Synthetic multivalent ligands in the exploration of cell-surface interactions

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Processes such as cell–cell recognition and the initiation of signal transduction often depend on the formation of multiple receptor-ligand complexes at the cell surface. Synthetic multivalent ligands are unique probes of these complex cell-surface-binding events. Multivalent ligands can be used as inhibitors of receptor-ligand interactions or as activators of signal transduction pathways. Emerging from these complementary applications is insight into how cells exploit multivalent interactions to bind with increased avidity and specificity and how cell-surface receptor organization influences signaling and the cellular responses that result.

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Current Opinion in Chemical Biology 2000, **4**:696–703

1367-5931/00/$ – see front matter

Abbreviations

LT-1	heat labile enterotoxin
MHC	major histocompatibility complex
NeuAc	*N*-acetyl neuraminic acid
SLT-1	Shiga-like toxin I
TCR	T-cell receptor

Introduction

The surface of a cell is a complex and dynamic milieu. Protruding from the lipid bilayer are proteins and saccharides of different shapes and sizes. The arrangement of these diverse molecules also is heterogeneous: they can be distributed evenly across a cell surface or localized within microdomains. This complex ensemble of molecules acts as a reporter for the cell; it can convey changes in the external environment such as the presence of nutrients, toxins, attractants, or foreign invaders. In addition, the cell surface serves as a site for the docking of other cells, the extracellular matrix, or pathogens. All of these functions depend on the binding of specific ligands to cell-surface receptors.

The ability of cell-surface receptors to recognize and respond to ligands provides impetus for the discovery of natural or non-natural ligands that interfere with these binding events. Most research on receptor–ligand interactions focuses on the interaction of molecules with single receptor binding sites. Ligands that display multiple copies of recognition elements are termed multivalent. Multivalent ligands are critical probes of cell-surface recognition, as cells often encounter naturally occurring multivalent arrays [1]. In cell–cell recognition events, for example, multiple copies of a ligand on one cell can be recognized by receptors on the apposing cell. Multivalent arrays can also elicit a cellular response. The chemoattractant chemokines, for example, interact with glycosaminoglycans to form multivalent arrays that recruit leukocytes through their interaction with receptors on the cell surface. The biologically important activities of these naturally occurring multivalent displays provide impetus for the chemist. Chemical synthesis can provide access to ligands that mimic endogenous multivalent arrays. Alternatively, it can yield new types of multivalent ligands that alter cellular function by mechanisms inaccessible to natural substances. In this review, we focus on recent examples in which synthetic multivalent ligands have been used to illuminate and manipulate cellular responses.

The advantages of using synthetic ligands to probe multivalent cell-surface binding events are numerous. For example, they can be used to dissect the contributions of various ligand features to a binding interaction that is naturally multivalent. Physiological multivalent ligands are often too scarce, structurally heterogeneous, or complex (such as a cell or viral surface) to identify the relevant underlying molecular mechanisms. Many features of these ligands, such as number (valency) and orientation of receptor binding sites and the flexibility, size, and shape of the scaffold from which these binding sites are displayed may contribute to binding [1,2•,3,4]. Synthetic multivalent ligands can be generated such that the scaffold structure, identity of binding elements, number of binding elements, and the spacing of binding elements can be varied systematically. Arrays that mimic natural multivalent displays and those that are unrelated have been generated; both can have intriguing biological activities.

Synthetic multivalent ligands as inhibitors and effectors

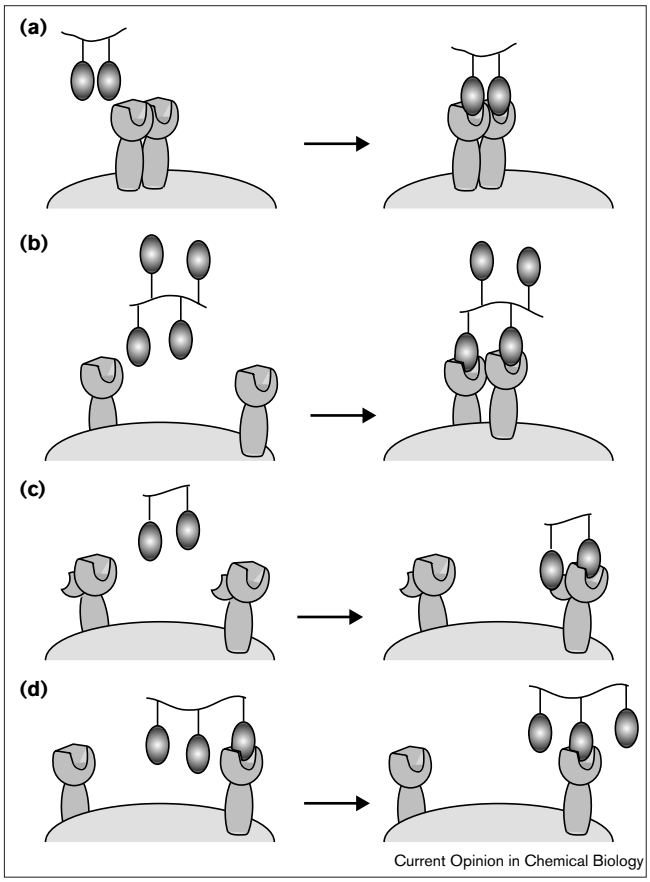
Synthetic multivalent ligands have been used to explore two primarily distinct cell-surface interactions: those in which the multivalent ligand prevents receptor–ligand binding and those in which binding of the multivalent ligand induces a cellular response. Ligands that have the former activity are inhibitors, and those with the latter are effectors. Multivalent inhibitors can interfere with a wide range of interactions including those involving cell–cell, cell–extracellular-matrix, cell–virus, and cell–toxin binding. Multivalent effectors can be applied to understand, dissect, and manipulate signal transduction pathways, especially those initiated by multiple receptor–ligand contacts at the cell surface. A range of responses can be elicited by multivalent ligand binding, in principle, and

these include cellular activation, migration, and differentiation. To provide a context for interpreting how multivalent ligands function, a brief overview of the mechanisms by which these ligands interact with their target receptors is provided (Figure 1).

