Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Force spectroscopy predicts thermal stability of immobilized proteins by measuring microbead mechanics

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Supporting Methods

Plasmids, bacterial strains, and supports: *Plasmids:* the gene of N-terminal His-tagged formate dehydrogenase from *Candida boidinii* (FDH), was synthesized and cloned into pET28b(+) (pET28-*fdh_cb*) by Genscript Inc. (Hong Kong). The other plasmid encoding, His-tagged superfolded green fluorescent protein (sGFP),[1] His-tagged glycerol dehydrogenase from *Bacillus stearothermophilus*[2] (GlyDH) and NADH oxidase from *Thermus thermophilus*[3] were previously described elsewhere. *Bacterial strains*: All recombinant proteins were produced in *E. coli* BL21 (DE3) cells. sGFP, FDH and GlyDH were purified by metal affinity chromatography with agarose activated with cobalt-chelates and eluted with 300 mM of imidazol in 25 mM sodium phosphate at pH 7. NOX was purified by thermal shock at 70ºC for 1 hour and further centrifuged to eliminate the precipitated meshophile proteins. *Supports*: agarose 10BCL® was activated with aldehyde groups (Ag-G) according to the protocol described by Guisan,[4] and activated with cobalt chelates (Ag-Co^{2+}) according to the protocol described by Armisen et al.[5]

Colorimetric assay of different enzymes. *Alcohol dehydrogenate enzymatic activity assays*: FDH and GlyDH activities were measured by the oxidation of formic acid and glycerol, respectively. Reaction mixture was composed of 100 mM of formic acid or glycerol and 1 mM NAD+ dissolved in 25 mM sodium phosphate at pH 7. The enzymatic reaction was triggered by adding 5 µL of properly diluted soluble or

immobilized enzyme to a 200 µL reaction mixture placed in one well of a 96-well microplate and maintained under agitation at 25ºC in a Varioskan™ Flash Multimode Reader (Thermo Scientific). Activity was calculated by the reduction of NAD+ into NADH by monitoring the increment in absorbance at 340 nm. One unit of either FDH or GlyDH activity was defined as the amount of enzyme required for release one µmol of NADH per minute. *NADH oxidase (NOX) activity assay*: NOX activity was measured by monitoring the oxidation of NADH. Reaction mixture was composed of 0.5 mM of NADH and 0.15 mM FAD+ dissolved in 25 mM sodium phosphate at pH 7. The enzymatic reaction was triggered by adding 5 µL of properly diluted soluble or immobilized enzyme to a 200 µL reaction mixture placed in one well of a 96-well microplate and maintained under agitation at 25ºC in a Varioskan™ Flash Multimode Reader (Thermo Scientific). Activity was calculated by the oxidation of NADH to NAD+ by monitoring the increment in absorbance at 340 nm. One unit of NOX activity was defined as the amount of enzyme required for release one µmol of NADH per minute.

Atomic force microscopy (AFM). The surface topography of the Ag-G-sGFP stabilized on the PEM coated glass is acquired on a Nanowizard II AFM (JPK, Berlin, Germany) microscope. Images are captured in the dynamic mode under buffer conditions with a silicon sharpened DNP-S10A cantilever (Bruker, Berlin, Germany) having a nominal spring constant of 0.350 N/m, a nominal radius of 20 nm, and resonant frequency at ~13 kHz.

Supporting Results

Table S1. Immobilization conditions yield, and absolute Young´s modulus of different protein immobilized on different agarose microbeads.

Protein/ Molecule	Agarose beads	Immobilization conditions	Immobilization time (hours)	Load (mg/g)	Immobilization Yield (%)	Absolute measured E (kPa)	Standard deviation +/- E	Standard error of mean +/- E
none	Ag-CB	-	**-**	-	-	329.48	112.73	11.11
none	Ag-Co^{2+}	-	**-**	-	-	198.36	77.80	4.24
none	Ag-G	-	**-**	-	-	216.20	77.33	12.54
Ethanolamine	Ag-CB	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1**	-	-	436.53	126.77	11.66
Imidazol	Ag-Co^{2+}	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1**	-	-	362.20	116.1368	14.75
Ethanolamine	Ag-G	100 mM $NaHCO_3$ pH 10, 25 ºC.	**3**	-	-	401.97	139.09	14.27
sGFP	Ag-CB	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1**	6.6	66	438.75	149.81	13.08
	Ag-Co^{2+}	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1**	10	100	359.64	119.34	6.68
	Ag-G	100 mM $NaHCO_3$ pH 10, 25 ºC.	**3**	10	100	1007.33	772.37	70.51
FDH	Ag-CB	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1.5**	3.6	36	385.18	122.30	5.98
	Ag-G	100 mM $NaHCO_3$ pH 10, 25 ºC.	**3**	9.7	97	946.99	398.38	22.00
GlyDH	Ag-CB	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1.5**	9.8	98	205.01	85.82	4.18
	Ag-G	100 mM $NaHCO_3$ pH 10, 25 ºC.	**3**	9.8	98	1044.37	589.33	39.73
NOX	Ag-CB	25 mM NaH_2PO_4 pH 7, 25 ºC.	**1.5**	9.5	95	374.30	185.54	9.95
	Ag-G	100 mM $NaHCO_3$ pH 10, 25 ºC.	**3**	9.5	5	711.68	398.15	32.51

