Ligand mobility suppresses membrane wrapping in passive endocytosis.

Lorenzo Di Michele,1 * Pritam Kumar Jana,2 and Bortolo Matteo Mognetti2,†

1 Biological and Soft Systems, Cavendish Laboratory, University of Cambridge, JJ Thomson Avenue, Cambridge CB3 0HE, United Kingdom
2 Université Libre de Bruxelles (ULB), Interdisciplinary Center for Nonlinear Phenomena and Complex Systems, Campus Plaine, CP 231, Bld. du Triomphe, B-1050 Brussels, Belgium
(Dated: August 15, 2017)

Receptor mediated endocytosis is an ubiquitous process through which cells internalize biological or synthetic nanoscale objects, including viruses, unicellular parasites, and nanomedical vectors for drug or gene delivery. In passive endocytosis the cell plasma membrane wraps around the “invader” particle driven by ligand-receptor complexation. By means of theory and numerical simulations, here we demonstrate how particles decorated by freely diffusing and non-mutually-interacting (ideal) ligands are significantly more difficult to wrap than those where ligands are either immobile or interact sterically with each other. Our model rationalizes the relationship between uptake mechanism and structural details of the invader, such as ligand size, mobility and ligand/receptor affinity, providing a comprehensive picture of pathogen endocytosis and helping the rational design of efficient drug delivery vectors.

The cell plasma membrane is a complex interface, optimized to regulate transport of small molecules and nanoscale carriers. Internalisation of particles ranging from a few to a few tens of nanometers, including viruses and artificial vectors for drug and nucleic acid delivery, typically occurs via endocytosis. In this process, a particle (invader) is wrapped by the membrane, which then buds off towards internalising the particle within an endosome [1]. Endocytosis is mediated by the specific binding of ligands, decorating the particle, to membrane receptors. The process is “active” if wrapping is aided by dedicated signaling pathways, as in clathrin-dependent [2] and caveolin-dependent endocytosis [3]. Wrapping, can however also be “passive”, if solely mediated by multivalent ligand-receptor interactions, without the activation of signaling pathways nor ATP consumption. Viruses are sometimes able to hijack active endocytosis pathways, but in other cases are passively uptaken [4, 5]. Artificial vectors including solid nanoparticles [6], liposomes [7–9] and polymerosomes [10], are often uptaken by passive endocytosis. A deep understanding on how the structure of the invader influences passive endocytosis is thus required to aid the design of synthetic vectors, but also to clarify the still poorly understood uptake mechanisms of some pathogens [4, 11, 12].

The modeling of passive endocytosis has traditionally relied on phenomenological approaches where the multivalent nature of the ligand-receptor interactions has been neglected [13–15], or considered only in the limit of irreversible ligand-receptor binding [16–19]. Thermodynamic models for multivalent interactions have instead been developed in the context of cell-cell adhesion [20, 21], membrane targeting [22–26], synapse formation [21, 27–29], and the self-assembly of synthetic ligand-functionalized particles [30–32]. These studies have repeatedly highlighted how multivalent interactions give rise to complex phenomena that can only be captured with a bottom-up modeling approach, and could be of paramount importance in endocytosis. Particularly rich is the phenomenology observed in the presence of mobile ligands/receptors, that can freely diffuse on the substrates, and thus accumulate within the adhesion spots, maximising the number of bonds [33, 34]. Cell-membrane receptors embedded in the fluid plasma membrane, and ligands decorating synthetic liposomes fall within this category [9]. Instead, ligands decorating solid nanoparticles or viruses are anchored to a fixed point [6, 35]. Even if linkers are mobile, however, steric interactions and membrane crowding limit the local concentration of ligands and receptors [36, 37].

In this letter we present a thermodynamic description of passive endocytosis that correctly accounts for the multivalent nature of the interactions in the relevant scenarios of fixed and mobile ideal (non-sterically-interacting) ligands. Moreover, we introduce new analytic and numerical strategies to describe mobile ligands/receptors interacting through excluded volume. We demonstrate how particles functionalized by fixed ligands are more easily wrapped by the membranes, while those functionalized by mobile ligands are the most prone to incomplete or partial wrapping. Furthermore, increasing excluded volume interactions between mobile ligands makes complete wrapping easier.

Our formalism can quantify the effect of ligand density, size, and mobility, on the wrapping tendency of biological invaders, and suggest whether or not active phenomena are needed to achieve full engulfment. Moreover, our findings can readily be applied to the design of synthetic vectors, which to optimize passive wrapping should either feature fixed ligands or bulky mobile ligands.

We model the invader particle as a sphere or a prolate ellipsoid with axis of rotation orthogonal to the cell surface, and innermost point penetrating to a depth $h$ (see Fig. 1). The invader has total surface area...
with \( \Gamma \) of our study [40]. The analytical expressions of specific contributions does not influence the outcomes deformations of the host, as including these (system line tension. In Eq. 1 we neglect non-local elastic effects.

