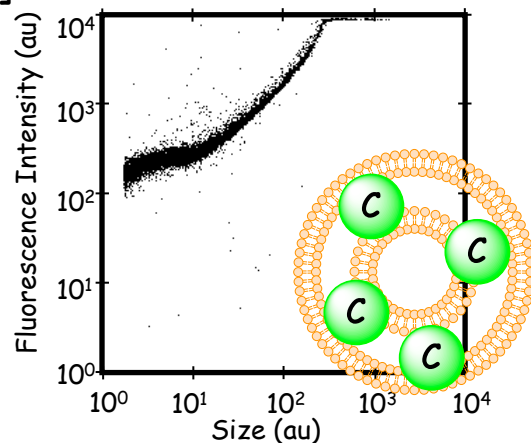


Particle Size Distribution: 0.2 - 1.0  $\mu\text{m}$   
(DLS analysis)

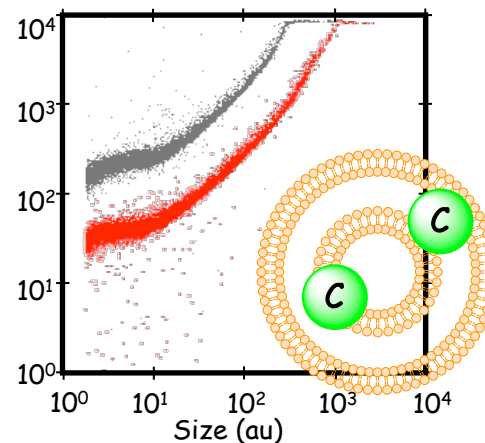
# Calibration of Amounts of Catalysts



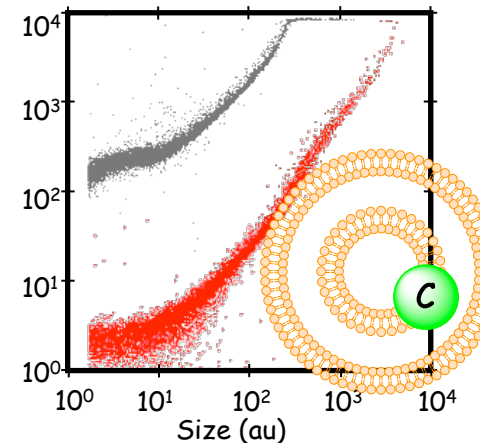
$[C_f]$  @GV = 1 mol%



0.1 mol%



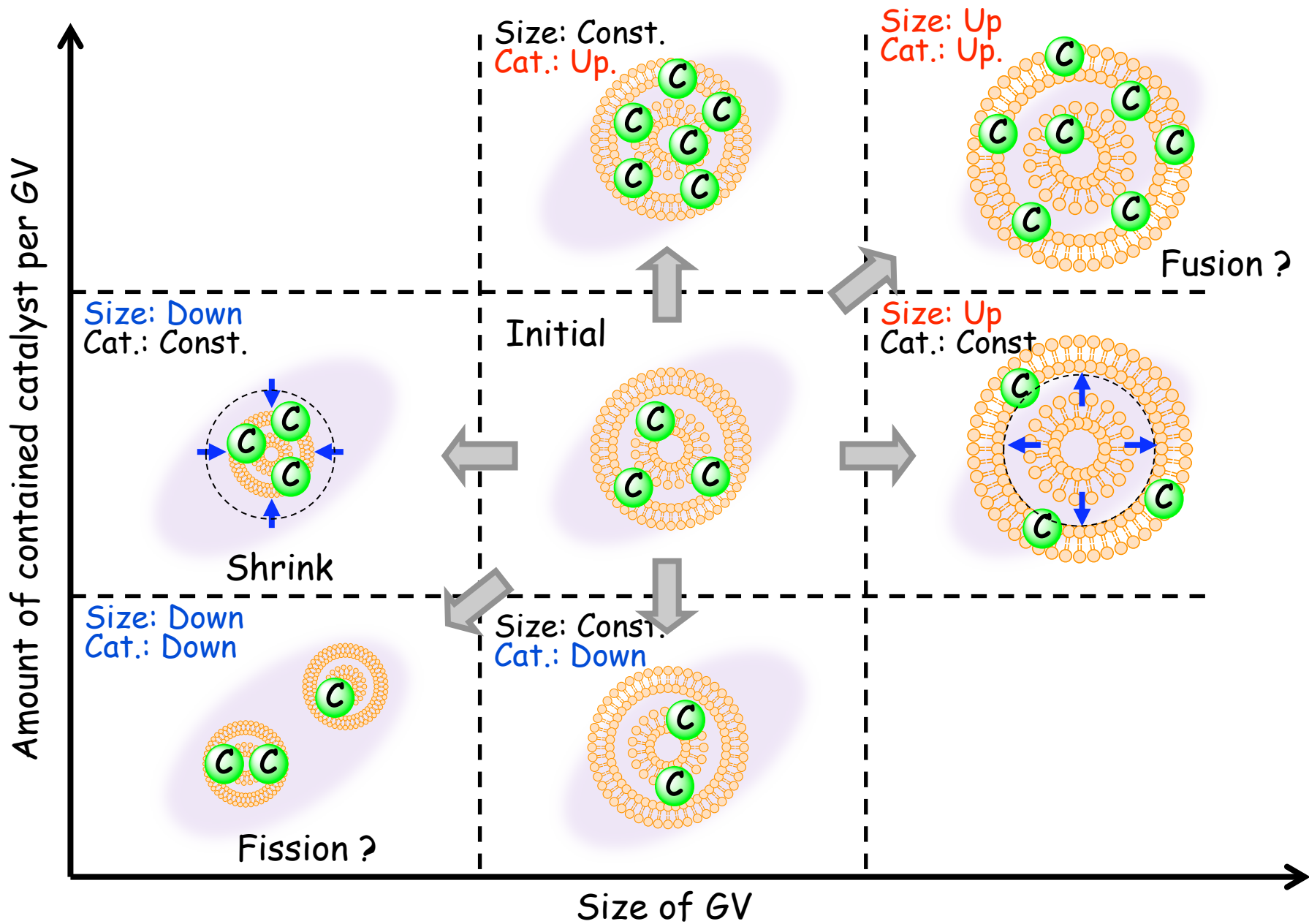
0.01 mol%



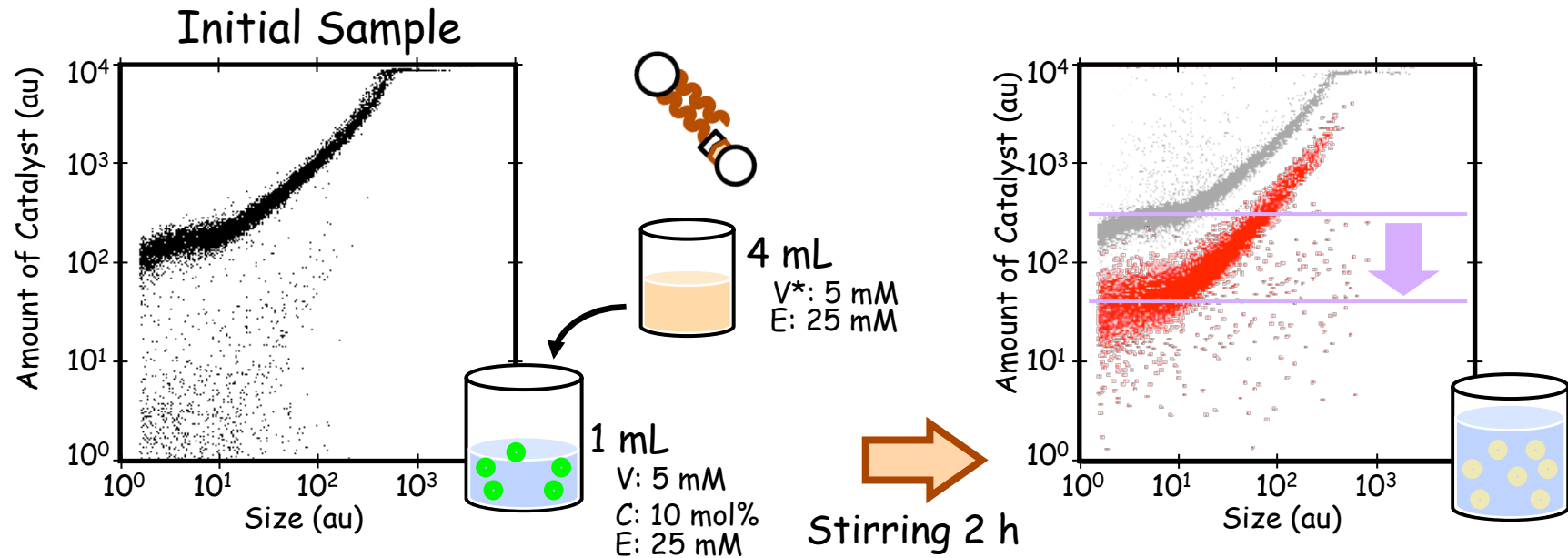
Distribution of fluorescence intensity depends on the amounts of  $C_f$ .

