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Article

Push-pull mechanics of E-cadherin ectodomains in biomimetic adhesions

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ABSTRACT E-cadherin plays a central role in cell-cell adhesion. The ectodomains of wild type cadherins form a crystalline-like two dimensional lattice in cell-cell interfaces mediated by both *trans* (apposed cell) and *cis* (same cell) interactions. In addition to these extracellular forces, adhesive strength is further regulated by cytosolic phenomena involving α and β–catenin–mediated interactions between cadherin and the actin cytoskeleton. Cell-cell adhesion can be further strengthened under tension through mechanisms that have not been definitively characterized in molecular detail. Here we quantitatively determine the role of the cadherin ectodomain in mechanosensing. To this end, we devise an E-cadherin-coated emulsion system, in which droplet surface tension is balanced by protein binding strength to give rise to stable areas of adhesion. To reach the honeycomb/cohesive limit, an initial emulsion compression by centrifugation facilitates E-cadherin *trans*-binding, while a high protein surface concentration enables the *cis*-enhanced stabilization of the interface. We observe an abrupt concentration dependence on recruitment into adhesions of constant crystalline density, reminiscent of a first-order phase transition. Removing the lateral *cis*-interaction with a "*cis* mutant" shifts this transition to higher surface densities leading to denser, yet weaker adhesions. In both proteins, the stabilization of progressively larger areas of deformation can be rationalized by a stiffening catch-bond, whose strength increases with tension. This catch bond may well correspond to one that has been identified in the cadherin “X-dimer".

SIGNIFICANCE The cytoskeletal role in reinforcing cell-cell adhesion is well known, but the contribution of the extracellular E-cadherin domains remains elusive. This work uses a biomimetic emulsion system to demonstrate the important ‘catch-bond’ behavior of E-cadherin ectodomains in response to push-pull mechanics. We find that the binding strength of E-cadherin adhesion increases with tension in both the WT and the *cis*-deficient MT proteins. Moreover, we observe abrupt recruitment into crystalline adhesions as a function of surface concentration, consistent with the proposal of a first-order phase transition at adhesion junctions. Our system is compatible with biological cells, opening the field to biophysical studies of the hybrid system.

INTRODUCTION

E-cadherin adhesion plays a crucial role in mechanical processes in biology, such as morphogenesis, development (1–3), maintenance of tissue structure (4–7) and tumor metastasis (8, 9). *In-vivo*, cadherins form cell-cell junctions through the cooperative action of *trans* and *cis* binding (10, 11). Extracellular *trans* dimers undergo a homophilic interaction with a free energy of binding that has been measured *in vitro* to be in the range of $9 - 10 k_B T$ (12) in 3-D bulk solution. These dimers laterally cluster via *cis* interactions on the cell surface into a 2D lattice at adhesion sites, with a 2D binding energy predicted to be on the order of 4 $k_B T$ by numerical simula-

tions (13). The clustering of cadherins at adherens junctions is driven by both intra- and extracellular interactions (14–16).

There is increasing evidence that cadherin adhesions are active mechanical sensors (17–23). More specifically, intracellular domains interact with the actin cytoskeleton through adaptor proteins, α and β catenin, which have been shown to be tension-dependent (24–26). Whether the mechanosensitive behavior of E-cadherin is due to its intrinsic molecular properties or due to those of the cadherin cytoskeletal complex is unclear. Of note, extracellular cadherin domains have been shown to exhibit mechanosensitive catch-bond properties in single-molecule force-spectroscopy experiments (27–29).

Simplified systems of cadherin-functionalized emulsions, liposomes, and model membranes, have served as useful probes of the mechanisms underlying *cis* and *trans* cooperativity (30–35). Nevertheless, the mechanosensing effects of extracellular cadherin adhesion and the relative contribution of *cis* and *trans* binding have not been quantified. Here, we investigate extracellular cadherin adhesion in a tissue-mimetic emulsion, where compression and relaxation, protein surface concentration, and the presence of *cis* interactions can be independently controlled.

Using emulsions, we mimic this cellular adherens junction formation by pushing the emulsions together through a calibrated pressure in the kPa range to facilitate protein recruitment and adhesion (36). This applied pressure mimics the protrusive pushing forces driven by the actin-based Arp2/3 complex, which are known to be necessary for cells to efficiently form and extend cadherin adhesions (37). After compression by centrifugation, the emulsion is allowed to relax back to mechanical equilibrium, in which there is a balance between surface tension and the adhesive energy of protein binding. This step aims to mimic the stabilization of adhesion by cellular pulling forces (38). The resulting droplet deformation allows us to estimate the average binding energy per cadherin dimer. Comparing wild type (WT) and *cis*-deficient mutant (MT) shows that *cis* interactions significantly contribute to the stabilization of adhesions and lead to larger areas of deformation (10).

For both WT and MT, we find a pressure-sensitive adhesion response. The larger the applied pressure and therefore droplet strain, the larger the equilibrium adhesion size. At the maximum pressure, we reach the honeycomb limit and study the effect of cadherin surface concentration. The WT self-assembles into adhesions with a constant density of 15.7×10^3 cadherins/ μm^2 independent of initial concentration. This finding is consistent with numerical simulations that propose that *cis* interactions drive crystallization in cadherin adhesion in 2-D (13, 39). The absence of *cis* interactions allows the MT to freely rearrange until it reaches a jamming density of 20.1×10^3 cadherins/ μm^2, which is higher than that of the WT crystal. The solid nature of these adhesions in both WT and MT is confirmed by fluorescence recovery after photobleaching (FRAP) measurements.

