Supplementary Information

Electrostatic term within the framework of the Dynamic Force Spectroscopy theory for the strength of molecular adhesion bonds.

The bond strength, f^*, defined as the most probable unbinding force or, equivalently, as the peak force from the unbinding force histogram, and measured in our experiments as the adhesion during the retract of the tip from the substrate, is usually plotted versus the logarithm of the mechanical loading rate, r. In most experiments, these strength spectra follow linear regimes[1]. Such strength spectra have been interpreted in pure mechanical loading using an off-rate expressed as in the frame of the Bell model[2]:

$$r = \left(\nu_m \, k_B \, T / a_m\right) exp\left(E_m / k_B \, T\right) \qquad \text{(si1)}$$

where k_B is the Boltzmann constant and T the absolute temperature. E_m is the height of the energy barrier allowing the likelihood of bond survival and is expressed as

$$E_m \equiv a_m f^* . \qquad \text{(si2)}$$

The off-rate, ν_m, and a_m, usually interpreted as the location of the energy barrier (a_m, has a length's dimension), are obtained from the fit of the $f^* \; vs. \; log_e(r)$ linear relationship as deduced from (si1) and (si2) equations. This linear relationship was proved to reasonably fit experimental results in various examples as in the case of biotin/streptavidin bond strengths[3], dissociation of P-selectin, an example of adhesion molecule from an antibody[4], or fibrinogen and colloid surfaces with variable hydrophobicity[5]. The typical values for f^* were found to be in the range of few tenths of nanonewtons. In these papers, it was assumed that the energy barrier only stemmed from a mechanical origin as evidenced through the variation of loading rate and had a magnitude in the order of the thermal energy (at room temperature : ~4pN.nm).

We postulate here that an electrostatic term has to be added in order to correctly explain our experimental results through the Dynamic Force Microscopy theory:

$$E = E_m + E_e \qquad \text{(si3)}$$

As in the mechanical case, it can be written, in a more general way:

$$E \equiv a f^* \qquad \text{(si4)}$$

For strength spectrum with a single barrier, from equations (si1) -where E_m is replaced by E- and (si4), it can be deduced that the peak, f^*, in the force distribution shifts to higher force in proportion to $log_e(r)$ with a slope, f_β, deduced from the following relationship:

$$f^* = f_\beta \, log_e(r) + c \qquad \text{(si5)}$$

where r, the loading rate, is calculated as $r = {\Delta f}/{\Delta t}$ and c is a constant related to the slope f_β and the off-rate, ν.

In our AFM experiments, the application of force's gradient only occurs during the contacting time of the double layers so that $\Delta f \cong \Delta f_0 \cong cst$, with Δf_0 equals to the force set-up (~1nN) and the effective contact time, $\Delta t = \tau$. Thus we can write: $r \cong {\Delta f_0}/{\tau}$ and deduce equation (1) in main text

$$f^* = - f_\beta \, log_e(\tau) + \left[c + log_e(\Delta f_0)\right] \quad (1).$$

Captions of Supplementary Figures

Figure SI1:

Typical evolution of the adhesion signal as detected on parts of the sample (ITO in the present case) away from the NR particles during the whole completion of AFM data with the same tip. The points around t=0 correspond to the initial large scan (scan size $(10\mu m)^2$). The other data are for $(1.5\mu m)^2$ scans (Im#1 or Im#3 images). This adhesion signal is constant, proving that no contamination of the AFM tip occurs, whatever the type of tip, the size of NR, the type of retract curve and the applied voltage. The shown example is for a Si_3N_4 tip. Typical values for the mean adhesion signal vary between 0.10nN and 0.45nN depending of the local shape (and contacting area) of the AFM tip.

Figure SI2:

The variation of height signal, for the images of the type Im#1 or Im#3, is plotted versus the pixel time (the time spent by the tip at a given position on the surface during the approach and retract phases) in figures SI2.A (black line) and SI2.B (grey line). Figure SI2.A is a magnification of figure SI2.B for small times. The corresponding height images –scan size $(1.5\mu m)^2$) are drawn as inserts. The upper envelopes of these height signals are plotted by orange lines. The zone investigated by the AFM tip during Im#2 (scan size $(50nm)^2$) is visualized by the green segment in figure SI2.A. The values for the height signal at the summit of the NR particles are noted in figure SI2.B.

