Supporting Information:

Developing a Novel Acidic Residue Reactive and Sulfoxide-containing MS-cleavable Homobifunctional Cross-linker for Probing Protein-Protein Interactions

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SUPPLEMENTAL METHODS

Dihydrazide Sulfoxide (DHSO) Synthesis

Disuccinimidyl sulfoxide (DSSO) was synthesized as previously published [1]. The 2-step synthesis scheme for DHSO from DSSO is depicted in Figure 1D. Briefly, *tert*-butyl carbazate (1.10 g, 8.32 mmol) was added to DSSO (1.41 g, 4.16 mmol) in DCM (50 mL). The resulting yellow solution was let stir at room temperature for 12 h, after which trifluoroacetic acid (2.20 mL, 28.7 mmol) was added. The resulting orange solution was let stir for 72 h before removing the solvent in vacuo. The resulting orange oil was dissolved in methanol, and then triethylamine was added. The resulting mixture was let stir for 20 mins, after which a white solid had precipitated. The solid was collected via centrifuge, and then stirred with fresh methanol for 20 mins. The solid was collected via centrifuge again, and this process of stirring with fresh methanol was repeated another two times. Drying the isolated white solid in vacuo afforded DHSO (0.375 g, 46%): mp 159–162 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.13 (s, 2H), 4.24 (s, 4H), 3.0 –2.97 (m, 2H), 2.83–2.75 (m, 2H), 2.43 (t, J = 7.5 Hz, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.3, 46.7, 26.2; IR (thin film): 3308, 3044, 1631, 1449, 1297, 1032 cm^{-1}; HRMS (ESI) m/z calculated for $C_6H_{15}N_4O_3S$ [M + H]$^+$ 223.0865, found 223.0857.

Digestion of DHSO Cross-linked Proteins

For in-gel digestion, cross-linked proteins were separated by SDS-PAGE and visualized by Coomassie blue staining. The selected cross-linked gel bands were excised, reduced with TCEP for 30 min, alkylated with iodoacetamide for 30 min in the dark, and then digested with trypsin at 37°C overnight. Peptide digests were extracted, concentrated, and reconstituted in 3% ACN/2% formic acid for MS^n analysis. For in-solution digestion, cross-linked proteins were first precipitated with TCA and then re-suspended in 8M urea buffer. Reduction and alkylation were

performed prior to Lys-C/trypsin digestion as previously described [2]. The resulting digests were desalted using Waters C18 Sep-Pak cartridges and fractionated by peptide size exclusion chromatography (SEC) based on the protocol by Leitner et al. [3]. The fractions containing cross-linked peptides were collected for subsequent MS^n analysis.

Liquid Chromatography-Multistage Tandem Mass Spectrometry (LC MS^n) Analysis

Each MS^n experiment consists of one MS scan in FT mode (350-1400 m/z, resolution of 60,000 at m/z 400) followed by two data-dependent MS^2 scans in FT mode (resolution of 7500) with normalized collision energy at 10% on the top two MS peaks with charges 4+ or higher, and three MS^3 scans in the LTQ with normalized collision energy at 35% on the top three peaks from each MS^2.

Data Analysis and Identification of DHSO Cross-linked Peptides

MS^n Data extraction and analysis were performed in the same way as previously described [4]. MS^3 data was subjected to a developmental version of Protein Prospector (v.5.16.0) for database searching, using Batch-Tag against SwissProt.2014.12.4.random.concat databases limited to either the *Bos taurus* or *Equus caballus* taxonomy with mass tolerances for parent ions and fragment ions set as ±20 ppm and 0.6 Da, respectively. Trypsin was set as the enzyme with four maximum missed cleavages allowed. Cysteine carbamidomethylation was set as a constant modification. A maximum of four variable modifications were also allowed, including protein N-terminal acetylation, methionine oxidation, and N-terminal conversion of glutamine to pyroglutamic acid. In addition, three defined modifications representing cross-linker fragment moieties on aspartic acid and glutamic acid were selected: alkene (A, $C_3H_4N_2$, +68 Da), sulfenic

acid (S, $C_3H_6N_2SO$, +118 Da), and unsaturated thiol (T, $C_3H_4N_2S$, +100 Da) modifications. Initial acceptance criteria for peptide identification required a reported expectation value ≤ 0.1. The in-house program XL-Discoverer, a revised version of previously developed Link-Hunter, was used to validate and summarize cross-linked peptides based on MS^n data and database searching [1].

