Supporting Information for: A Practical Guide to Single Protein AFM Nanomechanical Spectroscopy Mapping: Insights and Pitfalls as Unraveled by All-Atom MD Simulations on Immunoglobulin G.

J. G. Vilhena,[*,†,§] Maria Ortega,[‡] Manuel R. Uhlig,[¶] Ricardo Garcia,[¶] and Rubén Pérez[*,‡,‖]

†*Department of Physics, University of Basel, Klingelbergstrasse 82, 4056 Basel, Switzerland*

‡*Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, E-28049 Madrid, Spain*

¶*Materials Science Factory, Instituto de Ciencia de Materiales de Madrid (ICMM), CSIC, 28049 Madrid, Spain.*

§*Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, E-28049 Madrid, Spain*

‖*Condensed Matter Physics Center (IFIMAC), Universidad Autónoma de Madrid, E-28049 Madrid, Spain*

E-mail: guilherme.vilhena@unibas.ch; ruben.perez@uam.es

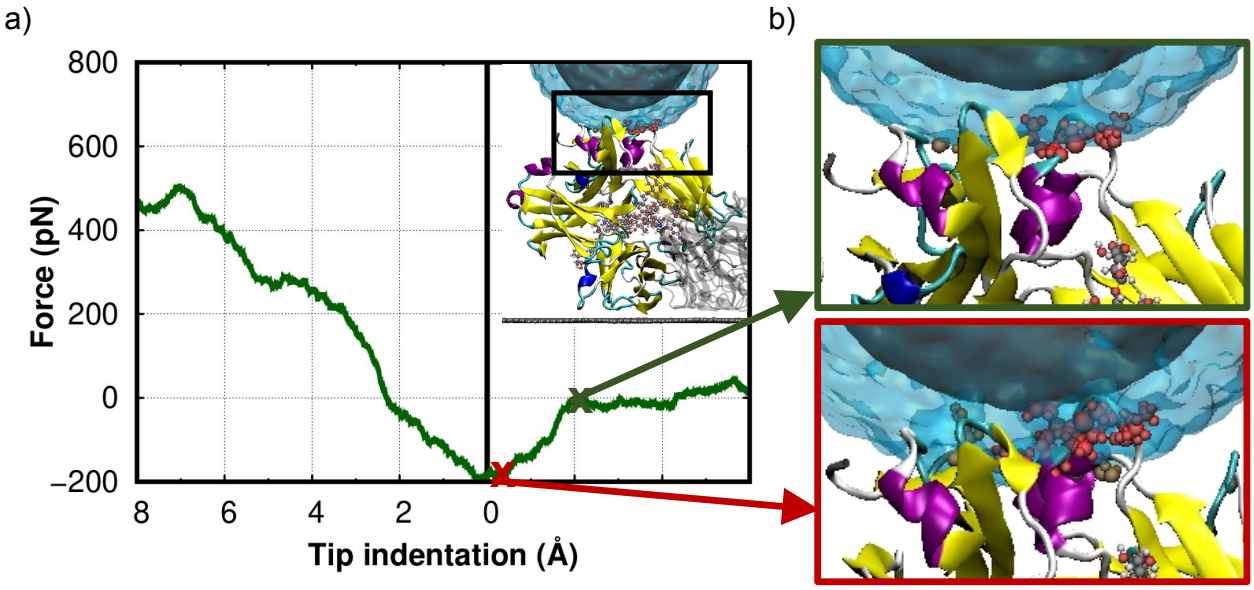

Figure S1: **Protein jumps to contact at F_c2 indentation site.** (**a**) F_c2 force distance curve. The inset shows the region of the protein underneath the tip. Protein, tip and surface representations are the same as in Fig. 1 of the main text. Additionally we represent the first layer of water molecules surrounding the tip. We represent them as a transparent Connolly surface colored in cyan. (**b**) Inside green box: Side view of the protein contact area just before it jumps to contact. Note the hydrophobic aminoacids represented with red ball-stick model. Inside red box: Side view of the protein contact area just after it jumps to contact. The aminoacids highlighted in red spontaneously break the tip hydration layer so to establish contact. This snapshot depicts the amino-acids reorganization occurring in this process.

