Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

How soft is a single protein?: Stress-strain curve of antibody pentamers with 5 pN and 50 pm resolutions

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Hybrid dynamic AFM method

A photothermal excitation is applied to drive the cantilever oscillation (Fig. S1). This method enables a proper tracking of the frequency shift of the AFM probe which in turns facilitates the accuracy in the measurements and the transformation of observables into forces. The thermal fit is showed in the Fig. S2.

High resolution images of IgM antibodies have been acquired by using excitation forces as close as possible to the one used to excite the cantilever far from the surface $V_{exc,0}$≈620 mV. To enlarge the force range, we have used two types of cantilevers. For applying very small forces, we have used cantilevers with very small force constants (k≈ 0.07 N/m) while to image the molecules in the plastic regime we used cantilevers with a force constant about one order of magnitude higher (k≈ 0.76 N/m).

The images at different forces were generated by choosing different set-point values in the driving force, usually in the 650 to 720 mV range. The driving force could be changed by 10 mV steps. During the imaging process, the dissipated power is calculated by

$$P_{ts} = P_0\left[\frac{V_{exc}}{V_{exc,0}} - \frac{f}{f_0}\right] \quad \text{(S1)}$$

where P_0 is the power dissipated by the microcantilever in the absence of tip-protein forces (for example, with the tip 10-100 nm above the protein surface) . In the experiments the power dissipated in the sample was in the 0.0003 to 0.012 pW range. The experimental parameters deduced here are consistent with the AFM dynamics provided by computer simulations.

Force reconstruction

To transform an AFM image into a force map, we have recorded the frequency shift Δf, the driving voltage V_{exc} , and the mean deflection of the tip z_o as a function of the tip-surface separation (Fig. S5). The above data is recorded by keeping the oscillation amplitude constant. The above curves have been acquired at the end of the imaging process to minimize tip damage.

From the force curve data we can reconstruct the force versus tip-antibody distance by using the Sader-Jarvis force reconstruction algorithm.

$$F_{ts}(d) = 2k\int_{d}^{\infty} X_{SJ}dx + 2k\int_{d}^{\infty} \frac{\sqrt{A}}{8\sqrt{\pi(x-d)}}X_{SJ}dx - 2k\int_{d}^{\infty} \left(\frac{A^{3/2}}{\sqrt{2(x-d)}}\frac{\partial X_{SJ}}{\partial d}\right)dx \qquad \text{(S2)}$$

$$X_{SJ} = \frac{\Delta f(d)}{f_0} \qquad \text{(S3)}$$

where k is the spring constant of the cantilever, F_{ts} is the interaction force between tip and sample, f_0 is the unperturbed resonant frequency, Δf is the change in the resonant frequency, □ is the amplitude of oscillation and d is the distance of closest approach between tip and sample in an oscillation. By combining the panels of Figure S5 we can assign a force to a given ΔV_{exc}.

Error analysis

The force is deduced from an integral expression which makes cumbersome to apply the standard method to determine the error. Here, we have estimated ΔF from the reconstructed force curve (see Fig. S6) at a given distance d by taking the force values at $d+\Delta d$ and $d-\Delta d$, with Δd=0.05 nm.

$$\Delta F = |F(d + \Delta d) - F(d - \Delta d)| \qquad \text{(S4)}$$

The errors in the stress-strain curve (Figure 5) have been calculated by error propagation. The equations used are the following:

$$\Delta\sigma = \sigma\sqrt{\left(\frac{\Delta F}{F}\right)^2 + \left(\frac{\Delta R}{R}\right)^2 + \left(\frac{\Delta\delta}{\delta}\right)^2} \qquad \text{(S5)}$$

$$\Delta\varepsilon = \varepsilon\sqrt{\left[\left(\frac{\Delta\delta}{\delta}\right)^2 + \left(\frac{\Delta h}{h}\right)^2\right]} \qquad \text{(S6)}$$

The uncertainty in the force increases with the force. This is because the slope of the frequency shift curve increases by increasing the indentation (Fig. S5).

