Force Spectroscopy of Single Receptor-Ligand Bond Using an Optical Trap

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Abstract: Here we developed an thermal fluctuation assay using optical trap technique, which enables to visualize consecutive binding–unbinding transition and to unravel the bond dissociation at low spring cosntant and low loading rate. This novel method provided further understandings in monitoring biophysics of receptor–ligand interactions.
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OCIS codes: (000.0000) General; (000.0000)

Cell adhesion mediated by receptor–ligand interactions is crucial to such biological processes as inflammatory reaction [1], tumor metastasis [2], arteriosclerosis [3], and wound healing [4]. To mediate cell adhesions, receptors and their ligands must be anchored onto two apposed surfaces, which is so-called two-dimensional (2D) interaction [5]. 2D association and dissociation kinetics governs the formation and rupture of surfacebound receptor–ligand bond. Direct measurement of 2D kinetics rates and force spectrum is indispensable to understand the biophysical bases of receptor–ligand interactions in regulating cell adhesions.

Here, the association and dissociation of interacting receptor–ligand molecules were investigated using two apposed functionalized microbeads in weak laser trap. Two specific issues have been addressed upon optical tweezer assay: 1) How is the association rate of receptor-ligand interaction measured directly? 2) How is the bond dissociation regulated at the low loading rate r_f ($<10^2$ pN/s) or the low spring constant k ($<10^{-1}$ pN/nm)?

As to the first issue, we developed a thermal fluctuation approach using optical trap set-up. The Brownian motion of a microbead in weak trap was monitored in real-time and the resulted displacement was used to identify sequential association and dissociation events of receptor-ligand bond (Fig. 1) which fits first-order association model (Eq.1) to obtain association rate k_f. Where $p_a(t_f)$ is the probability having a bond at time no less than t_f ($t \leq t_f$), $\overline{A_c}$ is the mean

$$p_a(t_f) = 1 - \exp\left[-\overline{A_c} m_r m_l k_f t_f\right] \qquad (1)$$

contact area since A_c varies in [0, t_f], and m_r and m_l are the site densities of receptor and ligand, respectively. Using
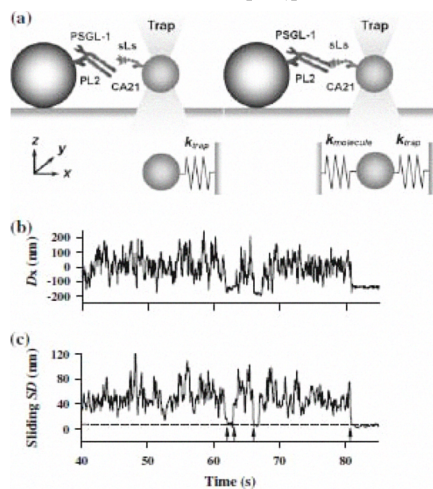

Fig. 1 (a) Schematic of thermal fluctuation approach using optical trap (not in scale). (b) Time course of displacement of selectin-coupled microbead along x-axis (D_x) on focus plane. (c) Time course of sliding standard deviation (SD) of D_x over a window size of ten frames.
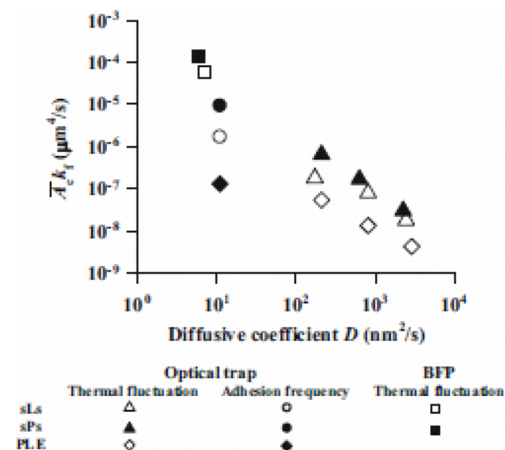
Fig. 2 Dependence of 2D effective forward rate $A_c k_f$ on diffusive coefficient D.

this approach, three selectin constructs (Soluble L-selectin (sLs), P-selectin (sPs) or PLE consisting of Lec-EGF domains) and their P-selectin glycoprotein ligand 1 (PSGL-1) were used to estimate the 2D association rate and the dependence of bond formation on carrier diffusion. Our results indicated that 2D association rate predicted upon first-order kinetics was in the order of sPs > sLs > PLE and enhancing the diffusivity of carrier reduced 2D association rate (Fig. 2).