Mechanisms of multivalent receptor–ligand interactions

Multivalent ligands often possess increased functional affinity (the apparent affinity for the interaction) for their targets compared with that of monovalent ligands [1,5,6]. Several different mechanisms can contribute to their potency. For example, multivalent ligands can bind oligomeric receptors on the cell surface. In this case, the translational entropy cost is paid with the first receptor–multivalent-ligand contact; subsequent binding interactions proceed without additional translational entropy penalties, although there is a conformational entropy cost (Figure 1a). Even with receptors that are not oligomeric, multivalent ligands can bind avidly to multiple receptors, a process that is facilitated by the two dimensional diffusion of receptors in the fluid bilayer (Figure 1b) Some proteins possess binding subsites in addition to the primary binding site, and these can be occupied by a multivalent ligand (Figure 1c). In addition, because multivalent ligands can display a higher local concentration of binding elements, they can have higher functional affinities even when only one receptor is engaged (Figure 1d) Another effect of multivalent-ligand binding that is relevant for inhibition of cell-surface interactions is steric stabilization. In steric stabilization, the size and hydration shell of the multivalent ligand precludes the engagement of a cell surface with an opposing viral particle or cell [7]. Together, these different modes of recognition impart unique activities to multivalent ligands.

Figure 1



Mechanisms by which multivalent ligands can interact with cell-surface receptors. **(a)** Multivalent ligands can bind oligomeric receptors by occupying multiple binding sites (chelate effect). **(b)** Multivalent ligands can cause receptors to cluster on the cell surface. This can lead to the activation of signaling pathways. **(c)** Multivalent ligands can occupy primary and secondary binding sites on a receptor. **(d)** Multivalent ligands display higher local concentrations of binding epitopes, which can result in higher apparent affinities.

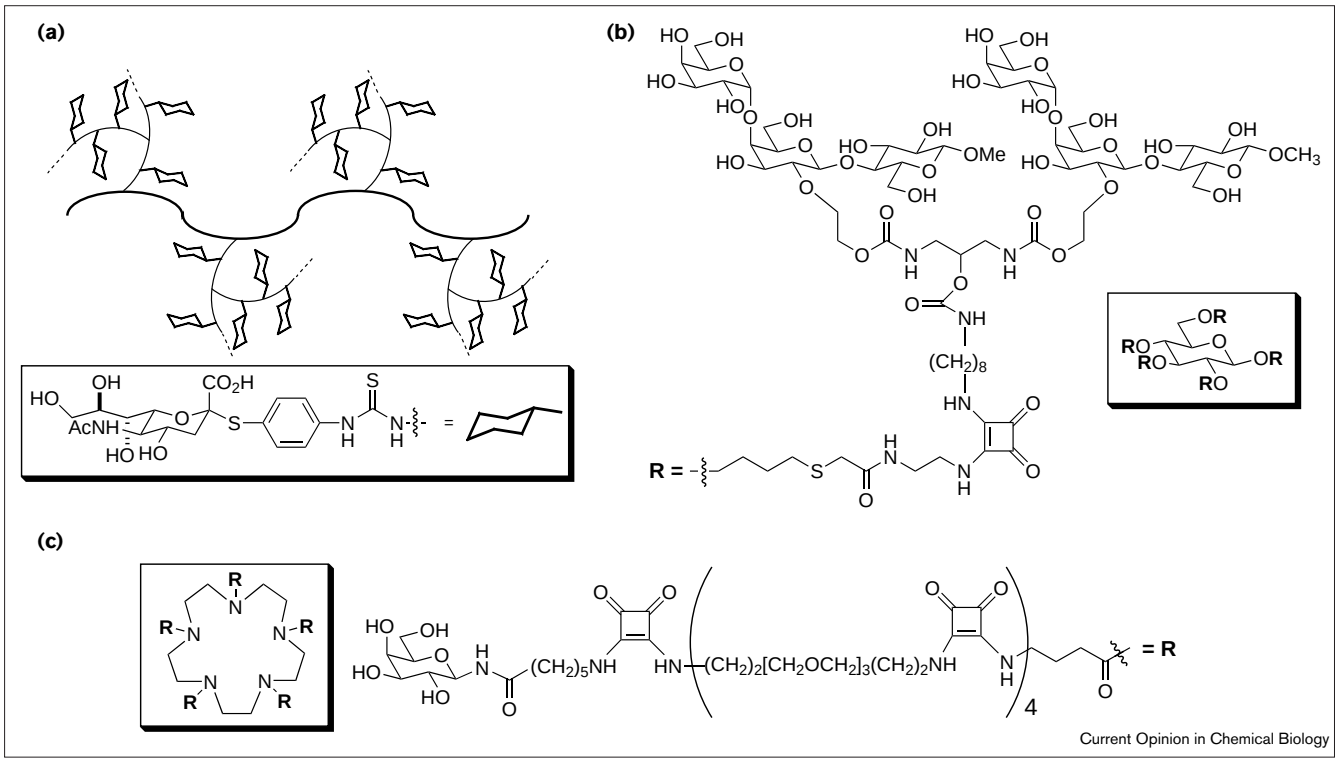
Multivalent inhibitors of cell-surface-receptor–ligand interactions

The high functional affinities of multivalent ligands have prompted efforts to develop potent inhibitors of cell-surface binding events. Examples of success using this approach range from the generation of inhibitors of virus binding to host cells, to compounds devised to block bacterial cell binding, to materials that inhibit the binding of leukocytes to endothelial cells. Advances in chemical synthesis are fueling these developments by providing access to a variety of platforms for binding-element presentation.

