Polymer-tethered ligand-receptor interactions between surfaces

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Ligand-receptor interactions between specific pairs of molecules or residues mediated by macromolecules represent a recurring theme in cell adhesion and related biological processes [1]. While the ligand-receptor binding is a short ranged lock-and-key type interaction between residues of specific chemical structures, the presence of macromolecules gives rise to a long-range component to the interaction that is usually related to certain generic characteristics such as chain lengths, electric charges, hydrophobicity, etc. Tethering ligand and receptor groups by polymer chains to the surfaces thus combines features of both types of interactions, and provides a versatile control over the range and strength of the overall surface interactions.

The ligand-receptor interactions between surfaces were first studied by Bell, Dembo and Bongrand in a model for cell-adhesion [2]. In this model, the specific ligand-receptor binding is treated as a chemical reaction with an equilibrium constant dependent on the surface separation. However, the interaction due to polymer-tethered ligands and receptors is different from a simple chemical reaction in at least two important respects: First, in most cases binding occurs between residues in two macromolecules or polymers; the apparent binding equilibrium constant must reflect the free energy associated with the chain conformation degrees of freedom. In a confined geometry as illustrated in Fig. 1 where ligands and receptors are tethered to the surfaces, how to relate the effective binding affinity in this geometry to an intrinsic binding energy, or equilibrium constant in bulk solutions, is nontrivial. Second, a chemical equilibrium treatment implicitly involves the translational entropy of one or more species. When the polymer tethers are immobilized on the surfaces, the translation degrees of freedom are lost and definition of an equilibrium constant becomes problematic.

In this paper we provide a rigorous thermodynamic analysis of the model as depicted in Fig. 1 and study the resulting interaction potential between the parallel surfaces due to the tethered ligand-receptor binding. Our general analysis properly accounts for different contributions to the binding affinity and applies to any specific model for the polymer chain. We examine the difference between the scenario when the whole surfaces are in contact with fixed density of molecules, and when only a small part of the surfaces are in contact and molecules can diffuse into the contact area. These two cases are treated as a closed system and an open system with fixed chemical potential, respectively.

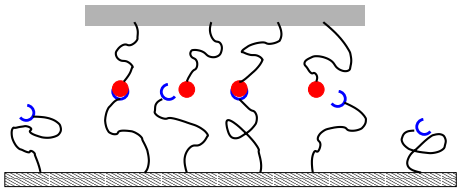


FIG. 1: Schematic view of the model for surfaces with tethered ligands and receptors.

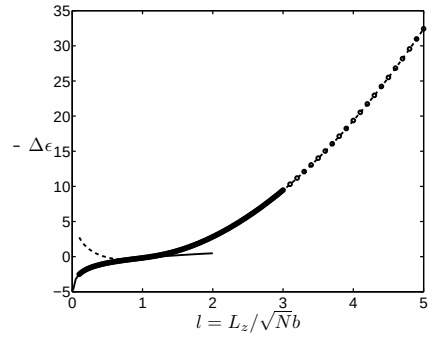
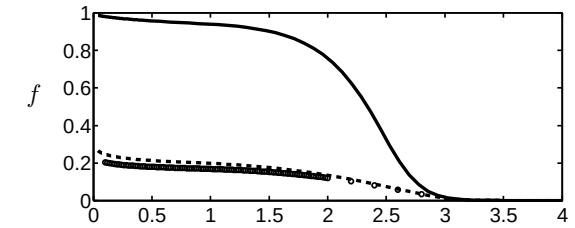


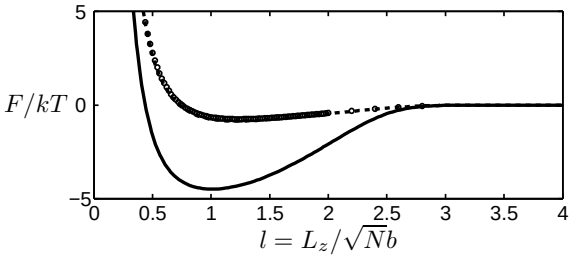
FIG. 2: Contribution to the effective binding energy from the tether polymer. l is the separation between surfaces scaled by the average end-to-end distance of the tethered ligand-receptor bridge. The circles represent results from numerical calculations; the dashed line and the solid line are from approximate expressions in the limit $l \ll 1$ and $l \gg 1$.

