

## **Supporting Information**

### **High-throughput formation of lipid bilayer membrane arrays with an asymmetric lipid composition**

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## **Supplementary Methods**

### **Analysis of dithionite leakage across lipid bilayer**

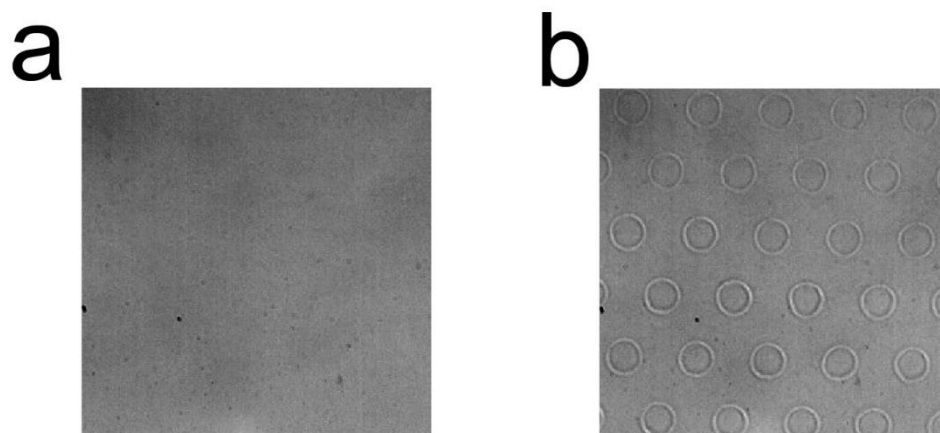
We tested the possibility of dithionite leakage across lipid bilayer (Supplementary Fig. 2).

For dithionite leakage test, the buffer (500 mM MOPS [pH 7]) was encapsulated in the chambers with 5  $\mu$ M Alexa 488, which exhibits increased fluorescence intensity upon reduction by 50 mM dithionite (Supplementary Fig. 2a). The buffer in the flow cells was then exchanged with the buffer containing 50 mM dithionite under a microscope (Supplementary Fig. 2b,c). Before and after the buffer exchange, the fluorescence intensity of Alexa 488 was stable, demonstrating that the hermetic seal of the lipid bilayer prevented dithionite leakage for 7 h.

### **Analysis of response of NBD-DPPE to dithionite**

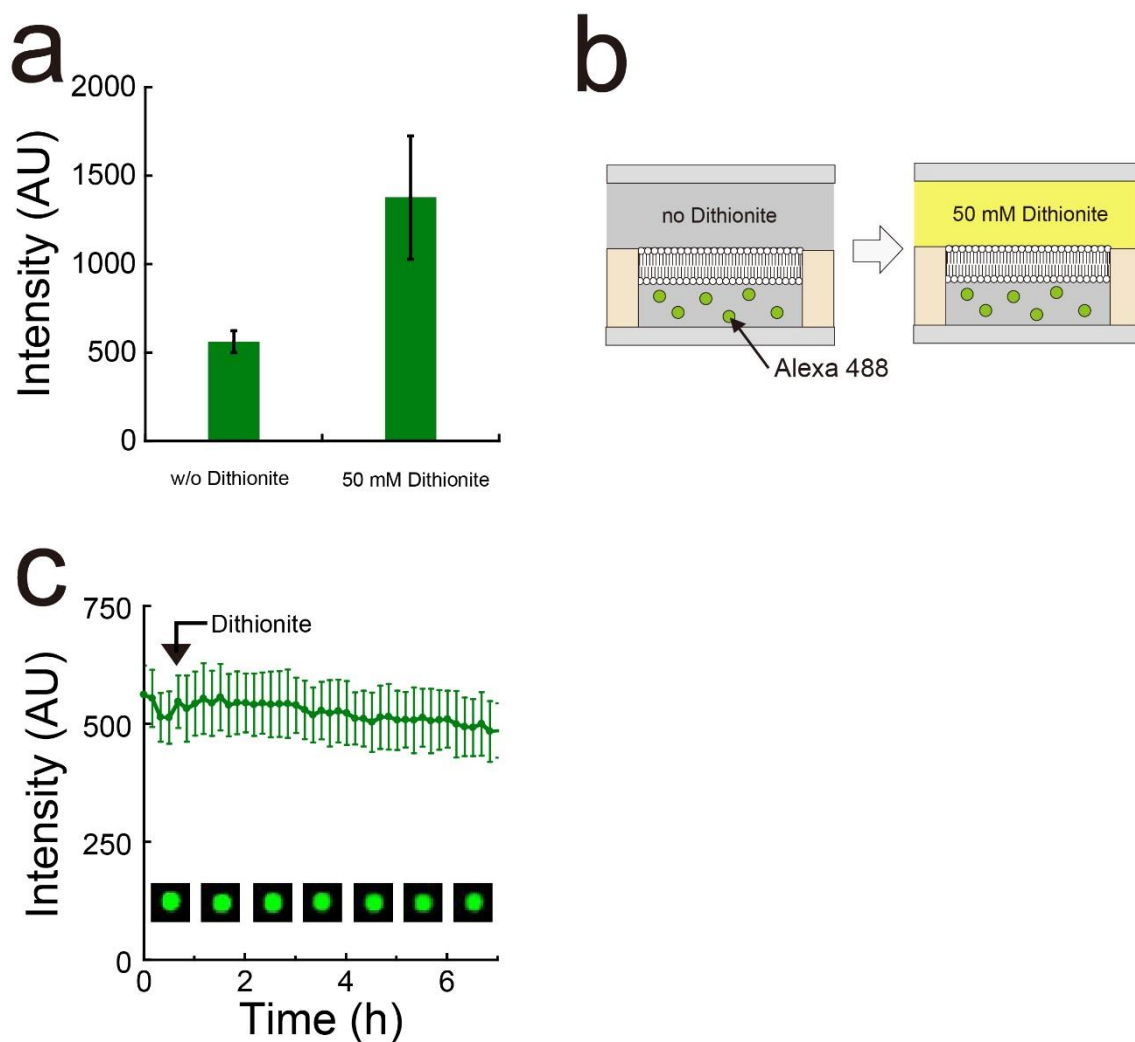
We checked the response of NBD-DPPE to dithionite (Supplementary Fig. 3a,b). For dithionite response test, the buffer (500 mM MOPS [pH 7]) was encapsulated in the chambers sealed with symmetric lipid bilayers (a 2:50:50 (w:w:w) mixture of NBD-DPPE, DOPE and DOPG). The buffer in the flow cells was then exchanged with the buffer containing 50 mM dithionite under a microscope (Supplementary Fig. 3a). After the buffer exchange, the fluorescence intensity of NBD-DPPE was decreased to almost