Distribution of size dose *not* depend on the amounts of  $C_f$ .

# How to read 2D plot



# Self-reproduction after 1<sup>st</sup> Feeding

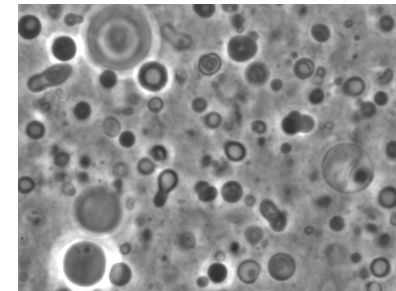
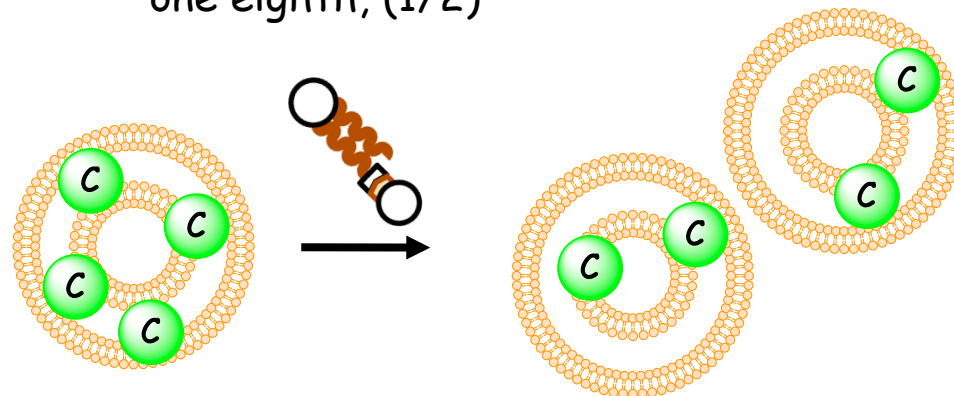
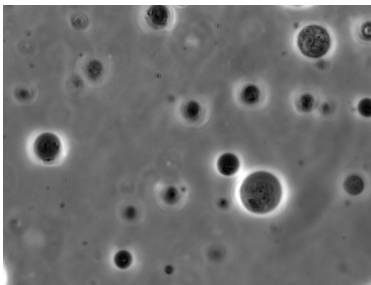


Distribution of size:

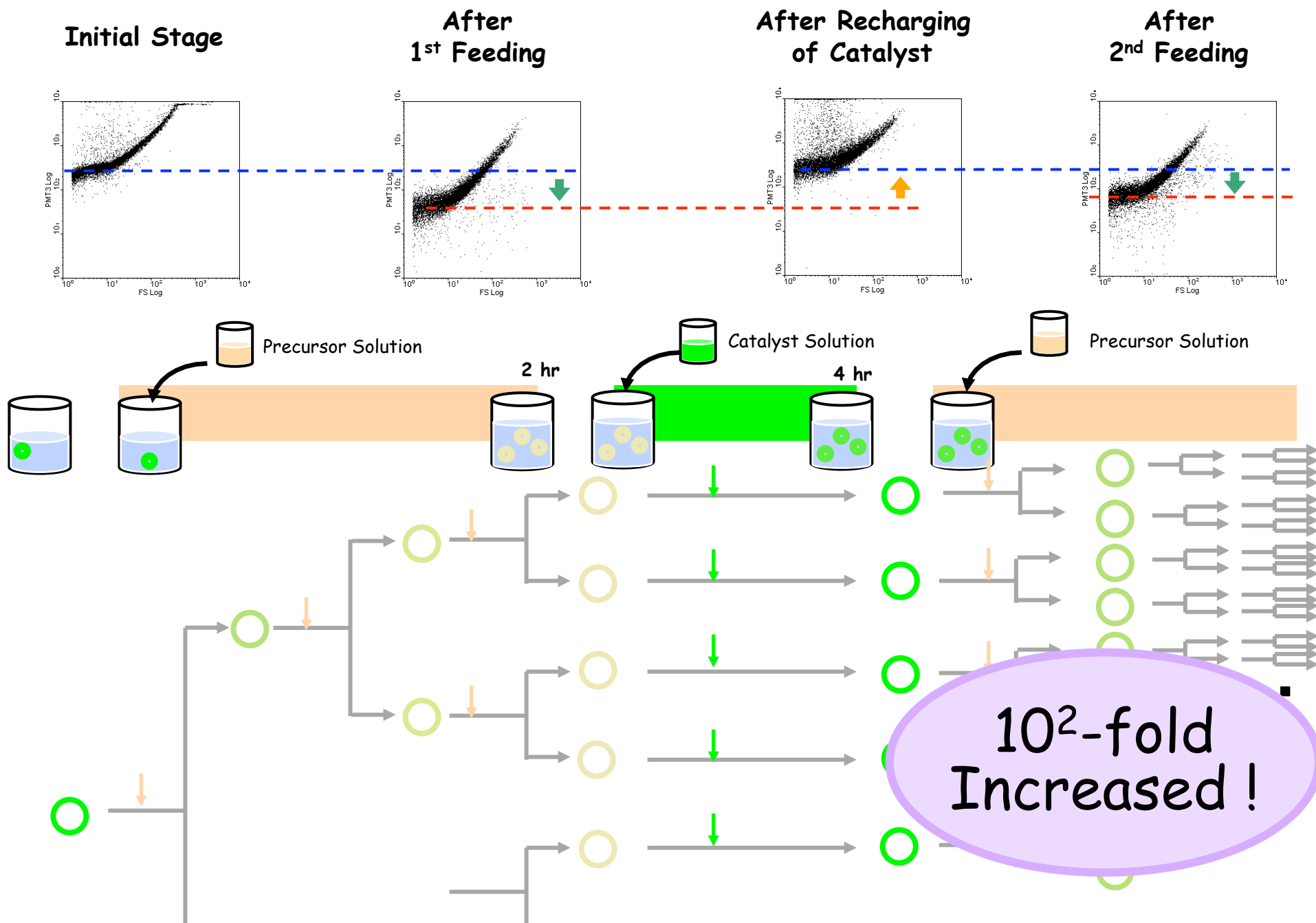
almost the same but larger part disappeared

Distribution of catalyst:

