

Site-Selective Anatomy of Streptavidin/Avidin-Biotin Bonding Processes Using Dynamic Force Spectroscopy

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Dynamic Force Spectroscopy (DFS) has enabled us to investigate the specific interactions between two molecules, such as ligand-receptor pairs [1]. In DFS measurement, the unbinding force applied to a molecular bonding is increased at a constant rate, and the force required to rupture the molecular pair is measured. The energy landscape of the interactions is derived from the relationship between the rupture force and the loading rate.

However, the constant loading rate required for DFS experiment has not been realized because the force probe, in general, is retracted from the substrate at a constant velocity instead of constant loading rate, where it is difficult to analyze the landscape in detail. In order to achieve precise measurement, we have developed a system that has a feedback loop to keep the loading rate constant. The unbinding force applied to a molecular pair is detected using Atomic Force Microscopy, and the probe-substrate distance is precisely controlled with the feedback [2] (Figure 1).

Here, we demonstrate the results of the selective analysis of molecular interactions obtained using this method for the Biotin-Streptavidin and the Biotin-Avidin molecular systems.

Figure 2 shows the most frequent rupture forces plotted against the logarithmic form of the loading rate for the Biotin-Streptavidin. The potential-barrier position in the energy landscape is estimated from the slope of the linear relationship between the rupture force and the loading rate. For a 0.01 M phosphate (pH 7.4) solution, the potential barrier position was estimated to be 0.68 nm, which is formed by the phosphate molecules in buffer solution bridging between a streptavidin and a biotin. We changed the buffer solution from the phosphate to the several solutions. For the 0.05 M sodium nitrate solution, the slope changed at a loading rate of about 10^2 - 10^3 pN/s as shown in Fig. 2, and the barrier position estimated from the steep slope is 0.26 nm. The potential barrier position of 0.26nm is attributed to the potential barrier formed by the direct bonding between the biotin and streptavidin molecules. In contrast, the potential barrier position of the avidin was estimated to be 0.43 nm, and the slope was not changed for several solutions.

Details will be discussed at the conference.

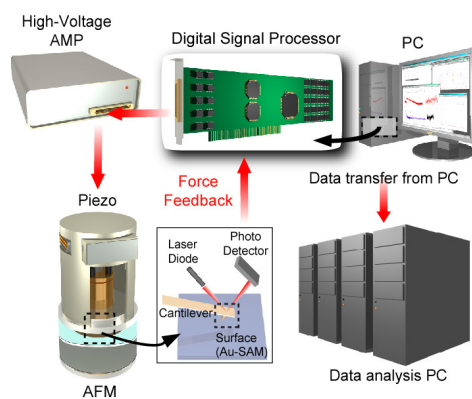


Figure 1 Schematic diagram of dynamic force spectroscopy system using a newly developed atomic force microscopy.

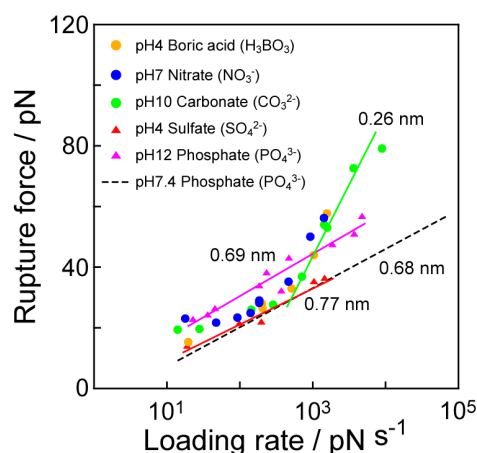


Figure 2 The most frequent rupture forces plotted against the loading rates.

References

- [1] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, *Nature* 397, (1999), 50.
- [2] O. Takeuchi, T. Miyakoshi, A. Taninaka, K. Tanaka, D. Cho, M. Fujita, S. Yasuda, S. P. Jarvis, H. Shigekawa *J. Appl. Phys.* 100, (2006), 074315.