Mixing of Biochemical Reagents Inside of Giant Vesicles by Centrifugation-Induced Fusion

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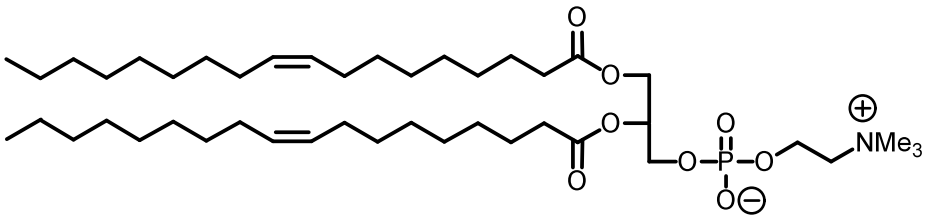
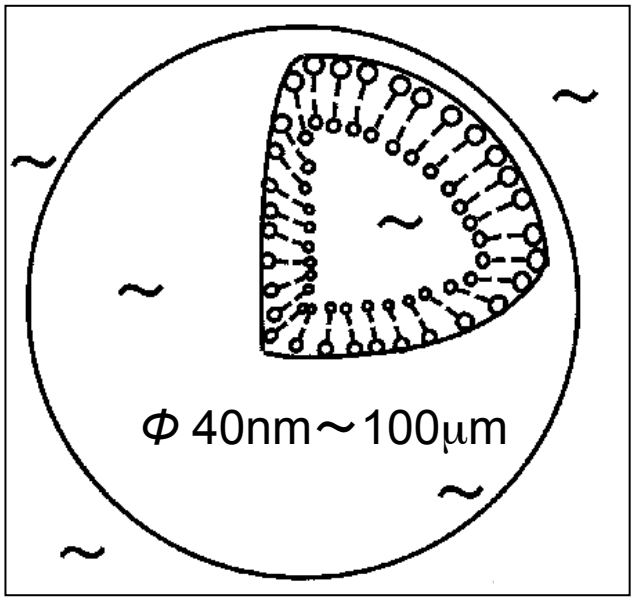
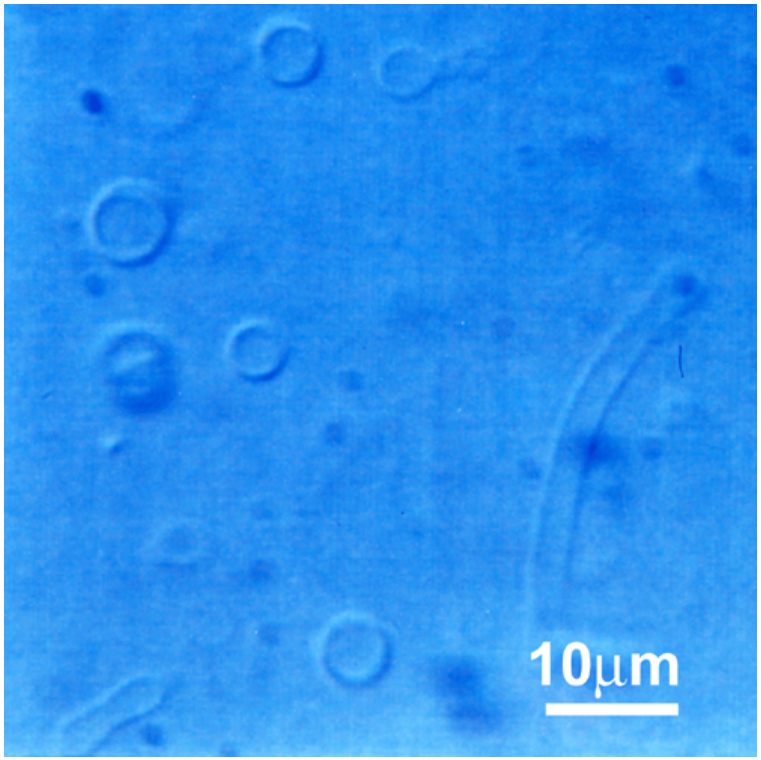
Giant vesicles

✓ Closed lipid bilayer membrane (lipid capsule)



1,2-dioleoyl-3-*sn*-glycero-phosphocholine





Differential interference contrast micrograph
Cross-section of giant vesicles

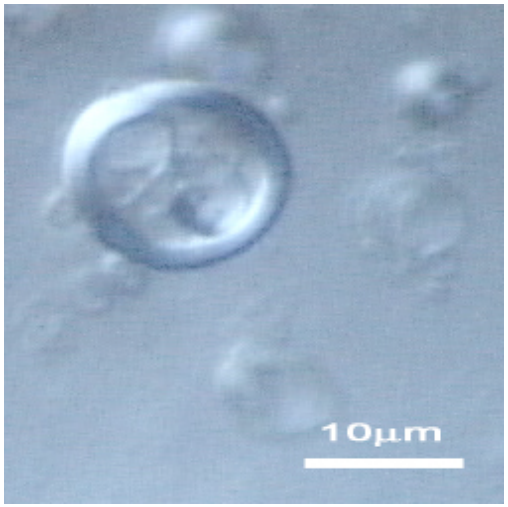
Construction of biomimetic reaction field for observing/measuring biochemical reaction network in sense of analytical chemistry!!

