膜融合の直接可視化

Direct observation of membrane fusion

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Membrane vesicles mediate various physiological functions upon fusion with biological membranes. Despite the physiological importance, biophysical features of membrane fusion remain elusive due to the technical difficulties to measure fusion events in high throughput manner. To address this issue, we here attempted to elucidate various biophysical features of membrane fusion by developing a novel microsystem, enabling single particle analysis of membrane fusion in quantitative manner.

The fusion events were visualized by using micro-chamber array sealed with bio-membrane [1, 2]. In this setup, fluorescent lipids are provided into a membrane on chamber upon the fusion of fluorescent liposome, and therefore, single fusion event is detectable as a stepwise fluorescent increase on chamber. Then, we measured the fusion efficiency by counting the number of chambers with membrane fusion. The efficiency was extremely low (≈ 0.1%) without any additives and increased more than 30-folds by fusion promoters, i.e., Ca^{2+} and PEG. Interestingly, the efficiency was decreased 8-folds when F_oF_1, a membrane protein with large hydrophilic domains, was reconstituted into liposomes. This result suggested that steric hindrance of membrane proteins would hamper membrane fusion.

In this study, we succeeded in developing a novel microsystem to visualize membrane fusion at single particle level, and moreover, quantitatively evaluating the effect of fusion promoter and inhibitor on membrane fusion. For further understating, we would like to measure the membrane fusions in more physiological conditions.

References

1, Watanabe et al., ***Proc. Natl. Acad. Sci. USA*** (2018) 115, 3066-3071

2, Watanabe et al., ***Nat. Commun.*** (2014) 5, 4519