Supporting Information

Title: Ideal, catch and slip bonds in cadherin adhesion

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Sliding-rebinding model that describes X-dimer catch-slip bonds: We fitted the catch-slip transition of W2A cadherins using a sliding-rebinding model for two pairs of pseudoatoms (1, 2). According to this model, interacting cadherins first form a pair of bonds of equal strength. When a tensile force is applied to detach the bonds, the interacting partners rearrange themselves to enhance the probability of forming a new pair of interactions. Kinetic rate equations were generated accordingly for four possible states: P_{11} (probability of original bound state with two pairs of pseudoatoms), P_{10}/P_{01} (probability of state where either one of the bound pairs dissociate before sliding), $P_{10}^{'}$ (probability of a bound state for the newly created interaction after sliding) and P_{00} (probability of dissociated state). The steps of the reactions are depicted schematically in Figure S1. The corresponding rate equations are:

$$\frac{dP_{11}}{dt} = 2k_{+1}P_{10} + k_{+2}P_{10}^{'} - k_{-2}P_{11}$$

$$\frac{dP_{10}}{dt} = k_{-2}P_{11} - 2\left(k_{+1} + k_{-1}\right)P_{10}$$

$$\frac{dP_{10}^{'}}{dt} = 2P_n k_{+1}P_{10} - \left(k_{+2} + k_{-1}\right)P_{10}^{'}$$

Where k_{+1} and k_{+2} are the rebinding rate constants independent of force, k_{-1} and k_{-2} are the force dependent off-rates for a single and double pseudoatom pair (each pseudoatomic interaction was assumed to be identical) and P_n is the probability of forming new interactions at a constant force.

The off-rates, k_{-1} and k_{-2} can be described by the equation

$$k_{-1} = k_{-1}^{0} e^{-fx/k_B T}$$

$$k_{-2} = 2k_{-1}\left(f/2\right)$$

where, k_{-1}^{0} is the intrinsic off-rate of a bond and x is the distance between the bound state and the transition state. The probability of forming new interactions P_n is described as

$$P_n = 0 \qquad if \quad f < 0;$$

$$P_n = \left\{0.5\left[1 + \sin\left(\frac{\pi f}{f_0} - \frac{\pi}{2}\right)\right]\right\}^n \qquad if \quad 0 \le f \le f_0;$$

$$P_n = 1 \qquad if \quad f > f_0;$$

where n was used as fitting parameter to account the interfacial angle of the X-dimer and f_0 is the force at which the catch bond transitions to a slip bond. We analytically solved the rate equations and obtained survival probability $\left(1 - P_{00}\right)$ as $P_{11} + P_{10} + P_{10}^{'}$.

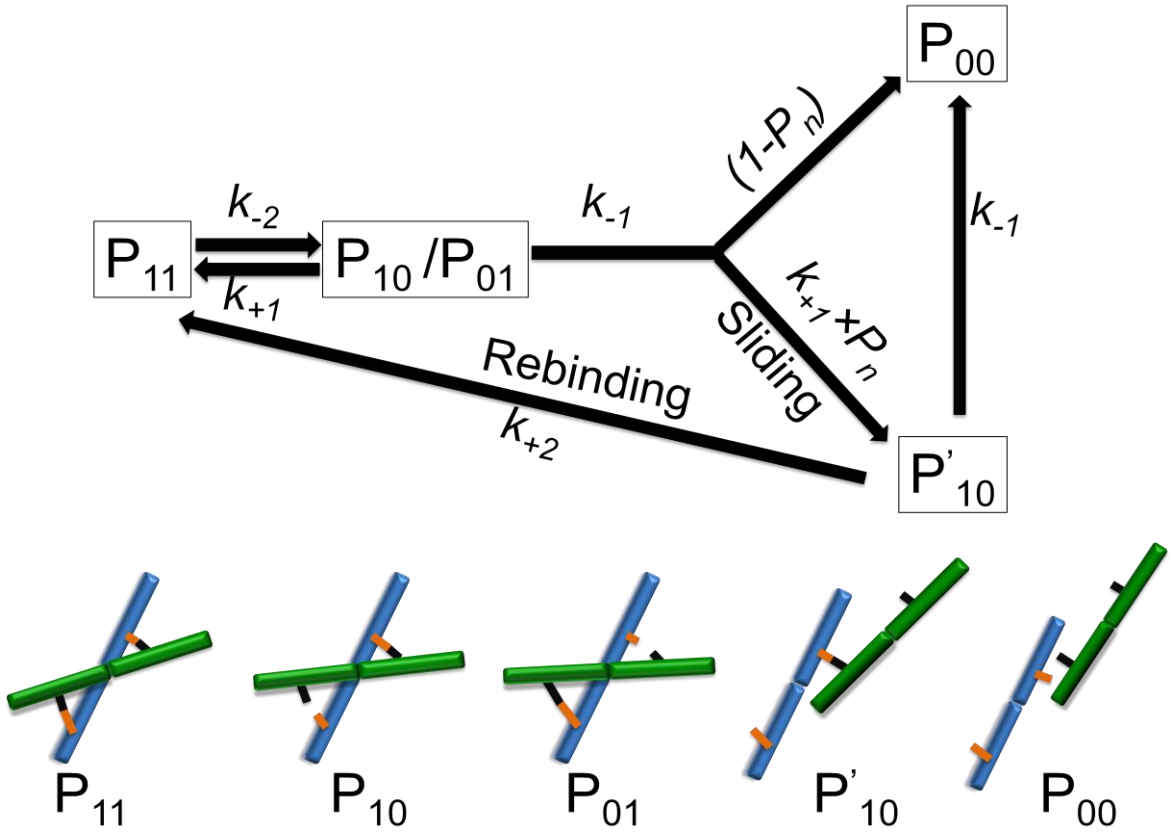

Figure S1: Sliding-rebinding model for catch-slip bond formation. For clarity, the cartoons depict only the two outer cadherin domains that participate in X-dimer formation. P_{11} corresponds to the probability of bound state with two pairs of pseudoatoms; P_{10}/P_{01} corresponds to probability of state where either one of the bound pairs dissociate before sliding; $P_{10}^{'}$ corresponds to probability of a bound state for the newly created interaction after sliding and P_{00} corresponds to probability of the dissociated state.

$k_{-1}^{0}\left(s^{-1}\right)$	$x(nm)$	$k_{+1}^{0}\left(s^{-1}\right)$	$k_{+2}^{0}\left(s^{-1}\right)$	$f_0(pN)$	n
30.4	0.34	5.3	1985.9	29.2	4.8

Table S1: Parameters of sliding-rebinding model fit to the force dependent lifetimes of W2A cadherin. W2A cadherin catch-slip bonds were fit to the sliding-rebinding model described in Figure S1.