GV preparation (Bottom-up type)

Film-swelling Bangham *et al.*

- Mostly multilamellar, nesting, aggregated etc
- Low encapsulation ratio
- Large number of GVs

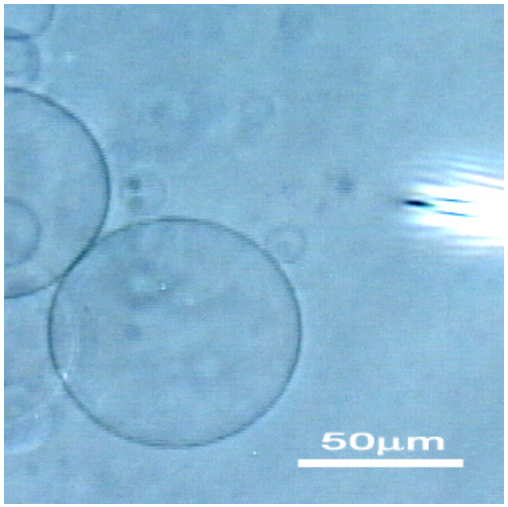
Differential interference contrast micrograph



Electroformation Angelova *et al.*

- Unilamellar, spherical
- High encapsulation ratio (by microinjection etc)
- Small number of GVs

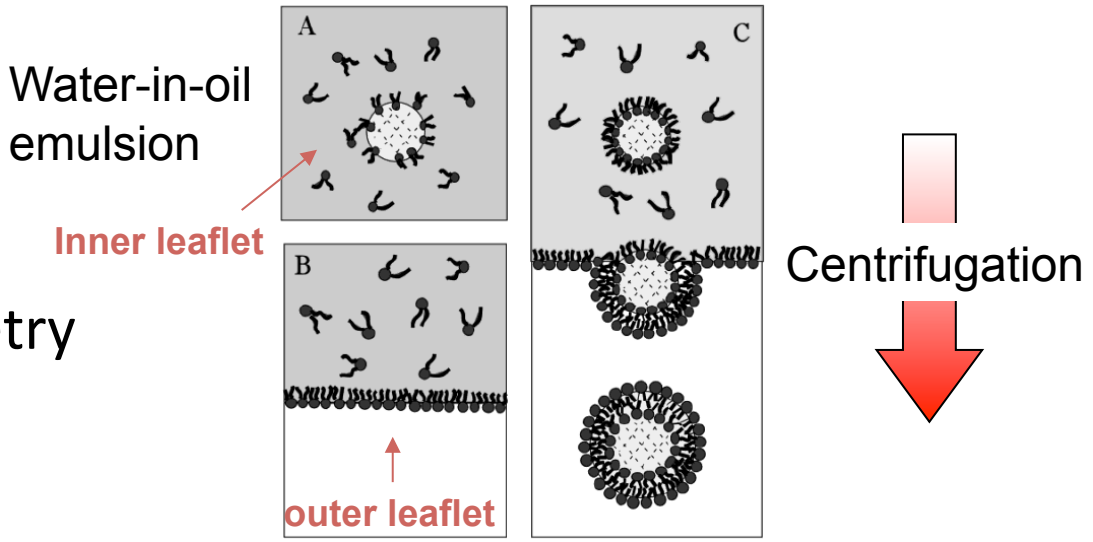
Phase contrast micrograph



W/O emulsion centrifugation method (Top-down type)

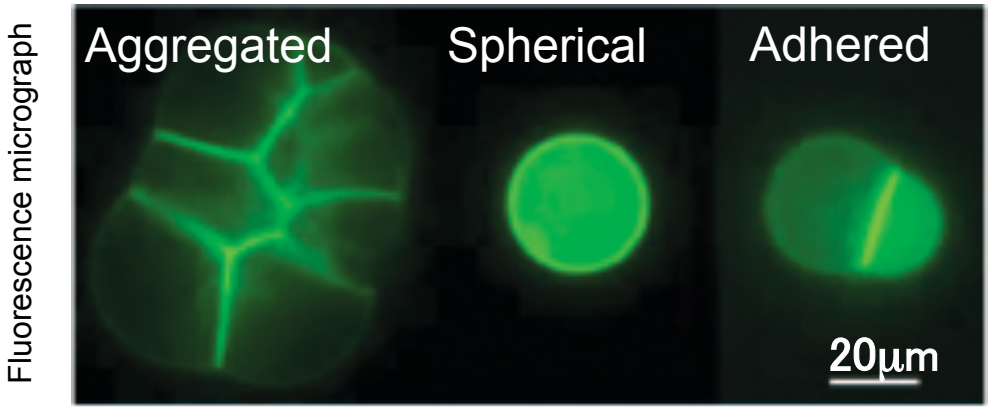
Pautot (2003)