Interestingly, we observe that a wide range of adhesion areas can be stabilized by a constant cadherin density, which we interpret via a tension-dependent binding free energy of cadherin dimers. Our experiments indicate that the binding energy progressively increases with droplet strain, offering a scale of binding energies from 1.6 k_BT in the weak-binding regime, up to 20 k_BT per molecule at the cohesive limit. Our results suggest an intrinsic bond strengthening due to applied tension, suggesting that E-cadherin mechanosensitivity is due in part to intrinsic molecular properties of E-cadherin.

MATERIALS AND METHODS

Emulsion Preparation

The protocol for the emulsion preparation is described in (30). Briefly, the oil droplets are co-stabilized with SDS (1 mM) and the following mixtures of lipids: EPC (egg phosphatidylcholine) and DGS-NTA (Ni) lipids at a molar ratio of 92:8. The lipids were purchased from Avanti Polar Lipids (St. Louis, MO). They were mixed to reach a total mass of about 14 mg and dried under nitrogen before the addition of 10 mL 50-cSt silicone oil purchased from Sigma Aldrich (St. Louis, MO). The lipid-containing oil was then sonicated for 30 min at room temperature and heated for 3 hours at 50°C. We thus obtained an oil saturated with phospholipids. The lipid-containing oil (10 mL) was then emulsified through a microfluidic chip. The resulting emulsion was mono-disperse with a diameter of about 20 μm. Brownian emulsion droplets were produced through membrane emulsification by a 0.5 μm pore membrane (SPG Technology, Miyazaki, Japan), with 10 mM SDS as the continuous phase, which resulted in droplets with a diameter of approximate $4 - 5$ μm. To functionalize these droplets with DGS-NTA(Ni), 0.5 mg of DGS-NTA(Ni) was dried and rehydrated with binding buffer and droplets at a $1:1$ ratio. The droplets were incubated with the phospholipid and binding buffer mix overnight at room temperature and gentle rotation.

Cadherin Grafting

Binding buffer was prepared containing 3.8mM calcium, 2 mM EDTA, 20 mM Tris, 10 mM NaCl, 10 mM KCl, pH = 7, and 1 mM SDS in a 50:50 glycerol/water solution for refractive index matching. The emulsion was mixed with binding buffer at 40% volume fraction and then incubated with varying concentrations of His-tagged E-cadherin ectodomains in 50 μL of binding buffer containing EDTA. Low concentrations of salts were added to reduce nonspecific adhesions between the droplets.

Cadherin Density Estimation

We experimentally measured the density of cadherins on the droplet surface at droplet saturation. The fluorescence intensity of a solution of 1 μM WT E-cadherin, containing 3.0×10^{13} molecules was measured through a fluorimeter (Horiba-PTI

QM-400 Fluorescent spectrometer). Upon adding droplets at 40% volume fraction and 1.8 mM calcium, we observed a 62% loss in fluorescence intensity of the 1 μM cadherin solution. This loss of intensity from the bulk solution indicated that approximately 1.8×10^{13} cadherin molecules migrated from the bulk solution on to the droplets. We estimated the droplet density to be about 3.2×10^4 /μl, corresponding to a total droplet count of about 1.6×10^6 in a 50μl sample. Therefore, there were approximately 1.2×10^7 cadherin molecules per droplet. For a droplet of radius 10 μm, the surface area is 1256 μm^2. The density of cadherins at saturation is approximately 9.3×10^3 /μm^2. Therefore, a saturation intensity of 150 A.U. for WT cadherin and 120 A.U. for MT cadherin corresponded to a cadherin surface density of 9.3×10^4 /μm^2. The fluorescence measurements are shown in Fig. S1.

Confocal Microscopy

The droplets were grafted with cadherin proteins which were fluorescently labeled with Alexa 488. The buffer was refractive index matched with glycerol for transparency, which allowed for imaging of the emulsion in 3-D. We used a fast-scanning confocal microscope (TCS SP5 II; Leica Microsystems, Buffalo Grove, IL)

Centrifugation and Pressure Measurement

The experimental setup involved cadherin-functionalized droplets contained in a capillary, 5mm and 100μm in depth (VitroTubes, NJ). The droplets in the capillary were compressed in a centrifuge at speeds ranging from 50g up to 800g, after which they were allowed to relax over a 48-hour period. The pressure inside the compressed aggregate was estimated through the relation $P = \Delta\rho g h$, where P is the hydrostatic pressure, $\Delta\rho$ is the density difference between silicon oil and water, and h is the height of the compressed aggregate pile which was measured after centrifugation and equilibration of droplets.

Fluorescence Recovery after Photobleaching (FRAP)

Droplets functionalized with cadherin were placed in a glass capillary. The droplet-containing capillaries were centrifuged at 800g to form a cohesive 3-D droplet aggregate, which was then visualized through confocal microscopy. Suitable adhesions and free perimeters were identified and marked as regions of interest. A laser pulse of ~ 200mW was applied for 1.5 minutes to the FRAP region of interest in order to photobleach. FRAP intensity trajectories were fit using custom MATLAB code that subtracted background, normalized the intensities to their pre-bleach level, and fit to an exponential model $I = I_{\max}(1 - e^{-t/\tau})$, where I is the normalized intensity, $I_{\max}$ is the pre-photobleach intensity, t is time, and τ is the characteristic recovery time.