Some of the first applications of multivalent inhibitors were focused on preventing the binding of influenza virus hemagglutinin to host cells [8–12], and this area of research remains active [13,14•]. The system is an excellent testing ground because the interaction of the virus with the cell surface naturally relies on multivalent binding. The virus contacts host cells through the interaction of the trimeric influenza virus hemagglutinin with *N*-acetyl neuraminic acid (NeuAc; a type of sialic acid) residues on the host-cell surface. Although viral strains arise each year with different antigenic epitopes, all invade their hosts through NeuAc binding. High concentrations of monovalent sialic acid derivatives are required to inhibit binding (ca 10^{-3} M) [15]; consequently, multivalent inhibitors with high functional affinities have been sought. A variety of different types of multivalent ligand have been identified as effective inhibitors of influenza virus hemagglutinin, including NeuAc-substituted acrylamide polymers [8–10], liposomes [16,17], and divalent ligands [11,12]. High molecular weight polyacrylamide polymers were found to be extremely effective inhibitors of the virus [18], yet other scaffolds continue to be investigated because of the toxicity of polyacrylamide-based materials.

The effects of a ligand's architecture on its inhibitory activity and specificity were explored recently with a series of synthetic inhibitors of hemagglutination. Reuter *et al.* [14•] generated a number of structurally distinct ligands bearing NeuAc residues, and these were tested for their ability to

Figure 2



Chemical structures of multivalent ligands that function as inhibitors. **(a)** Schematic depiction of a dendrigraft polymer substituted with sialic acid epitopes. The polymer backbone was generated by grafting linear polyethyloxazoline chains onto linear polyethyleneimine (PEI) to first produce a comb-branched structure. This was then subjected to hydrolysis and grafting reactions to afford dendrigraft polymers. The resulting primary and secondary amine groups were then conjugated to sialic acid through reaction with a protected isothiocyanate derivative of NeuAc. **(b)** Structure of a decavalent ligand that is a highly potent inhibitor of SLT-1. **(c)** Structure of a pentavalent ligand that is an extremely effective inhibitor of LT-1.

prevent hemagglutinin binding and viral infection *in vitro* (Figure 2a; [14•]). The investigations revealed that the inhibitory potency of the multivalent ligand depends on its three-dimensional structure, and the different ligands are effective against various viral strains. In one striking example, branched polymers displaying NeuAc-substituted dendrimers were 50,000-fold more potent than monovalent NeuAc against sendai virus, but their increase in activity for influenza virus strain H2N2 was only twofold. This discrepancy may be due to differences in the three-dimensional structure of the viral hemagglutinin or to the distribution of viral hemagglutinin on the surface of the virus. Both of these variables may play important physiological roles in determining host–pathogen specificity *in vivo*.

The power of multivalent ligand design is illustrated by two studies describing the development of potent inhibitors of bacterial toxin binding to host cells [19••,20••]. Heat labile enterotoxin (LT-1) from *Escherichia coli* and the Shiga-like toxin I (SLT-1) from *Shigella dysenteriae* type 1 are members of the class of bacterial AB_5 toxins, and many fatalities result from the diseases caused by this class of proteins. The toxins gain access to cells by multivalent binding to the saccharide portion of gangliosides on mammalian cell surfaces. The structures of LT-1 [21] and SLT-1 [22] bound to monovalent carbohydrate derivatives have been determined by X-ray crystallography. In the case of LT-1, five monovalent trisaccharides were found to bind to a single face through their interactions with the five B subunits. The structure of SLT-1 is similar, although it differs in that 15 saccharide residues interact, such that each B subunit binds three saccharides. The structural information regarding the location of the saccharide-binding sites was used to design multivalent inhibitors of the toxins.

A modular strategy for the assembly of pentavalent ligands that target LT-1 was devised by Fan *et al* ([20••]; Figure 2c). A pentacyclen core was used to present galactose residues such that the connecting linker could readily be varied. By evaluating compounds possessing linkers that varied in length (variations ranging from 32 to 83 atoms), the authors identified an inhibitor of LT-1 that is 10^5-fold more active than galactose. Dynamic light scattering experiments suggest that this inhibitor forms a 1:1 complex with the toxin. These results, along with the high potency of the ligand, suggest that the inhibitor acts by the chelate effect (see Figure 1a). This is an important example of a compound that appears to use chelation to block a

multivalent cell surface. Moreover, this systematic investigation demonstrates the value of a flexible synthetic route for generating highly efficacious multivalent ligands.

Kitov *et al.* [19••] initiated their studies of SLT-1 inhibition with a different plan. The structure of SLT-1 suggested that multivalent ligands could be designed that occupy multiple binding sites within a single B subunit [22]. Thus, the strategy for design of SLT-1 inhibitors focused initially on generating divalent derivatives of the P^k trisaccharide that would target two adjacent trisaccharide-binding sites of the toxin (Figure 2b). The divalent ligand target was 40-fold more active than the trisaccharide, but the authors reasoned this gain would not be sufficient to inhibit the binding of the toxin to cells. Consequently, they appended their lead to a glucose scaffold through a linker (31 atoms) to display five copies of the divalent binding element. The resulting compound is a powerful inhibitor. It is 10^6-fold more active than is the P^k trisaccharide at inhibiting SLT-1, and it was able to protect cells in culture from the toxin. To better understand the source of their ligand's potent activity, the authors conducted X-ray crystallographic studies on the complex of the multivalent ligand and the toxin. Their data indicate that the ligand acts by a binding mode not anticipated in the ligand design. The solid-state structure reveals a complex containing two copies of the toxin to one of the ligand. All five subunits on each toxin are occupied with a single trisaccharide, and the two trisaccharide units within the divalent binding element act to dimerize the toxin. Thus, the multivalent ligand appears to act by the chelate effect but also by receptor clustering. This important study highlights the benefits of multivalent ligand design, and demonstrates the potential of incorporating different binding modes into ligand design.

The number of examples in which multivalent ligands have been found to be potent inhibitors is growing. The identification of efficacious inhibitors of bacterial toxins suggests blueprints for inhibitor design that perhaps can be generalized to create inhibitors of other toxins in this family. These advances and other recent results [23–29] underscore the interplay between chemistry and biology in the investigation and prevention of cell-surface interactions.

