Minimal encounter time and separation determine ligand-receptor binding in cell adhesion
SUPPLEMENTARY INFORMATION

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Molecular extension

In this section, we justify our approximation that the reactive site is diffusing on an hemisphere of radius given by the maximal extension of the molecular tether. The structure of antibodies and immunoglobulin domains is described in (1). Antibodies are composed of a straight Fc-domain of 8 nm linked to the 8 nm Fab domain through a freely rotating hinge. Fc-ICAM-1 is represented by a 8 nm Fc segment connected to a 20 nm ICAM-1 segment through an elbow allowing 50° rotation. We compute the distance between the anchored end and the free end for the molecular construction composed of one antibody and the Fc-ICAM-1 chimera. The result presented on fig. 1 shows that a large proportion of configurations exhibit an end to end distance close to the maximal extension of 36 nm. As a comparison, we have plotted the end-to-end distance of a flexible polymer anchored to the surface and exhibiting an extended length of 36 nm. We have used the approximate description by a gaussian chain of radius of gyration $R_g \simeq aN^{3/5} \simeq 9$ nm, with $a = 1$ nm and N=36 subunits.

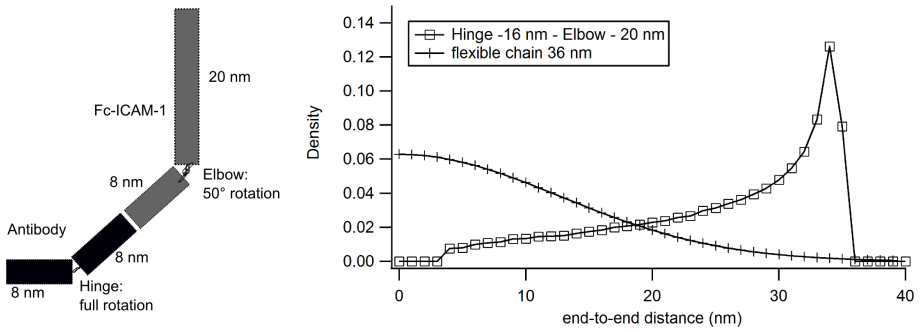

Figure 1: Calculation of the spatial density of the tip of an anchored antibody-Fc-ICAM-1 complex (squares) or of a gaussian chain of identical length (crosses)

First bead-ligand encounter

In the sliding carpet method, we compute the probability that a ligand encounters for the first time any receptor on a given bead, assuming a spherical geometry of the interaction regions of both the ligand and the receptor. We

note M the center of the bead. M goes from altitude $(a + z(t))$ to altitude $(a + z(t + \Delta t))$ during a time step Δt. In order to count for a new interaction, the bead must be outside the interaction zone of the ligand at time t and inside at time $t + \Delta t$. For such a displacement from $M(t)$ to $M(t + \Delta t)$, we calculate the area over which one must find a ligand so that the interaction is possible. In order that they interact, the distance between the ligand and some point at the periphery of the bead must be below L, the total molecular chain length. So the ligand must be within a disk of radius $R = \sqrt{(a + L)^2 - (a + z)^2}$, centered on the position of the bead, projected in the plane of the wall. For a first encounter, the ligand must be in the area delimited by the exclusion of the disk of radius R(t) from the disk of radius $R(t + \Delta t)$. Fig. 2A-D summarizes the different cases. The hatched areas of Fig. 2 are:

$$S_a = \pi R(t + \Delta t)^2 \tag{1}$$

$$S_b = \pi R(t + \Delta t)^2 - [R(t + \Delta t)^2 \arccos\left(\frac{Dr^2 + R(t + \Delta t)^2 - R(t)^2}{2DrR(t + \Delta t)}\right) + R(t)^2 \arccos\left(\frac{Dr^2 + R(t)^2 - R(t + \Delta t)^2}{2DrR(t)}\right)] + \mathcal{A}$$

$$\text{with } \mathcal{A} = \frac{1}{2}\sqrt{2Dr^2(R(t + \Delta t)^2 + R(t)^2) - Dr^4 - (R(t + \Delta t)^2 - R(t)^2)^2}$$

$$S_c = 0 \tag{2}$$

$$S_d = \pi(R(t + \Delta t)^2 - R(t)^2) \tag{3}$$

The position of the ligand is randomly chosen in the hatched area. Whether there is eventually an encounter is determined by the probability of finding a ligand in this area: $P = \sigma_L S_i$, where i stands for cases a, b, c or d (see Fig. 2), and σ_L is the surface density of the ligands. Once the ligand is set up, a receptor is randomly chosen on the bead at a distance equal or below the total molecular tether length, L. Choosing the receptor that is the closest to the ligand gives a difference of less than 5% in the frequency of adhesion at G=10 s^{-1}.

