Receptor clustering is a common signaling mechanism for cell surface receptors. Exogenous ligands such as antibodies or synthetic analogues can be used to artificially induce clustering. New studies using defined synthetic ligands suggest that the spatial organization of these clusters attenuates signaling in one pathway but has no effect in another. From the point of view of a biologist seeking to understand receptor clustering at a molecular level: How close do the receptors need to be to be “clustered”? How many receptors form an active cluster? Must they be in a particular orientation? Although a bivalent antibody may be able to induce an active cluster, it provides no mechanism to ask these subtle structural questions.

Chemists have addressed these questions by using synthetic ligands with defined characteristics—by changing valency, distance between sites, and orientation—to examine the physical process involved in clustering (2). Baird et al. (3) present a comprehensive study of structurally defined multivalent ligands and their effect on the signaling of the FcεRI immunoreceptor. Building on a large body of pioneering work on this receptor system (4), the authors use rigid trivalent ligands to induce receptor clustering and probe the effects of altered clustering on downstream signaling. They test the effect of altering the distances between receptors and observe that two pathways appear to be in play: one that is sensitive to the distance between ligand sites (ligand-length dependent) and one that is not (ligand-length independent).

Immunoreceptor Signaling. The FcεRI immunoreceptor is a well-studied complex involved in the allergic response mediated primarily by mast cells (5). The complex consists of four subunits: an α, β, and two γ chains (see Figure 1). Many of the details of FcεRI downstream signaling remain obscure. Activation of FcεRI results in degranu-
through binding of multiple IgE molecules, 

dimensionality that obscures the organi- 

tors triggers signaling, and the formation of 

Cluster Organization. Clustering of the 

The signaling pathway downstream of 

which then induce clustering of the recep-

tifs (ITAMs) found in the FcεRI β and γ 

tors leading to degranulation (10). Clustering 

Figure 1. FcεRI signaling pathways. Schematic representation of key signaling elements 

downstream of the FcεRI complex. a) The receptor complex is composed of an α, β, and 

tory level. 

ation of mast cells, releasing inflammatory 

mediators from secretory granules that lead 

to inflammation. The receptor binds to IgE 

antibody molecules; however, binding to in- 

dividual IgE will not lead to activation. The 

α-chain of the receptor recognizes IgE mol- 

eles in complex with multivalent antigens 

through binding of multiple IgE molecules, 

which then induce clustering of the recep-

tor complex (see Figure 1).

The signaling pathway downstream of 

FcεRI begins with the phosphorylation of 

components of the receptor complex by Src 

family kinases Syk (spleen tyrosine kinase), 

Lyn, and Fyn. The Src kinases interact with 

the immunoreceptor tyrosine activation mo-


tors that IgE antibodies are known. Although 

useful, these conjugates suffer from struc-

tural heterogeneity that obscures the organi-

zation of the receptor cluster at the molecu-

lar level.

Cluster Organization. Clustering of the 

FcεRI induces downstream signaling 

through phosphorylation of the ITAM sites 

in the complex by Src kinases. Using antibi-

dodies and antibody fragments, research-

ers have shown that dimerization of recep-

tors triggers signaling, and the formation of 

larger clusters leads to increased signaling 

and receptor immobilization (11). Antibodi-

es are inherently limited by their structure. 

Synthetic ligands provide a powerful alter-
native for testing the properties of clusters with altered properties (such as distance between receptors).

Synthetic bivalent ligands for FcεRI have previously been prepared by the construction of DNA fragments that assemble into rigid double-stranded DNA complexes bearing antibody epitopes at the 5′ ends (12). Using these bivalent ligands, it was found that dimeric ligands are less active than multivalent BSA-DNP conjugates and that the distance between the receptors affects activity (4). The distance dependence of these ligands suggested that effective triggering of FcεRI signaling requires a structured cluster.

In the present study, Baird et al. use a similar strategy to prepare trivalent ligands to interrogate the FcεRI system. Increasing the valency of the ligand causes more complex clustering in the membrane (see Figure 2). By comparing the activities of bivalent and trivalent ligands with similar spacing, this strategy provides a way to change the organization of the receptor cluster. Varying the architecture of multivalent ligands (13) has been found to have dramatic effects on a variety of clustering systems (2); therefore, this class of ligands is an ideal tool for understanding the structural requirements of the cluster.