Effectors: multivalent ligands that activate biological systems

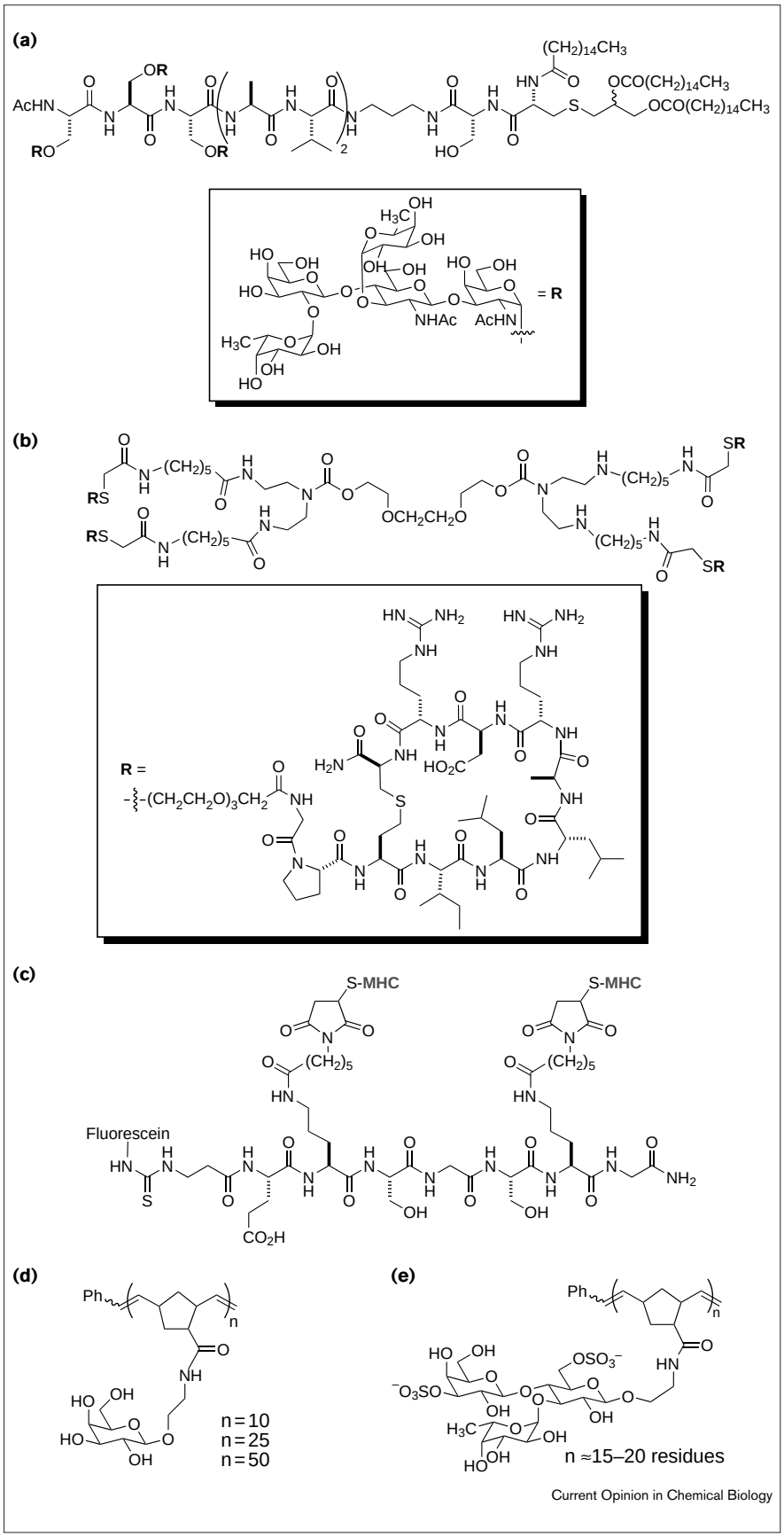
The ligand-binding modes that result in the initiation of a biological signal can be similar to those that are involved in inhibiting binding interactions. Specifically, when a ligand induces the dimerization of a cell surface receptor, the change in receptor proximity can initiate a particular signaling pathway [30,31]. Physiological multivalent ligands for cell-surface receptors often cause cellular responses through this mode of binding. Ligand-promoted receptor clustering (Figure 1d) is important in many biological processes, some of which include growth factor signaling [31], immune system function [32,33], and neuronal cell communication. Synthetic divalent ligands that promote receptor clustering have been used to initiate cellular responses [34] and to explore the role of receptor proximity in signal transduction and the cellular responses that result. Recent chemical strategies have opened the possibilities of exploring higher valency ligands as effectors of biological function.

The most common synthetic multivalent effectors are those that provoke an immune response; partially or fully synthetic vaccines are examples. These materials display multiple copies of an antigen on a synthetic scaffold or carrier protein. In a poorly understood process, these arrays can induce potent immune responses. The generation of anti-cancer vaccines is one area in which chemical synthesis is playing a major role [35–38,39••]. The development of anti-cancer vaccines is challenging because tumor cells can differ from normal cells in their cell-surface composition, but normal immune responses can be inhibited or circumvented by the tumor. Thus, attempts to develop anticancer vaccines have focused on taking the tumor antigens out of the cellular context and presenting them as multivalent arrays on different scaffolds. Although peptide antigens are being investigated for generating anti-cancer vaccines [38,40], it is the attempts to generate immune responses to carbohydrate antigens that have prompted many recent synthetic efforts [35–37,39••,41].

The utility of the synthetic oligosaccharide and glycopeptide epitopes that are commonly found on tumor-cell surfaces to serve as anticancer vaccines is an active area of exploration (Figure 3). In recent investigations by Danishefsky, Lloyd and co-workers [42,43•], multivalent conjugates composed of synthetic oligosaccharides linked to a standard carrier protein were successful at eliciting both humoral and cellular responses (IgM and IgG antibodies). These synthetic vaccines are now in clinical trials, and the synthetic approach taken to generate these immunogens provides opportunities for optimizing their activities. For example, the clustering of antigens can influence the effectiveness of a vaccine [44•]. These issues also are under investigation in the development of vaccines based on dendrimeric scaffolds [45], which display multiple copies of T cell and antigenic epitopes [46,47]. Together with studies that use synthetic multivalent ligands to explore the clustering of specific receptors in the immune system [48•,49], these investigations are illuminating the molecular requirements involved in immune-cell–antigen recognition.