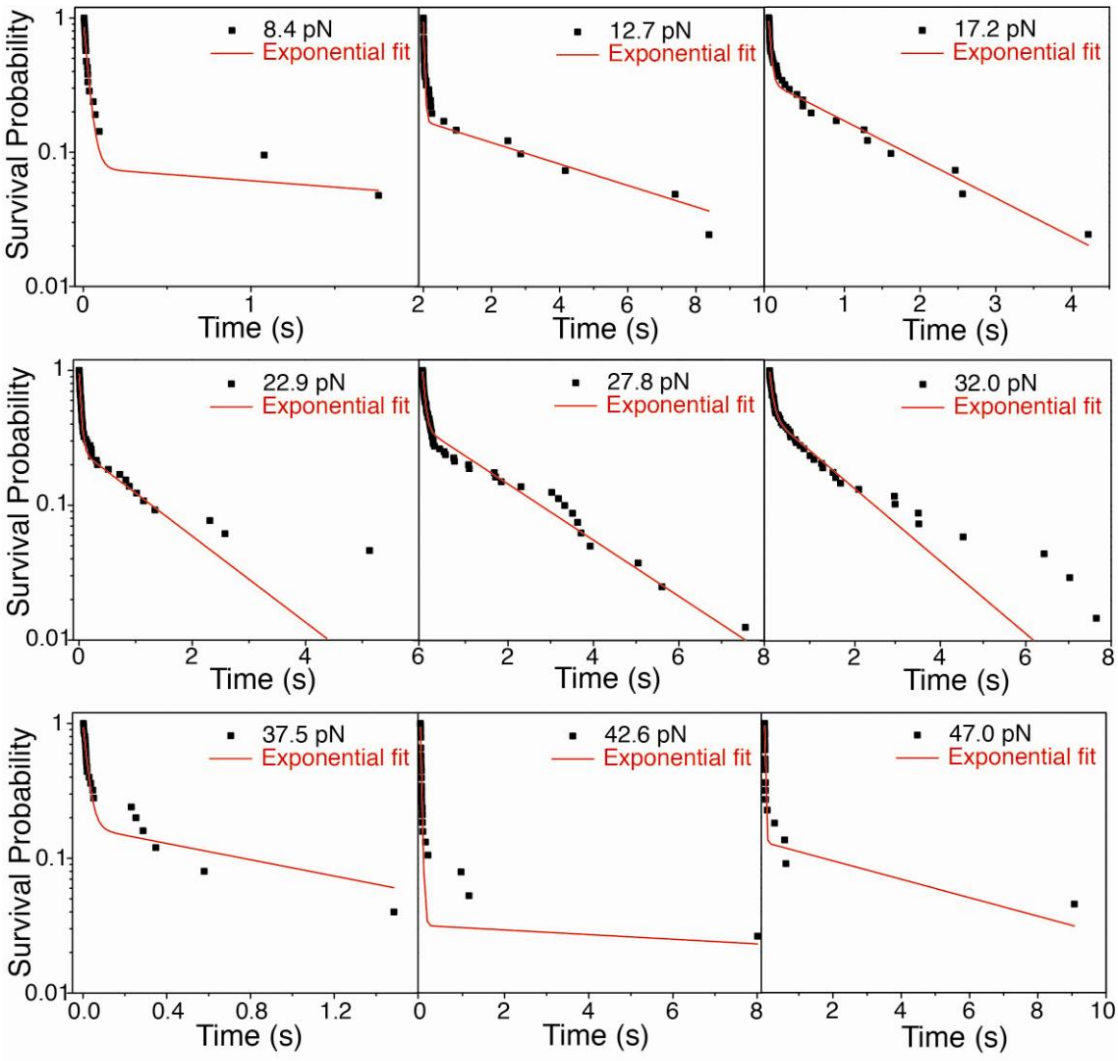

Figure S2: Survival probabilities of W2A cadherins decay as a double exponential. Bond lifetimes of W2A cadherin X-dimers interacting for 0.3 s were measured at nine different clamping forces. A statistical F-test showed that the probability that the bond survives in the bound state for a majority of the clamping forces was described by the sum of 2 exponentials suggesting the cadherins interact in 2 bound states. The bond survival probabilities were fit to a function given by $P(t) = P_1(0) \times e^{-k_1(f).t} + [1 - P_1(0)] \times e^{-k_2(f).t}$ where $k_1(f)$ and $k_2(f)$ are the off-rates and the bond lifetimes are $1/k_1(f)$ and $1/k_2(f)$. We ascribed the lower lifetime adhesive state to the unbinding of X-dimers and the higher lifetime state to non-specific adhesion.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
8.4	21	0.93367	0.93942	0.93184
12.7	41	0.86176	0.96551	0.96358
17.2	41	0.76629	0.97494	0.97608
22.9	65	0.90948	0.99359	0.99334
27.8	80	0.87508	0.98682	0.99395
32.0	68	0.84843	0.9957	0.9974
37.5	25	0.90336	0.97404	0.97144
42.6	38	0.94674	0.94824	0.94491
47.0	22	0.93579	0.97295	0.96889

Table S2: Goodness of fit statistics for W2A cadherin survival probabilities. Bond survival probabilities of W2A cadherin X-dimers at different clamping forces were fit to a single exponential decay model with one parameter, a double exponential decay model with three parameters and a triple exponential decay model with five parameters. An F-test between the single *vs.* double and double *vs.* triple exponential decay model showed that the double exponential decay model fits significantly better than the triple exponential decay in the 95% confidence interval limit. In a few cases, the triple exponential decay model was superior to the double-exponential decay; we ascribe this to an artifact of combining results from different experiments.

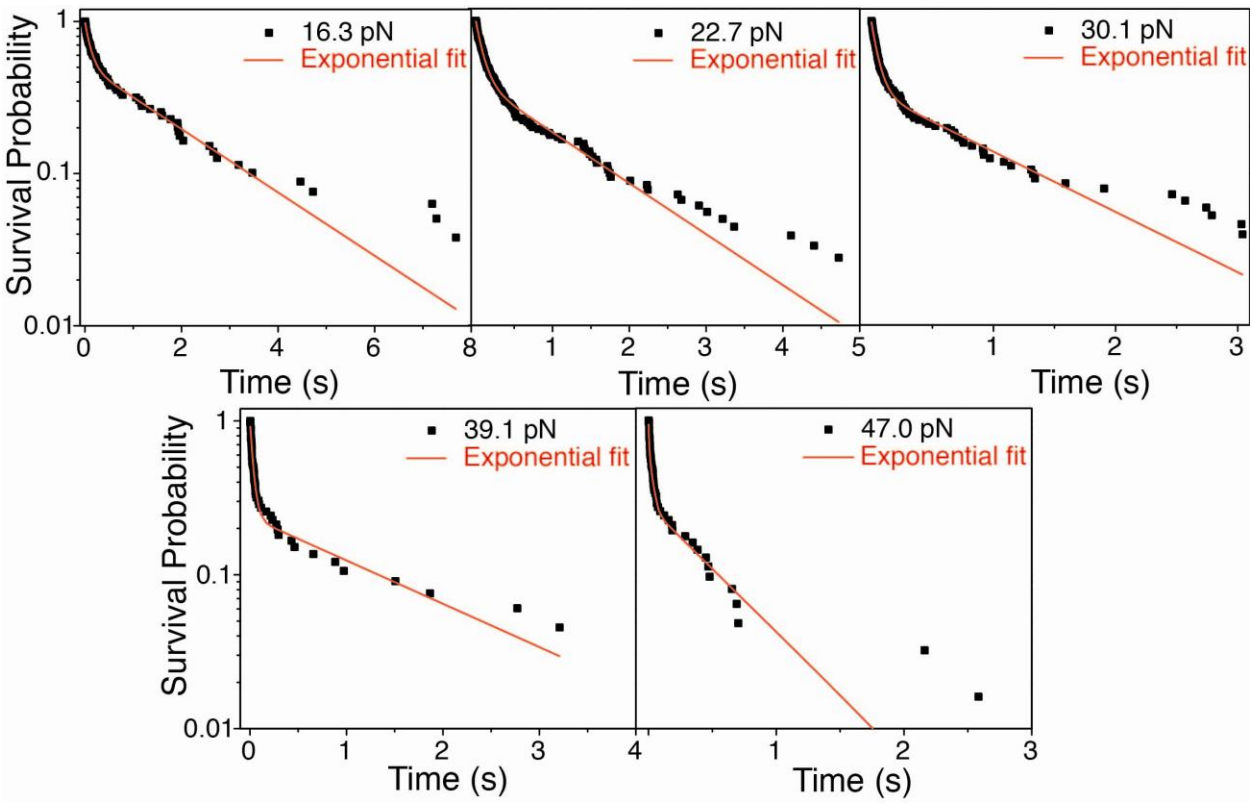

Figure S3: Survival probabilities of K14E cadherins interacting for 3 s decay as a double exponential. Bond lifetimes of K14E cadherin strand-swap dimers interacting for 3 s were measured at five different clamping forces. A statistical F-test showed that the bond survival probability for a majority of the clamping forces was described by the sum of 2 exponentials suggesting the cadherins interact in 2 bound states.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
16.3	79	0.85952	0.99613	0.99761
22.7	180	0.92424	0.9966	0.99855
30.1	152	0.90588	0.99665	0.99728
39.1	66	0.9082	0.98011	0.97944
47.0	62	0.91527	0.97974	0.97903

Table S3: Goodness of fit statistics for the survival probabilities of K14E cadherins interacting for 3 s. F-tests between the single *vs.* double and double *vs.* triple exponential decay fits showed that for a majority of the data, a double exponential decay fits significantly better than the triple exponential decay in the 95% confidence interval limit.