SUPPLEMENTAL FIGURES:
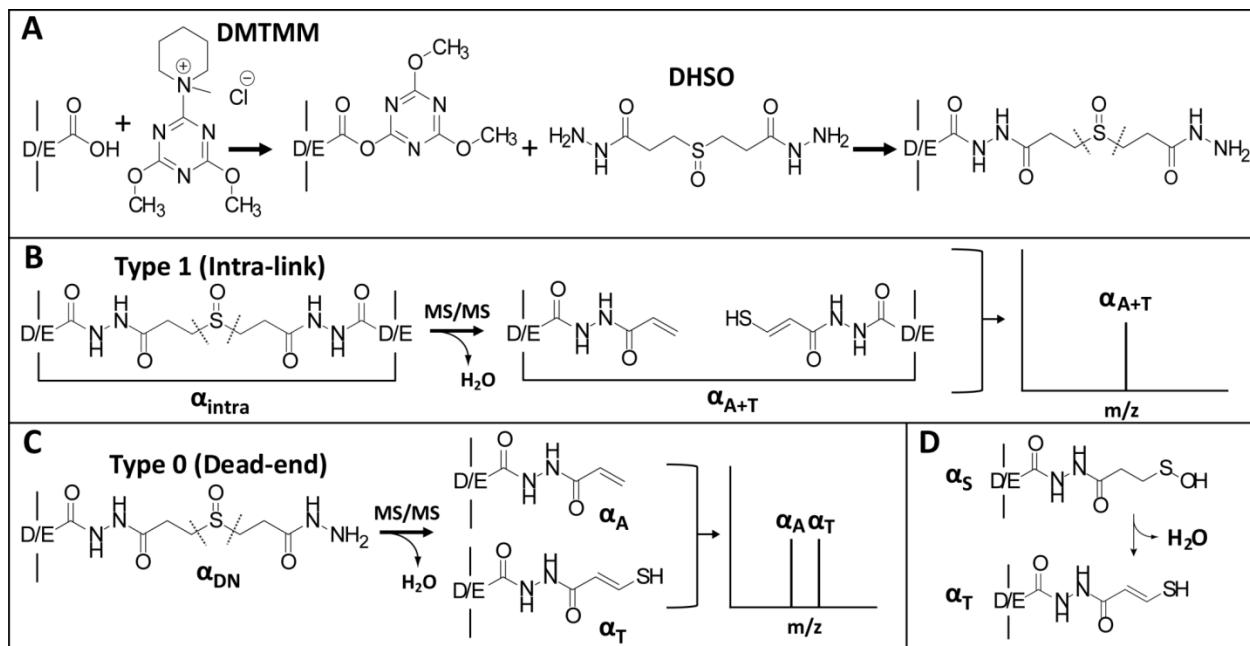
Figure S-1. Characteristic MS^2 fragmentation patterns for DHSO cross-linked peptides. (A) The scheme of peptide cross-linking by DHSO in the presence of DMTMM. MS^2 fragmentation of (B) DHSO intra-linked peptide α_{intra}. (C) Dead-end modified peptide α_{DN}. (D) The conversion scheme of α_S to α_T. Note: S* (sulfenic acid moiety) can be converted to the more stable unsaturated thiol moiety (T) via water loss as shown in (D).
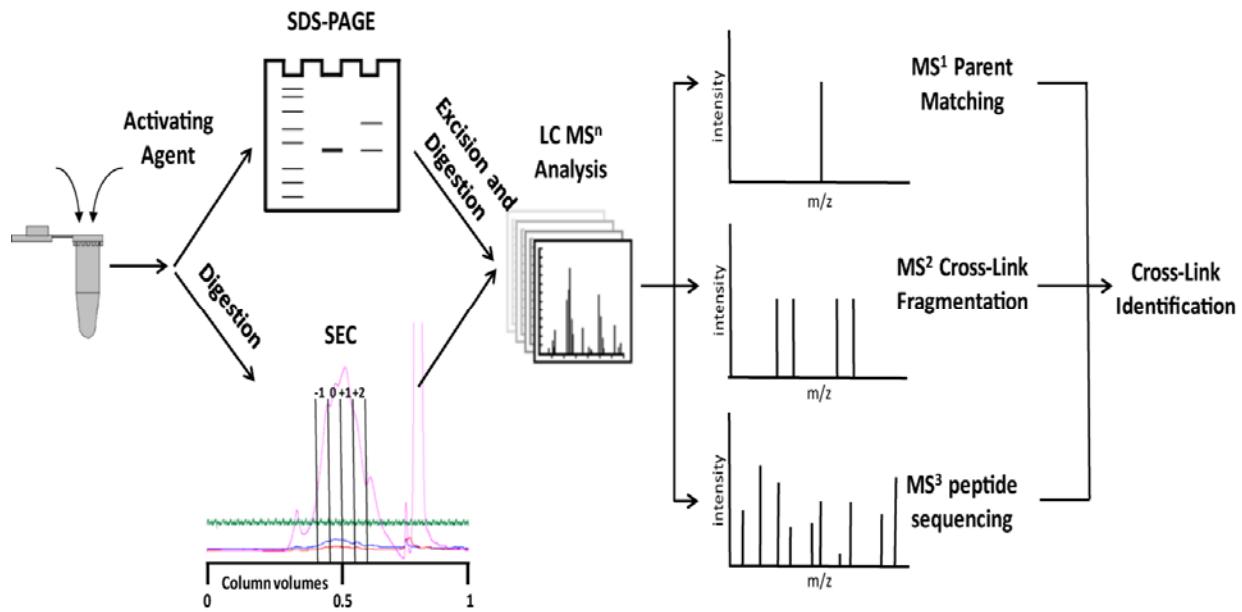
Figure S-2. The general XL-MS workflow for the identification of cross-linked DHSO peptides from proteins.
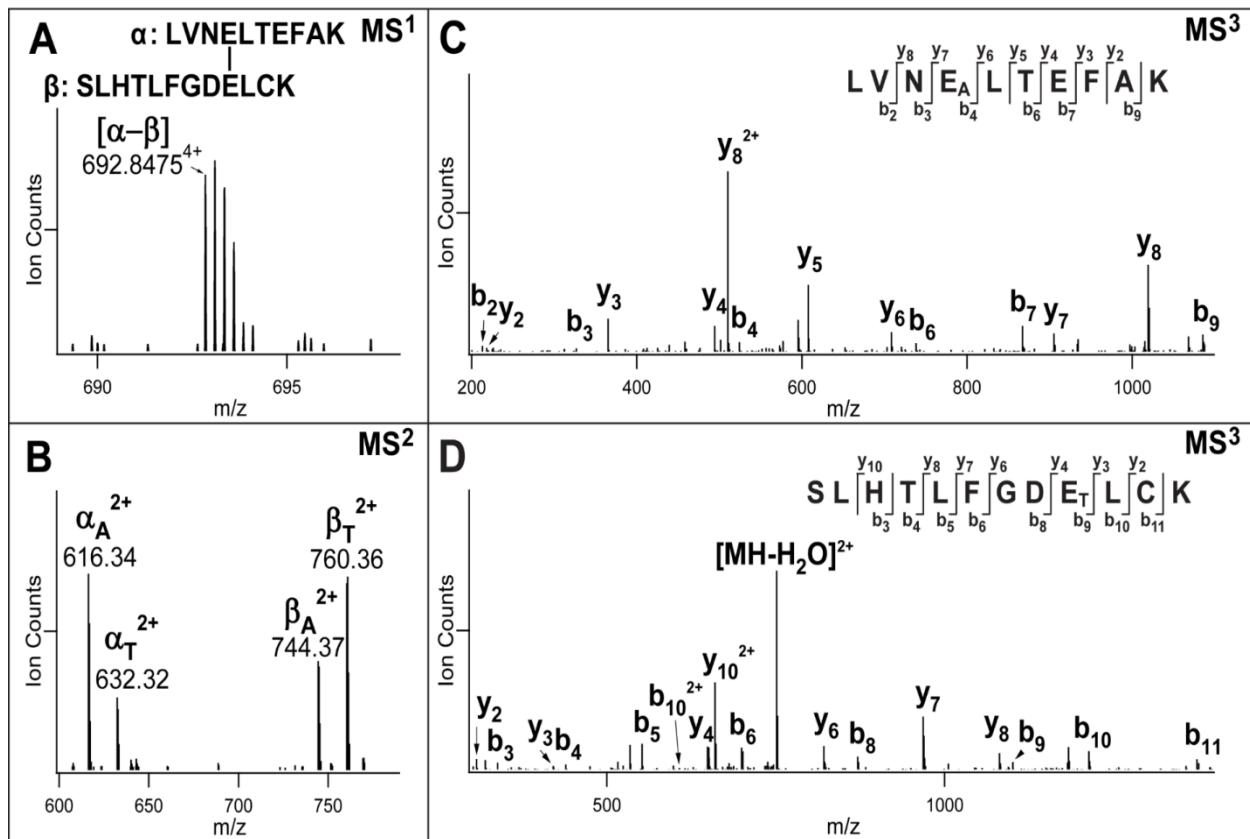
Figure S-3. MSⁿ analysis of a representative DHSO inter-linked BSA peptide. (A) MS spectrum of a DHSO interlinked BSA peptide $\alpha-\beta$ (m/z 692.8475^{4+}). (B) MS^2 spectrum of the cross-linked peptide detected in (A). MS^3 spectra of MS^2 fragment ions (C) α_A (m/z 616.34^{2+}) and (D) β_T (m/z 760.36^{2+}).