Multivalent ligands can be used to ameliorate as well as generate an immune response [50•,51]. The production of a specific antibody can be diminished in the presence of a ligand that presents multiple copies of an antigen to the relevant B cells. The induction of B-cell tolerance to the specific antigen depends on the inability of the scaffold to activate T cells. A recent study explored the ability of tetravalent antigen displays to suppress the production of antibodies to a cyclic peptide antigen [50•]. A simple scaffold that displays four copies of the peptide antigen suppressed

Figure 3



Chemical structures of multivalent ligands that elicit biological responses. **(a)** A glycoconjugate based on the carbohydrate tumor antigen Lewis Y. This synthetic vaccine elicits antibodies that bind Lewis-Y-displaying cells. **(b)** Structure of a tetravalent peptide derivative that results in a decrease in B cell production of anti-cyclic peptide antibodies. **(c)** Structure of a divalent conjugate of a peptide-bound MHC used to explore the importance of TCR clustering in T cell activation. The MHC complex was appended to the peptide backbone through conjugate addition of an MHC cysteine side chain to maleimide groups. **(d)** Multivalent galactose derivatives that act as chemoattractants for *E. coli*. **(e)** Multivalent 3′,6-disulfo Lewis X derivatives that inhibit L-selectin and promote L-selectin downregulation from the surface of human neutrophils.

the production of 93% of the anti-cyclic peptide antibodies in mice (Figure 3b). Some of the conjugates tested were significantly less effective, and these results indicate that both the structure of the antigen and that of the scaffold can influence the tolerogenic activity of the display. A detailed understanding of how specific structural features influence activity awaits further elucidation of the molecular interactions that lead to a decrease in antibody production. This study provides an illustration of how a multivalent display can be used to down-regulate an immune response.

Defined, synthetic multivalent displays have been used to explore the importance of T-cell receptor (TCR) clustering in T cell activation ([48•]; see also Update). T cells can become activated when they interact with cells displaying peptide-occupied major histocompatibility complexes (MHCs). TCR clustering has been implicated in the activation process, but different conclusions have been drawn about how many TCRs must be clustered for activation. To explore the role of TCR clustering in activation, Cochran *et al.* [48•] synthesized divalent, trivalent and tetravalent arrays of peptide-bound MHCs as TCR ligands (Figure 3c). When these were assayed for their ability to stimulate T cells, the monovalent ligand failed to induce activation. Ligand-induced dimerization of the TCR was found to be sufficient for triggering, and the extent of activation did not appear to depend on ligand valency.

The clustering of cell-surface receptors is probably an important mechanism for controlling immune responses, as the above examples suggest. Moreover, ligand-induced receptor clustering can activate diverse signaling pathways that control critical cellular responses. For example, receptor clustering has been implicated in controlling the chemotaxis of bacteria toward attractants [52,53]. A recent study addresses whether the number of receptors incorporated into a cluster influences the biological activity elicited by a multivalent ligand that results [54••]. Ligands that vary systematically in the number of copies of chemoattractant groups they display were synthesized by the ring-opening metathesis polymerization (ROMP; Figure 3d). Galactose- and glucose-bearing multivalent ligands were shown to be effective synthetic chemoattractants for the bacteria *E. coli* and *Bacillus subtilis*. The chemotactic response elicited by a ligand depended on its valency: ligands with higher valencies were the most potent. Fluorescent derivatives of the multivalent ligands [54••,55] were used to visualize the location of a multivalent ligand and its receptors on the cell surface. These experiments suggest that the differences in activities of the multivalent chemoattractants are a result of their differential abilities to cluster the chemoreceptors. The results indicate that cell-surface receptor responses can be not just initiated but also modulated by changes in receptor proximity.

Synthetic multivalent ligands with multiple functions

Effector and inhibitor functions of a multivalent ligand can be intertwined. For example, if the effector promotes receptor down-regulation, it can inhibit the function of the receptor by this unique mechanism. An application of this strategy is in the down-regulation of L-selectin, a cell-surface receptor that mediates the transient attachment and rolling of leukocytes to endothelial cell glycoproteins during leukocyte homing and the inflammatory response. Physiologic ligands of L-selectin are sulfated glycoprotein mucins, which present multiple copies of carbohydrate epitopes to the protein. Inhibitors of L-selectin may act as anti-inflammatory therapeutics, and a number of monovalent and multivalent synthetic L-selectin ligands have been generated [55–58]. In addition to inhibiting L-selectin by generating competitor ligands, another strategy can be envisioned. The alternative approach exploits the observation that the concentration of L-selectin on the cell surface can be altered by a proteolytic cleavage event (shedding), a process that results in release of a soluble form of L-selectin into circulation [59]. Gordon *et al.* [60,61] have reported that multivalent ligands designed to mimic physiologic L-selectin ligands can down-regulate the amount of L-selectin on human neutrophils. These glycoprotein mimics act as inhibitors, blocking L-selectin-mediated leukocyte rolling [62], and as effectors that decrease concentration of cell surface L-selectin [60,61]. Thus, both effector and inhibitor functions are present in one ligand.

Conclusions

Multivalent ligands possess elements of structural complexity not present in their traditional monovalent counterparts. The identity of the binding elements, structure of the scaffold, number of binding groups, and density of binding elements are all parameters that influence the mechanisms by which a ligand acts. Given these diverse variables, it is clear that synthetic multivalent ligands have unique potential as inhibitors and effectors. Thus, chemists and biologists are now poised to ask new fundamental questions. What are the binding modes that give rise to the best inhibitors? How does ligand architecture influence ligand function? How does the extent of clustering of a receptor influence its function? Does the pattern of engaged receptors on a cell influence the output response? Synthetic multivalent ligands of high complexity can now be generated to address such questions.

