

Supporting Information

ClipsMS: An Algorithm for Analyzing Internal Fragments Resulting from Top-Down Mass Spectrometry

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Joseph A. Loo

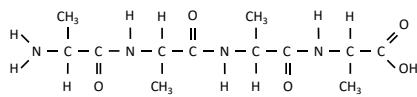
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Theoretical Peptide AAAA



Terminal Fragments

Internal Fragments

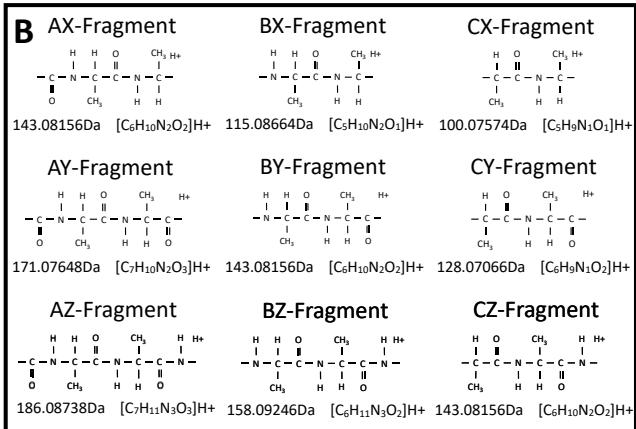
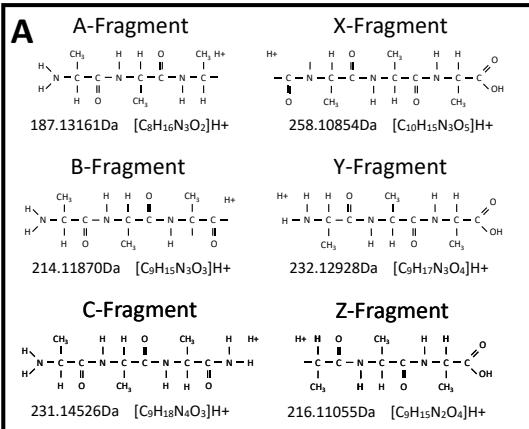


Figure S1. Examples of theoretical (A) terminal and (B) internal fragments and masses that are searched in ClipsMS.