Accounting for bead rotation

For the sake of simplicity, the rotation of the bead was not implemented in the simulation. However, the shear-induced rotation reduces the velocity of the receptor relative to the ligand, w, compared to the velocity of the center

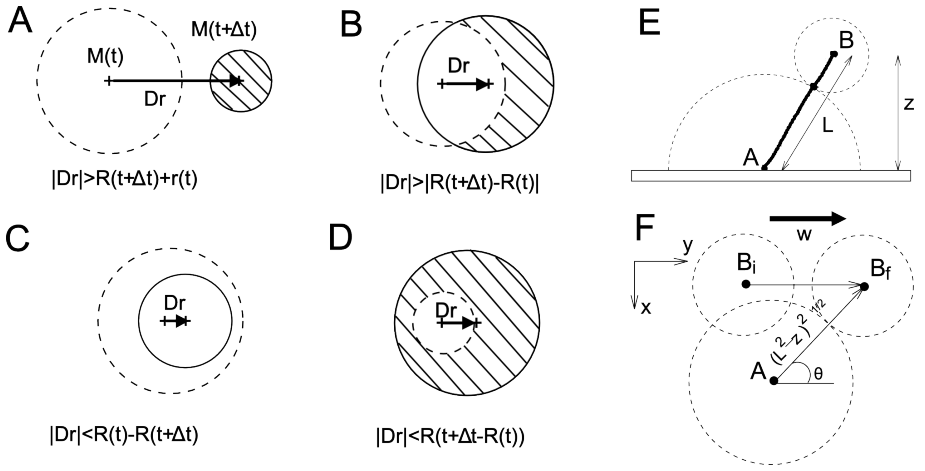

Figure 2: (A-D). The dashed circle limits the region of interaction at time t, with radius $R(t)$, and the full circle is for time $t+\Delta t$, with radius $R(t+\Delta t)$. The hatched area is the area where ligands must be in order that there is a first encounter with some receptor on the bead that has moved from $M(t)$ to $M(t+\Delta t)$. Dr is the in plane projection of the displacement of the bead. (E-F). The receptor B at altitude z moves with velocity w. It interacts with the ligand A as long as it stays in the disk of radius $\sqrt{L^2-z^2}$ from the ligand. A. Side view. B. Top view showing the position of the receptor at two different times.

of mass of the bead, V, as $w \simeq 0.43\,V$ (2, 3). Random rotation of the sphere limits the ligand-receptor interaction duration to $t_{rot} = \frac{\phi^2}{D_r}$ with the rotational diffusion coefficient $D_r = \frac{kT}{8\pi\nu a^3} \sim 1/70$ s^{-1} (ν is the viscosity of the medium) and ϕ the maximal angle of which the bead can turn without disrupting an interaction (4). Evaluating $\phi \simeq \cos(\theta)\sqrt{L^2-z^2}/a$ for a receptor at altitude z and averaging on the azimuthal angle θ defining the receptor position on the bead with respect to the flow direction (see figure and detailed calculation in last paragraph of the supplementary information), one finds $t_{rot}(z) = \frac{L^2-z^2}{2a^2D_r}$. Using the estimate for the encounter duration imposed by convection in supplementary information, $t_{conv}(z) = (\pi/4)\frac{\sqrt{L^2-z^2}}{w}$, one shows that $t_{conv}(z) \ll t_{rot}(z)$ for shear rates $G \geq 10$ and most altitudes z. Therefore, the encounter time is mostly limited by the convection. This situation contrasts with previous studies involving ligand-receptor binding mediated by submicron magnetic bead-bead contacts, which have shown to

be limited by rotational diffusion of the colloids (4). Finally effects of rotation on the translational friction coefficient or effects of random rotation on translation are negligible, as shown previously (3).

