Supplementary data

Deciphering the energy landscape of unbinding process for [uranyl-DCP]-antibody complex using dynamic force spectroscopy

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MATERIAL & METHODS

Experimental setups

Force-displacement curves were recorded with a Dimension 3100 AFM microscope, a Nanoscope IV controller (Digital Instrument Veeco, Santa Barbara, CA) equipped with the force curve mode and an open loop scanner. Spring constants of gold-coated tips (Olympus Biolever, Olympus and Veeco NPG) were determined as previously described (1). A wide range of loading rates was obtained by controlling either the retracting speed of the piezo scanner and using different spring constants of the cantilevers. Loading rate values prior to rupture were corrected as previously described (2). The contact time was controlled in the range of 50 to 500 ms using a deflection threshold around 15-25 nm for soft Olympus tips and about 2-10 nm for other tips. Four systems were constructed: system 1, DCP was attached on the cantilever and Mab PHE03S was deposited on the flat gold substrate; system 2, Ni-DCP was attached on the cantilever and the same for Mab PHE03S as in system 1; system 3, same as system 1 except that Mab PHE03S was replaced by the anti-GST monoclonal antibody (negative control); system 4, Cu-DCP was attached on the cantilever while Mab U08S was deposited on the substrate.

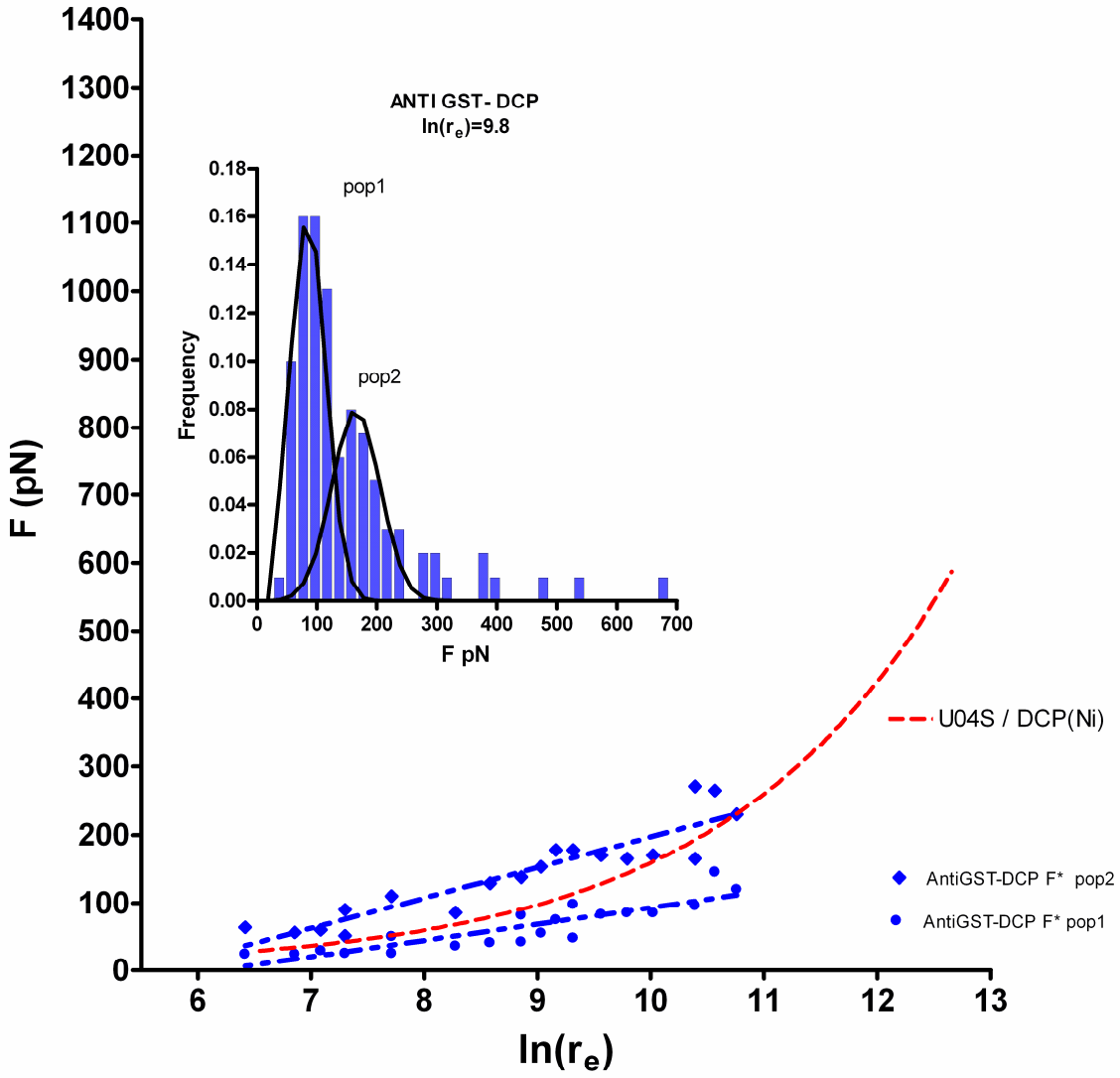
Functionalization of the tip coupled with DCP and [Metal-DCP]

