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Flavin catalysed desulfurization of peptide and protein in aqueous media

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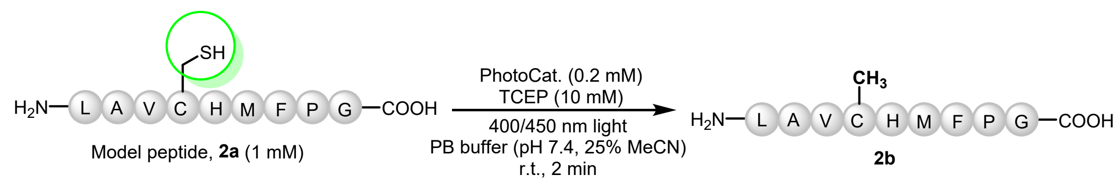
1. Supplementary tables and figures

Table S1. Screening of photocatalysts for desulfurization of peptide cysteine.

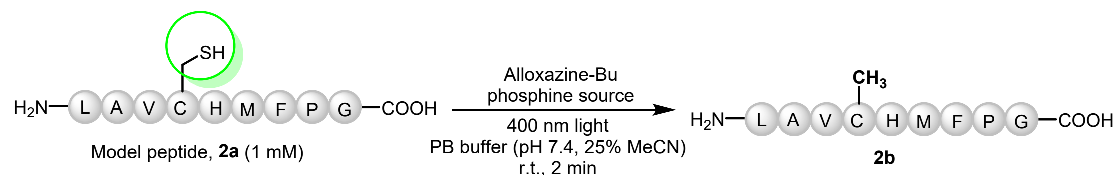


Entry	Photocatalyst	Conversion (%)
1	Riboflavin	53
2	Riboflavin tetrabutyrate (RFTB)	77
3	Flavin mononucleotide (FMN)	51
4	Riboflavin tetraacetate (RFTA)	61
5	Rose Bengal (RB)	46
6	$MesAcrClO_4$	14
7	$Ru(bpy)_3Cl_2$	16
8	$Ru(bpz)_3(PF6)_2$	22
9	$[Ir(ppy)_2(dtbbpy)](PF_6)$	16
10	Alloxazine-Bu (400 nm)	99
11	-	N.D.
12	400 nm/450 nm (absence of PhotoCat.)	N.D.
13	Alloxazine-Bu (absence of light)	N.D.

Conditions: **2a** (1 mM), TCEP (10 mM) and photocatalyst (0.2 mM) under light irradiation (400 nm or 450 nm) for 2 min at rt using PB buffer (pH 7.4, 25% MeCN) as solvent. Conversion was determined by LC-MS.

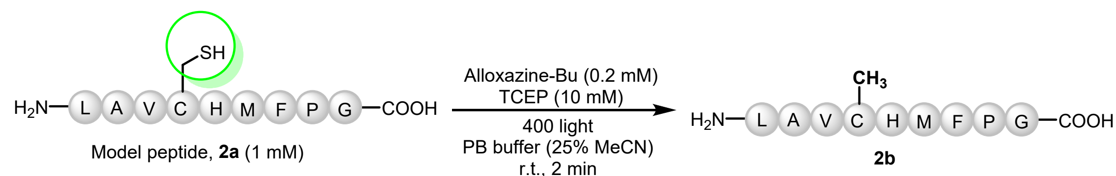
Table S2. Optimization of catalyst loading and phosphine loading.

Entry	Catalyst or phosphine	Catalyst or phosphine loading (mM)	Conversion (%)
1	Alloxazine-Bu	1	99
2		0.5	99
3		0.2	99
4		0.1	56
5	TCEP	20	99
6		10	99
7		5	72
8	$P(OEt)_3$	10	N.D.



Conditions: **2a** (1 mM), phosphine source and alloxazine-Bu (0.2 mM) under light irradiation (400 nm) for 2 min at rt using PB buffer (pH 7.4, 25% MeCN) as solvent. Conversion was determined by LC-MS.

Table S3. Optimization of buffer pH for desulfurization of peptide cysteine.



Entry	pH	Conversion (%)
1	5.5	92
2	6.0	95
3	6.5	97

4	7.0	98
5	7.4	99
6	8.0	99

Conditions: **2a** (1 mM), TCEP (10 mM) and alloxazine-Bu (0.2 mM) under light irradiation (400 nm or 450 nm) for 2 min at rt using PB buffer (25% MeCN) as solvent. Conversion was determined by LC-MS.