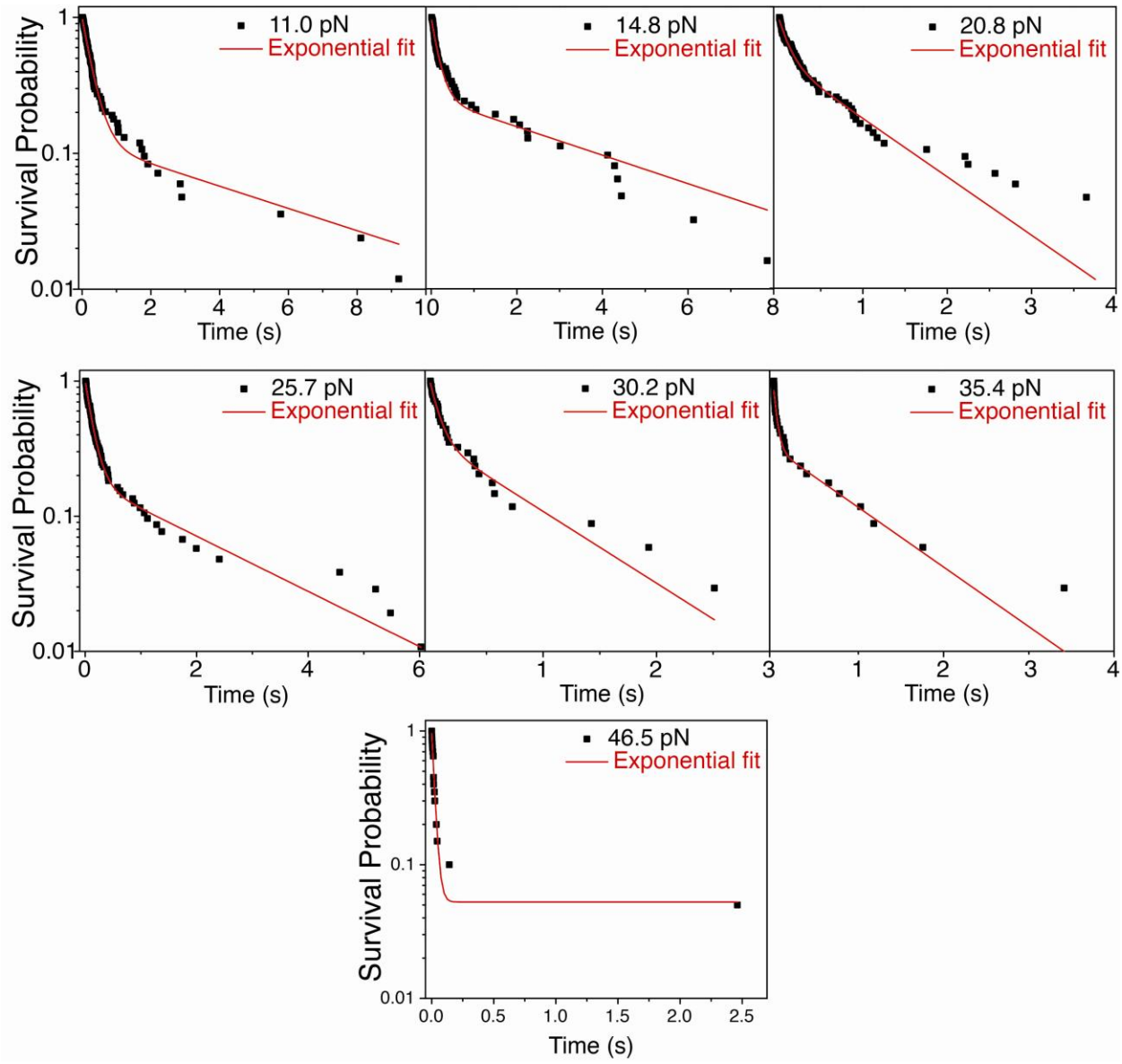

Figure S4: Survival probabilities of K14E cadherins interacting for 0.3 s decay as a double exponential. Bond lifetimes were measured at seven different clamping forces. A statistical F-test showed that the probability that the bond survives in the bound state for all of the clamping forces was described by the sum of 2 exponentials suggesting the cadherins interact in 2 bound states.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
11.0	84	0.97516	0.98975	0.98949
14.8	62	0.8986	0.98312	0.98252
20.8	85	0.94056	0.99167	0.99319
25.7	104	0.96976	0.99368	0.99368
30.2	34	0.93227	0.99168	0.99192
35.4	34	0.86005	0.96156	0.95916
46.5	20	0.96196	0.96176	0.95615

Table S4: Goodness of fit statistics for K14E cadherins interacting for 0.3 s. F-tests between the single *vs.* double and double *vs.* triple exponential decay model showed that the double exponential decay fits significantly better than the triple exponential decay in the 95% confidence interval limit.

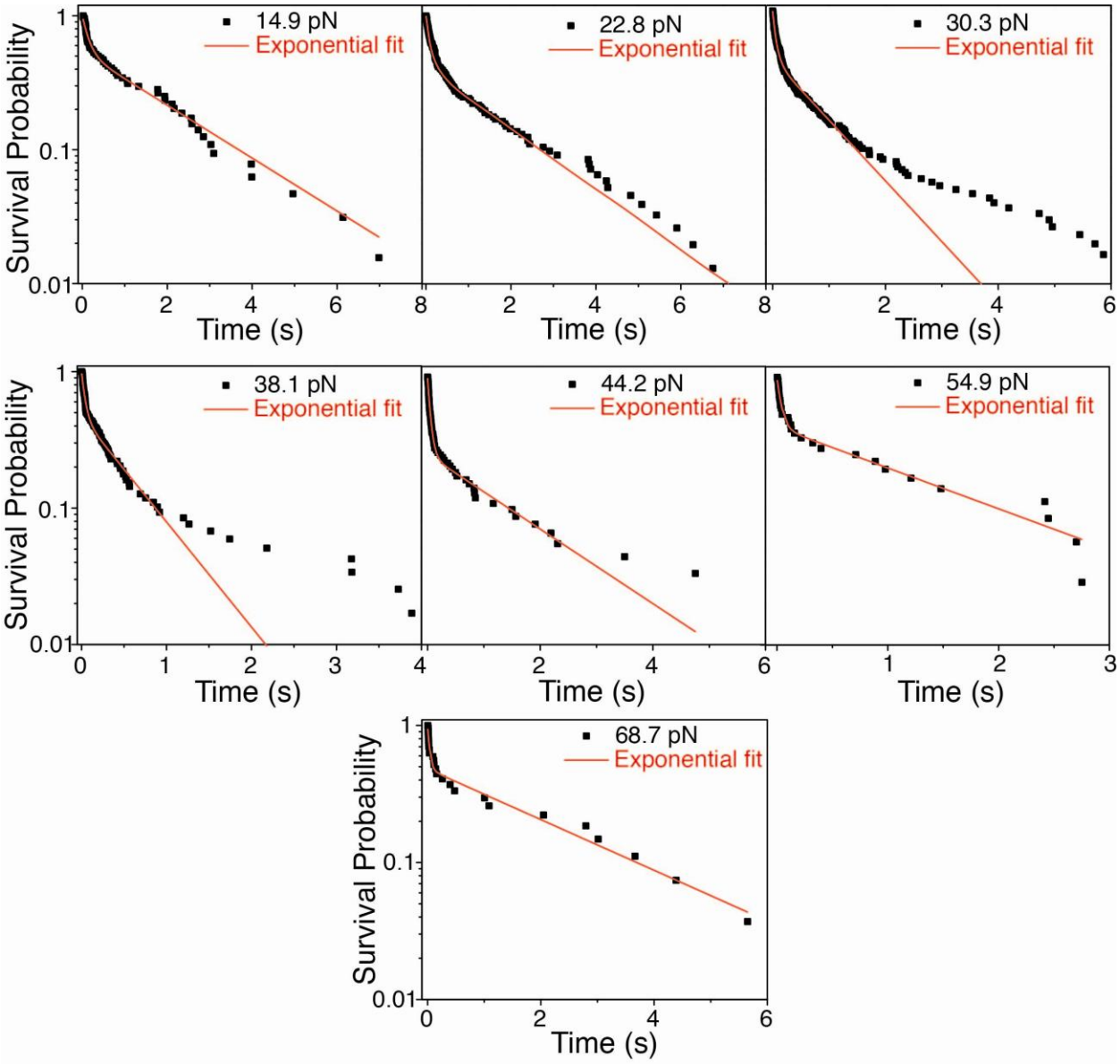

Figure S5: Survival probabilities of WT cadherins interacting for 3 s decay as a double exponential. Bond lifetimes were measured at seven different clamping forces. A statistical F-test showed that the probability that the bond survives in the bound state for a majority of the clamping forces was described by the sum of 2 exponentials suggesting the cadherins interact in 2 bound states.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
14.9	64	0.83993	0.98338	0.98281
22.8	153	0.90833	0.99801	0.99868
30.3	305	0.89832	0.99568	0.99801
38.1	118	0.8663	0.98821	0.98884
44.2	88	0.91447	0.98948	0.98922
54.9	34	0.78996	0.98076	0.97957
68.7	27	0.7337	0.97481	0.97907

Table S5: Goodness of fit statistics for WT cadherins interacting for 3 s. F-tests between the single *vs.* double and double *vs.* triple exponential decay model showed that, for most cases, the double exponential decay model fits better than the triple exponential decay in the 95% confidence interval limit.