RESULTS

Our biomimetic emulsion system mimics the outer cell membrane, but replaces the cytoskeleton with silicone oil, as shown in Fig. 1A. The oil-in-water interface has a surface tension of 7mN/m in Fig. S2 A and B. The monolayer membrane is stabilized by a mixture of SDS, Egg-PC (EPC), and DGS-NTA-Ni phospholipids, which serve to functionalize the droplets with E-cadherin (30, 41). Droplets are generated using microfluidics, which gives rise to monodisperse ($D = 20 \pm 1$ µm) emulsions that are stable against coalescence. This synthesis allows for their compression into a biliquid foam structure resembling cohesive tissues, as shown in Fig. 1 B, C.

These emulsions are visualized under a confocal microscope, revealing uniform cadherin fluorescence on the surface of the droplets, as shown in the inset in Fig. 2A. Cadherins depend on calcium ions for their adhesive function (42). Specifically, the ectodomains are comprised of tandem immunoglobulin-like domains that bind calcium, which increases their rigidity to form a crescent shape (43). This conformation enables them to bind other cadherins through *trans* interactions across cells and *cis* interactions on the same cell. Histograms of fluorescence intensity on the droplet surface reveal that adding calcium $[Ca^{2+}]$ increases cadherin surface adsorption. Converting the intensity to protein density ρ (see Methods) allows us to quantify the surface adsorption of bulk cadherin [cad], as shown in Fig. 2B. Both WT and MT data are well fit with the Langmuir adsorption isotherm

$$\frac{\rho}{\rho_{\max}} = \frac{[\text{cad}]K_{eq}}{1 + [\text{cad}]K_{eq}}, \tag{1}$$

where $\rho_{\max}$ is the cadherin saturation density and K_{eq} is the equilibrium constant for the His-tag-nickel interaction, whose value in the presence of calcium was estimated to be 4×10^{-12}. Note that in the presence of calcium, cadherin preferentially partitions into the aqueous phase above [cad] = 1 μM, likely due to protein aggregation. We find that adding physiological 1.8mM for $[\text{Ca}^{2+}]$ increases $\rho_{\max}$ by 25%, which corresponds to a decrease in inter-cadherin distance from 9.1 to 8.1 nm, almost reaching the crystalline packing limit at 7.2 nm (10). The ratio of K_{eq} with and without calcium yields a free energy difference of 0.3 k_BT. This adsorption enhancement may be a consequence of the entropy loss upon calcium stiffening of the proteins, as illustrated in Fig. 2C. The protein concentration does not affect the surface tension because the proteins are not adsorbed at the interface, but are carried by the functionalized lipids (44).

Having characterized the density of proteins on the surface, we demonstrate their adhesive function via an aggregation assay (45). Thermal emulsion droplets of diameter 5 μm, functionalized with either WT or MT, do not adhere in the absence of calcium, nor do they adhere in the presence of calcium without cadherins, as shown in Fig. S3. However, they do bind to form diffusion-limited aggregates (DLA) (46) in the presence of proteins and calcium, as shown in Fig. 2D. These

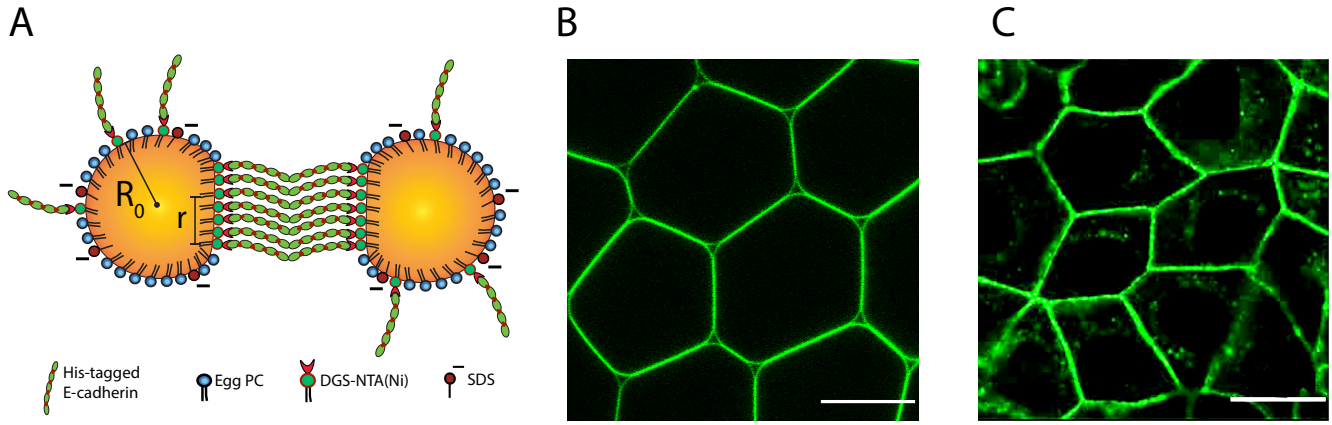

Figure 1: (A) Schematic representation of biomimetic emulsion droplets. Droplets consist of silicon stabilized through phospholipids and SDS. Lipids with a NTA(Ni) group allow for the binding of His-tagged cadherin ectodomains. (B) Biomimetic emulsion droplets functionalized with fluorescent E-cadherin extracellular domain form a confluent tissue-like structure. (C) Cadherin-expressing living cells form a confluent packing through cadherin binding (40). Scale bar: 20μm.

branched structures have on average three droplet neighbors in WT or MT adhesion, consistent with DLA theory, as shown in Fig. 2E.

