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Supporting Information

Structural Dynamics and Catalytic Properties of a Multi-Modular Xanthanase

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Author Contributions

OVM, PFJ & SPM contributed equally. SPM performed enzymology, MS, and NMR experiments, analyzed the resulting data, and co-wrote the article. OVM and EB carried out the crystal screening and structure analysis. NM assisted with MS data acquisition and analysis. HB supervised research at UBC, analyzed data, and co-wrote the article. DRS, LA, SMV, VPR, THS and LG performed the experiments (cloning, expression, variant generation, purification, detergent stability and CBM binding); RNM, AS and JEN planned the experiments and co-wrote the article. PFJ planned and performed HDX-MS experiments, analyzed data and co-wrote the article. GK analyzed HDX-MS data and co-wrote the article. KDR planned HDX-MS experiments, analyzed data and supervised research at UCPH. KSW and GJD supervised the structural biology and co-wrote the article.

FIGURES

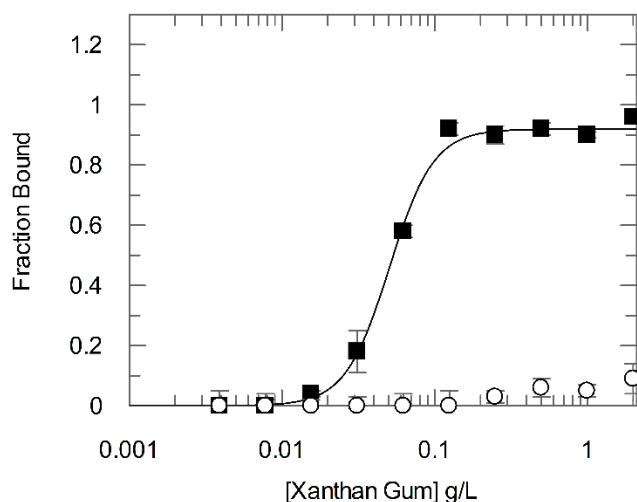


Figure S1 – The C terminal domain of PspXan9 defines a new family of xanthan-binding modules, termed CBM84. Fraction of Xanthan-bound protein (Protein-Xanthan) as function of xanthan gum concentration for the CBM84 domain (filled squares) with the purified GH12 endoglucanase EG3 from *Hypocrea jecorina* shown as a control (unfilled circles). Protein at 0.2 mg/ml was incubated with 0.004 – 2 mg/ml Xanthan gum (50 mM NaAcetate pH 5) and the fraction of bound *Hypocrea jecorina* EG3 (GH12) and CBM84 to Xanthan gum were estimated by measuring protein intrinsic fluorescence at $\lambda_{EX} \sim 280$ nm $\lambda_{EM} \sim 303$ nm (CBM84) and $\lambda_{EX} \sim 280$ nm $\lambda_{EM} \sim 345$ nm (*Hypocrea jecorina* EG3). Data for CBM84 were fit using a Hill equation to give $K=0.05$ g/L and a Hill coefficient of 2.9.

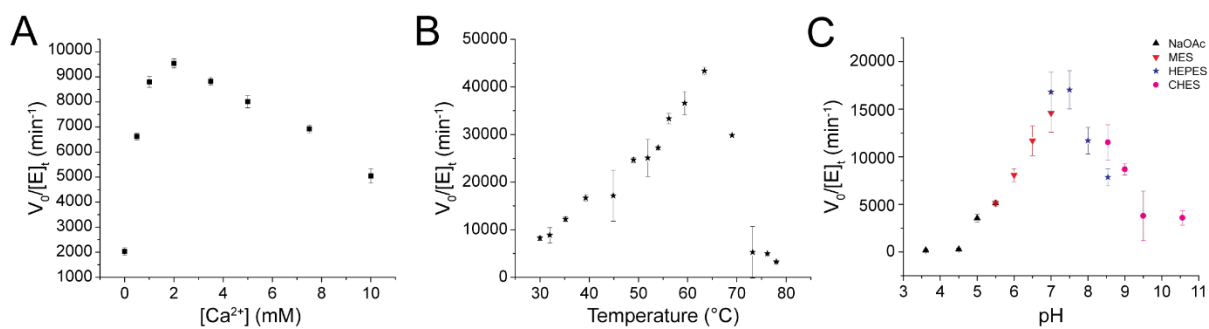


Figure S2 – Optimization of *PspXan9* activity. *A.* The effect of calcium concentration on the hydrolytic activity of *PspXan9* in 50 mM HEPES-NaOH buffer, pH 7.0. *B.* Temperature-rate profile for 10 min assays in the presence of 2 mM CaCl_2 . Error bars represent the standard deviation over three replicates. *C.* pH-rate profile. For each, the rate of reducing-end formation with $0.5 \text{ mg} \cdot \text{mL}^{-1}$ of lyase-treated xanthan as the substrate was determined after a 10 min incubation.

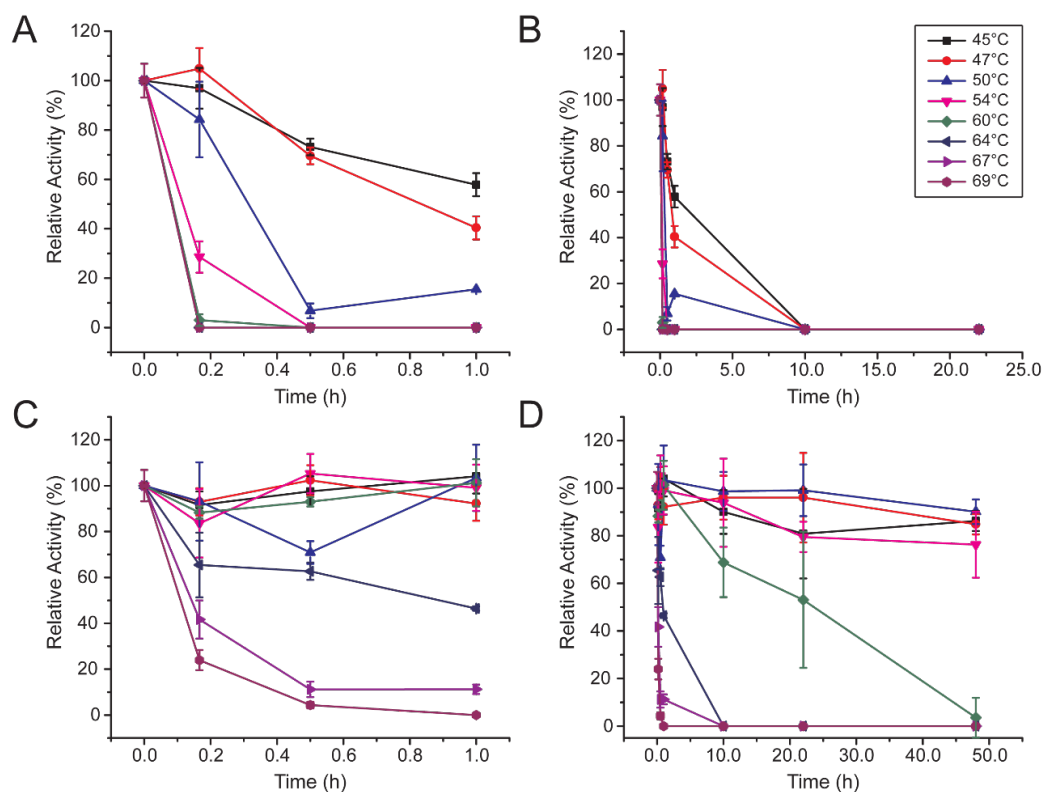


Figure S3 – The effect of Ca^{2+} ions on thermostability of *PspXan9*. *A.* Remaining activity of 16 nM *PspXan9* following incubation at the indicated temperatures; *B.* shows an expansion of the data in panel A to cover a longer time period. *C.* remaining activity of 16 nM *PspXan9* in the presence of 20 mM CaCl_2 following incubation at the indicated temperatures; *D.* shows an expansion of the data in panel C to cover a longer time period. After dilution, enzyme activity assays contained a final concentration of 2 mM CaCl_2 (*cf.* Fig. S003B). Error bars represent the standard deviation over three replicates.

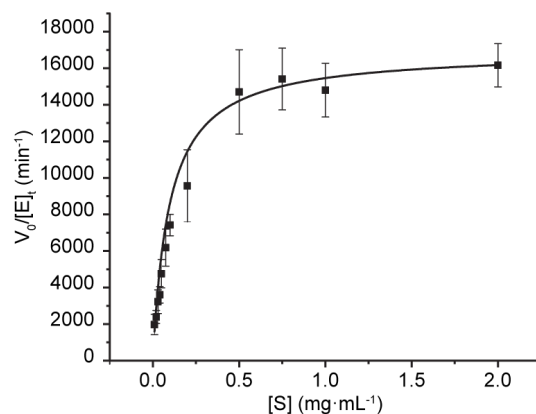


Figure S4 – Michaelis-Menten kinetics of *PspXan9* activity on lyase-treated xanthan. *PspXan9* kinetics were determined by quantifying activity after a 10 min incubation with lyase-treated xanthan concentrations between 0.01-2 $\text{mg} \cdot \text{mL}^{-1}$. Error bars represent the standard deviation over three replicates.