half within 2 min (Supplementary Fig. 3b), showing that the NBD-DPPE located in outer leaflet of bilayer was quenched. Accordingly, we found that NBD-DPPE can response to dithionite within 2 min, which is much faster than flip-flop of NBD-DPPE.



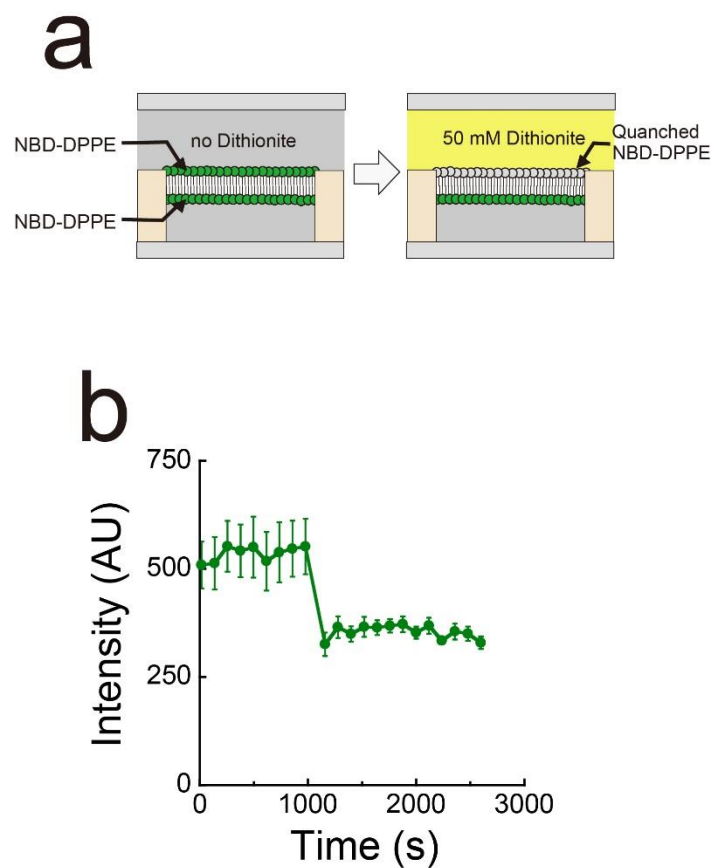
**Figure S1. Bright-field image of lipid bilayers**

Bright-field image before (**a**) and after (**b**) lipid membrane formation.



**Figure S2. Leakage of dithionite**

(a) Time course of fluorescent intensity of 5  $\mu$ M Alexa 488 in the presence or absence of 50 mM Dithionite. (b) Schematic illustration of the buffer exchange after the formation of lipid membranes. (c) Time course of 5  $\mu$ M Alexa 488 encapsulated in micro-chambers before and after injection of 50 mM Dithionite in the flow channel. Insets show fluorescent images of Alexa 488 in the representative micro-chamber.



**Figure S3. Response of NBD-DPPE to Dithionite**

(a) Schematic illustration of the buffer exchange after the formation of symmetric lipid membranes. (b) Time course of fluorescent intensity of symmetric membrane containing 2 % NBD-DPPE before and after injection of 50 mM dithionite in the flow channel.