Supporting Information

Lipid bilayer spreading velocity and molecular filtration characteristics

Experimental Procedures: The spreading dynamics and the filtering effect of TR-DHPE in the self-spreading DLPC lipid bilayer at the nano-gate was monitored in-situ using an epi-fluorescence microscope. In this experiment, TR-DHPE was doped with a concentration of 0.2 mol%. In the fluorescence microscope images (Fig. S1a), the self-spreading lipid bilayer was observed as the bright region due to the incorporation of TR-DHPE in the bilayer. During the spreading at the nano-gate region (Fig. S1a bottom), the Au nano-obstacle was observed clearly as the dark area. This observation proves that the lipid bilayer spread through the nano-gates.

Results and Discussion: The effect of the molecular filtering can be evaluated quantitatively from the decrease in fluorescence intensity at the front edge of the spreading bilayer. As shown in Fig. S1b and c, the self-spreading bilayer containing TR-DHPE molecules shows an exponentially decaying profile of fluorescence intensity. The profile reflects an accumulation of TR-DHPE at the front edge of the self-spreading DLPC lipid bilayer. Although the fluorescence intensity at the front edge, I_0, remained almost constant, independent of the spreading distance in the control channel (Fig. S1b), I_0 in the gate channel gradually decreased as the spreading progressed. This decrease in I_0 at the gate channel region indicates that TR-DHPE molecules were filtered while passing through the nano-gates.

The filtering phenomenon of spreading bilayers at nano-gates was discussed in regard to a local structural change of the self-spreading bilayer at the nano-gate. The structural change of the lipid bilayer leads to the modification of the chemical potential at the nano-gate, resulting in a highly localized difference in the molecule's solubility at that region, which is thought to be the origin of the molecular filter at the nano-gate. The dissipation energy of the self-spreading at the gate contributed to the structural change was appeared as a decrease in the velocity of self-spreading. In the present system, averaged velocities through the nano-gate region were estimated as 245, 196, 167, and 165 nm/s for the control, 218-nm Gate, 128-nm Gate, and 82-nm Gate channels, respectively.

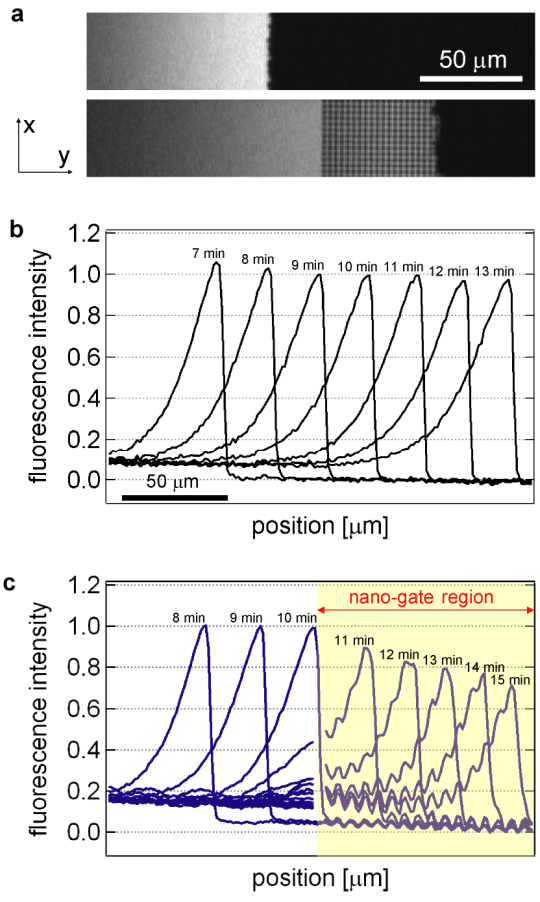

Figure S1. **Fluorescence microscopy observation of self-spreading lipid bilayer through Au nanogates.** a, Fluorescence microscope images of the leading edge of the spreading lipid bilayer (top) before arrival at the nano-gate and (bottom) during passage through the 128-nm Gate. b,c, Fluorescence intensity line profiles in (b) control channel and (c) 82-nm Gate channel.