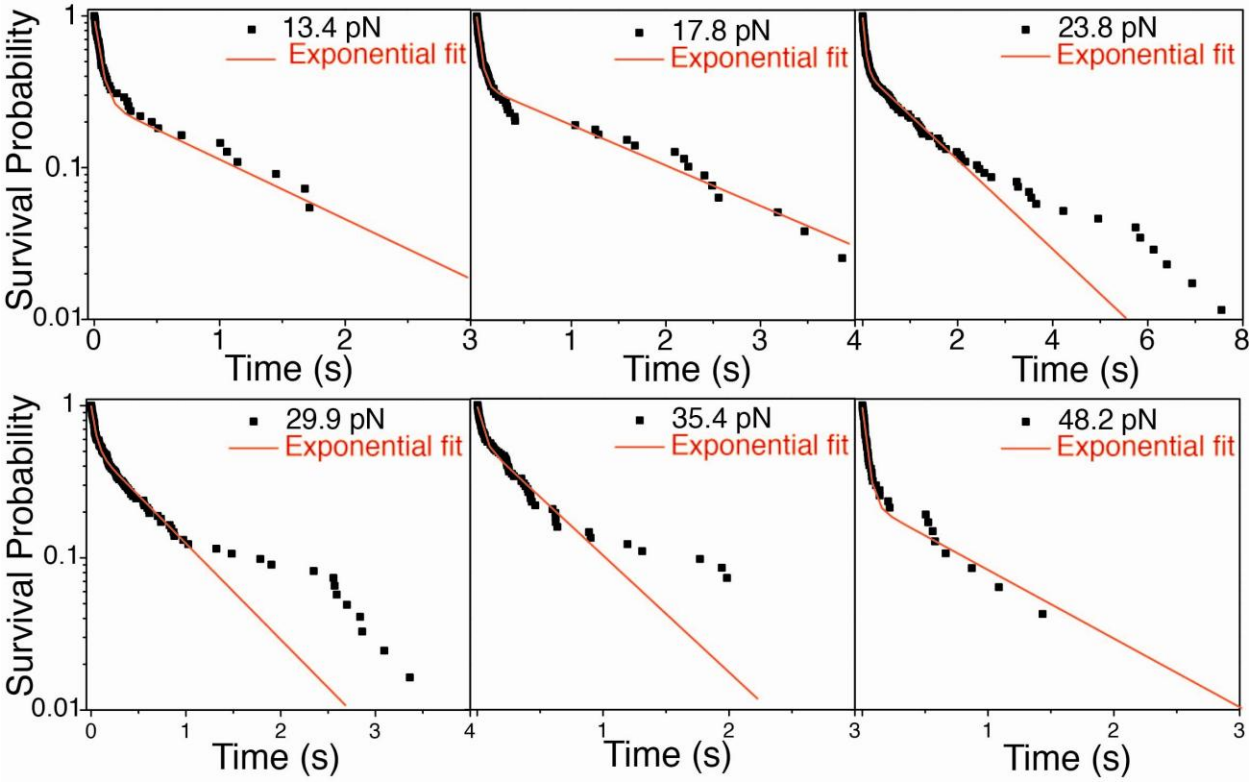

Figure S6: Survival probabilities of WT cadherins interacting for 0.3 s decay as a double exponential. Bond lifetimes were measured at six different clamping forces. A statistical F-test showed that the probability that the bond survives in the bound state for a majority of the clamping forces was described by the sum of 2 exponentials suggesting the cadherins interact in 2 bound states.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
13.4	55	0.91247	0.98963	0.98921
17.8	78	0.87789	0.99396	0.99718
23.8	173	0.83405	0.99706	0.99826
29.9	122	0.88589	0.9942	0.99753
35.4	82	0.89299	0.99152	0.99595
48.2	47	0.93035	0.99181	0.99139

Table S6. Goodness of fit statistics for WT cadherins interacting for 0.3 s. F-tests between the single *vs.* double and double *vs.* triple exponential decay model showed that, for most cases, the double exponential decay model fits better than the triple exponential decay in the 95% confidence interval limit.

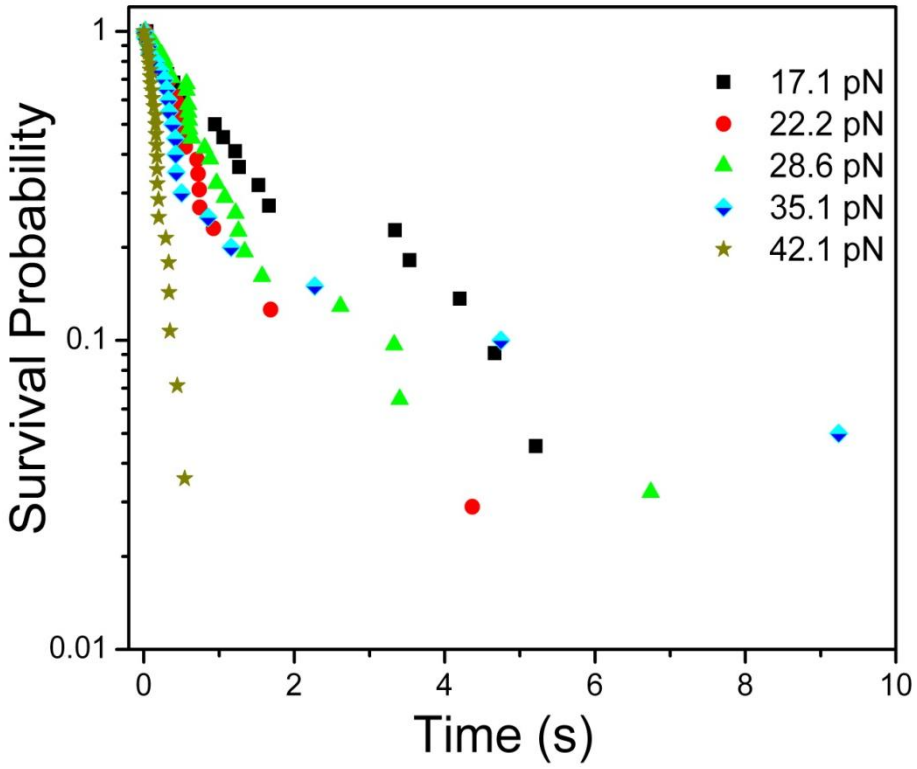

Figure S7: Survival probabilities of W2A-K14E double mutants interacting for 0.3 s follow a single exponential decay. Bond lifetimes measured for different clamping forces are similar to the long-lifetime, low-probability state measured with WT, W2A and K14E cadherin.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
17.1	22	0.9823	0.9955	0.9911
22.2	26	0.9591	0.9730	0.9704
28.6	31	0.9712	0.9692	0.9668
35.1	20	0.9461	0.9548	09519
42.1	28	0.9411	0.9364	0.9308

Table S7. Goodness of fit statistics for W2A-K14E double mutant cadherins interacting for 0.3 s. F-tests between the single *vs.* double and double *vs.* triple exponential decay model showed that, for most cases, the single exponential decay model fits the data better than a double exponential decay in the 95% confidence interval limit.

