

Figure S1. Illustration of peptide fragment separation. Proteolytic peptides (from maltose binding protein) obtained as in Fig. 1 are separated roughly by C18 reversed phase HPLC using a shaped gradient to spread eluant peptides. Eluant is fed by electrospray into the mass spectrometer which repeatedly scans the entire mass spectrum (~1 sec/scan). From the total ion chromatogram in panel A, one scan is shown in B and expanded in C.

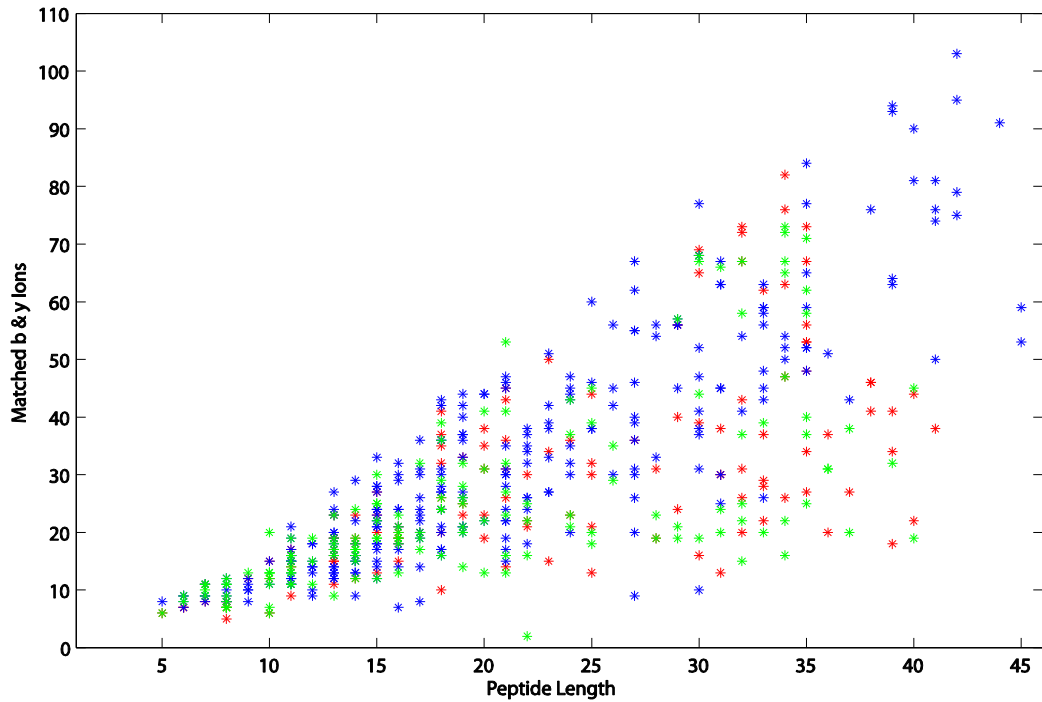
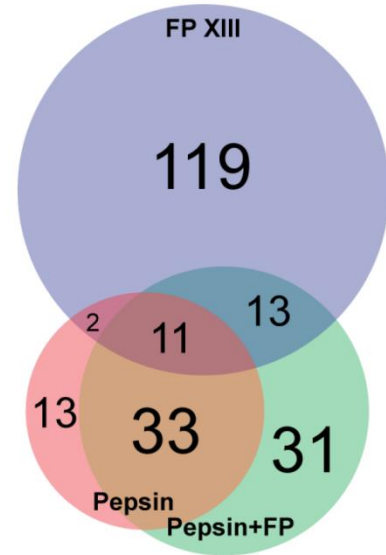


Figure S2. On the quality of MS/MS peptide identification. The number of b and y ions found for α -synuclein peptide fragments is plotted against the size of the peptide. Data sets obtained with the three different proteolysis conditions are merged (pepsin – red; fungal protease XIII – blue; the two in tandem – green). The large number of matching b and y ions found speaks for the quality of the MS/MS identifications. Of the 543 peptides found (all three protease combinations), only one has a suspiciously low number of daughter ions.

Table S1. The steps of peptide recognition and filtering used and the peptide survival record at each step. MS/MS runs used a CID top four method with dynamic exclusion and two replicates with static exclusion of peptides previously identified. MS/MS data were analyzed by SEQUEST (Bioworks) to create a peptide list to be used in ExMS analysis of MS data. The peptide list was culled at $P_{\text{pep}} < 0.990$ to reduce false identifications to 1/1000, and culled further by removing peptides not definitively found by the ExMS program in trial MS runs with all-H and 50% D-exchanged protein. Items marked “merge” reflect the culling of redundantly found peptide ions. “Unique” means that peptides in more than one charge state are only counted once. The progression of peptide MS/MS identification shows that more peptides could be obtained. additional MS/MS steps and by additional proteolytic manipulations (different exposure time, other proteases).