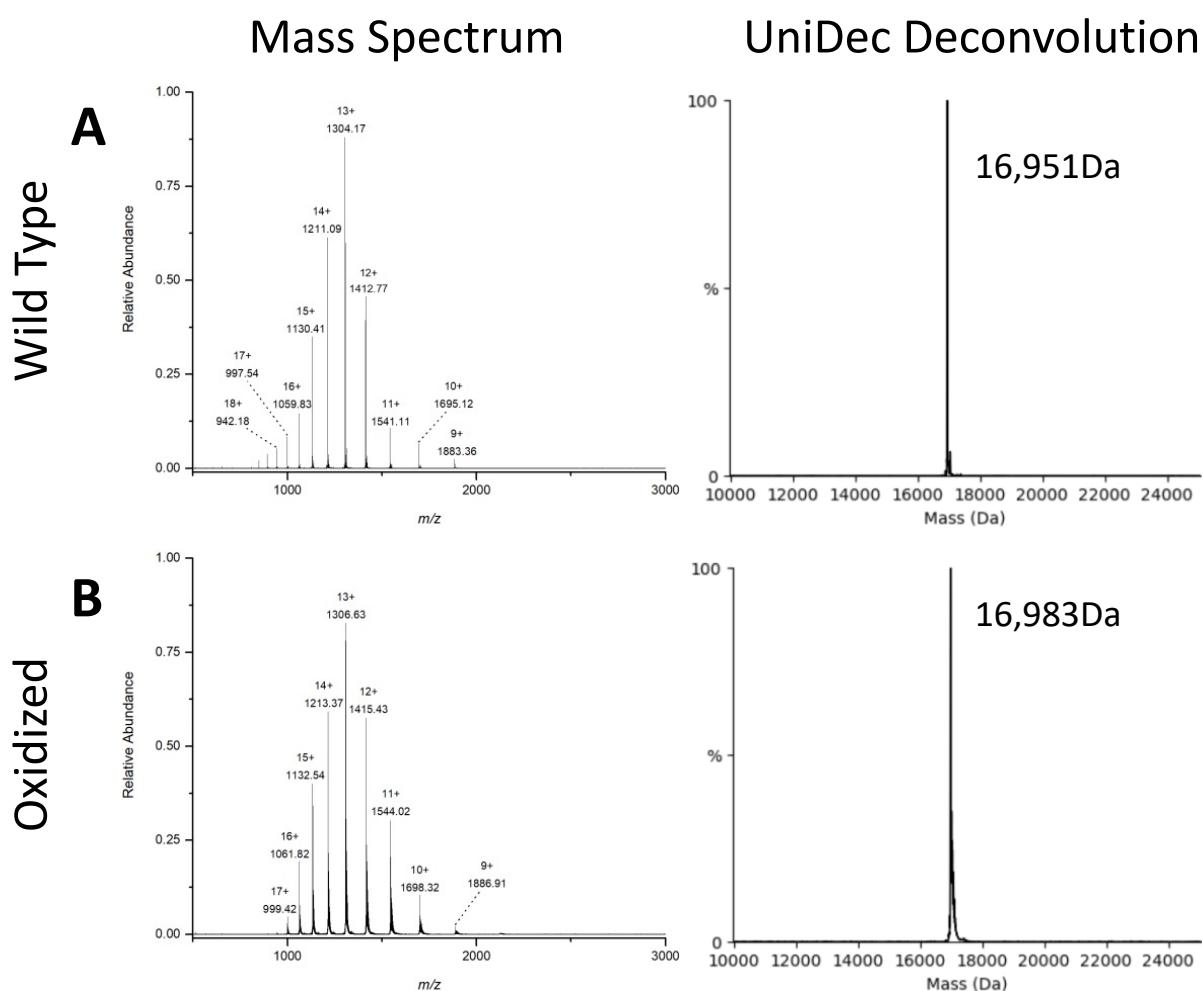


Figure S2. (A) MS1 spectrum of apo-myoglobin with the corresponding UniDec spectrum confirming the mass of wt apo-myoglobin (16,951Da), and (B) a spectrum of oxidized apo-myoglobin with the corresponding UniDec spectrum confirming the mass of oxidized apo-myoglobin (16,983 kDa).¹ The mass shift of 32 Da on oxidized apo-myoglobin indicates the presence of two oxidation sites on the protein.