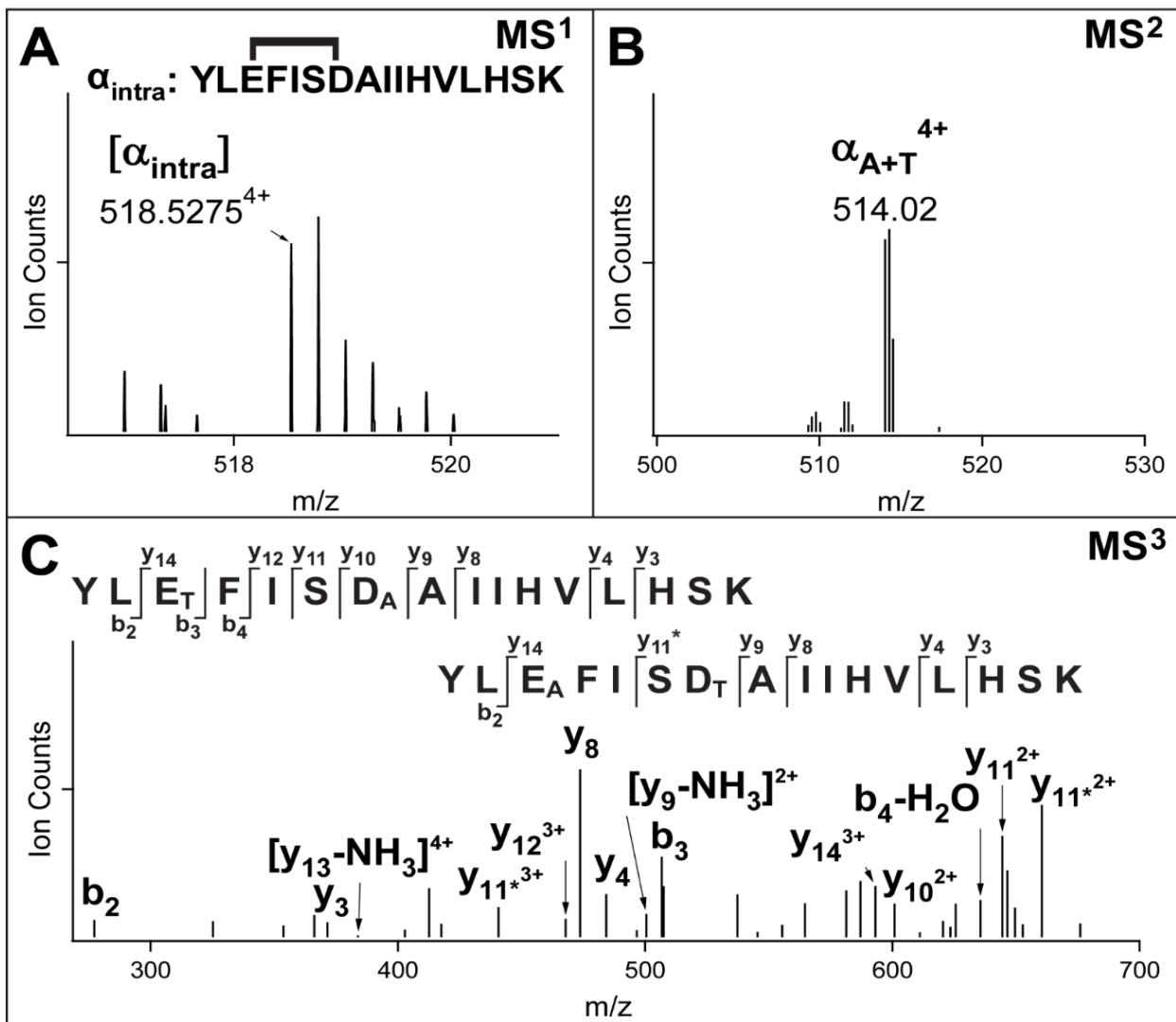
Figure S-4. MS^n analysis of a DHSO intra-linked myoglobin peptide. (A) MS spectrum of a DHSO intra-linked myoglobin peptide α_{intra} (m/z 518.5275^{4+}). (B) MS^2 spectrum of the intra-linked peptide in (A). (C) MS^3 spectrum of the MS^2 fragment ion α_{A+T} (m/z 514.02^{4+}).
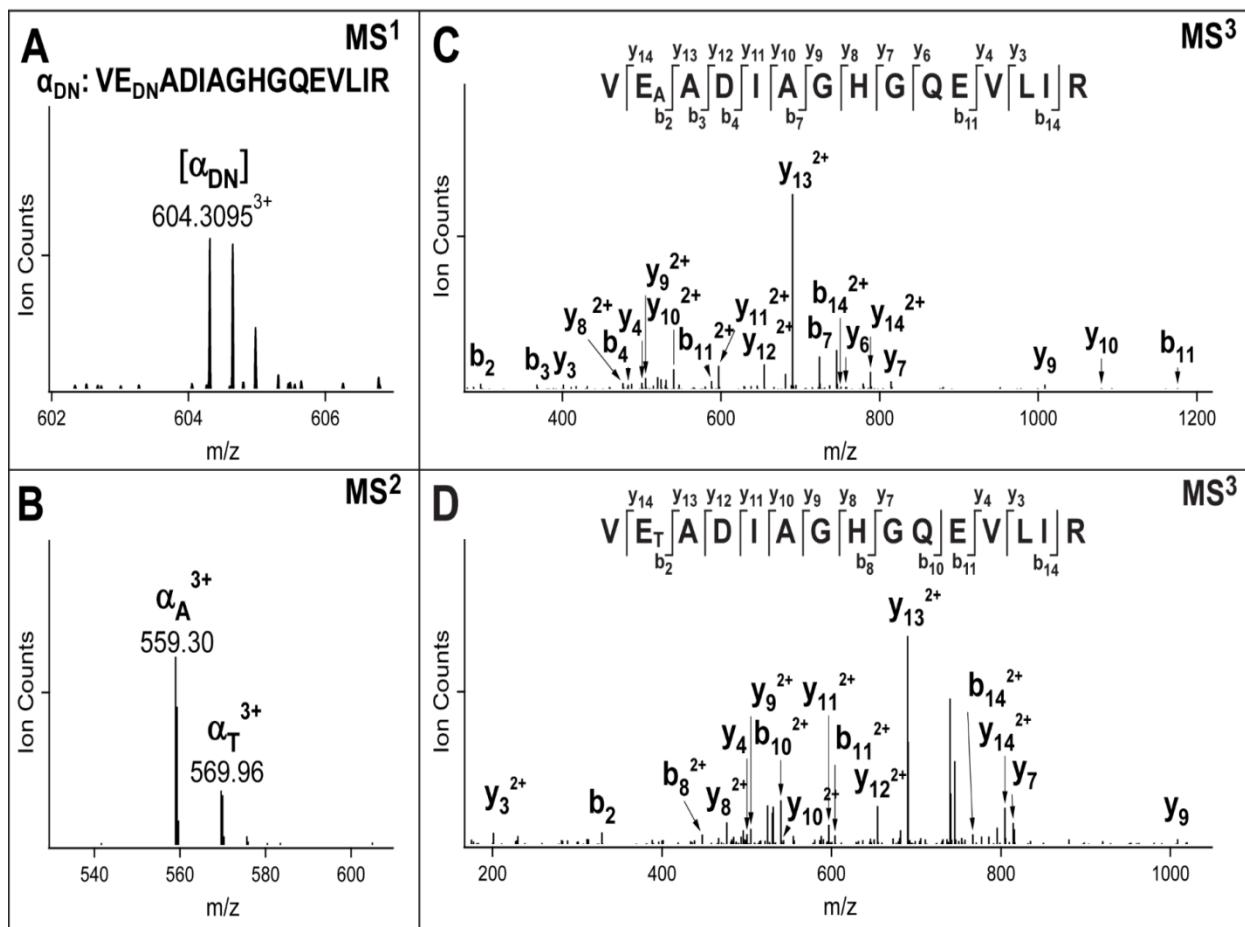
Figure S-5. MS^n analysis of a DHSO dead-end modified myoglobin peptide. (A) MS spectrum of a DHSO dead-end modified myoglobin peptide α_{DN} (604.3095^{3+}). (B) MS^2 spectrum of the parent ion detected in (A). MS^3 spectra of MS^2 fragment ions (C) α_A (m/z 559.30^{3+}) and (D) α_T (m/z 569.96^{3+}).
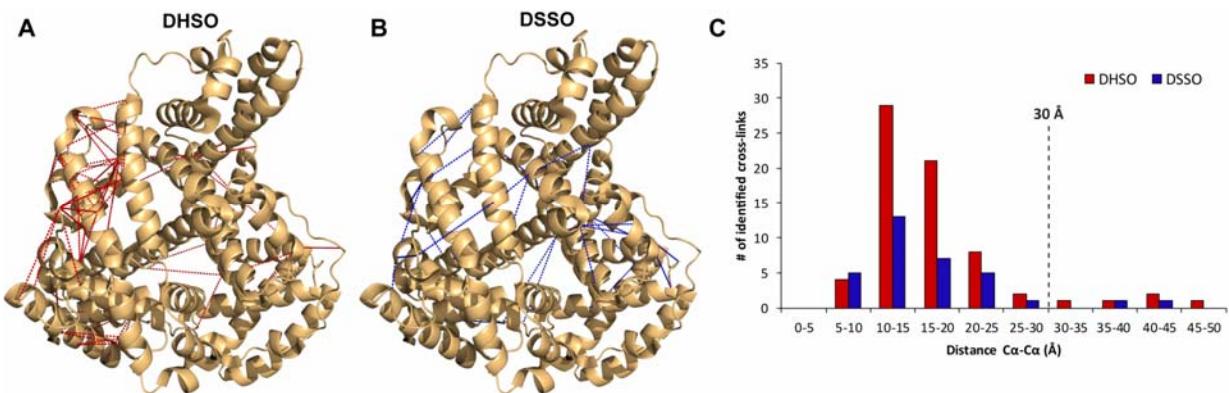
Figure S-6. BSA cross-link maps on its crystal structure (PDB: 4F5S). (A) with DHSO cross-link map (red). (B) DSSO cross-link map (blue). (C) The distribution plot of identified linkages vs. their spatial distances between D|E-D|E for DHSO (red) or K-K for DSSO (blue) in BSA structure.

