

Supplementary Information for

Serine protease dynamics revealed by NMR analysis of the thrombin-thrombomodulin complex

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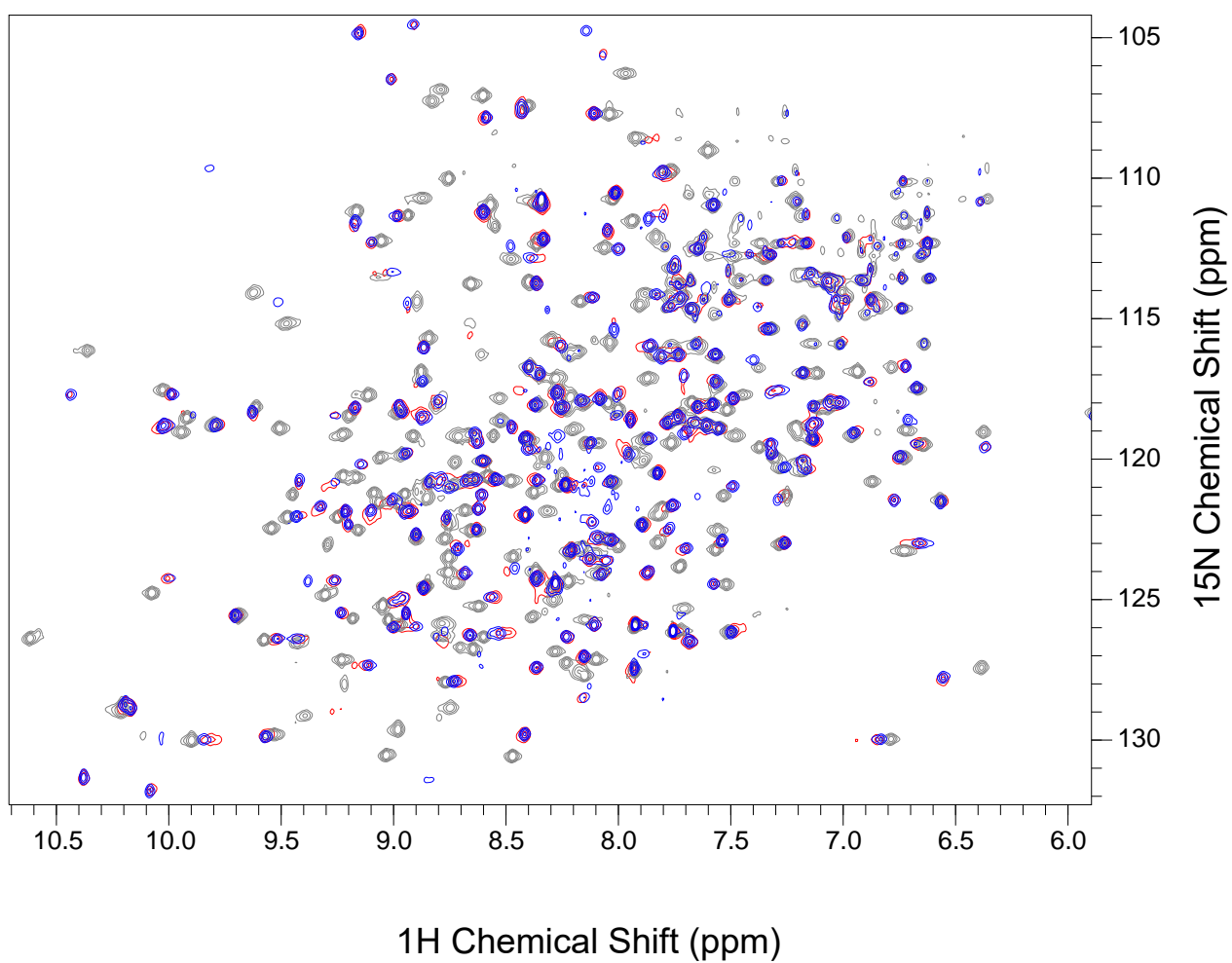


Fig. S1. TROSY HSQC Spectra of apo thrombin (blue), thrombin inhibited with D-Phe-Pro-Arg chloromethylketone (PPACK) (grey) and the thrombin-TM456 complex (red). All spectra were acquired at 800 MHz, room temperature, and a concentration of 0.12 mM.