Fragment location

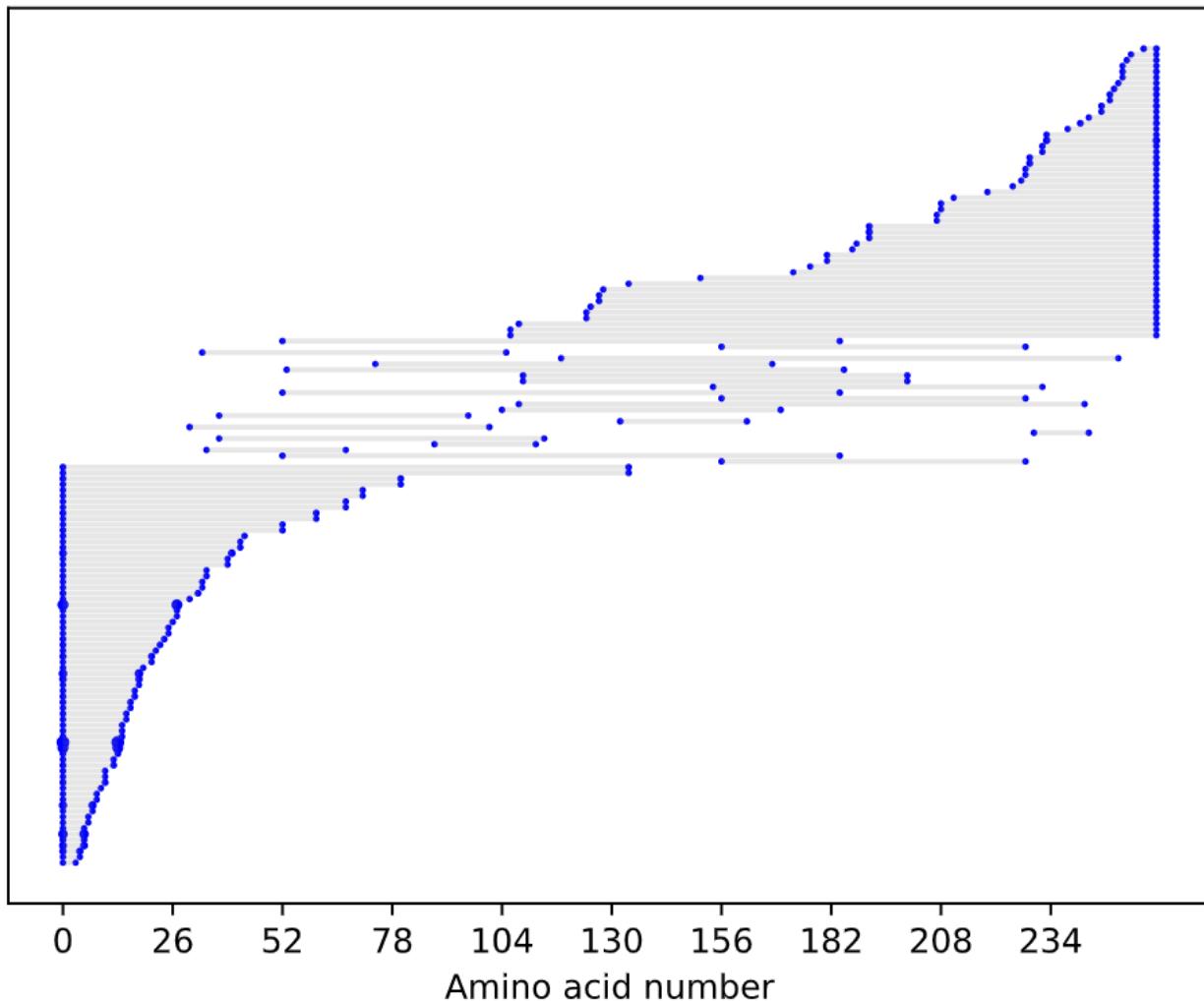


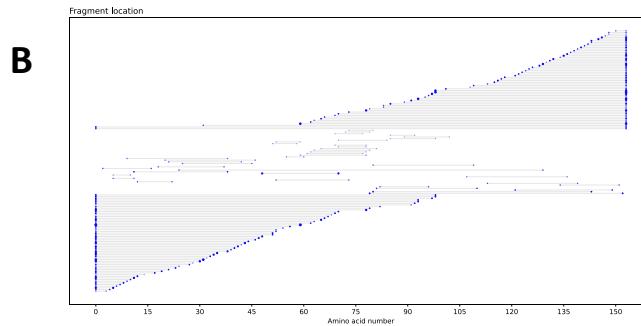
Figure S3: The fragment location figure for a top-down mass spectrum of carbonic anhydrase II. Internal fragments are shown to increase sequence coverage in the center of the protein sequence complementing the terminal fragments.

ECD MS/MS Apo Myoglobin

A

Refer to Figure 2

Fragment Location



Sequence Coverage Map

C

Terminal Fragments

```
GLS DGEWQQLN VWMCKV EADIA G H C
QEVLILR I G H P E T L E K F D K F K H L K
T E A E M K A S E D L K K H G T V V L T I A L G G I
L K K K G H H E A E L K P V A Q S H A T K H R I P
I K Y L E I S D A I I H V L H S K H P G D F G A
D A Q G A M T K A L E L F R N D I A A K Y K E L G
F Q G
```

D

Fragment Cleavage Map

Terminal Fragments

```
G L S D | G | E W | Q | Q | V | L | N | V | W | G | K | V | E | A | D | I | A | G | H | G
Q E | V | L | I | R | L | F | T | G | H | P | E | T | L | E | K | F | D | K | F | K | H | L | K
T | E | A | E | M | K | A | S | E | D | L | K | K | H | G | T | V | V | L | T | A | L | G | G | I
L | K | K | K | G | H | H | E | A | E | L | K | P | L | A | Q | S | H | A | T | K | H | K | I | P
I | K | Y | L | E | F | I | S | D | A | I | X | H | V | L | H | S | K | H | P | G | D | F | G | A
D | A | Q | G | A | M | T | K | A | L | E | L | F | R | N | D | I | A | A | K | Y | K | E | L | G
F | Q | G
```

Internal Fragments

```
GLS DGEWQQLN VWMCKV EAD I D I N G H G
QEVLILR I G H P E T L E K F D K F K H L K
T E A E M K A S E D L K K H G T V V L T I A L G G I
L K K K G H H E A E L K P V A Q S H A T K H R I P
I K Y L E I S D A I I H V L H S K H P G D F G A
D A Q G A M T K A L E L F R N D I A A K Y K E L G
F Q G
```

