Probing the Interaction of Individual Amino Acids with Inorganic Surfaces Using Atomic Force Spectroscopy

Supporting Information

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Materials and Methods

Chemical modification of the AFM Tip

The chemical modification of the AFM tip is based on a process described before.[1, 2] Silicon Nitride (Si_3N_4) AFM cantilevers with silicon tips (MSNL10, nominal tips radius ~ 2 nm, Bruker) were cleaned and oxidized using O_2 Plasma (Atto, Diener Electronic) for 5 min in order to produce Si-OH groups on the surface. To modify the tips with a NH_2 monolayer, they were suspended above (3 cm) a solution of 8% (vol\vol) 3-aminopropyltriethoxysilane in toluene in a desicator filled with a dry nitrogen atmosphere for 1.5 hours. Then, the tips dried on a hot plate for 10 min at 100°C under atmospheric conditions. After cooling the amine-functionalized tips to RT, they were modified with a mixture of methoxy-PEG-N-hydroxysuccinimide, 2000 Da (mPEG-NHS, Nanocs), and Fluorenylmethyloxycarbonyl-PEG-N- hydroxysuccinimide, 5000 Da (Fmoc-PEG-NHS, Iris Biotech) in a molar ratio of 7:1 respectively. The reaction was performed at a total PEG concentration of 5 mM in chloroform containing 0.5% (vol\vol) triethylamine, at room temperature for 1 hour. Following an extensive wash with chloroform and N-Methyl-2-pyrrolidone (NMP), the Fmoc protecting groups were cleaved by treating the tips with 20% piperidine (vol/vol in N-methyl-2-pyrrolidone) for 30 min. After extensive wash (three times) with NMP, the amine groups were then coupled with the desired acetylated amino acid (AA). Coupling was done using Ac-AA-COOH/diisopropylethylamine/ 2-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HBTU), molar ratio of 1:1:1 at a total concentration of 30 mM in 5 ml NMP for 1.5 hour, followed by a wash with NMP. To block the remaining free amine groups the tips were acetylated using aceticanhydride/ diisopropylethylamine at a molar ratio 4:1 and a total concentration of 0.65 M in NMP. Finally, the tips were washed with NMP, chloroform, EtOH, and water in order to remove any uncoupled residues. In the case of glutamate and lysine, a further cleavage of the OtBu and Boc protecting group respectively was done using 90% (vol\vol) trifluoroacetic acid (TFA)\water for 30 min followed by an extensive wash with water.

Characterization of the functionalized AFM tip

In order to monitor and verify the chemical modification of the tip, a silicon wafer (100) was modified using the same procedure that was used for the AFM tips. Either a fluorinated phenylalanine (GL biotech) or a phenylalanine (Acros organics) coupled to the substrate. The surfaces at different stages of the chemical modification were analyzed using X-ray photoelectron spectroscopy (XPS) and contact angle measurements.

X-ray photoelectron spectroscopy (XPS)

The X-ray Photoelectron Spectroscopy (XPS) measurements were performed using a Kratos Axis Ultra X-ray photoelectron spectrometer (Karatos Analytical Ltd., Manchester, UK) using an Al Kα monochromatic radiation source (1,486.7 eV) with 90° takeoff angle (normal to analyzer). The high-resolution XPS spectra were collected for C 1s, B 1s, F 1s, O 1s and Si 2p levels with pass energy 20 eV and step 0.1 eV. The binding energies were calibrated relative to C 1s peak energy position as 285.0 eV when needed. Data analyses were performed using Casa XPS (Casa Software Ltd.) and Vision data processing program (Kratos Analytical Ltd.).