Using large cadherin-coated droplets the size of biological cells, we study the effects of concentration and applied pressure on cadherin-cadherin adhesion strength in both WT and MT proteins. Gravity alone is insufficient to deform the droplets away from spherical because the kinetic barrier to adhesion is too high. Instead, applying a centrifugation rate of 800g, corresponding to a compression of 4.8 kPa, deforms the droplets into a foam and facilitates cadherin recruitment into adhesions. We subsequently allow the system to relax for 48h to reach mechanical equilibrium where the surface tension force is balanced by the cadherin binding forces, as shown in Figs. 3A, B. Droplet relax back to spheres in the absence of calcium within 15 minutes, Fig. S4.

Control experiments show that cadherin adhesion is only activated in the presence of calcium, otherwise the droplets return to their spherical shape. With calcium, increasing the initial concentration of cadherins on the surface, ρ_i, stabilizes progressively larger adhesions in both the WT and MT proteins, as shown in Fig. 4A. For each concentration, the adhesive emulsions are refractive index matched for visualization in 3-D (47), as shown in Fig. 4B, C. Image analysis identifies the average intensity and the adhesion area A, which in turn give the density and a corresponding number of cadherins N in each adhesion patch. Here N is measured directly from the cadherin fluorescence intensity, and converted to density after mechanical equilibrium is reached. The broad distribution of patch radii arises from the heterogeneous droplet packing geometry and stresses therein (47). Figure 4D shows that the WT on average stabilizes larger patches than the MT under the same experimental conditions.

From these data, we estimate the free energy of binding per molecule e_b. First, we estimate the total deformation energy E_d stored in a system consisting of two droplets in contact. In the limit of small deformations and given a constant surface tension $\gamma = 7 \pm 0.5$ mN/m across the droplet interface (48), we have

$$E_d = \frac{2\gamma}{\mathcal{S}_0} A^2, \tag{2}$$

where $\mathcal{S}_0 = 4\pi R_0^2$ denotes the area of the spherical droplet of radius R_0 and A is the contact area of adhesion. In mechanical equilibrium, this energy of deformation is balanced by the total free energy of binding $N \times e_b$ of N cadherin molecules. Using (2), the binding energy per cadherin bond e_b is thus given by

$$e_b = \frac{2\gamma A^2}{\mathcal{S}_0 N}. \tag{3}$$

This result predicts $A = \left(\frac{e_b \mathcal{S}_0}{2\gamma}\right)^{1/2} N^{1/2}$ or equivalently $A = \frac{e_b \mathcal{S}_0}{2\gamma}\rho$, where ρ is the cadherin density in the patch. In Fig. 5A, this linear increase of area with density holds in the limit of low initial concentrations ρ_i (under 4.8 kPa compression) and gives $e_b = 1.6 \pm 0.3$ k_BT in the case of the MT. This free energy of *trans* binding alone is in the range predicted by computational studies that take into account the rotational and vibrational entropy loss upon confinement and dimerization in 2D (49).

Above a threshold ρ, the cadherins jam into patches of a constant density ($A \propto N$) $\rho_{MT} = 20.1 \times 10^3/\mu m^2$. Increasing ρ_i enhances the probability to make larger adhesion patches, but does not change their density. This MT density corresponds to an average inter-cadherin distance of 6.9 ± 0.1nm, which agrees with the jamming limit of the random packing of disks (50). On the other hand, WT adhesions are comprised of both *cis* and *trans*-interacting cadherins, which forces them to crystallize at a lower density of $\rho_{WT} = 15.7 \times 10^3/\mu m^2$,

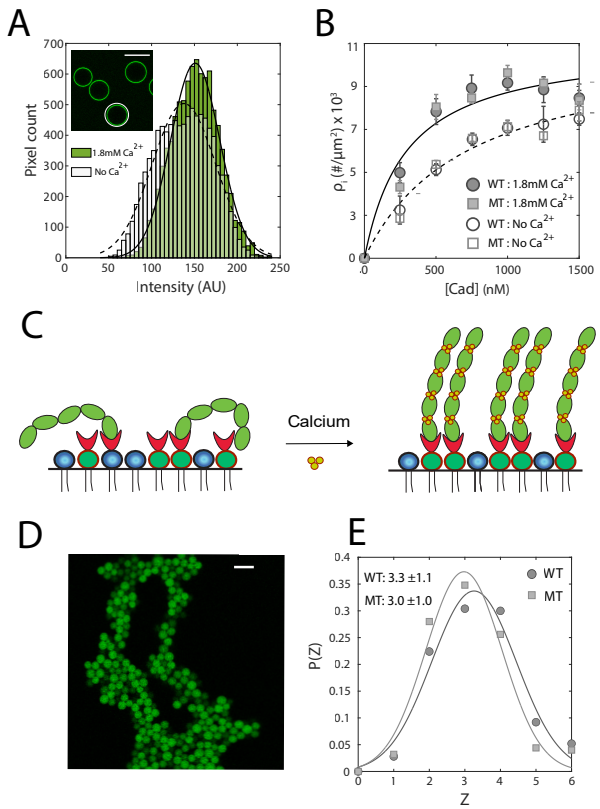

Figure 2: (A) Distribution of pixel brightness for a given droplet, measured on the focal plane that displays the larger ring diameter. The average intensity in presence of calcium (green histogram) is larger that the average intensity without (white histogram), indicating that calcium favors the recruitment of cadherins at the droplet surface. (B) Cadherin surface adsorption density ρ_i as a function of cadherin bulk concentration [Cad]. Adsorption at the droplet surface increases when bulk density increases, and saturates when [Cad]$\simeq 10^3$nM. Addition of calcium in the medium yields a higher cadherin surface density up to $\rho_i \simeq 9.3\times10^3$ cad/ µm^2. (C) Schematic representation of calcium binding to cadherins. Calcium binding rigidifies cadherins, giving them a crescent shape, which is crucial to forming *trans* dimers. Rigidified cadherins also leave more surface area open for the binding of additional cadherins to the droplet. (D) Cadherin-coated Brownian droplets (green disks) form large aggregates upon calcium addition, confirming homophilic adhesion. Scale bar: 20 µm.(E) Distribution of coordination number for WT and MT cadherin-coated droplets. Both WT and MT form droplet aggregates of mean coordination number $\langle Z\rangle = 3$.