Figure SI3:

In a first step, a linear voltage ramp is applied (figure SI3.A) between working and counter electrodes. The corresponding cyclic voltammogram $I(V)$ is plotted in figure SI3.B. Then the electrical circuit is opened and the variation of the so-called Open Circuit Potential (OCP) with time is measured (figure SI3.C black line). The best exponential fit is plotted by the dashed red line. This last step lasts 45 minutes.

Figure SI4:

Three different types of retract curves are observed on the NR particles whatever the nature of the AFM tip SiO_2 or Si_3N_4 and the voltage. Type I (figure SI4.A) is characterized by a low adhesion (in the range of few tenths of nN) and is also systematically observed on the bare part of the substrate (i.e. away from the NR particles). Type II and III are characteristics of pixels on NR and are associated to a higher adhesion (in the range of few tens of nN). Type II (figure SI4.B) differs from type III (figure SI4.C) by the quasi-instantaneous return to state of null force. Instead, type III (figure SI4.C) adhesion curves possess two or more plateaus after the main rupture of adhesion.

Figure SI5:

In case of SiO_2 tip and a 0mV voltage applied to the ITO substrate, large scan images $(1.5\mu m)^2$ -Im#1 and Im#3, of small (figure SI55.A-D) and large (figure SI5.E-H) NR particles, are plotted. The left and right columns are AFM data for Im#1 and Im#3 respectively. Images A, B, E, F are height data and images C, D, G, H are for adhesion.

Figure SI6:

The violet markers represent the difference in adhesion between Im#1 and Im#3 (data from figure 6.B) of NR particles for Si_3N_4 tip (voltage: 0mV). The orange markers are for the mean value of stiffness as measured at the top of the studied NR particle for the first large scan at $(1.5\mu m)^2$ (Im#1). The orange arrow schematizes the frontier[6] between the so-called SRP and LRP.

Figure SI7:

A: Time evolution of the raw adhesion AFM signal (orange thin line) for small scanned zone – $(50nm)^2$-(Im#2) in the case of a small NR investigated by a Si_3N_4 tip. This signal is then smoothed (orange bold line in figure SI7.A). Please note the transition from a high-level of adhesion to a lower one at a transition time marked by the magenta dashed line.

B: Time evolution of the smoothed height signal (black line) acquired simultaneously with the adhesion signal (figure SI7.A). The blue signal corresponds to the time variation of height signal of a $(50nm)^2$ scanned zone on a bare part of the substrate (it means: out of the NR particles): this signal is due to residual thermal drifts. Its slope is identical to that of the *δ(Height)* signal on NR particles after the transition (at the right of the magenta dashed line). The smoothed height signal, as corrected from this residual thermal drift, ("*Height_TD*") is plotted in figure SI7.B (green curve).

Supplementary References

1 E. Evans and K. Ritchie, *Biophys. J.*, 1997, **72**, 1541–1555.

2 G. I. Bell, *Science*, 1978, **200**, 618–627.

3 R. Merkel, P. Nassoy, A. Leung, K. Ritchie and E. Evans, *Nature*, 1999, **397**, 50–53.

4 B. T. Marshall, K. K. Sarangapani, J. Lou, R. P. McEver and C. Zhu, *Biophys. J.*, 2005, **88**, 1458–1466.

5 L.-C. Xu and C. A. Siedlecki, *Langmuir*, 2009, **25**, 3675–3681.

6 K. Berthelot, S. Lecomte, Y. Estevez, B. Coulary-Salin and F. Peruch, *Biochim. Biophys. Acta BBA - Proteins Proteomics*, 2014, **1844**, 473–485.