Evaluation of the layer thickness by XPS

For the layer and substrate, the thickness *d* (nm) may be expressed as:

$$d = \lambda_o \sin\theta \; ln\left(\frac{N_s \lambda_s I_o}{N_o \lambda_o I_s} + 1\right)$$

Where I_s and I_o are the intensities of the peaks from the substrate and the layer respectively, the substrate is the 2p signal from silicon and layer is the sum of the intensities of C 1s, O 1s and N 1s peaks, θ is the takeoff angle (in our case $sin\ \theta = 1$) and N_s and N_o are the volume densities. The inelastic mean free paths (IMFPs) parameters for substrate λ_s and layer λ_o assumed as 3.1 nm and 3.3 nm respectively (calculated using *QUASES-IMFP-TPP2M* software).[3]

Quantitative XPS analysis

During XPS measurements, soft X-rays illuminate a region of the sample and photoelectrons originated from it are detected. Only electrons, emitted from atoms

near the surface of the sample can escape from the material and be analyzed. The depth of a typical analysis is about 1-10 nm, which enables the study of very thin surfaces such as monolayers. Slight changes in peak intensities can provide quantitative information on the surface chemistry and the ability to determine the precise chemical composition. Using an identical aperture of the detector and analyzing the same area in the sample, we were able to determine the variations in molecular density on the substrate.

Contact angle measurements

Conatct angle measurements were carried out using a Theta Lite optical tensiometer (Attension, Finland).

Preparation of substrates

A silicon wafer (100) was cut into 0.5 cm squares using a diamond pen. The substrates were then cleaned in acetone followed by isopropanol in an ultrasonic bath, and oxidized in a piranha solution (sulfuric acid/H_2O_2, vol/vol 7:3) for 30 min. To remove residual piranha solution, the substrates were washed in water and dried under nitrogen gas. Mica disks (V1 12mm, Ted Pella USA) were freshly cleaved before each use.

Single molecule force spectroscopy measurements

Unless specified otherwise force spectroscopy measurements were carried out in water (Mili-Q, ~20 MΩ) at 25 $^{\circ}$C, using commercial AFM, a NanoWizard® 3 (JPK Instruments, Germany). Si_3N_4 (MSNL-10 series, Bruker) cantilevers with spring constants ranging from 10 to 30 pN nm^{-1} were calibrated by the thermal fluctuation method (included in the AFM software) with an absolute uncertainty of approximately 10%. [4] Measurements were obtained by bringing the amino acid functionalized tip into contact with the substrate with a compression force of ~200 pN and then retracting the tip at various speeds, from 0.1 to 1.5 μm/sec, for a distance of ~200 nm.

Data Analysis

Prior to analysis, the deflection values (V) are converted to force by multiplying the photodiode sensitivity (V/m) and by the experimentally determined spring constant.[5] Only single adhesion events were taken into account (between 10%-30 % of the curves) ensuring that >95% probability that the adhesion event was mediated by a single bond.[6] The apparent loading rate was experimentally measured. The force curves were plotted vs. time and the slope just prior to the rupture point was analyzed statistically from at least 10 different curves. The unbinding forces of individual amino acid were derived from the jump in force following the separation of the cantilever from the substrate using software for data processing (JPK Instruments, Germany).

Worm Like Chain (WLC) fitting

An adhesion event which exhibits a force curve was fitted using a WLC model:

$$F(x) = \frac{k_b T}{l_p}\left[\frac{1}{4}\left(1 - \frac{x}{L}\right)^{-2} + \frac{x}{L} - \frac{1}{4}\right]$$

Where T is the temperature, k_b is the Boltzmann constant and l_p and L are the persistence length and the contour length respectively.

References

(1) Ratto, T. V.; Langry, K. C.; Rudd, R. E.; Balhorn, R. L.; Allen, M. J.; McElfresh, M. W., Force spectroscopy of the double-tethered concanavalin-a mannose bond. *Biophys. J.* **2004,** 86, 2430-2437.

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(3) Briggs, D.; Seah, M. P., *Practical surface analyses*. 2 ed.; Wiley: New York, NY, USA, 1990; Vol. 1.

