Binding Cooperativity of Membrane Adhesion Receptors

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Cell adhesion processes are essential for the distinction of self and foreign in immune responses, the formation of tissues, or the signal transduction across the synaptic cleft of neurons. The adhesion processes are mediated by the specific binding of receptor and ligand proteins anchored in the cell membranes. Because of the importance of these processes, the binding of cells to other cells or to surface-supported lipid membranes with anchored ligand molecules (see **Fig. 1**) has been studied intensively.

Binding Affinity of Receptors and Ligands

A central question is how to characterize and measure the binding affinity of the membrane-anchored receptor and ligand molecules that are involved in cell adhesion. For *soluble* receptor and ligand molecules, the binding affinity can be characterized by the binding equilibrium constant K_{3D}, defined by

$$[RL]_{3D} = K_{3D}[R]_{3D}[L]_{3D} \quad (1)$$

where $[RL]_{3D}$ is the volume concentration of bound receptor-ligand complexes, and $[R]_{3D}$ and $[L]_{3D}$ are the volume concentrations of unbound receptors and unbound ligands in the solution. The equilibrium constant K_{3D} is determined by the binding free energy of the complex and can be measured with standard experimental methods.

An often considered two-dimensional analogue for *membrane-anchored* receptors and ligands is the quantity

$$K_{2D} = \frac{[RL]}{[R][L]} \quad (2)$$

where [RL], [R], and [L] are the *area* concentrations of bound receptor-ligand complexes, unbound receptors, and unbound ligands. However, different experimental methods to measure K_{2D} lead to values that differ by several orders of magnitude, which indicates that K_{2D} is not a proper constant **[1]**.

Membrane Fraction within Receptor Binding Range

Quantifying the affinity of membrane-anchored receptor and ligand molecules is complicated by the fact that the binding process depends on the local separation and, thus, the conformations of the two apposing membranes. A receptor molecule can only bind an apposing ligand if the local membrane separation is comparable to the length of the receptor-ligand complex. A central quantity therefore is the fraction P_b of the apposing membranes with a separation within the binding range of the receptor-ligand interaction. The concentration of bound receptor-ligand complexes

$$[RL] = P_b K_b [R][L] \quad (3)$$

is proportional to P_b as well as to the concentrations [R] and [L] of unbound receptors and ligands **[1,2,4]**. Here, K_b is the well-defined two-dimensional equilibrium constant for membrane segments within the binding range of the receptors and ligands.

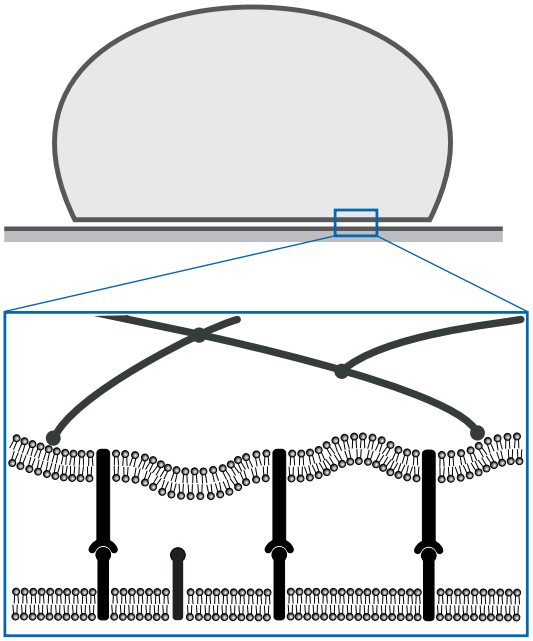

Fig. 1: A cell adhering to a supported membrane with anchored ligands that bind to receptors in the cell membrane. The binding of receptors and ligands in the cell adhesion zone is affected by membrane shape deformations and fluctuations on nanometer scales, which are dominated by the bending rigidity of the cell membrane. The adhesion receptors of immune cells are typically mobile along the membrane only weakly, coupled to the cytoskeleton, if at all.

Thermal shape fluctuations of the membranes on nanometer scales lead in general to values of P_b smaller than 1. For cell membranes, these nanometer scale fluctuations are not, or only weakly, suppressed by the cell cytoskeleton, in contrast to large-scale shape fluctuations.

