Supplementary Information for
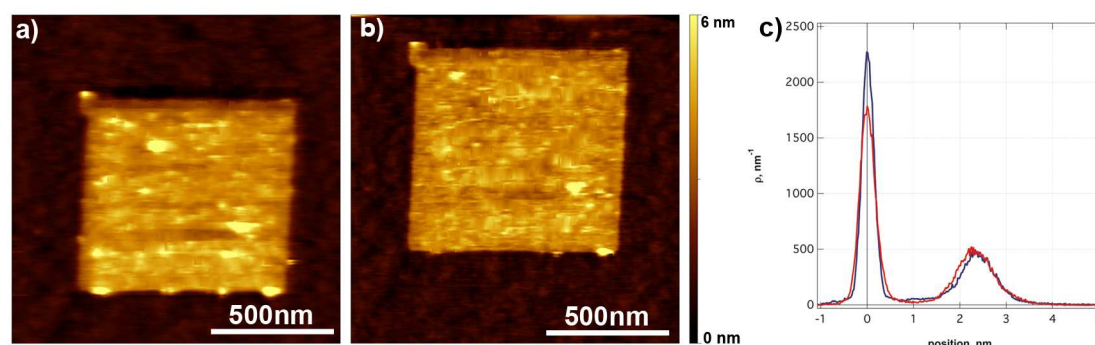
Oriented Immobilization of Prion Protein Demonstrated *via* Precise Interfacial Nanostructure Measurements

Barbara Sanavio, [†,‡,||] *Denis Scaini,* [†,||] *Christian Grunwald,* [§] *Giuseppe Legname,* [‡,||,#,¶]

Giacinto Scoles, [†,‡,#,·] *Loredana Casalis,* [†,||,#,·]

Supplementary Figure S1.

Supplementary Figure S1 shows how the NTA patches do not react with the protein to be immobilized without the presence of Nickel, and how the surrounding surface remains clean when challenged with protein solution. a) Nanografted NTA patch imaged in TBS (20mM Tris buffer, 150 mM NaCl, pH 7.4) after nanofabrication. b) The same patch after incubation with 300nM CloneP but without previous exposure to Ni (II). The patch remains clean, no protein deposition is observed, suggesting that proteins stick neither on the patch nor on the surrounding surface without a specific interaction. Panel c) shows the histogram distribution of heights before (blue) and after (red) challenge with protein solution (note the superimposition of the two curves). The first Gaussian peak centered on 0 refers to the surrounding carpet, the right one refers to the nanografted patch.

Supplementary Figure S2.
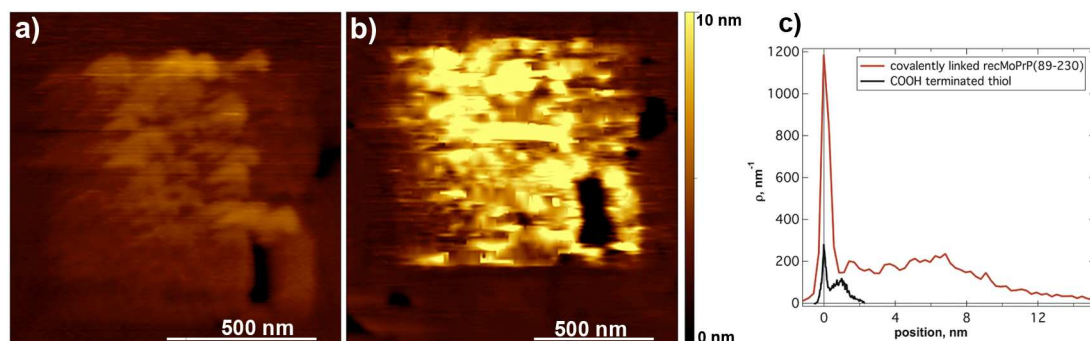
Supplementary Figure S2 shows a nanostructured COOH terminated thiols on panel a), and the same patch after activation with DCC/NHS to react with recMoPrP (89-230) 216 nM in panel b). Panel c) reports the histogram height distribution for the two cases. Patches were very non-uniform compared to NTA-Ni-His mediated immobilization, and varying reaction condition like protein concentration, incubation time, didn't improve substantially the outcome. Since this immobilization strategy didn't provide homogeneous orientation, it was not pursued for binding studies.

Supplementary table S1

Supplementary Table S1. Fit results for Figure 4.		
	Δh recMoPrP (89-230) over CloneP nanopattern	Rq recMoPrP (89-230) over CloneP nanopattern
Kd (app)	2.9 ± 1.1 nM	1.5 ± 1.3 nM
R^2	0.9242	0.8924
χ square	0.9301	0.1045

Supplementary table S2

Supplementary Table S2. Fit results for Figure 5, panel a).		
	Δh recMoPrP (89-230) over CloneP nanopattern 450 μM NTA	Δh recMoPrP (89-230) over CloneP nanopattern 143 μM NTA
Kd (app)	2.0 ± 0.8 nM	1.6 ± 0.9 nM
R^2	0.9346	0.8888
χ square	0.2685	0.2844

Supplementary table S3

Supplementary Table S3. Fit results for Figure 5, panel a).		
	Δh recMoPrP (89-230) over D18 nanopattern 450 μM NTA	Δh recMoPrP (89-230) over D18 nanopattern 90 μM NTA
Kd (app)	2.3 ± 1.4 nM	1.9 ± 1.3 nM
R^2	0.9186	0.8761
χ square	0.2867	0.3611