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Reference: Yang, T., Baryshnikova, O. K., Mao, H., Holden. M. A., Cremer, P. S. "Investigations of Bivalent Antibody Binding on Fluid-Supported Phospholipid Membranes: The Effect of Hapten Density" *J. Am. Chem. Soc.*, **2003**, *125*, 4779-4784.

Bioanalysis and Sample: Cremer's group investigates the bivalent binding system between bivalent antibody, polyclonal anti-dinitrophenyl-KLH lgG antibody and monovalent hapten with N-dinitrophenyl-aminocaproyl phosphatidylethanolamine served as the hapten moiety. The interaction between antibody and hapten is a sequential two-step binding process. Apparent dissociation constant K_{Dapp} is determined at different hapten densities. Fraction of bivalent binding increases with hapten density. A very simple model is used to calculate the first and second dissociation constant K_{D1} ($2.46*10^{-5}$ M) and K_{D2} ($1.37*10^{-8}$ mol/m^2).

Importance: many diseases involve the attack of immune system. Some of these biological processes are multivalent binding process between antibody and antigen. So it is important to learn the mechanism of these processes and develop strategies for inhibiting them. The bivalent interaction between antibody and hapten has been studied intensely. However, previous research of this system is always time-consuming, expensive and the model used to calculate K_D is complicated which slow down the study. So the demand to develop a high-throughput and less expensive assay as well as a simple model is increasing. The author of this paper develops a microfluidic device which can run 12 samples simultaneously. The small volume of sample reduces the cost of the experiment. Also the model in the paper to calculate K_{D1} and K_{D2} is much simpler than former research.

Technique: the author uses a 12-channel microfluidic device as the platform of the assay. Glass and poly-(dimethylsiloxane) (PDMS), which are two main materials for microdevice, are also good supports for bio-molecule. A lipid bilayer with a function to mimic cell surface rearrangements is formed on the surface of each channel by infusing the solution containing the lipid bilayers. The haptens also in the solution associate with the bilayer and locate on the surface of bilayers. Then continuous flow of antibody with fluorescent tag is infused into the channel. Antibody interacts with the hapten and is attached to the surface. After reaching the equilibrium, the fluorescence intensity is measured by epifluorescence microscopy. By increasing the concentration of antibody in the solution, the density of attached antibody increases as well as fluorescence intensity. When all the surface sites are binding with the antibody, the fluorescence intensity reaches its maximum. Apparent dissociation constant K_{Dapp} equals the antibody concentration when the fluorescence intensity is half of the maximum intensity. K_{Dapp} decreasing with hapten density indicates the increasing of bivalent binding fraction. The first and second dissociation constants are determined by studying the relationship between K_{Dapp} and hapten density. It is interesting that K_{D2} is lower than K_{D1} which is different from bulk phase. The bigger K_{D1} is due to high extropic cost for antibody and hapten orientation.

Example of Results: Figure 1 shows that K_{Dapp} decreases with hapten density. This is because the decrease of monovalent binding fraction and increase of bivalent binding fraction. The data can fit the non-linear regression equation 1 derived in the literature.

$$K_{\mathrm{Dapp}} = \frac{K_{\mathrm{D1}}K_{\mathrm{D2}}}{K_{\mathrm{D2}} + 2[\mathrm{L}]_{\mathrm{s}}} \quad \textbf{(1)}$$

where [L]s is the surface density of available binding sites.

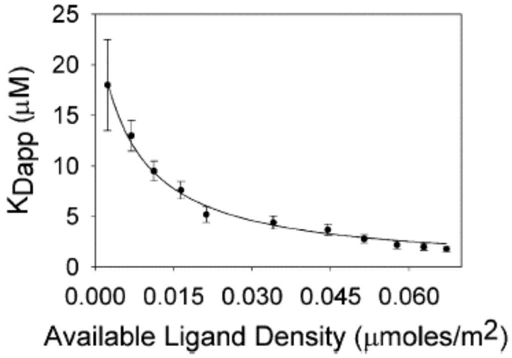

Figure 1 relationship between K_{Dapp} and [L]s

Opinion: the author takes the advantage of microfluidic system to build a platform for quick and less expensive biointeraction study. The K_D determination is much simpler. However, the high background fluorescence causes a big error when hapten density is small which probably affect the accuracy of the measurement.