F_{adh}(h) \propto S_{CR}(h) \) while here we propose a representation of the adhesive free energy that fully accounts for the multivalent nature of the interactions. Since the invader is typically much smaller than the host cell, we can model the contact region as a finite surface of area \( S_{CR} \) in contact with an infinite reservoir of ideal membrane receptors. We indicate with \( \rho_R \) the average receptor density on the host cell. If no ligand-receptor complexes (dimers) are formed, and assuming uniform receptor distribution on the cell, the contact region should have receptor density \( \rho_R \). In our model \( \rho_R \) is controlled by the density of the ideal reservoir \( \rho_R^{(0)} \), and by the extent of steric interactions between the receptors in the contact region. The assumption of ideal receptors produces \( \rho_R = \rho_R^{(0)} \), while increasing steric repulsion causes \( \rho_R \) to decrease below \( \rho_R^{(0)} \). The equilibrium density of receptors in the contact region will generally differ from both \( \rho_R \) and \( \rho_R^{(0)} \), and depend on the steric interactions, the formation of bonds, and \( \rho_R^{(0)} \). Under the assumption of fluid cell membrane we allow free diffusion of receptors. A number \( N_L \) of either fixed or mobile ligands is present on the invader.

The equilibrium constant \( K_{2D}^{(eq)} = \exp(-\beta \Delta G_0)/\rho_{\alpha} \), where \( \Delta G_0 \) is the ligand-receptor interaction free energy and \( \rho_{\alpha} = 1 M \) is the standard concentration, controls dimerization in a 2D solution. For linkers confined to a surface, a 2D equilibrium constant can be written as \( K_{2D}^{(eq)} = \exp(-\beta \Delta G_0)/\rho_{\alpha} \), where the \( \delta \) is a length comparable with the size of the ligands/receptors, which accounts for entropic costs hindering dimerization simply in terms of \( K_{2D}^{(eq)} \).

As demonstrated in Fig. 1(b), ligands, receptors and dimers (linkers) are modeled as hard disks of diameter \( \alpha \), which thus determines the extent of steric interactions. The same \( \alpha \) is assumed for ligand-ligand, receptor-receptor, dimer-dimer, ligand-dimer and receptor-dimer interactions. Unbound ligands and receptors are modeled as non-interacting.

Under these assumptions the system of linkers is fully determined by the parameters \( \{ N_L, \rho_R^{(0)}, S_{CR}, S_{OR}, K_{2D}^{(eq)}, \alpha \} \). For ideal linkers with \( \alpha = 0 \), the adhesive free energy \( F_{adh}(h) \) can be derived for both fixed and mobile ligands, analogously to what previously done in the context of linker-mediated particle interactions (see Refs. [31, 33, 34] and SM Sec. II.B [38]).

\[
\beta F_{adh, \alpha=0} = \frac{S_{CR}(h)}{S_{Tot}} \log \left[ 1 + K_{2D}^{(eq)} \rho_R \right] 
\]

\[
\beta F_{adh, \alpha=0} = \frac{S_{CR}(h)}{S_{Tot}} \log \left[ 1 + K_{2D}^{(eq)} \rho_R \right] 
\]

These expressions correctly account for combinatorial and confinement entropic contributions specific to mult-
tivalent interactions. For the case of ideal fixed ligands, Eq. 2 recovers the phenomenological assumption $F_{adh}(h) \propto S_{CR}(h)$, with the advantage that our expression allows one to link the proportionality constant to the microscopic details of the system. The trend determined in Eq. 3 for the case of mobile ligands is instead strikingly different, as shown in Fig. 2 (a). The logarithmic dependence on $S_{CR}$ translates into a sharp onset of adhesion, with the free energy flattening out as more of the invader gets wrapped. This behavior is intuitively explained with the accumulation of ligands in the contact region that leads to the formation of many bonds as soon as the invader contacts the host.

For ideal linkers, the accumulation of ligands in the contact region is limited uniquely by confinement entropy, but for $\alpha > 0$ steric interactions start playing a role. For non-ideal linkers the adhesive free energy can be written as $\beta F_{adh}^{mob,\alpha>0}(h) = \beta F_{adh}^{mob,\alpha=0}(h) + \beta F_{adh}^{mob,ex}(h)$. The excess free energy $\beta F_{adh}^{mob,ex}(h)$ can be evaluated through a second-order virial expansion (see SM Sec. I.B [38])

$$\beta F_{adh}^{mob,ex}(h) = N_L B_2 K^{eq}_{2D}(0) \rho_R^2 S_{CR} \times$$

$$\frac{N_L K^{eq}_{2D} S_{OR}/S_{Tot} + 2S_{Tot} + 2S_{CR} K^{eq}_{2D}(0) \rho_R^2}{(S_{Tot} + S_{CR} K^{eq}_{2D}(0) \rho_R^2)^2},$$

where $B_2 = \pi \alpha^2 / 2$ is the second virial coefficient of hard disks of diameter $\alpha$. In Fig. 2 we demonstrate the effect of excluded volume, quantified by the ligand packing fraction $\phi = \pi \alpha^2 N_L / S_{Tot}$. As $\phi$ (or equivalently $\alpha$) increases, the sharp adhesion onset as a function of $S_{CR}$ becomes less evident. This is a direct consequence of excluded volume interactions hindering the accumulation of ligands in the contact region.

The analytical expansion in Eq. 4 is only accurate in the limit of small packing fraction: $\phi \lesssim 0.05$. To access $F_{adh}^{mob,\alpha>0}$ at higher $\phi$ we adopt a numerical Monte Carlo approach based on the model sketched in Fig. 1(b). The adhesion free energy is determined by thermodynamic integration [43]

$$\beta F_{adh}^{mob,\alpha>0} = \int_0^{K^{eq}_{2D}} dK^{eq}_{2D} \frac{\langle n_D \rangle}{K^{eq}_{2D}},$$

where $\langle n_D \rangle$ is the average number of dimers estimated by MC at a given $K^{eq}_{2D}$.