Comparison with Chang and Hammer

The earlier work by Chang and Hammer (5) studies the effect of relative motion between the surfaces on the forward rate of binding of tethered reactants. They consider a two-dimensional model where reactive species can diffuse on the surface, but where convection usually dominates diffusion ($Pe \ll 1$). In contrast, our model is three-dimensional and considers the diffusion of the bead, while reactants are immobile on the surface, a description which is perfectly matching our experimental situation. In both approaches, the relative motion between the surfaces increases the rate (number per unit of time) of encounters linearly with the relative velocity, but this effect is integrated by us by using the frequency of adhesion per unit of distance travelled by the beads along the flow. Second, while Chang and Hammer calculate an average encounter duration, we establish instead the complete distribution of encounter durations in order to test the binding law. Indeed, for the non-linear erfc function which fits our data, the use of an average encounter duration is not appropriate and leads to a large error on the adhesion frequency.

Analytical estimate of the adhesion frequency

We estimate the typical encounter duration, t_e^*, while neglecting diffusion. The effect of bead diffusion is finally estimated by comparing the frequency of adhesion obtained from the simulation, which includes diffusion effects, and in the convective limit, as represented by the thin plain line in fig. 3A. Diffusion reduces the adhesion frequency by about 50% at a shear rate G=10 s^{-1}. t_e^* can be found by calculating the mean duration of convection driven encounters. We note $w \simeq 0.43aGK_v(z)$ the velocity of the receptor on the bead, taken to be parallel to the flow. The maximal time spent by a receptor B at position (x, z) in the interaction region of a ligand A is (see Fig. 2): $t(x(\theta), z) = 2\frac{\sqrt{L^2-z^2}\cos\theta}{w}$. Taking into account that receptors bind randomly to the ligand, a factor 0.5 is introduced when averaging on the successive receptors interacting successively with the same ligand. A first average on $x(\theta) = \sqrt{L^2 - z^2}\sin\theta$ positions of the receptor gives $t_{conv}(z) = \int_{\theta=-\pi/2}^{-\pi/2} 0.5t(x,z)dx / \int_{\theta=-\pi/2}^{-\pi/2} dx = (\pi/4)\frac{\sqrt{L^2-z^2}}{w}$ which is

compared with the rotation time in order to justify why rotational diffusion was neglected (see Numerical simulation). The typical encounter duration is found by integrating on altitudes z. Using z_0 the most probable bead altitude in absence of hyaluronan, the typical encounter duration reads $t_e^*(G,L) = (\pi/4)\frac{\sqrt{L^2-z_0^2}}{0.43aGK_v(z_0)}$. The numerical simulation gives, for a bead at altitude z_0, $t_e^*(G,L) \simeq \frac{\sqrt{L^2-z_0^2}}{0.43aGK_v(z_0)}$.

Assuming pure convection of the bead, one can also estimate the number of encounters per unit length, λ. Since the bead flows straight, one bead at altitude z_0 meets on average $n_1 = 2r\sigma_L$ ligands per unit length of bead displacement. $r \simeq 2\sqrt{2a(L-z_0)}$ is the length on the substrate perpendicular to the flow where ligands are at distance less than L of the bead. A given ligand meets successively $n_2 \sim r/\sqrt{L^2-z_0^2}$ receptors on the same bead. Since we assume single bond formation, only one receptor interacts at one time and one obtains $\lambda = n_1 n_2 \sim 8a\sigma_L\sqrt{\frac{L-z_0}{L+z_0}}$.

One approximation previously mentioned considers only the first ligand available at a distance less than L from the bead. In practice, however, there is a significant probability p that more than one ligand is at less than L from the bead. p is given by the Poisson distribution with parameter $s = \pi\sigma_L 2a(L-z_0) \simeq 1.64$ for $L = 76$ nm, z_0=18 nm: no ligand p_0=0.19, 1 ligand p_1=0.32, 2 ligands or more $p_{\geq 2}$=0.49. Therefore, the present estimate of encountered ligands should be multiplied by $\tilde{p} = p_1/(p_1+p_{\geq 2}) \simeq 0.45$. Finally, for a given shear rate and molecular length, the frequency of adhesion of a bead at altitude z_0 can be written as:

$$FA \simeq \tilde{p}\lambda\alpha\,\mathrm{erfc}(\sqrt{t_{on}/t_e^*}) = p8a\sigma_L\sqrt{\frac{L-z_0}{L+z_0}}\alpha\,\mathrm{erfc}(\sqrt{\frac{0.43aGK_v(z_0)t_{on}}{\sqrt{L^2-z_0^2}}}) \quad (4)$$

where α and t_{on} are parameters intrinsic to the bond.

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