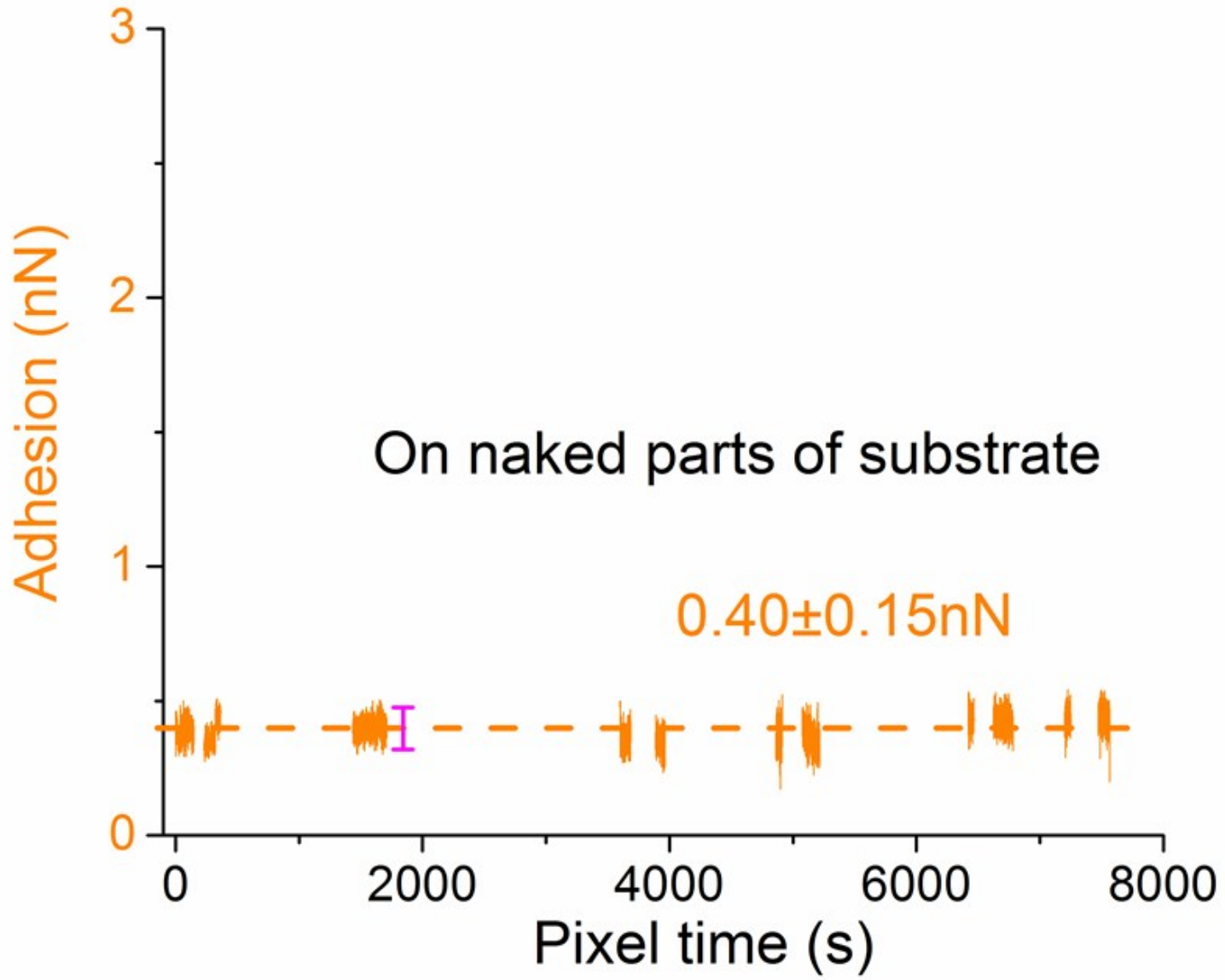
Adhesion (nN)
3
2
1
0
On naked parts of substrate
0.40±0.15nN
0
2000
4000
6000
8000
Pixel time (s)

