

論文の内容の要旨

論文題目

Liposome MANSIONs: quantitative and statistical analysis of the dynamics of cell-sized liposomes under steady flow environment

(リポソーム MANSIONs：定常流れ場における

細胞サイズのリポソームの動態の定量・統計解析)

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Cell-sized liposomes are a promising scaffold for developing constructive biology to clarify the essence of chemical logic behind the complexity of the current living cells by reconstituting the cellular dynamics from scratch. The emergent technology of microfluidics is a promising tool to study cell-sized liposomes. Microfluidic devices enabled the accurate manipulation of the external environment and high-throughput measurements for cell-sized liposomes. In the development of elaborate microfluidic devices, the external flow has been regarded as an artifact to be carefully addressed. Despite the significant interests to the flow-mediated properties of living cells in cell biology, less is elucidated for the behaviors of the lipid membrane under a steady flow environment. The purpose of the thesis is to establish the platform for the quantitative and statistical analysis of cell-sized liposomes under a steady flow environment. In the thesis, we first describe the development of a machine-assisted observation platform of the cell-sized liposome, termed MANSIONs as an abbreviation of Machine-Assisted Numerous Simultaneous and Interactive Observation of Non-equilibrium self-assemblies. Then, we describe the unique behaviors of cell-sized liposomes under the steady flow environment found and elucidated by MANSIONs.

Chapter 2 described the design and performance of MANSIONs (Figure 1). MANSIONs included two newly designed microfluidic devices (arraying device and mixing device, respectively) and the peripheral of microfluidic experiments integrated via the in-house-developed Python program. The

arraying device enabled the trap of cell-sized liposomes in juxtaposed large circular spaces (termed nests) (diameter: $12.7 \pm 1.3 \mu\text{m}$). The mixing device mixed two aqueous solutions with a dynamic range of 30–70%. The performance of MANSIONs was verified by measuring the behaviors of the liposomes exposed to the osmotic stress whose concentration was optimized in advance by the automated repetition of the protocols. Noteworthy as the system implemented the sudden stoppage of the external flow at an arbitrary timing, liposomes can be measured under the condition closely similar to the bulk experiments. The measured water permeability under the dynamic condition and static condition was $86.4 \pm 48.8 \mu\text{m s}^{-1}$ and $77.9 \pm 41.2 \mu\text{m s}^{-1}$, respectively, which indicated that the external flow did not affect the water permeability significantly. Besides, the morphological analysis of the liposomes during the osmotic stress revealed that the liposomes escaped from the nest when the diameter reached 92% of the initial value. The influence of the absolute diameter was not significant. The result indicated that MANSIONs was applicable to the deformability assay of the liposomes under a steady flow environment. Thus, we deduced that the developed machine-assisted observation platform, MANSIONs, was a promising tool to investigate liposomes under a steady flow environment quantitatively and statistically.

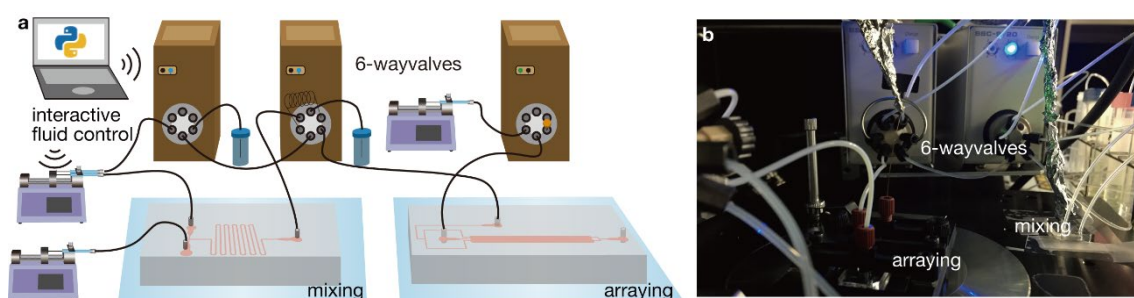


Figure 1 The schematic illustration (a) and the picture (b) of the whole setup.

Chapter 3 presented a technical improvement of the microfluidic device to accomplish the observation of cell-sized liposomes prepared by the water-in-oil emulsion transfer (WOET) method. The WOET method is useful to prepare cell-sized liposomes encapsulating even macromolecules and micrometer-sized colloidal particles at a high yield. We showed that the innate microfluidic device did not suit the liposomes prepared by the WOET method because of the strong adhesion on the surface. We suspected contaminated oil residues in the membrane as a cause of the undesired adhesion. We showed that the microfluidic device whose surface was modified by the perfluoroalkyl group could stably capture the cell-sized liposomes prepared by the WOET method. We found convection inside the liposomes when we observed the liposomes encapsulating fluorescent microbeads with the microfluidic device (Figure 2). As the convection ceased by the stoppage of the external flow, the driving force of the convection was the external flow. Besides, we visualized that the long-tailed distribution of the encapsulation yield was not biased even under the microfluidic investigation. The

result was critical to ensure the compatibility of the microfluidic exploration to the traditional experimental data as the encapsulation yield was the core of the reconstructed system in the liposomes. On the contrary, we clarified that the water permeability of the liposomes prepared by the WOET method was $10.2 \pm 5.0 \mu\text{m s}^{-1}$, which was almost 10 % of the previously known value. It was the first quantitative identification of the reduced water permeability for the liposomes prepared by the WOET method, to the best of our knowledge. The result should be noted as an inevitable feature of the liposomes prepared by the WOET method. The convection observed in the liposomes was also noteworthy: the steadily stirred inner water phase could provide a unique reaction property inside the cell-sized liposomes encapsulating bio-functional molecules prepared by the WOET method.