For each sample, 5 different beads were measured, collecting 200 f-d curves per bead. We need a large number of f-d curves since we find some curves where the indentation occurs too far from apex of bead due to slight rolling, where the bead is wrongly contacted, too noisy curves etc. All these curves must be discarded for analysis since they don't represent the mechanical properties of the material but are inevitable in this type of measurements. For these reason we need a large number of curves in order to have reliable data. The standard error expresses how accurate the mean E value is regarding the experimental data; this error varies with number of f-d curves considered for analysis while standard deviation is not affected by the number of analyzed curves.

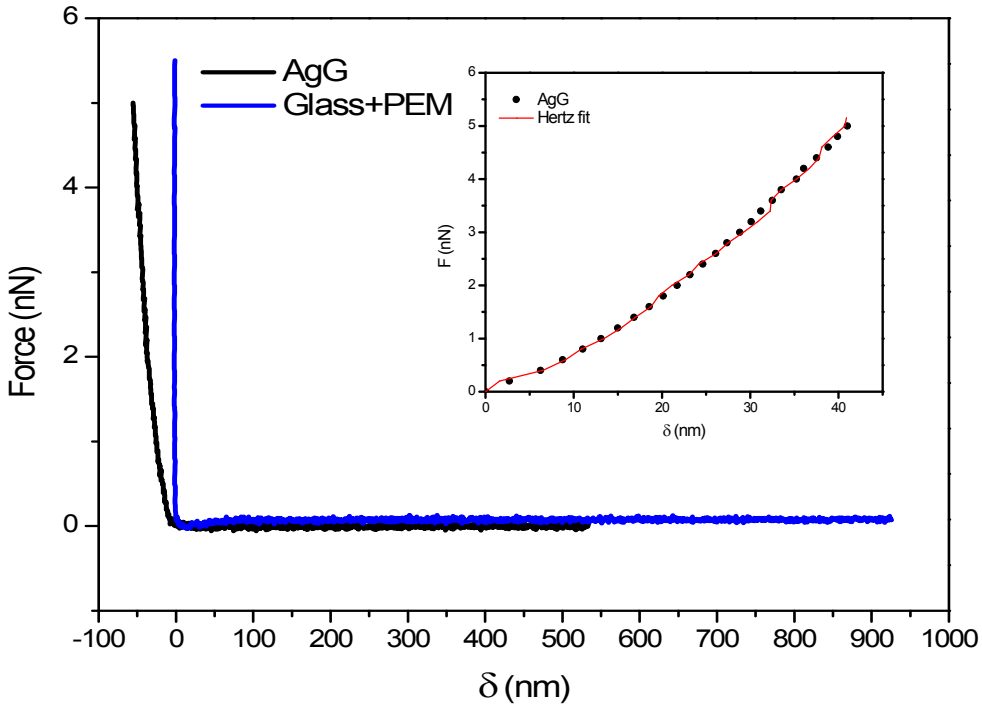

Figure S1. **Force-displacement curves in approach half-live of the colloidal probe on the reference "hard" surface (blue) and the Ag-G bead loading sGFP (black).** δ is the indentation depth observed for the Ag-G microbeads immobilizing sGFP. The higher force is applied the higher δ value. The inset shows the quality of the fitting of the indentation versus force data with the Hertz model.