FIGURE SI1

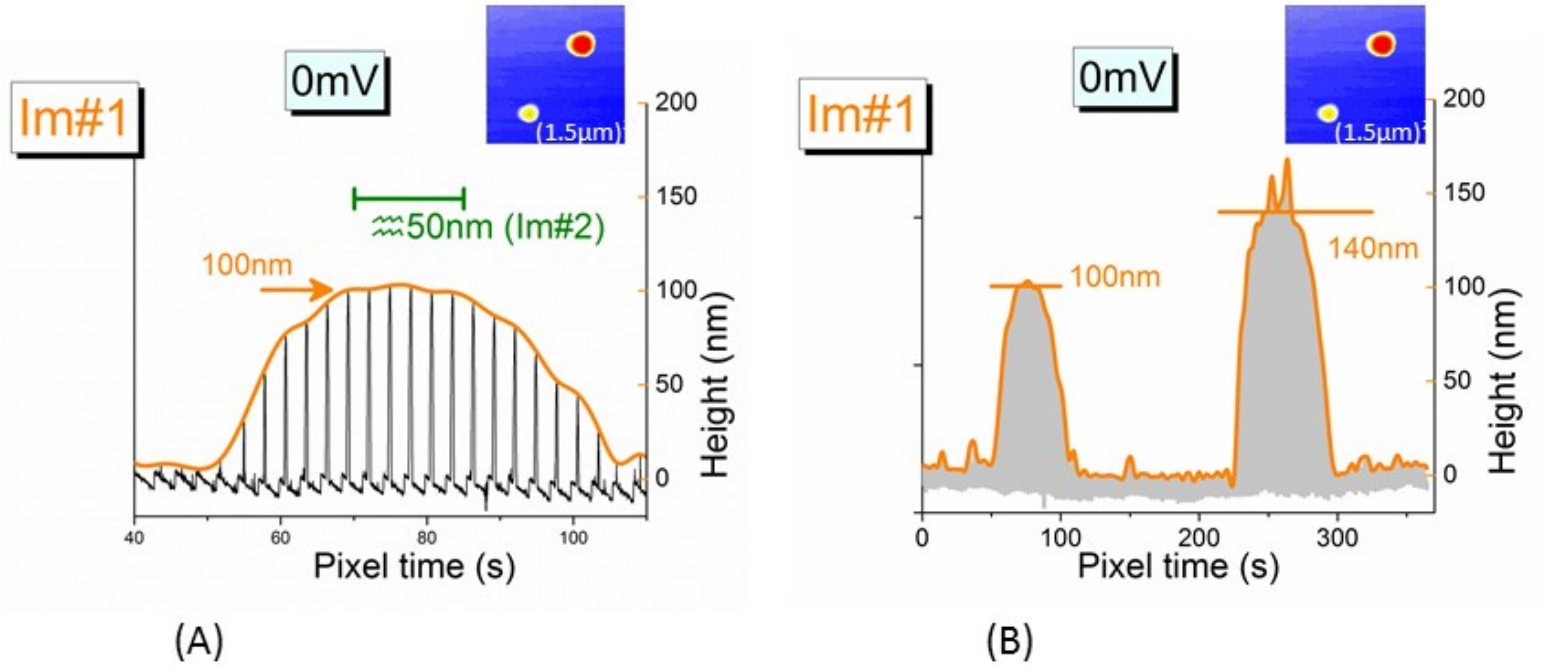
Im#1
0mV
(1.5µm)
50nm (Im#2)
100nm
200
150
100
50
0
Height (nm)
40
60
80
100
Pixel time (s)
(A)
Im#1
0mV
(1.5µm)
100nm
140nm
200
150
100
50
0
Height (nm)
0
100
200
300
Pixel time (s)
(B)