Many studies of protein function focus on elucidating the function of a single protein, yet the amount of information available from genomics and proteomics is now leading to a shift in perspective: there is a focus on understanding how systems of interacting proteins control cellular responses. Despite this interest in systems, the design of ligands has focused traditionally on inhibiting or augmenting the function of a single protein. Multivalent ligands with their ability to organize, activate, or inhibit multiple receptors, are uniquely suited to address emerging questions in systems biology.

Update

The use of synthetic scaffolds to display peptide-loaded MHCs has been explored further by Cochran and Stern [63]. The authors use different scaffolds to generate a variety of

multivalent displays of MHC molecules loaded with HLA–DR1 peptide fragments. The resulting conjugates, which were isolated as monomer, dimers, trimers, and tetramers, were characterized by their sizes. Those conjugates capable of dimerizing cell-surface T-cell receptors (having valencies of two of greater) were able to activate HA1.7 T cells.

Acknowledgements

We thank past and present members of the Kiessling group for their contributions to our thinking in this area. LLK. acknowledges the Dreyffus Foundation, the NIH (GM55984) and the NSF for support. JEG thanks the Biotechnology Training Program for predoctoral fellowship (T32GM08349). LES was supported by an NIH predoctoral fellwoship (Gm8750).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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

2. • Kiessling LL, Strong LE, Gestwicki JE: **Principles for multivalent ligand design.** *Annu Rep Med Chem* 2000, **35**:321-330.
This review outlines the design principles used for devising multivalent ligands and discusses how ligand structure influences the properties and activities of multivalent ligands.

3. Roy R, Page D, Perez SF, Bencomo VV: **Effect of shape, size, and valency of multivalent mannosides on their binding properties to phytohemagglutinins.** *Glycoconjugate J* 1998, **15**:251-263.

4. Lee YC, Lee RT: **Carbohydrate-protein interactions: basis of glycobiology.** *Acc Chem Res* 1995, **28**:321-327.

5. Kiessling LL, Pohl NL: **Strength in numbers: non-natural polyvalent carbohydrate derivatives.** *Chem Biol* 1996, **3**:71-77.

6. Rao JH, Lahiri J, Isaacs L, Weis RM, Whitesides GM: **A trivalent system from vancomycin•D-Ala-D-Ala with higher affinity than avidin•biotin.** *Science* 1998, **280**:708-711.

7. Mammen M, Dahmann G, Whitesides GM: **Effective inhibitors of hemagglutination by influenza virus synthesized from polymers having active ester groups. Insight into the mechanism of inhibition.** *J Med Chem* 1995, **38**:4179-4190.

8. Matrosovich MN, Mochalova LS, Marinina VP, Byramova NE, Bovin NV: **Synthetic polymeric sialoside inhibitors of influenza virus receptor-binding activity.** *FEBS Lett* 1990, **272**:209-212.

9. Spaltenstein A, Whitesides GM: **Polyacrylamides bearing pendant α-sialoside groups strongly inhibit agglutination of erythrocytes by influenza virus.** *J Am Chem Soc* 1991, **113**:686-687.

10. Gamian A, Chomik M, Laferriere CA, Roy R: **Inhibition of influenza A virus hemagglutinin and induction of interferon by synthetic sialylated glycoconjugates.** *Can J Microbiol* 1991, **37**:233-237.

11. Sabesan S, Duus JO, Domaille P, Kelm S, Paulson JC: **Synthesis of cluster sialoside inhibitors for influenza virus.** *J Am Chem Soc* 1991, **113**:5865-5866.

12. Glick GD, Knowles JR: **Molecular recognition of bivalent sialosides by influenza virus.** *J Am Chem Soc* 1991, **113**:4701-4703.

13. Kamitakahara H, Suzuki T, Nishigori N, Suzuki Y, Kanie O, Wong C-H: **A lysoganglioside/poly-L-glutamic acid conjugate as a picomolar inhibitor of influenza hemagglutinin.** *Angew Chem Int Ed Engl* 1999, **37**:1524-1527.

14. • Reuter JD, Myc A, Hayes MM, Gan Z, Roy R, Qin D, Yin R, Piehler LT, Esfand R, Tomalia DA *et al.*: **Inhibition of viral adhesion and infection by sialic-acid-conjugated dendritic polymers.** *Bioconjugate Chem* 1999, **10**:271-278.
This paper describes the syntheses and activities of a range of polyvalent sialic acid derivatives that vary in shape. The testing of these ligands against a variety of viral proteins provides insight into how ligand structure influences biological activity and specificity.

15. Sauter NK, Bednarski MD, Wurzburg BA, Hanson JE, Whitesides GM, Skehel JJ, Wiley DC: **Hemagglutinins from two influenza virus variants bind to sialic acid derivatives with millimolar dissociation constants: a 500 MHz proton nuclear magnetic resonance study.** *Biochemistry* 1989, **28**:8388-8396.

16. Kingery-Wood JE, Williams KW, Sigal GB, Whitesides GM: **The agglutination of erythrocytes by influenza virus is strongly inhibited by liposomes incorporating an analog of sialyl gangliosides.** *J Am Chem Soc* 1992, **114**:7303-7305.

17. Spevak W, Nagy JO, Charych DH, Schaefer ME, Gilbert JH, Bednarski MD: **Polymerized liposomes containing *C*-glycosides of sialic acid: potent inhibitors of influenza virus *in vitro* infectivity.** *J Am Chem Soc* 1993, **115**:1146-1147.

18. Choi S-K, Mammen M, Whitesides GM: **Generation and *in situ* evaluation of libraries of poly(acrylic acid) presenting sialosides as side chains as polyvalent inhibitors of influenza-mediated hemagglutination.** *J Am Chem Soc* 1997, **119**:4103-4111.