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Table S-1. Detailed Summary of DHSO Inter-Linked Myoglobin Peptides Identified by LC MS^n

#	Peptide Seq	AA location	MS m/z	z	Δ(PPM)	Mod. Position	MS2 m/z	z	Distance (Cα-Cα)
1	ALELFR ALELFR	A135-R140 A135-R140	421.2357	4	0.186	E_T137 E_A137	424.726 408.740	2 2	-
2	HPGDFGADAQGAM(ox)TK ALELFR	H120-K134 A135-R140	613.7913	4	-2.547	$D_A123\|D_A127$ E_A137	793.885 408.740	2 2	21.6Å|15.4Å
3	HPGDFGADAQGAMTK ALELFR	H120-K134 A135-R140	609.7944	4	.435	D_A127 E_A137	785.859 408.741	2 2	15.4Å
4	KKGHHEAELKPLAQSHATK ELGFQG	K79-K97 E149-G154	737.1354	4	1.188	$E_A84\|E_A86$ E_A149	726.736 718.335	3 1	14.1Å|16.8Å
5	LFTGHPETLEK ALELFR	L33-K43 A135-R140	441.8353	5	-0.494	E_T42 E_A137	457.895 408.740	3 2	24.4Å
6	LFTGHPETLEK ALELFR	L33-K43 A135-R140	441.8364	5	1.996	$E_A39\|E_A42$ E_A137	447.239 408.740	3 2	22.0Å|24.4Å
7	LFTGHPETLEK TEAEM(ox)K	L33-K43 T52-K57	437.0133	5	2.713	$E_T39\|E_T42$ E_A53	437.895 396.682	3 2	14.6 Å|14.7Å
8	LFTGHPETLEK TEAEMK	L33-K43 T52-K57	542.0137	4	-1.652	E_T42 $E_A53\|E_A55$	686.342 388.685	2 2	14.7Å|15.4Å
9	LFTGHPETLEK TEAEM(ox)K	L33-K43 T52-K57	546.2628	4	-3.499	E_T39 E_A53	686.341 396.682	2 2	14.6Å
10	LFTGHPETLEK TEAEM(ox)K	L33-K43 T52-K57	546.0119	4	-2.068	E_T42 E_A53	686.341 396.682	2 2	14.7Å
11	LFTGHPETLEK TEAEMK	L33-K43 T52-K57	433.8133	5	0.389	E_T42 E_A55	457.896 388.685	3 2	15.4Å
12	LFTGHPETLEK LFTGHPETLEK	L33-K43 L33-K43	546.4794	5	-3.081	$E_T39\|E_T42$ $E_A39\|E_A42$	686.340 447.238	2 3	-
13	LFTGHPETLEK LFTGHPETLEK	L33-K43 L33-K43	683.1003	4	-0.109	$E_A39\|E_A42$ E_T42	670.355 686.339	2 2	-
14	LFTGHPETLEK LFTGHPETLEK	L33-K43 L33-K43	682.8481	4	-2.906	E_A42 E_T42	670.354 686.340	2 2	-
15	LFTGHPETLEK LFTGHPETLEK	L33-K43 L33-K43	683.3470	4	-6.165	E_A39 E_T42	670.355 686.339	2 2	-
16	TEAEM(ox)K ALELFR	T52-K57 A135-R140	415.2066	4	0.561	E_A53 E_T137	396.681 424.725	2 2	28.0Å
17	TEAEMK TEAEMK	T52-K57 T52-K57	401.1799	4	0.611	E_A53 E_T53	388.684 404.67	2 2	-
18	TEAEMK ASEDLK	T52-K57 A58-K63	421.7067	4	1.031	$E_A53\|E_A55$ $E_T60\|D_T61$	388.684 445.722	2 2	13.0Å|9.8Å 14.0Å|11.0Å
19	TEAEMK ASEDLKK	T52-K57 A58-K64	421.7063	4	-0.392	E_A53 $E_T60\|D_T61$	388.684 445.724	2 2	13.0Å|9.8Å
20	TEAEMK HPGDFGADAQGAMTK	T52-K57 H120-K134	599.7642	4	-3.253	E_A53 D_T127	388.684 801.844	2 2	32.1Å
21	VEADIAGHGQEVLIR TEAEMK	V18-R32 T52-K57	500.8524	5	18.47	E_T28 E_A53	569.627 388.685	3 2	10.5Å
22	VEADIAGHGQEVLIR TEAEM(ox)K	V18-R32 T52-K57	629.8124	4	0.950	E_T28 $E_A53\|E_A55$	853.939 396.681	2 2	10.5Å|13.0Å
23	VEADIAGHGQEVLIR ASEDLK	V18-R32 A58-K63	614.5657	4	-2.085	$E_A19\|D_A21$ E_A60	558.970 730.374	3 1	18.4Å|14.7Å
24	VEADIAGHGQEVLIR ASEDLK	V18-R32 A58-K62	614.3143	4	-2.107	$E_A19\|D_A21$ $E_T60\|D_T61$	558.969 730.374	3 1	18.4Å|18.8Å 14.7Å|16.1Å
25	VEADIAGHGQEVLIR ASEDLKK	V18-R32 A58-K63	517.2736	5	1.308	D_T21 $E_T60\|D_T61$	569.627 445.725	3 2	14.7Å|16.1Å
26	VEADIAGHGQEVLIR ASEDLKK	V18-R32 A58-K64	517.2736	5	1.308	D_T21 E_A60	569.627 429.738	3 2	14.7Å
27	VEADIAGHGQEVLIR HPGDFGADAQGAMTK	V18-R32 H120-K134	659.7219	5	1.861	D_T21 D_A123	569.627 785.858	3 2	15.7Å
28	VEADIAGHGQEVLIR HPGDFGADAQGAMTK	V18-R32 H120-K134	659.7219	5	1.861	D_T21 $D_T123\|D_T127$	569.627 801.847	3 2	15.7Å|22.3Å