FIGURE SI2

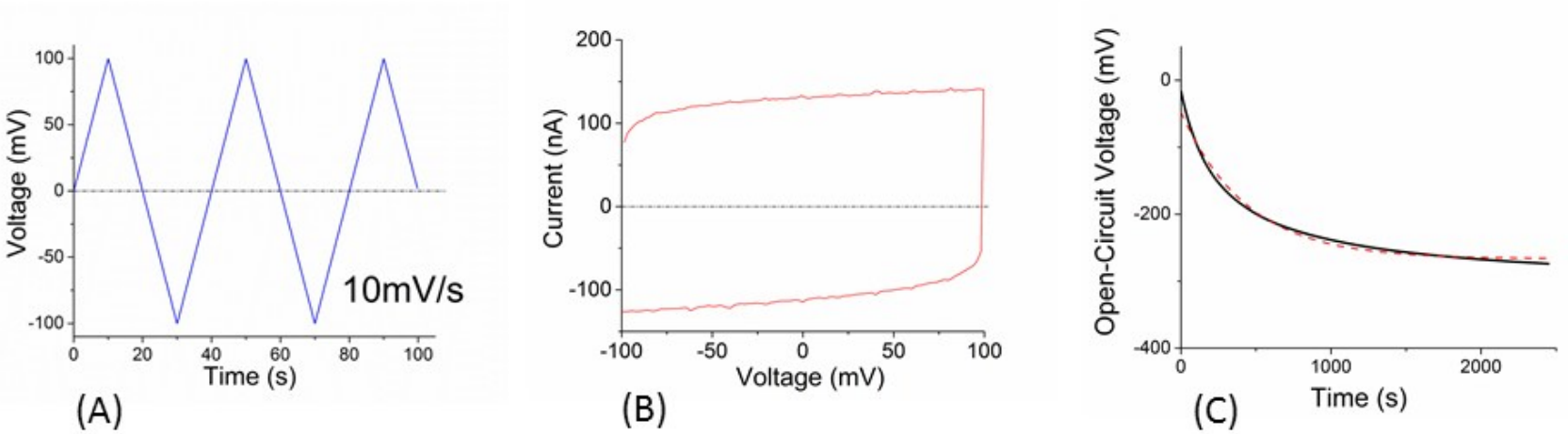
Voltage (mV)
Time (s)
10mV/s
(A)
Current (nA)
Voltage (mV)
(B)
Open-Circuit Voltage (mV)
Time (s)
(C)