Asymmetric distribution of TR-DHPE on the top layer.

Experimental Procedures: TR-DHPE chloroform solution was added to DMPC chloroform solution so that the final molecular fraction becomes 1/99 (TR-DHPE/DMPC). A small amount of mixed solution was deposited on a cleaned glass substrate. After evaporating the chloroform, the substrate was immersed in 100 mM Na_2SO_4 aqueous solution. The self-spreading behavior was observed by epi-fluorescence microscopy with high-pressure mercury lamp. After the immersion for 15 min, KI aqueous solution was added to the solution. Resulting KI concentration in the solution was ca. 500 mM.

Results and Discussion: Figure S2 demonstrated fluorescence microscope images and corresponding intensity line profiles before and after KI addition. As can be seen clearly from intensity line profiles, the fluorescence intensity of TR-DHPE was almost quenched by the KI addition. On the other hand, similar experiment using 1-palmitoyl-2-{12-[(7-nitro-2-1,3-benzoxadiaz ol-4-yl)amino]lauroyl}-sn-glycero-3-phosphocholine (NBD-PC, Avanti) revealed that the fluorescence from NBD was quenched only by half. Since iodide ion approaches and quenched the dye molecules on the top layer, the quenching ratio directly reveals the distribution ratio between top and bottom layers. Therefore, almost 90 % quenching of TR-DHPE indicates that 90 % of TR-DHPE are distributed on the top layer, whereas half quenching of NBD indicates that NBD distribute almost equally on both top and bottom layers. The difference between these two dye molecule are caused by a difference in bulkiness of dye moiety. Bulky TR group has high repulsive interaction with solid surface when they are situated in the bottom layer, thus causing a preferential distribution in the top layer. These results observed on the self-spreading lipid bilayer are in consistent with previous results on supported membrane system, indicating there is little or no effect of self-spreading on the dye distribution between the top and bottom layers.

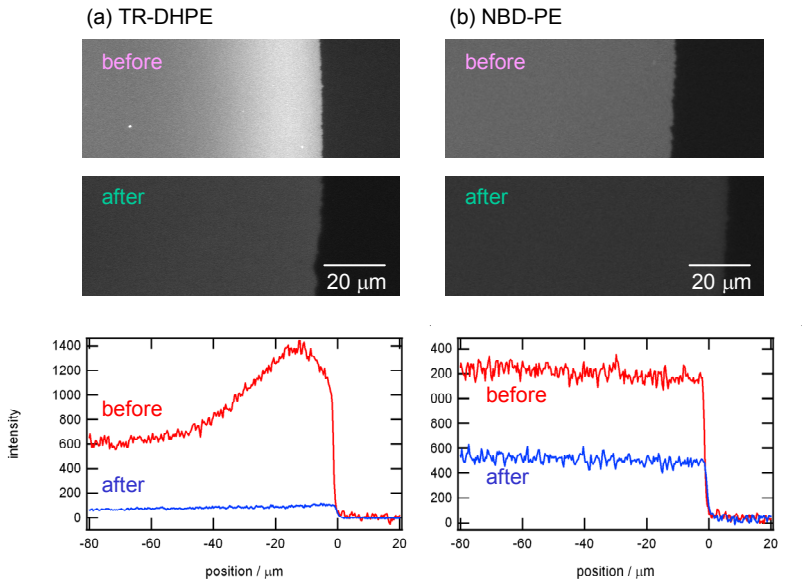

Figure S2. **Fluorescence microscopy observation of self-spreading lipid bilayer containing (a) TR-DHPE and (b) NBD-PE.** Fluorescence microscope images of the leading edge of the spreading lipid bilayer (top) before and (bottom) after KI addition are shown. Note that the image brightness and contrast were modified for clear appearance. Quantitative analysis can be made by intensity line profiles shown in the bottom panels.