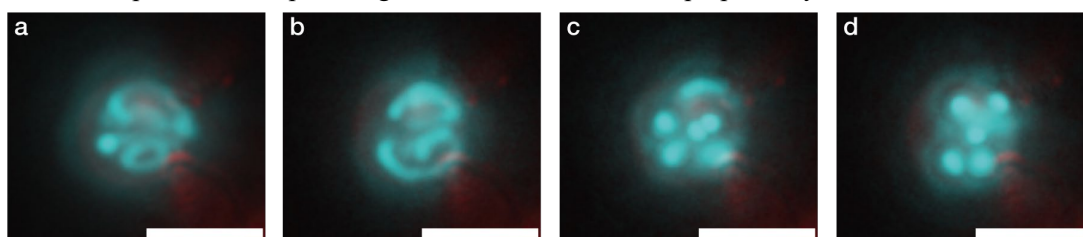


Figure 2 Shear-induced bipolar convection in the liposome. Lipid was depicted in red (emission: 600–690 nm), and fluorescent microbeads were shown in cyan (emission: 515–545 nm). Images were taken under constant flow ($40 \mu\text{L h}^{-1}$) (a) and a static condition (b–d): 5 sec (b), 9 sec (c), and 13 sec (d) after the manipulation of the valve. Optical exposure time was 500 msec. Scale bars: $10 \mu\text{m}$.

Chapter 4 explained a unique transportation by which molecules were accumulated into the cell-sized liposomes under a steady flow environment against a concentration gradient. Namely, when liposomes were exposed to a solution of uranine, a water-soluble fluorescent molecule, the concentration of uranine inside the liposomes could be higher than that of the outside (Figure 3). The liposomes did not contain any proteins or additional components other than phospholipid. The kinetic investigation and time-resolved measurement with the support of the mathematical toy model revealed that the imbalanced kinetics of intake and release of uranine across the liposomal membrane appeared under the steady flow environment. We observed that the accumulation already started during the size-sorting region, and the passive permeation became the dominant transportation when the external flow ceased. Thus, considering that the newly elucidated transportation across the liposomal membrane was deeply related to the external steady flow, we termed it as hydrodynamic accumulation (HDA). Noteworthy, HDA was first demonstrated by uranine, but later we found that other molecules, fluorescein, and fluorescein-tagged adenosine triphosphate, could also be accumulated. Since adenosine triphosphates are used as the chemical energy source in living systems, the potential of HDA was significant for constructive biology and also the origin of life research.

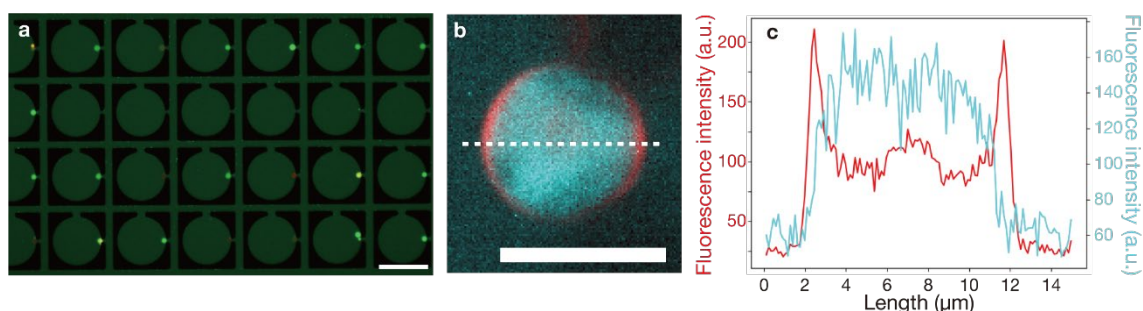


Figure 3 Liposomes accumulated uranine against the concentration gradient. (a, b) Representative images of the liposomes trapped in the arraying device taken by (a) epi-fluorescence microscopy (scale bar: 100 μm) and (b) spin-disc confocal laser microscopy (scale bar: 10 μm). (c) Line profile corresponding to the white dashed line in (b).

Chapter 5 focused more on the detailed picture of membrane disturbance that occurred in a steady flow environment by discussing the mechanism of HDA. First, we showed that HDA was determined by the electrochemical effect brought by the anionic phospholipids. HDA did not occur in the absence of anionic phospholipids, but at least three different anionic phospholipids equally contributed to the emergence of HDA. The electrochemical effect was more significant than previously known factors to affect the permeation, such as cholesterol or temperature. Noteworthy, we observed a non-monotonic contribution of the anionic phospholipid to the efficiency of HDA. Namely, at low mole fraction of the anionic phospholipid, the efficiency increased along with the increase of the mole fraction of the anionic phospholipid but decreased when the mole fraction of PG was over 50 %. We discussed that an unexpected heterogeneity at the liposomal membrane was generated, and the most plausible candidate was the asymmetric distribution of the anionic phospholipid. Besides, we reported that polyethylene glycol (PEG) was also accumulated swiftly against the concentration gradient. The passive permeation of PEG was known to depend on the molecular weight strongly. However, the kinetics of HDA was much less significant to the molecular weight of PEG, while we found that the other hydrophilic polymer, dextran, did not permeate at the finite observation time, even in the presence of PEG. These results indicated that the enhancement of the permeation was not accompanied by the pore formation. By observing the modulation of the fluorescence emission of laurdan, a solvatochromic fluorophore, we showed that the membrane exposed to a steady flow environment became hydrophilic compared to the bulk condition.

The thesis achieved the automated observation platform enabling a high-throughput, accurate, and reproducible study of cell-sized liposomes under the steady flow environment. We found and elucidated the hydrodynamic accumulation, useful and unique transportation across the liposomal membrane, using the developed automatic system. Thus, the thesis contributes to constructive biology and further opens a new research interest in the relevant research field.