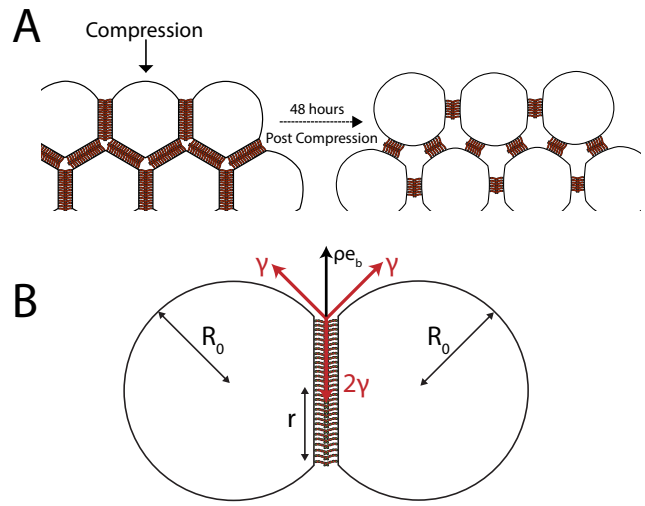

Figure 3: (A) Droplets adopt a foam-like structure through centrifugation. Over 48 hours, the system relaxes to a foam under tension, where cohesion is maintained by the cadherin-populated patches between neighbouring droplets. Compressed droplets attempt to relax back to spheres under the influence of droplet surface tension and the re-entry of aqueous buffer. (B) Expanded view of the force balance between two adhesive droplets of uncompressed radii R_0 and adhesion radius r, with cadherin density ρ. Mechanical equilibrium is maintained in adhesive droplets through the balance between cadherin binding energy e_b and droplet surface tension γ.

also shown in the figure. This patch density is independent of initial concentration. It corresponds to an inter-cadherin distance of 7.9 nm, which is in good agreement with the reported crystal lattice distances of 7.2 to 7.9 nm, as measured by crystallography or electron microscopy of liposome and cell-cell adhesions (10, 51).

Assuming a constant e_b per molecule in (3), a given cadherin density fixes the area of deformation. However, our data shows that patches of the same density give rise to increasing adhesion sizes as a function of the applied pressure. We therefore interpret our experimental results in terms of the e_b increase with droplet deformation, quantified as the area strain of a droplet,

$$\xi = \frac{\mathcal{S} - \mathcal{S}_0}{\mathcal{S}_0}, \tag{4}$$

where $\mathcal{S}$ denotes the area of the deformed droplet and, in the limit of small deformations, $\mathcal{S} - \mathcal{S}_0 = \sum_{i=1}^{n} A_i^2/\mathcal{S}_0$ (52), where index i labels the patches on a droplet with n contacts. In the limit of maximum compression into a biliquid foam, we measure the number of contacts per droplet $n = 12$ (53, 54), and we can thus set the local strain of a contact patch of area A to be $\xi \simeq 12A^2/\mathcal{S}_0^2$.

To maximize the extent of adhesion we saturate ρ_i to $9.3 \times 10^3/\mu m^2$, and test the effect of the applied pressure ranging from 0.3 kPa to 4.8 kPa. In Fig. 5B, we show the same linear increase of A vs. N, i.e., constant cadherin packing density, for the WT and MT as those observed in Fig. 5A,