one eighth,  $(1/2)^3$

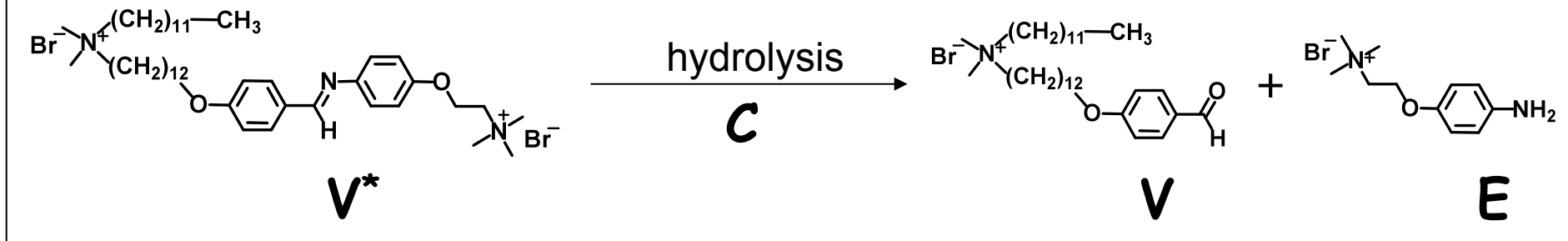


# Robustly Self-reproducing GV is Realized!

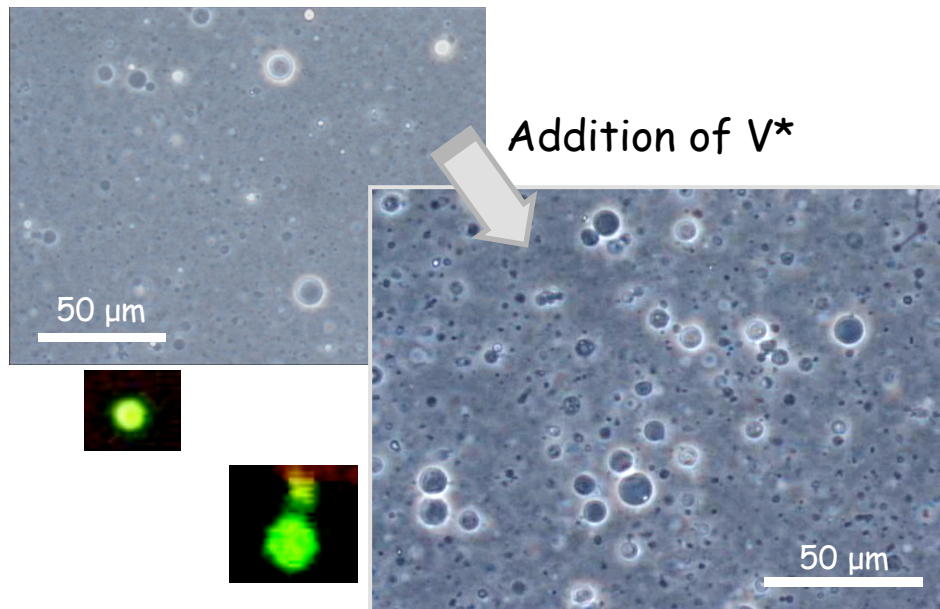


# Construction of Self-reproducing GVs Tolerant to a Highly Ionic Medium

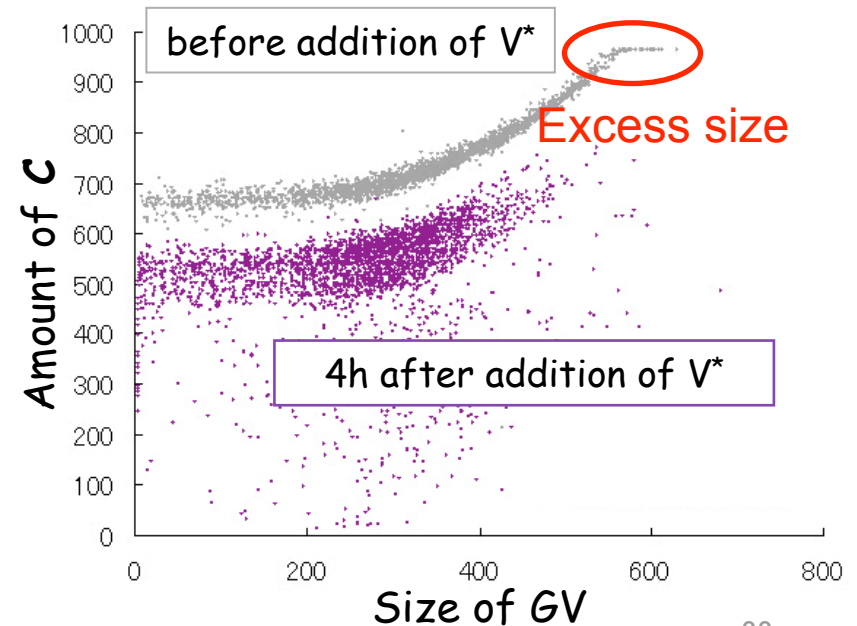
Hydrolysis of membrane precursor  $V^*$



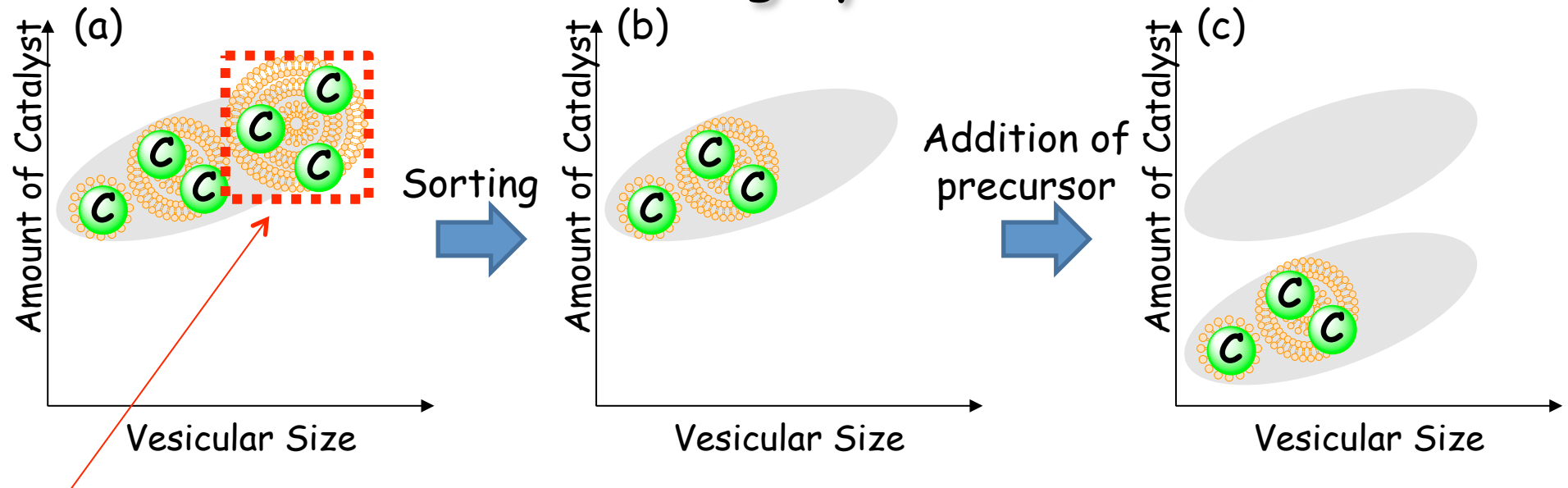
phase-contrast microscopic image



**Self-reproduced !!**

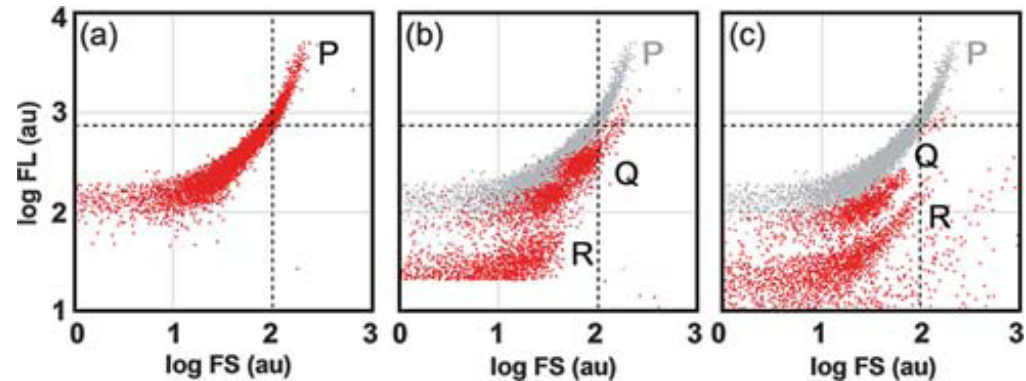


# Sorting of Self-reproducing GVs Tolerant to a Highly Ionic Medium



Irregular ensemble of giant vesicles with diameter of over 20  $\mu\text{m}$ , which was always arisen at swelling, was eliminated by sorting.