The simulated adhesion free energy is shown in Fig. 2. For ideal linkers ($\alpha = 0$), we recover the result of Eq. 3, while for small excluded volume the numerical curves overlap with the theoretical predictions of Eq. 4. As anticipated, deviations from the theory are observed at $\phi \gtrsim 0.05$. When $\phi$ is further increased the adhesive free energy changes drastically, developing a linear region at low $S_{CR}$ and converging towards the trend observed for fixed ligands. This behavior is a consequence of the excluded volume interactions frustrations the accumulation of ligands in the contact region. Indeed, for large enough $K^{eq}_{2D}$, the highest possible number of ligands get recruited in the contact region and dimerizes, forming a dense packing. In this regime the adhesion energy is simply proportional to the number of dimers that can fit in the contact region, and thus to $S_{CR}$. Surprisingly, at high $\phi$ (e.g. $\phi = 0.4$) and large $S_{CR}$ we observe that $F_{adh}^{mob,\alpha>0}$ becomes more attractive than $F_{adh}^{mob,\alpha=0}$. This effect is caused by a reduction in the overall steric hindrance following dimerization: the area excluded to each dimer by an unbound ligand-receptor pair is larger than the area excluded by a single dimer.

To study the effect of ligand mobility on endocytosis, we

FIG. 2. The influence of ligand mobility and steric interactions on the adhesion free energy. (a) Adhesion free energy as a function of the contact-area fraction calculated for fixed and mobile ideal ligands (solid lines, Eqs. 2 and 3) and non-ideal mobile ligands (dashed lines, Eq. 4). Symbols connected by thin lines show the results of MC simulations. For the ideal cases we use $K^{eq}_{2D}(0) = 5.5066 \times 10^3$. In the presence of steric interactions ($\phi > 0$) we increase the reservoir receptor density $\rho_R$ to maintain a constant $\rho_R = N_L / S_{Tot}$. For $\phi = 0.01, 0.02, 0.05, 0.1, 0.2, 0.4$ we scale $\rho_R(0)$ (and thus $K^{eq}_{2D}(0)$) by a factor 1.04, 1.09, 1.24, 1.57, 2.84, 20.5, as calculated by dedicated MC simulations. In all cases we use $N_L = 500$. (b) Deviation of the non-ideal adhesion free energy from the ideal case.
combine Eq. 1 with the analytical expressions for $F_{adh}$ in the regimes of fixed and mobile ideal ligands (Eqs. 2 and 3), taken as the limiting cases for the systems with $\phi > 0$ that, as discussed above, display intermediate behavior. We consider two different invader shapes: a sphere of radius $a$, and a prolate ellipsoid with semi-major axis $a$ and semi-minor axis $b = a/2$. The former mimics artificial nanoparticles, liposomes and many enveloped viruses including HIV and influenza [35, 44], the latter is arranged with the major axis normal to the host surface, resembling the shape and invasion geometry of the malaria plasmodium [11]. The overall free energy is minimized as a function of the invader penetration depth $h \in [0, 2a]$, and the equilibrium values of $h$ are shown in Fig. 3 as a function of $K_{2D}^{(eq)} \rho_R^{(0)}$ (cf. Eqs. 2 and 3) and all the other relevant system parameters $N_L$, $\sigma$, $\kappa$, $\gamma$. For generality, membrane tension, bending modulus, and line tension are expressed in reduced units $\tilde{\sigma} = \sigma/[k_BT a^{-2}]$, $\tilde{\gamma} = \gamma/[k_BT a^{-1}]$ and $\tilde{\kappa} = \kappa/[k_BT]$. For temperature $T = 37^\circ C$, and considering invader similar in size to a typical virus, i.e. $a = 50$ nm, the range of parameters covered in Fig. 3 spans biologically relevant intervals $\kappa \in [0, 30] \times k_BT$ [45–47], $\sigma \in [0, 34] \times 10^{-6}$ J m$^{-2}$ [45, 46, 48], $\gamma \in [0, 12] \times 10^{-13}$ J m$^{-1}$ [11, 49], and $N_L \in [10, 1000]$ [44, 50].

For the case of fixed ligands, spherical invaders always display a first-order transition between fully unwrapped ($h = 0$) and fully wrapped ($h = 2a$) configurations. Partially wrapped states do not occur, as previously observed when neglecting long-range elastic deformations of the host membrane [40]. As intuitively expected, the wrapping transition occurs at lower $K_{2D}^{(eq)} \rho_R^{(0)}$ for “softer” membranes (lower $\tilde{\sigma}$ and $\tilde{\kappa}$) and higher number of ligands on the invader. No $\gamma$-dependence is observed, since in both the fully wrapped and fully unwrapped states the triple line has zero length.

The scenario changes drastically for the case of mobile ligands, were we observe the emergence of several partially wrapped configurations. The phase boundary marking the onset of wrapping differs only marginally from the case of fixed ligands, but the range of conditions where full wrapping is achieved is significantly reduced. For instance, while with fixed ligands and $K_{2D}^{(eq)} \rho_R^{(0)} = 10^2$ full wrapping is reached at $N_L \approx 140$, for mobile ligands $N_L$ needs to be as large as 540. Likewise, for the same ligand-receptor affinity, fixed ligands induce full wrapping at all tested values of $\tilde{\kappa}$ and $\tilde{\sigma}$, while for mobile ligands $\tilde{\kappa} < 22$ and $\tilde{\sigma} < 9$ are required, values that can easily be exceeded in typical biological cells [46, 48].

