Journal of Structural Biology 168 (2009) 90–94

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Multivalent ligand–receptor binding on supported lipid bilayers

Hyunsook Jung, Aaron D. Robison, Paul S. Cremer *

Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, TX 77843-3012, USA

ARTICLE INFO

Article history:
Received 4 November 2008
Received in revised form 22 April 2009
Accepted 27 May 2009
Available online 7 June 2009

Keywords:
Supported lipid bilayers
Multivalent ligand–receptor binding
Microfluidic platforms
Equilibrium dissociation constants
Total internal reflection fluorescence microscopy

ABSTRACT

Fluid supported lipid bilayers provide an excellent platform for studying multivalent protein–ligand interactions because the two-dimensional fluidity of the membrane allows for lateral rearrangement of ligands in order to optimize binding. Our laboratory has combined supported lipid bilayer-coated microfluidic platforms with total internal reflection fluorescence microscopy (TIRFM) to obtain equilibrium dissociation constant (K_D) data for these systems. This high throughput, on-chip approach provides highly accurate thermodynamic information about multivalent binding events while requiring only very small sample volumes. Herein, we review some of the most salient findings from these studies. In particular, increasing ligand density on the membrane surface can provide a modest enhancement or attenuation of ligand–receptor binding depending upon whether the surface ligands interact strongly with each other. Such effects, however, lead to little more than one order of magnitude change in the apparent K_D values. On the other hand, the lipophilicity and presentation of lipid bilayer-conjugated ligands can have a much greater impact. Indeed, changing the way a particular ligand is conjugated to the membrane can alter the apparent K_D value by at least three orders of magnitude. Such a result speaks strongly to the role of ligand availability for multivalent ligand–receptor binding.

1. Introduction

Phospholipid membranes demarcate the interface between the inside of living cells and their external environment. Their two-dimensional fluidity enables a great variety of complex ligand–receptor binding processes to occur. This includes pathogen attack, the trafficking of lymphocytes, the response of the immune system, and the signaling of cells (Heldin, 1995; Kiessling and Pohl, 1996; Mammen et al., 1998; Sabesan et al., 1992). Multivalent ligand–receptor binding interactions have been thought to augment weak individual binding events to achieve tighter binding with higher selectivity (Choi, 2004; Kiessling et al., 2006). Indeed, multivalent binding creates expanded contact between binding surfaces (Mammen et al., 1998). As such, multivalent strategies have been used clinically to improve immunotherapeutics for cancer treatment (Sulchek et al., 2005).

The binding of proteins and viruses with multiple binding sites to membrane interfaces typically results in the reorganization of membrane-bound components. Many inhibitory drugs function by disrupting these interactions, especially by binding to components within the membrane (Liu et al., 2006; Morgenstern, 1998). Understanding multivalent interactions as a function of membrane chemistry should provide critical insight into biological recognition. The information gleaned from such work could be used to improve drug design including the inhibition of viral entry and the termination of cancer metastasis (Long et al., 2008). A proper understanding of multivalent ligand–receptor interactions is also of great importance for the development of highly selective biosensor devices that exploit these interactions.

Various model systems have been used to mimic the cell surface environment in multivalent binding studies. Three common platforms are self-assembled monolayers (SAM) (Rao et al., 1999; Smith et al., 2003), phospholipid vesicles (Lee et al., 2005), and supported lipid bilayers (Doyle et al., 2003; Pisarchick and Thompson, 1990; Yang et al., 2003). Both vesicles and supported bilayers are composed of the same lipid molecules found in the plasma membranes of living cells. Moreover, they possess the same two-dimensional fluidity as cell membranes (Cremer and Boxer, 1999), and are capable of mimicking the lateral rearrangements that take place *in vivo* on the cell surface (Hlavacek et al., 1999). For studies of multivalency, the supported bilayer systems are particularly useful because they can be interrogated by a wide variety of surface specific microscopies and spectroscopies. It is known that a thin water layer (approximately 0.5–1.5 nm in thickness) generally resides between the lower leaflet of a supported bilayer and the underlying substrate. This enables individual lipid molecules to facilely translate along the surface (Kim et al., 2001). Therefore, several ligand molecules within the membrane can bind to a single aqueous protein with multiple binding sites simply by undergoing two-dimensional rearrangement (Fig. 1). Since a wide variety of lipid-conjugated ligands can be incorporated into the membrane, it is possible to study the effects of their specific chemistry and presentation on multivalent binding in a highly controlled manner.

* Corresponding author. Fax: +1 979 845 7561.
E-mail address: cremer@mail.chem.tamu.edu (P.S. Cremer).

