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Supporting Material

Title: Single-cell adhesion tests against functionalized microspheres arrayed

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Materials and Methods

Bead preparation and measurement of cadherin surface densities

E- and N-cadherin chimeric proteins (consisting of the extracellular domain of human cadherin fused to the Fc region of human IgG) were purchased from R&D Systems (Minneapolis, MN). The Fc regions allowed binding of the chimera to protein-A-coated polystyrene beads (4.8 μm diameter; Corpuscular Inc., Cold Spring, NY). Negative control beads were prepared by replacing the cadherin chimera with plain Fc fragments (cleaved from human IgG; Jackson ImmunoResearch Laboratories, West Grove, PA).

The Protein A beads were first washed twice in phosphate buffered saline with 0.01% (v/v) Tween-20 (PBST) and then incubated in either a cadherin solution (20 μg/mL of E- or N-cadherin in PBST) or an Fc-fragment solution (~50 μg/mL in PBST) for 1 h. Excess and weakly bound protein was removed by washing the beads three times in PBST. To cover bare regions on the beads and block nonspecific binding, the beads were further incubated in phosphate buffered saline with 1% (w/v) bovine serum albu-

min (BSA) overnight and then washed with calcium-free Hank's Balanced Salt Solution (HBSS).

The densities of E- and N-cadherin on the beads were quantified using the Quantum Simply Cellular kit from Bangs Laboratories (Fishers, IN) following the manufacturer's protocol. The monoclonal primary antibodies were HECD-1 against E-cadherin and GC-4 against N-cadherin (both by Abcam, Cambridge, MA). After incubation with a fluorescent secondary antibody (Alexa 488 goat anti-mouse, Invitrogen, Carlsbad, CA), the bead fluorescence intensities were measured by flow cytometry (Attune Acoustic Focusing Cytometer, Applied Biosystems, Foster City, CA). The surface densities of E- and N-cadherin were similar at ~3400 and ~4000 cadherins/μm^2, respectively.

E- and N-cadherin transfected L cells

L cells were stably transfected with either DsRed-tagged E-cadherin (1) or GFP-tagged N-cadherin (gift from Dr. Kathleen Green, Northwestern University) and FACS-sorted based on fluorescence intensity. Cadherin-expressing cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (Invitrogen) with 10% Fetal Bovine Serum (Atlanta Biologicals, Lawrenceville, GA) using standard cell culture techniques. Immediately before experiments, the adherent cells were detached by incubation in EDTA-free trypsin solution containing 1.8 mM Ca^{2+} for 5 minutes and then re-suspended in culture medium.

Setup for adhesion measurements using cantilever-based bead arrays

Details of our horizontal AFM/micropipette instrument have been described previously (2). The AFM cantilevers used in this study were Olympus Bio-Levers supplied on conventional AFM cantilever chips (Asylum Research, Santa Barbara, CA; Fig. 1 *C* of the main text, Movie S1). For each experiment, a chip was plasma-cleaned for 1 min (Harrick Plasma, Ithaca, NY) and clamped into the measurement chamber (2). The chamber was then gently filled with HBSS with Ca^{2+}. The cantilever spring constants were calibrated using the Sader method (3). Both the local cantilever stiffness as well as the optical lever sensitivity are functions of the position along the length of the cantilever (2). Accordingly, our calibration determined these parameters for all bead locations of a given bead array.

Micropipettes (~4 μm inner diameter) were fabricated as described previously (4). To reduce nonspecific adhesion between micropipette tips and beads or cells, the micropipettes were backfilled with HBSS containing 1% (w/v) BSA. Both types of cadherin-coated beads and control Fc beads were carefully deposited onto the bottom of the experiment chamber in three distinct regions using gel-loading pipette tips. Individual probe beads were selected from each population using the micropipette and attached to the cantilever as described in the main text (Movie S1). Cadherin beads from the same populations were later used as test beads in bead-bead adhesion measurements (Fig. 2 *A, B* and *D* of the main text, Movie S2).

Alternatively, small quantities of E- and N-cadherin-expressing cells were deposited into the chamber for use in cell-bead experiments (Fig. 2 *C* and *E* of the main text, Movie S2). The L cells are naturally adherent and tend to spread on untreated coverslips. To inhibit cell spreading and facilitate the manipulation of individual cells with micropipettes, the bottom coverslips of our experiment chamber were pre-coated with BSA as follows. First, the coverslips were sonicated in 2% Hellmanex solution (Sigma-Aldrich, St. Louis, MO), rinsed in DI water, dried, and plasma-cleaned for 1 min. The clean coverslips were siliconized by submersion in a solution of 2% SurfaSil (Thermo Scientific, Rockford, IL) in acetone for ~20 s. Excess SurfaSil was removed by rinsing first in acetone and then in methanol. The SurfaSil layer was cured by drying the coverslips at 110°C for 15 min. SurfaSil-treated coverslips were then soaked in HBSS containing 1% (w/v) BSA for 30 min to allow BSA to physisorb to the coverslip surface. Finally, the BSA-coated coverslips were rinsed in DI water and dried under a stream of argon.

