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

T. Toyota,¹,²,³,⁴ M. Matsunaga,⁴ M. Fujinami⁴

¹ University of Tokyo, Komaba,
² Research Center for Life Science as Complex Systems
³ PRESTO, JST
⁴ Chiba University
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

**Electroformation**  
Angelova *et al.*  
- Unilamellar, spherical  
- High encapsulation ratio  
(by microinjection etc)  
- Small number of GVs

---

Differential interference contrast micrograph

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 with Stokes’ Law

Frictional force = \( 3 \pi d \eta \nu \)

- \( d \): diameter of particle,
- \( \eta \): **viscosity** of solution,
- \( \nu \): speed of descending particle

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

- \( d \): diameter of particle,
- \( \sigma, \rho \): **density** of particle and solution,
- \( r, \omega \): rotation radius and speed
Fluorescein-containing GVs formed by centrifugation

Typical recipe

- Centrifugation
- W/O emulsion

TrisHCl buffered solution containing sugars (1 M) and fluorescein

Giant vesicles

35μm
Flow cytometric (FCM) analysis on GV shape

Flow cytometer (EPICS ALTRA)
Detector for side light scatter and fluorescence
Detector for forward light scatter
Sample

Scheme
Irradiation laser

Forward light scatter ~ Cross-section area
Fluorescein ~ Volume

From website of Bechmann Coulter
Quantitative population analysis on GV shape


**Fluorescence intensity of vesicular membrane**

\[
\frac{\text{Fluorescence intensity of vesicular membrane}}{\text{Fluorescence intensity per one fluorophore}} = N_{\text{HPC}}
\]

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

- **Vesicular membrane**
  - allophtycocyanin
    - (Ex: He-Ne laser 633 nm, Em: 650-670 nm Bandpass)

- **Inner water region**

- **Molar Ratio of cholesterol and fluorophore to phospholipids**: \( r_{\text{chol}}, r_{\text{HPC}} \)

- **Surface area of polar heads of phospholipid and cholesterol**: 0.65 nm², 0.28 nm²

- **Lipid membrane area / \( \mu \text{m}^2 \)**

\[
S_n = \frac{1}{2} \times \left( \frac{N_{\text{HPC}}}{r_{\text{HPC}}} \times 0.65 + N_{\text{HPC}} \times \frac{r_{\text{chol}}}{r_{\text{HPC}}} \times 0.28 \right) \times 10^{-6}
\]
Nominal lamellarity of GV

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

- W/O emulsion centrifugation method
  - Slope: 3/2
  - Nominal lamellarity: 2~3

- Film swelling method
  - Slope: --
  - Nominal lamellarity: 10~20

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

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

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

Our strategy:

Vesicle Fusion or Hemi-fusion for mixing internal contents
Mixing internal contents inside of GV

Centrifugation → Fusion → Hemifusion
Why does it work?

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

Membrane fluidity for transformation at contacting site
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.
Acknowledgements

Analytical Chemistry Lab. in Chiba University
  Mr. Tomohiro Hosoi
  Prof. Koichi Oguma

Information Bioscience Lab. in Osaka University
  Mr. Kazuya Nishimura
  Dr. Takeshi Sunami
  Prof. Hiroaki Suzuki
  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