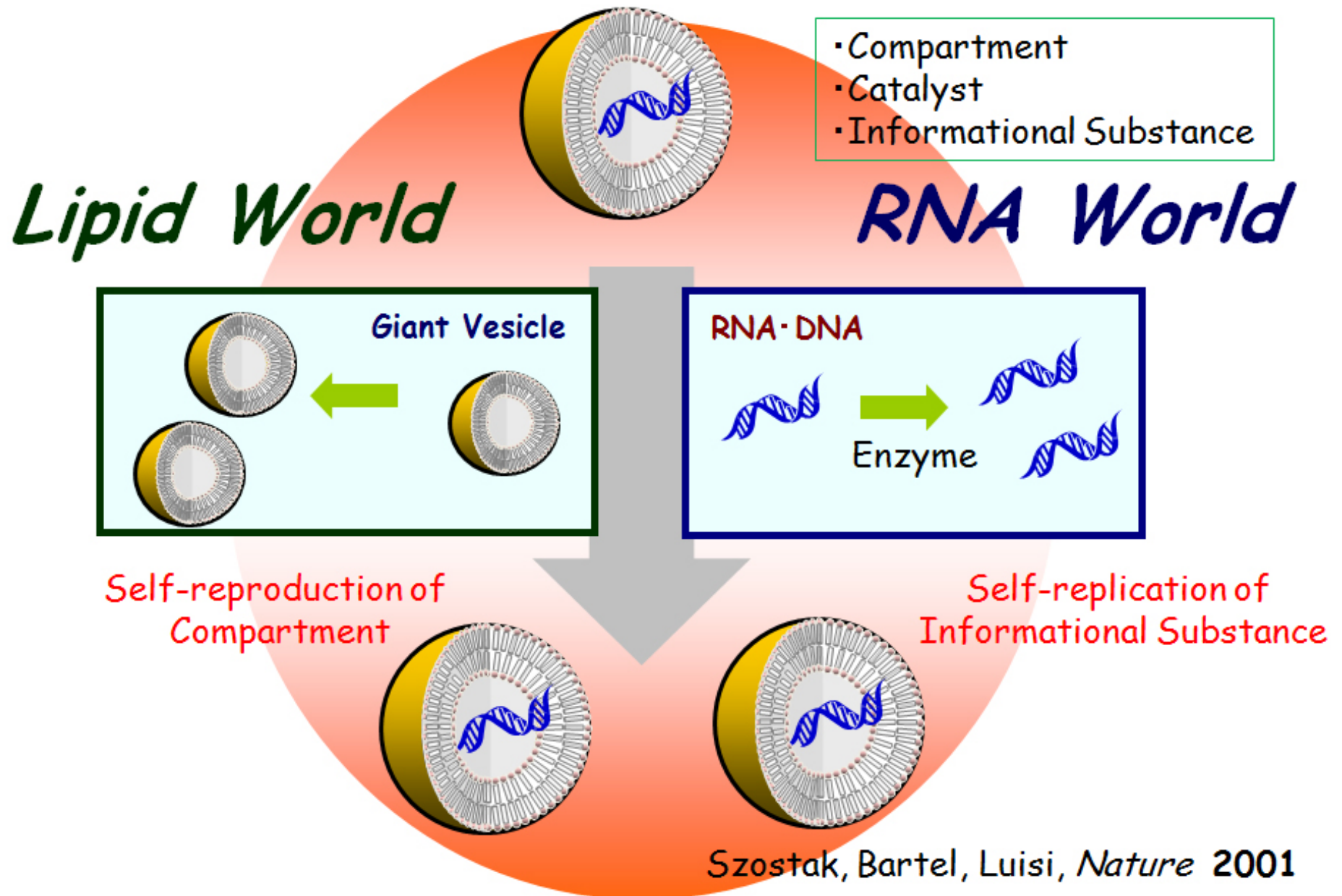
Distribution after addition of membrane precursor was discrete and similar to that of original one.



**New vesicular ensemble R grew !**

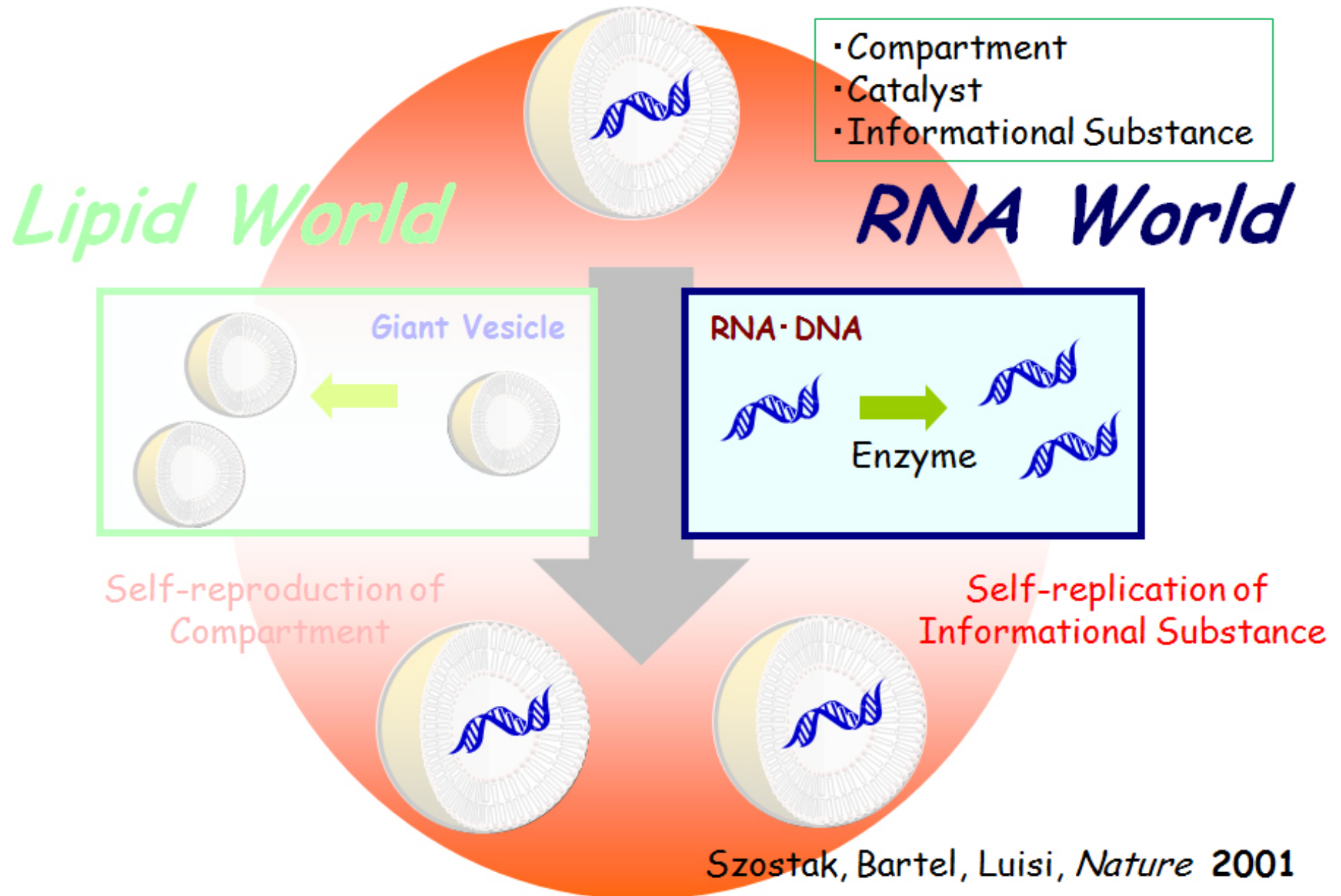
K. Kurihara, *et al.*, *Soft Matter* **6**, 1888 (2010)  
→ P202 (25<sup>th</sup> Aug)