1047-8477/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2009.05.010

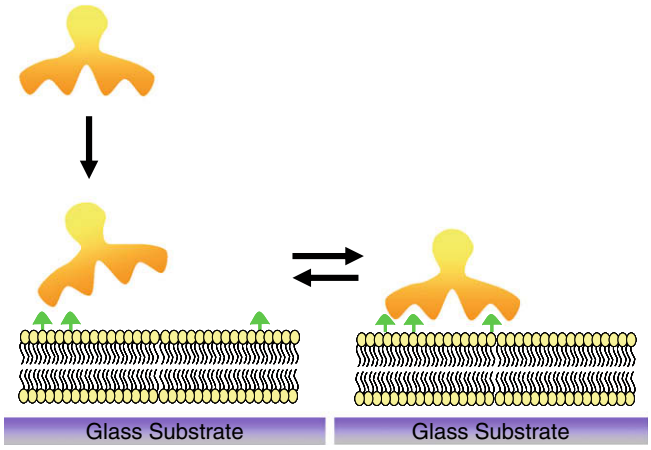

Fig. 1. Schematic illustration of a fluid supported lipid bilayer facilitating a bivalent ligand–receptor binding event. The ligands (in green) undergo lateral rearrangement within the fluid lipid bilayer to bind to an antibody (in orange) in a two-step process.

Results for several different hapten–antibody and ganglioside–toxin interactions are provided below. These model system studies demonstrate that ligand presentation is more important than ligand density in determining the overall protein affinity for the membrane surface.

2. High-throughput microfluidic devices

Exploiting microfluidic devices for the quantitative investigation of multivalent ligand–receptor interactions in lipid membranes was established by our laboratory over the past decade (Yang et al., 2001). Traditional binding measurements had previously been done using a standard flow cell geometry (Kalb et al., 1990). Such experiments usually required long periods of time to make sequential binding measurements as well as large sample volumes of protein solutions. Consequently, limited information about ligand–receptor interactions could be abstracted from a given set of measurements. By contrast, microfluidic platforms provided a high-throughput/low sample volume approach to such measurements. Moreover, binding data at multiple protein concentrations can be collected simultaneously. Therefore, these methods often avoid several sources of noise associated with temporal variations in illumination intensities from an arc lamp source as well as detector drift.

It should be noted that multivalent ligand–receptor binding interactions have been studied by a wide variety of techniques from total internal reflection fluorescence microscopy (Pisarchick and Thompson, 1990) and isothermal titration calorimetry (Goins and Freire, 1988) to surface plasmon resonance spectroscopy (Terrettaz et al., 1993) and quartz crystal microbalance analysis (Janshoff et al., 1997). Flow cytometry (Lauer et al., 2002), fluoroimmunoassays (Singh et al., 2000), fluorescence resonance energy transfer (Ma and Cheng, 2006), atomic force microscopy (Rinker et al., 2008; Sulchek et al., 2005), and a colloid particle phase transition method (Baksh et al., 2004) have also been used. We have largely relied on fluorescence-based methods, which are compatible with our microfluidic approach. The typical setup employed in these ligand–receptor binding studies is illustrated in Fig. 2. Generally, the same bilayer chemistry is present in each channel, but the bulk solution contains various protein concentrations. By imaging all the microchannels simultaneously, it is possible to watch an entire binding curve evolve over time (Jung et al., 2008).

3. The influence of ligand density of multivalent protein binding

The specific binding of antibodies to their target antigens on cell membrane surfaces is a key step in immune response. A common approach for studying antibody–antigen interactions has been to use phospholipid membranes containing hapten-conjugated phosphatidylethanolamine (Thompson et al., 1993). Common model haptens include dinitrophenyl (DNP), trinitrophenyl (TNP), nitroxide, dinitrophenyl nitroxide, fluorescein, and biotin. All of these moieties have been found to be active in antibody binding studies (Hafeman et al., 1981; Pisarchick and Thompson, 1990; Tamm, 1988; Tamm and Bartoldus, 1988; Wright et al., 1988).

Antibodies may bind either monovalently or bivalently to the corresponding antigenic surface. McConnell and co-workers first claimed that IgG molecules interact with the membrane through