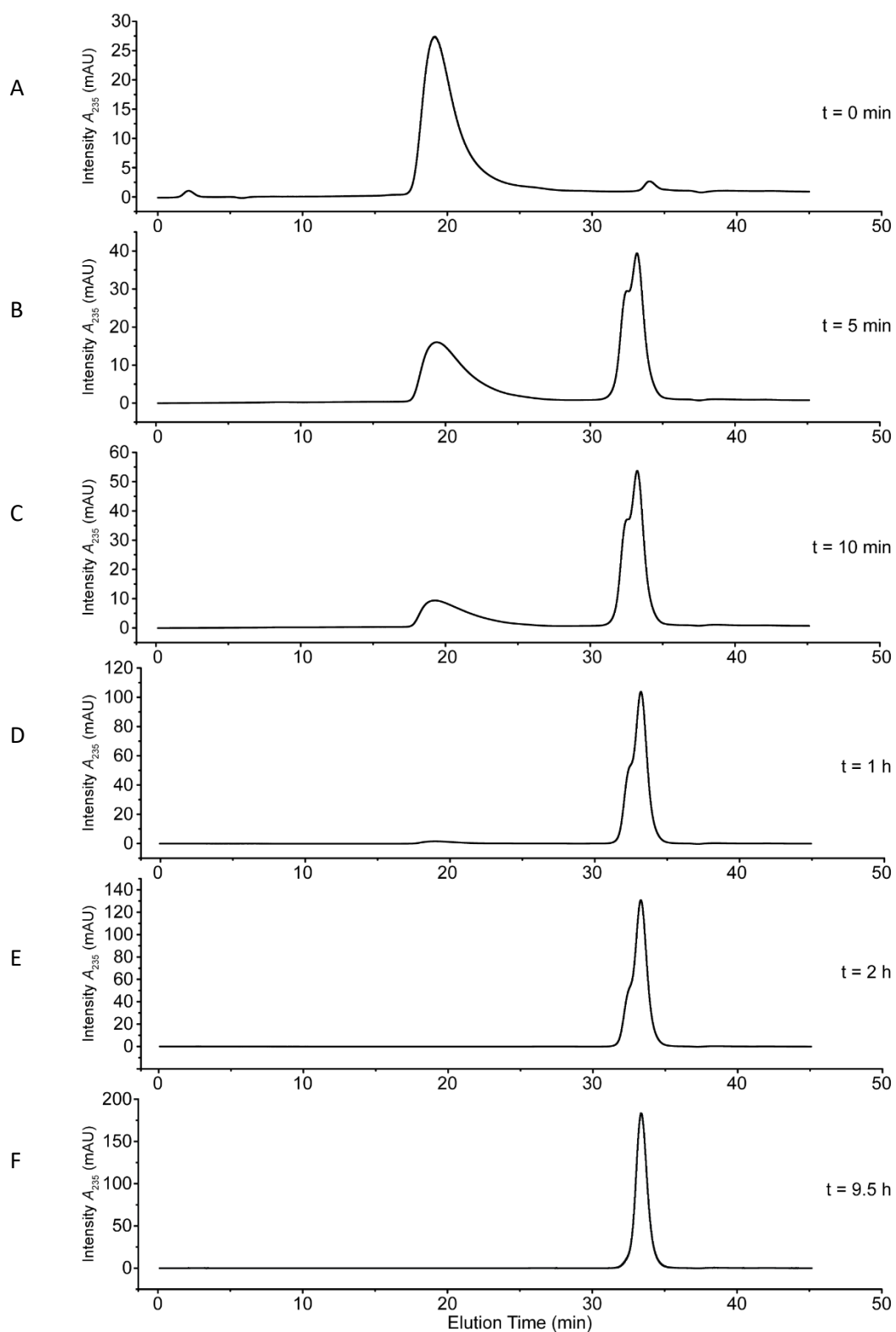


Figure S5 – HPLC-SEC-UV analysis of xanthan oligosaccharide products from a time course digestion with *PspXan9*. 2 mg·mL⁻¹ of lyase-treated xanthan (A) was incubated with 16 nM *PspXan9* at 55°C and sampled at various time points (B-F) for analysis by HPSEC.

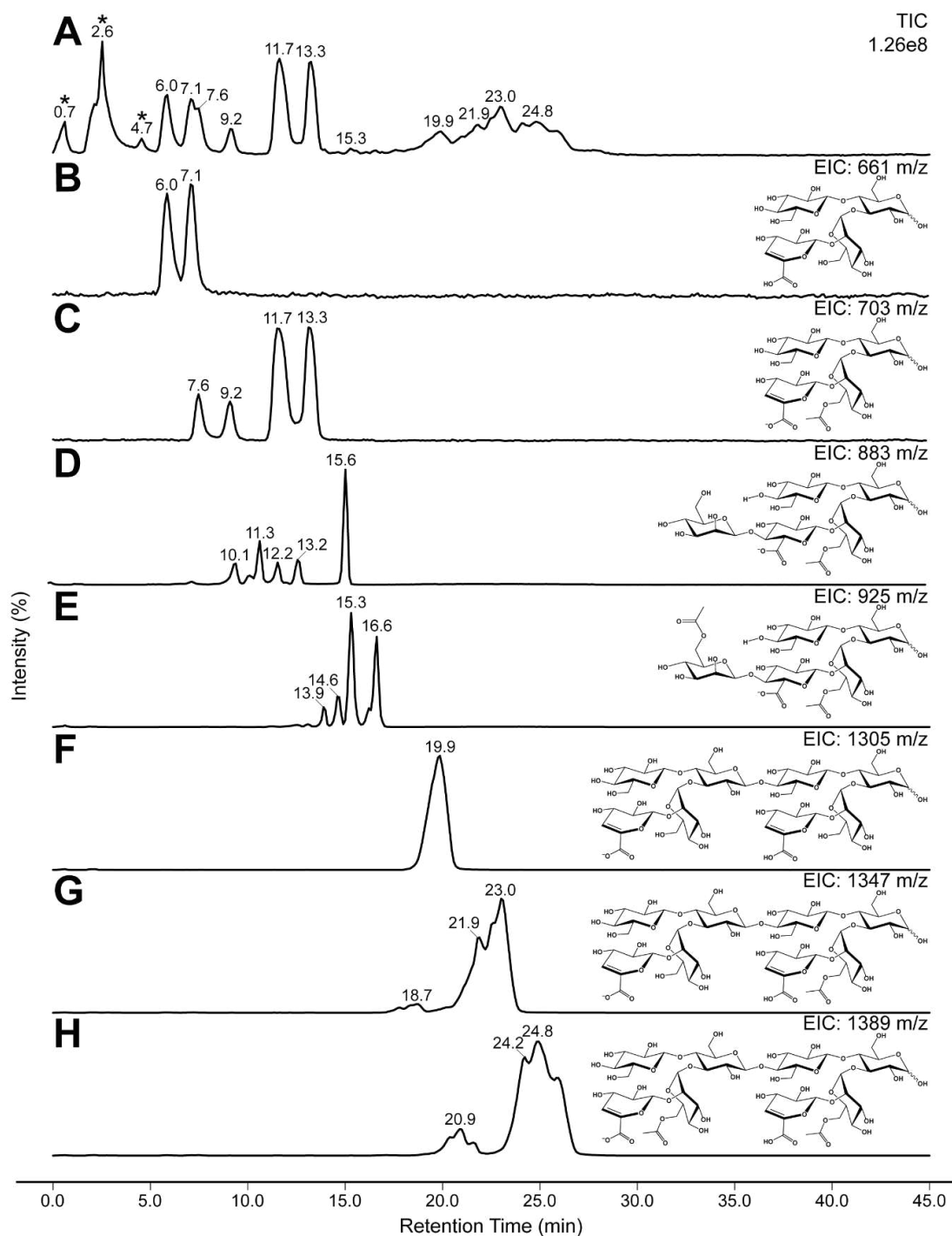


Figure S6 – LCMS chromatograms of partial digest products from *PspXan9*. Products from 2 h of degradation by 16 nM *PspXan9* were separated over 45 min by liquid chromatography and analyzed using mass spectrometry with a scan range of 100 – 2000 m/z in negative-ion mode. The resulting total ion chromatogram (A) and extracted ion chromatograms (B-H) of expected products are displayed above, with corresponding structures and m/z values indicated on the right. Resolution of reducing-end anomers and acetylated variants is observed for some products. Non-carbohydrate peaks in the TIC are denoted with an asterisk symbol.

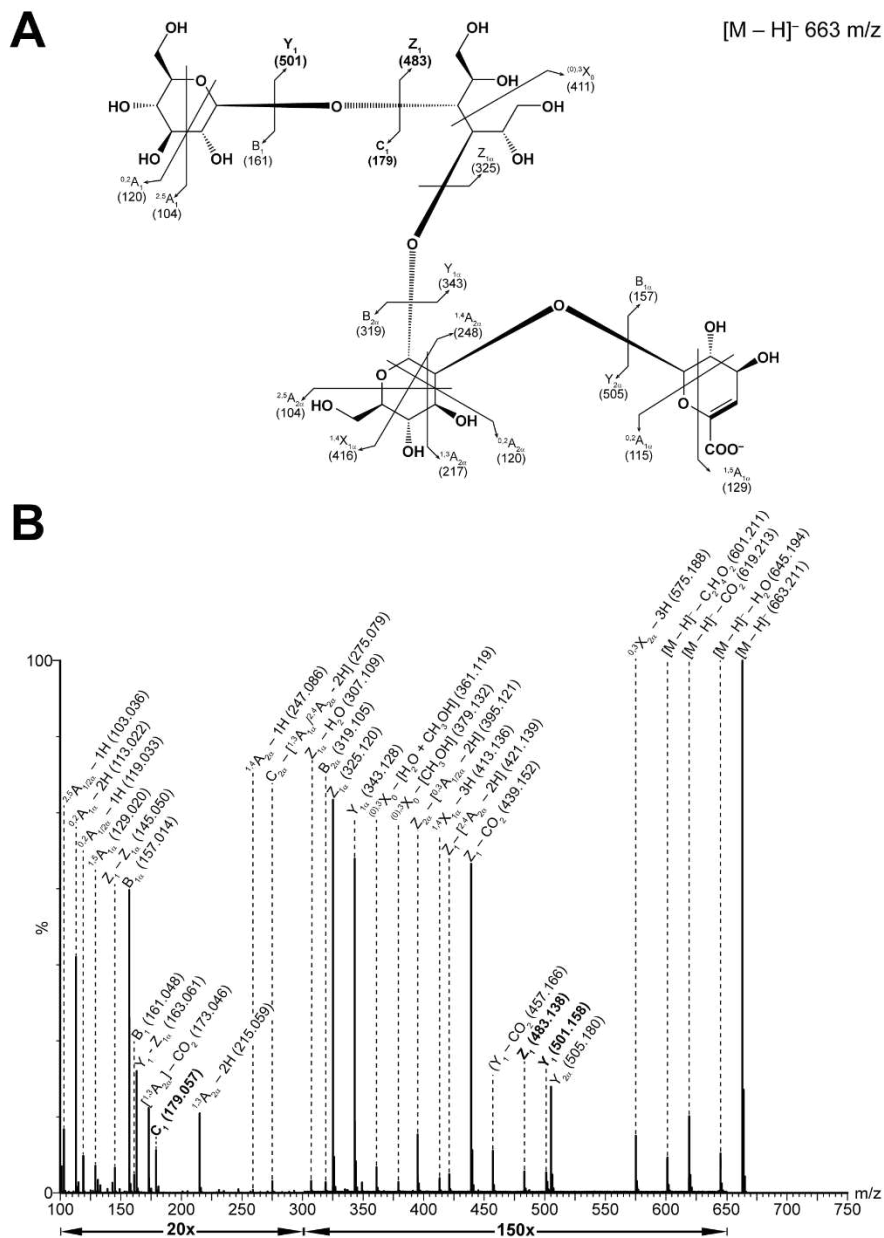


Figure S7 – MS/MS of the 663 m/z precursor ion, corresponding to the reduced, lyase-treated xanthan tetrasaccharide, from lyase-treated xanthan degradation by *PspXan9*. *A*. The fragmentation pattern of the 663 m/z precursor ion; *B*. The MSMS spectrum of the product ions from the fragmentation of 663 m/z. Selected regions are magnified and diagnostic peaks labelled in bold. Standard carbohydrate fragment nomenclature is used¹. The presence of a forward slash in a peak label indicates the presence of alternative assignments. Regions within the bounds of arrows found below the x-axis, indicate the amount of magnification (if any) the region is being displayed at.