The gold-coated tip was activated with 10 nM 5-thiouredoethanethiol-DCP or DCP-thiol (ERAS Labo, St. Nazaire les Eymes, France) in dimethyl formamide (DMF) at room temperature (RT) for 30 min, and then rinsed in 10 mM phosphate, 50 mM KCl buffer, pH 7.5. For DCP-chelated tips, the cantilever was incubated in 2 µM $NiCl_2$ or $CuCl_2$ with a phosphate buffer (pH 7) at RT for 30 min. Unbinding events were systematically carried out prior any physical measurement to ensure the correct functionalization of tips.

Functionalization of gold-coated glass slides

The gold-coated surface was pre-treated with 10 nM mercapto-undecanoic acid (Sigma Chemical, St Louis, MO, USA) in ethanol. After three rinses, the sample was incubated for 10 min in a v/v aqueous solution of 0.4 mg/mL ethyl-N-[3-diethylaminopropyl] carbodiimide and 0.6 mg/mL N-hydroxysuccinimide (Pierce Biotechnology, Rockford, IL, USA), followed by three rinses with acetate buffer and then submerged in 1 nM Protein A (protA, Pierce) in 10 mM sodium acetate buffer (pH 4.5) followed by three rinses. Monoclonal mouse antibodies (Mabs) were added as drops in 0.1 nM protein solutions and 10 mM phosphate buffer, pH 7.5 for 15 min. ProtA and Mab were cross-linked via a bifunctional cross-linker using 1 mM dimethyl adipimidate (DMA, Pierce) in 10 mM borate buffer for 15 min at pH 8, followed by three rinses in 10 mM phosphate, 50 mM KCl buffer at pH 7.5. Finally, the slide surface was saturated with bovine serum albumin (BSA, Sigma) at 1µg/ml for 30 min in 10 mM phosphate buffer (pH 7.5). Loosely bound proteins were removed by rinsing several times with this buffer.