Figure S4. A. Broadband ECD MS of 20 μ M apo-myoglobin formed from acidic denaturing conditions. B. A fragment location map indicating the region of the protein sequence covered by terminal and internal fragments including fragments with the addition of a hydrogen atom (1.00783Da). C. A sequence coverage map for the terminal and internal fragments also including fragments with the addition of a hydrogen atom (1.00783Da). Darker regions indicate more coverage. D. A fragment cleavage map indicating the location of inter-amino acid cleavage sites for terminal and internal fragments including fragments containing an additional hydrogen atom (1.00783Da).

Table S1. Amino acid residue masses used to calculate theoretical fragments in ClipsMS.²

Amino Acid	Molecular Weight
A	71.037114
R	156.101111
N	114.042928
D	115.026944
C	103.009185
E	129.042594
Q	128.058578
G	57.021464
H	137.058912
I	113.084064
L	113.084064
K	128.094963
M	131.040485
F	147.068414
P	97.052764
S	87.032029
T	101.047679
W	186.079313
Y	163.063329
V	99.068414

Table S2. Sample output of ClipsMS including the fragment type, localized modifications, unlocalized modifications, terminal modifications, observed mass, theoretical mass, start amino acid, end amino acid, error, sequence, intensity, and molecular formula.

Frag Type	Localized Mod	Unlocalized Mod	Term Mod	Observed Mass	Theoretical Mass	Start AA	End AA	Error	Sequence	Intensity	Formula
C Fragment	0	0	0	6675.415628	6675.409316	1	59	0.94556	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGHPETLEKFDKFHKLKEAEMKASE	39886720	C300 H466 N81 O90 S1
C Fragment	0	0	0	3402.755322	3402.755322	1	31	0.03673	GLDGEWQVQLNWGKVEADIGHGQEVLR	277860608	C151 H237 N44 O45 S1
C Fragment	0	0	0	3246.652926	3246.654211	1	30	-0.39579	GLDGEWQVQLNWGKVEADIGHGQEVLI	198412896	C145 H225 N40 O45 S1
C Fragment	0	0	0	8647.629036	8647.629036	1	78	1.11996	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGHPETLEKFDKFHKLKEAEMKASEDLKKHGTVLALTGGILKK	194605216	C39 H627 N101 O113 S1
C Fragment	0	0	0	4802.465087	4802.468927	1	43	-0.79959	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGHPETLEKFDKFHKLKEAEMKASEDLKKHGTVLALTGGILKK	191463006	C218 H334 N59 O64 S1
C Fragment	0	0	0	1230.609567	1230.611331	1	11	-1.43343	GLDGEWQVQL	177542192	C54 H84 N15 O18 S1
C Fragment	0	0	0	447.2195446	447.219797	1	5	-0.54872	GLDG	165365232	C17 H31 N6 O8 S1
C Fragment	0	0	0	3820.976943	3820.976943	1	35	-0.87543	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	160233120	C172 H267 N48 O51 S1
C Fragment	0	0	0	7353.79476	7353.790626	1	65	0.56216	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGHPETLEKFDKFHKLKEAEMKASEDLKKH	148447040	C330 H516 N91 O98 S1
C Fragment	0	0	0	3763.8952	3763.895479	1	34	-0.92429	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	140507136	C170 H264 N47 O50 S1
C Fragment	0	0	0	5457.813483	5457.813123	1	48	0.05696	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	129385312	C249 H379 N68 O71 S1
C Fragment	0	0	0	4184.12768	4184.131213	1	38	-0.84438	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	13538350	C188 H288 N53 O56 S1
C Fragment	0	0	0	4527.910635	4527.910559	1	41	-0.86475	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	122917280	C20 D13 H31 N65 O62 S1
C Fragment	0	0	0	5320.755681	5320.754211	1	47	0.27628	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	104063632	C243 H372 N65 O70 S1
C Fragment	0	0	0	1344.654492	1344.654259	1	12	0.17328	GLDGEWQVQL	89008484	C58 H91 N17 O20 S1
C Fragment	0	0	0	4398.256538	4398.262956	1	40	-0.145921	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	86289096	C199 H336 N55 O59 S1
C Fragment	0	0	0	5800.036508	5800.033929	1	51	-0.57258	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	77548496	C265 H405 N72 O75 S1