29	VEADIAGHGQEVLIR HPGDFGADAQGAMTK	V19-R32 H120-K134	659.7193	5	-2.080	E_A19 D_T127	558.969 801.843	3 2	19.8Å
30	VEADIAGHGQEVLIR TEAEM(ox)K	V18-R32 T52-K57	504.0492	5	-2.495	$D_T21|E_T28$ E_A53	569.627 396.681	3 2	20.1Å|10.5Å
31	YLEFISDAIIHVLHSK ALELFR	Y104-K119 A135-R140	564.5054	5	-3.321	D_T110 E_A137	662.346 408.739	3 2	10.1Å
32	YLEFISDAIIHVLHSK ALELFR	Y104-K119 A135-R140	705.3836	4	1.880	D_A106 E_A137	651.691 816.475	3 1	8.4Å
33	YLEFISDAIIHVLHSK ALELFR	Y104-K119 A135-R140	564.5085	5	2.171	$D_T106|D_T110$ E_A137	662.350 408.741	3 2	8.4Å|10.1Å

Note: "|" means or; "&" means and; "T": unsaturated thiol moiety; "A": alkene moiety.

Table S-2. Detailed Summary of DHSO Inter-Linked BSA Peptides Identified by LC MS^n

#	Peptide Seq	AA location	MS m/z	z	Δ(PPM)	Mod. Position	MS2 m/z	z	Distance (Cα-Cα)
1	AEFVEVTK LVTDLTK	A249-K256 L257-K263	475.0076	4	-0.713	E_A250 D_A260	495.765 429.257	2 2	16.1Å
2	AEFVEVTK LVTDLTK	A249-K256 L257-K263	475.0078	4	-0.292	E_T253 D_A260	511.752 429.257	2 2	10.9Å
3	AEFVEVTK QNCDQFEK	A249-K256 Q413-K420	544.7503	4	-0.181	E_A250 E_T419	495.768 584.731	2 2	45.1Å
4	AEFVEVTK YICDNQDTISSK	A249-K256 Y286-K297	638.5493	4	-1.936	$E_A250|E_A253$ D_T292	495.767 772.330	2 2	11.5Å|11.6Å
5	AEFVEVTK YICDNQDTISSK	A249-K256 Y286-K297	638.5493	4	-1.936	E_A250 D_T292	495.767 772.330	2 2	11.5Å
6	AEFVEVTK YICDNQDTISSK	A249-K256 Y286-K297	638.5522	4	2.606	E_A253 $D_T289|D_T292$	495.767 772.331	2 2	12.8Å|11.6Å
7	ATEEQLK TVM(ox)ENFVAFVDK	A562-K568 T569-K580	601.5462	4	-0.098	$E_A564|E_A565$ E_T572	443.735 750.354	2 2	12.4Å|11.0Å
8	ATEEQLK TVMENFVAFVDK	A562-K568 T569-K580	601.5485	4	1.611	E_A565 E_T572	443.735 750.356	2 2	11.0Å
9	DTHKSEIAHR VHKECCHGDLLECADDRADLAK	D25-R34 V264-K285	499.8580	8	-1.939	D_T25 $D_A278|D_A279|D_A282$	431.877 536.846	3 5	21.2Å|20.9Å|21.7Å
10	DTHKSEIAHR VHKECCHGDLLECADDRADLAK	D25-R34 V264-K285	570.9825	7	4.106	D_T25 $D_A279|D_A282$	431.877 670.808	3 4	21.2Å|21.7Å
11	DTHKSEIAHR VHKECCHGDLLECADDRADLAK	D25-R34 V264-K285	499.7346	8	2.110	$D_T25|E_T30$ $D_A278|D_A279|D_A282$	431.876 536.847	3 5	21.2Å|20.9Å|21.7Å, 12.0Å|13.9Å|16.7Å
12	DTHKSEIAHR VHKECCHGDLLECADDRADLAK	D25-R34 V264-K285	570.9843	8	-0.169	$D_T25|E_T30$ $D_A278|D_A282$	6431.876 536.847	3 5	21.2Å|21.7Å, 12.0Å|16.7Å
13	DTHKSEIAHR VHKECCHGDLLECADDRADLAK	D25-R34 V264-K285	570.9797	7	-0.798	E_T30 $D_A278|D_A279|D_A282$	647.311 536.848	2 5	12.0Å|13.9Å|16.7Å
14	DTHKSEIAHR LVTDLTK	D25-R34 L257-K263	542.7869	4	0.787	D_A25 D_A260	631.324 429.259	2 2	22.7Å
15	DTHKSEIAHR TCVADESHAGCEK	D25-R34 T76-K88	711.5669	4	0.368	D_T25 $D_A80|E_A81$	647.310 766.318	2 2	18.8Å|15.4Å
16	DTHKSEIAHR TCVADESHAGCEK	D25-R34 T76-K88	711.3146	4	-1.686	D_T25 E_A87	647.311 766.316	2 2	14.6Å
17	DLGEEHFK ADEKK	D37-K44 A152-K156	438.2119	4	1.889	E_T41 E_A154	537.738 329.680	2 2	16.3Å|13.0Å
18	GLVLIAFSQYLQQCPFDEHVK YLYEIAR	G45-K65 Y161-R167	902.9565	4	-1.045	$D_A61|E_A62$ E_T164	1280.660 514.256	2 2	12.4Å|13.7Å
19	HLVDEPQNLIK CCTKPESER	H402-K412 C460-R468	665.0706	4	.496	D_T405 E_A465	703.368 617.770	2 2	13.6Å
20	HLVDEPQNLIK CCTKPESER	H402-K412 C460-R468	665.0706	4	.496	$D_A405|E_A406$ E_A465	687.382 617.770	2 2	13.6Å|15.8Å
21	HLVDEPQNLIK CCTKPESER	H402-K412 C460-R468	532.4614	5	5.743	D_T405 $E_A465|E_A467$	703.367 412.182	2 3	13.6Å|13.1Å
22	IETMR LGEYGFQNALIVR	I205-R210 L421-R433	579.3010	4	1.167	E_A206 E_T423	359.19 790.405	2 2	17.8Å
23	IETMR VPQVSTPTLVEVSR	I205-R210 V438-R451	587.3107	4	-2.486	E_A206 E_T448	359.19 806.428	2 2	18.8Å
24	LKPDPNTLCDEFK YLYEIAR	L139-K151 Y161-R167	673.0840	4	1.077	E_T149 E_A164	838.893 498.269	2 2	14.9Å
25	LKPDPNTLCDEFKADEK YLYEIAR	L139-K155 Y161-R167	627.5087	5	-0.588	$D_T153|E_T154$ E_A164	707.332 498.269	3 2	16.7Å|15.6Å
26	LVTDLTK VHKECCHGDLLECADDRADLAK	L257-K263 V264-K285	897.4279	4	0.795	D_A260 E_A282	857.511 894.072	1 3	9.1Å
27	LVTDLTK VHKECCHGDLLECADDRADLAK	L257-K263 V264-K285	513.2476	7	0.730	D_T260 $D_A279|D_A282$	445.245 536.848	2 5	7.8Å, 9.1Å
28	LVTDLTK VHKECCHGDLLECADDRADLAK	L257-K263 V264-K285	513.2481	7	1.704	D_T260 $D_A278| D_A279|D_A282$	445.245 536.848	2 5	11.6Å, 7.8Å, 9.1Å
29	LVTDLTK YICDNQDTISSK	L257-K263 Y286-K297	605.4583	4	1.684	D_A260 D_A292	429.259 772.330	2 2	18.9Å
30	LKECCDKPLLEK SHCIAEVEK	L298-K309 S310-K318	558.8729	5	-1.841	$E_A300|D_A303$ E_T317	534.278 586.764	3 2	11.2Å|15.8Å
31	LKECCDKPLLEK SHCIAEVEK	L298-K309 S310-K318	465.8965	6	0.744	E_A300 E_T317	400.961 586.764	4 2	11.2Å
32	LKECCDKPLLEK SHCIAEVEK	L298-K309 S310-K318	698.3413	4	1.014	E_T300 E_A315	816.901 570.778	2 2	11.9Å
33	LKECCDKPLLEK SHCIAEVEK	L298-K309 S310-K318	698.3408	4	0.298	E_A300 $E_A315|E_A317$	800.915 570.778	2 2	11.9Å|11.2Å
34	LKECCDKPLLEK CCTKPESER	L298-K309 C460-R468	721.8368	4	0.393	E_A300 E_A467	800.914 617.769	2 2	26.4Å