When interacting ligands and receptors are immobile (i.e., lack lateral translation degrees of freedom), we show that a simple chemical reaction model fails to represent the physics of the interaction [3]. This "quenched" problem is relevant when the diffusivity of molecules is slow compared to the macroscopic adhesion and motion of the cell. We study the thermodynamics of the quenched system using a density expansion for the interaction free energy and the average number of bound pairs per molecule. Illustrative calculations are performed for the ideal Gaussian chain model, which, while ignoring excluded volume interactions, captures the chain connectivity and polymer-surface interactions. The results, especially the scaling dependence on the chain lengths, can provide crude guide for bioengineering design of surface interactions using polymers.

For polymer-tethered ligands and receptors between the surfaces, binding occurs as surfaces approach each other: at large surface separations binding incurs a free



(a)



(b)

FIG. 3: (a) The interaction profile between the surfaces. (b) The average fraction of bound ligands or receptors. The receptors and ligands both have a scaled density $\phi = 0.01$ and have a molecular binding energy $\epsilon = 10kT$. The solid line represents a closed system for mobile ligand and receptor molecules, and the dashed line and circles are for the system with immobile ligands and receptors from leading order and up to $O(\phi^4)$ terms in the density expansion.

energy cost due to chain stretching. Fig. 2 shows this free energy cost as a function of the dimensionless surface separation. $\Delta\epsilon$ can be interpreted as the difference between the effective binding energy and the molecular binding energy. The effective binding energy is directly related to the fraction of bound molecules. Hence from Fig. 2 we can infer the microscopic binding energy from measurements of the surface interactions, or determine the fraction of bound molecules at any surface separation from the molecular binding energy.

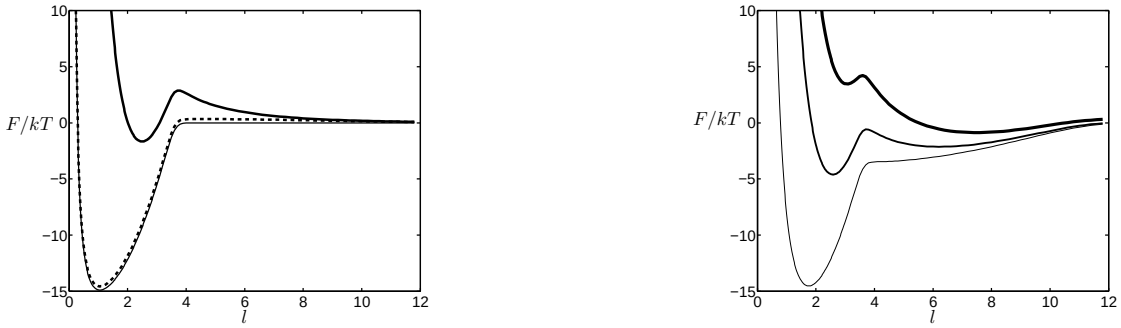
In addition to binding between ligands and receptors, the polymers also induce repulsion between the surfaces due to confinement when surfaces are close. These two effects result in a net interaction potential as shown in Fig. 3(b).

Using scaling arguments one can estimate the different length scales and energetic scales of the interaction potential. For the Gaussian chain model, we find that the onset of binding, where ligand-receptor bridges start to form, scales as $L_1 \sim \sqrt{\beta\epsilon N}b$ as a balance between chain stretching and binding, where $\beta\epsilon$ is the molecular binding energy and Nb^2 is the mean square end-to-end distance of a tethered ligand-receptor bridge. For $\beta\epsilon \gg 1$ most molecules are bound at the potential minimum, the free energy minimum is located at $L_0 \sim \sqrt{N}b$ as a balance between chain stretching and confinement, and the depth of the free energy minimum is approximately ϵ plus a constant correction independent of the chain length. In addition, the quasi-equilibrium critical tension for breaking the adhesion, which is found from the maximum of the derivative of the free energy with respect to the surface separation (or from the inflection point of the free energy curve directly) scales as $\tau \propto N^{-1/2}$. All these results are verified in numerical calculations for the Gaussian chain model. For a different chain model, these results still hold if the $N^{1/2}b$ factor is replaced by the average end-to-end distance of the polymer in that model. In Fig. 3 we show the results for cases with mobile ligands and receptors and with both ones immobile. For the immobile case, as alluded to earlier, the chemical reaction picture becomes inapplicable due to the lost of translational entropy of the molecules. Instead the binding free energy is given by an average over the random distribution of the receptor and ligand molecules, essentially a "quenched" average. The dashed line and the circles represent results from leading order (quadratic in the density) and up to quartic order terms in the density expansion. For the low densities we are studying the leading order result is already quite accurate.