19. •• Kitov PI, Sadowska JM, Mulvey G, Armstrong GD, Ling H, Pannu NS, Read RJ, Bundle DR: **Shiga-like toxins are neutralized by tailored multivalent carbohydrate ligands.** *Nature* 2000, **403**:669-672.
The authors identify a potent multivalent inhibitor of SLT-1. An X-ray crystallography study of the complex suggests that the ligand causes dimerization of the toxin, and that each of the galactose residues occupies a single binding site on the toxin. This contribution and [20••] are outstanding examples of the value and importance of ligand design.

20. •• Fan EK, Zhang ZS, Minke WE, Hou Z, Verlinde C, Hol WGJ: **High-affinity pentavalent ligands of *Escherichia coli* heat-labile enterotoxin by modular structure-based design.** *J Am Chem Soc* 2000, **122**:2663-2664.
See also annotation [19••]. A systematic approach to multivalent ligand design is described. Using this strategy, the authors find a potent inhibitor of enterotoxin.

21. Sixma TK, Pronk SE, Kalk KH, van Zanten BAM, Berghuis AM, Hol WGJ: **Lactose binding to heat-labile enterotoxin revealed by X-ray crystallography.** *Nature* 1992, **355**:561-564.

22. Ling H, Boodhoo A, Hazes B, Cummings MD, Armstrong GD, Brunton JL, Read RJ: **Structure of the shiga-like toxin I B-pentamer complexed with an analogue of its receptor Gb_3.** *Biochemistry* 1998, **37**:1777-1788.

23. Wang J-Q, Chen X, Zhang W, Zacharek S, Chen Y, Wang PG: **Enhanced inhibition of human anti-gal antibody binding to mammalian cells by synthetic α-gal epitope polymers.** *J Am Chem Soc* 1999, **121**:8174-8181.

24. Horan N, Yan L, Isobe H, Whitesides GM, Kahne D: **Nonstatistical binding of a protein to clustered carbohydrates.** *Proc Natl Acad Sci USA* 1999, **96**:11782-11786.

25. Maheshwari G, Brown G, Lauffenburger DA, Wells A, Griffith LG: **Cell adhesion and motility depends on nanoscale RGD clustering.** *J Cell Sci* 2000, **113**:1677-1686.

26. Lindhorst TK, Dubber M, Krallmann-Wenzel U, Ehlers S: **Cluster mannosides as inhibitors of type 1 fimbriae-mediated adhesion of *Escherichia coli*: pentaerythritol derivatives as scaffolds.** *Eur J Org Chem* 2000:2027-2034.

27. Mrksich M: **A surface chemistry approach to studying cell adhesion.** *Chem Soc Rev* 2000, **29**:267-273.

28. Saxon E, Bertozzi CR: **Cell surface engineering by a modified Staudinger reaction.** *Science* 2000, **287**:2007-2010.

29. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE: **Micropatterned surfaces for control of cell shape, position, and function.** *Biotechnol Prog* 1998, **14**:356-363.

30. Klemm JD, Schreiber SL, Crabtree GR: **Dimerization as a regulatory mechanism in signal transduction.** *Annu Rev Immunol* 1998, **16**:569-592.

31. Heldin C-H: **Dimerization of cell surface receptors in signal transduction.** *Cell* 1995, **80**:213-223.

32. Germain RN, Stefanova I: **The dynamics of T cell receptor signaling: complex orchestration and the key roles of tempo and cooperation.** *Annu Rev Immunol* 1999, **17**:467-522.

33. Healy JI, Goodnow CC: **Positive versus negative signaling by lymphocyte antigen receptors.** *Annu Rev Immunol* 1998, **16**:645-670.

34. Spencer DM, Wandless TJ, Schreiber SL, Crabtree GR: **Controlling signal transduction with synthetic ligands.** *Science* 1993, **262**:1019-1024.

35. Koganty RR, Reddish MA, Longenecker BM: **Glycopeptide- and carbohydrate-based synthetic vaccines for the immunotherapy of cancer.** *Drug Discov Today* 1996, **1**:190-198.

36. Toyokuni T, Singhal AK: **Synthetic carbohydrate vaccines based on tumor-associated antigens.** *Chem Soc Rev* 1995, **24**:231.

37. Hakomori S-i, Zhang Y: **Glycosphingolipid antigens and cancer therapy.** *Chem Biol* 1997, **4**:97-104.

38. Bellone M, Iezzi G, Imro MA, Protti MP: **Cancer immunotherapy: synthetic and natural peptides in the balance.** *Immunol Today* 1999, **20**:457-462.

39. •• Danishefsky SJ, Allen JR: **From the laboratory to the clinic: a retrospective on fully synthetic carbohydrate-based anticancer vaccines.** *Angew Chem Int Ed Eng* 2000, **39**:837-863.
This is an excellent overview of the progress made by one group in developing synthetic and semisynthetic anti-cancer vaccines.

40. Ben-Yedidia T, Arnon R: **Design of peptide and polypeptide vaccines.** *Curr Opin Biotechnol* 1997, **8**:442-448.

41. Ragupathi G: **Carbohydrate antigens as targets for active specific immunotherapy.** *Cancer Immunol Immunother* 1996, **43**:152-157.

42. Glunz PW, Hintermann S, Schwarz JB, Kuduk SD, Chen X-T, Williams LJ, Sames D, Danishefsky SJ, Kudryashov V, Lloyd KO: **Probing cell surface 'glyco-architecture' through total synthesis, immunological consequenses of a human blood group determinant in a clustered mucin-like context.** *J Am Chem Soc* 1999, **121**:10636-10637.

43. • Wang Z-G, Williams LJ, Zhang X-F, Zatorski A, Kudryashov V, Ragupathi G, Spassova M, Bornmann W, Slovin SF, Scher HI *et al.*: **Polyclonal antibodies from patients immunized with a globo H-keyhole limpet hemocyanin vaccine: isolation, quantification, and characterization of immune responses by using totally synthetic immobilized tumor antigens.** *Proc Natl Acad Sci USA* 2000, **97**:2719-2724.
A synthetic strategy for an affinity matrix displaying carbohydrates used in cancer vaccines was developed. This new matrix was then used to analyze the range and specificities of antibodies produced from vaccines bearing the relevant carbohydrates. This provides a new method for characterizing the immune response to cancer vaccine therapy.