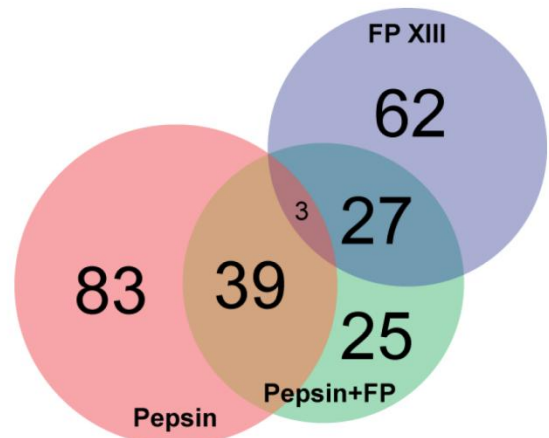
Alpha-Synuclein (140 residues)

Tandem MS Analysis	Pepsin	FP XIII	Pepsin+FP		
MS/MS Run 1 (P=0.990)	84	154	94		
MS/MS Run 2 (P=0.990)	53	93	51		
MS/MS Run 3 (P=0.990)	39	66	51		
MS/MS Merge	129	267	147	Merge	418
Unique Peptides	78	163	97		255
Unique Peptides / Residue	0.6	1.2	0.7		1.8
ExMS Analysis	Pepsin	FP XIII	Pepsin+FP		
Survive All H Trial (Unique)	77	163	96		
Survive 50% D Trial 1 (Unique)	62	150	89		
Survive 50% D Trial 2 (Unique)	62	150	91	Merge	
Survive All Above (Unique)	59	145	88		222
Unique Peptides / Residue	0.4	1.0	0.6		1.6
		Average Coverage			29



Apolipoprotein A-I (243 residues)

Tandem MS Analysis	Pepsin	FP XIII	Pepsin+FP		
MS/MS Run 1 (P=0.5)	107	92	98		
MS/MS Run 2 (P=0.5)	65	42	62	Merge	
MS/MS Merge	172	134	160		411
Unique Peptides	145	124	143		298
Unique Peptides / Residue	0.6	0.5	0.6		1.2
ExMS Analysis	Pepsin	FP XIII	Pepsin+FP		
Survive All H Trial (Unique)	141	112	123		
Survive 50% D Trial 1 (Unique)	129	111	106		
Survive 50% D Trial 2 (Unique)	129	102	115	Merge	
Survive All Above (Unique)	125	92	94		239
Unique Peptides / Residue	0.5	0.4	0.4		1.0
		Average Coverage			11

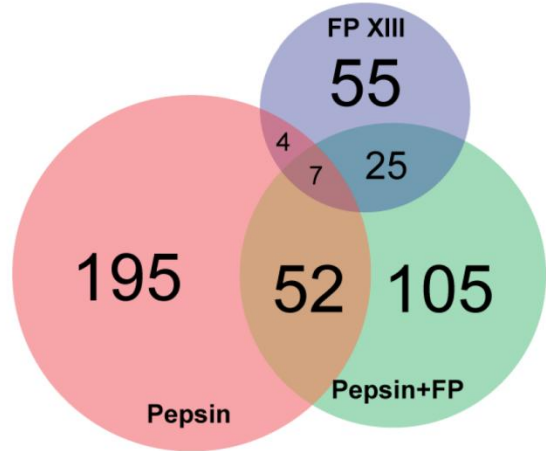


Maltose Binding Protein (370 residues)

<u>Tandem MS Analysis</u>	<u>Pepsin</u>	<u>FP XIII</u>	<u>Pepsin+FP</u>	
MS/MS Run 1 (P=0.990)	274	148	217	
MS/MS Run 2 (P=0.990)	195	92	105	
MS/MS Run 3 (P=0.990)	42	40	57	Merge
MS/MS Merge	493	242	379	847
Unique Peptides	439	137	105	608
Unique Peptides / Residue	1.2	0.4	0.7	1.6

<u>ExMS Analysis</u>	<u>Pepsin</u>	<u>FP XIII</u>	<u>Pepsin+FP</u>	
Survive All H Trial (Unique)	438	137	251	
Survive 50% D Trial 1 (Unique)	365	122	245	
Survive 50% D Trial 2 (Unique)	380	127	230	Merge
Survive All Above (Unique)	258	91	189	443
Unique Peptides / Residue	0.7	0.3	0.5	1.2

Average Coverage 14



Hsp104 (908 residues)

<u>Tandem MS Analysis</u>	<u>Pepsin</u>	<u>FP XIII</u>	<u>Pepsin+FP</u>	
MS/MS Run 1 (P=0.990)	402	205	390	
MS/MS Run 2 (P=0.990)	240	92	287	
MS/MS Run 3 (P=0.990)	232	61	284	Merge
MS/MS Merge	618	320	564	1200
Unique Peptides	395	262	441	840
Unique Peptides / Residue	0.4	0.3	0.5	0.9

<u>ExMS Analysis</u>	<u>Pepsin</u>	<u>FP XIII</u>	<u>Pepsin+FP</u>	
Survive All H Trial (Unique)	395	262	439	
Survive 50% D Trial 1 (Unique)	384	191	320	
Survive 50% D Trial 2 (Unique)	375	162	337	Merge
Survive All Above (Unique)	373	154	312	664
Unique Peptides / Residue	0.4	0.2	0.3	0.7

Average Coverage 10