Figure S1

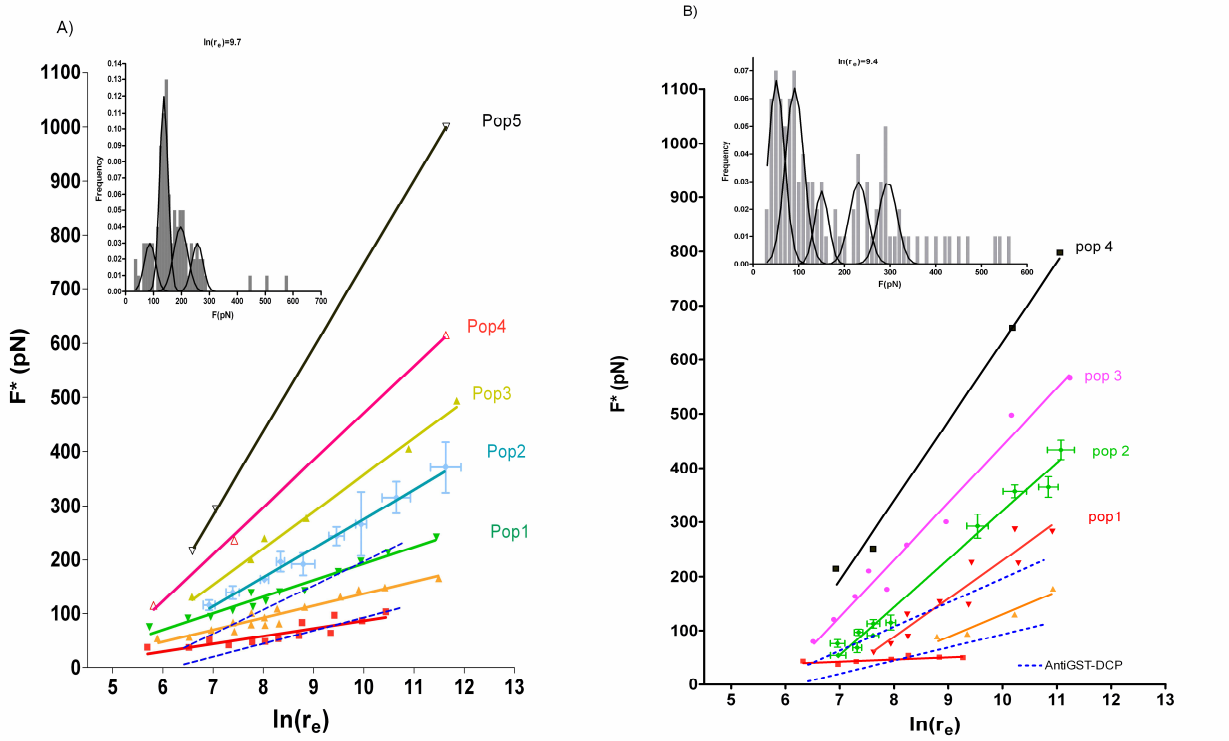


Plot of F^* vs. ln (r_e) for Mab antiGST-DCP (system 3) unbinding in comparison with that for Mab U04S-[Ni-DCP] plotted with the red dashed line. System 3 was used for observing typical non specific unbinding events. The plot exhibits clearly two distinct major populations (blue filled circles and diamonds) contributed from the interactions with chelator. We used the corresponding energy to distinguish specific and non specific events from experiments for all the study systems. There are a very few number of force-displacement curves (n=86) observed for Mab U04S-[Ni-DCP] interactions. The red dashed line corresponds to the best fit

of the experimental data. We found no specific rupture events occurred in the dissociation of Ni-DCP from Mab U04S.

Inset shows the histogram of unbinding events of system 3 at the loading rate r_e = 18,033 $pN.s^{-1}$. The bin size of force is 20 pN. The histogram was fitted with Gaussian distribution functions, as described previously (2) where the most probable unbinding forces F* was set to equal the maximum of the fitted curve.