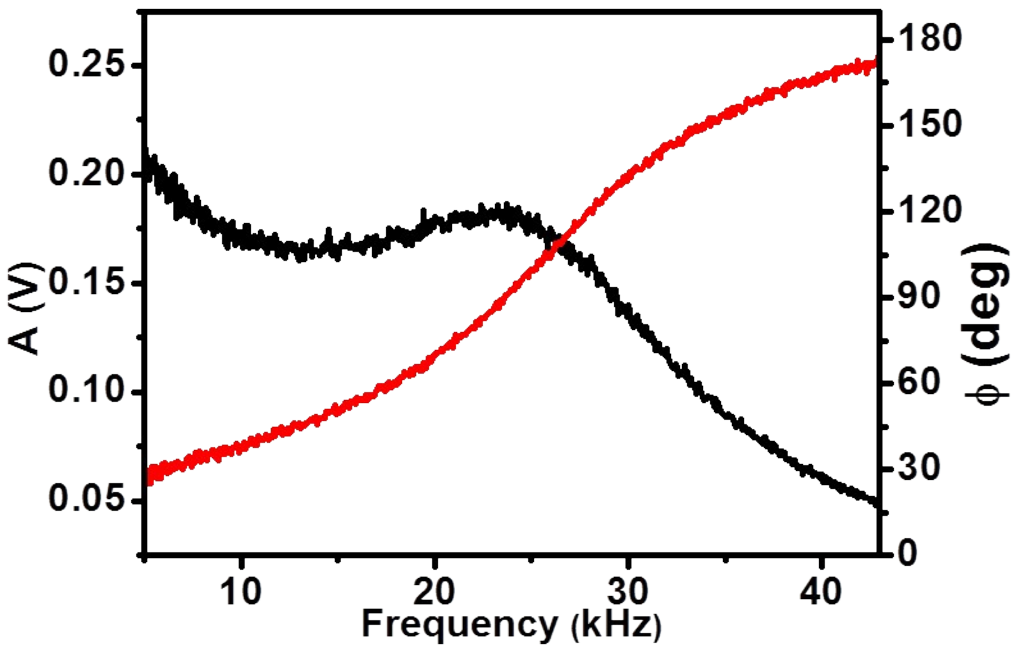

Figure S1. Resonance and phase shift (red) curves obtained in liquid with a photothermal excited AC40TS cantilever.

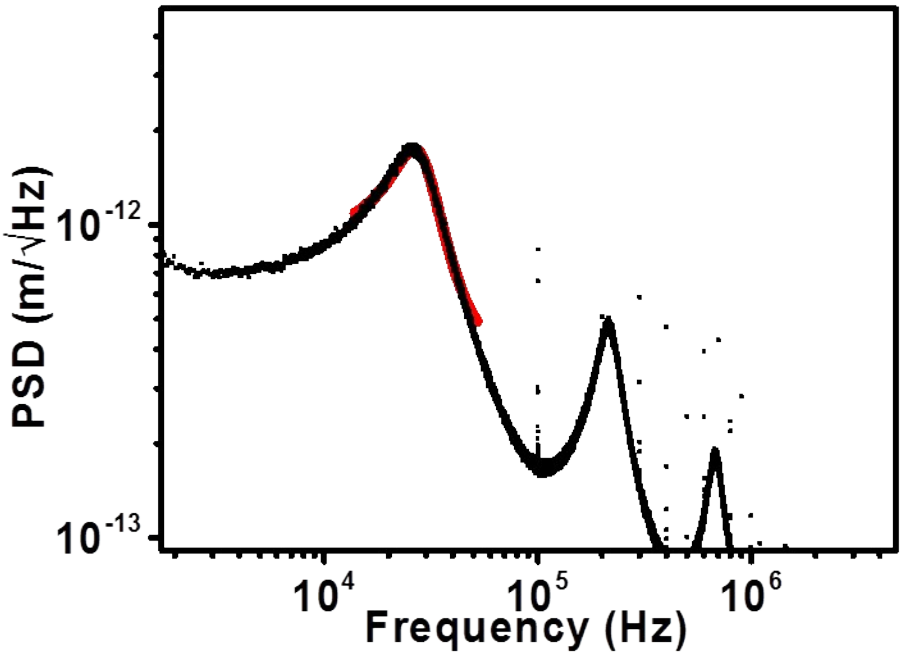

Figure S2. Power spectral density of the thermal motion of an AC-40TS cantilever (Olympus, Japan). In the red is the fitting applied to deduce the quality factor and the force constant of the cantilever (1st flexural mode), respectively, 1.9 and 0.077 N/m.

