Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

Force spectroscopic detection of peptide cleavage by thrombin exploiting biotin-streptavidin interactions in a bio-sensing context

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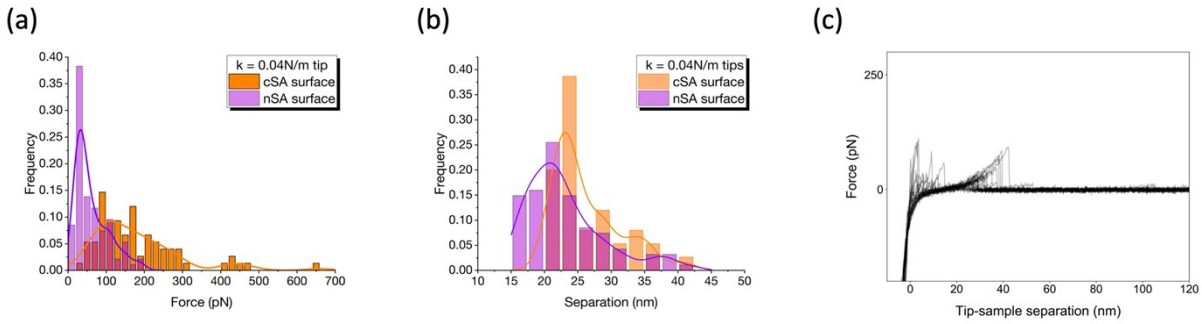

Fig. S1 Force (a) and separation (b) histograms obtained in Fig3b are re-plotted in comparison with the distributions obtained on the cSA surface (reported in Fig2b) without removing cluster 2. (c) Superposition of force distance curves forming cluster 2.

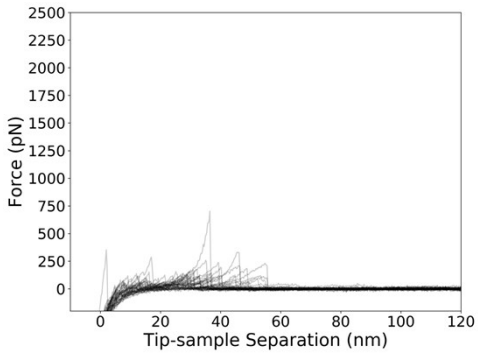

Fig. S2 Overlapped single unbinding force curves collected in 0.57μM thrombin solution with a tip without TEES spacer molecules against the cSA surface.

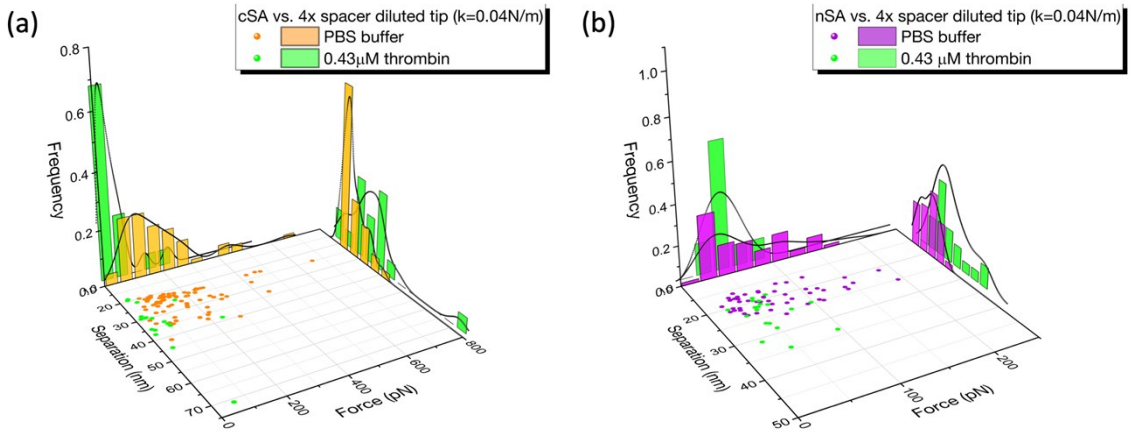

Fig. S3 comparison of the unbinding force and separation distribution of filtered single unbinding events between PBS and thrombin. The results are obtained with the softer tip and with TEES spacer, against either the cSA surface (a) or the nSA surface (b).