For ellipsoidal invaders, Fig. 3 demonstrates the presence of partially wrapped states also for the case of fixed ligands. However, mobile ligands cause the regions of stable fully wrapped configurations to shrink significantly, and in some cases disappear altogether from the tested parameter range. Moreover, the partially wrapped states found with fixed ligands tend to be close to full wrapping (lighter shades in Fig. 3), while mobile ligands tend to stabilize marginally wrapped configurations (darker shades in Fig. 3).

In view of the trends shown in Fig. 2 for the adhesion free energy in the presence of steric interactions, it is expected that invaders with high-packing fraction of mobile ligands would be prone to complete wrapping, much like those with fixed ligands.

In summary, we apply state of art modeling of multivalent ligand-mediated interactions to the problem of passive endocytosis, and demonstrate how membrane wrapping of invader particles is drastically affected by ligand mobility and steric interactions. If ligands are diffusive and have negligible steric interactions, complete
membrane wrapping is hindered, and the invading particle is often found in a partially engulfed state. In turn, complete membrane wrapping is facilitated if ligands are immobile or their accumulation is substantially limited by steric interactions. These effects may have important implications in understanding the relationship between the structure of biological invaders and their ability to induce passive endocytosis. Regardless of the capsid shape, many viruses are enveloped by a (near) spherical lipid bilayer, decorated with glycoprotein complexes (spikes), whose role is targeting cell receptors and driving endocytosis [44]. Despite being embedded in a fluid membrane, these ligands are anchored to a protein matrix present underneath the bilayer, which makes them immobile. Our results suggest that ligand anchoring may be crucial to allow or at least facilitate membrane wrapping in enveloped viruses. Indeed, influenza A has ∼ 375 spikes on its surface [44, 50], which according to our model may not be sufficient to induce complete wrapping if the ligands were mobile (Fig. 3). In turn we predict that, with only ∼ 73 ligands on its surface, HIV virions would struggle to achieve passive engulfment even in the regime of fixed ligands, suggesting that active endocytosis pathways may be a strict requirement [12].

Our findings apply as well to artificial delivery vectors relying on passive endocytosis. Ligand-receptor interactions are often exploited to improve the uptake efficiency and selectivity of liposomes, routinely used for intracellular delivery of water insoluble drugs [9]. We predict that passive endocytosis of liposomes can be enhanced by choosing high-viscosity lipid formulations that hinder ligand mobility, or choosing high-molecular weight ligands to boost their packing fraction.

The authors thank Pietro Cicuta for fruitful discussions and comments on the manuscript. The work of BMM and PKJ was supported by the Fonds de la Recherche Scientifique de Belgique - FNRS under grant n° MIS F.4534.17. LDM acknowledges support from Emmanuel College Cambridge, the Leverhulme Trust, and the Isaac Newton Trust through an Early Career Fellowship (ECF-2015-494) and the EPSRC Programme Grant CAPITALS number EP/J017566/1. Computational resources have been provided by the Consortium des Equipements de Calcul Intensif (CECI), funded by the Fonds de la Recherche Scientifique de Belgique - FNRS under grant n° 2.5020.11.
Supplemental Materials: Ligand mobility suppresses membrane wrapping in passive endocytosis.

I: PASSIVE MEMBRANE WRAPPING OF INVADERS

As shown in Fig. 1 of the main text, we model the invader as a prolate ellipsoid with axis ($z$) orthogonal to cell surface, defined by the equation

$$\frac{x^2}{a^2} + \frac{y^2}{a^2} + \frac{z^2}{b^2} = 1,$$

(S1)

with $b > a$ and eccentricity defined by

$$e^2 = 1 - \frac{a^2}{b^2}.$$

(S2)

The case of spherical invader is simply recovered in the limit $a = b$ and $e = 0$. In polar coordinates $\theta$ and $\phi$, the surface of the invader is parametrised as

$$x = a \cos \theta \cos \phi,$$

(S3)

$$y = a \cos \theta \sin \phi,$$

(S4)

$$z = b \sin \theta.$$

(S5)

As detailed in Eq. 1 of the main text, the free energy of the system, in which the innermost point of the invader penetrates to a depth $h$, comprises a membrane stretching term ($F_{\text{stretch}}$), membrane bending term ($F_{\text{bend}}$), a line tension term ($F_{\text{lt}}$), and an adhesion term ($F_{\text{adh}}$). The latter is discussed in the next section. Below we calculate the energy terms associated with the mechanical deformation of the membrane [S1].