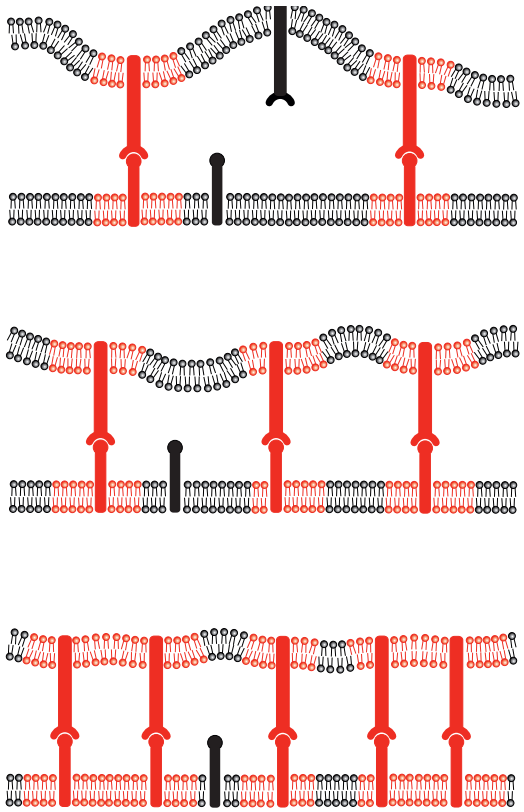

Fig. 2.: An important quantity is the area fraction P_b of the membranes within binding separation of the receptors and ligands. The area fraction P_b (shown in red) increases with the concentrations of receptors and ligands, since the formation of receptor-ligand bonds 'smoothens out' thermal membrane shape fluctuations. The 'smoothening' facilitates the formation of additional receptor-ligand bonds and, thus, leads to a binding cooperativity **[1,2,4]**.

We have developed a statistical-mechanical model of membrane adhesion in which the membranes are described as discretized elastic surfaces and the adhesion receptors and ligands as individual molecules diffusing on these surfaces **[2]**. In our model, the fraction P_b of the membranes within binding range of the receptors and ligands turns out to be much smaller than 1 for typical lengths and concentrations of receptors and ligands in cell adhesion zones. Scaling analysis and Monte Carlo simulations lead to the relation

$$P_b \approx c(\kappa/k_BT)l_{we}^2 K_b[R][L] \qquad (4)$$

which indicates that the membrane fraction P_b within the binding range of the receptors and ligands is proportional to $[R]$ and $[L]$. Here, $c \approx 13$ is a dimensionless prefactor, $\kappa = \kappa_1\kappa_2/(\kappa_1 + \kappa_2)$ is the effective bending rigidity of the two apposing membranes with rigidities κ_1 and κ_2, k_BT is Boltzmann's constant times temperature, and l_{we} is the interaction range of the receptor-ligand bonds.

Cooperative Binding of Membrane Receptors

A direct consequence of the equations (3) and (4) is the quadratic dependence

$$[RL] \approx c(\kappa/k_BT)l_{we}^2 K_b^2 [R]^2[L]^2 \qquad (5)$$

of the bond concentration $[RL]$ on the area concentrations $[R]$ and $[L]$ of free receptors and ligands. This quadratic dependence indicates cooperative binding. The binding cooperativity results from a 'smoothening' of the thermally rough membranes and, thus, an increase of P_b with increasing concentrations $[R]$ and $[L]$ of receptors and ligands, which facilitates the formation of additional receptor-ligand complexes (see **Fig. 2**).

A consequence of eq. (5) is that the quantity K_{2D} defined in eq. (2) is not constant, but depends on the concentrations of the receptors and ligands. Eq. (5) thus helps to understand why different experimental methods to measure K_{2D} can lead to significantly different results **[1]**.

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References:

[1] Krobath, H., Rozycki, B., Lipowsky, R. and Weikl, T. R.: Binding cooperativity of membrane adhesion receptors. Soft Matter **5**, 3354-3361 (2009).

[2] Weikl, T. R., Asfaw, M., Krobath, H., Rozycki, B. and Lipowsky, R.: Adhesion of membranes via receptor-ligand complexes: Domain formation, binding cooperativity, and active processes. Soft Matter **5**, 3213-3224 (2009).

[3] Rozycki, B., Lipowsky, R. and Weikl, T. R.: Adhesion of surfaces via particle adsorption: exact results for a lattice of fluid columns. J. Stat. Mech., 211006 (2009).

[4] Rozycki, B., Lipowsky, R. and Weikl, T. R.: Segregation of receptor-ligand complexes in cell adhesion zones: phase diagrams and the role of thermal membrane roughness. New. J. Phys. **12**, 095003 (2010).