As to the second issue, we used optical trap approach to quantify P-selectin and PSGL-1 bond rupture at $r_f \leq 188$ pN/s with low k (~10^{-3}-10^{-2} pN/nm) (Fig. 3). Our data indicated that most probable rupture force f^* retained the similar values when r_f increased up to 20 pN/s (Fig. 4 a). These data were different from those described previously at high r_f where f^* increased piecewise with r_f, implying that bond dissociation might follow distinctive mechanisms at low loading rates with low spring constants. It was also found that bond rupture force f varied with different combinations of k and v even at same r_f and most probable force f^* was enhanced with spring constant when $k <$ 47.0×10^{-3} pN/nm (Fig. 4 b), indicating that the bond dissociation at low r_f was spring constant-dependent and that bond rupture force depended on both the loading rate and the mechanical compliance of force transducer.
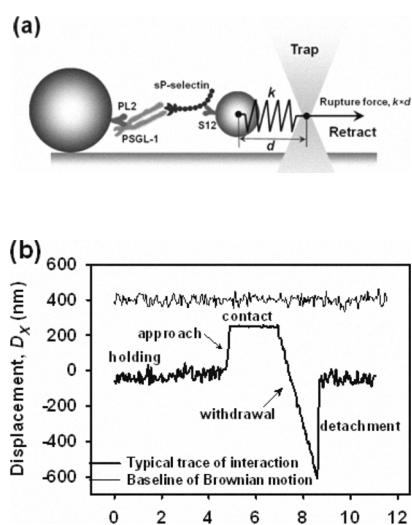

Fig. 3 (a) Illustration of functionally coated molecule pair for rupture force measurements. (b) Typical time course of off-center displacement for rupturing the P-selectin-PSGL-1 bond.
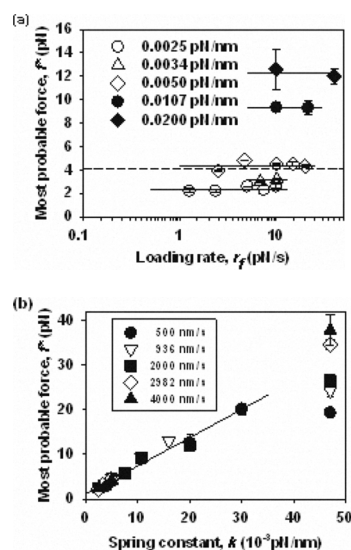
Fig. 4 (a) Independence of most probable force, f^*, on loading rates ($r_f \leq 20$ pN/s). (b) Dependence of the most probable force on spring constant.

These findings further quantitative understandings of cell interactions mediated by adhesive molecules.

References

[1] T. A. Springer, "Traffic Signals on Endothelium for Lymphocyte Recirculation and Leukocyte Emigration," Annual Review of Physiology 57, 827-872 (1995).

[2] S. M. Albelda, "Role of integrins and other cell adhesion molecules in tumor progression and metastasis," Lab Invest 68(1), 4-17 (1993).

[3] E. Galkina and K. Ley, "Vascular adhesion molecules in atherosclerosis," Arterioscler Thromb Vasc Biol 27(11), 2292-2301 (2007).

[4] F. Grinnell, "Wound repair, keratinocyte activation and integrin modulation," J Cell Sci 101 (Pt 1), 1-5 (1992).

[5] D. A. Hammer and D. A. Lauffenburger, "A dynamical model for receptor-mediated cell adhesion to surfaces," Biophys J 52(3), 475-487 (1987).