- Single-wall (unilamellar)
- Large number of GVs
- Engineering leaflet asymmetry



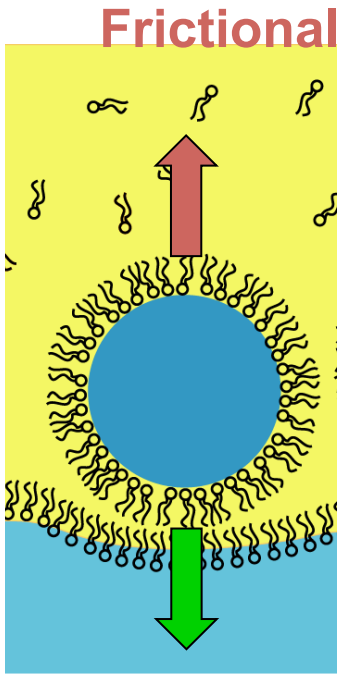
Noireaux (2004)

- Gene expression of Hemolysin-GFP complex in GVs



- Does encapsulation ratio of content reach 100 %?
- How is the population of GVs in shape distributed?

Centrifugation principle



Centrifugation Principle with Stokes' Law

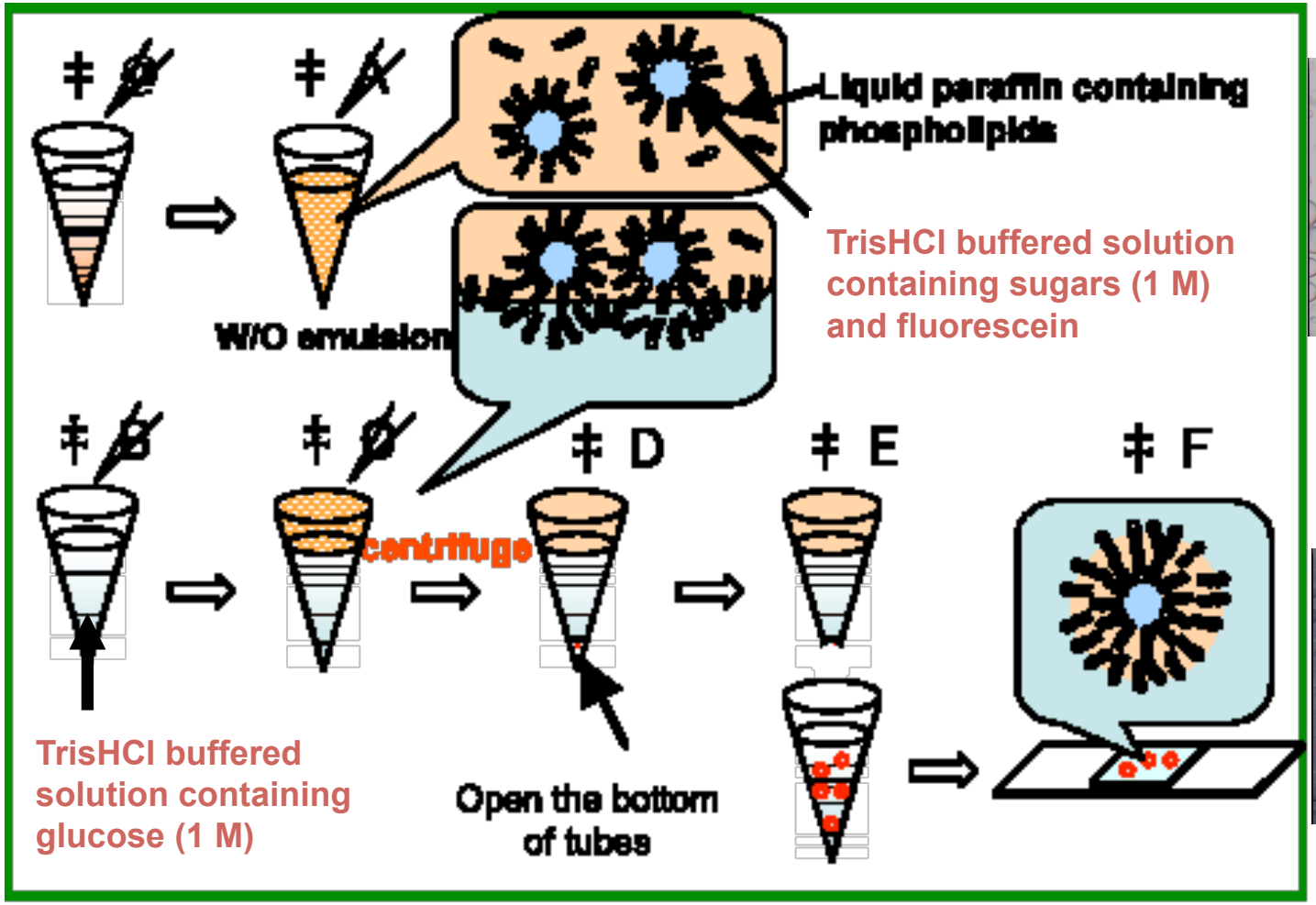
Frictional force = $3 \pi d \eta v$

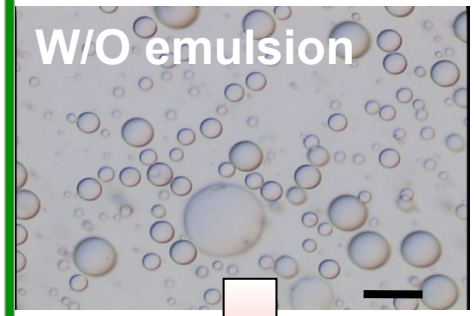
d: diameter of particle,
η: **viscosity** of solution,
v: speed of descending particle

Centrifugal force = $\frac{1}{6} \pi d^3 (\sigma - \rho) r \omega^2$

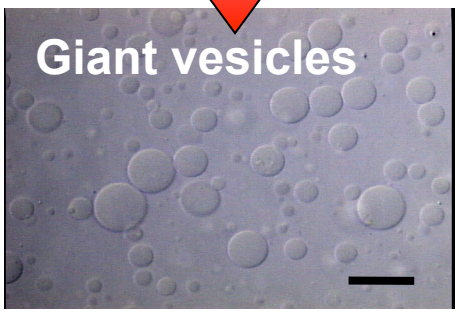
d: diameter of particle,
σ,ρ: **density** of particle and solution,
r, ω: rotation radius and speed