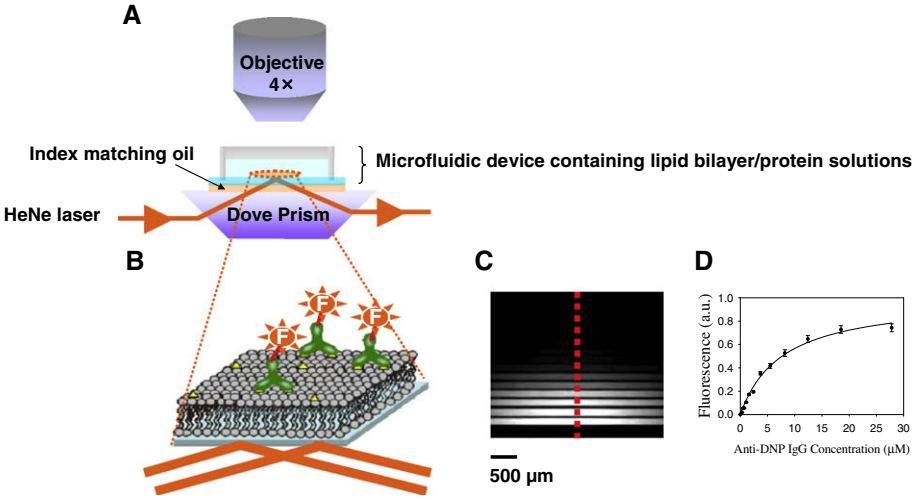

Fig. 2. (A) Schematic diagram of the experimental setup for performing ligand–receptor binding measurements in microfluidic devices. The device consists of a polydimethylsiloxane (PDMS)/glass multi-channel microfluidic device. The index matching oil is introduced between the device and the prism to allow TIRFM measurements to be made. (B) Schematic representation of dye-labeled proteins adsorbed on the supported bilayer surface. The evanescent field generated at the liquid/solid interface (orange lines) excites fluorescent molecules adjacent to the interface with high specificity. (C) Total internal reflection fluorescence micrograph of a working device. The amount of adsorbed protein within a given channel is linearly proportional to the intensity of fluorescence. (D) A plot of fluorescence intensity (from red dotted line in (C)) vs. bulk protein concentration can be used to construct a standard binding isotherm from which K_D values can be abstracted.

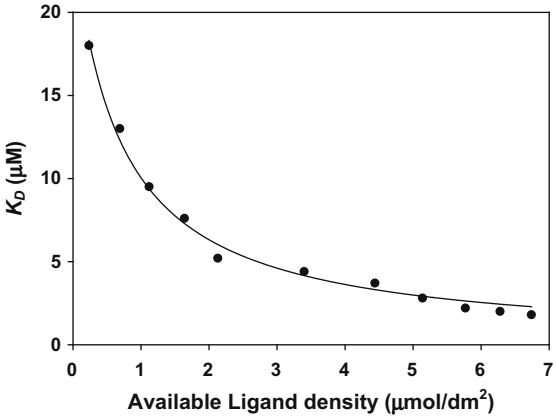

Fig. 3. K_D vs. available DNP ligand density for anti-DNP binding to hapten-conjugated membranes. Reproduced from Yang et al. (2003).

both of their binding sites (Parce et al., 1979). Thompson and co-workers estimated that 10–50% of the IgGs were bound monovalently in their studies (Pisarchick and Thompson, 1990). These investigators also found that the apparent equilibrium dissociation constant (K_D) of the anti-DNP antibody was ~10-fold stronger than the corresponding Fab fragment under conditions with very high lipid-conjugated DNP concentrations. It should be noted, however, that ligand density should play a role in ligand–receptor binding affinity since both the ligand distribution and inter-ligand distance (Huskens et al., 2004) might affect the ability of IgG molecules to become bivalently bound to the membrane.

The dependence of K_D values for antigen–antibody binding on supported membranes was first noted by Tamm and co-workers from studies at three different ligand densities (Kalb et al., 1990; Tamm and Bartoldus, 1988). Our laboratory made binding isotherm measurements for the DNP/anti-DNP system at 11 different ligand densities ranging from 0.1 to 5 mol% by employing microfluidic platforms (Yang et al., 2003). These experiments demonstrated that the K_D value tightened in a systematic manner according to a hyperbolic function as the ligand densities was increased from 0.1 to 5.0 mol% (Fig. 3). Specifically, we were able to abstract the individual binding constants for the sequential two-step binding process: $K_{D1} = 25\ \mu M$ and $K_{D2} = 1.38 \times 10^{-6}$ mol/dm^2. Such results clearly indicate that the binding of an antibody to a membrane surface is enhanced by the second binding event. This corresponds to a positively cooperative binding effect with an entropic difference between the two binding events of $\Delta S = 24.1$ J/(mol K). Although IgG molecules are largely monovalently bound at low hapten density (74% at 0.1 mol% of DNP), nearly all the antibodies are bivalently bound at higher ligand density (91% at 5 mol% DNP). Moreover, it was found that increasing the density of DNP-PE in phospholipid membranes from 0.1 to 5.0 mol % strengthened the apparent K_D value by roughly a factor of 13 (Yang et al., 2003). This is a rather modest increase given the fact that the ligand density was increased by a factor of 50.