C Fragment	0	0	0	890.399615	890.400275	1	8	-0.74517	GLDGEWQ	74813128	C38 H56 N11 O14 S1
C Fragment	0	0	0	2114.062323	2114.066535	1	19	-1.19237	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	72110784	C95 H145 N26 O29 S1
C Fragment	0	0	0	4917.496342	4917.495871	1	44	0.09578	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	72101728	C222 H339 N60 O67 S1
C Fragment	0	0	0	10310.904928	10310.888028	1	93	1.15446	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	71896952	C464 H741 N130 O134 S1
C Fragment	0	0	0	10875.82815	10875.82171	1	98	0.915179	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	71311528	C499 W78 N139 O140 S1
C Fragment	0	0	0	7766.059605	7766.059197	1	69	0.05254	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	69683056	C350 H552 N95 O103 S1
C Fragment	0	0	0	7454.839638	7454.838305	1	66	0.18485	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	66992788	C334 H52 N93 O101 S1
C Fragment	0	0	0	2470.235109	2470.236121	1	23	-0.40968	GLDGEWQVQLNWGKVEADIGHG	66749252	C110 H169 N30 O35 S1
C Fragment	0	0	0	10747.73932	10747.72675	1	97	1.17001	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	63984386	C487 H277 N137 O139 S1
C Fragment	0	0	0	1913.985537	1913.986827	1	17	-0.67399	GLDGEWQVQLNWGKVEADIGHG	63687832	C87 H133 N24 Q50 S1
C Fragment	0	0	0	3662.904928	3662.9078	1	33	-0.78408	GLDGEWQVQLNWGKVEADIGHGQEVLR	60314392	C166 H257 M46 O48 S1
C Fragment	0	0	0	6388.294862	6388.297579	1	56	-0.42531	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	5869976	C289 H449 N78 O84 S1
C Fragment	0	0	0	10173.482917	10173.482917	1	92	-0.18679	GLDGEWQVQL	58782672	C458 H734 N127 O133 S1
C Fragment	0	0	0	5192.649935	5192.659248	1	46	-1.79349	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	46571404	C237 H360 N63 O69 S1
C Fragment	0	0	0	2921.416012	2921.417669	1	27	-0.56719	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	46013272	C238 H119 N37 O42 S1
C Fragment	0	0	0	7552.906906	7553.906719	1	67	0.02476	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	44378872	C39 H52 N93 O101 S1
C Fragment	0	0	0	2342.174462	2342.177543	1	21	-1.31544	GLDGEWQVQLNWGKVEADIGHG	40127252	C105 H161 N28 O33 S1
C Fragment	0	0	0	1117.526602	1117.527267	1	10	-0.55956	GLDGEWQVQ	38401180	C48 H73 N14 017 S1
C Fragment	0	0	0	7031.625444	7031.615287	1	62	0.14448	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	38162766	C316 H449 N85 O95 S1
C Fragment	0	0	0	762.3415546	762.341567	1	7	-0.18679	GLDGEW	36994952	C33 H46 N9 011 S1
C Fragment	0	0	0	390.1985072	390.198326	1	41	0.46438	GLDSD	33560796	C15 H26 N5 07 S1
C Fragment	0	0	0	576.2620209	576.262384	1	61	-0.63009	GLDSDGE	30180068	C228 H87 N7 011 S1
C Fragment	0	0	0	4285.174033	4285.178892	1	39	-1.13391	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	29675962	C192 H295 N54 O58 S1
C Fragment	0	0	0	9106.849359	9106.863287	1	82	-0.5294	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	28001424	C411 H656 N115 O117 S1
C Fragment	0	0	0	1018.458484	1018.458853	1	9	-0.39766	GLDGEWQWO	25162260	C43 H64 N13 016 S1
C Fragment	0	0	0	5929.083034	5929.082423	1	52	0.14859	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	20696572	C270 H416 N73 O78 S1
C Fragment	0	0	0	6129.173701	6129.162133	1	54	0.17496	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	20245026	C278 H423 N75 O82 S1
C Fragment	0	0	0	7159.709381	7159.71025	1	63	-0.12137	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	19655936	C322 H506 N87 O96 S1
C Fragment	0	0	0	2607.293471	2607.295033	1	24	-0.55909	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	13978781	C116 H176 N33 O36 S1
C Fragment	0	0	0	275.171929	275.171382	1	31	1.36568	GLS	10444626	C11 H23 N4 04 S1