35	LKECCDKPLLEK CCTKPESER	L298-K309 C460-R468	721.8368	4	0.393	E$_T$300\|D$_T$303 E$_A$467	816.901 617.769	2 2	26.4Å|31.1Å
36	LKECCDKPLLEK NYQEAK	L298-K309 N341-K346	494.8425	5	-2.149	E$_T$300\|D$_T$303 E$_A$344	544.935 410.702	3 2	40.9Å|41.7Å
37	LKHLVDEPQNLIK CCTKPESER	L400-K412 C460-R468	580.4977	5	7.275	D$_T$405\|E$_T$406 E$_A$465\|E$_A$467	823.959 412.183	2 3	13.6Å|13.1Å 15.8Å|15.3Å
38	LCVLHEK TPVSEKVTK	L483-K489 T490-K498	518.7810	4	1.484	E$_T$488 E$_T$494	499.749 544.793	2 2	10.7Å
39	LVNELTEFAK SLHTLFGDELCK	L66-K75 S89-K100	692.8486	4	-.732	E$_A$69 E$_A$97	616.337 744.370	2 2	9.3Å
40	LVNELTEFAK SLHTLFGDELCK	L66-K75 S89-K100	692.8496	4	0.712	E$_A$72 E$_A$97	616.337 744.370	2 2	13.3Å
41	LVNELTEFAK SLHTLFGDELCK	L66-K75 S89-K100	692.8486	4	-.732	E$_A$69 D$_T$96\|E$_T$97	616.338 760.357	2 2	9.3Å|12.1Å
42	LVNELTEFAK SLHTLFGDELCK	L66-K75 S89-K100	692.8502	4	1.578	E$_A$72 D$_T$96\|E$_T$97	616.338 760.357	2 2	15.2Å|13.3Å
43	NECFLSHKDDSPDLPK KVPQVSTPTLVEVSR	N123-K138 K437-R451	746.1718	5	-7.481	D$_A$135 E$_A$448	657.309 854.493	3 2	13.5Å
44	QNCDQFEK ATEEQLK	Q413-K420 A562-K568	518.7349	4	.292	E$_T$416 E$_A$564	584.731 443.736	2 2	16.9Å
45	QNCDQFEK CCTKPESER	Q413-K420 C460-R468	605.7531	4	2.460	E$_T$419 E$_T$467	568.745 633.756	2 2	16.8Å
46	QNCDQFEK CCTKPESER	Q413-K420 C460-R468	605.7520	4	.645	E$_A$419 E$_T$465	584.730 633.755	2 2	14.0Å
47	QNCDQFEK CCTKPESER	Q413-K420 C460-R468	605.7518	4	.314	D$_T$419 E$_T$465\|E$_T$467	584.730 633.756	2 2	14.0Å|16.8Å
48	QNCDQFEK CCTKPESER	Q413-K420 C460-R468	605.7521	4	0.810	D$_A$416 E$_T$465	568.744 633.756	2 2	12.6Å
49	QNCDQFEK CCTKPESER	Q413-K420 C460-R468	484.8036	5	1.768	D$_T$416 E$_A$465\|E$_A$467	584.730 412.183	2 3	12.6Å|15.7Å
50	QNCDQFEK LGEYGFQNALIVR	Q413-K420 L421-R433	684.0776	4	0.508	E$_A$419 E$_A$423	568.744 774.420	2 2	8.2Å
51	RPCFSALTPDETYVPK ATEEQLK	R507-K523 A562-K568	721.8555	4	1.171	E$_A$518 E$_A$564	974.982 443.735	2 2	17.8Å
52	SEIAHR VHKECCHGDLLECADDRADLAK	S29-R34 V264-K285	585.9390	6	1.516	E$_T$30 D$_A$282	406.695 670.804	2 4	16.7Å
53	SHCIAEVEK CCTKPESER	S310-K318 C460-R468	606.7698	4	2.091	E$_T$315\|E$_T$317 E$_T$467	586.763 633.755	2 2	18.1Å|15.8Å
54	SHCIAEVEK CCTKPESER	S310-K318 C460-R468	606.7702	4	2.751	E$_A$317 E$_T$467	570.778 633.756	2 2	15.0Å
55	SHCIAEVEK CCTKPESER	S310-K318 C460-R468	485.6177	5	2.924	E$_T$317 E$_A$465\|E$_A$467	586.764 412.183	2 3	20.3Å|15.0Å
56	SHCIAEVEK NYQEAK	S310-K318 N341-K346	503.4868	4	2.604	E$_T$315 E$_A$344	586.764 410.701	2 2	35.7Å
57	VHKECCHGDLLECADDRADLAK SHCIAEVEK	V264-K285 S310-K318	645.9636	6	4.495	E$_A$275\|D$_A$278\|D$_A$279\|D$_A$282 E$_T$317	670.808 586.765	4 2	27.6Å|24.5Å|22.8Å|21.2Å
58	VHKECCHGDLLECADDRADLAK YICDNQDTISSK	V264-K285 Y286-K297	707.8174	6	1.732	D$_A$278\| D$_A$279\|D$_A$282 D$_T$289	670.807 772.330	4 2	16.7Å|15.2Å|10.6Å
59	YICDNQDTISSK ECCDKPLLEK	Y286-K297 E300-K309	730.8301	4	1.435	D$_T$289 D$_T$303	772.331 680.325	2 2	10.9Å
60	YICDNQDTISSK LKECCDKPLLEK	Y286-K297 L298-K309	791.1262	4	3.024	D$_T$292 D$_T$303	772.331 816.902	2 2	15.0Å
61	YICDNQDTISSK LKECCDKPLLEK	Y286-K297 L298-K309	791.1237	4	-0.136	D$_A$292 E$_T$300\|D$_T$303	756.334 816.900	2 2	13.4Å|15.0Å
62	YICDNQDTISSK SHCIAEVEK	Y286-K297 S310-K318	676.0577	4	2.777	D$_A$292 E$_T$317	756.345 586.764	2 2	15.5Å