4. GM_1 clustering inhibits cholera toxin binding

In addition to studies of antibody–hapten binding, we also explored the binding of ganglioside GM_1 to cholera toxin, which is an AB_5 toxin (Cuatrecasas, 1973; Gill, 1976). The reason this system was chosen was because the valency of binding was five instead of two as was the case for DNP/anti-DNP. Therefore, one might expect a much more substantial strengthening of the K_D value as the ligand density is increased. Experiments were conducted inside microfluidic channels using phospholipid bilayers containing 0.02–10.0 mol% GM_1 (Shi et al., 2007). Instead of showing strengthening, however, the equilibrium dissociation constant actually weakened by about one order of magnitude as the ligand density was increased 50-fold (Fig. 4A).

These results could be explained by ligand–ligand interactions involving the clustering of the glycolipids into islands within the bilayer (Fig. 4B). Indeed, corresponding atomic force microscopy studies confirmed the presence of ganglioside islands. The diameter of these islands increased with increasing ligand concentration. In fact, the change in mean island diameter was highly correlated with the weakening of the K_D value found from the binding measurements. Such a finding is strong evidence that ligand–ligand interactions within the membrane can actually inhibit binding for strongly interacting ligands. GM_1–GM_1 interactions are undoubtedly related to hydrogen bonding between adjacent oligosaccharide headgroups (Peters and Grant, 1984; Sharom and Grant, 1978).

The results for the GM_1–cholera toxin system are highly significant because they demonstrate that merely increasing ligand density within a membrane is no guarantee that the binding affinity for a multivalent protein will increase. It has been hypothesized that ligand densities within cell membranes can vary. For example, certain ligands may be present at higher concentrations within lipid raft regions. Such higher number densities may increase the number of proteins that are bound, even if the free energy of binding were somewhat attenuated (van der Goot and Harder, 2001; Wolf et al., 1998).

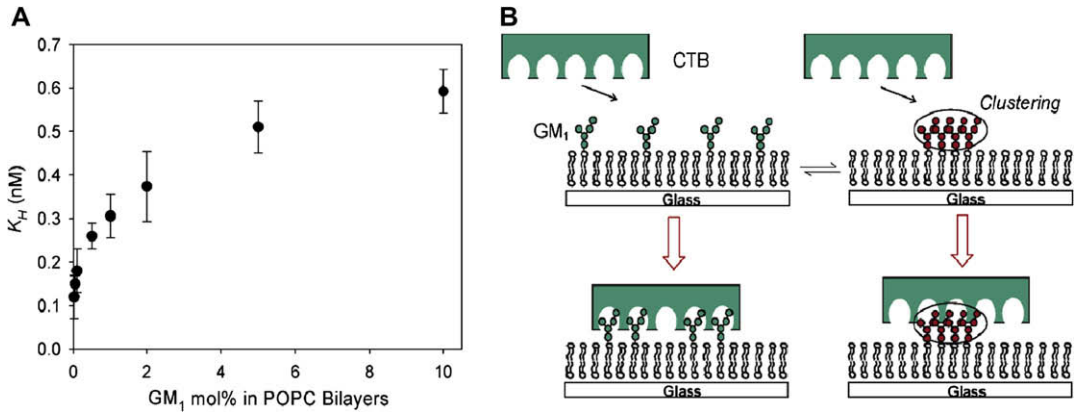

Fig. 4. (A) K_H vs. GM_1 concentration. K_H is the apparent equilibrium dissociation constant obtained by fitting to a Hill–Waud binding model (Lencer et al., 1987). (B) Schematic representation of the ligand clustering effect for cholera toxin binding to GM_1 in supported lipid bilayers. Reproduced from Shi et al. (2007).

5. Ligand presentation can strongly modulate protein–ligand binding

Although our cholera toxin–GM_1 studies showed evidence for binding inhibition with increased ligand density, the effects were not very pronounced. Indeed, one order of magnitude weakening or tightening in the dissociation constant is rather modest. Moreover, relatively large changes in ligand density were required to achieve such effects. Therefore, one must search for other factors that can influence the strength of multivalent binding between aqueous proteins and membrane surfaces. To address this question, studies of ligand presentation and lipophilicity were undertaken. It should be noted that the cell membrane interface itself has been thought to affect ligand presentation (Leckband et al., 1995). In fact, ligand molecules may interact with the interior of the fluid lipid bilayers, rendering them less available to incoming proteins (Balakrishnan et al., 1982a,b). Moreover, since cell surfaces are typically covered by a glycocalix (Schneider et al., 2002), interference with binding by aqueous proteins may occur (Albersdörfer et al., 1997; Dori et al., 2000; Kim et al., 2000; Moore and Kuhl, 2006). These qualitative notions led us to ask several questions. First, how large of an influence can ligand lipophilicity have upon protein binding? Second, how large could the effect of a glycocalix mimic be on protein binding? Finally, is it possible to substantially alter the free energy of binding through the placement of ligands at various positions within the membrane?