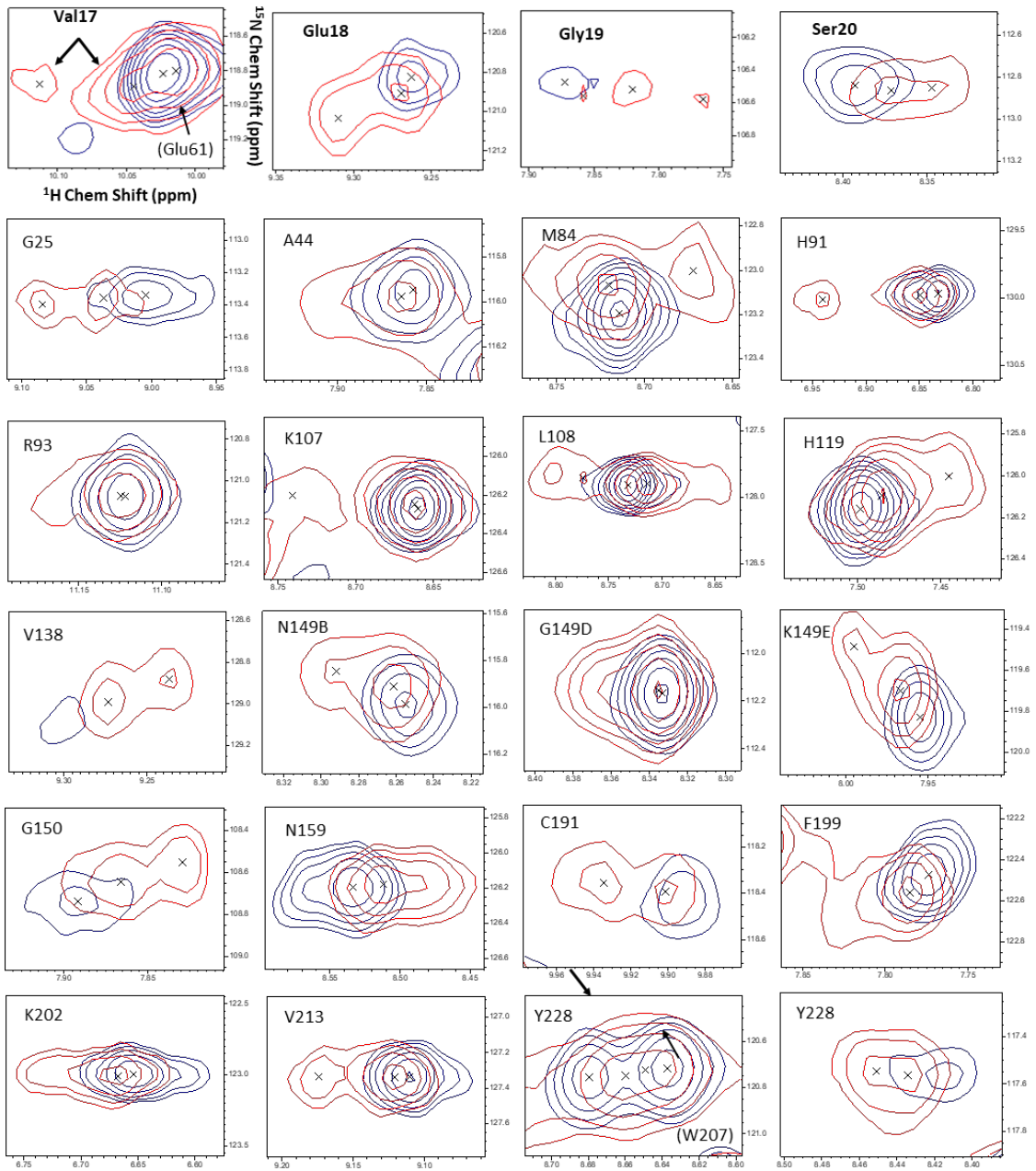


Fig. S2. All thrombin resonances that appeared as doublets or multiplets in the thrombin-TM456 HSQC spectrum. Residues are numbered according to the CT numbering scheme. The red traces correspond to the thrombin-TM456 HSQC spectrum taken at 800 MHz, while the blue traces correspond to the apo-thrombin spectrum taken at 800 MHz.