FIGURE SI3

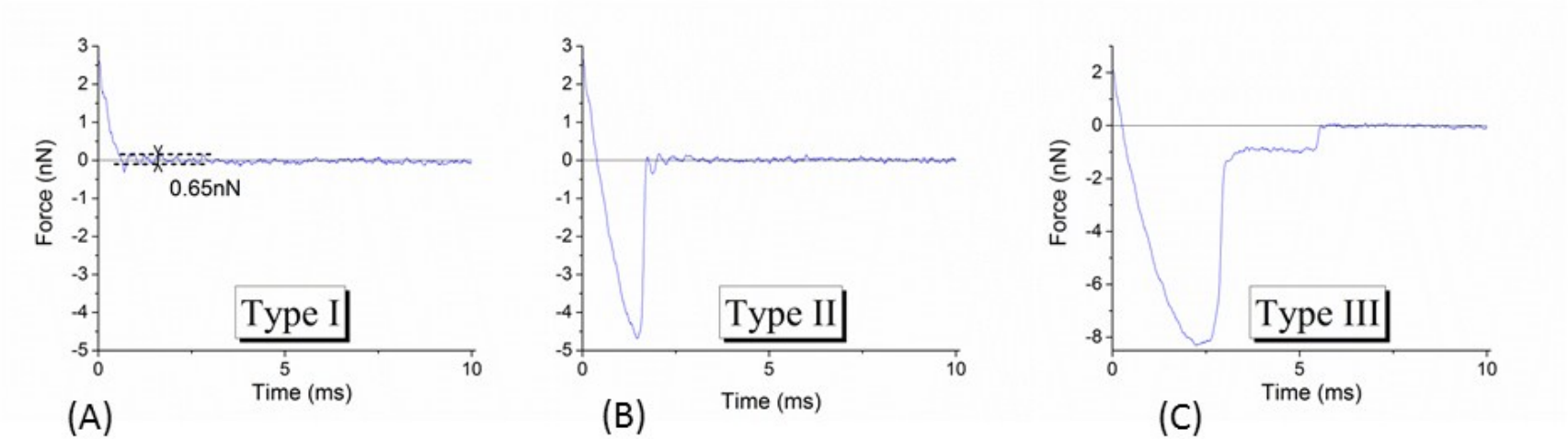

FIGURE SI4

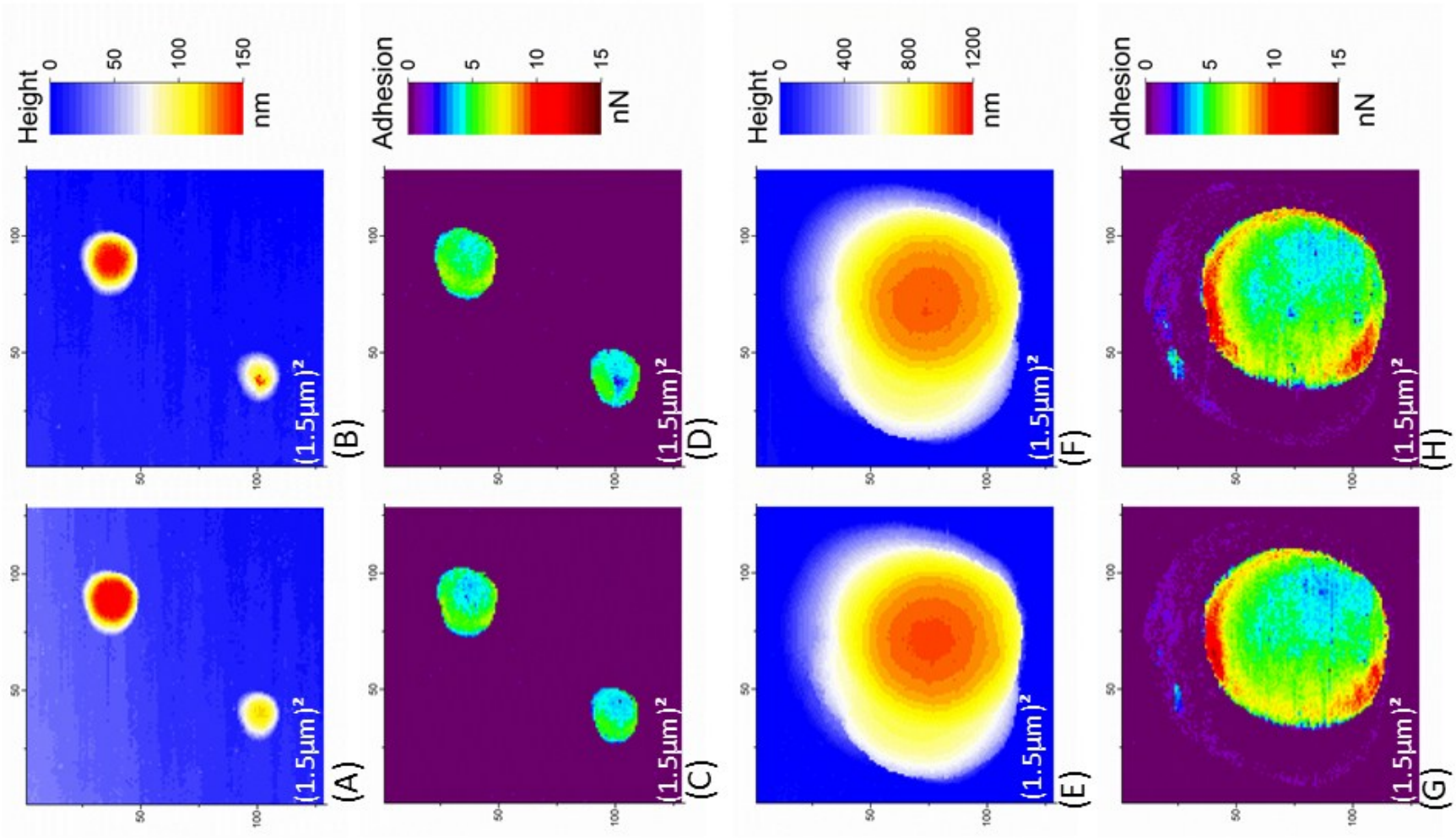
Height
0
50
100
150
nm
Adhesion
0
5
10
15
nN
Height
0
400
800
1200
nm
Adhesion
0
5
10
15
nN
(1.5µm)²
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)