Note: "|" means or; "&" means and; "T": unsaturated thiol moiety; "A": alkene moiety.

Table S-3. Detailed Summary of DSSO Inter-Linked Myoglobin Peptides Identified by LC MS^n

#	Peptide Seq	AA location	MS m/z	z	Δ(PPM)	Mod. Position	MS2 m/z	z	Distance (Cα-Cα)
1	FDKFK KKGHHEAELKPLAQSHATKHK	F44-K48 K79-K99	618.5516	5	0.116	K_T46 K_T97	770.355 584.053	1 4	14.6Å
2	FDKFKHLKTEAEMK KHGTVVLTALGGILK	F44-K57 K64-K78	715.5761	5	-0.108	$K_A46\&K_A48\&K_T51$ K_T64	649.311 796.963	3 2	11.9Å|15.7Å|18.3Å
3	FKHLK GHHEAELKPLAQSHATKHK	F47-K51 G81-K99	492.2618	6	1.093	K_A48 $K_T88\|K_T97$	363.719 544.037	2 4	27.1Å|17.1Å
4	FKHLK GHHEAELKPLAQSHATKHK	F47-K51 G81-K99	590.5117	5	-0.608	K_T48 K_A97	758.406 544.037	1 4	17.1Å
5	FKHLK HKIPIK	F47-K51 H98-K103	391.9812	4	-0.157	K_T48 K_A99	363.719 411.239	2 2	15.6Å
6	FKHLKTEAEMKASEDLKK YLEFISDAIIHVLHSKHPGDFGADAQGAMTK	F47-K64 Y104-K134	970.3080	6	0.463	$K_A48\&K_T51\&K_A57$ K_T119	776.385 1152.221	3 3	26.1Å|24.4Å|15.4Å
7	HGTVVLTALGGILKK KGHHEAELKPLAQSHATK	H65-K79 K80-K97	608.5053	6	1.553	K_A78 K_T80	520.987 690.017	3 3	5.3Å
8	KHGTVVLTALGGILK NDIAAKYK	K64-K78 N141-K148	647.3630	4	-0.850	K_T64 K_A146	796.963 488.759	2 2	27.0Å
9	KKGHHEAELKPLAQSHATK NDIAAKYK	K79-K97 N141-K148	837.6671	4	-1.356	$K_A79\&K_T80\&K_A88$ K_T146	768.724 976.510	3 1	18.0Å, 18.4Å, 7.1Å
10	KKGHHEAELKPLAQSHATK NDIAAKYK	K79-K97 N141-K148	638.7356	5	0.486	$K_T80\|K_T88$ K_T146	549.789 1008.485	4 1	18.4Å|7.1Å
11	KGHHEAELKPLAQSHATK NDIAAKYK	K80-K97 N141-K148	766.1448	4	1.629	K_T88 K_A146	690.018 976.511	3 1	7.1Å
12	LFTGHPETLEKFDK FKHLK	L33-K46 F47-K51	416.0513	6	0.971	K_A43 K_A48	429.721 363.719	4 2	8.0Å
13	LFTGHPETLEKFDK HKIPIK	L33-K46 H98-K103	639.3386	4	-1.892	K_T43 K_A99	874.421 395.253	2 2	7.7Å
14	LFTGHPETLEKFDK HKIPIKYLEFISDAIIHVLHSK	L33-K46 H98-K119	737.5630	6	.054	K_T43 $K_A99\|K_A103$	874.423 664.631	2 4	7.7Å|13.0Å
15	LFTGHPETLEKFDK KKGHHEAELKPLAQSHATKHKIPIK	L33-K46 K79-K103	801.4203	6	2.184	K_T43 $K_A88\&K_T97\&K_A99$	874.421 755.913	2 4	7.7Å, 20.3Å, 10.5Å
16	LFTGHPETLEKFDK IPIKYLEFISDAIIHVLHSK	L33-K46 I100-K119	831.8438	5	.575	K_T43 K_A103	874.421 797.454	2 3	13.0Å
17	LFTGHPETLEKFDKFKHLK HKIPIK	L33-K51 H98-K103	561.7958	6	-1.21	$K_T43\&K_A46\&K_T48$ K_T146	636.063 395.253	4 2	7.7Å,15.0Å, 15.6Å
18	TEAEMKASEDLK KHGTVVLTALGGILK	T52-K63 K64-K78	754.6490	4	0.028	K_A57 K_T64	703.330 796.963	2 2	11.6Å
19	TEAEMKASEDLKK YLEFISDAIIHVLHSKHPGDFGADAQGAMTK	T52-K65 Y104-K134	100.0855	5	-2.117	K_A57 K_A119	767.378 1141.568	2 3	15.4Å

Note: "|" means or; "&" means and; "T": unsaturated thiol moiety; "A": alkene moiety.