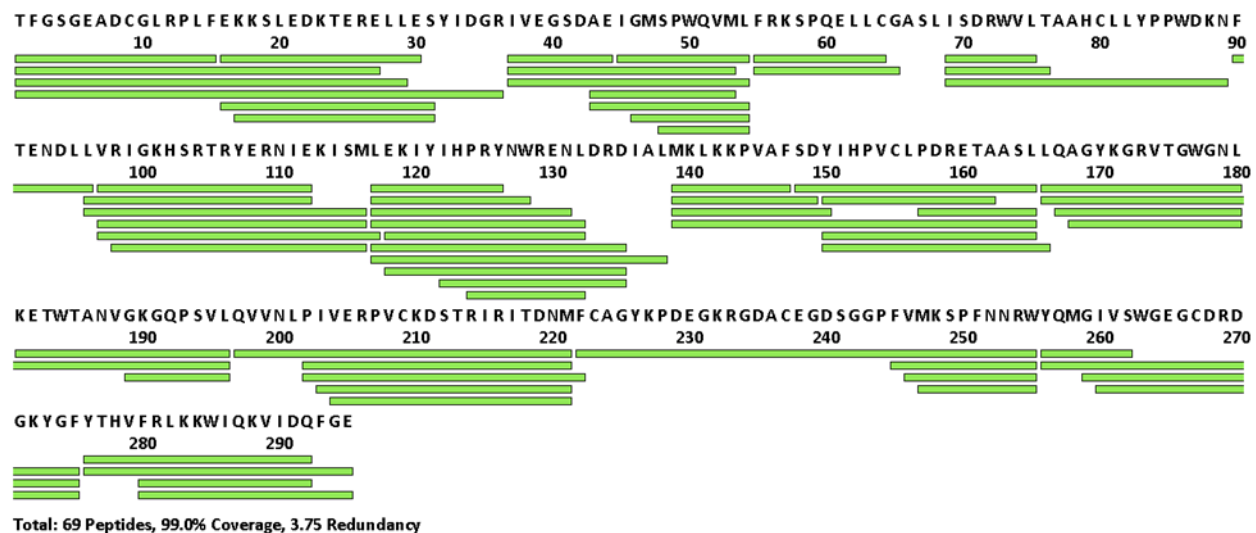


Fig. S3. Coverage map showing the peptides identified and evaluated during HDX-MS experiments on WT thrombin and thrombin-TM456.