FIGURE SI5

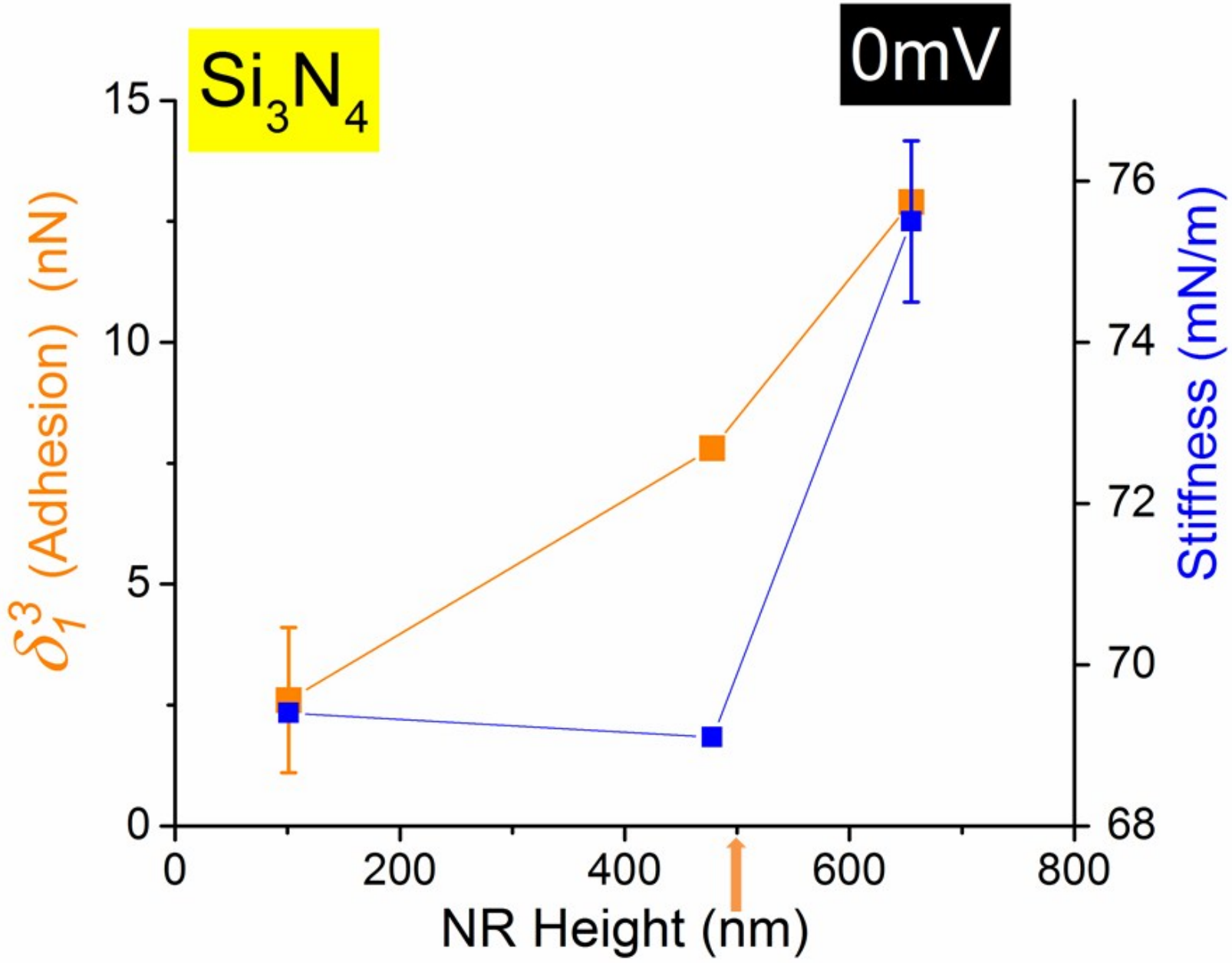
Si3N4
0mV
15
10
5
0
0
200
400
600
800
NR Height (nm)
δ13 (Adhesion) (nN)
76
74
72
70
68
Stiffness (mN/m)