44. • Glunz PW, Hintermann S, Williams LJ, Schwarz JB, Kuduk SD, Kudryashov V, Lloyd KO, Danishefsky SJ: **Design and synthesis of Le^y-bearing glycopeptides that mimic cell surface Le^y mucin glycoprotein architecture.** *J Am Chem Soc* 2000, **122**:7273-7279.
A series of complex saccharides were synthesized and appended to a peptide display in varying densities. Immunological studies demonstrated that the extent of clustering had a direct impact on the ability of the antigen to stimulate antibody production.

45. Tam JP: **Synthetic peptide vaccine design — synthesis and properties of a high-density multiple antigenic peptide system.** *Proc Natl Acad Sci USA* 1988, **85**:5409-5413.

46. Bay S, Lo-man R, Osinaga E, Nakada H, Leclerc C, Cantacuzene D: **Preparation of a multiple antigen glycopeptide (MAG) carrying the Tn antigen.** *J Pept Res* 1997, **49**:620-625.

47. Veprek P, Jezek J: **Peptide and glycopeptide dendrimers. Part 1.** *J Pept Sci* 1999, **5**:5-23.

48. • Cochran JR, Cameron TO, Stern LJ: **The relationship of MHC-peptide binding and T cell activation probed using chemically defined MHC class II oligomers.** *Immunity* 2000, **12**:241-250.
This paper describes a useful approach to exploring the clustering of the TCR using defined multivalent ligands. Using a variety of analytical methods, the authors conclude that the dimerization of the TCR causes receptor activation and that the extent of activation does not depend on the size of the TCR cluster.

49. He X-S, Rehermann B, López-Labrador FX, Boisvert J, Cheung R, Mumm J, Wedermeyer H, Berengeur M, Wright TL, Davis MM *et al.*: **Quantitative analysis of hepatitis C virus-specific $CD8^+$ T cells in peripheral blood and liver using peptide-MHC tetramers.** *Proc Natl Acad Sci USA* 1999, **96**:5692-5697.

50. • Jones DS, Coutts SM, Gamino CA, Iverson GM, Linnik MD, Randow ME, Ton-Nu H-T, Victoria EJ: **Multivalent thioether-peptide conjugates: B cell tolerance of an anti-peptide immune response.** *Bioconjugate Chem* 1999, **10**:480-488.
This paper provides a clear illustration of the value of multivalent ligands for inhibiting antibody production.

51. Jones DS, Barstad PA, Field MJ, Hachmann JP, Hayag MS, Hill KW, Iverson GM, Livingston DA, Palanki MS, Tibbetts AR *et al.*: **Immunospecific reduction of antioligonucleotide antibody-forming cells with a tetrakis-oligonucleotide conjugate (LJP-394), a therapeutic candidate for the treatment of lupus nephritis.** *J Med Chem* 1995, **38**:2138-2144.

52. Bray D, Levin MD, Morton-Firth CJ: **Receptor clustering as a cellular mechanism to control sensitivity.** *Nature* 1998, **393**:85-88.

53. Liu Y, Levit M, Lurz R, Surette MG, Stock JB: **Receptor-mediated protein kinase activation and the mechanism of transmembrane signaling in bacterial chemotaxis.** *EMBO J* 1997, **16**:7231-7240.

54. •• Gestwicki JE, Strong LE, Kiessling LL: **Tuning chemotactic responses with synthetic ligands.** *Chem Biol* 2000, **7**:583-592.
Bacterial chemotactic responses were shown to vary based on the valency of the multivalent ligands. This is the first demonstration that a cellular response can be varied systematically using a multivalent ligand.

55. Gordon EJ, Gestwicki JE, Strong LE, Kiessling LL: **Synthesis of end-labeled multivalent ligands for exploring cell-surface-receptor-ligand interactions.** *Chem Biol* 2000, **7**:9-16.

56. Simanek EE, McGarvey GJ, Jablonowski JA, Wong C-H: **Selectin-carbohydrate interactions: from natural ligands to designed mimics.** *Chem Rev* 1998, **98**:833-862.

57. Nishida Y, Uzawa H, Toba T, Sasaki K, Kondo H, Kobayashi K: **A facile synthetic approach to L- and P-selectin blockers via copolymerization of vinyl monomers constructing the key carbohydrate modules of sialyl $lewis^x$ mimics.** *Biomacromol* 2000, **1**:68-74.

58. Toppila S, Renkonen R, Penttila L, Natunen J, Salminen H, Helin J, Maaheimo H, Renkonen O: **Enzymatic synthesis of alpha 3′-sialylated and multiply alpha 3-fucosylated biantennary polylactosamines — a bivalent [sialyl diLe(x)]-saccharide inhibited lymphocyte-endothelium adhesion organ-selectively.** *Eur J Biochem* 1999, **261**:208-215.

59. Kishimoto TK, Jutila MA, Berg EL, Butcher EC: **Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors.** *Science* 1989, **245**:1238-1241.

60. Gordon EJ, Strong LE, Kiessling LL: **Glycoprotein-inspired materials promote the proteolytic release of cell surface L-selectin.** *Bioorg Med Chem Lett* 1998, **6**:1293-1299.

61. Gordon EJ, Sanders WJ, Kiessling LL: **Synthetic ligands effect cell surface strategies.** *Nature* 1998, **392**:30-31.

62. Sanders WJ, Gordon EJ, Beck PJ, Alon R, Kiessling LL: **Inhibition of L-selectin-mediated leukocyte rolling by synthetic glycoprotein mimics.** *J Biol Chem* 1999, **274**:5271-5278.

63. Cochran JR, Stern J: **A diverse set fo oligomeric class II MHC-peptide complexes for probing T-cell receptor interactions.** *Chem Biol* 2000, **7**:683-696.