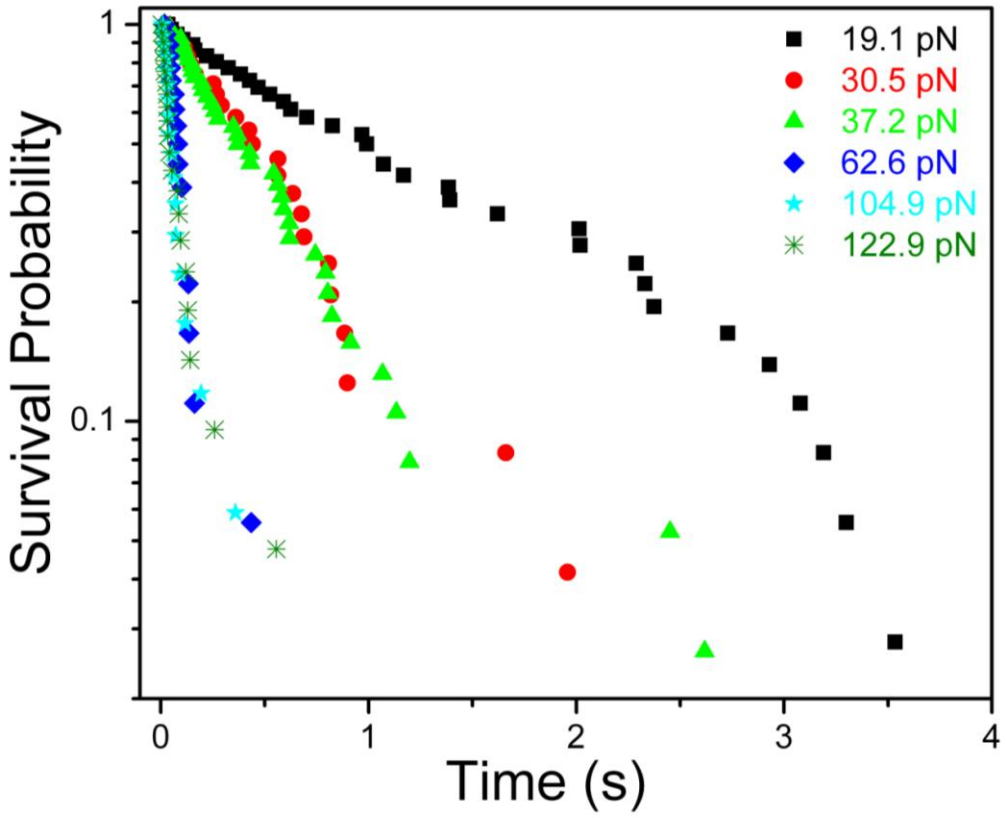

Figure S8: Survival probabilities for non-specific interactions also follow a single exponential decay. We measured the force dependent lifetimes of non-specific interactions between WT cadherins immobilized on an AFM tip and an identically treated substrate lacking cadherins. The tip and surface were functionalized with Polyethylene Glycol (PEG) linkers, some of which were decorated with Streptavidin molecules. Biotinylated cadherin monomers were attached only to the Streptavidins on the AFM tip. Bond lifetimes measured for different clamping forces were similar to the long-lifetime, low-probability state measured with WT, W2A, and K14E cadherins and also to the single bound state of the W2A-K14E double mutant.

Force (pN)	Number of events	Adj-R^2 (Single Fit)	Adj-R^2 (Double Fit)	Adj-R^2 (Triple Fit)
19.1	17	0.9357	0.9264	0.9142
30.5	21	0.9697	0.9739	0.9707
37.2	36	0.9921	0.9935	0.9931
62.6	24	0.9819	0.9801	0.9780
104.9	38	0.9885	0.9879	0.9871
122.9	18	0.8763	0.8598	0.8382

Table S8. Goodness of fit statistics for non-specific interactions. F-tests between the single *vs.* double and double *vs.* triple exponential decay model showed that the single exponential decay model fits the data better than a double exponential decay in the 95% confidence interval limit.

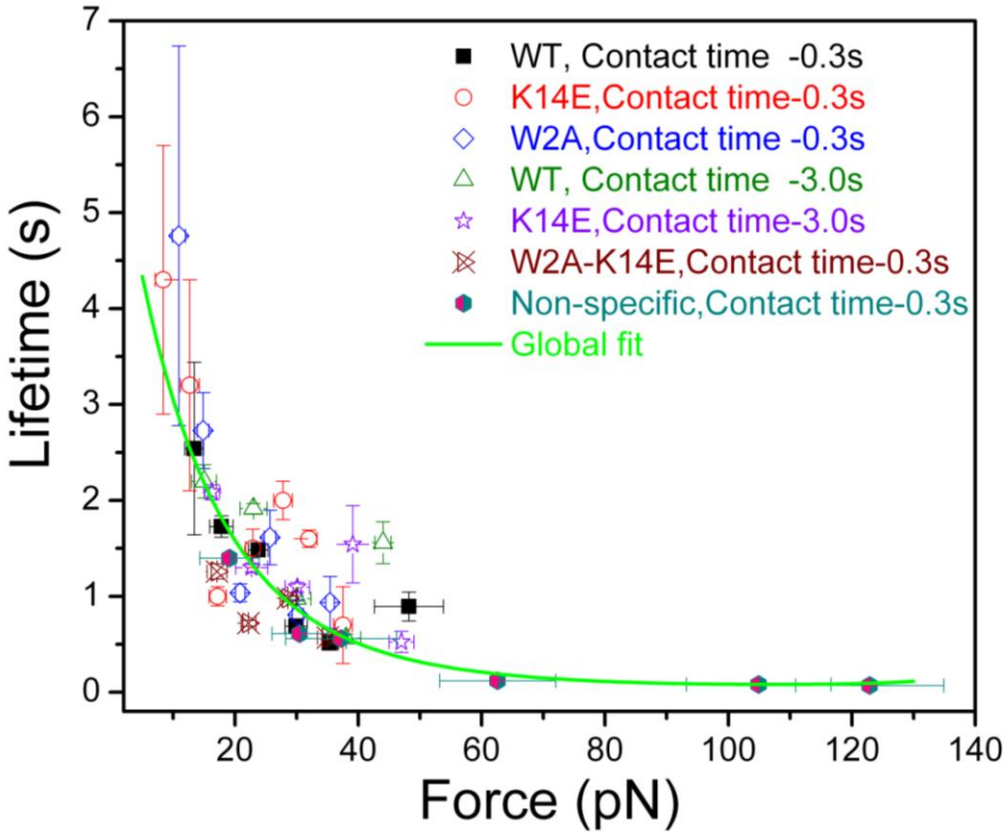

Figure S9: Force-lifetime curves of non-specific interactions, W2A-K14E double mutant binding and the long lifetime states of WT, W2A and K14E cadherins show identical slip bond behavior. The survival probability of both non-specific interactions and the W2A-K14E cadherin double mutant binding decayed as a single exponential indicating that they formed a single bound state. In contrast, the survival probabilities of W2A cadherin, K14E cadherin and WT cadherin were described by the sum of 2 exponentials suggesting the cadherins interact in 2 bound states: a short lifetime state with high probability of occurrence and a longer lifetime state with lower probability of occurrence. The longer lifetime state was identical to the single bound state formed by both non-specific interactions and the W2A-K14E double mutant; all the constructs formed identical slip bonds. The data was globally fit to a microscopic, slip bond model given by the equation (3)

$$\tau(F) = \tau_0\left(1 - \nu F x^{\#}/\Delta G^{\#}\right)^{1-1/\nu} e^{-\Delta G^{\#}\left[1-\nu F x^{\#}/\Delta G^{\#}\right]^{1/\nu}}$$

where, τ_0 is the intrinsic lifetime of a bond, $x^{\#}$ is the distance between the bound state and the transition state, $\Delta G^{\#}$ is the free energy of activation in absence of external force, and ν is a scaling factor that specifies the free-energy profile.

$\tau_0(s)$	$x^{\#}(nm)$	$\Delta G^{\#}(k_B T)$	ν
6.2	0.3	25.1	0.5

Table S9: Parameters for the fit to longer lifetime state. The force dependent bond lifetimes in Figure S9 were globally fit to a microscopic, slip bond model (3). The parameters obtained from the fit are shown in this table where the value of ν corresponds to a harmonic well escape-energy landscape.

$\tau_0(s)$	$x^{\#}(nm)$	$\Delta G^{\#}(k_B T)$	ν
0.63	0.46	20.6	0.5

Table S10: Parameters for the fit to cadherin strand-swap dimers. The force dependent short lifetime state of WT cadherin interacting for 3 s and K14E mutants interacting for 3 s and 0.3 s were globally fit to the microscopic, slip bond model (3). The parameters obtained from the fit are shown in this table where the value of ν corresponds to a harmonic well escape-energy landscape.