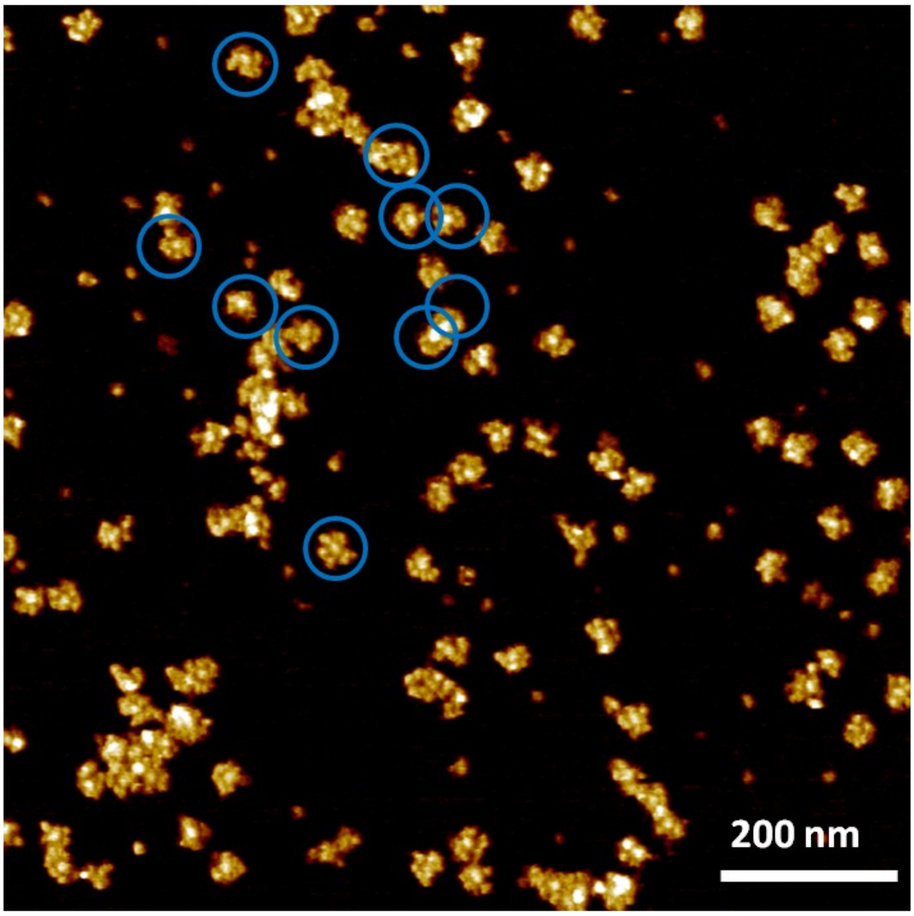

Figure S3. AFM image of a distribution of pentameric antibodies on mica. Enclosed by a circle are the IgM antibodies selected to gather the force versus deformation data.

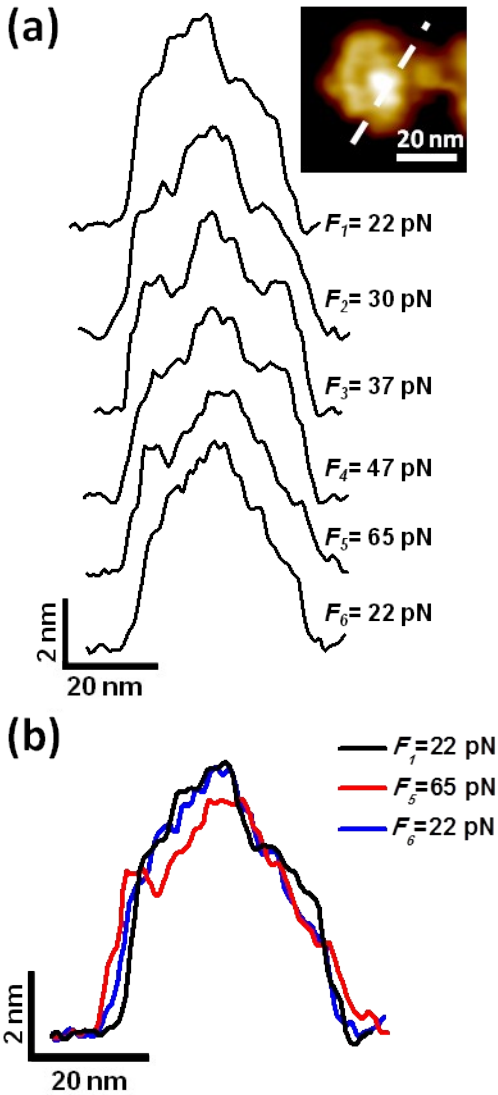

Figure S4. Forces and deformation on a single IgM. Additional example of the change of the height profile of an IgM as a function of the force exerted by the tip. (a). Height profiles as a function of the applied force. (b). Comparison of the height profiles before and after the application of a force of 65 pN. The height profiles obtained at F_1 and F_6 are very similar. This indicates that the application of a force of 65 pN generates elastic deformations.