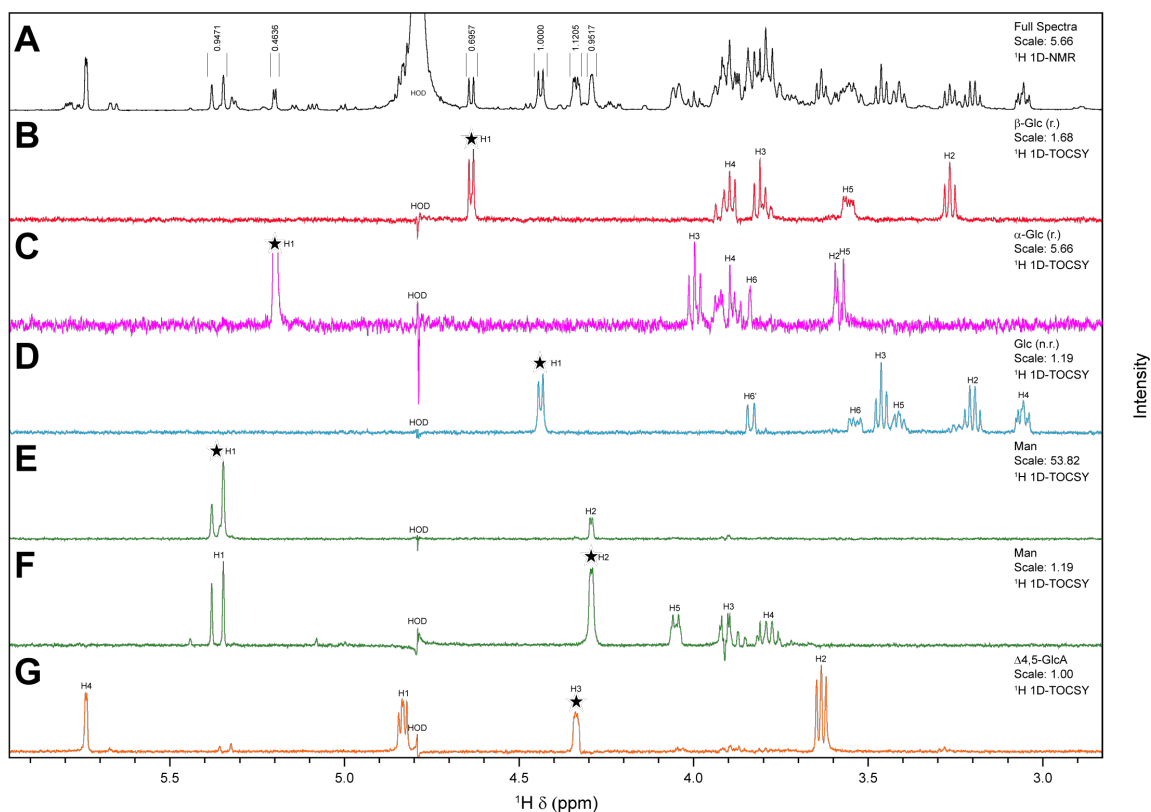


Figure S9 – Selective ^1H 1D-TOCSY experiment of deacetylated lyase-treated xanthan tetrasaccharides generated from lyase-treated xanthan degradation by *PspXan9*. *A.* Anomeric protons were targeted based on the full 1D-NMR spectrum, using the established anomeric proton region from between 4.3-5.5 ppm. Spectra corresponding to individual spin systems are displayed in various colors with: *B.* the reducing end (r.) β -glucose in red. *C.* the reducing end (r.) α -glucose in magenta. *D.* the nonreducing end (n.r.) β -glucose in light blue. *E.* α -mannose in green with irradiation on H1 at δ 5.36 ppm. *F.* α -mannose in green with irradiation on H2 at δ 4.29 ppm. *G.* β - Δ 4,5-ene-glucuronic acid in orange. Proton integrations are presented on the full ^1H 1D-NMR spectrum above their corresponding peak. Irradiations from ^1H 1D-TOCSY experiments are displayed by black stars above corresponding peaks.

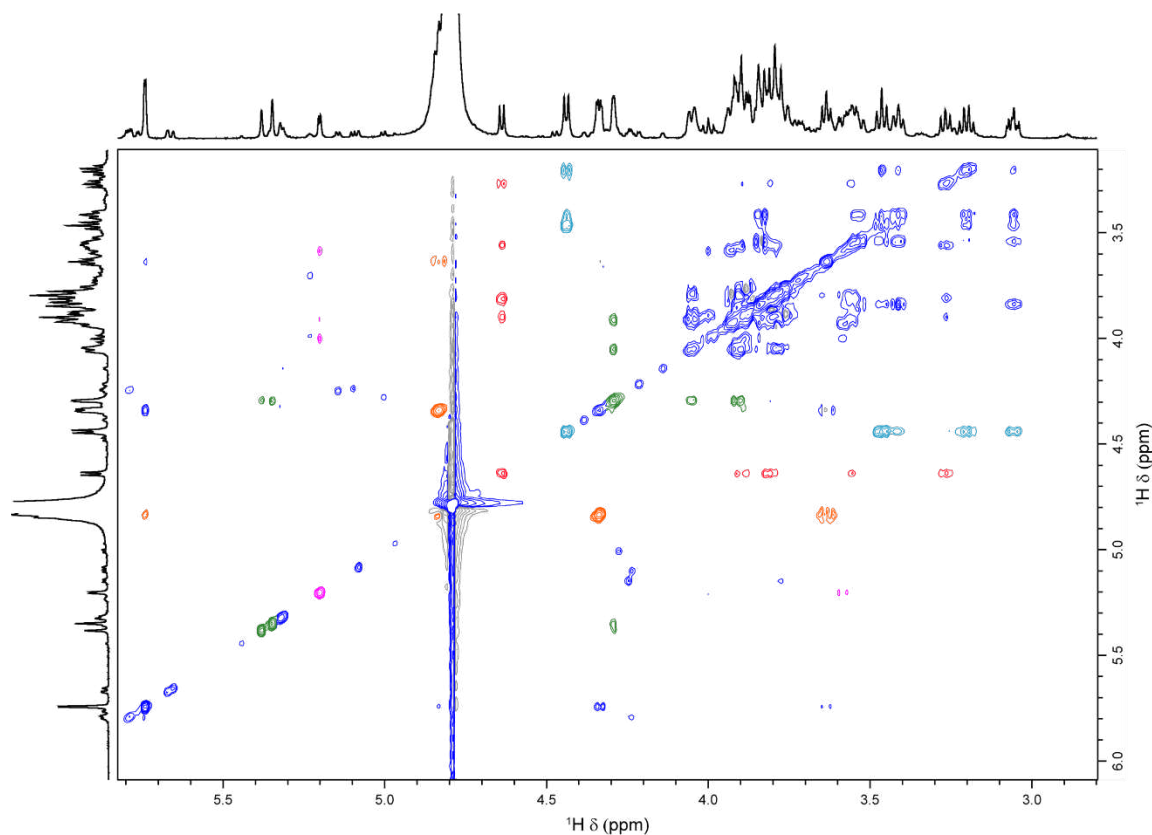


Figure S10 – ^1H 2D-TOCSY experiment of deacetylated lyase-treated xanthan tetrasaccharides generated from lyase-treated xanthan degradation by *PspXan9*. Each crosspeak indicates coupling between protons through magnetization transfer. Crosspeaks originating from the same spin system are shown in matching colours specific to each monosaccharide with the α/β reducing end glucose shown in magenta and red, respectively; the non-reducing end β -glucose shown in light blue; non-reducing α -mannose in green and β - $\Delta 4,5$ -ene-glucuronic acid in orange. The remaining positive peaks are shown in dark blue and negative peaks are shown in grey. The crosspeaks that couple with the H2 diagonal peak of mannose are also shown in green due to the poor magnetization transfer from the anomeric proton.

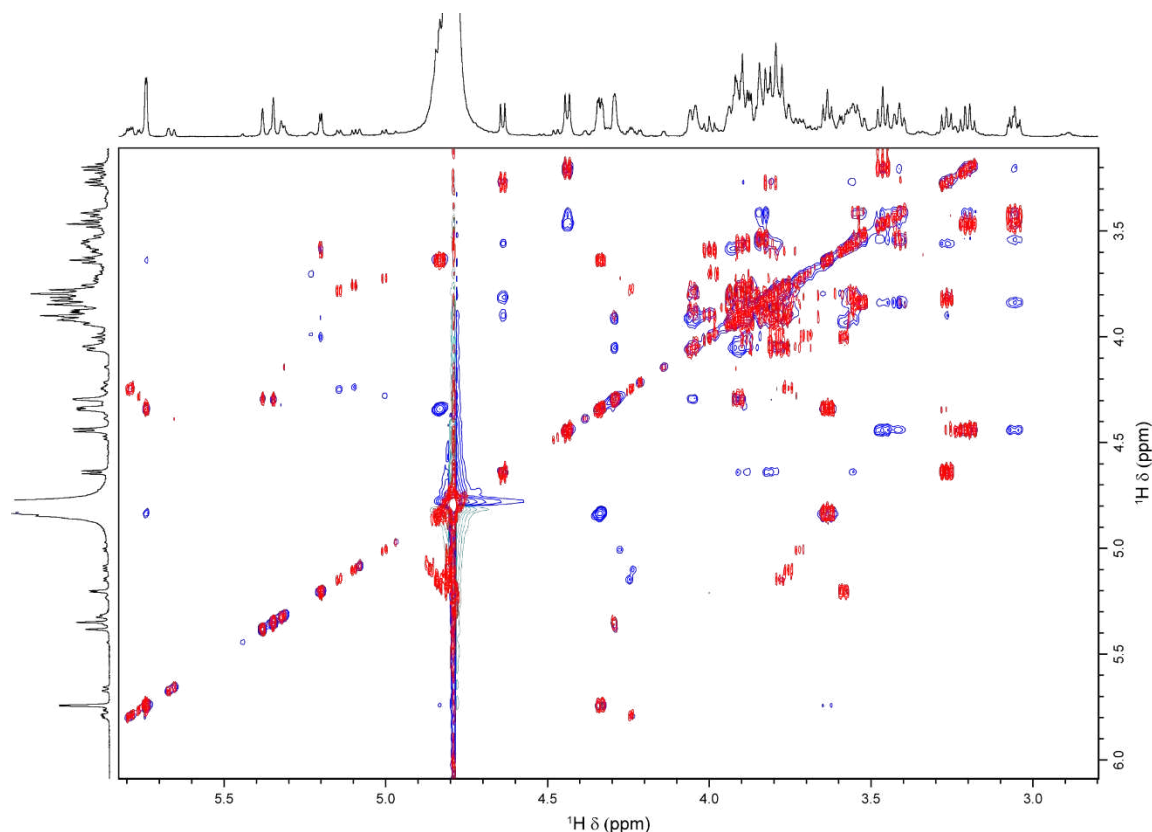


Figure S11 – ^1H 2D-TOCSY experiment overlaid with a ^1H 2D-COSY experiment of deacetylated lyase-treated xanthan tetrasaccharides generated from lyase-treated xanthan degradation by *PspXan9*. The ^1H 2D-TOCSY spectrum (see Figure S10 for more details) is overlaid with data from ^1H 2D-COSY allowing visualization of the crosspeaks that are 3J -coupled to neighboring vicinal protons. Peaks from ^1H 2D-TOCSY are presented in dark blue, whereas ^1H 2D-COSY peaks are shown in red.