Estimation of the ratio between on-rate constants

We consider adhesion events between surface-bound reactants of kind "a" and "b". The adhesion frequency $p^{(a:b)}$ measured in repeated contacts between the two surfaces can be related to the average number $\langle n^{(a:b)} \rangle$ of individual bonds forming per contact through (5),

$$\left\langle n^{(a:b)} \right\rangle = -\ln\left(1 - p^{(a:b)}\right). \tag{1}$$

This relationship assumes that only specific bonds form between the two reactants. If negative control experiments exhibit non-negligible adhesion, the measured adhesion frequency $p_m^{(a:b)}$ encompasses specific and nonspecific events. Assuming that surface "b" had been replaced with a suitable nonreactive surface (denoted by "ctl") in the control experiments, one may infer the specific adhesion frequency using (6),

$$p^{(a:b)} = \frac{p_m^{(a:b)} - p_m^{(a:ctl)}}{1 - p_m^{(a:ctl)}}, \tag{2}$$

where $p_m^{(a:ctl)}$ is the adhesion frequency measured in the control experiments.

The average number of bonds is a function of the contact area A_c between the two surfaces, the surface densities ρ_a and ρ_b of the two reactants, and the kinetic on- and off-rate constants, k_{on} and k_{off}, respectively. This functional dependence is given by (5),

$$\langle n \rangle = A_c \, \rho_a \, \rho_b \, \frac{k_{on}}{k_{off}} \left(1 - e^{-k_{off} t}\right), \tag{3}$$

where t denotes the contact time (and superscripts have been dropped for the moment). Previous measurements of the stress-free bond lifetime of E-cadherin interactions gave values greater or equal than ~100 s, i.e., $k_{off} \leq 0.01$ s^{-1} (7). The contact time in our experiments was $t = 0.026 \pm 0.005$(SD) s. Thus the exponent in Eq. 3 is much smaller than 1 for our experiments, allowing us to linearize the exponential:

$$\langle n \rangle \cong A_c \, \rho_a \, \rho_b \, k_{on} t \, . \tag{4}$$

The contact time t was kept constant throughout the experiments included here. The nominal (feedback-controlled) touch force—and thus the contact area—was constant as well. We did not notice a reduction of the adhesion frequency with time for a given test bead, indicating that the protein surface densities did not appreciably decrease due to cadherin detachment from the beads. Therefore, the ratio between the average bond numbers in adhesion tests using the same pipette-held bead (of kind "a") against two different cantilever-probe beads (of kind "a" and "b", respectively) simplifies to

$$\frac{\left\langle n^{(a:a)} \right\rangle}{\left\langle n^{(a:b)} \right\rangle} \cong \frac{\rho_a \, k_{on}^{(a:a)}}{\rho_b \, k_{on}^{(a:b)}}. \tag{5}$$

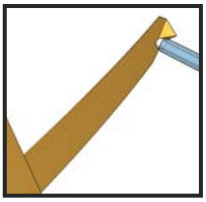
Combined with Eqs. 1 and 2, it follows that the ratio between the on-rate constants of "homophilic" (a:a) and "heterophilic" (a:b) interactions is given in terms of measured quantities by

$$\frac{k_{on}^{(a:a)}}{k_{on}^{(a:b)}} \cong \frac{\rho_b}{\rho_a} \frac{\ln\left(1 - \dfrac{p_m^{(a:a)} - p_m^{(a:ctl)}}{1 - p_m^{(a:ctl)}}\right)}{\ln\left(1 - \dfrac{p_m^{(a:b)} - p_m^{(a:ctl)}}{1 - p_m^{(a:ctl)}}\right)} = \frac{\rho_b}{\rho_a} \frac{\ln\left(1 - p_m^{(a:a)}\right) - \ln\left(1 - p_m^{(a:ctl)}\right)}{\ln\left(1 - p_m^{(a:b)}\right) - \ln\left(1 - p_m^{(a:ctl)}\right)}. \tag{6}$$

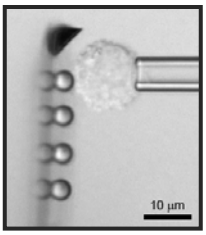
The measured ratio of cadherin densities on our beads was $\rho_E : \rho_N \approx 0.85$ (cf. Materials and Methods section). Examples of measured adhesion frequencies are shown in Fig. 2 *D*. Using these values, we obtain $k_{on}^{(E:E)} \big/ k_{on}^{(E:N)} \approx 2.3 \pm 1.0 (\mathrm{SD})$ and $k_{on}^{(N:N)} \big/ k_{on}^{(E:N)} \approx 5.3 \pm 2.7 (\mathrm{SD})$ as crude estimates of the ratios of on-rate constants of the respective cadherin bonds.

Movie Legends

Movie S1. Assembly of bead arrays on AFM cantilevers. Starting from a sketch of a commercial AFM chip, this animation zooms in on a single cantilever. It then illustrates how a micropipette is used to attach a bead to the flat of the cantilever. Additional beads are attached in a similar fashion to form a one-dimensional array along the centerline of the cantilever. Finally, the animation transitions to a videomicrograph of a completed five-bead array. It shows the cantilever in the side view that is characteristic for the horizontal configuration of our custom-built AFM.



Movie S2. Testing cadherin binding using functionalized bead arrays. An array of cadherin and control beads was assembled on a cantilever. A micropipette-held cadherin bead is repeatedly moved to/from contact with individual probe beads of the array. Because the local stiffness of the cantilever depends on the probe-bead position, the micropipette retraction speed is adjusted to maintain a constant force-loading rate. In a separate experiment featuring a similar bead array, a cadherin-expressing cell is sequentially tested against different beads of the array.



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