(4) Hutter, J. L.; Bechhoefer, J., Calibration of atomic-force microscope tips. *Rev. Sci. Instrum.* **1993,** 64, 1868-1873.

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(6) Evans, E.; Kinoshita, K.; Simon, S.; Leung, A., Long-lived, high-strength states of ICAM-1 bonds to β_2 integrin, I: Lifetimes of bonds to recombinant $\alpha_1\beta_2$ under force. *Biophys. J.* **2010,** 98, 1458-1466.

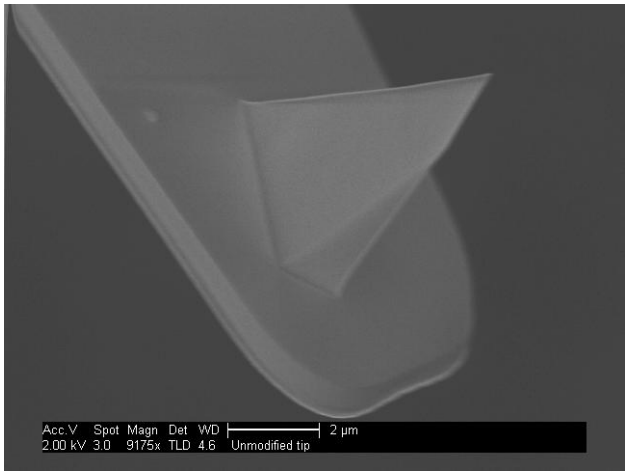

Figure S1. SEM micrograph of a bare MSNL10 silicon tip. The tip has a nominal radius of ~ 2 nm.

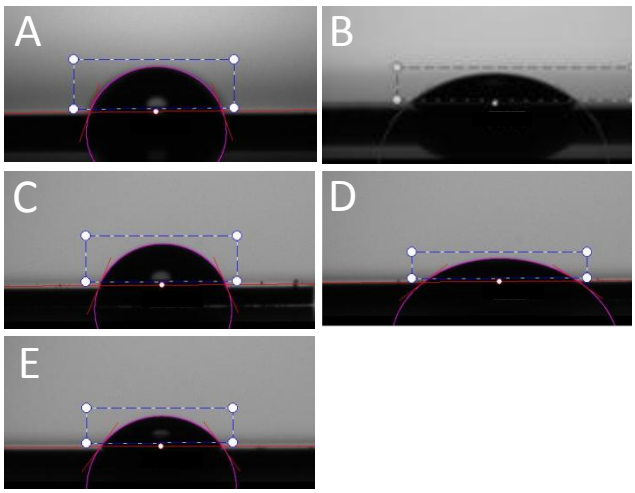

Figure S2. Contact angle measurements at different stages of the chemical modification of the surface. **(A)** A bare silicon surface had an angle of 70°. **(B)** After treatment with piranha for 30 min the angle changed to 41°. **(C)** Modification of the surface with 3-aminopropyltriethoxysilane increased the contact angle to 67°. **(D)** PEGylation of the surface reduced the contact angle to 35° **(E)** Coupling phenylalanine to the PEG molecules increased the contact angle to 53°.