Membrane stretching. The stretching energy is calculated as $F_{\text{stretch}} = \sigma S_{\text{CR}}(h)$, where $S_{\text{CR}}(h)$ is the contact area between invader and cell and $\sigma$ is the cell-membrane stretching modulus. Defining $y(h) = (h - b)/b$, in the general case we find

$$F_{\text{ellips stretch}}(h) = 2\pi ab\sigma \int_{-1}^{y(h)} dy \sqrt{1 - e^2 y^2}$$

$$= 2\pi ab\sigma \left[ \frac{y(h)}{2} \sqrt{1 - e^2 y(h)^2} + \frac{\arcsin(ey(h))}{2e} + \frac{1}{2} \sqrt{1 - e^2} + \frac{\arcsin e}{2e} \right],$$

(S6)

$$\frac{dF_{\text{ellips stretch}}(h)}{dh} = 2\pi ab\sigma \sqrt{1 - e^2 y(h)^2}.$$

(S7)
while for spherical invaders we obtain

\[ F_{\text{stretch}}^{\text{sph}}(h) = 2\pi ah\sigma \quad \frac{dF_{\text{stretch}}^{\text{sph}}(h)}{dh} = 2\pi a\sigma. \]  

S8

Membrane beiding. The bending energy of the membrane calculated as the integral over the contact area of \(2\kappa H^2\) where \(\kappa\) is the bending modulus and \(H\) is the average curvature \(H = 1/(2c_1) + 1/(2c_2)\), where \(c_1\) and \(c_2\) are the principal radii of curvature at a given point \(r = (x, y, z)\). These radii are equal to \(\alpha^2/p\) and \(\beta^2/p\) where \(\alpha\) and \(\beta\) are the semi-axes of the ellipse obtained intersecting the ellipsoid with the central plane parallel to the plane tangent to \(r\), while \(p\) is the distance between the center of the ellipsoid and the tangent plane [S2]. We find

\[ \alpha^2 = a^2 \quad \beta^2 = a^2 \sin^2 \theta + b^2 \cos^2 \theta \quad p = \frac{ab}{\sqrt{a^2 \sin^2 \theta + b^2 \cos^2 \theta}}, \]  

S9

resulting in

\[ H = \frac{ab}{\sqrt{a^2 \sin^2 \theta + b^2 \cos^2 \theta}} \left[ \frac{1}{2a^2} + \frac{1}{2(a^2 \sin^2 \theta + b^2 \cos^2 \theta)} \right]. \]  

S10

The bending contribution to the energy is then written as

\[ F_{\text{bend}}(h) = \pi ka^3 b \int_{-1}^{w(h)} \frac{dy}{\sqrt{1 - e^2 y^2}} \left[ \frac{1}{b^2} - \frac{1}{a^2} - \frac{1}{2a^2} + \frac{1}{2(a^2 \sin^2 \theta + b^2 \cos^2 \theta)} \right]^2. \]  

S11

\[ \frac{dF_{\text{bend}}(h)}{dh} = \frac{\pi ka^3}{\sqrt{1 - e^2 y(h)^2}} \left[ \frac{1}{b^2} - \frac{1}{a^2} + \frac{1}{2(a^2 \sin^2 \theta + b^2 \cos^2 \theta)} \right]^2. \]  

S12

For spherical invaders the equations reduce to

\[ F_{\text{bend}}^{\text{sph}}(h) = 4\pi \frac{hk}{a} \quad \frac{dF_{\text{bend}}^{\text{sph}}(h)}{dh} = 4\pi \frac{h\kappa}{a}. \]  

S13

Line tension. This contribution describes the energy associated to the deformation of the membrane at the junction (triple line) between the contact region and the non-adhering surface of the host cell. As such

\[ F_{\text{lt}}^{\text{sph}}(h) = F_{\text{lt}}^{\text{sph}}(h) = 2\pi \gamma \Gamma(h) = 2\pi \gamma a \sqrt{1 - y(h)^2} \]  

S14

\[ \frac{dF_{\text{lt}}^{\text{sph}}(h)}{dh} = -\frac{2\pi \gamma a}{b} \frac{y(h)}{\sqrt{1 - y(h)^2}}, \]  

S15

where \(\gamma\) is the line tension and \(\Gamma(h)\) is the length of the triple line.

II: CALCULATION OF THE ADHESION FREE ENERGY: MOBILE LIGANDS

We consider an invader of area \(S_{\text{Tot}}\) carrying \(N_l\) ligands, interacting with a cell surface functionalized by receptors. \(S_{\text{CR}}\) denotes the area of the contact region (CR) between the cell and the invader, while \(S_{\text{OR}}\) is the area of the invader outer region (OR) (see Fig. 1 of the main text). The density of receptors in the CR is controlled by the areal density \(\rho_{R(0)}\) of an ideal receptor reservoir in contact with the CR, related to a receptor chemical potential \(\mu_R\) by the relation \(\mu_R \sim \log \rho_{R(0)}\). Ligands and receptors are modeled as freely diffusing hard disks. Ligands can reversibly bind receptors forming connections between the invader and the cell membrane (see Fig. 1 of the main text). Reaction dynamics is controlled by the equilibrium constant \(K_{\text{21D}}^{\text{eq}}\) (see main text).