# Giant Vesicle-based Artificial Cell

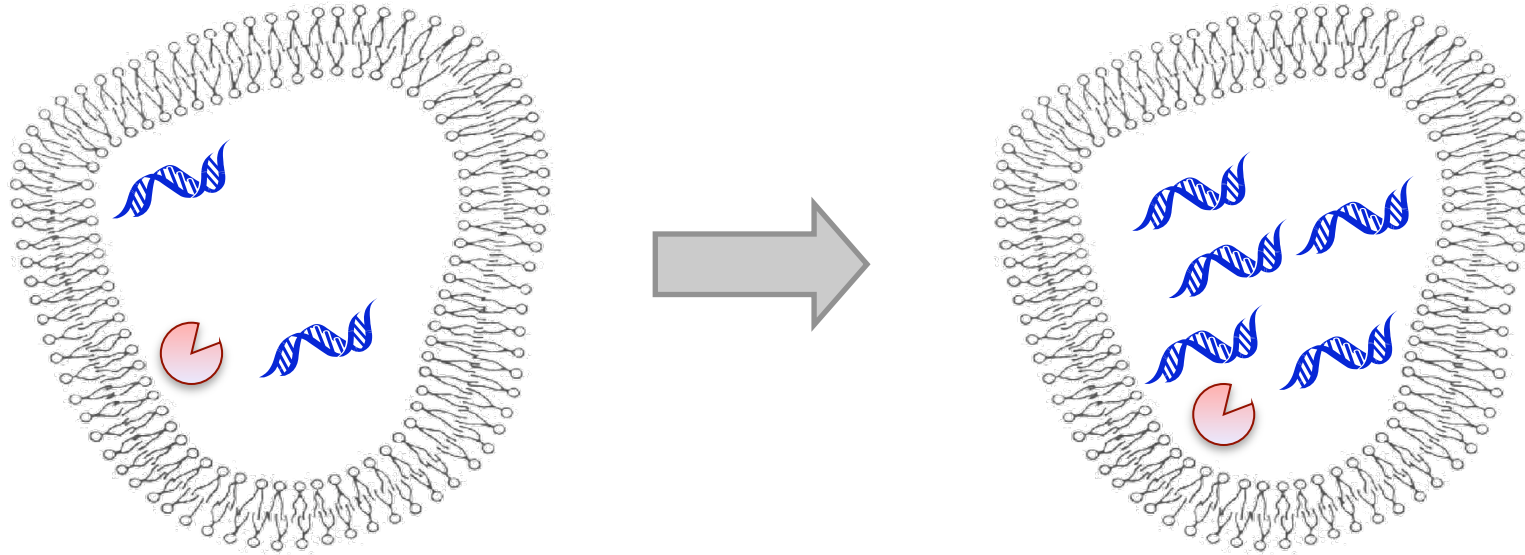




# Giant Vesicle-based Artificial Cell

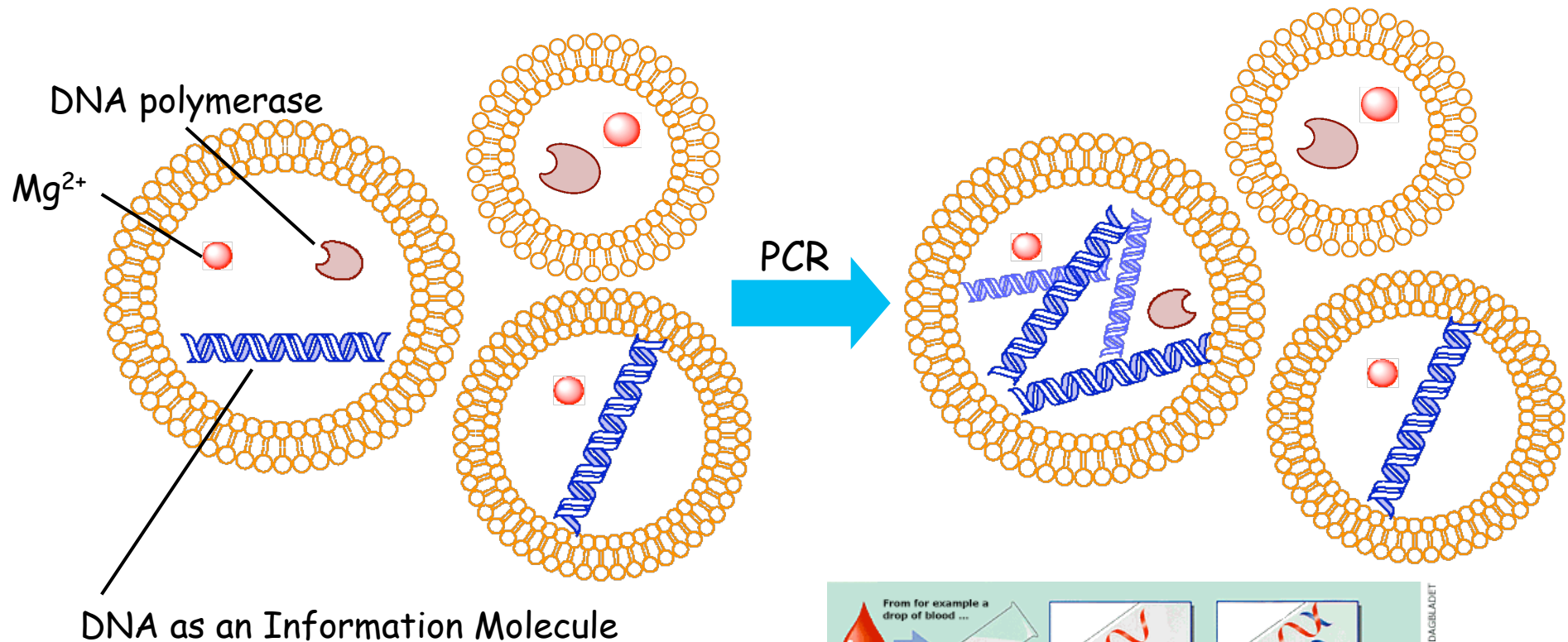


# Enzymatic Reaction in Vesicle

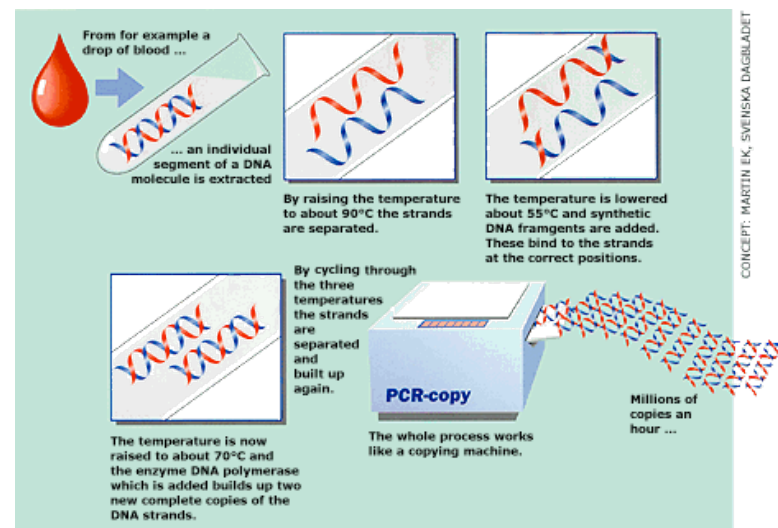


- **Polyadenine Synthesis (LV):** P. Walde *et al.*, *JACS*, 1994.
- **Polymerase Chain Reaction (LV):** T. Oberholzer *et al.*, *Chem. Biol.*, 1995.
- **Protein Synthesis (LV):** P. L. Luisi *et al.*, *BBRC*, 1999.
- **Transcription Reaction (GV):** K. Yoshikawa *et al.*, *Langmuir*, 2001.
- **Genetic Network (GV):** T. Yomo *et al.*, *FEBS Lett.*, 2004.

# Amplification of Information Molecule in GV



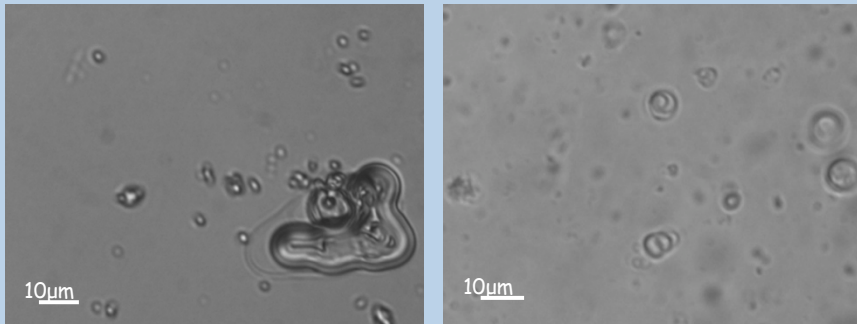
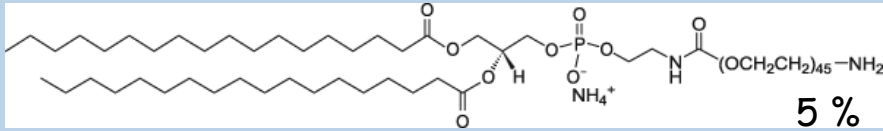
Competition between sufficient and insufficient vesicles in respect of contents of replication reagents.



DNA can be amplified by PCR method easily.

# Tunings for PCR in GV

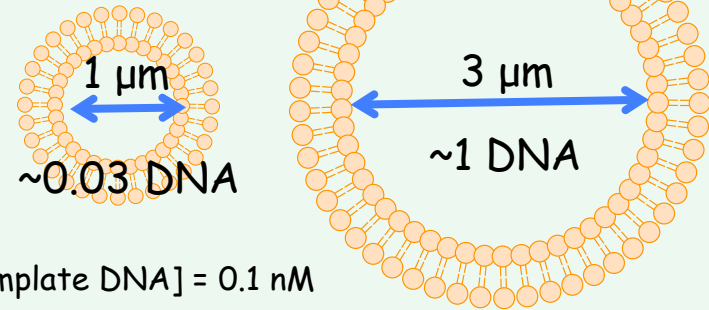
## Stabilization of Vesicular Membrane



in highly ionic aqueous dispersion

## Distribution of Template DNA in GVs

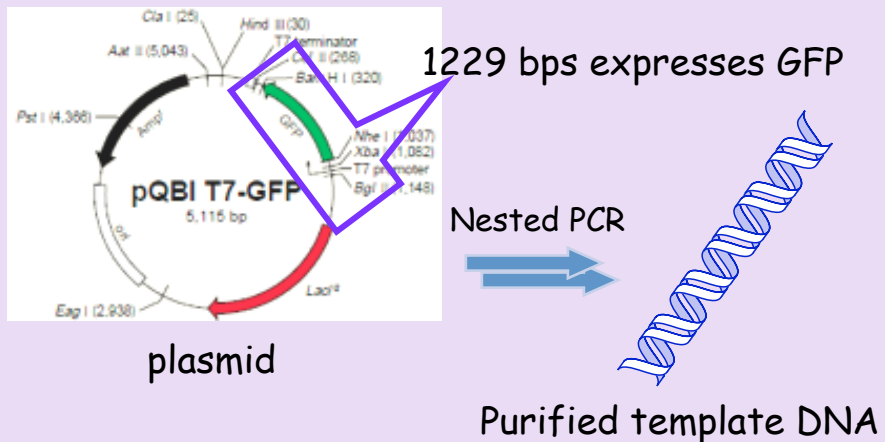
“Size Effect”