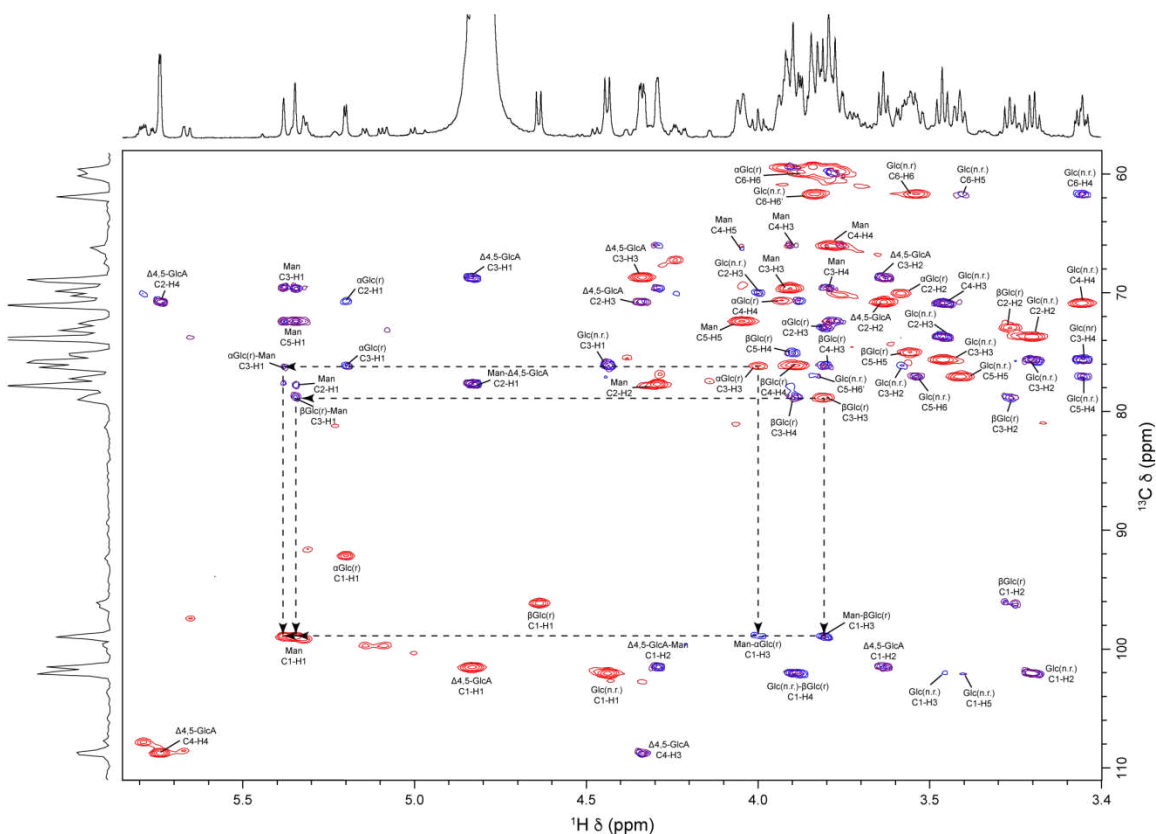


Figure S12 – Complete assignment of proton and carbon nuclei from deacetylated lyase-treated xanthan tetrasaccharides generated from lyase-treated xanthan degradation by *PspXan9* using ^1H , ^{13}C HSQC and ^1H , ^{13}C HMBC experiments. Each red crosspeak corresponds to a $^1J_{\text{C,H}}$ -coupling interaction from the HSQC experiment. $^2J_{\text{C,H}}$ - or $^3J_{\text{C,H}}$ -couplings were generated with HMBC experiments optimized for couplings at 5 Hz or 10 Hz, and are represented by blue and purple crosspeaks, respectively. The dotted arrows aid in tracing the path between the red HSQC crosspeaks of both of the C3-H3 peaks from the α/β -glucose reducing end (Glc(r.)) glucose through blue/purple HMBC crosspeaks to the red HSQC crosspeak of the C1-H1 of mannose. The notation states the sugar residue and its corresponding atom(s) below it. The sugar residue containing the carbon atom is always listed first, followed by the residue containing the proton atom. However, if both carbon and proton atoms exist within the same residue, the residue is only listed once. Abbreviations used: r., reducing end; n.r., nonreducing end; Glc, glucose; Man, mannose; $\Delta 4,5$ -GlcA; $\Delta 4,5$ -ene-glucuronic acid.