As expected from previous work, degranulation triggered by the trivalent ligands showed a strong dependence on distance between epitope sites (3). However, further examination of the signaling pathway provided some interesting results. The initial event of the signaling pathway was expected to be phosphorylation of the FcεRI β and γ chains along with LAT, and these events all showed a clear dependence on length. Kinetic resolution of these events confirmed that phosphorylation of the β chain precedes that of the γ chain and that the shorter ligands have a more pronounced effect on both events. Thus, the organization of the complex is a determining factor that controls signal amplification via the β- to γ-chain phosphorylation. In contrast to these results, it was also shown that phosphorylation of PLCγ was ligand-length independent. Therefore, two mechanisms of signaling appear to be in play: one that is sensitive to the distance between receptors and one that is not.

Baird et al. proceeded to study the details of the signaling pathway downstream of cluster formation. A common step in cellular activation is a rapid increase in intracellular calcium concentration. Calcium ions may be held in intracellular stores or can be allowed into the cell through ion channels in the plasma membrane. Experiments using calcium indicator dyes allowed quantification of the changes in intracellular Ca^{2+} from either source. Although the kinetics are slightly faster for the shortest and most potent ligand, the total Ca^{2+} released is similar for all of the ligands. Signaling components thought to be responsible for inducing intracellular calcium in mast cells are the enzymes PLCγ and PI3K (see Figure 1). Specific inhibitors of each of these enzymes are known; wortmannin is an inhibitor of PI3K and U73122 is an inhibitor of PLCγ (14, 15). These inhibitors were employed to test the involvement of each enzyme in FcεRI downstream signaling. It was found that in the presence the PLCγ inhibitor U73122, intracellular Ca^{2+} release is completely inhibited for both trivalent ligands tested and the BSA-DNP conju-

![Figure 2. Cluster architecture. The valency and structure of receptor ligands can influence the formation of clusters and arrays on the cell surface. The cluster architecture is dependent on the valency, orientation, and distance between sites. A multivalent receptor and multivalent ligand can combine to form a range of possible structures. Some hypothetical structures are shown for clustering of a) divalent, b) trimeric, and c) tetrameric ligands with a divalent receptor. In addition to dimer structures, larger clusters and extended arrays are possible.](image-url)
gate. In contrast, the DNP-BSA conjugate is still able to evoke a response in the presence of the PI3K inhibitor wortmannin, whereas the trivalent ligands are not. This result suggests that the DNP-BSA conjugate is better able to utilize the principal pathway of signaling. It is possible that both pathways are involved for the trivalent ligands but that the intensity of signal provided by the DNP-BSA conjugate is able to partly overcome inhibition of the complementary pathway.

How does the structure of the cluster manifest these dramatic changes in signaling? The existence of both a length-dependent pathway (principal pathway) and a length-independent pathway (complementary pathway) suggests different aspects of the cluster are recognized by each. If this is the case, we might expect that any clustering of FcεRI could activate one pathway to provide a basal “on” signal. To provide greater dynamic range, a second pathway could discriminate between small and large clusters, providing a mechanism more sensitive to the number or structure of the antigen. Models of signaling for clustered systems have suggested that one purpose of receptor clustering may be a massive improvement in dynamic range (16). The mechanism responsible for these effects may involve conformational change of the receptor (17) or a change in the proximity of active sites (18). In the system under investigation, it seems likely that small dimer or trimer complexes are active in both pathways; however, very large clusters induced by ligands like the DNP-BSA conjugate would be significantly more potent through a pathway that recognizes cluster size or receptor proximity.

The study by Baird et al. provides an exciting window into both the complexity and dynamics of receptor clustering. These results reinforce that receptor clustering is far from a nonspecific aggregation process within the membrane. Instead, it appears that the number and proximity of receptors within the cluster manifest specific molecular changes. These data suggest that the availability of defined multivalent ligands will continue to provide us with crucial data for understanding the molecular organization of membrane complexes.

REFERENCES