Typical recipe





Centrifugation

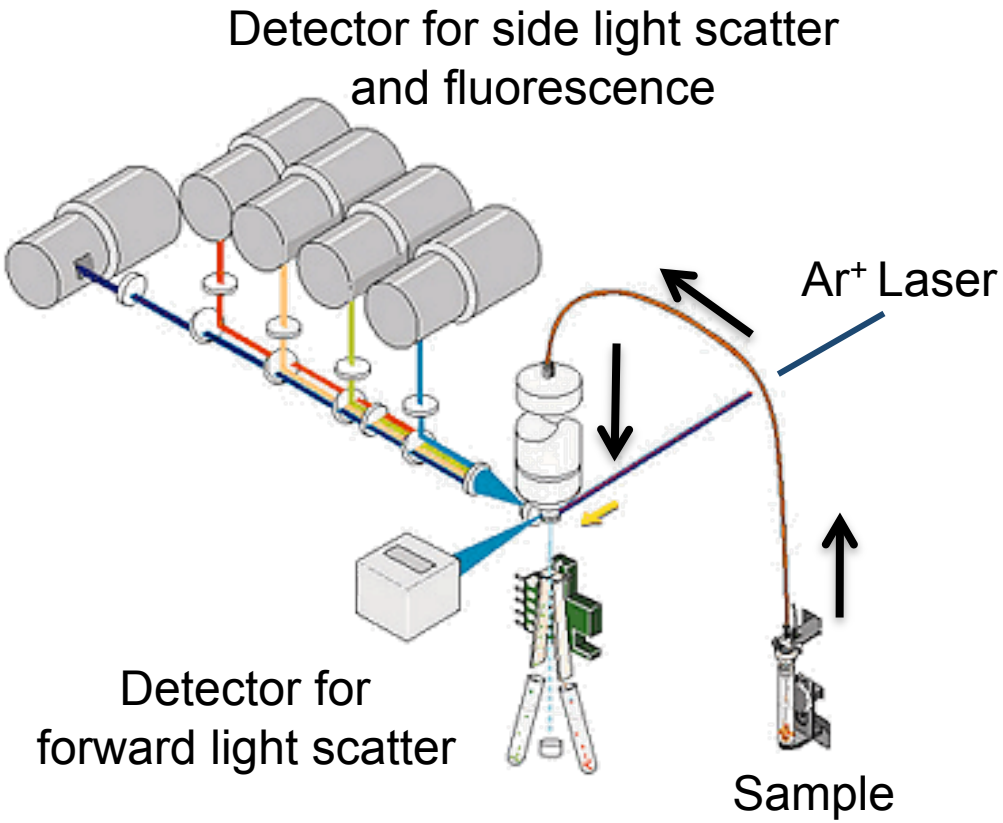


35μm

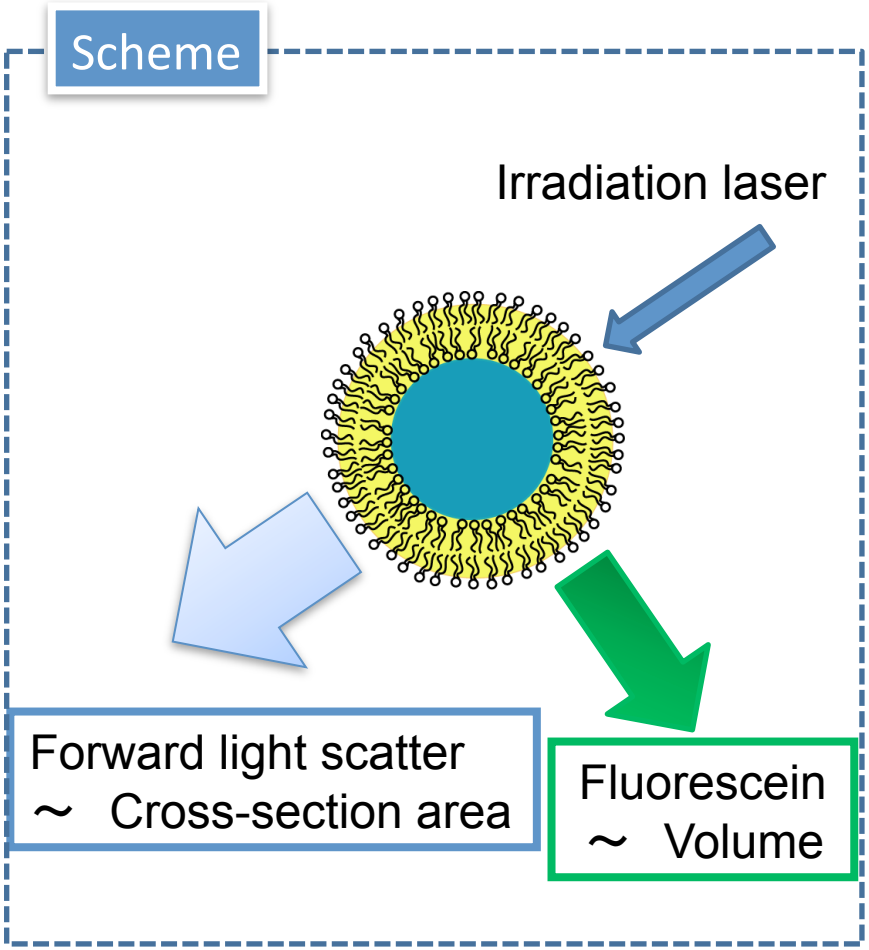
- Fluorescein-containing GVs formed by centrifugation

Flow cytometric (FCM) analysis on GV shape

Flow cytometer (EPICS ALTRA)

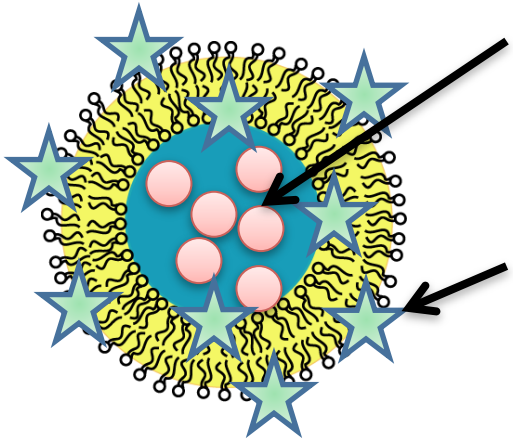


From website of Bechmann Coulter



Quantitative population analysis on GV shape

K. Nishimura, T. Hosoi *et al*., *Langmuir*, **25**, 10439 (2009).



Inner water region

allophycocyanin
（Ex: He-Ne laser 633 nm, Em: 650-670 nm Bandpass）

Vesicular membrane

BODIPY-tagged phospholipid
（Ex: Ar^+ laser 488 nm, Em: 515-545 nm Bandpass）

$$\frac{\text{Fluorescence intensity of vesicular membrane}}{\text{Fluorescence intensity per one fluorophore}} = \text{Number of fluorophore per GV: } N_{\text{HPC}}$$

- Molar Ratio of cholesterol and fluorophore to phospholipids: r_{chol}, r_{HPC}
- Surface area of polar heads of phospholipid and cholesterol 0.65 nm^2, 0.28 nm^2