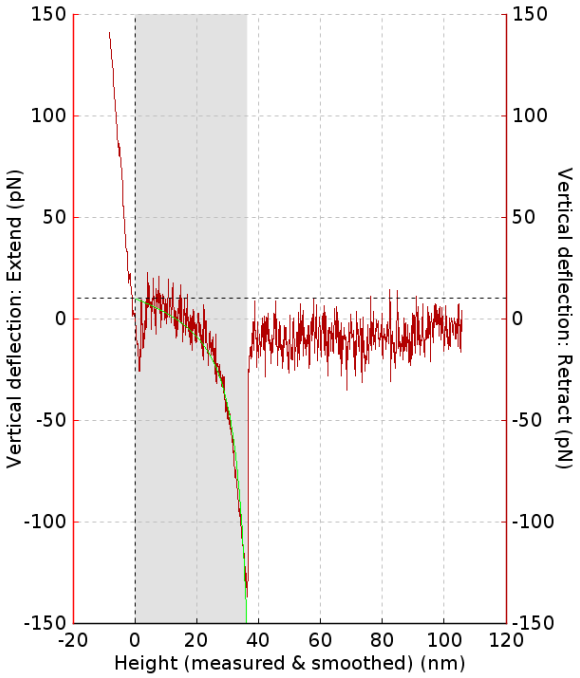

Figure S3. A fit based on the worm-like chain (WLC) model for a typical F-D curve. The fit indicates on a contour length of 44.6 nm and a persistence length of 187 pm.

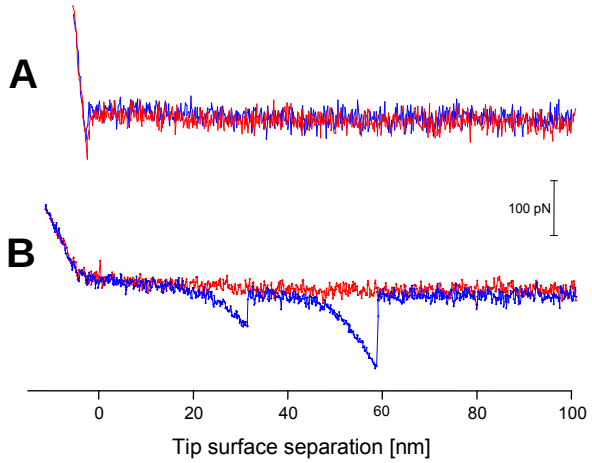

Figure S4. Representative F-D curves that were discarded from the force histograms. The red and blue traces indicate approach and retract signals, respectively. (**A**) a F-D curve without any peak. (**B**) a F-D curve with two peaks both indicating on adhesion events.

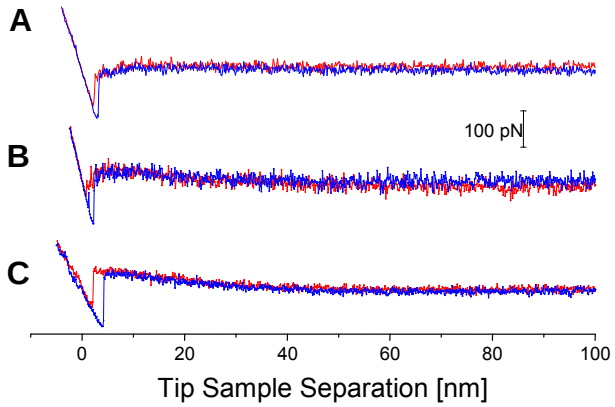

Figure S5. Representative F-D curves for a tip functionalized with **(A)** mPEG-NHS (without coupling of an amino acid), **(B)** glutamate or **(C)** glutamine.