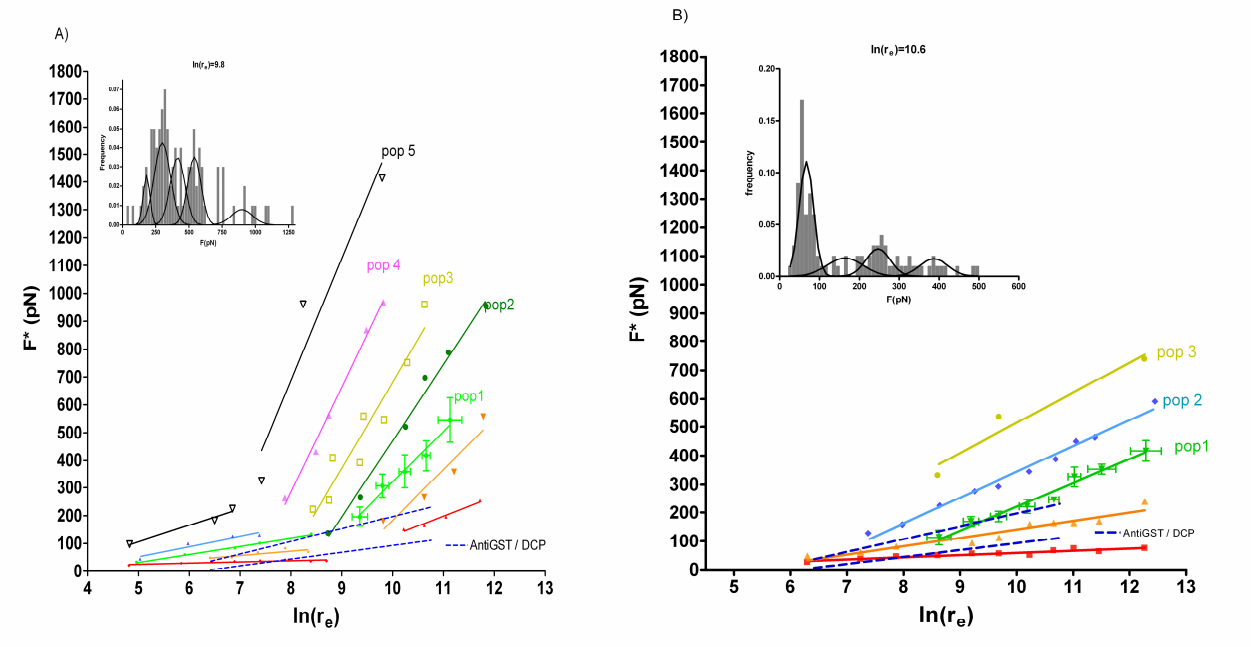
Figure S2



Plots of the most probable forces F^* vs. ln (r_e). (A) The results for system 1 are associated with seven fits, the first two falls in the region of non-specific interactions and thus discarded (in red and orange). Standard deviations are only indicated for fit 2 that pop2 was retained for the clarity in the main text. Inset shows the distribution of unbinding events of Mab PHE03S-DCP at the loading rate r_e = 17,230 pN.s^{-1}. The bin size of force is 10 pN.

(B) The results for system 2, Mab PHE03S-[Ni-DCP} complex. Standard deviations are only indicated for fit 2 corresponding to pop2 for the clarity. Inset shows the distribution of unbinding events of system 2 at the loading rate r_e = 11,944 pN.s^{-1}. The bin size of force is 10 pN.

Figure S3



A) Plot of F^* vs. ln (r_e) for the system of Mab U08S-[UO_2-DCP] as previously published (3). Five and seven fits were made in the regions of low and high loading rates, respectively. Based on the non-specific unbinding measurements in this work (blue dotted lines), populations were re-attributed as indicated so that red and orange curves are associated with non-specific events. For the sake of clarity in this work, only pop1 was retained in the main text. Inset displays the unbinding event distribution for UO_2-DCP dissociated from Mab U08S at the loading rate r_e = 18,291 pN.s^{-1}. The force bin size is 10 pN.

B) Plot of F^* vs. ln (r_e) for the system of Mab U08S-[Cu-DCP]. Among the five fits, the first two are in the region of non-specific interactions (blue dotted lined) and thus discarded. Standard deviations are only indicated for pop1. Inset shows the histogram of unbinding events between Cu-DCP and Mab U08S at the loading rate r_e = 42,256 pN.s^{-1}. The force bin size is 10 pN.

References

1. Odorico, M., J.-M. Teulon, O. Berthoumieu, S.-w. W. Chen, P. Parot, and J.-L. Pellequer. 2007. An integrated methodology for data processing in Dynamic Force Spectroscopy of ligand-receptor binding. *Ultramicroscopy.* 107:887-894.
2. Odorico, M., J.-M. Teulon, L. Bellanger, C. Vidaud, T. Bessou, S.-w. W. Chen, E. Quéméneur, P. Parot, and J.-L. Pellequer. 2007. Energy landscape of chelated uranyl – antibody interactions by Dynamic Force Spectroscopy. *Biophys. J.* 93:645-654.
3. Teulon, J. M., M. Odorico, S. W. Chen, P. Parot, and J. L. Pellequer. 2007. On molecular recognition of an uranyl chelate by monoclonal antibodies. *J. Mol. Recogn.* 20:508-515.