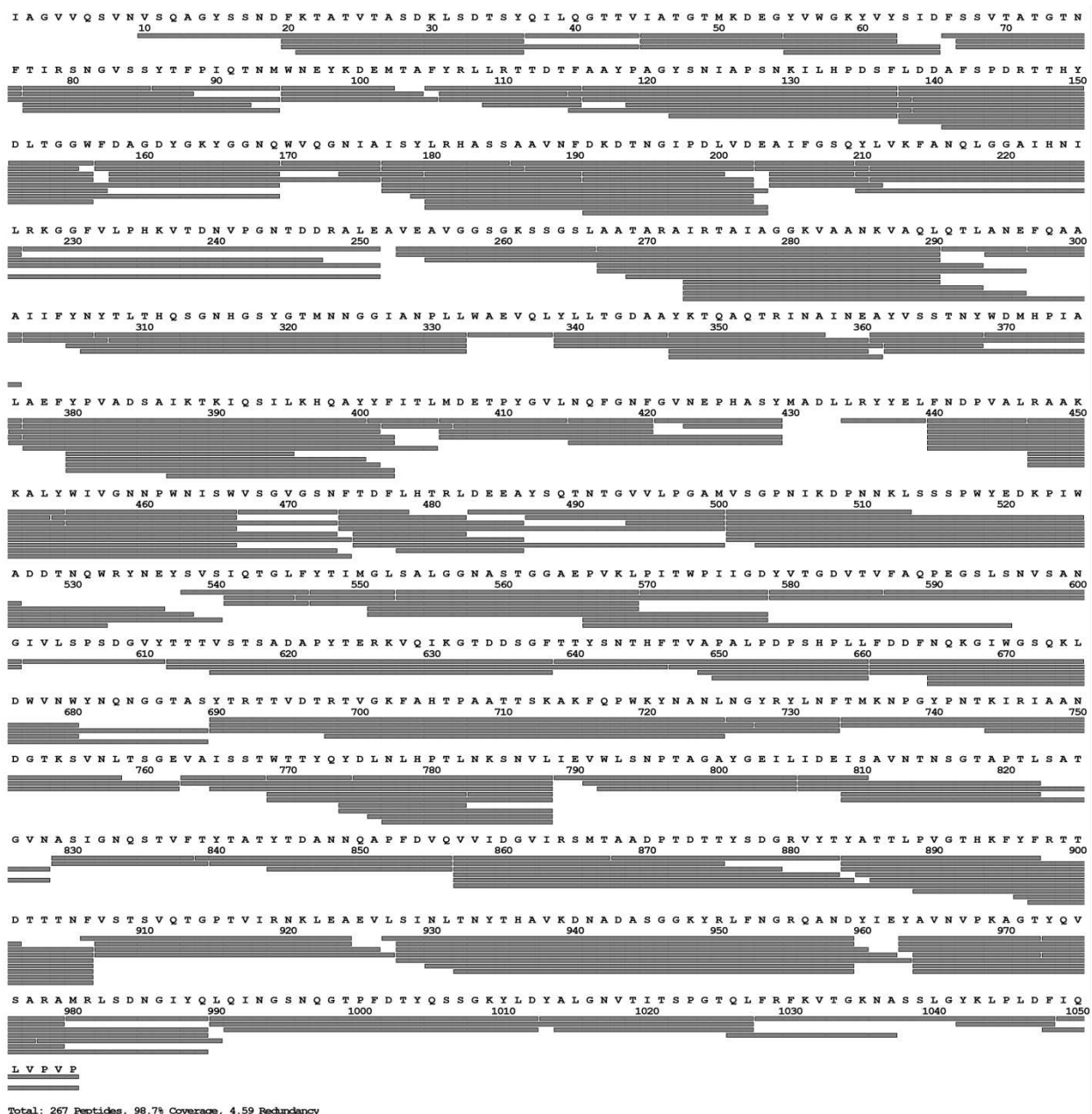
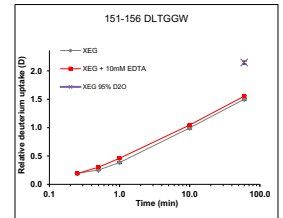
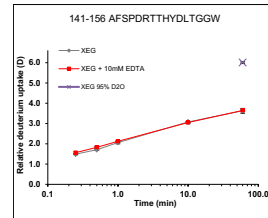
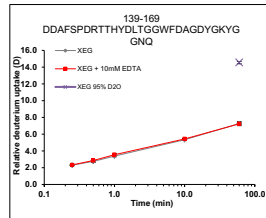
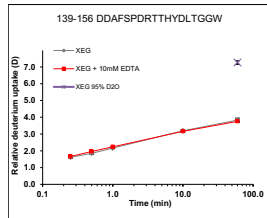
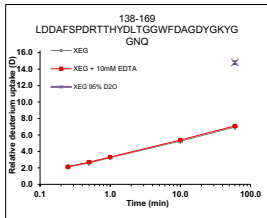
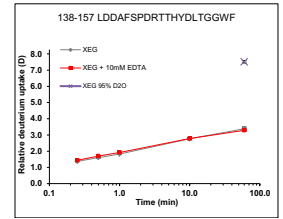
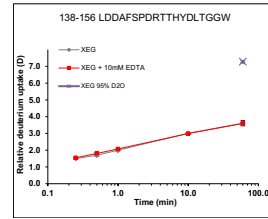
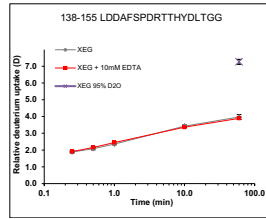
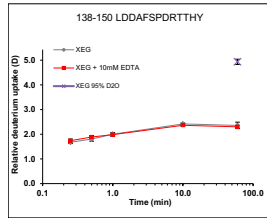
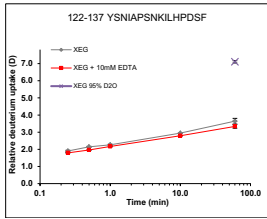
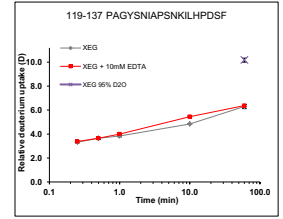
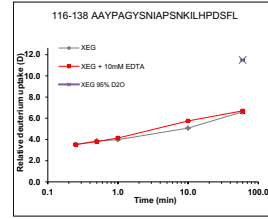
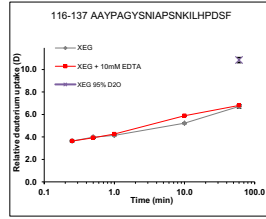
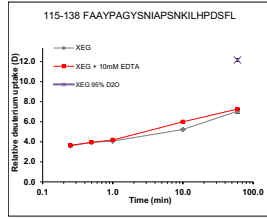
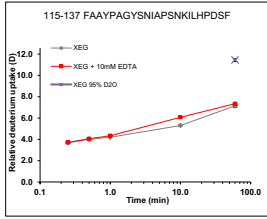
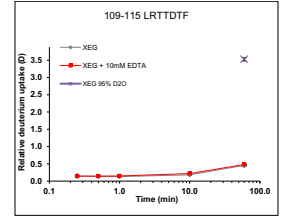
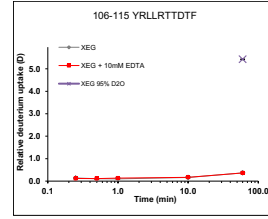
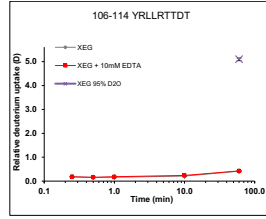
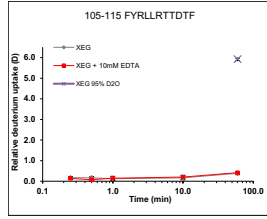
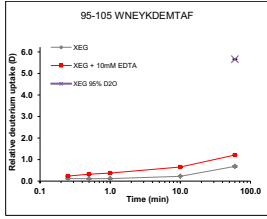
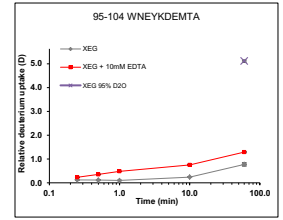
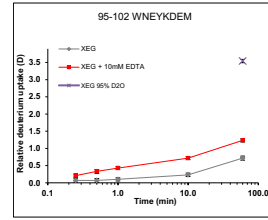
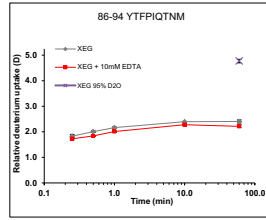
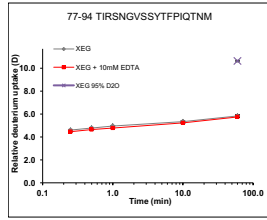
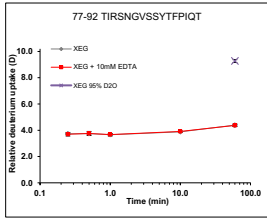
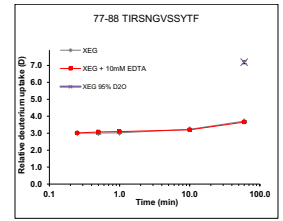
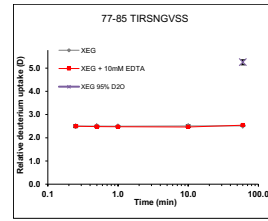
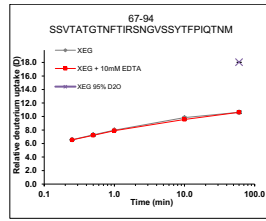
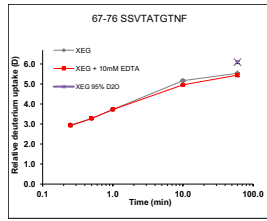
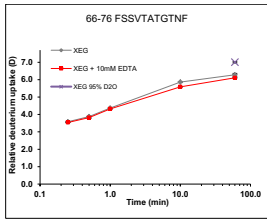
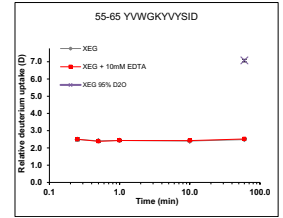
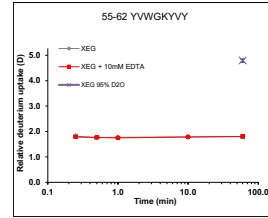
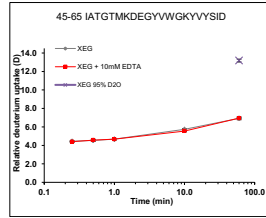
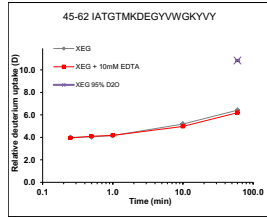
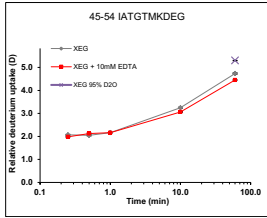
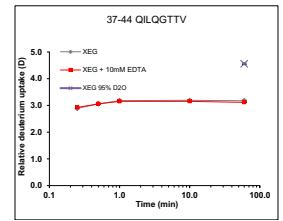
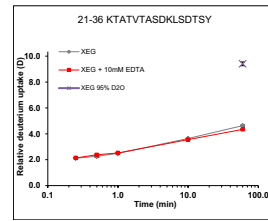
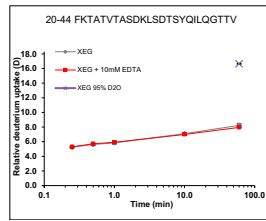
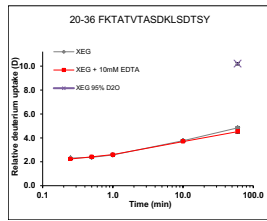
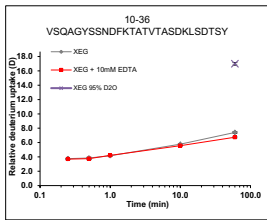
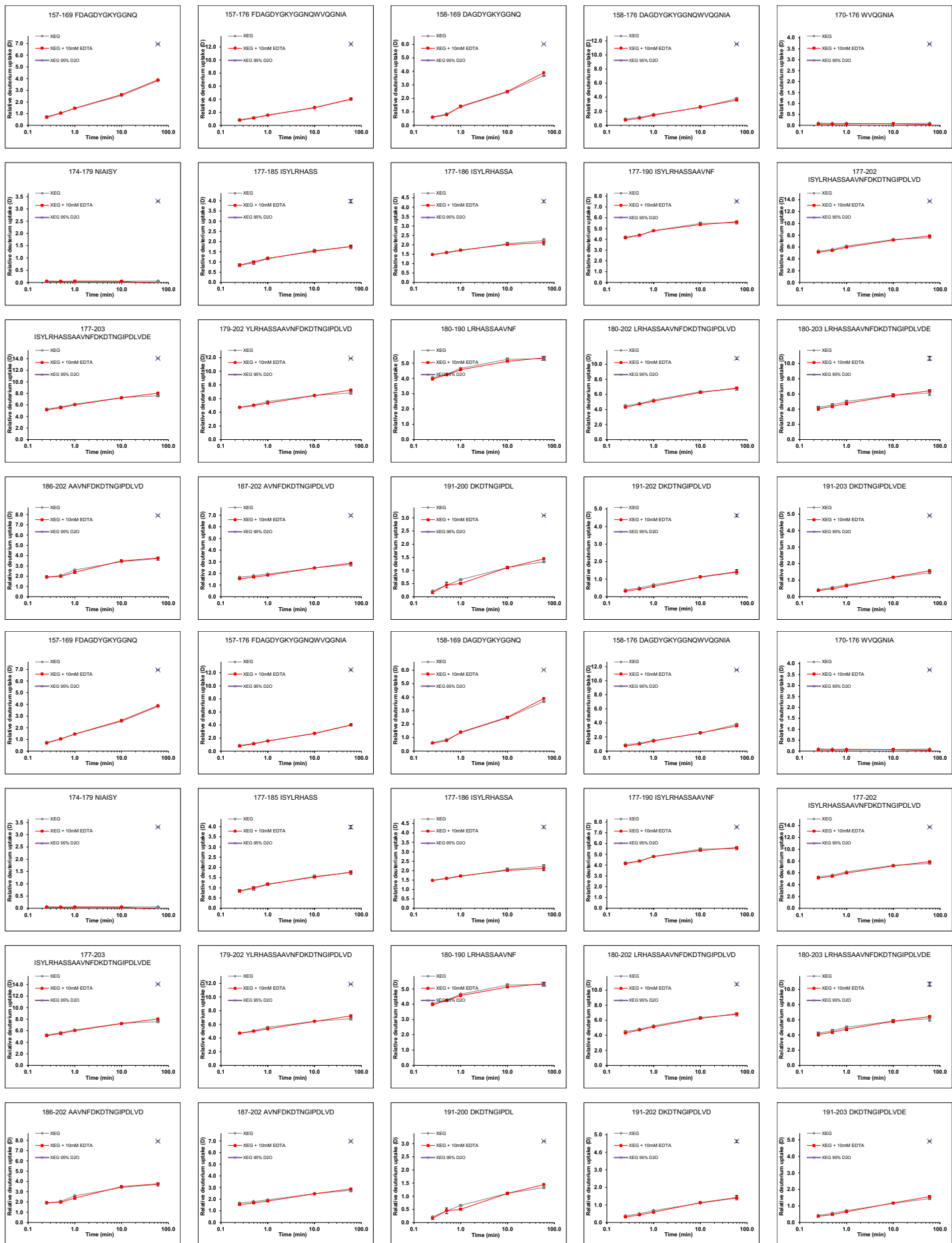
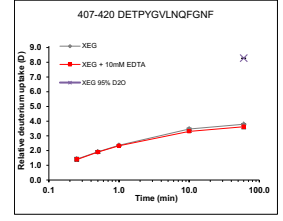
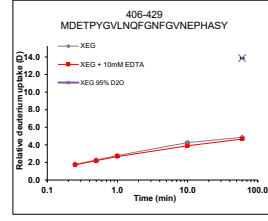
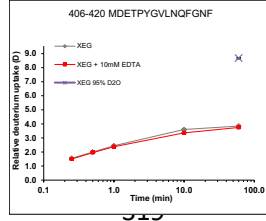
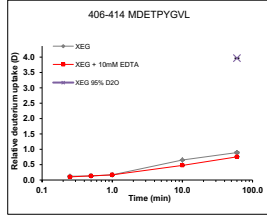
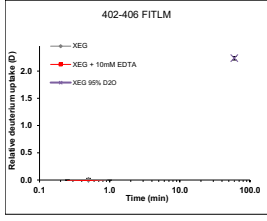
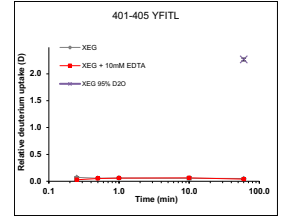
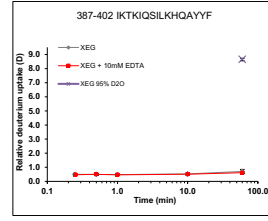
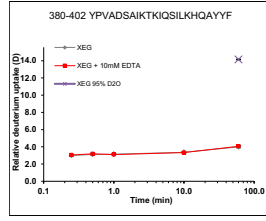
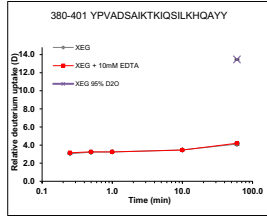
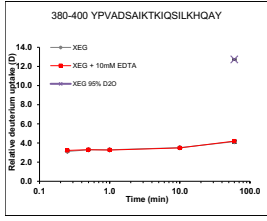
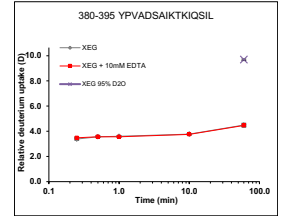
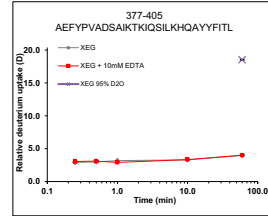
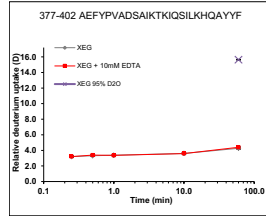
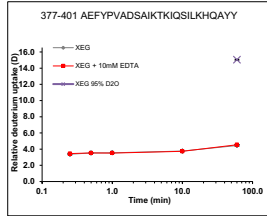
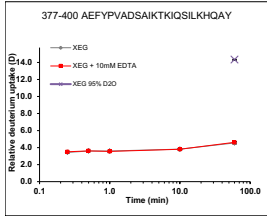
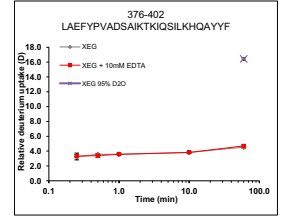
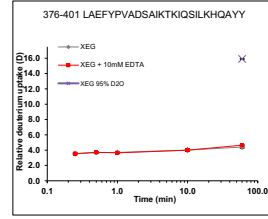
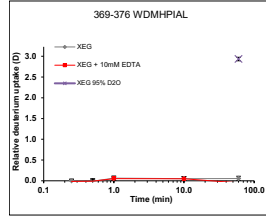
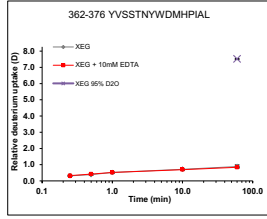
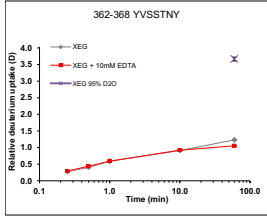
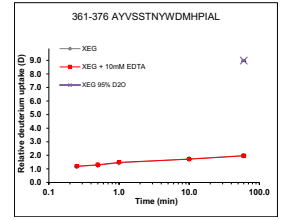
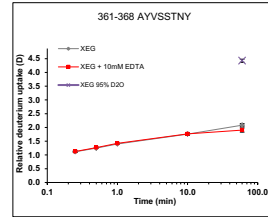
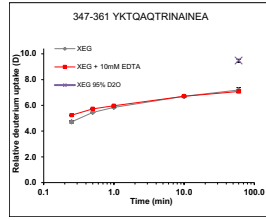
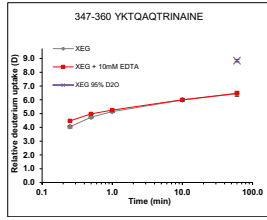
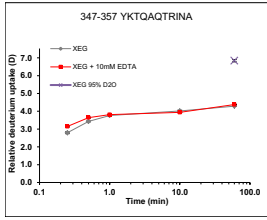
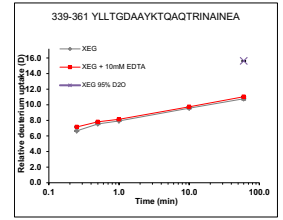
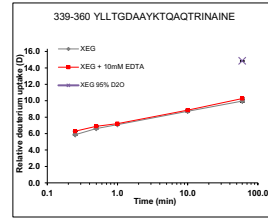
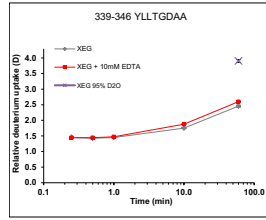
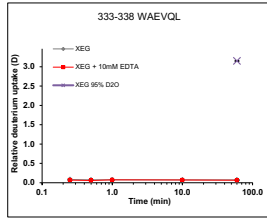
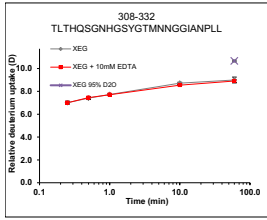
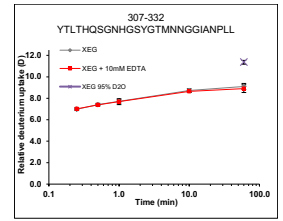
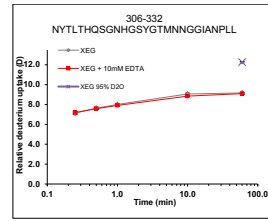
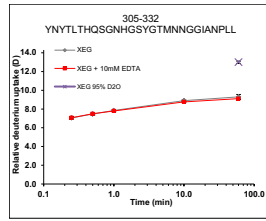
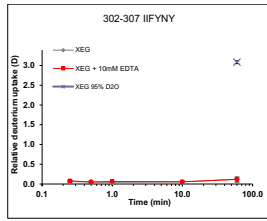
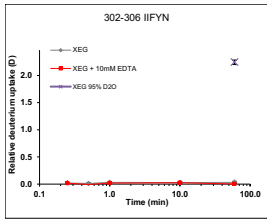
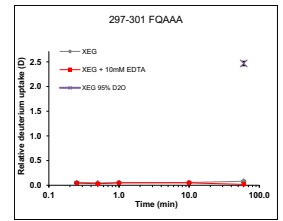
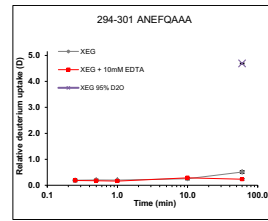
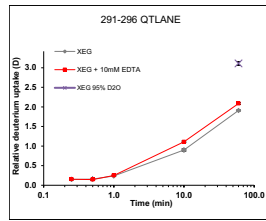
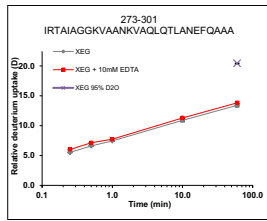
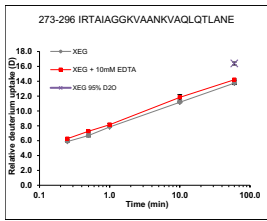
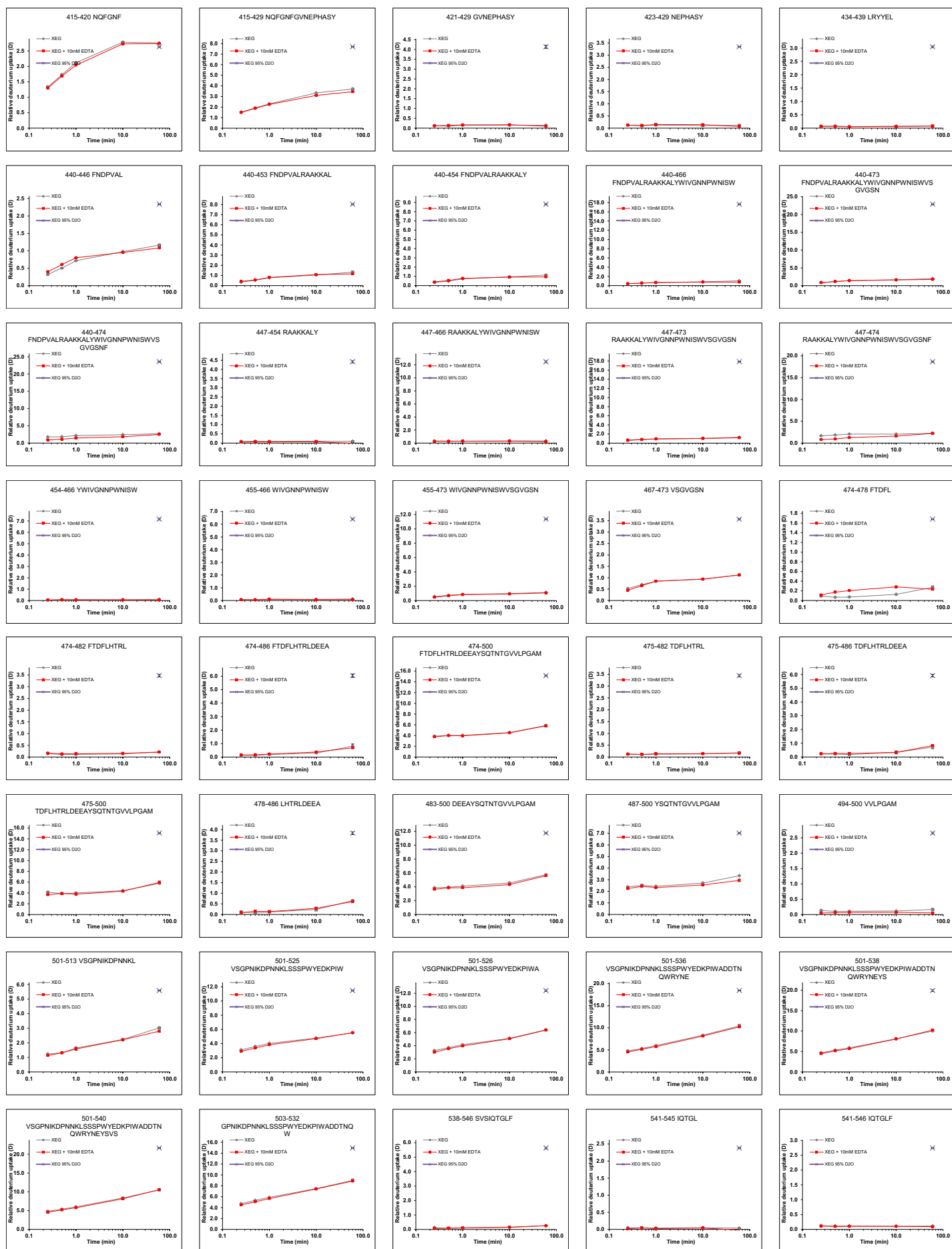