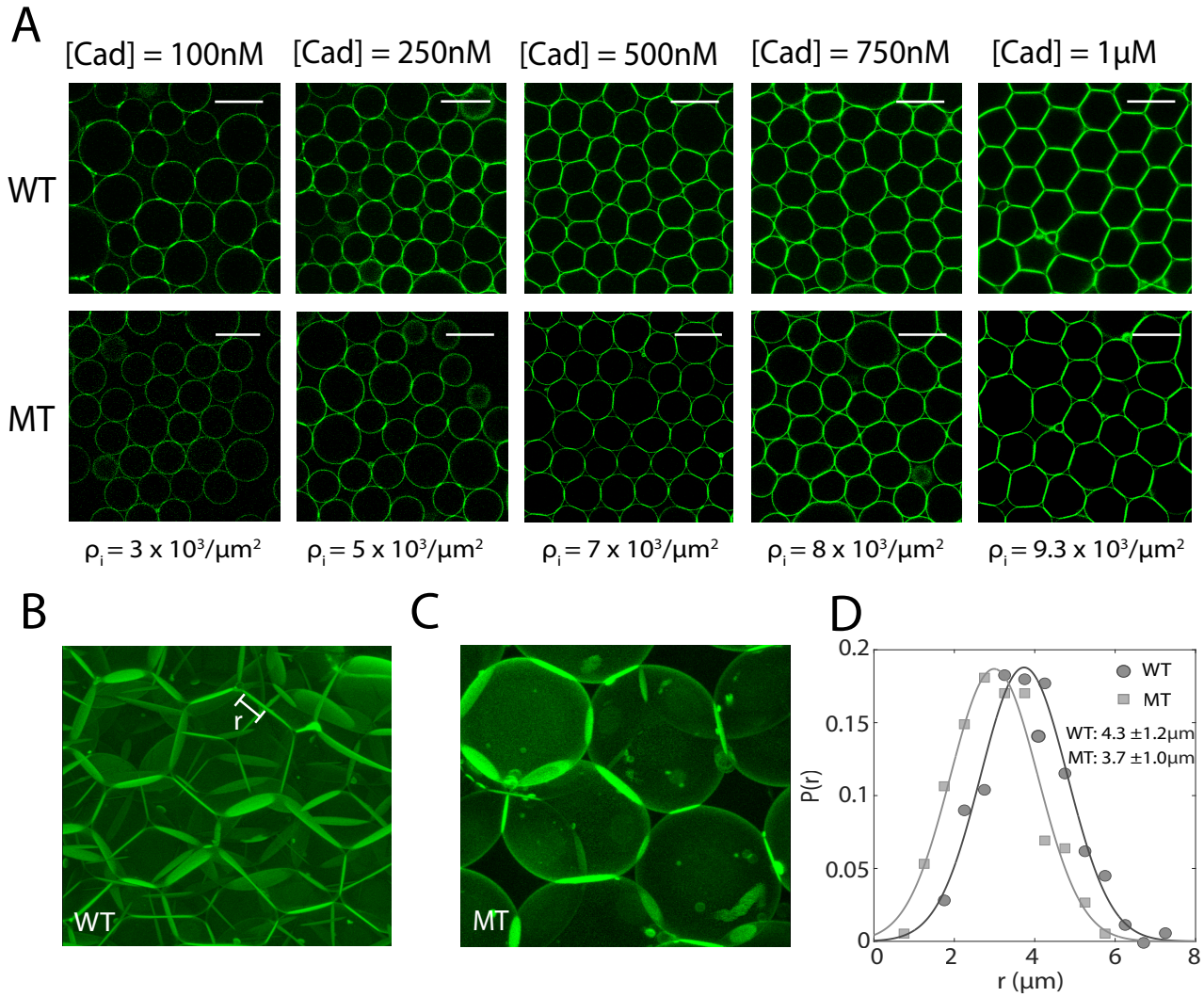

Figure 4: (A) Final configuration of the system after compression at 4.8kPa and 48h relaxation, for different concentrations of bulk cadherins, both for WT (upper panels) and MT (lower panels). Droplets of varying cadherin surface densities were centrifuged, resulting in the formation of irreversible adhesions and deformations. Scale bar: 20 μm. (B, C) 3-D reconstruction of compressed droplets for WT (panel B) and MT (panel C). Cadherins are concentrated at sites of adhesion upon compression. For each patch, the radius r is measured, and yields the patch area A. Panel width corresponds to 40μm. (D) Distribution of patch radii for both WT and MT systems. For (B), (C) and (D): surface density $\rho_i = 9.3 \times 10^3$cad/ μm^2 and compression $P = 4.8$kPa.

confirming protein crystallization or jamming, respectively. Increasing the pressure forms progressively larger adhesion areas, similar to the effect of increasing ρ_i at constant pressure in Fig. 5A. Both ρ_i and the applied pressure, therefore, play an important role in stabilizing adhesions.

We find $e_b \propto \xi^{1/2}$, see Fig. 5C. This result is indicative of a force-dependent adhesion response, where the cadherin-dimer bond is strengthened in response to deformation, suggesting a catch-bond behavior (29). While both WT and MT show a continuously increasing binding free energy, the WT stabilizes larger adhesion patches than the MT and reaches higher binding energies of up to 20k_BT at the cohesive droplet limit. The amplitude of the stress response of the MT is 3/4 of that of the WT, highlighting the importance of *cis* and *trans* cooperativity in stabilizing adhesion.

Next, we plot the percentage of recruited proteins into adhesions as a function of both pressure and concentration, see Fig. 5D. Since increasing pressure linearly increases the area of adhesion, it also linearly increases the fraction of recruited molecules, consistent with a random attachment model at fixed concentration. Conversely, increasing ρ_i at fixed pressure, we observe a sharp non-linear increase in recruitment for both WT and MT above a common threshold density, see Fig. 5D. This behavior is reminiscent of a density-