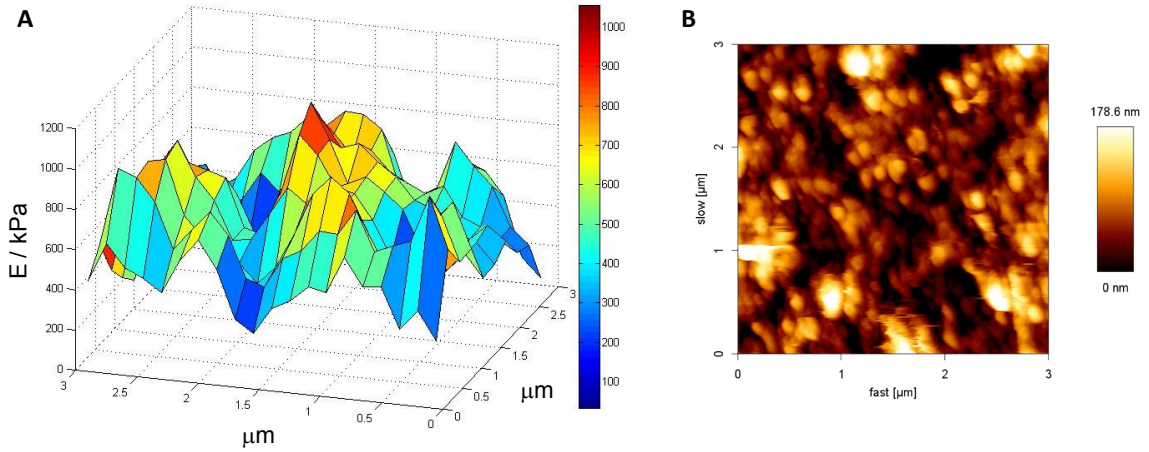

Figure S2. Force (A) and topographical (B) maps of agarose beads immobilizing sGFP taken under buffer conditions. The topographical and force maps were obtained: in the dynamic mode with pyramidal tips (B) and in the force volume mode with colloidal tips (A), respectively. In both cases, sGFP was immobilized on Ag-G at pH 10 and 25ºC during 24 hours. Immobilized proteins decorate the fibers increasing the roughness of the surface. Topographical maps of plain agarose beads without the proteins were not shown since the pyramidal standard tip exclusively used for the topographical studies dived into the very soft material making impossible to acquire reliable data to construct the corresponding topographical map. Only when proteins were attached to the beads, the latter were stiff enough to avoid the tip diving resulting in reliable topographical maps.

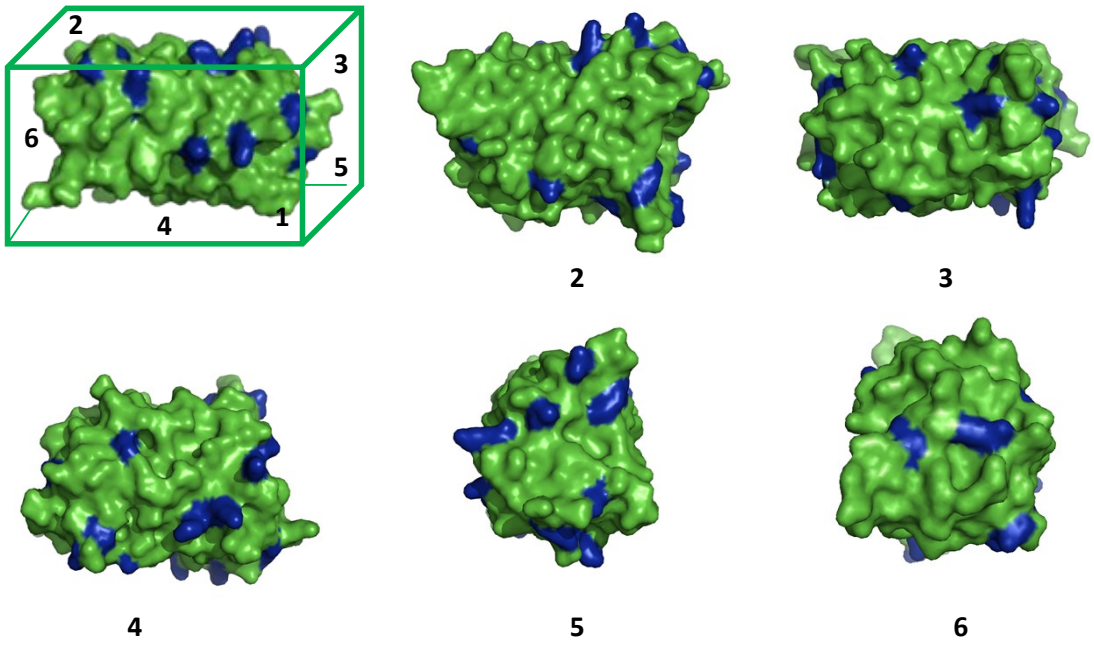

Figure S3. Different faces of the sGFP. Surface representation of the X-ray structure of sGFP. The 20 Lysines residues found in the surface are highlighted in blue. The figure was created with pymol 0.99 (DeLano, USA) using the PDB ID: 2B3P

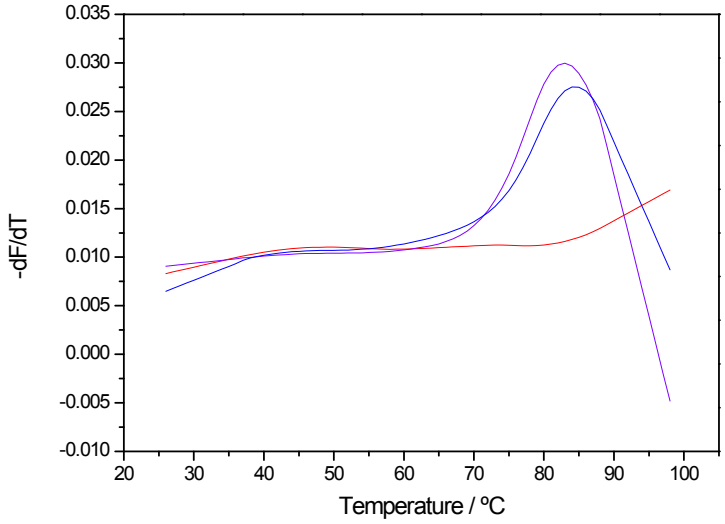

Figure S4. Derivative of the sGFP fluorescence at different temperatures. sGFP was immobilized on Ag-G (red line), on Ag-CB (blue) and Ag-Co^{2+} (purple line)

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