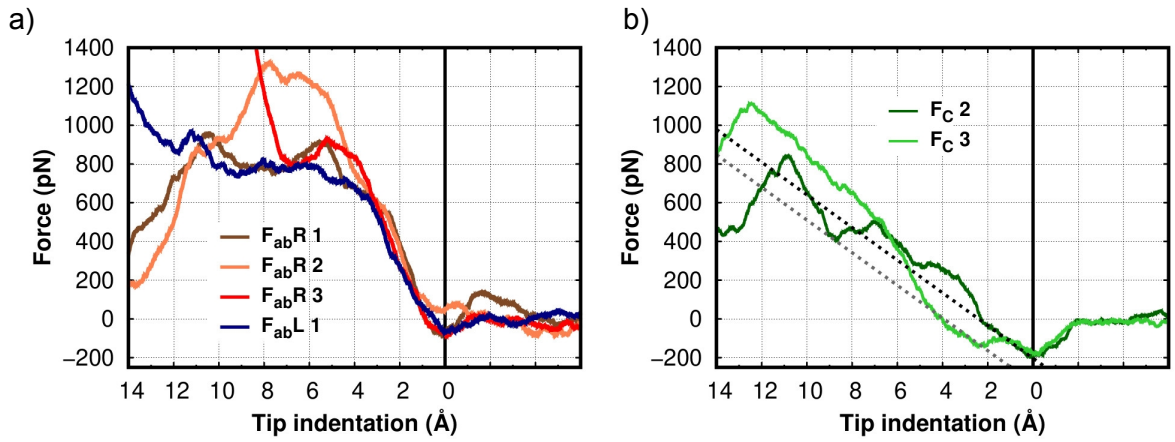

Figure S2: **Stability of the protein stiffness at different indentation sites.** (**a**) Force curves obtained at all different $F_{ab}R$ sites and $F_{ab}L1$ site. This plot shows that regardless of the indentation site, we always obtain similar stiffness on the $F_{ab}R$ – evidenced by similar slope between 0-4 Å. Interestingly such stiffness is also similar to the one obtained on the other identical domain, i.e. $F_{ab}L$. All in all, this curve corroborates that stiffness if a domain property, independent of the indentation site. (**b**) Similarly, for the F_c domain, at the two stable indentation sites we also obtain similar stiffness values. At the F_c1 site, owing to the lack of aminoacids in direct contact with the surface, the protein always initiates a on-surface sliding thus impeding to directly probe the stiffness at this site.