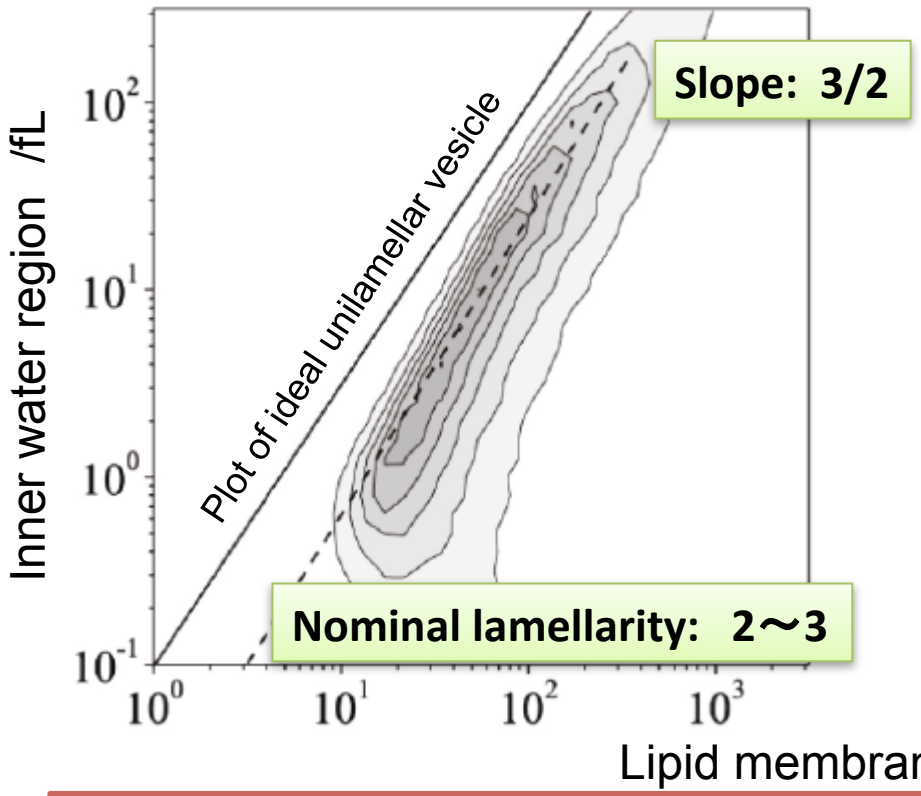
Lipid membrane area / μm^2

$$S_n = \frac{1}{2} \times \left(\frac{N_{\text{HPC}}}{r_{\text{HPC}}} \times 0.65 + N_{HPC} \times \frac{r_{\text{chol}}}{r_{\text{HPC}}} \times 0.28 \right) \times 10^{-6}$$

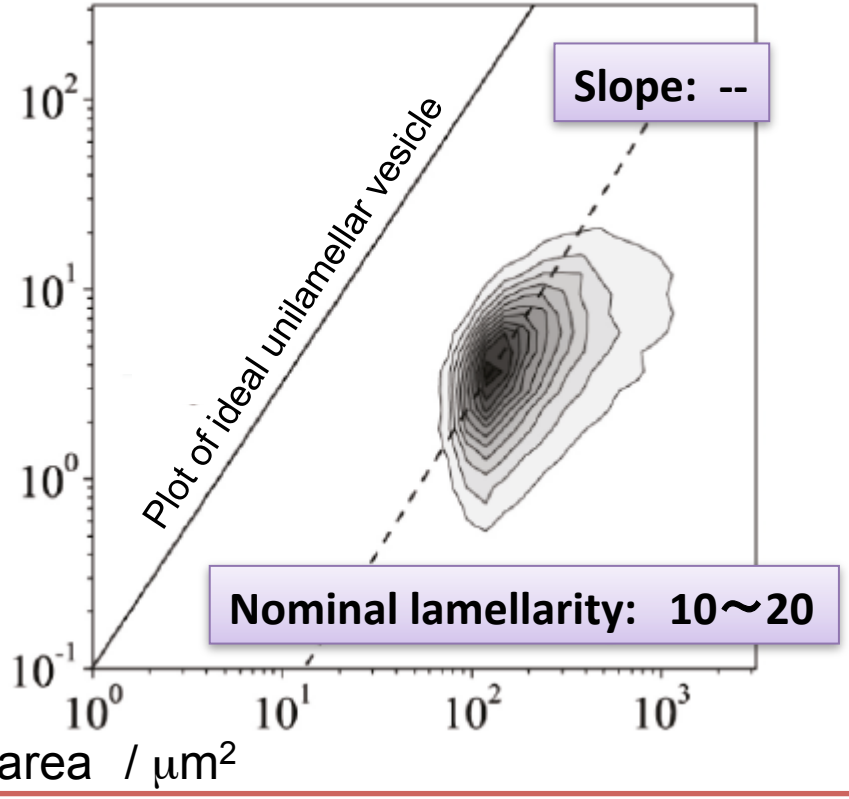
Nominal lamellarity of GV

$$\text{Nominal lamellarity} = \frac{\text{Lipid membrane area per volume}}{\text{Lipid membrane area per volume of an unilamellar vesicle}}$$

W/O emulsion centrifugation method



Film swelling method



Almost all GVs were spherical and their nominal lamellarity was quite low (2~3).