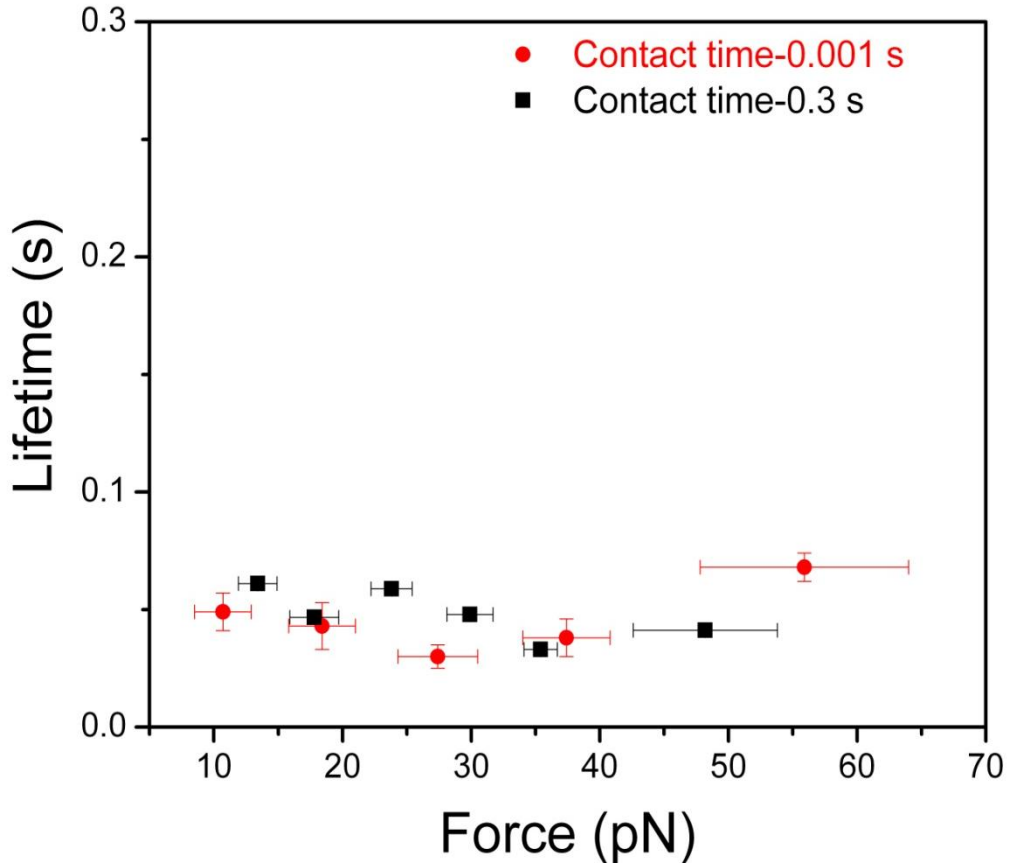

Figure S10: WT cadherins form ideal bonds even at a very short interaction time of 0.001 s. Even at a short interaction time of 0.001 s, the WT cadherins form ideal bonds suggesting that the transition from X-dimer to the intermediate state occurs at a very rapid rate. This interaction time corresponds to the fastest data acquisition rate of our force measurement apparatus.

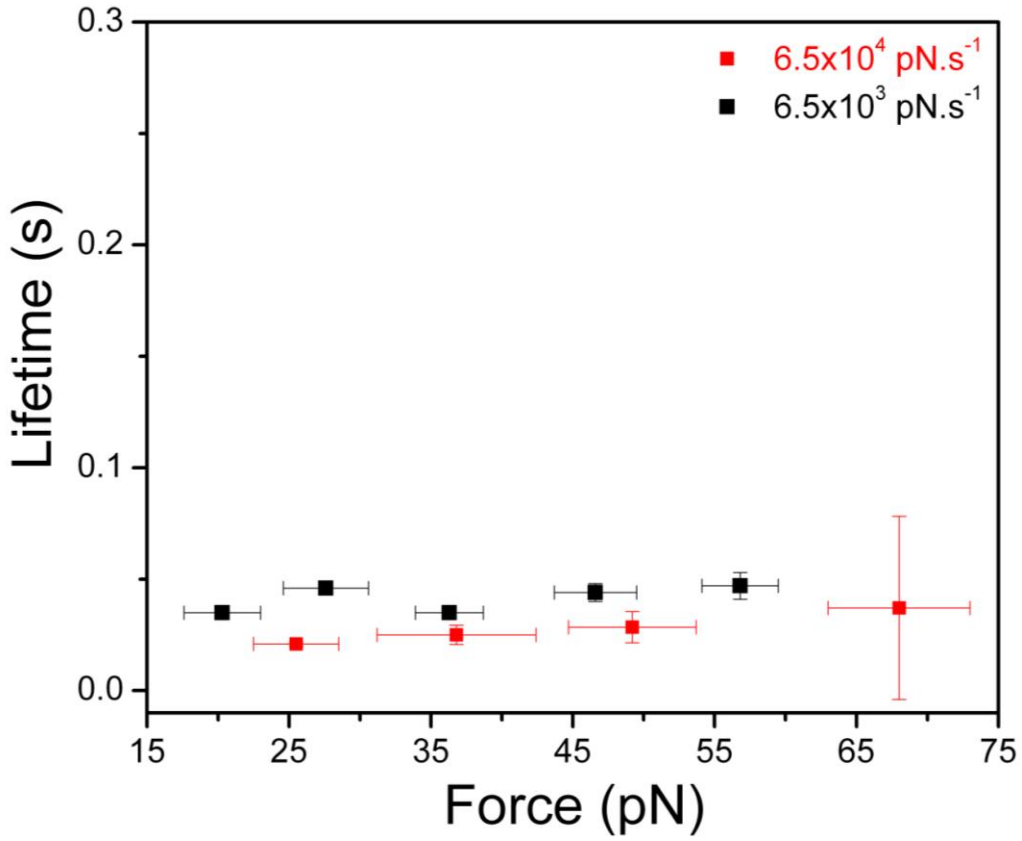

Figure S11: Effect of ramp-rate on WT cadherin ideal bond. In our experiments, the cadherins are loaded at a constant ramp-rate to a constant hold-force. We tested the effect of ramp-rate on the ideal bond behavior of WT cadherins; a recent study has shown that a mechanical bond that behaves as a catch-slip bond at low ramp-rates may transform to a slip-only bond at high ramp-rates (4). Force-clamp experiments with WT cadherins that were permitted to interact for 0.3 s, were done at two different ramp rates, 6.5×10^3 pN/s and 6.5×10^4 pN/s. In both cases the bonds behaved as ideal bonds, their lifetimes did not vary with clamping force.

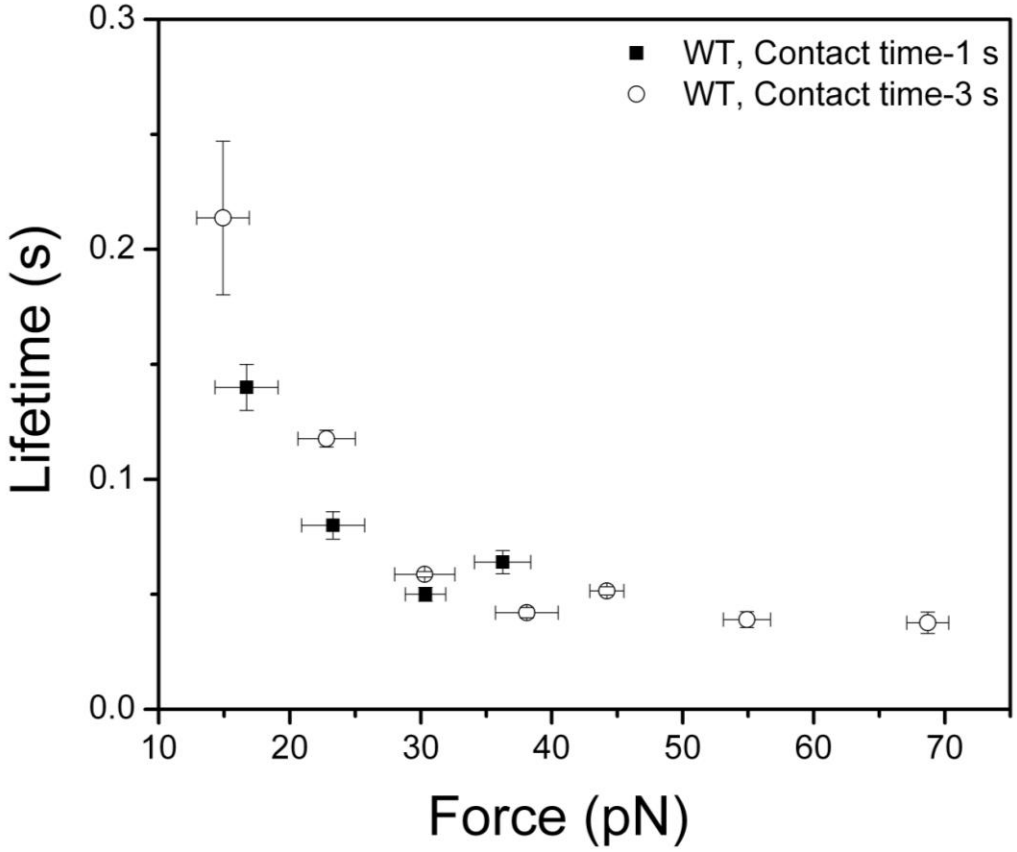

Figure S12: WT cadherin interacting for 1 s and 3 s form identical slip bonds.