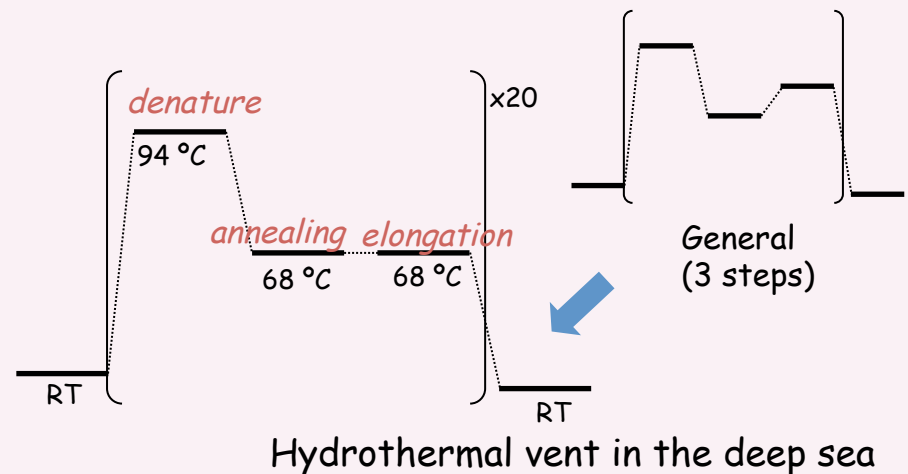
[Template DNA] = 0.1 nM

Encapsulation by a freezing-thawing method

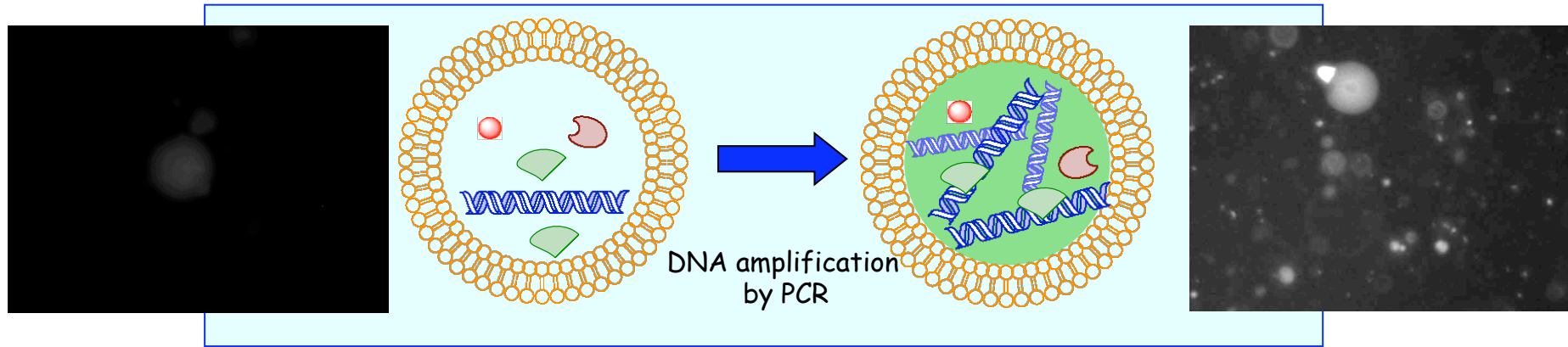
## Purification of template DNA



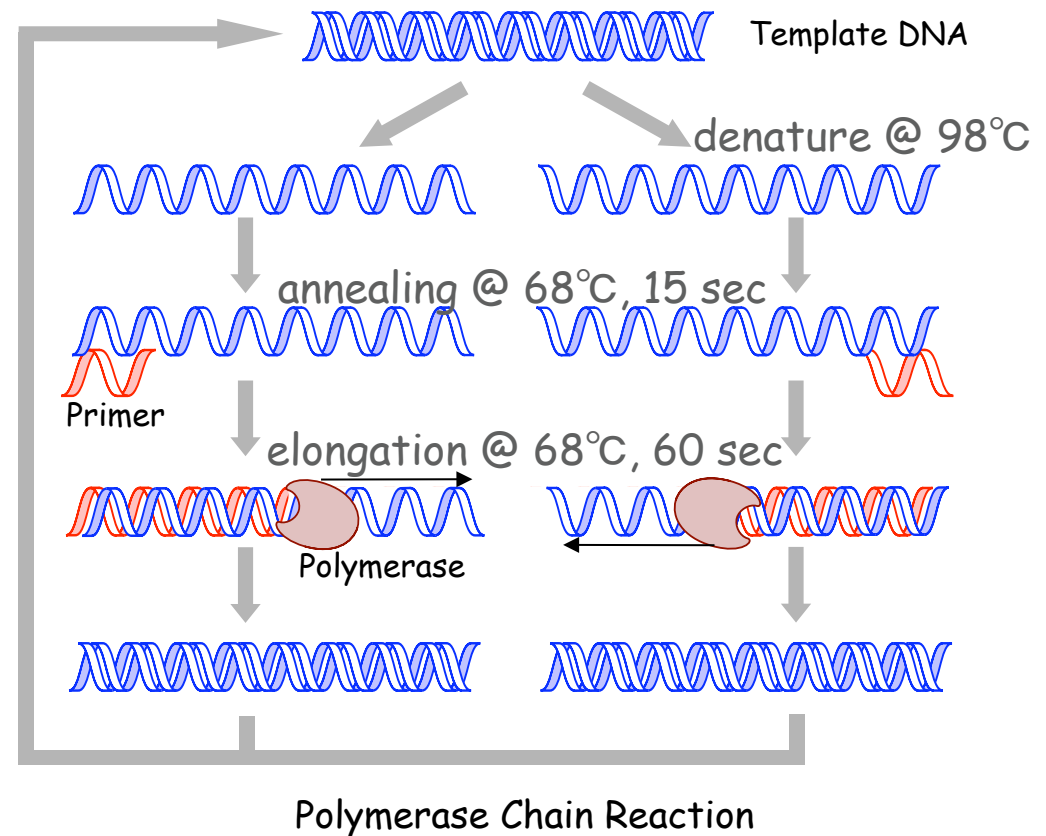
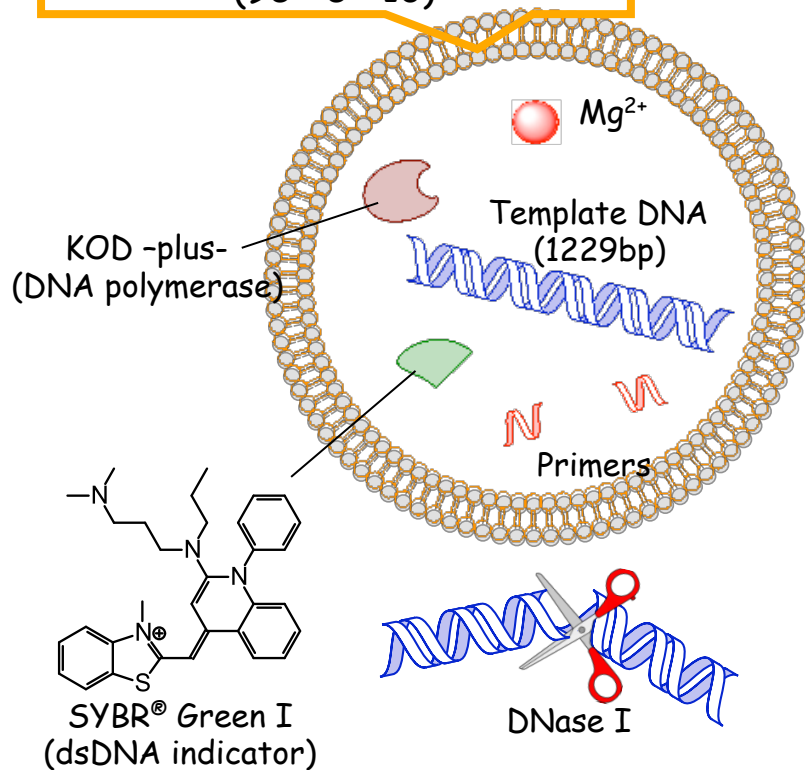
## Two-Step PCR



