

## Single Molecule Diffusion on Hard, Soft and Fluid Surfaces

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### Supplemental Information

To determine the lateral diffusion of lipid bilayers, fluorescent 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) ammonium (LR-PE), purchased from Avanti Polar Lipids, is used to mix with L- $\alpha$ -phosphatidylcholine ( $\alpha$ -PC) at a molar ratio of 1:10<sup>5</sup> to form a smooth and uniform lipid bilayer, whose morphology is displayed in Supplemental Fig. 1b. By using fluorescence correlation spectroscopy (FCS), an auto-correlation function of LR-PE in  $\alpha$ -PC lipid bilayer in Phosphate buffered saline (PBS) buffer solution is obtained as shown in Fig. 1c, yielding  $D_s = 3.0 \mu\text{m}^2/\text{s}$  in PBS aqueous solution as the lateral diffusion coefficient of  $\alpha$ -PC lipid bilayer by fitting  $G(\tau)$  with Eq 1.

**Supplemental Figure 1.** (a) The chemical structure of  $\alpha$ -PC and LR-PE, (b) fluorescence micrograph of mixed  $\alpha$ -PC and LR-PE lipid bilayer, and (c) measured normalized  $G(\tau)$  by  $G(0)$  of LR-PE in the mixed lipid bilayer.