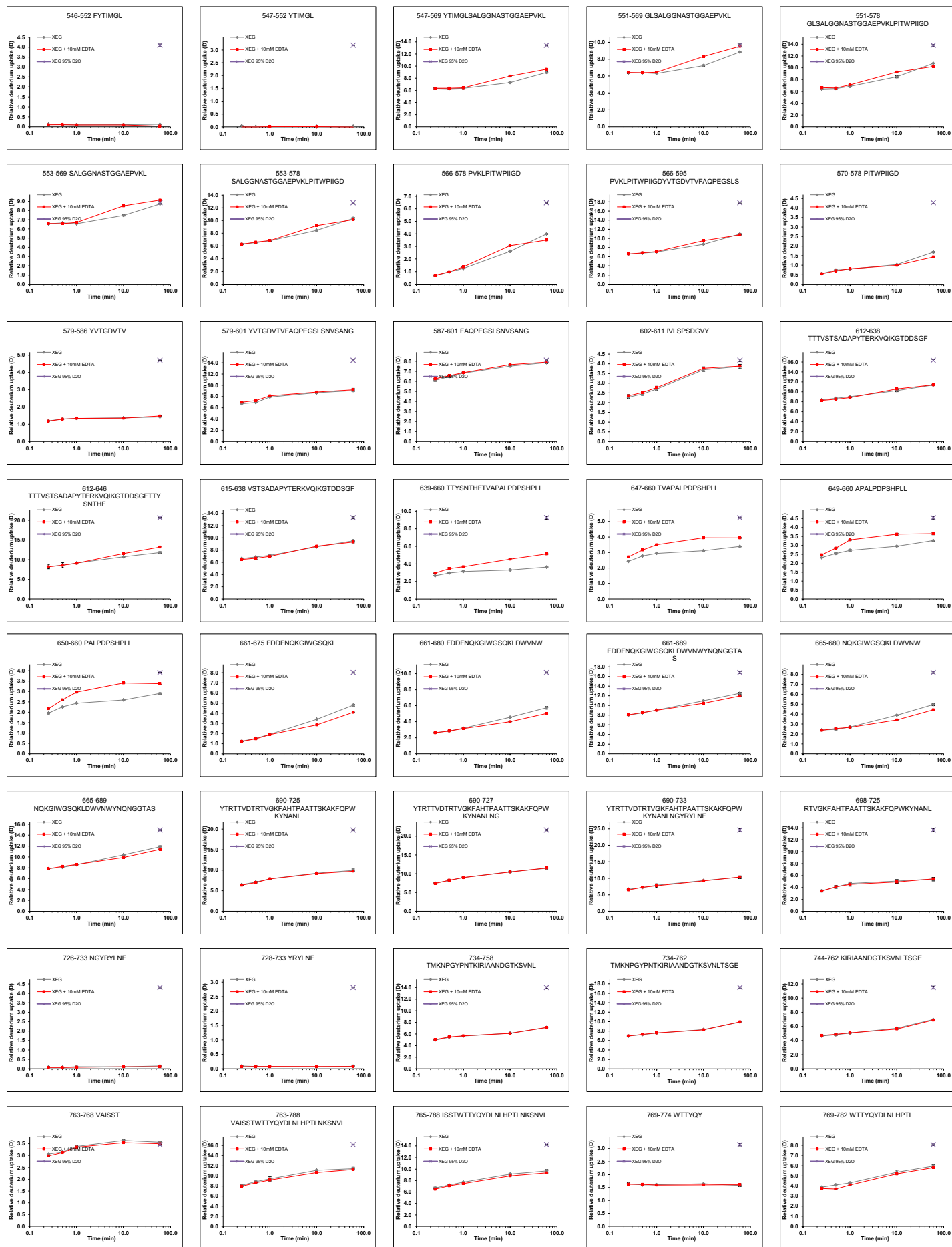
Figure S14 – Sequence coverage of *PspXan9* in HDX-MS experiments. Peptic peptides identified by tandem MS which yielded MS signals of suitable quality to report on HDX are indicated by grey bars.