To answer the questions posed above, antibody–antigen binding experiments were performed at phospholipid membrane surfaces with two different haptens (Jung et al., 2008). The first hapten was biotin, which has an octanol–water partition coefficient (log *P* value) of 0.11. The second hapten was DNP with a partition coefficient of 1.5. Therefore, the DNP is expected to be far more lipophilic than the biotin moiety. In a first set of experiments, K_D values for IgG binding to the respective hapten moieties were determined in bulk solution by fluorescence anisotropy measurements (Fig. 5A). The results clearly showed that both haptens bound with almost the identical K_D values to their respective antibodies. Moreover, these bulk K_D values were in the low nanomolar range as expected. On the other hand, measurements made with lipid-conjugated haptens on supported bilayers revealed that the equilibrium dissociation constant was three orders of magnitude tighter for anti-biotin than for anti-DNP (Fig. 5B). This pronounced difference can be attributed to the relative propensity of the DNP moiety to become buried within the aliphatic portions of the lipid bilayer and thereby be unavailable to the incoming anti-DNP antibody. By contrast, the biotin contains an ureido moiety (–NH–CO–NH–), which should lead to a preference for residing in the aqueous phase.

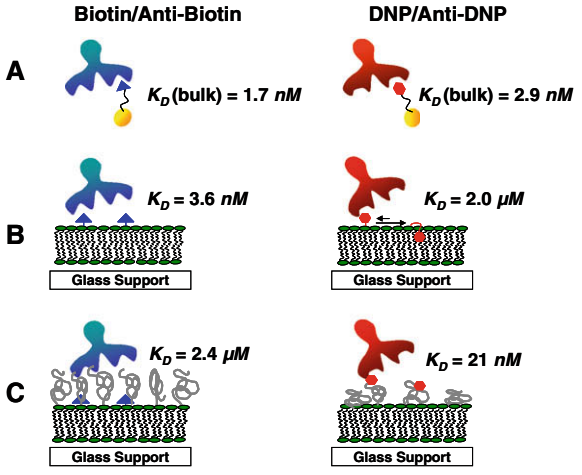

Fig. 5. Schematic diagram presenting the influence of various parameters on anti-DNP/DNP and anti-biotin/biotin interactions. (A) Binding between the IgG molecules and their respective haptens in bulk solution. (B) Binding between IgG molecules and haptens on supported bilayers. (C) PEG-coated lipid bilayer surfaces effectively hindering biotin/anti-biotin binding while enhancing the DNP/anti-DNP binding because of specific hapten presentation. Reproduced from Jung et al. (2008).

In a second set of experiments, we wished to see whether the binding affinities for anti-biotin and anti-DNP could be reversed by changing the way their respective haptens are presented on the surface of the phospholipid bilayer. To weaken anti-biotin binding, a polyethylene glycol (PEG) cushion was added to the membrane through direct conjugation of the polymer chain to phosphatidylethanolamine headgroups (Fig. 5C). Results from this system clearly showed that the K_D value for biotin/anti-biotin binding was weakened by approximately three orders of magnitude. On the other hand, DNP/anti-DNP binding could be strengthened by the inclusion of the polymer film. In this case, however, the DNP moiety was conjugated at the end of the polymer chain instead of on a lipid headgroup (Fig. 5C). This strengthened the K_D value by roughly two orders of magnitude. Such results are in line with the notion that that a sufficiently long, flexible, and hydrophilic ethylene oxide linker can maximize the binding interaction (Ahlers et al., 1992; Leckband et al., 1995). On the other hand, short hydrophobic spacers should weaken binding (Cooper et al., 1981; Kimura et al., 1990).

The results described in the studies outlined above clearly demonstrate that ligand presentation in simple model systems is generally more effective for altering interfacial binding events, as compared to modulating ligand density or clustering. These findings might have implications for the binding of proteins at the surfaces of cell membranes. However, future work will need to be undertaken *in vivo* to test the hypothesis that the ligand's lipophilicity or its presentation within the cell membrane's glycocalix are indeed more important factors in determining binding strength than either its number density or its ability to cluster with neighboring moieties.