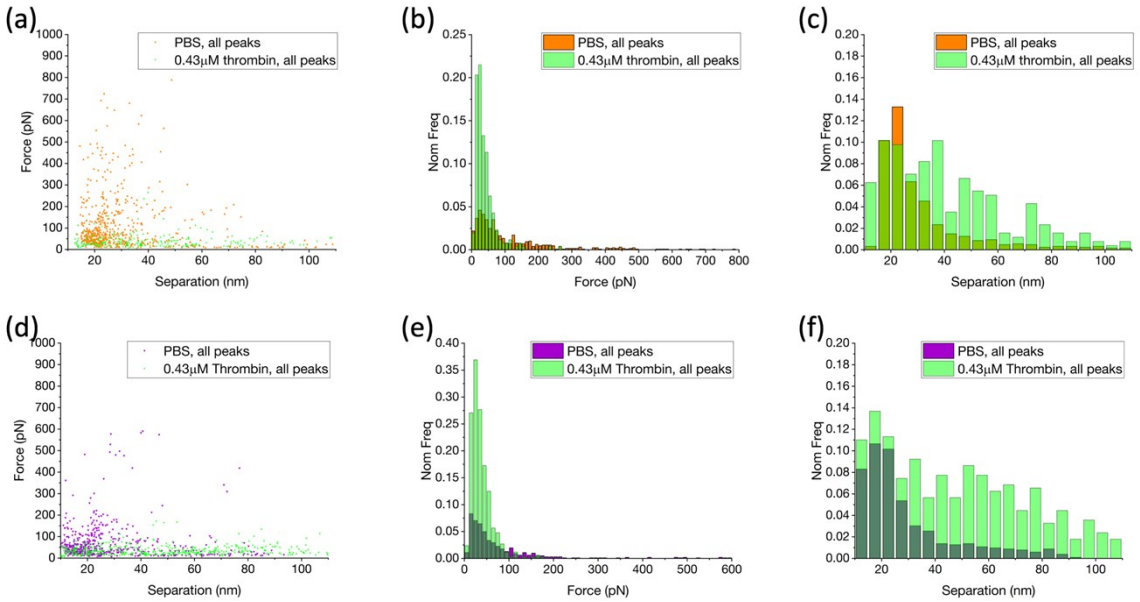

Fig. S4 Maps of the forces and separation distances relative to all the unbinding peaks obtained without and with 0.43μM thrombin, using a soft tip with TEES spacer against the cSA (a) and nSA (d) surfaces, up to a separation of 110 nm. Force (b, e) and tip-sample separation histograms (c, f) of all the peaks detected in (a) and (d).

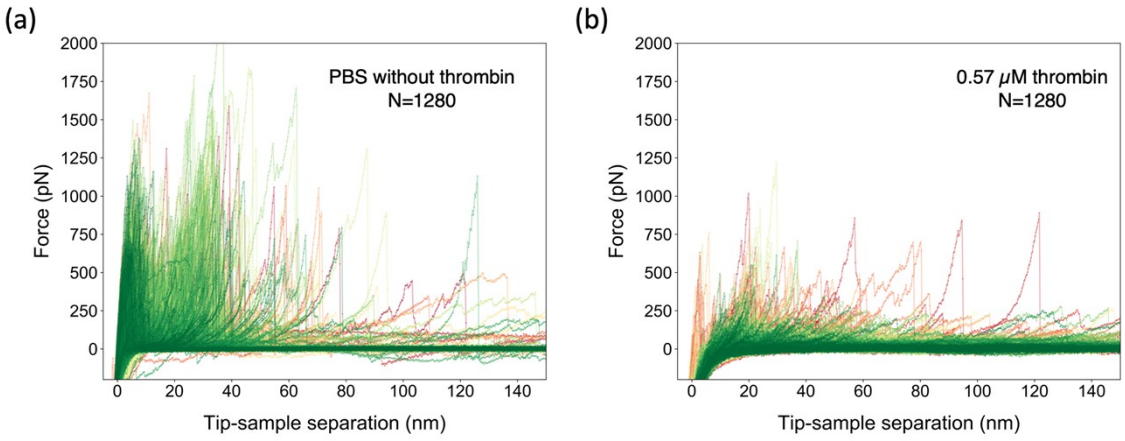

Fig. S5 A replot of Fig. 6a, b with a gradually changing red-yellow-green color code representing the force-curve acquisition time.

Table S1 Comparison of the unbinding frequencies obtained in pure PBS and in the presence of thrombin using different surfaces and tips systems, in their corresponding specific confining windows (last two columns).

Systems	$c_{thrombin}$	Frequency of peaks in the confining window		Separation window	Force window
	(μM)	PBS	Thrombin	(nm)	(pN)
cSA, stiff tip	0.04	24.0%	19.7%	20～40	400～1500
cSA, stiff tip	0.21	18.2%	5.5%	20～40	400～1500
cSA, soft tip	0.43	12.0%	2.0%	20～40	> 90*
nSA, soft tip	0.43	8.2%	0.9%	15～35	> 90*

**** The lower force limit of our thrombin detection window is selected around the end point of the first peak region from the unbinding force distribution obtained in PBS. (Fig. S4)***