The partition function of the system \(Z\) is derived summing over all the possible configurations of the system, specified
by the number of dimers \((n_D)\), of receptors \((n_R, n_R - n_D)\) of which unbound), and ligands \((n_L, n_L - n_D)\) of which unbound) present in the CR

\[
Z = \sum_{n_L=0}^{N_L} \sum_{n_R \geq 0} \min[n_R, n_L] \sum_{n_D=0}^{\min[n_R, n_L]} Z(n_L, n_R, n_D)
\]

\[
Z(n_L, n_R, n_D) = \exp[-\beta F(n_L, n_R, n_D)]
\]

\[
= \left( \frac{N_L}{n_L} \right) (S_{OR})^{n_L-n_l} (S_{CR})^{n_R+n_l-n_D} Z_{OR}^{(excl)} (N_L - n_L) \times
\]

\[
\frac{(p_R^{(0)})^{n_R}}{n_R!} \frac{n_R! n_L!}{n_D! (n_R - n_D)! (n_L - n_D)!} (K_{2D}^{(eq)})^{n_D} Z_{CR}^{(excl)} (n_R - n_D, n_L - n_D, n_D).
\]

In Eq. S16, \((p_R^{(0)})^{n_R}/n_R!\) is the grand-canonical weight of having \(n_R\) receptors in the CR while the following combinatorial term accounts for the number of ways \(n_D\) dimers can be formed starting from \(n_L\) ligands and \(n_R\) receptors in the contact region [S3, S4]. \(F\) is the free-energy at fixed number of complexes in the CR, and \(Z_{OR}^{(excl)}\) is the non-ideal part of the partition function of \(N_L - n_L\) ligands confined in an area equal to \(S_{OR}\), and can be written as

\[
Z_{OR}^{(excl)} = \frac{1}{(S_{OR})^{N_L-n_L}} \int d^2 r_1 \cdots d^2 r_{N_L-n_L} \exp[\beta \sum_{i<j} V_{LL}(|r_i - r_j|)],
\]

where \(r_i\) are ligand coordinates spanning the outer region of the invader, and \(V_{LL}\) models excluded volume interactions between ligands. We neglect curvature effects and calculate \(Z_{OR}^{(excl)}\) using flat surfaces with periodic boundary conditions. This approximation is valid in the limit of big invaders and allows sampling the non-ideal properties of the system using small simulation boxes at given ligand and receptor densities. \(Z_{CR}^{(excl)}\) is the non-ideal part of the partition function in the contact region and is defined similarly to Eq. S17. However \(Z_{CR}^{(excl)}\) also includes excluded volume interactions between dimers and ligands/receptors as specified by the potentials \(V_{LL}, V_{LD}\) and \(V_{RD}\). Without loss of generality in this study we have neglected ligand-receptor steric interactions. If one chose \(V_{LR} \neq 0\), however, the thermodynamic integration procedure defined in Eq. 5 of the main text should have included an extra contribution due to the fact that ligands and receptors interact also in absence of dimerization. In this work we sampled micro-states distributed as in Eq. S17 using Monte Carlo simulations (Sec. ) and a virial expansion as detailed in Sec. II.A:

II.A: Monte Carlo Algorithm

The Monte Carlo moves we implemented are sketched Fig. 1(b) of the main text. The acceptance rules presented below satisfy detailed balance conditions calculated using Eq. S16.

Ligands are moved between the CR and the OR by means of a semi-grand canonical move that conserves the total number of ligands. The flow chart of the algorithm is the following:

- With equal probability we decide whether to attempt a displacement from the CR to the OR or vice versa.
- A ligand is randomly chosen from the CR (OR).
- A new position for the ligand is randomly selected in the OR (CR).
- We check whether the new position satisfies excluded volume constraints (\(i.e.\) the ligand does not overlap with another ligand or a dimer).
- If excluded volume constraints are satisfied the move is accepted with probability

\[
\text{acc} = \min \left[ 1, \frac{n_o S_n}{(N_L - n_o + 1)S_o} \right],
\]

where \(n_o\) is the number of ligands in the region from which we attempt to remove a binder, and \(S_o/S_n\) \((o/n=CR\) or OR\) is the area of the old/new region.
Receptors are exchanged between the CR and an ideal reservoir with areal density $\rho_{R}^{(0)}$ by means of a grand-canonical move, implemented as follows:

- With equal probability we decide whether to attempt an insertion or removal of a receptor from the CR.
- If an insertion move is chosen we randomly select a position for the new receptor in the CR.
- We check excluded volume constraints in the CR.
- If excluded volume constraints are satisfied we accept the insertion move with probability
  \[
  \text{acc}(m \rightarrow m + 1) = \min \left[ 1, \frac{\rho_{R}^{(0)} S_{CR}}{m + 1} \right],
  \]
  where $m$ is the number of receptors in the CR prior the move.
- For removal moves we chose a random receptor to remove from the CR.
- Removal moves are accepted with probability
  \[
  \text{acc}(m \rightarrow m - 1) = \min \left[ 1, \frac{m}{\rho_{R}^{(0)} S_{CR}} \right].
  \]
  \[\text{(S20)}\]
  \[\text{(S21)}\]

**Reaction moves** in which dimers are formed from a dissociated ligand-receptor pair in the CR, or an existing dimer is split into a ligand and a receptor, are implemented as follows:

- With equal probability we decide whether to form or break a dimer.
- If a dimer formation is attempted, we randomly chose a ligand and a receptor from the CR.
- A position for the newly formed dimer is chosen randomly in the CR.
- Excluded volume constraints are checked.
- If excluded volume constraints are satisfied, the dimerisation is accepted with probability
  \[
  \text{acc}\{n, m, d\} \rightarrow \{n - 1, m - 1, d + 1\} = \min \left[ 1, \frac{nm K_{\text{eq}}^{(2D)}}{d + 1 S_{CR}} \right],
  \]
  where $n$, $m$, and $d$ are respectively the number of ligands, receptors and dimers in the contact area prior the move.
- If a dimer breakup is attempted, we randomly chose a dimer in the CR.
- New positions for the freed ligand and receptor are chosen randomly in the CR.
- Excluded volume constraints are checked for the ligand and the receptor.
- If excluded volume constraints are satisfied the breakup move is accepted with probability
  \[
  \text{acc}\{n, m, d\} \rightarrow \{n + 1, m + 1, d - 1\} = \min \left[ 1, \frac{d}{(n + 1)(m + 1) K_{\text{eq}}^{(2D)}} \right].
  \]
  \[\text{(S23)}\]
II.B: Virial expansion of the adhesion free energy

We estimate the partition function of the system (Eq. S16) by using a second virial approximation of the excluded volume part of for the OR ($Z_{OR}^{(excl)}$) and the CR ($Z_{CR}^{(excl)}$) partition functions [S5]. In terms of Mayer factors ($f_{LL}(r_{ij}) = \exp[V_{LL}(|r_i - r_j|)] - 1$) $Z_{OR}^{(excl)}$ (Eq. S17) can be written as

$$Z_{OR}^{(excl)}(N_L - n_L) = \frac{1}{(S_{CR})_{N_L-n_L}} \int dr_1 \cdots dr_{N_L-n_L} \prod_{i<j} (f_{ij}(r_{ij}) + 1)$$

$$= 1 + \frac{1}{S_{CR}} \sum_{i<j} \int dr_i dr_j f_{ij}(r_{ij}) + \cdots,$$

(S24)

from which we obtain

$$Z_{OR}^{(excl)}(N_L - n_L) = 1 - B_2 \frac{(N_L - n_L)(N_L - n_L - 1)}{S_{OR}} + \cdots,$$

(S25)

where $B_2 = \pi \alpha^2/2$ is the second virial coefficient of disks with diameter $\alpha$. Note that corrections to the previous expressions are of the order of $\phi^2$. Similarly

$$Z_{CR}^{(excl)}(n_R^0, n_L^0, n_D) = 1 - B_2 \frac{n_L^0(n_L^0 - 1)}{S_{CR}} - B_2 \frac{n_R^0(n_R^0 - 1)}{S_{CR}} - B_2 \frac{n_D(n_D - 1)}{S_{CR}} - 2B_2 \frac{n_L^0 n_R^0}{S_{CR}} - 2B_2 \frac{n_L^0 n_D}{S_{CR}}$$

(S26)

where $n_L^0$ and $n_R^0$ are the numbers of free ligands and receptors in the CR ($n_L^0 = n_L - n_D$ and $n_R^0 = n_R - n_D$). By inserting Eqs. S25 and S26 into Eq. S16 we can explicitly estimate the free energy $\mathcal{F}$ as function of $n_L$, $n_D$, $n_R$, and $B_2$. In the saddle-point approximation we identify most probable values for the average numbers of ligands, receptors, and dimers by setting

$$\frac{d\mathcal{F}}{dn_L} = 0, \quad \frac{d\mathcal{F}}{dn_R} = 0, \quad \frac{d\mathcal{F}}{dn_D} = 0$$

(S27)

obtaining, respectively

$$\log \left[ \frac{N_L - n_L}{S_{OR}} \frac{S_{CR}}{n_L - n_D} \right] = 2B_2 \left[ \frac{n_L - n_D}{S_{CR}} - \frac{N_L - n_L}{S_{OR}} \right] + 2B_2 \frac{n_D}{S_{CR}}$$

(S28)

$$\log \left[ \frac{\rho_R \frac{S_{CR}}{n_R - n_D}}{S_{CR}} \right] = 2B_2 \frac{n_R - n_D}{S_{CR}} + 2B_2 \frac{n_D}{S_{CR}} = 2B_2 \frac{n_R}{S_{CR}}$$

(S29)

$$\log \left[ \frac{(n_R - n_D)(n_L - n_D)}{n_D} \frac{K_{2D}^{(eq)}}{S_{CR}} \right] = 2(B_2 - B_2) \frac{n_R - n_D}{S_{CR}} + 2(B_2 - B_2) \frac{n_L - n_D}{S_{CR}} + 2(B_2 - B_2) \frac{n_D}{S_{CR}} = -2B_2 \frac{n_D}{S_{CR}}.$$

(S30)

By setting $B_2 = 0$ in Eqs. S28, S29 and S30 we obtain the number of ligands, receptors, and dimers ($n_{L,0}$, $n_{D,0}$, and $n_{R,0}$) in the limit of ideal linkers ($\alpha = 0$) [S4]

$$n_{L,0} = N_L \frac{S_{CR}(1 + K_{2D}^{(eq)} \rho_R^{(0)})}{S_{OR} + S_{CR}(1 + K_{2D}^{(eq)} \rho_R^{(0)})}$$

(S31)

$$n_{D,0} = N_L \frac{S_{CR}K_{2D}^{(eq)} \rho_R^{(0)}}{S_{OR} + S_{CR}(1 + K_{2D}^{(eq)} \rho_R^{(0)})}$$