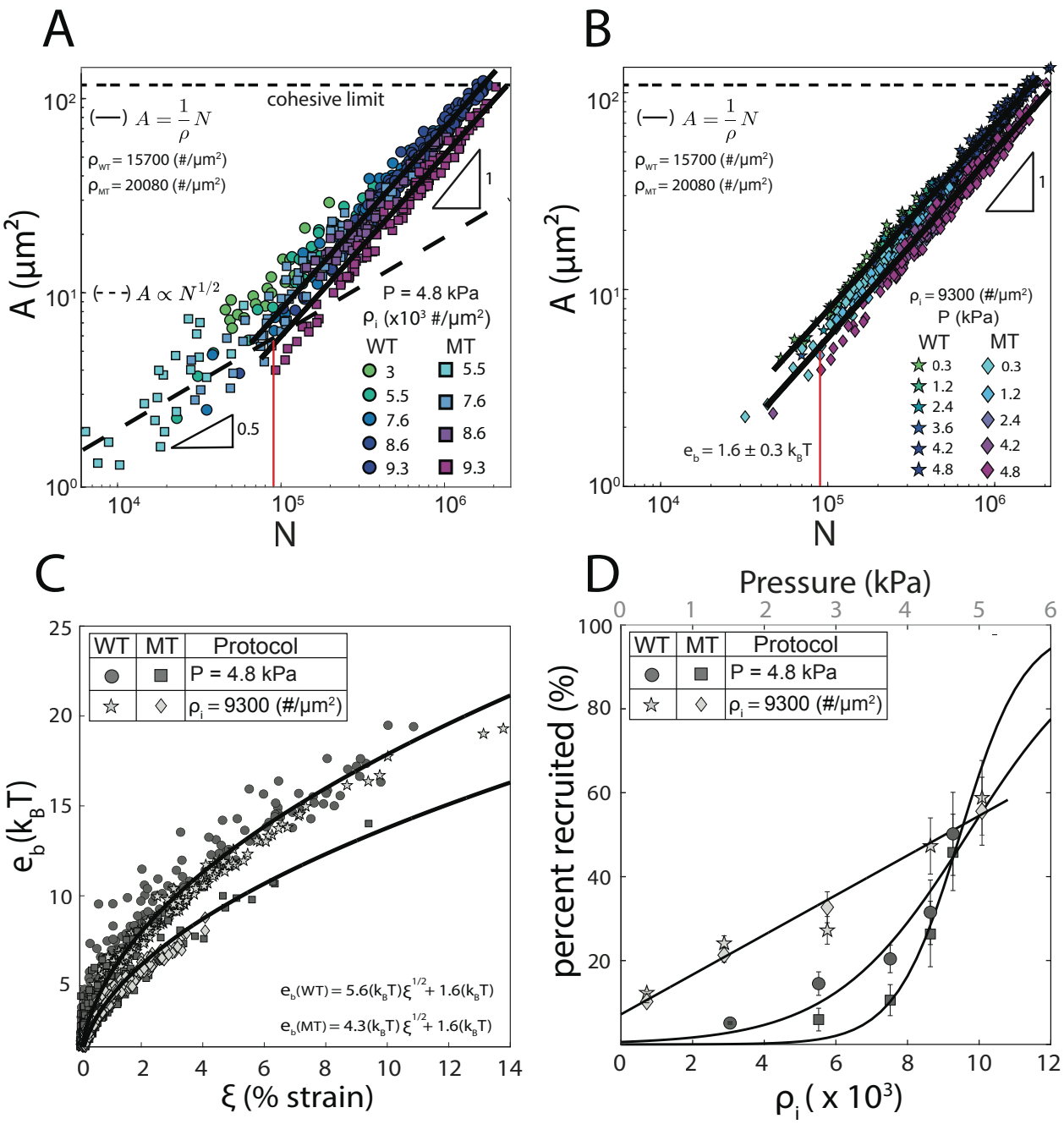

Figure 5: (A) Area A of patches as a function of cadherin number N in the patch for different cadherin surface concentration ρ_i in WT and MT systems, at a given compression $P = 4.8$kPa. We identify two regimes. For low cadherin number, the data follows $A \propto N^{1/2}$ (black dashed line), yielding an estimate of $e_b = 1.6 \pm 0.3\ k_BT$. For larger N, the data follows $A \propto N$, indicating constant density in the patches. (B) Area of patches A, as a function of the number of cadherins N in the patch for fixed cadherin surface concentration ρ_i and different values of compression. The compression ranges from about 0.3 to 4.8 kPa. For both WT and MT, we observe $A \propto N$, indicating that adhesions are growing at a constant density. For the WT the density is $\rho_{\mathrm{WT}} = 15.7 \times 10^3 \mathrm{cad}/\mu\mathrm{m}^2$, while the MT adhesions are denser at about $\rho_{\mathrm{MT}} = 20.1 \times 10^3 \mathrm{cad}/\mu\mathrm{m}^2$. (C) Binding energy e_b per cadherin bond as a function of the area strain ξ of the deformed droplets, for both pressure and density evolution as shown in in panel A and B. Since $e_b = 2\gamma A^2/(N\mathcal{S}_0)$ and $A = \rho N$ with constant density, e_b varies with deformation (D) Fraction of cadherins recruited as a function of varying cadherin density and pressure. Circles (WT) and squares (MT) show cadherin recruitment as a function of cadherin density (ρ_i). An empirical fit demonstrates the sigmoidal nature of the recruitment with an inflection point around 9500cad/ $\mu\mathrm{m}^2$. Stars (WT) and diamonds (MT) show recruitment evolution as a function of applied pressure (top axis). Varying the pressure from 0.3 to 4.8 kPa leads to a linear growth in recruitment for both WT and MT.