Motivation for mixing internal contents

Enzymatic Reactions in Cells

Compartmentalized by micrometer-sized closed membrane

- Confinement effect
- Surface activity of membrane

etc

Construction of biomimetic reaction space inside of closed lipid membrane

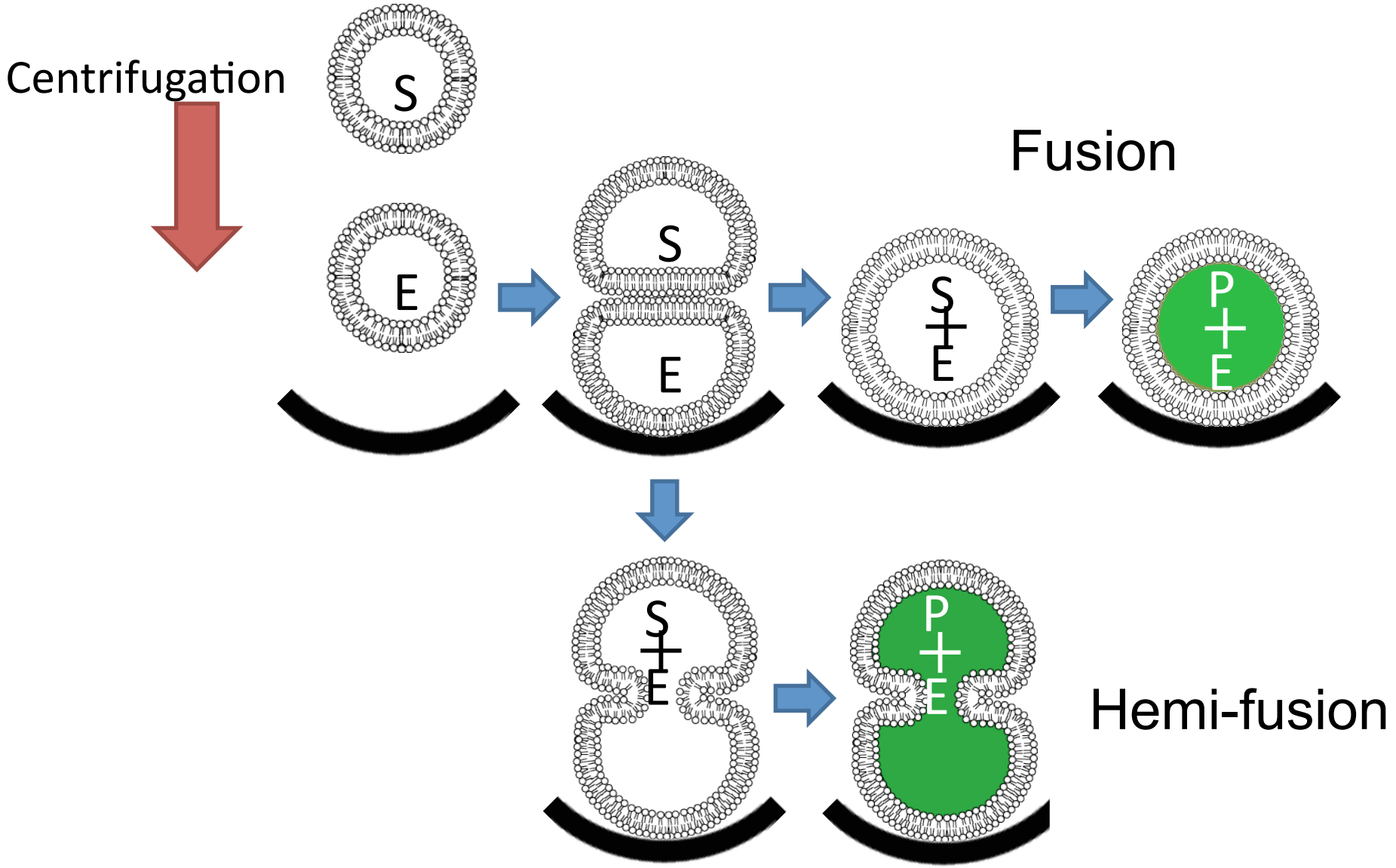
Subject

- Space inside of GV ~ 1 fL→ depletion of substrates
- Addition of non-permeable substrates into GVs is difficult.