(S32)

$$n_{R,0} = \rho_R^{(0)} S_{CR} + n_{D,0}$$

(S33)
Using Eqs. S31, S32, and S33 with Eqs. S28, S29, and S30 we can calculate the leading order corrections to the ideal terms \((n_{L,1}, n_{D,1}, n_{R,1})\). These satisfy

\[
\begin{align*}
    n_{D,1} - n_{L,1} & \left[ \frac{S_{CR}}{S_{OR}} + 1 \right] = \frac{2B_2n_{D,0}}{S_{CR}} (n_{L,0} - n_{D,0}) & \text{(S34)} \\
    n_{D,1} - n_{R,1} & = 2\rho_R^{(0)} B_2 n_{R,0} & \text{(S35)} \\
    \frac{n_{R,1}}{\rho_R^{(0)} S_{CR}} + \frac{n_{L,1}}{n_{L,0} - n_{D,0}} - n_{D,1} & = -\frac{2B_2 n_{D,0}}{S_{CR}} & \text{(S36)}
\end{align*}
\]

The free energy of the system can then be calculated using the equilibrium concentrations of the complexes (Eqs. S31-S36) in the perturbative expansion of \(\mathcal{F}\) (Eqs. S16, S25, and S26)

\[
\beta \mathcal{F} = \beta \mathcal{F}(n_{L,0} + n_{L,1}, n_{R,0} + n_{R,1}, n_{D,0} + n_{D,1}, n_{L,0} + n_{R,0}, n_{D,0}) \\
= K + N_L \log \left[ \frac{N_L - n_{L,0}}{S_{OR}} \right] + \frac{B_2}{S_{CR}} \left[ (n_{L,0} - n_{D,0})(N_L + n_{D,0}) + n_{R,0}^2 \right] \\
F_{\text{adh}}^{\text{mob,}\alpha} = F(K_{2D}^{(\text{eq})}) - F(0) & \text{(S37)}
\]

where \(K = -N_L \log \left[ \frac{N_L - \rho_R^{(0)} S_{CR}}{S_{OR}} \right]\), \(F_0\) is the reference value of the free-energy that is calculated using \(K_{2D}^{(\text{eq})} = 0\), and \(S_{\text{Tot}}\) is the total area of the invader (\(S_{\text{Tot}} = S_{OR} + S_{CR}\)).

Note that because of the saddle-point equations S27, at the leading order in \(\phi\), only the ideal number densities contribute to the free energy.

Also, \(F_0\) should be subtracted from \(\mathcal{F}\) when calculating adhesion free energies. Using Eqs. S33, S31, S32 in Eq. S37 we can derive Eqs. 3 and 4 of the main text.

### III: Calculation of the Adhesion Free Energy: Fixed Ligands

Here we adapt calculations of the previous section to the case of invaders decorated by fixed ligands binding ideal mobile receptors. In this case the number of ligands in the CR is fixed and equal to \(n_{L}(h) = N_L S_{CR}(h)/S_{\text{Tot}}\). Similarly to Eq. S16 the partition function is then given by

\[
Z = \sum_{n_R \geq 0} \sum_{n_D=0}^{n_{\text{min}}[n_R,n_L]} Z(n_R, n_D) \\
Z(n_R, n_D) = \exp[-\beta \mathcal{F}(n_R, n_D)] \\
= \left( \frac{S_{CR} \rho_R^{(0)}}{n_R} \right)^{n_R} \frac{n_{R,0}! n_{L,0}!}{n_{R,0}! n_{L,0}!(n_{R,0} - n_{D,0})(n_{L,0} - n_{D,0})!} \left( \frac{K_{2D}^{(\text{eq})}}{S_{CR}} \right)^{n_D}. & \text{(S38)}
\]

Using the previous equations we can calculate the average number of ideal receptors and dimers by solving the saddle–point equations

\[
\frac{d\mathcal{F}(n_{R,0}, n_{D,0})}{dn_R} = 0 \quad \frac{d\mathcal{F}(n_{R,0}, n_{D,0})}{dn_D} = 0. & \text{(S39)}
\]

obtaining

\[
n_{R,0} - n_{D,0} = \rho_R^{(0)} S_{CR} \quad \frac{n_{D,0}}{(n_{R,0} - n_{D,0})(n_{L,0} - n_{D,0})} = \frac{K_{2D}^{(\text{eq})}}{S_{CR}}. & \text{(S40)}
\]

Using Eqs. S40 into Eq. S38 we can calculate the free energy and adhesion free energy as follows

\[
\beta \mathcal{F} = \beta \mathcal{F}(n_{R,0}, n_{L,0}) = -n_L(h) \log(1 + K_{2D}^{(\text{eq})} \rho_R^{(0)}) + \rho_R^{(0)} S_{CR} \\
F_{\text{adh}}^{\text{fix,}\alpha=0} = F(K_{2D}^{(\text{eq})}) - F(0) & \text{(S41)}
\]

Eq. S42 corresponds to Eq. 2 of the main text.
[S1] W. Helfrich, Zeitschrift für Naturforschung C 28, 693 (1973).
[S2] N. M’Arthur, Edinburgh Mathematical Notes 24, xvi (1929).
[S3] F. J. Martinez-Veracoechea and D. Frenkel, Proc. Natl. Acad. Sci. USA 108, 10963 (2011).
[S4] S. Shimobayashi, B. M. Mognetti, L. Parolini, D. Orsi, P. Cicuta, and L. Di Michele, Phys. Chem. Chem. Phys. 17, 15615 (2015).
[S5] T. L. Hill, Statistical mechanics: principles and selected applications (Courier Corporation, 2013).