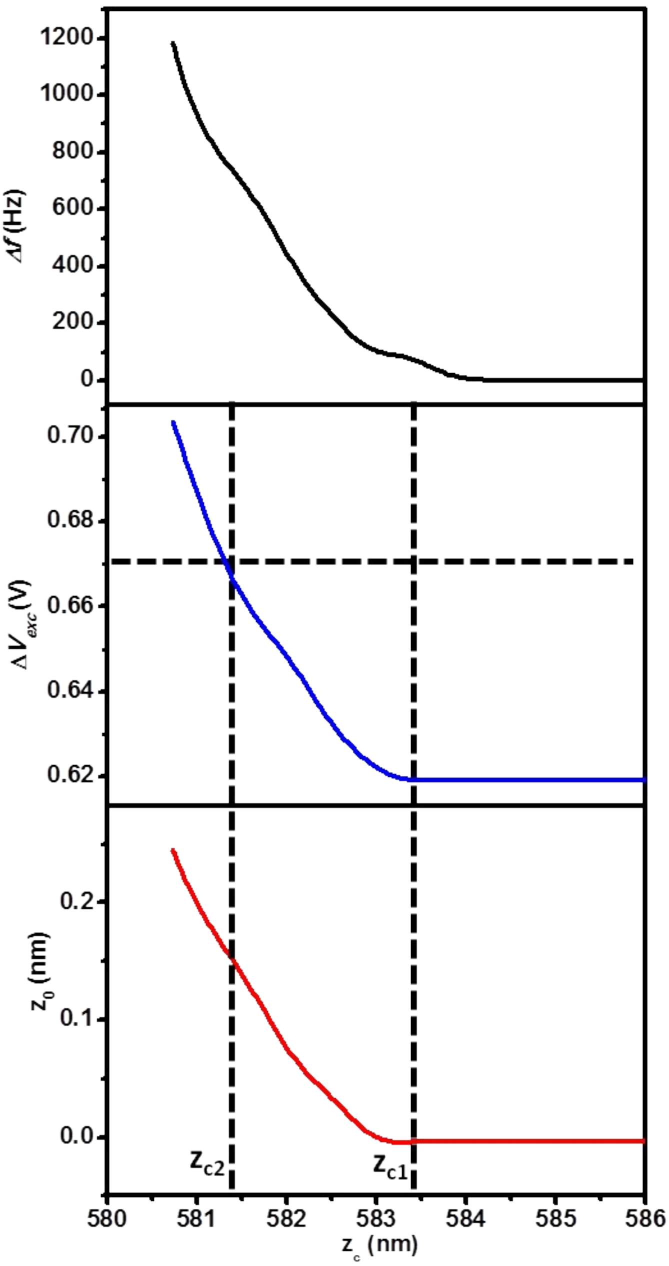

Figure S5. Hybrid dynamic AFM observables dependence on the tip-sample separation. Top to bottom, frequency shift, drive voltage and mean cantilever deflection curves taken on top of a single IgM antibody; z_{c1} represents the piezo position at the contact point and z_{c2} is the piezo displacement during imaging.

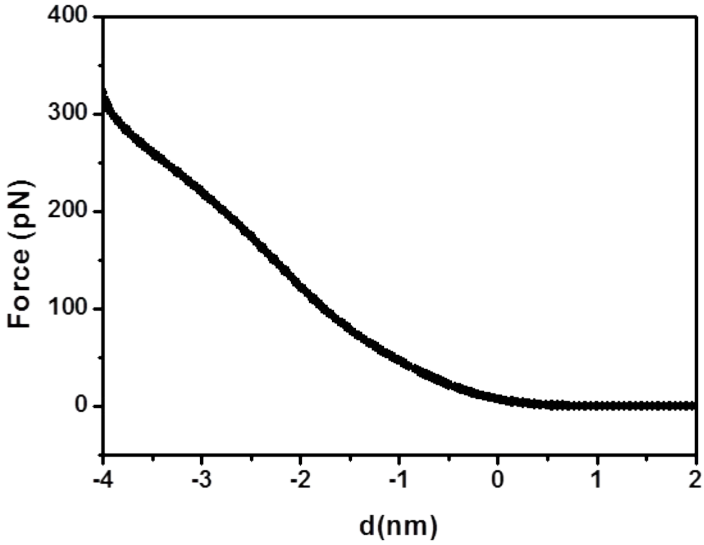

Figure S6. Force versus distance curve reconstructed from the data of Fig. S5.