6. Summary and outlook

The thermodynamic binding process is now well understood for a few model systems such as the ones discussed here. Nevertheless, a general understanding of the molecular level mechanisms governing multivalent binding interactions and the associated lateral rearrangements which take place within membranes is still far from complete. One intriguing direction is to try to obtain data at the single molecule level as individual binding events are inherently stochastic. Recently, multivalent biomolecular interactions have been visualized at the single molecule level (Rinker et al., 2008). Moreover, individual monovalent and bivalent events have been separately detected using picoampere current measurements in association with protein pores (Howorka et al., 2004). Finally, it should be noted that cell membranes represent complex heterogeneous mixtures of ligands and receptors with the potential for many simultaneous interactions. Therefore, future studies with model systems will need to ever more closely mimic the actual cellular environment in order to fully appreciate the role that lipid and protein chemistry play in these binding events.

Acknowledgments

We thank Dr. Tinglu Yang for useful discussions. This work was funded by the National Institutes of Health (GM070622) and the Robert A. Welch Foundation (Grant A-1421).

References

Ahlers, M., Grainger, D.W., Herron, J.N., Lim, K., Ringsdorf, H., Salesse, C., 1992. Quenching of fluorescein-conjugated lipids by antibodies. Quantitative recognition and binding of lipid-bound haptens in biomembrane models, formation of two-dimensional protein domains and molecular dynamics simulations. Biophys. J. 63, 823–838.

Albersdörfer, A., Feder, T., Sackmann, E., 1997. Adhesion-induced domain formation by interplay of long-range repulsion and short-range attraction force. A model membrane study. Biophys. J. 73, 245–257.

Baksh, M.M., Jaros, M., Groves, J.T., 2004. Detection of molecular interactions at membrane surfaces through colloid phase transitions. Nature 427, 139–141.

Balakrishnan, K., Mehdi, S.Q., McConnell, H.M., 1982a. Availability of dinitrophenylated lipid haptens for specific antibody binding depends on the physical properties of host bilayer membranes. J. Biol. Chem. 257, 6434–6439.

Balakrishnan, K., Hsu, F.J., Cooper, A.D., McConnell, H.M., 1982b. Lipid hapten containing membrane targets can trigger specific immunoglobulin E-dependent degranulation of rat basophil leukemia cells. J. Biol. Chem. 257, 6427–6433.

Choi, S.K., 2004. Synthetic Multivalent Molecules. Wiley, Hoboken, NJ.

Cooper, A.D., Balakrishnan, K., McConnell, H.M., 1981. Mobile haptens in liposomes stimulate serotonin release by rat basophil leukemia cells in the presence of specific immunoglobulin E. J. Biol. Chem. 256, 9379–9381.

Cremer, P.S., Boxer, S.G., 1999. Formation and spreading of lipid bilayers on planar glass supports. J. Phys. Chem. B 103, 2554–2559.

Cuatrecasas, P., 1973. *Vibrio cholerae* choleragenoid. Mechanism of inhibition of cholera toxin action. Biochemistry 12, 3577–3581.

Dori, Y., Bianco-Peled, H., Satija, S.K., Fields, G.B., McCarthy, J.B., Tirrell, M., 2000. Ligand accessibility as means to control cell response to bioactive bilayer membranes. J. Biomed. Mater. Res. 50, 75–81.

Doyle, E.L., Hunter, C.A., Phillips, H.C., Webb, S.J., Williams, N.H., 2003. Cooperative binding at lipid bilayer membrane surfaces. J. Am. Chem. Soc. 125, 4593–4599.

Gill, D.M., 1976. The arrangement of subunits in cholera toxin. Biochemistry 15, 1242–1248.

Goins, B., Freire, E., 1988. Thermal-stability and intersubunit interactions of cholera-toxin in solution and in association with its cell-surface receptor ganglioside GM_1. Biochemistry 27, 2046–2052.

Hafeman, D.G., Von Tscharner, V., McConnell, H.M., 1981. Specific antibody-dependent interactions between macrophages and lipid haptens in planar lipid monolayers. Proc. Natl. Acad. Sci. USA 78, 4552–4556.

Heldin, C.H., 1995. Dimerization of cell surface receptors in signal transduction. Cell 80, 213–223.

Hlavacek, W.S., Posner, R.G., Perelson, A.S., 1999. Steric effects on multivalent ligand–receptor binding: exclusion of ligand sites by bound cell surface receptors. Biophys. J. 76, 3031–3043.

Howorka, S., Nam, J., Bayley, H., Kahne, D., 2004. Stochastic detection of monovalent and bivalent protein–ligand interactions. Angew. Chem. Int. Ed. 43, 842–846.

Huskens, J., Mulder, A., Auletta, T., Nijhuis, C.A., Ludden, M.J.W., Reinhoudt, D.N., 2004. A model for describing the thermodynamics of multivalent host–guest interactions at interfaces. J. Am. Chem. Soc. 126, 6784–6797.