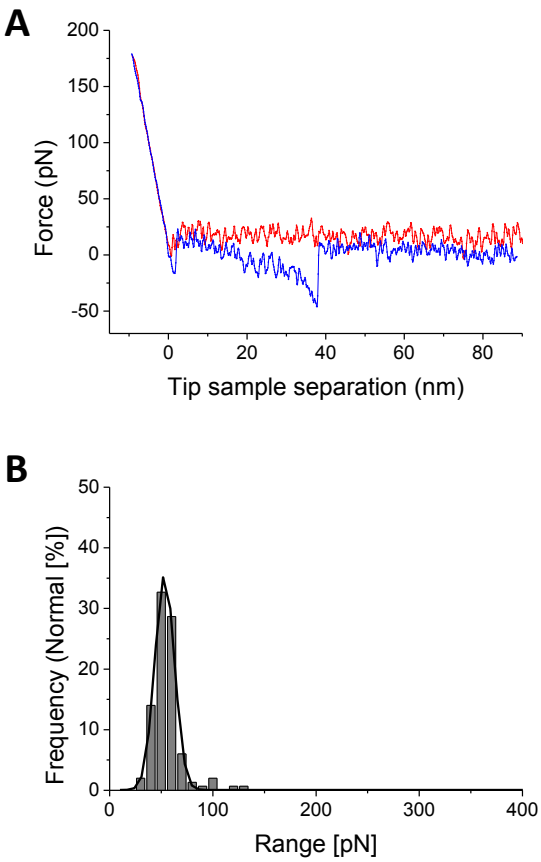

Figure S6. (**A**) A representative F-D curve for a tip functionalized with a mix of mPEG-NHS and fmoc-PEG-NHS (without cleaving and coupling of an amino acid) at a loading rate of ~6 pN/s. (**B**) A force histogram at a loading rate of ~6 pN/s with a MPF of 53.2±9.4 pN (n=100).

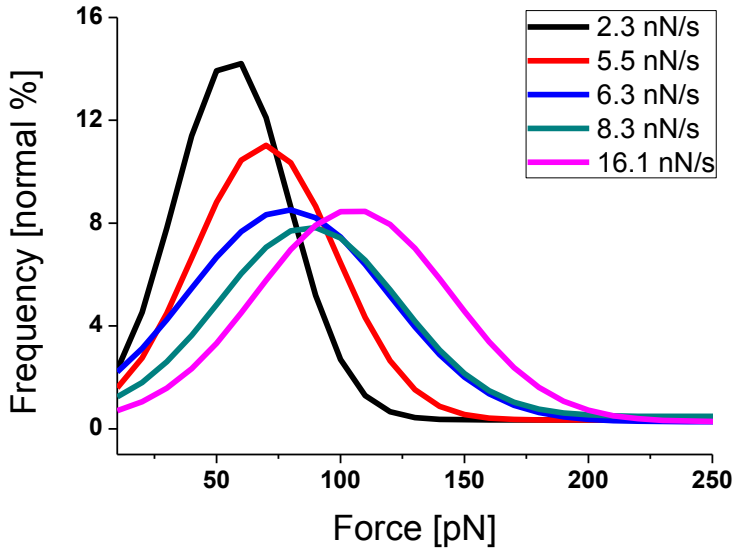

Figure S7. A Gaussian fit for the adhesion histograms of lysine to silicon at different loading rates, 2.3-16.1 nN/s.

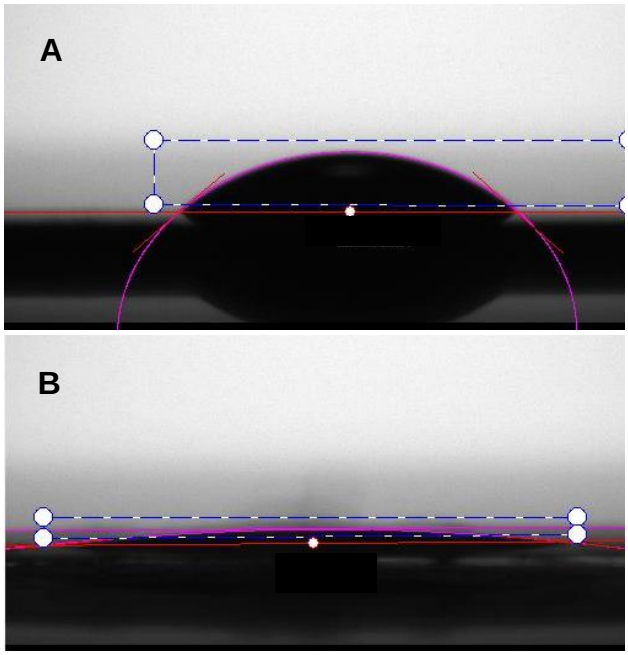

Figure S8. Contact angle images of **(A)** a silicon surface treated with piranha had a contact angle of 40^{o}, and **(B) a** freshly cleaved mica surface had a contact angle of 6^{o}.