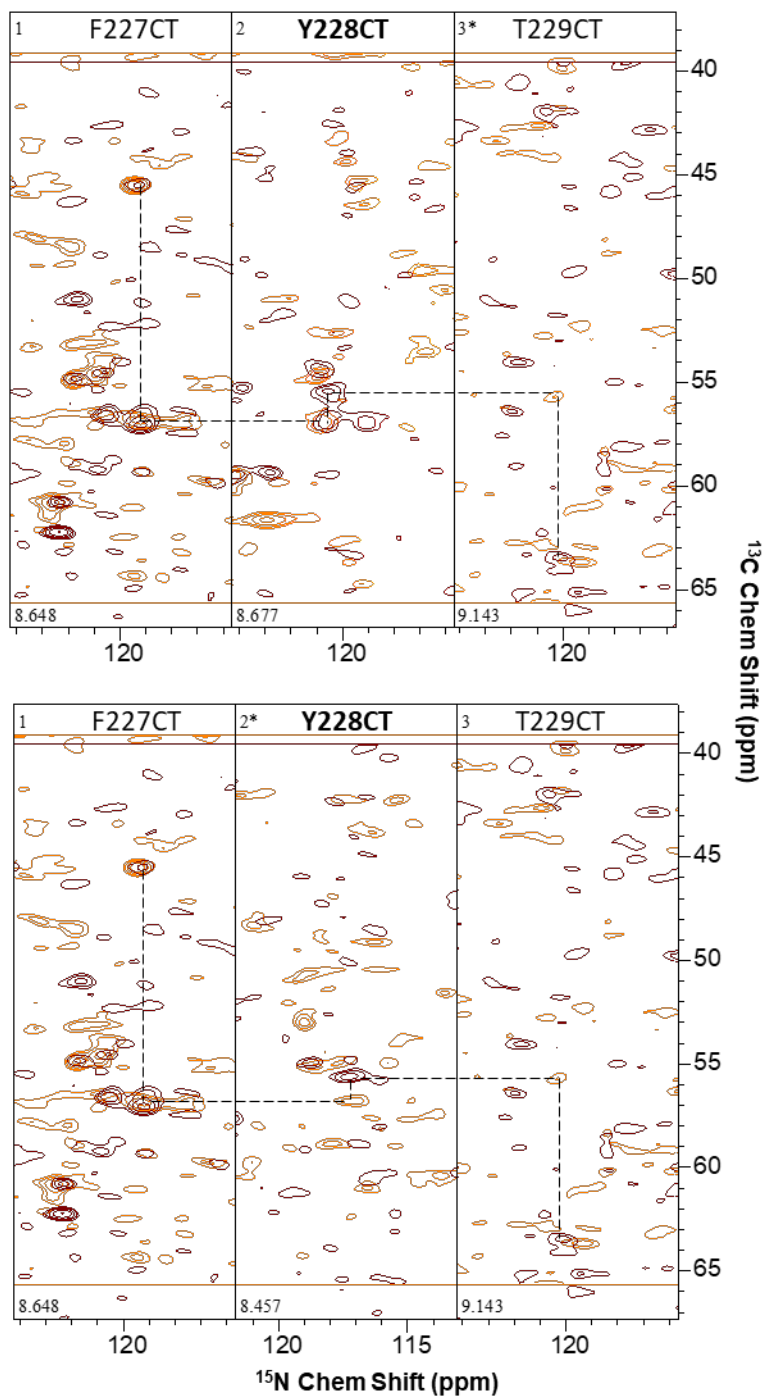


Fig. S4. Backbone walking from Ca_i to Ca_i HNCA peaks for both residue 228 assignments. The brown and orange spectra correspond to the HNCA and HNcoCA spectra taken at 800 MHz for thrombin-TM456.

Table S1. Experimental details for 3D spectra collected for backbone assignments and 2D relaxation dispersion spectra.

Experiment	# Scans	Spectral Window (ppm) $^1\text{H}\times(^{13}\text{C}\times)^{15}\text{N}$	Complex Points $^1\text{H}\times(^{13}\text{C}\times)^{15}\text{N}$	Offset (ppm) $^1\text{H}\times(^{13}\text{C}\times)^{15}\text{N}$	NUS sampling rate (%)
3D HNCA-TROSY*	64	17.3×31.6×36.6	1024×58×35	4.77, 54.7, 118.1	38
3D HN(CO)CA-TROSY	80	20×26.5×36.2	1024×56×48	4.77, 52.4, 118.6	36
3D HNCO-TROSY	64	20×14.6×36.2	1024×44×44	4.77, 175.5, 118.6	38
2D ^{15}N -HSQC-TROSY*	16	17.3×36.6	1024×128	4.77, 118.1	N/A
2D ^{15}N -CPMG-TROSY	96	16.7×34.6	1024×128	4.77, 118.7	N/A
2D ^{15}N -CPMG-TROSY [‡]	80	16.3×34.6	1024×98	4.77, 118.1	N/A

^aAll spectra were collected with a cryogenic probe at 600, 800[‡] and 900* MHz (^1H) at a temperature of 298 K.