The difference between the immobile case and the mobile case can be summarized as two main effects. First, in the immobile case tether chains are stretched between the anchoring ends of a bound ligand and its receptor, resulting in a higher stretching energy in the lateral direction. Second, since molecules are fixed, there could be certain number of molecules that are too far away to bind with each other. If the densities of molecules are low, both effects cause a considerable increase in the energetic cost of binding, resulting in a smaller fraction of bound molecules and shallower free energy minimum, as is shown in Fig. 3.

Of particular interest is the difference between closed systems and open systems having a chemical potential of the species equal to the corresponding closed systems. Such a comparison is relevant to the initial and final stages of cell adhesion: the system behaves more like an open system when only a small part of the surfaces are in contact, and becomes a closed system when the surfaces are in full contact. Thus although our study concerns only systems at full equilibrium, comparing the behavior between the open and closed systems can provide information on the end points of a dynamic process. As expected, at the same chemical potential corresponding to the concentration of ligands and receptors of a closed system, the number density of bound ligand-receptor pairs is much higher in the open system, with a much deeper free energy minimum. This result may be relevant to the observation that a stable adhesion is formed even in the initial stage of cell adhesion, despite that only a small fraction of the cell membrane is in contact.

Cell membranes are coated by a hydrated layer of long-



(a) (b)

FIG. 4: (a) The interaction potential between surfaces with ligands and receptors, and additional repelling polymers: the dashed line represents the case of mobile repellers, the solid line for immobile repellers, and the thin line without repellers. The repeller's length is 16 times the length of a tethered ligand-receptor bridge, and repeller's density is 1/3 of the ligands or receptors (both of which are mobile and have equal density $\phi = 1$). (b) The interaction potential for the case when two types of ligands and receptors are present with additional repellers. The length of the longer tether is 16 times the shorter one; the length of the repeller is 36 times (thick line) and 16 times (intermediate line) the length of the shorter-tethered bridge. The thin line is for the case without repellers.

chain polymers (e.g., glycocalyx), which presents a steric barrier that must be overcome by ligand-receptor binding. Such an effect can be mimicked by adding a layer of grafted polymers that are longer than the combined lengths of the ligand and receptor tethers. The combination of an attractive well due to tethered ligand-receptor binding and steric repulsions due to the additional polymers can result in the appearance of a free energy barrier that separates the attractive part at intermediate surface separation and the repulsive part at larger surface separation. The thick solid line in Fig. 4(a) is a free energy profile with a fixed density of repeller polymers. For comparison, we also show the case where the repeller polymers can diffuse away when the two surfaces are brought together (dashed line). The latter case results in a much softer barrier.

More complex behavior is obtained when we introduce several different tether lengths together with repeller molecules. Fig. 4(b) shows free energy profiles for a system having two different polymer tether lengths for the ligand and receptor each. We see that there are two minima due to each type of ligand-receptor pair; the magnitude and location of each minimum can be individually controlled by adjusting the tether lengths, the binding affinity of the corresponding ligand-receptor pair, and the length and density of the repellers. The double well potential as shown in Fig. 4(b) also has many implications. For the system of colloidal particles, we expect a richer phase diagram as well as more complicated kinetics of phase separation due to the intermediate bound state. Such interaction potentials also suggest new directions for bioengineering design. The longer-tethered ligand-receptor bridge can serve as a precursor to shorter-tethered but stronger ligand-receptor binding; the presence of repellers gives us an additional degree of freedom in controlling desired binding, for example, by preventing unwanted binding when multiple types of ligand-receptor pairs are present.

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[3] Martin, J. I., Zhang, C.-Z., and Wang, Z.-G., *J. Polym. Sci. Part B: Polym. Phys.* 2006, 44, 2621-2637.