C Fragment	0	0	0	4655.396281	4655.400513	1	42	-0.90505	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	10153317	C40 H355 N85 O63 S1
C Fragment	0	0	0	7652.967559	7652.975133	1	68	-0.38968	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	8005857	C344 H514 N94 0102 S1
C Fragment	0	0	0	3020.485035	3020.486083	1	28	-0.34696	GLDGEWQVQLNWGKVEADIGHGQEVLR	7187482	C133 H203 N8 043 S1
C Fragment	0	0	0	3515.837402	3515.839386	1	32	-0.5643	GLDGEWQVQLNWGKVEADIGHGQEVLRFTGH	6456200	C157 H248 N45 O47 S1
C Fragment	0	0	0	1629.799519	1629.801986	1	14	-1.51368	GLDGEWQVQLNW	2774463	C74 H109 N20 O22 S1
Z Fragment	0	0	0	6633.499352	6633.499352	94	153	-0.02713	ATKHKPIKYLFISDAIIHLVSKHKGPDGFQDGAQGMATKALEFRNDIAAKYKELFGQ	245264208	C305 H474 N80 O84 S1
Z Fragment	0	0	0	6196.264101	6196.260684	98	153	0.05546	ATKHKPIKYLFISDAIIHLVSKHKGPDGFQDGAQGMATKALEFRNDIAAKYKELFGQ	199922288	C236 H443 N73 O79 S1
Z Fragment	0	0	0	2698.415629	2698.41476	130	153	0.33935	ATMKALEFRNDIAKYKELFGQ	199808704	C123 H619 N31 O35 S1
Z Fragment	0	0	0	6068.163995	6068.165721	99	153	-0.28444	IPKYLEFISDAIIHLVSKHKGPDGFQDGAQGMATKALEFRNDIAAKYKELFGQ	193644056	C280 H443 N71 O78 S1
Z Fragment	0	0	0	2083.073297	2083.073297	136	153	0.12626	ELFRNDIAKYKELFGQ	150563984	C96 H46 N24 Q28 S1
Z Fragment	0	0	0	762.390658	762.390658	147	153	0.86124	KELFGQ	140075296	C35 H58 N8 011 S1
Z Fragment	0	0	0	925.4514015	925.453987	146	153	0.12372	YKELFGQ	131402606	C44 H66 N9 013 S1
Z Fragment	0	0	0	2395.290142	2395.289438	133	153	0.29391	KALELFRNDIAAKYKELFGQ	123460160	C111 H174 N28 O31 S1
Z Fragment	0	0	0	2755.438676	2755.436186	129	153	0.90585	GMATKALELFRNDAIAKYKELFGQ	112769520	C125 H198 N32 O36 S1
Z Fragment	0	0	0	3965.966715	3965.972884	117	153	-1.55548	SKHKGPDGDAQAMATKALELFRNDIAAKYKELFGQ	100691576	C177 H272 N48 O54 S1
Z Fragment	0	0	0	1954.030764	1954.030703	137	153	-0.17349	LFRNDIAAKYKELFGQ	9279708	C91 H139 N23 O25 S1
Z Fragment	0	0	0	6857.599054	6857.599293	92	153	1.27756	SHATKHPIKYLFISDAIIHLVSKHKGPDGFQDGAQGMATKALELFRNDIAAKYKELFGQ	86512464	C43 H464 N86 O87 S1
Z Fragment	0	0	0	7508.0501024	7508.001884	86	153	1.10243	LXPLASHTKHPIKYLFISDAIIHLVSKHKGPDGFQDGAQGMATKALELFRNDIAAKYKELFGQ	86432406	C345 H540 N92 O94 S1
Z Fragment	0	0	0	1423.733177	1423.734186	141	153	-0.73679	DIAKYKELFGQ	80702824	C66 H101 N15 O20 S1
Z Fragment	0	0	0	3750.842668	3750.8454592	119	153	-0.85954	HKGPDGFQDGAQGMATKALELFRNDIAAKYKELFGQ	59094216	C168 H255 N45 O51 S1
Z Fragment	0	0	0	4103.026667	4103.031976	116	153	-0.25005	HSKHPGDFQDGAQGMATKALELFRNDIAAKYKELFGQ	5887560	C136 H219 N15 O51 S1
Z Fragment	0	0	0	9590.5993033	9590.596803	66	153	0.67778	TVLTLALGGILKKKGHHAEELPLAQASHATKHPIKYLFISDAIIHLVSKHKGPDGFQDGAQGMATKALELFRNDIAAKYKELFGQ	56931200	C439 H699 N119 O120 S1
Z Fragment	0	0	0	2496.341564	2496.337117	132	153	1.18341	TKALELFRNDIAAKYKELFGQ	5520912	C115 H181 N29 O33 S1
Z Fragment	0	0	0	3459.709664	3459.712752	122	153	-0.18256	DFGDAQAMATKALELFRNDIAAKYKELFGQ	55053328	C155 H228 N40 O48 S1
Z Fragment	0	0	0	5744.936969	5744.944829	102	153	-1.36816	YKELFISDAIIHLVSKHKGPDGFQDGAQGMATKALELFRNDIAAKYKELFGQ	52084328	C263 H402 N68 O75 S1
Z Fragment	0	0	0	3344.68812	3344.685808	123	153	0.693125	FGADAGAMATKALELFRNDIAAKYKELFGQ	49979400	C151 H23 N39 O45 S1
Z Fragment	0	0	0	3878.940855	3878.940855	118	153	-0.6806	KHPGDFQDGAQGMATKALELFRNDIAAKYKELFGQ	45241468	C174 H267 N47 O52 S1
Z Fragment	0	0	0	9912.384769	9912.372142	63	153	1.27386	KGTVLTALGGILKKKGHHAEELPLAQASHATKHPIKYLFISDAIIHLVSKHKGPDGFQDGAQGMATKALELFRNDIAAKYKELFGQ	42212112	C453 H72 N125 O123 S1
Z Fragment	0	0	0	3069.556017	3069.558816	126	153	-0.91186	DAQGMATKALELFRNDIAAKYKELFGQ	40466522	C127 H216 N26 O42 S1
Z Fragment	0	0	0	1195.620887	1195.621378	143	153	-1.91616	TKALELFRNDIAAKYKELFGQ	3836912	C56 H85 N13 O16 S1
Z Fragment	0	0	0	2954.528513	2954.531872	1					