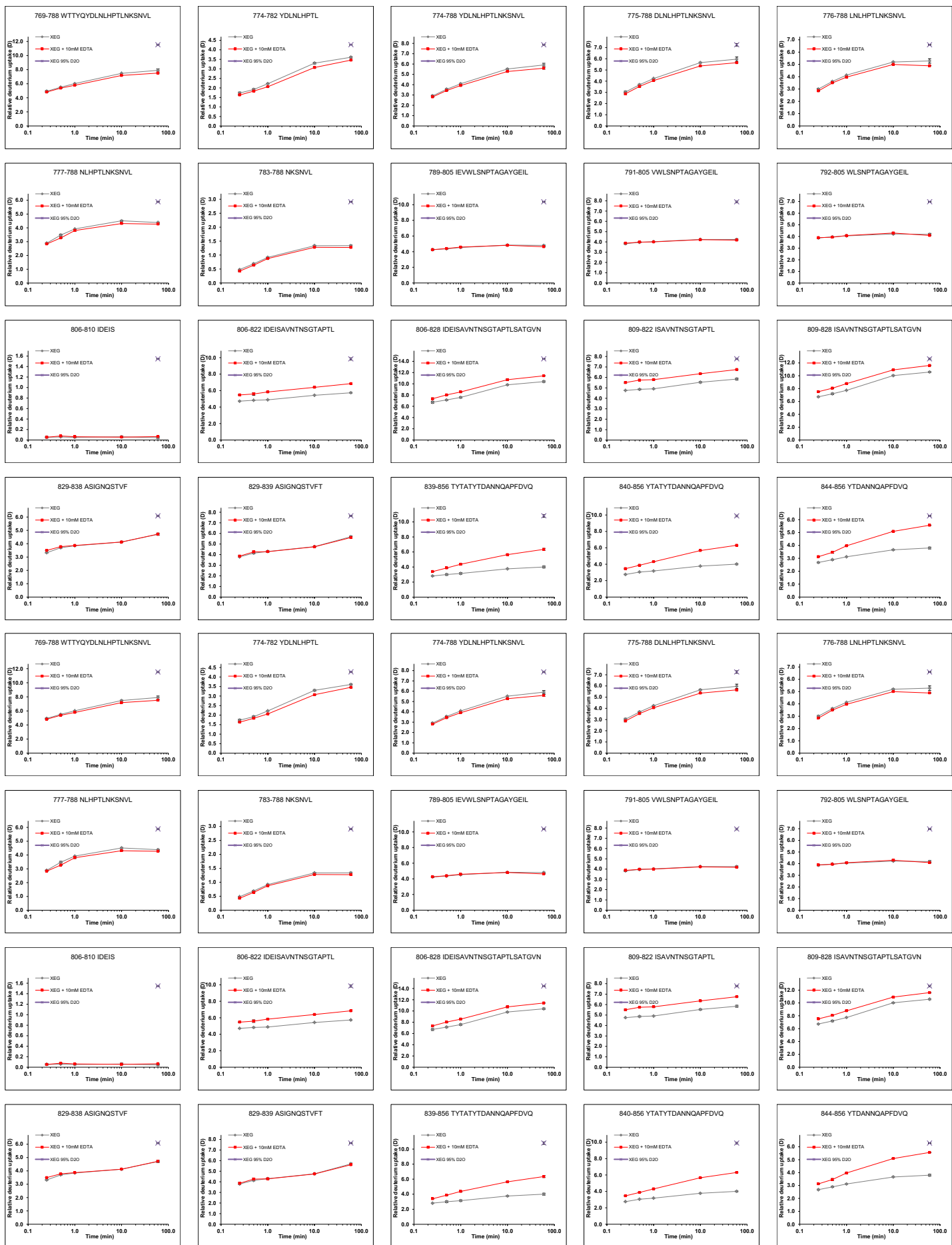












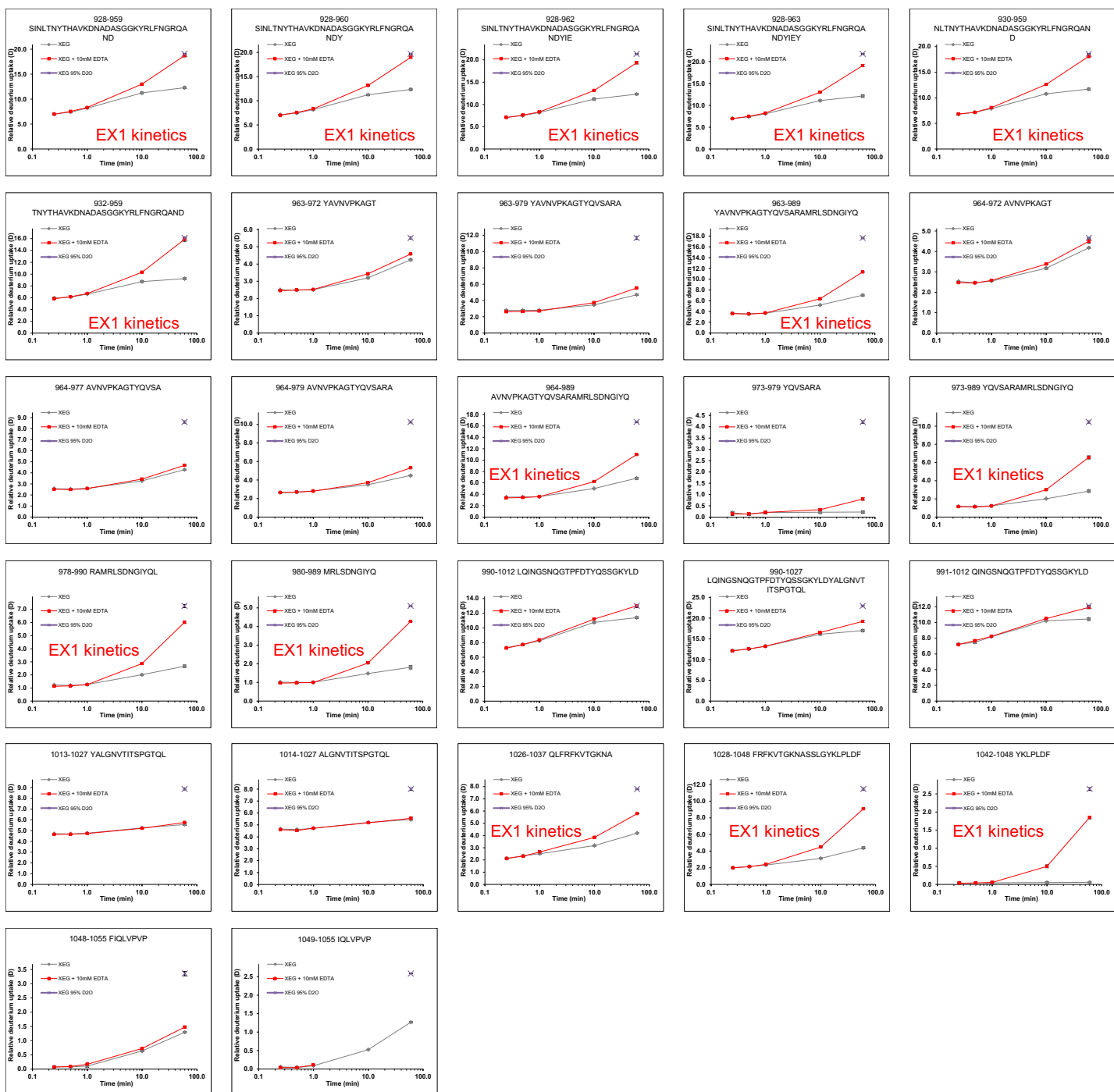


Figure S15 – Deuterium uptake plots of *PspXan9*. The deuterium incorporation of *PspXan9* peptic peptides in the absence (grey) and presence of 10 mM EDTA (red) was determined in triplicate HDX-MS experiments at 15 s, 30 s, 1 min, 10 min and 1 h and plotted with 1 x standard deviation (black). The fully deuterated control (95% D₂O) is shown in purple at the last time point (1 h). Note: *PspXan9* is labelled as XEG.