Janshoff, A., Steinem, C., Sieber, M., elBaya, A., Schmidt, M.A., Galla, H.J., 1997. Quartz crystal microbalance investigation of the interaction of bacterial toxins with ganglioside containing solid supported membranes. Eur. Biophys. J. 26, 261–270.

Jung, H., Yang, T., Lasagna, M.D., Shi, J., Reinhart, G.D., Cremer, P.S., 2008. Impact of hapten presentation on antibody binding at lipid membrane interfaces. Biophys. J. 94, 3094–3103.

Kalb, E., Engel, J., Tamm, L.K., 1990. Binding of proteins to specific target sites in membranes measured by total internal reflection fluorescence microscopy. Biochemistry 29, 1607–1613.

Kiessling, L.L., Pohl, N.L., 1996. Strength in numbers: non-natural polyvalent carbohydrate derivatives. Chem. Biol. 3, 71–77.

Kiessling, L.L., Gestwicki, J.E., Strong, L.E., 2006. Synthetic multivalent ligands as probes of signal transduction. Angew. Chem. Int. Ed. 45, 2348–2368.

Kim, D.H., Klibanov, A.L., Needham, D., 2000. The influence of tiered layers of surface-grafted poly(ethylene glycol) on receptor–ligand-mediated adhesion between phospholipid monolayer-stabilized microbubbles and coated glass beads. Langmuir 16, 2808–2817.

Kim, J., Kim, G., Cremer, P.S., 2001. Investigations of water structure at the solid/liquid interface in the presence of supported lipid bilayers by vibrational sum frequency spectroscopy. Langmuir 17, 7255–7260.

Kimura, K., Arata, Y., Yasuda, T., Kinosita, K., Nakanishi, M., 1990. Location of membrane-bound hapten with different length spacers. Immunology 69, 323–328.

Lauer, S., Goldstein, B., Nolan, R.L., Nolan, J.P., 2002. Analysis of cholera toxin–ganglioside interactions by flow cytometry. Biochemistry 41, 1742–1751.

Leckband, D.E., Kuhl, T., Wang, H.K., Herron, J., Mueller, W., Ringsdorf, H., 1995. 4-4-20 Anti-fluorescyl IgG Fab' recognition of membrane-bound hapten: direct evidence for the role of protein and interfacial structure. Biochemistry 34, 11467–11478.

Lee, H.K., Park, K.M., Jeon, Y.J., Kim, D., Oh, D.H., Kim, H.S., Park, C.K., Kim, K., 2005. Vesicle formed by amphiphilic cucurbit[6]uril: versatile, noncovalent modification of the vesicle surface, and multivalent binding of sugar-decorated vesicles to lectin. J. Am. Chem. Soc. 127, 5006–5007.

Lencer, W.I., Chu, S.H.W., Walker, W.A., 1987. Differential binding kinetics of cholera toxin to intestinal microvillus membrane during development. Infect. Immunol. 55, 3126–3130.

Liu, X.J., Liang, A.Y., Shen, Z., Liu, X., Zhang, Y., Dai, Z.P., Xiong, B.H., Lin, B.C., 2006. Studying drug–plasma protein interactions by two-injector microchip electrophoresis frontal analysis. Electrophoresis 27, 5128–5131.

Long, D.D., Aggen, J.B., Christensen, B.G., Judice, J.K., Hegde, S.S., Kaniga, K., Krause, K.M., Linsell, M.S., Moran, E.J., Pace, J.L., 2008. A multivalent approach to drug discovery for novel antibiotics. J. Antibiot. 61, 595–602.

Ma, G.Y., Cheng, Q., 2006. Manipulating FRET with polymeric vesicles: development of a "mix-and-detect" type fluorescence sensor for bacterial toxin. Langmuir 22, 6743–6745.

Mammen, M., Choi, S.K., Whitesides, G.M., 1998. Polyvalent interactions in biological systems: implications for design and use of multivalent ligands and inhibitors. Angew. Chem. Int. Ed. 37, 2754–2794.

Moore, N.W., Kuhl, T.L., 2006. Bimodal polymer mushrooms: compressive forces and specificity toward receptor surfaces. Langmuir 22, 8485–8491.

Morgenstern, R., 1998. A simple alternate substrate test can help determine the aqueous or bilayer location of binding sites for hydrophobic ligands/substrates on membrane proteins. Chem. Res. Toxicol. 11, 703–707.

Parce, J.W., Schwartz, M.A., Owicki, J.C., McConnell, H.M., 1979. Kinetics of antibody association with spin-label haptens on membrane surfaces. J. Phys. Chem. 83, 3414–3417.