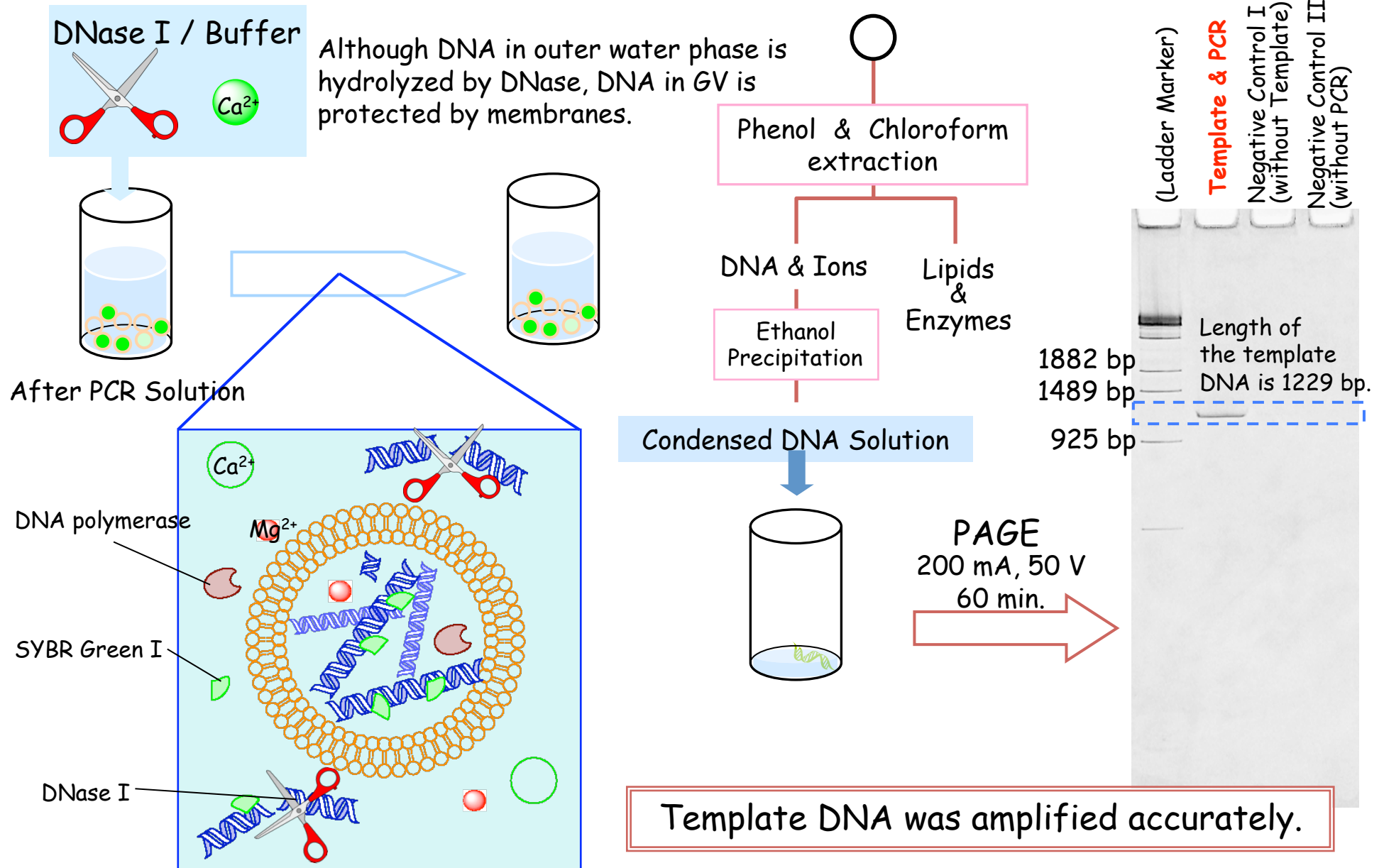
# Replication of DNA in Giant Vesicles



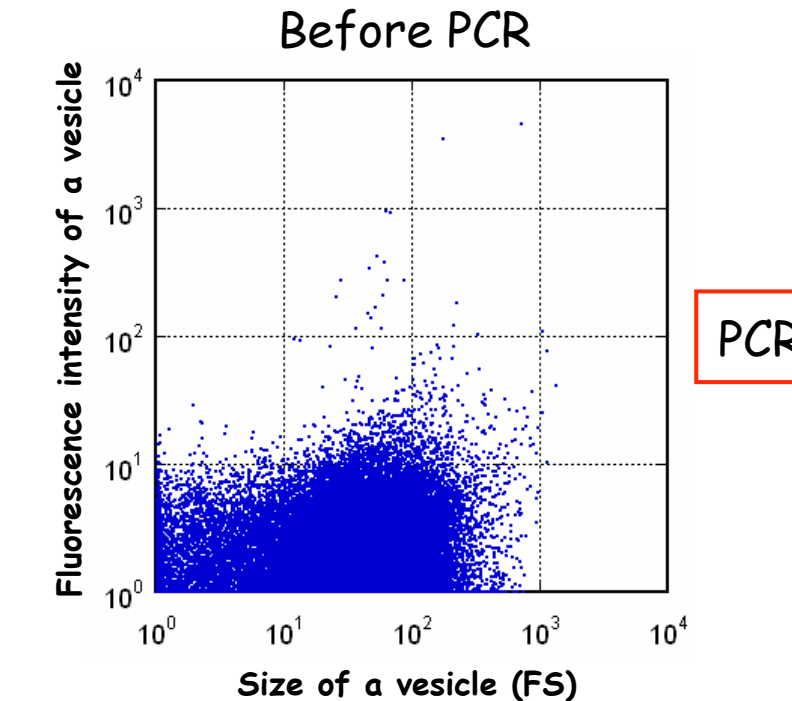
POPC, DSPE-PEG<sub>5000</sub>, Cholesterol  
(95 : 5 : 10)



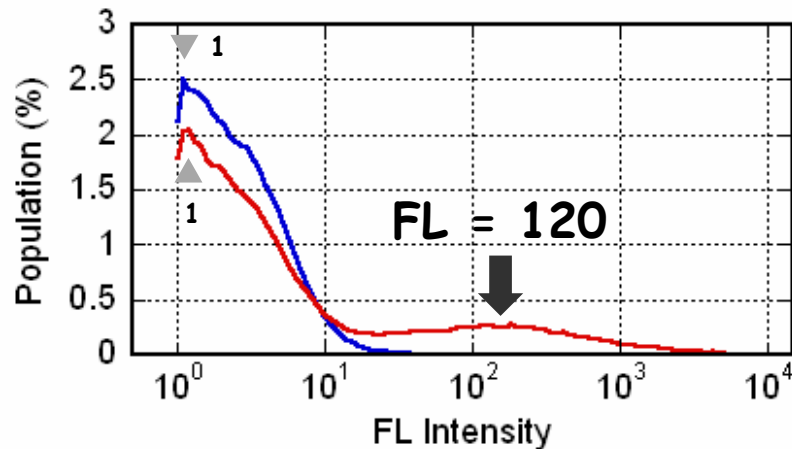
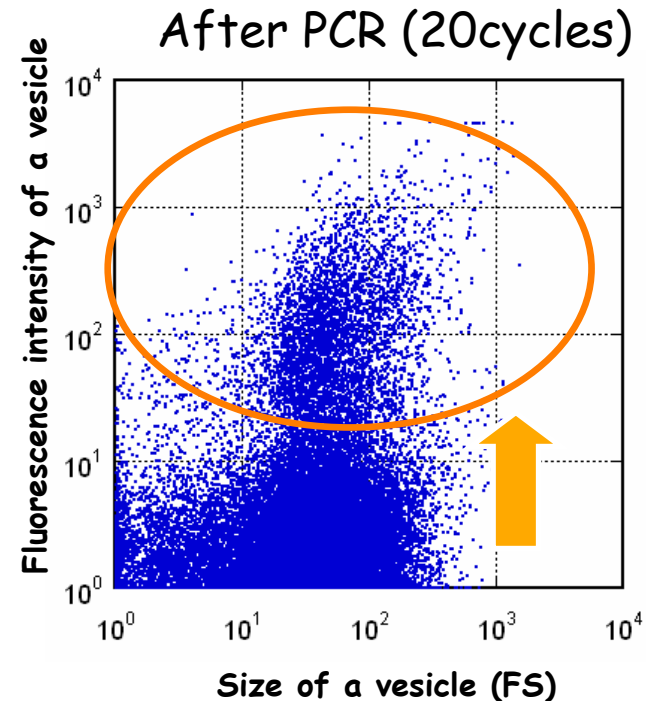
# Extraction of Replicated DNA for Gel Electrophoresis