Table S-4. Detailed Summary of DSSO Inter-Linked BSA Peptides Identified by LC MS^n

#	Peptide Seq	AA location	MS m/z	z	Δ(PPM)	Mod. Position	MS2 m/z	z	Distance (Cα-Cα)
1	ALKAWSVAR LAKEYEATLEECCAK	A233-R241 L372-K386	744.1126	4	4.399	K_A235 K_T374	528.305 950.911	2 2	13.5Å
2	ALKAWSVAR VHKECCHGDLLECADDRADLAK	A233-R241 V264-K285	754.9589	5	3.924	K_A235 K_T266	528.306 900.056	2 3	9.7Å
3	ALKAWSVAR LVTDLTKVHK	A233-R241 L257-K266	578.8257	4	0.636	K_T235 K_A263	544.291 604.357	2 2	9.0Å
4	AEFVEVTKLVTDLTK ADLAKYICDNQDTISSK	A249-K263 A281-K297	948.9763	4	5.128	K_A256 K_T285	873.984 1014.458	2 2	13.8Å
5	ADLAKYICDNQDTISSK ECCDKPLLEKSHCIAEVEK	A281-K297 E300-K318	889.6097	5	1.632	K_T285 K_A309	1014.458 800.374	2 3	9.1Å
6	ADLAKYICDNQDTISSK ECCDKPLLEKSHCIAEVEK	A281-K297 E300-K318	889.6097	5	1.632	K_T285 K_T304\|K_T309	1014.458 811.031	2 3	11.3Å|9.1Å
7	CASIQKFGER LAKEYEATLEECCAK	C223-R232 L372-K386	792.6119	4	3.328	K_A228 K_T374	625.306 950.910	2 2	16.4Å
8	CASIQKFGER LCVLHEKTPVSEK	C223-R232 L483-K495	723.8583	4	2.115	K_A228 K_T489	625.305 813.408	2 2	13.2Å
9	CASIQKFGER VTKCCTESLVNR	C223-R232 V496-R507	705.5812	4	3.067	K_A228 K_T498	641.290 776.852	2 2	13.1Å
10	CASIQKFGER LCVLHEKTPVSEKVTKCCTESLVNR	C223-R232 L483-K507	900.6266	5	0.080	K_A228 K_T489&K_T495&K_A498	625.305 1071.836	2 3	13.2Å|17.6Å|13.1Å
11	CASIQKFGER SLGKVGTR	C223-R232 S452-R459	543.2742	4	0.293	K_T228 K_A455	641.290 436.254	2 2	27.2Å
12	DTHKSEIAHR FKDLGEEHFK	D25-R34 F35-K44	520.8516	5	3.487	K_A28 K_T36	416.544 668.308	3 2	13.4Å
13	DTHKSEIAHR LVTDLTKVHK	D25-R34 L257-K266	501.6659	5	3.313	K_A28 K_T263	416.544 620.343	3 2	21.4Å
14	FPKAEFVEVTK LKECCDKPLLEK	F246-K256 L298-K309	746.8786	4	3.617	K_T248 K_A299	674.864 809.889	2 2	15.6Å
15	FPKAEFVEVTK YICDNQDTISSKLK	F246-K256 Y286-K299	784.8886	4	3.457	K_A248 K_T297	674.863 885.909	2 2	11.7Å
16	FKDLGEEHFK LVNELTEFAKTCVADESHAGCEK	F35-K44 L66-K88	803.7743	5	5.714	K_A36 K_A75	652.321 888.078	2 3	9.2Å
17	FKDLGEEHFK ADLAKYICDNQDTISSK	F35-K44 A281-K297	837.8948	4	5.217	K_A36 K_T285	652.320 1014.458	2 2	16.7Å
18	HPYFYAPELLYYANKYNGVFQECCQAEDK ECCDKPLLEK	H169-K197 E300-309	1014.2459	5	-3.074	K_A183 K_A304	1224.555 673.313	3 2	13.6Å
19	HPYFYAPELLYYANKYNGVFQECCQAEDK GACLLPKIETM(ox)R	H169-K197 G198-R209	1036.6772	5	2.428	K_T183 K_A204	1235.200 728.878	3 2	10.1Å
20	HKPKATEEQLK HKPKATEEQLK	H558-K568 H558-K568	555.8971	5	0.899	K_A561 K_T561	454.918 697.863	3 2	-
21	KQTALVELLK ATEEQLKTVM(ox)ENFVAFVDK	K548-K557 A562-K580	879.4573	4	0.408	K_A548 K_T568	598.868 1151.104	2 2	14.3Å
22	LKPDPNTLCDEFK FWGKYLYEIAR	L139-K151 F157-R167	795.8878	4	1.369	K_T140 K_A160	831.881 750.391	2 2	14.7Å
23	LKPDPNTLCDEFK SLGKVGTR	L139-K151 S452-R459	638.5690	4	0.425	K_T140 K_A455	831.880 436.255	2 2	21.3Å
24	LSQKFPK CCTKPESER	L242-K248 C460-R468	543.5061	4	4.415	K_A245 K_T463	451.263 626.744	2 2	20.5Å
25	LKHLVDEPQNLIK HKPKATEEQLK	L400-L412 H558-K568	603.3317	5	3.556	K_T401 K_A559\|K_A561	816.948 454.920	2 3	42.1Å|38.3Å
26	LFTFHADICTLPDTEKQIK KQTALVELLK	L529-547 K548-K557	894.9782	4	6.080	K_A544 K_A548	1166.089 598.868	2 2	6.4Å
27	NECFLSHKDDSPDLPKLKPDPNTLCDEFK SLGKVGTR	N123-K151 S452-R459	739.8589	6	2.696	K_T138 K_A455	887.159 436.254	4 2	19.9Å
28	DDSPDLPKLKPDPNTLCDEFK SLGKVGTR	D131-K151 S452-R459	773.1790	5	3.565	K_T138\|K_T140 K_A455	991.79 436.254	3 2	19.9Å|21.3Å
29	QNCDQFEKLGEYGFQNALIVR ATEEQLKTVM(ox)ENFVAFVDK	Q413-R433 A562-K580	1226.3392	4	4.267	K_T420 K_A568	1308.109 1135.061	2 2	14.6Å
30	QNCDQFEKLGEYGFQNALIVR KVPQVSTPTLVEVSR	Q413-R433 K437-R451	1082.5466	4	1.824	K_T420 K_A437	1308.113 847.479	2 2	22.3Å
31	SLGKVGTR CCTKPESER	S452-R459 C460-R468	536.0019	4	3.445	K_A455 K_T463	436.255 626.743	2 2	13.5Å
32	TPVSEKVTK HKPKATEEQLK	T490-K498 H558-K568	491.6657	5	3.475	K_T495 K_A559\|K_A561	537.780 454.918	2 3	19.8Å|18.7Å
33	TPVSEKVTK HKPKATEEQLK	T490-K498 H558-K568	614.3301	4	3.141	K_A495 K_T561	521.794 697.861	2 2	18.7Å

Note: "|" means or; "&" means and; "T": unsaturated thiol moiety; "A": alkene moiety.