Peters, M.W., Grant, C.W., 1984. Freeze-etch study of an unmodified lectin interacting with its receptors in model membranes. Biochim. Biophys. Acta 775, 273–282.

Pisarchick, M.L., Thompson, N.L., 1990. Binding of a monoclonal-antibody and its Fab fragment to supported phospholipid monolayers measured by total internal reflection fluorescence microscopy. Biophys. J. 58, 1235–1249.

Rao, J.H., Yan, L., Xu, B., Whitesides, G.M., 1999. Using surface plasmon resonance to study the binding of vancomycin and its dimer to self-assembled monolayers presenting D-Ala-D-Ala. J. Am. Chem. Soc. 121, 2629–2630.

Rinker, S., Ke, Y.G., Liu, Y., Chhabra, R., Yan, H., 2008. Self-assembled DNA nanostructures for distance-dependent multivalent ligand–protein binding. Nat. Nanotechnol. 3, 418–422.

Sabesan, S., Duus, J.O., Neira, S., Domaille, P., Kelm, S., Paulson, J.C., Bock, K., 1992. Cluster sialoside inhibitors for influenza virus: synthesis, NMR, and biological studies. J. Am. Chem. Soc. 114, 8363–8375.

Schneider, M.F., Lim, K., Fuller, G.G., Tanaka, M., 2002. Rheology of glycocalix model at air/water interface. Phys. Chem. Chem. Phys. 4, 1949–1952.

Sharom, F.J., Grant, C.W., 1978. Model for ganglioside behavior in cell membranes. Biochim. Biophys. Acta 507, 280–293.

Shi, J., Yang, T., Kataoka, S., Zhang, Y., Diaz, A.J., Cremer, P.S., 2007. GM_1 clustering inhibits cholera toxin binding in supported phospholipid membranes. J. Am. Chem. Soc. 129, 5954–5961.

Singh, A.K., Harrison, S.H., Schoeniger, J.S., 2000. Gangliosides as receptors for biological toxins: development of sensitive fluoroimmunoassays using ganglioside-bearing liposomes. Anal. Chem. 72, 6019–6024.

Smith, E.A., Thomas, W.D., Kiessling, L.L., Corn, R.M., 2003. Surface plasmon resonance imaging studies of protein–carbohydrate interactions. J. Am. Chem. Soc. 125, 6140–6148.

Sulchek, T.A., Friddle, R.W., Langry, K., Lau, E.Y., Albrecht, H., Ratto, T.V., DeNardo, S.J., Colvin, M.E., Noy, A., 2005. Dynamic force spectroscopy of parallel individual mucin1–antibody bonds. Proc. Natl. Acad. Sci. USA 102, 16638–16643.

Tamm, L.K., 1988. Lateral diffusion and fluorescence microscope studies on a monoclonal antibody specifically bound to supported phospholipid bilayers. Biochemistry 27, 1450–1457.

Tamm, L.K., Bartoldus, I., 1988. Antibody binding to lipid model membranes. The large ligand effect. Biochemistry 27, 7453–7458.

Terrettaz, S., Stora, T., Duschl, C., Vogel, H., 1993. Protein binding to supported lipid membranes—investigation of the cholera toxin–ganglioside interaction by simultaneous impedance spectroscopy and surface plasmon resonance. Langmuir 9, 1361–1369.

Thompson, N.L., Poglitsch, C.L., Timbs, M.M., Pisarchick, M.L., 1993. Dynamics of antibodies on planar model membranes. Acc. Chem. Res. 26, 567–573.

van der Goot, F.G., Harder, T., 2001. Raft membrane domains: from a liquid-ordered membrane phase to a site of pathogen attack. Semin. Immunol. 13, 89–97.

Wolf, A.A., Jobling, M.G., Wimer-Mackin, S., Ferguson-Maltzman, M., Madara, J.L., Holmes, R.K., Lencer, W.I., 1998. Ganglioside structure dictates signal transduction by cholera toxin and association with caveolae-like membrane domains in polarized epithelia. J. Cell Biol. 141, 917–927.

Wright, L.L., Palmer III, A.G., Thompson, N.L., 1988. Inhomogeneous translational diffusion of monoclonal antibodies on phospholipid Langmuir–Blodgett films. Biophys. J. 54, 463–470.

Yang, T., Jung, S.Y., Mao, H., Cremer, P.S., 2001. Fabrication of phospholipid bilayer-coated microchannels for on-chip immunoassays. Anal. Chem. 73, 165–169.

Yang, T., Baryshnikova, O.K., Mao, H., Holden, M.A., Cremer, P.S., 2003. Investigations of bivalent antibody binding on fluid-supported phospholipid membranes: the effect of hapten density. J. Am. Chem. Soc. 125, 4779–4784.