# Population Analysis of the Performance of PCR in GV

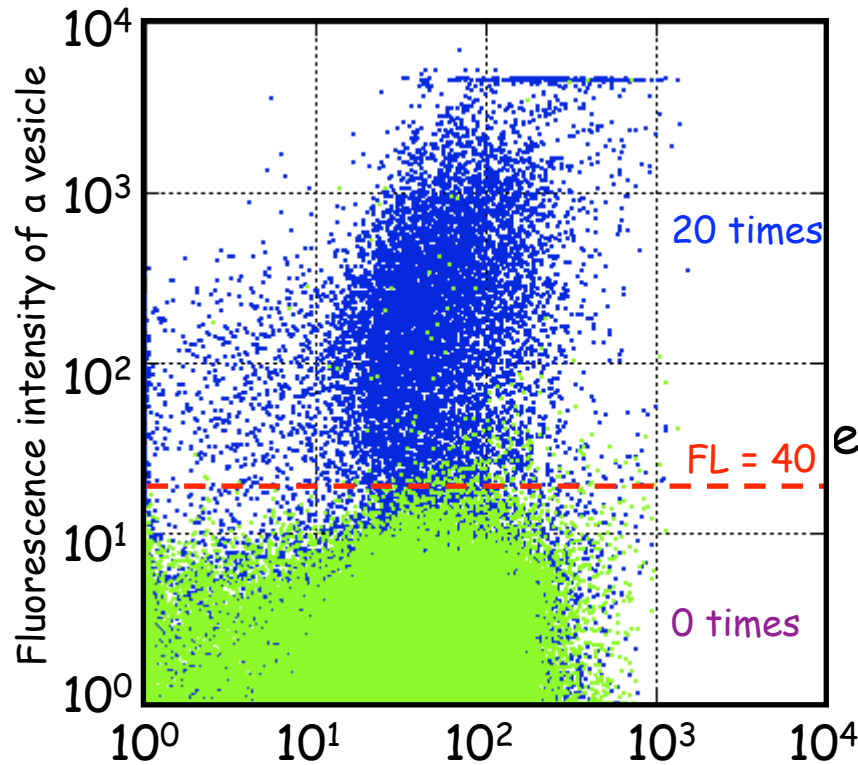


PCR cycles



- A group having a local maximum at FL=120 appeared after 20-PCR cycles.
- Fluorescence intensity of GV (DNA) increased by ca.100 times (average)
- Ratio of vesicles to replicate DNA is 9.2% (FL>25)

# Size of Giant Vesicle vs. Ratio of PCR-Performed GV

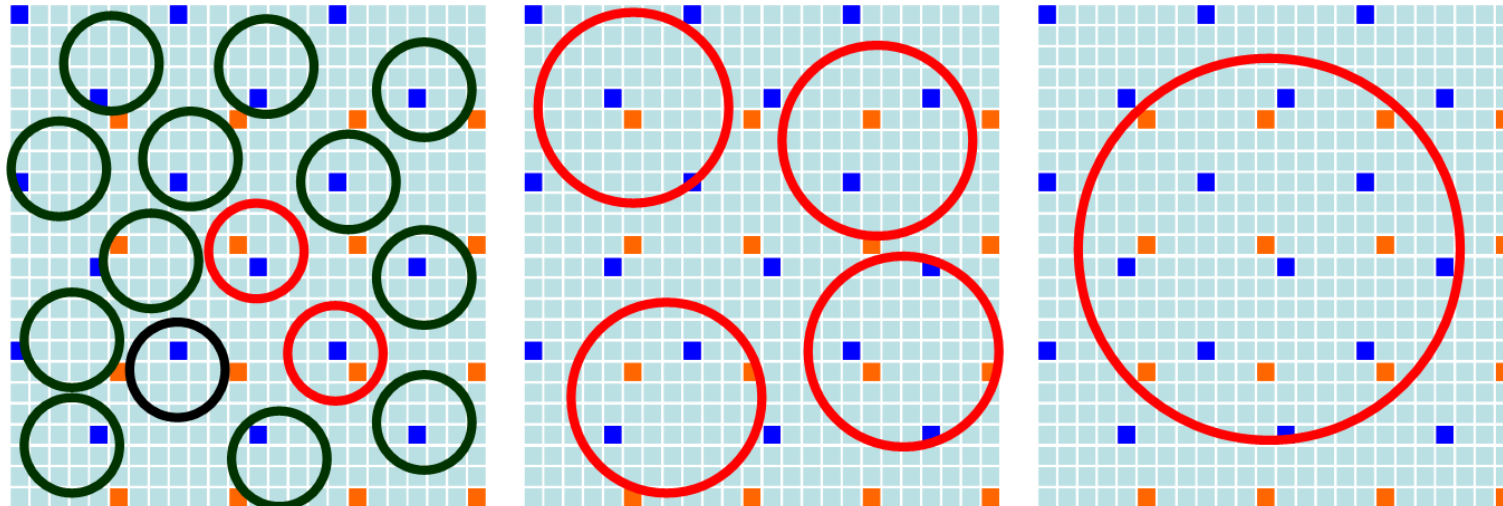


The survival race has already started !

ex) Mean number of capsulated DNA in GV

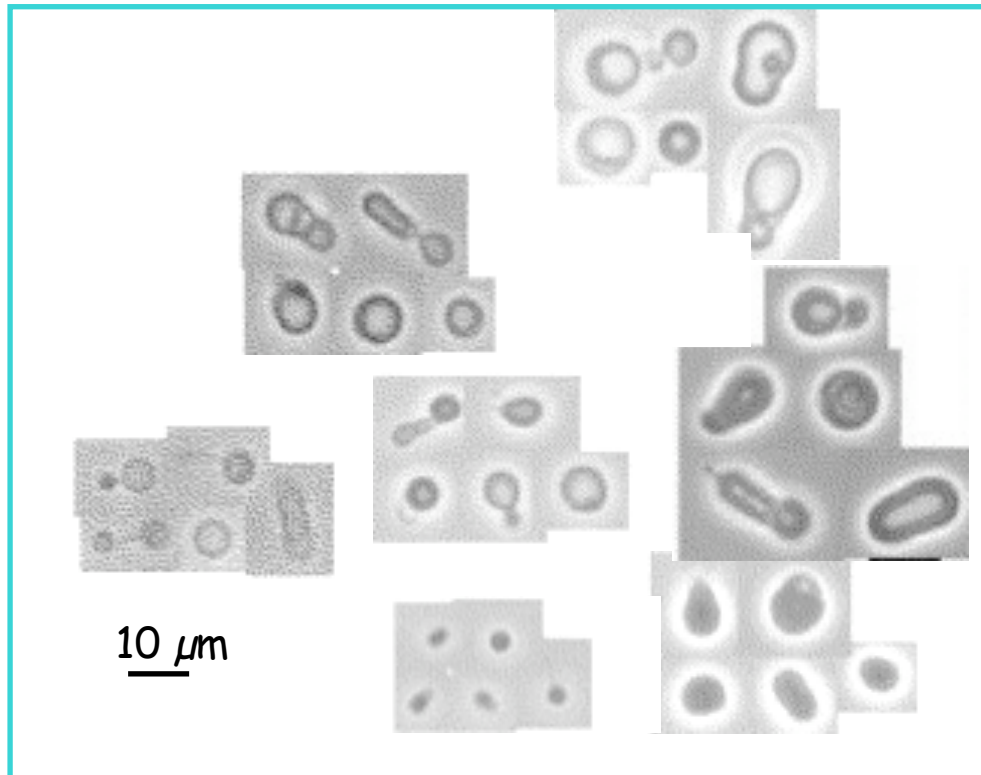
10 $\mu\text{m}$ GV	30 DNAs
5 $\mu\text{m}$ GV	4 DNAs
1 $\mu\text{m}$ GV	0.3 DNAs

Ratio of PCR-performed GV: Av. 10%

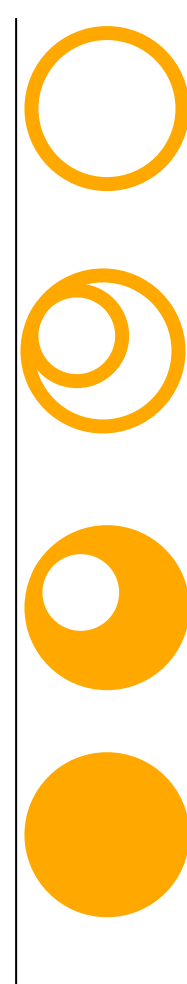




# Influence of Lamellarity of GVs on the ratio of PCR-Performed GVs



K. Sato, K. Obinata, T. Sugawara, I. Urabe, T. Yomo, *J. Biosci. Bioeng.*, **102**, 171-178 (2006).



Even a similar sized vesicle can have various effective volumes due to the internal structure.

## Meaning of Enzymatic Reaction in GV

- 1) Entrapment of precious component
- 2) Effective volume in GV -Lamella structure-
- 3) Electrostatic interaction between membrane and ingredients
- 4) Lamella structure and PCR performance