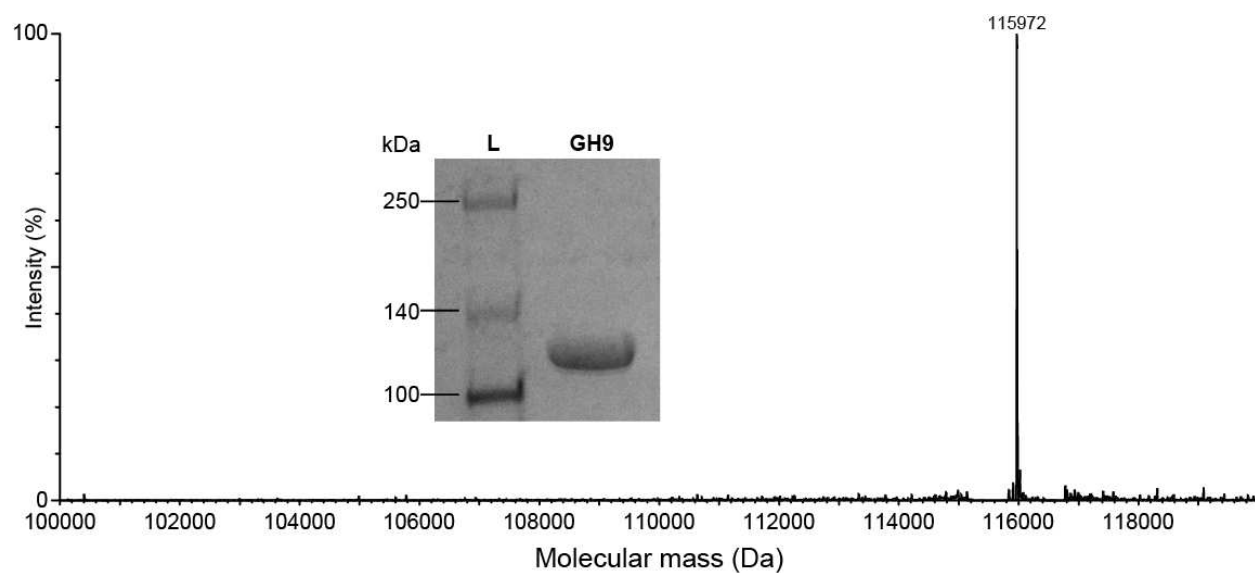


Figure S16 – Purity and molecular mass analysis of recombinant *PspXan9*. *Inset:* SDS-PAGE analysis of *PspXan9* (lane ‘GH9’) versus a molecular weight ladder (lane ‘L’). Intact-protein mass spectrometry indicates a single peak of mass 115972 versus 115969 calculated from the mature amino acid sequence.

TABLES

Table S1 – ^1H and ^{13}C assignments of the lyase-treated xanthan tetrasaccharide.

Monosaccharide	δ	Multiplicity	Coupling constant (J)	Monosaccharide	δ
^1H	(ppm)		(Hz)	^{13}C	(ppm)
<i>Glc(r)</i> —				<i>Glc(r)</i> —	
α H1	5.20	d	3.74	α C1	92.13
β H1	4.64	d	7.76	β C1	96.13
α H2	3.59	m	n.a.	α C2	70.05
β H2	3.26	dd	8.71; 8.71	β C2	72.92
α H3	4.00	t	9.47	α C3	76.11
β H3	3.81	t	9.67	β C3	78.84
α/β H4	3.90	t	9.48	α/β C4	76.11
α H5	3.58	m	n.a.	α C5	70.05
β H5	3.56	ddd	n.a.	β C5	75.01
α H6	3.87; n.a.	m	n.a.	α C6	59.08
β H6	n.a.; n.a.	n.a.	n.a.	β C6	n.a.
<i>Glc(n.r.)</i> —				<i>Glc(n.r.)</i> —	
H1	4.44	d	7.59	C1	102.07
H2	3.20	dd	8.78; 17.5	C2	73.67
H3	3.47	t	9.25	C3	75.61
H4	3.05	m	n.a.	C4	70.86
H5	3.41	dd	9.34; 6.39	C5	77.07
H6/6'	3.54; 3.84	m; d	n.a.; 11.38	C6	61.66
<i>Man</i> —				<i>Man</i> —	
H1	5.35; 5.38	d; d	<1; <1	C1	98.96
H2	4.29	dd	n.a.	C2	77.67
H3	3.90	m	n.a.	C3	69.66
H4	3.79	t	10.35	C4	66.03
H5	4.05	m	n.a.	C5	72.40
H6/6'	n.a.; n.a.	n.a.	n.a.	C6	
$\Delta 4,5\text{-ene-GlcA}$				$\Delta 4,5\text{-ene-GlcA}$ —	
—					
H1	4.82; 4.83	d; d	8.26; 8.26	C1	101.50
H2	3.63	dd	7.96; 7.96	C2	70.77
H3	4.34	dd	7.18; 2.80	C3	68.68
H4	5.74	d	2.26	C4	108.73
				C5	145.57
				C6	168.65

n.a. (not assigned) indicates that an unambiguous assignment was not possible due to overlapping peaks.

Table S2 – Data quality and structure refinement statistics

Data set	Native	Pt
PDB code	6FHJ	6FHN
Beamline	Diamond I04-1	Diamond I03
Wavelength (Å)	0.92	1.07
Space group	P3 ₂ 21	P3 ₂ 21
Cell parameters (Å)	a=103.35 b=103.35 c=212.5	a=103.27 b=103.27 c=210.35
Resolution range (Å) ^a	27.50-2.09 (2.09-2.04)	52.59-2.00 (2.05-2.00)
Number of reflections	858487	877195
Unique reflections	84289	88513
Monomers in asymmetric unit	1	1
Completeness %	99.9 (99.9)	100.0 (100.0)
I/I(σ)	15.8(3.6)	16.0 (3.5)
CC(1/2) ^c	0.999(0.878)	0.999 (0.876)
Average multiplicity	10.2 (10.5)	9.9 (9.8)
R _{merge} (%) ^b	9.9 (65.0)	8.2 (64.0)
Refinement statistics		
Percentage of R _{free} reflections	4.98	5.01
(%)R _{cryst} =Σ Fo - Fc / Σ Fo (%)	17.6	17.3
Free R factor (%)	20.3	20.8
Bond distances (Å):	0.013	0.012
Bond angles (°):	1.15	1.58
Average protein B values (Å ²)	34	39
Molprobity score	1.4	1.75
<i>Ramachandran Plot</i> ^d		
Most favoured	90.0	89.8
Additionally allowed	9.2	9.3
Generously allowed	0.6	0.5
Disallowed	0.2	0.4

^a Values in parentheses correspond to the highest resolution shell.

^b R_{merge} is defined as 100x Σ |I - <I>| / Σ I, where I is the intensity of the reflection.

^c CC(1/2) values for I_{mean} are calculated by splitting the data randomly in half.

^d Ramachandran plot analysis was carried out using PROCHECK².

Table S3 – Differential relative deuterium incorporation in percent of *PspXan9* in the absence and presence of chelating agent. Increases and reductions in differential relative deuterium uptake are written in red and blue respectively

Domain	Region	15s	30s	1min	10min	60 min
X229	46-54	-	-	-	>-2.5%	> -5%
	68-76	-	-	-	>-2.5%	-
	87-94	-	>-2.5%	>-2.5%	-	-
GH9	95-105	>2.5%	>5%	>7.5%	> 10%	> 10%
	116-122	-	-	-	>5%	-
	152-156	-	-	>2.5%	-	-
	192-200	-	-	>-2.5%	-	> 2.5%
	228-251	-	-	-	-	> 5%
	274-290	-	>2.5%	>2.5%	-	-
	292-296	-	-	-	>5%	> 5%
	297-301	-	-	-	-	> -2.5%
	340-346	-	-	-	>2.5%	> 2.5%
	348-357	>5% (348-361)	>2.5%	-	-	-
	362-368	-	-	-	-	> -5%
	370-376	-	-	-	-	> -2.5%
	406-414	-	-	-	> -2.5%	> -2.5%
	441-454	>2.5%	>2.5	>2.5%	-	> -2.5 %
	475-478	-	>5%	>7.5%	-	-
	488-513	-	-	-	-	>- 5%
	542-545	-	-	-	-	>-2.5%
β1	553-566	-	-	-	>10%	>5%
	567-570	-	-	-	>5%	> -7.5%
	571-578	-	-	-	-	> - 5%
	640-647	>2.5%	>5%	>5%	>12.5%	> 15%
	648-660	>5%	>7.5%	>12.5%	>20%	>10%
β2	662-680	-	-	-	>-5%	>-5%
	681-689	-	-	-	>-2.5%	>-2.5%
	775-781	-	-	>-2.5%	>-5%	>-2.5
β3	811-822	>7.5%	>10%	>10%	>10%	>10%
	823-828	>5%	>5%	>7.5%	>5%	> 5%
	841-844	>5%	>7.5%	>10%	>17.5%	>20%
	845-856	>5%	>7.5%	>12.5%	>22.5%	>25%
	869-883	>10%	>10%	>7.5%	-	>5%
	897-906	>5%	>12.5%	>20%	>55%	>55%
CBM84	907-924	-	-	-	>5%	>7.5%
	925-927	-	-	-	>10%	>15%
	928-959	-	-	-	EX1	EX1
	973-989	-	-	-	EX1	EX1
	991-1012	-	-	-	-	>10%
	1028-1048	-	-	-	EX1	EX1
	1049-1055	-	-	-	>5%	>5%

Table S4– Differential relative deuterium incorporation for selected regions (absolute values) of *PspXan9* in the absence and presence of chelating agent. For the time-point where the most

Peptide 95-105	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,11	0,21	0,26	0,43	0,52
Peptide 227-251	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,00	0,04	0,04	0,05	0,67
Peptide 291-296	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,01	-0,01	0,01	0,21	0,18
Peptide 551-569	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,05	0,00	0,15	1,08	0,68
Peptide 639-660	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,29	0,50	0,54	1,23	1,49
Peptide 809-822	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,75	0,88	0,88	0,82	0,91
Peptide 840-856	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,71	0,82	1,13	1,90	2,30
Peptide 897-906	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	0,36	0,70	1,11	2,95	2,97
Peptide 907-927	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	-0,04	0,08	0,20	1,47	2,66
Peptide 928-959	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	-0,03	0,13	0,15	1,75	6,42
Peptide 980-989	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	-0,05	-0,01	-0,01	0,56	2,46
Peptide 1028-1048	Time (min)	0,25	0,5	1	10	60
	Δ HDX (<i>PspXan9</i> EDTA- <i>PspXan9</i>) (Da)	-0,01	-0,02	0,07	1,37	4,68

pronounced difference occurs the value is highlighted in red.

Table S5 - Composition of liquid model detergent used

Ingredient	Amount (g)
Sodium dodecylbenzenesulfonate	174.7
Sodium lauryl ether sulfate (28% in water)	1146.4
Soy fatty acid (based on C16-18 fatty acids)	30
BIO-SOFT N25-7 (alcohol ethoxylate with 7 moles ethoxylate; based on C12-15 alcohol)	159
Water	1400
Triethanolamine	12
Sodium hydroxide	15.7
Sodium citrate	60
Calcium chloride	0.6
Total	2998.4

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