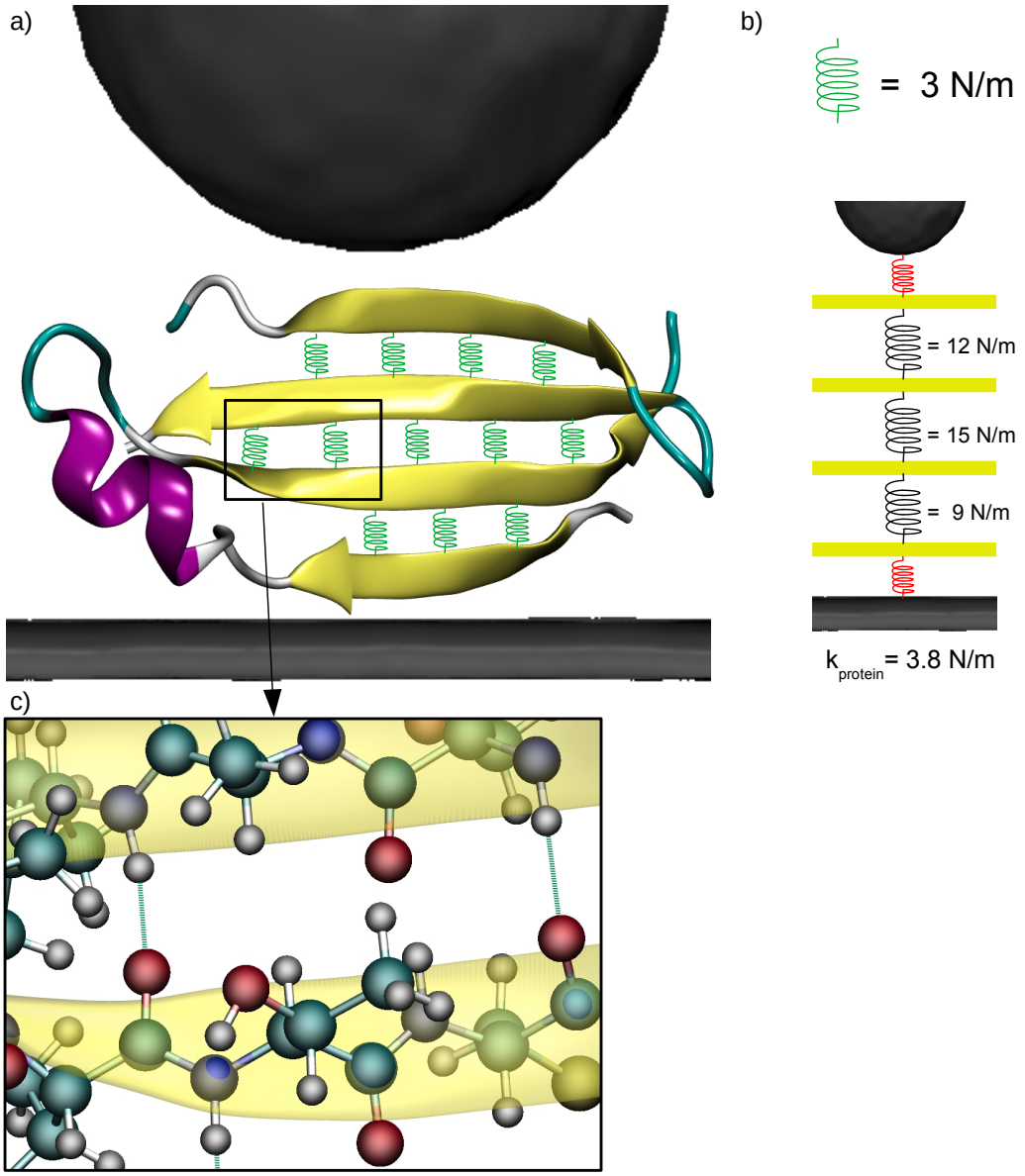

Figure S3: **Schematic representation of the link between protein stiffness and its hydrogen-bond network.** (**a**) Schematic representation of a β-sheet being indented by an AFM tip. This β-sheet corresponds to a fragment of the F_c domain. The protein, tip and surface are represented as in Fig. 1 of the main text. Additionally, hydrogen bonds are represented as green springs between the different strands. The number of springs/h-bonds is approximately the same than the actual number of h-bonds existing between the strands. (**b**) Mechanical equivalent of the protein. Considering each hydrogen bond with a stiffness of 3 N/m (see main text), then by summing all spring within the same strand (in parallel) we can estimate the compliance of each strand. Then by summing these springs in series we obtain a total stiffness of 3.8 N/m for the protein. Note that here we neglect the stiffness associated with the non-specific interactions between the tip/surface and the amino-acids in direct contact with them – both interactions represented as red springs. (**c**) Atomic view of a selected region of the β-sheet so to visualize the h-bonds between the strands. The secondary structure is shown in transparent, and all atoms (except the water molecules) are explicitly represented with ball-stick model.