

Supplementary Tables

Table S1: Deuterium Incorporation for FXIII at One Minute

Residues	Theo. D _{max} ^a	Zymogen	FXIIIa ^{Ca}	K9 DON FXIIIa ^{Ca}	IAA FXIIIa ^{Ca}	FXIIIa ^{Ila}	K9 DON FXIIIa ^{Ila}	IAA FXIIIa ^{Ila}
98-104	6.5	4.4 ± 0.0	2.9 ± 0.1	3.4 ± 0.1	2.4 ± 0.1	n/a	n/a	n/a
220-230	10.8	2.4 ± 0.1	2.9 ± 0.1	3.1 ± 0.1	2.5 ± 0.1	2.8 ± 0.1	3.0 ± 0.0	3.1 ± 0.1
240-247	7.5	1.2 ± 0.0	1.7 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.6 ± 0.0	1.7 ± 0.0	1.7 ± 0.1
248-264	15.9	2.9 ± 0.0	n/a	n/a	n/a	3.8 ± 0.0	3.8 ± 0.0	3.9 ± 0.1
513-522	9.7	3.8 ± 0.0	2.7 ± 0.0	2.9 ± 0.0	2.3 ± 0.1	3.3 ± 0.1	3.1 ± 0.0	3.1 ± 0.2
526-546	21.2	12.8 ± 0.1	7.7 ± 0.2	n/a	n/a	12.3 ± 0.1	10.7 ± 0.3	12.0 ± 0.5
535-541	6.5	0.6 ± 0.0	0.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1

FXIII, Factor XIII and IAA, Iodoacetamide. ^aThe maximum number of exchangeable protons within the indicated peptide, assuming 100% deuteration. This value accounts for all exchangeable backbone amide protons and a slight fraction of N-terminal, C-terminal, and side chain exchangeable protons which are dependent on the final percentage of D₂O in solution under quench conditions (approximately 4.5%). A fully deuterated peptide would theoretically have acquired this amount of deuterons.

Table S2: Deuterium Incorporation for FXIII at Ten Minutes

Residues	Theo. D _{max} ^a	Zymogen	FXIIIa ^{Ca}	K9 DON FXIIIa ^{Ca}	IAA FXIIIa ^{Ca}	FXIIIa ^{Ila}	K9 DON FXIIIa ^{Ila}	IAA FXIIIa ^{Ila}
98-104	6.5	4.8 ± 0.1	3.8 ± 0.1	2.8 ± 0.1	2.5 ± 0.0	n/a	n/a	n/a
220-230	10.8	2.9 ± 0.1	3.9 ± 0.3	3.7 ± 0.1	3.6 ± 0.2	3.7 ± 0.0	3.6 ± 0.1	3.7 ± 0.1
240-247	7.5	1.4 ± 0.0	1.9 ± 0.1	1.8 ± 0.0	2.0 ± 0.1	1.9 ± 0.0	1.9 ± 0.1	2.1 ± 0.2
248-264	15.9	3.5 ± 0.0	n/a	n/a	n/a	4.0 ± 0.1	3.9 ± 0.0	n/a
513-522	9.7	3.9 ± 0.0	2.8 ± 0.1	2.7 ± 0.0	2.3 ± 0.2	3.3 ± 0.0	2.9 ± 0.3	2.9 ± 0.0
526-546	21.2	13.0 ± 0.1	7.5 ± 0.2	6.1 ± 0.3	n/a	12.5 ± 0.2	10.5 ± 0.8	11.0 ± 0.2
535-541	6.5	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.1

FXIII, Factor XIII and IAA, Iodoacetamide. ^aThe maximum number of exchangeable protons within the indicated peptide, assuming 100% deuteration. This value accounts for all exchangeable backbone amide protons and a slight fraction of N-terminal, C-terminal, and side chain exchangeable protons which are dependent on the final percentage of D₂O in solution under quench conditions (approximately 4.5%). A fully deuterated peptide would theoretically have acquired this amount of deuterons.

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1 SETSRTAFGG RRAVPPNNSN AAEDDLPTVE LQGVVPRGVN LQEFNLVTSV HLFKERWDTN 60
61 KVDHHTDKYE NNKLIVRRGQ SFYVQIDFSR PYDPRRDLEF VEYVIGRYPQ ENKGTYPVP 120
121 IVSELQSGKW GAKIVMREDR SVRLSIQSSP KCIYVKFRMY VAVWTPYQVL RTSRNPETDT 180
181 VILFNPWCED DAVYLDNEKE REEYVLNDIG VIFYGEVNDI KTRSWSYQGF EDGILDTCLY 240
241 VMDRAQMDLS GRGNPIKVSF VGSAMVNAKD DEGLVVGSWD NIYAYGVPPS AWTGSVDILL 300
301 EYRSSENPVR YGQCWVFAGV ENTFLRCLGI PARIVTNYFS AHDNDANLQM DIFLEEDGNV 360
361 NSKLTKDSVW NYHCWNEAWM TRPDLPVGEF GWQAVDSTPQ ENSDGMVRCG PASVQAIRHG 420
421 HVCFQFDAPF VFAEVNSDLI YITAKKDGTG VVENVDATHI GKLIVTKQIG GDGMMDITDT 480
481 VKFQEGQEEE RLALLETALMY GAKKPLNTEG VMKSRSNVDM DFEVENAVLG KDFKLSITFR 540
541 NNSHNRVLT AYL SANITFY TGVPKAEFKK ETFDVTLEPL SFKKEAVLIQ AGEYMGQLE 600
601 QASLHFFVTA RINETRDVLA KQKSTVLTIPI EIIKVRGTQ VVGSDMTVTV QFTNPLKETL 660
661 RNVVWHLGGP GVTRPMKKMF REIRPNSTVQ WEEVCRPWVS GHRKLIASMS SDSLRHVYGE 720
721 LDVQIQRRPS M
— Pepsin Trypsin Chymotrypsin Both

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Figure S1: Sequence Coverage Map of FXIII. Pepsin fragments monitored during HDX experiments are underlined (45% sequence coverage) (22). Residues highlighted in red represent trypsin coverage (68%), in blue indicates chymotrypsin coverage (48%), and in green displays regions where the proteases overlap (81%).