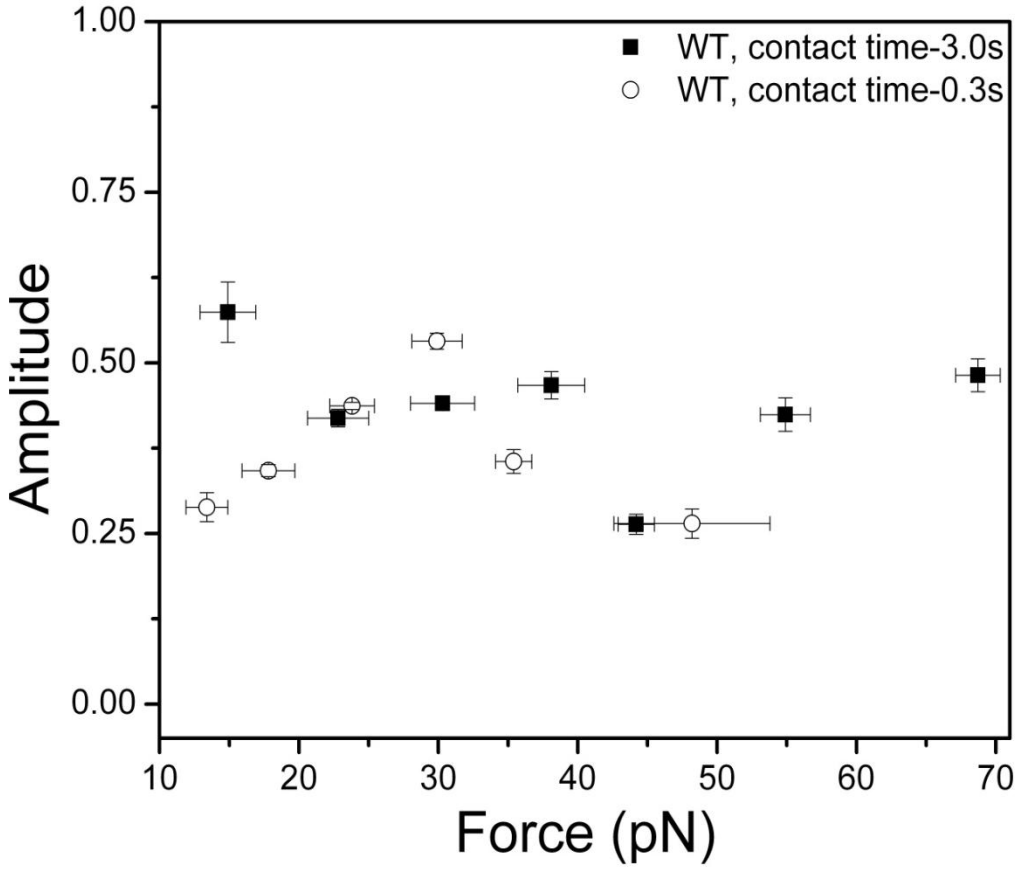

Figure S13: The amplitude or pre-exponential term of the longer lifetime state remains relatively constant with force for WT cadherins.

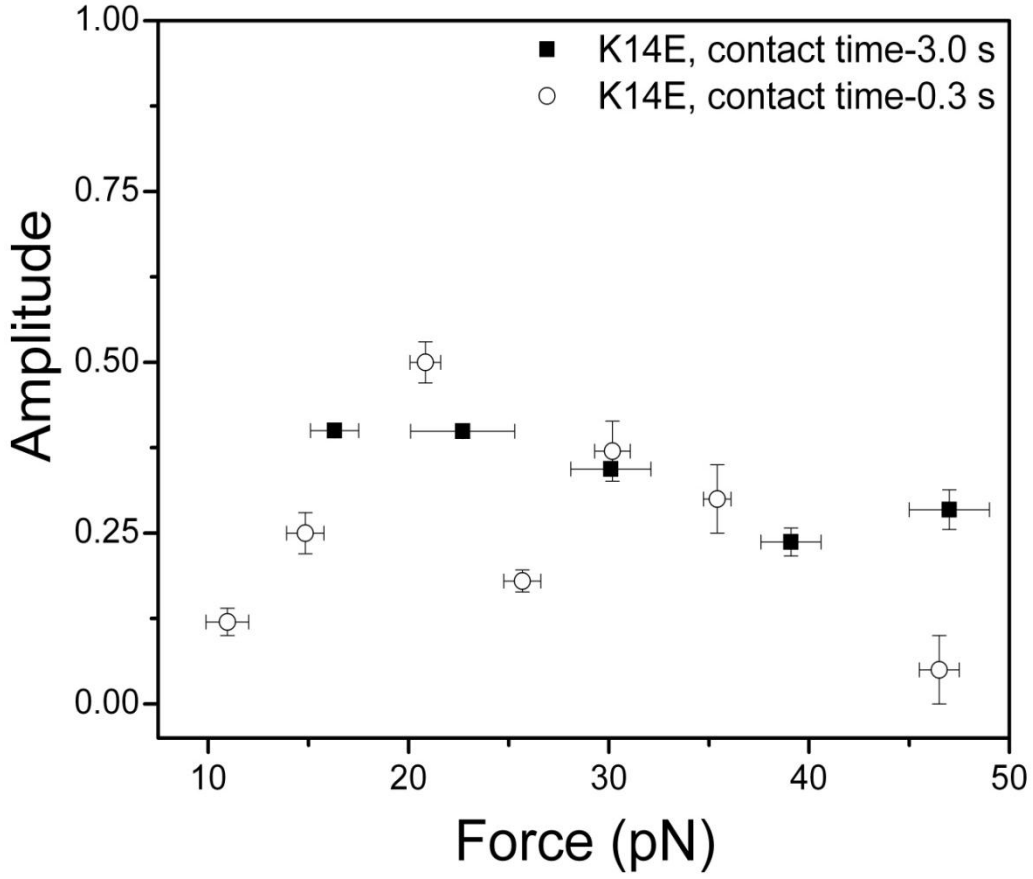

Figure S14: For K14E mutants, the amplitude or pre-exponential term of the longer lifetime state remains relatively constant with force.

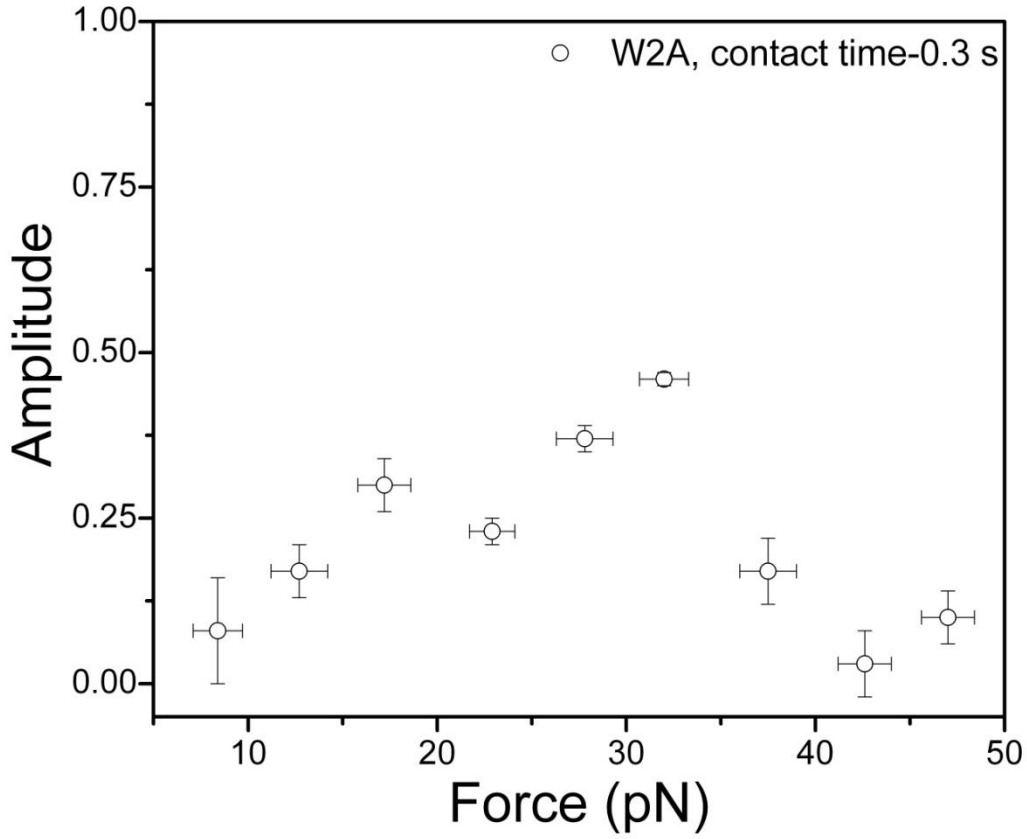

Figure S15: The amplitude or pre-exponential term of the longer lifetime state remains relatively constant with force for W2A mutants.