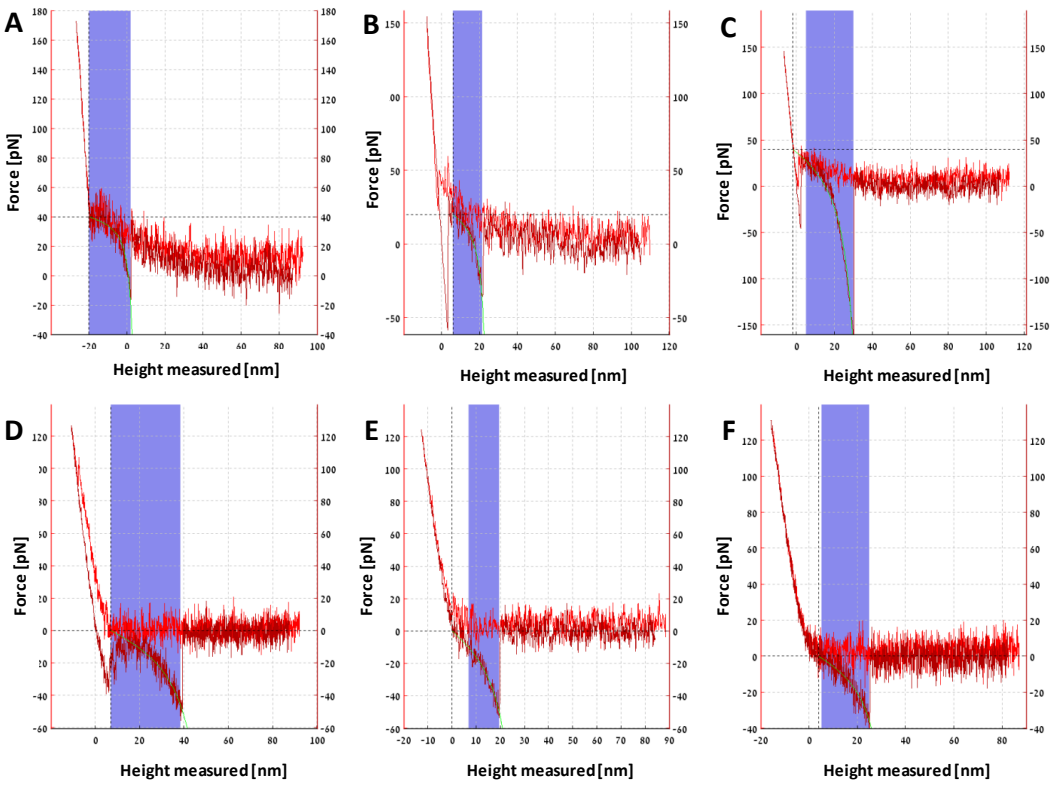

Figure S9. Representative WLC fit curves for the 5000 Da PEG (with a polydispersity of D=1.03) and the extracted averaged (from n events) contour length (C_l) and persistence length (p_s) for the different conditions: (A) pH 11.6 ; C_l=40.9±8.3 nm ; p_s=190.5±65.4 pm ; n=75, (B) pH 3.3 ; C_l=63.4±15.7 nm; p_s=183.2±64.7 pm ; n=72, (C) pH 7 ; C_l=53.2±12.9 nm ; p_s=122.3±43.9 pm ; n=90, (D) ionic strength 10 mM ; C_l=53.4±14.9 nm ; p_s=160.2±64.1 pm ; n=83, (E) ionic strength 20 mM ; C_l=33.9±12.5 nm ; p_s=155.7±66.7 pm ; n=62, (F) ionic strength 50 mM ; C_l=35.9±8.7 nm ; p_s=209.5±72.1 pm ; n=70.