Z.Fragment	0	0	0	1537.776592	1537.777114	140	153	-0.33945	NDIAAKYKELGFQG		22523802	C70 H107 N17 O22 S0
Z.Fragment	0	0	0	1840.945689	1840.946639	138	153	-0.51604	FRNDIAAKYKELGFQG		22165450	C85 H128 N22 O24 S0
Z.Fragment	0	0	0	1124.585088	1124.586064	144	153	-0.86787	AKYKELGFQG		22041524	C53 H80 N12 O15 S0
Z.Fragment	0	0	0	3140.595896	3140.59593	125	153	-0.01083	ADAGAMKTAKALEFRNDIAAKYKELGFQG		20005332	C140 H221 N37 O43 S1
Z.Fragment	0	0	0	4315.184274	4315.184274	114	153	-0.24518	VLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQG		18964876	C149 H299 N53 O57 S1
Z.Fragment	0	0	0	9784.185342	9784.217719	64	153	0.8323	HGTIVLTLGGILKKKGHEAELPLAQSHATKHIPKYLEFISDAIIHLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQG		12819766	C447 H709 N123 O122 S1
Z.Fragment	0	0	0	7708.078568	7708.081548	84	153	-0.38661	AELKPLAQSHATKHIPKYLEFISDAIIHLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQG		12603535	C35 H552 N94 O98 S1
Z.Fragment	0	0	0	8168.267095	8168.26343	80	153	0.44869	GHHEAELPLAQSHATKHIPKYLEFISDAIIHLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQG		12411123	C372 H576 N102 O104 S1
Z.Fragment	0	0	0	2196.154896	2196.157361	135	153	-1.12342	LELFRNDAIAKYKELGFQG		11926676	C102 H157 N25 O29 S0
Z.Fragment	0	0	0	4864.470212	4864.475372	109	153	-1.06075	DAIIHLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQG		7441562	C219 H338 N60 O64 S1
Z.Fragment	0	0	0	2627.37608	2627.377602	131	153	-0.57928	MTKAELFRNDIAAKYKELGFQG		6900706	C120 H190 N20 O24 S1
Z.Fragment	0	0	0	505.252515	505.253101	149	153	-1.08757	LGFGQ		6823282	C24 H35 N5 O7 S0
CZ.Int.Fragm	0	0	0	1125.592217	1125.593889	105	114	-1.48544	EHSDAIHV		86469552	C53 H81 N12 O15 S0
CZ.Int.Fragm	0	0	0	8109.24979	8109.250125	80	152	-1.86747	GHHEAELPLAQSHATKHIPKYLEFISDAIIHLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQG		7733236	C370 H573 N102 O102 S1
CZ.Int.Fragm	0	0	0	6987.649429	6987.663178	81	143	-1.96761	HHHEAELPLAQSHATKHIPKYLEFISDAIIHLHSKHPGDFGADAQAMTKALEFRNDIA		58335840	C317 H494 N89 O88 S1
CZ.Int.Fragm	0	0	0	8776.734388	8776.724606	74	152	1.11454	GILKKKGHHEAELPLAQSHATKHIPKYLEFISDAIIHLHSKHPGDFGADAQAMTKALEFRNDIAAKYKELGFQ		55233628	C40 H634 N111 O108 S1
CZ.Int.Fragm	0	0	0	3068.549188	3068.550991	122	149	-0.58757	DFGADAGAMTKALEFRNDIAAKYKEL		31020380	C137 H215 N36 O42 S1
CZ.Int.Fragm	0	0	0	3296.772817	3296.779016	82	110	-1.88032	HEAELPLAQSHATKHIPKYLEFISDA		25602240	C156 H239 N40 O42 S0
CZ.Int.Fragm	0	0	0	1504.813291	1504.811822	83	96	0.9762	EAELPLAQSHATK		15867519	C66 H110 N19 O21 S0
CZ.Int.Fragm	0	0	0	2009.089113	2009.085478	135	151	1.80928	LELFRNDAIAKYKELGF		15034973	C95 H146 N23 O25 S0
CZ.Int.Fragm	0	0	0	2778.412683	2778.414436	114	139	-0.63094	VLHSKHPGDFGADAQAMTKALEFR		14319574	C124 H193 N36 O35 S1
CZ.Int.Fragm	0	0	0	1069.567559	1069.567674	13	22	-0.10752	VWVGKVEADIA		13795592	C50 H77 N12 O14 S0
CZ.Int.Fragm	0	0	0	3363.806619	3363.809331	44	74	-0.80623	DIFKHLKTEAE MKASEDLKKHGTVLTLGG		12841460	C149 H248 N41 O45 S1
CZ.Int.Fragm	0	0	0	2180.174515	2180.174371	53	73	0.06605	AEMKASEDLKKHGTVLTLG		12616580	C95 H163 N26 O30 S1
CZ.Int.Fragm	0	0	0	784.398022	784.398817	6	11	-1.01352	EWQQL		12085659	C37 H54 N9 O10 S0
CZ.Int.Fragm	0	0	0	2998.489568	2998.482394	108	136	1.86561	SDAIHVLHSKHPGDFGADAQAMTKALE		7827239	C131 H205 N38 O41 S1
CZ.Int.Fragm	0	0	0	671.3144366	671.314753	6	10	-0.47131	EWQQL		4593338	C31 H43 N8 O9 S0

Table S3: ClipsMS Processing Times for Myoglobin with Different Parameters

Protein	Fragments Searched	PPM Error	Minimum Internal AA length	Localized Modification	Unlocalized Modification	Time(sec)
Myoglobin	c/z/cz	2	5	None	None	26.31
Myoglobin	c/z/cz	5	5	None	None	29.02
Myoglobin	c/z/cz	2	3	None	None	27.22
Myoglobin	c/z/cz	2	5	None	H+	49.6
Myoglobin	c/z/cz	5	5	None	H+	57.61
Myoglobin	c/z/cz	2	3	None	H+	51.84
Myoglobin	All Fragments	2	5	None	H+	427.95
Myoglobin	All Fragments	5	5	None	H+	636.12
Myoglobin	All Fragments	2	3	None	H+	483.52
Myoglobin	c/z/cz	2	5	2 Oxidation sites	H+	80.09
Myoglobin	All Fragments	2	5	2 Oxidation Sites	H+	657.99

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