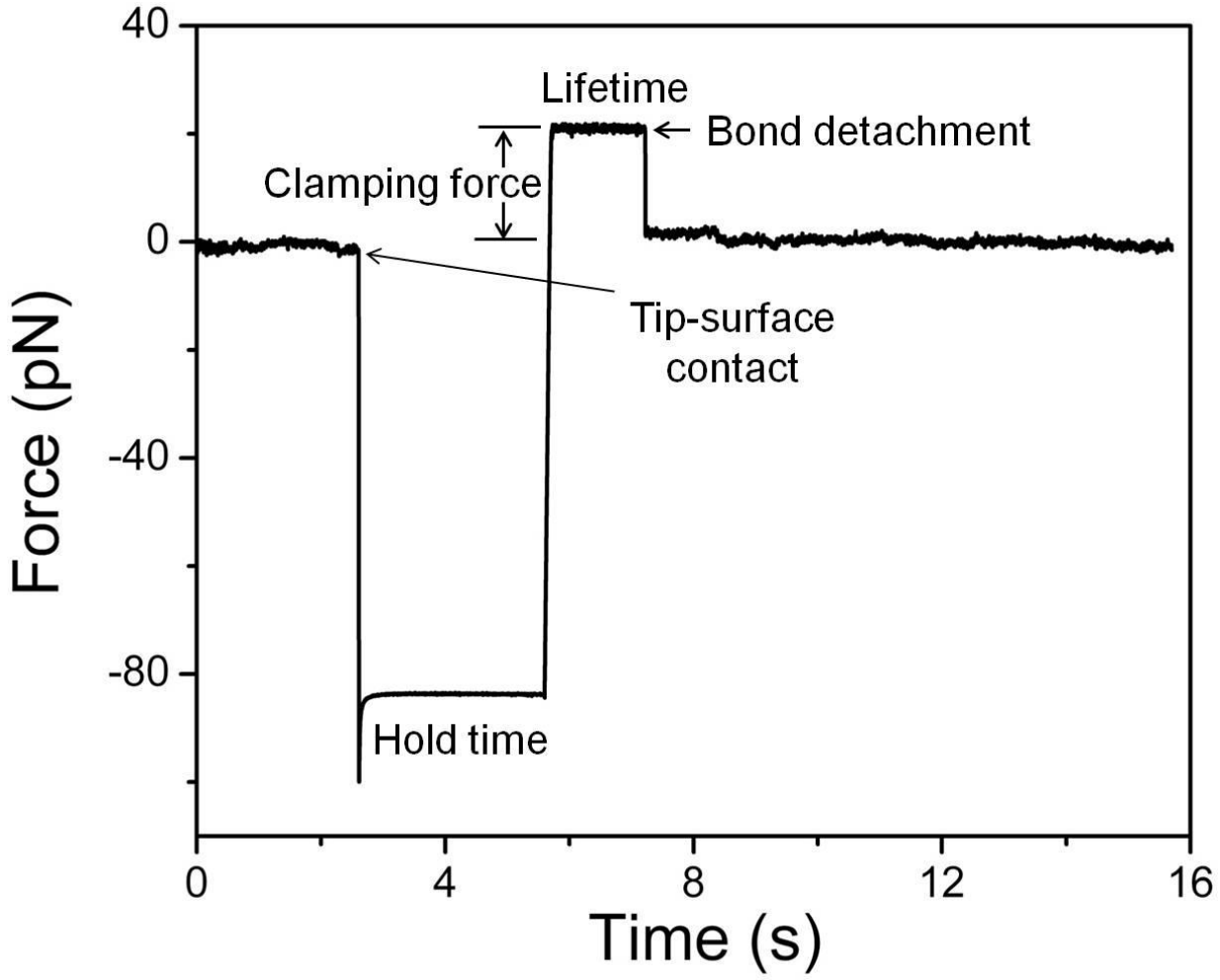

Figure S16: Typical AFM force clamp data. The cadherin functionalized substrate and AFM tip were first brought into contact. The tip was pressed against the surface at a force of 80 pN and held for different interaction times (3 s in this trace). The AFM tip was then rapidly withdrawn from the surface and 'clamped' at a pre-determined force so that a constant pulling force was applied to the cadherin-cadherin bond.

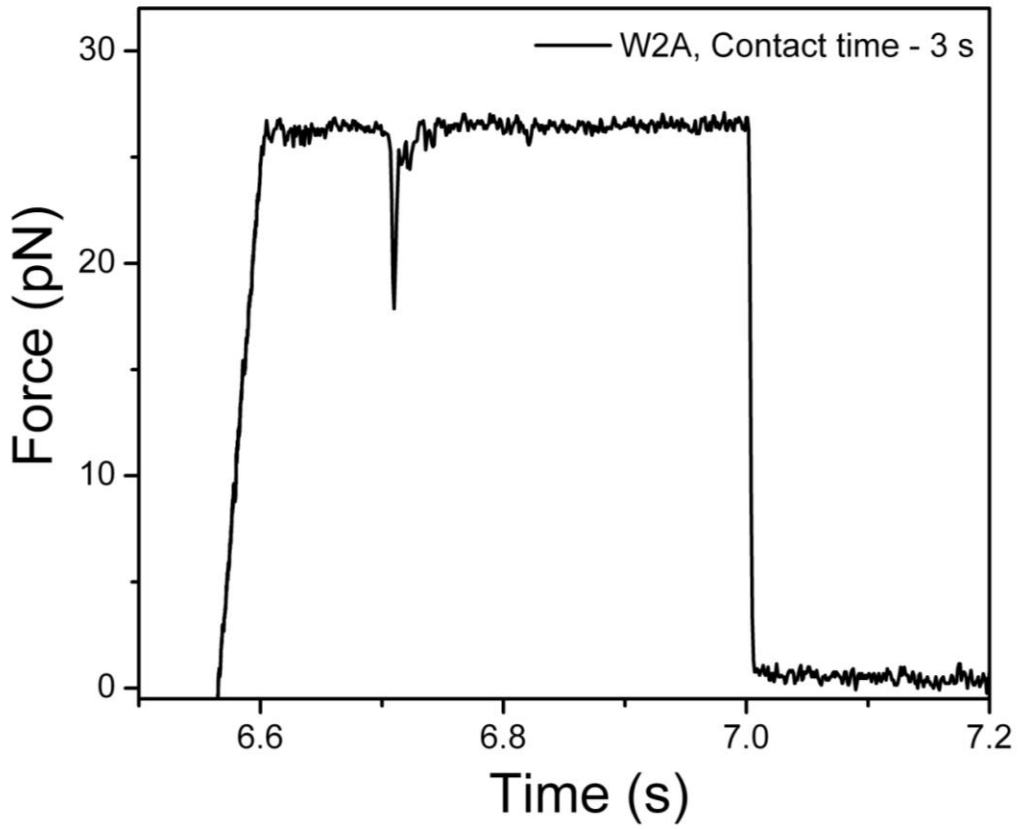

Figure S17: Typical AFM force clamp data for W2A cadherin interacting for 3 s shows multiple-steps. The length increase in each step (protein extension) corresponds to the contour length of an individual cadherin domain. Consequently, we ascribe these steps to the unfolding of individual domains in the cadherin extracellular region. Since similar data traces were observed for nonspecific interactions, this suggests that the steps arise due to non-specific adhesion of W2A cadherin with the opposing substrate/AFM tip. Consequently, we did not analyze the data for W2A cadherins interacting for 3.0 s.

References

1. Lou JZ, *et al.* (2006) Flow-enhanced adhesion regulated by a selectin interdomain hinge. *J. Cell Biol.* 174(7):1107-1117.

2. Lou JZ & Zhu C (2007) A structure-based sliding-rebinding mechanism for catch bonds. *Biophys. J.* 92(5):1471-1485.

3. Dudko OK, Hummer G, & Szabo A (2006) Intrinsic rates and activation free energies from single-molecule pulling experiments. *Phys. Rev. Lett.* 96(10).

4. Sarangapani KK, *et al.* (2011) Regulation of Catch Bonds by Rate of Force Application. *J. Biol. Chem.* 286(37):32749-32761.