Our strategy:

Vesicle Fusion or Hemi-fusion for mixing internal contents

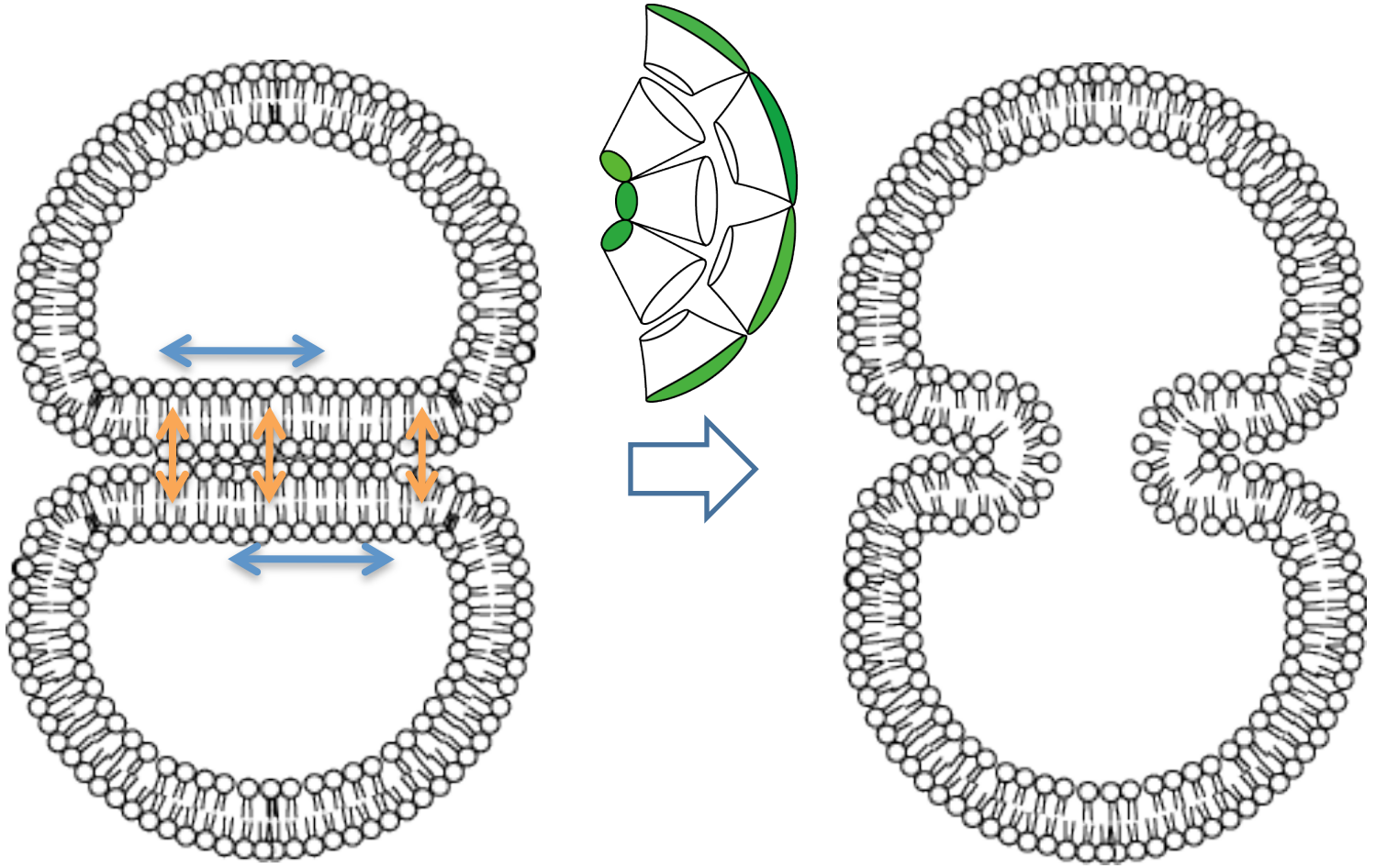
Mixing internal contents inside of GV



Why does it work?

- ✓ **Number density of lipid molecule in membrane**
- ✓ **Oil phase molecules**

Membrane fluidity for transformation at contacting site



Summary

- High encapsulation ratio of content!
  - Lipid conc. in oil phase
  - Temperature during centrifugation
  - Density difference
  - 10 mol% cholesterol
  - Encapsulation ratio (so far) : 63%

- Flow cytometry revealed
  - Almost all are spherical and have low nominal lamellarity (2 or 3).

- GVs fused/hemi-fused for mixing internal content
  - Mixing of internal content of GV was realized without any additives for fusion/hemifusion by collecting GVs through centrifugation.

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Prof. Tetsuya Yomo

More for W/O-EC GV…

Poster session (Tomorrow)

A. Shiga (P203)

Manipulation of W/O-EC GV by optical trapping

T. Furuya (P204)

W/O-EC GV dynamics observed by reflective interference contrast microscopy