FIGURE SI6

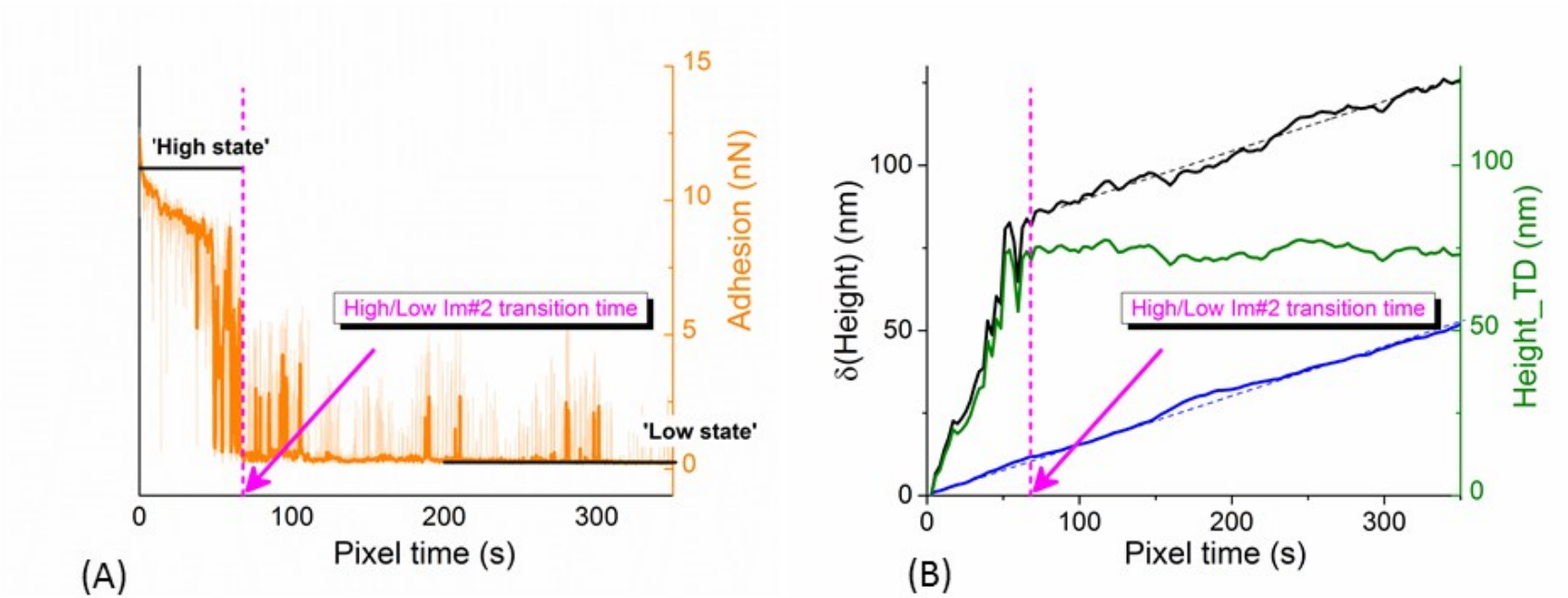
'High state'
High/Low Im#2 transition time
'Low state'
Adhesion (nN)
Pixel time (s)
(A)
δ(Height) (nm)
Height_TD (nm)
High/Low Im#2 transition time
Pixel time (s)
(B)

FIGURE SI7