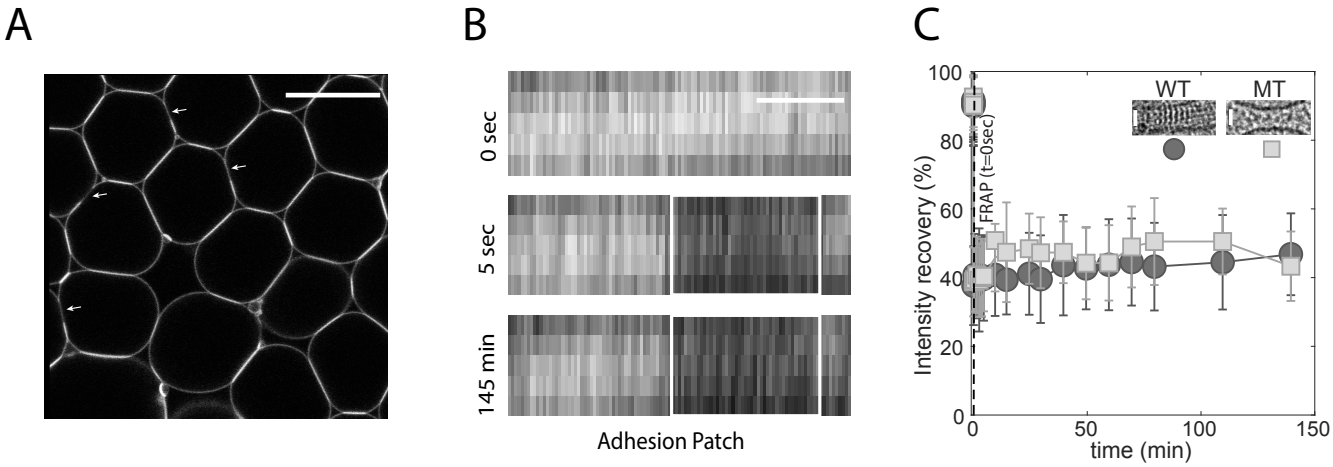

Figure 6: (A) Focus on a loosely packed region in the capillary for WT cadherin-saturated droplets ($\rho_i = 9.3 \times 10^3 \mathrm{cad}/\mu\mathrm{m}^2$) and maximal compression at $P = 4.8$kPa. Arrows indicate photobleached regions on adhesion patches. Scale bar: 20μm. (B) Magnified view of an adhesion before and after FRAP. Scale bar: 1μm. The photobleached regions of patches do not recover initial brightness, whether WT or MT, indicating a jammed state in the patches in both situations. (C) Quantitative measurement of recovery intensity after FRAP, for both WT and MT. Inset: electron-microscopy of patches made of WT or MT, reproduced from (10).

dependent phase-transition that has been predicted to occur in cadherin ectodomains (13, 39).

Our density measurements in Fig. 5 suggest that WT and MT adhesions are solid-like, either in a crystalline or a randomly jammed array. To test this hypothesis, we FRAP sections of many adhesion zones, as shown in Fig. 6. Both protein types do not fully bleach, but preserve 40% of the initial fluorescence after a one minute laser pulse. We do not observe any subsequent fluorescence recovery, consistent with solid adhesions. However, bleaching the proteins that are outside the adhesions uncovers two cadherin populations that recover over significantly different timescales, $\tau_1 = 44$ s and $\tau_2 = 523$ s, respectively. Their diffusion constants, given by $D = r_b^2/(4\tau)$, where r_b is the radius of the circular photobleached region, yield $D_1 = 2.2 \times 10^{-2}\ \mu\mathrm{m}^2/\mathrm{s}$ and $D_2 = 2.0 \times 10^{-3}\ \mu\mathrm{m}^2/\mathrm{s}$, Fig. S5. The fast recovery is consistent with the diffusion of lipids on the surface of a silicon oil emulsion (55), while the slow recovery may be a result of lateral crowding interactions at the saturation cadherin ρ_i used in these experiments.

DISCUSSION

In this paper we have established that our biomimetic emulsion system can recapitulate a number of known E-cadherin dependent phenomena and thus constitutes a useful platform to study a range of phenomena associated with cadherin-mediated cell-cell adhesion. First, we quantify the effect of calcium on the packing density of cadherins at droplet interfaces and recapitulate its role in enabling *trans* dimerization. Second, as observed in cells, WT cadherins coated on droplet surfaces spontaneously organizes into adhesions with a crystalline density, driven by both *trans* and *cis* interactions. Third, we find that the MT does not form a crystalline lattice, but, rather, jams into clusters with a higher density than the WT. This tendency of the MT to form dense adhesions through non-specific lateral interactions has also been observed previously in lipid bilayer systems (56). Our most important finding is that both WT and MT are mechanosensitive, and that this is a property of the ectodomain alone. It is known that cadherins are capable of mechanosensitive force-dependent tuning of their adhesion by acting like catch-bonds under tensile stress(27–29, 57, 58). The formation of this catch bond is a property of the cadherin X-dimer (59) which corresponds to an alternate conformation to the canonical strand-swapped cadherin dimer. A number of studies have detected X-dimers on cell surfaces (60, 61). In addition FRET-based measurements of *cis* and it trans-cooperativity have shown that bond lifetimes are greatly increased due to *cis* interactions (32). Our results suggest a similar *cis*-contribution to the binding energies, where WT cadherins are able to reach higher binding energies compared to the *cis* mutant. Here we establish that catch bonds are formed as a consequence of applied pressure and with increasing droplet deformation, we estimate increasing binding energy to reach $20\mathrm{k_BT}$ for WT and $14\mathrm{k_BT}$ for MT. Most notably, our experiments provide a clear demonstration that catch bonds can be formed under conditions that mimic cellular forces thus opening the door to experiments, for example with mutant protein aimed at directly establishing a role for X-dimers in the E-cadherin mechanosensitive response and, more generally, in regulating the transmission of forces between cells.

AUTHOR CONTRIBUTIONS

L.S., B.H., L.L.P., and J.B. designed the study and wrote the paper. K.N. designed and performed experiments, analyzed experimental data and wrote the paper, A.I. analyzed experimental data, R.Z. developed theory, L.F. analyzed experimental data, O.J.H. expressed and purified proteins.

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COMPETING INTERESTS

The authors declare no competing interests

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SUPPLEMENTARY MATERIAL

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