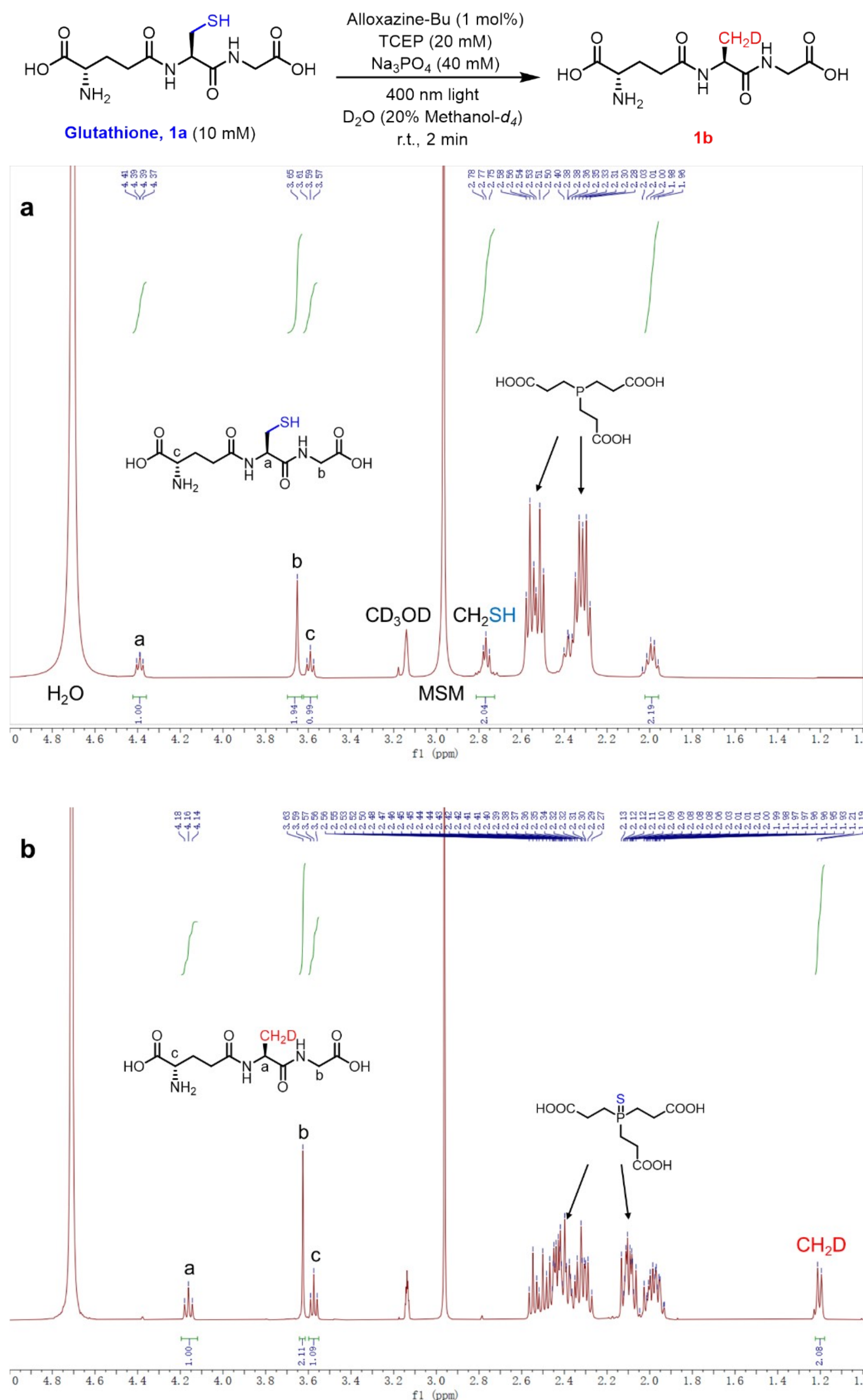


Figure S1. The ^{1}H NMR analysis of the desulfurization reaction of GSH. (**a**) Assignment of the reaction before light irradiation. (**b**) Assignment of the reaction after 2 min light irradiation. MSM: dimethyl sulfone.

2. NMR analysis of desulfurization

2.1. General information

All chemical reagents are commercially available from *Energy Chemical* without purification. **Nuclear Magnetic Resonance (NMR)** spectra were recorded on Bruker 400 MHz spectrometer under ambient temperature (20 °C).

2.2. NMR analysis of desulfurization of GSH

A typical desulfurization reaction of GSH. To a 2 mL vial was added GSH (1.5 mg, 10 mM), alloxazine-Bu (0.2 mg, 0.1 mM), TCEP (2.5 mg, 20 mM), Na_3PO_4 (3.3 mg, 40 mM), and 500 uL D_2O (20% Methanol-d_4). Dimethyl sulfone (MSM, 1.9 mg, 40 mM) was added as internal standard. The vial was then caped and equipped with magnetic bar. The reaction was stirred and irradiated with 400 nm LED light (40 W) for 2 min. The resulting solution was then transferred to an NMR tube, and analyzed by ^{1}H NMR on a 400 MHz spectrometer. As shown in Figure S1, the NMR spectra are consistent with the reported literature.[1]

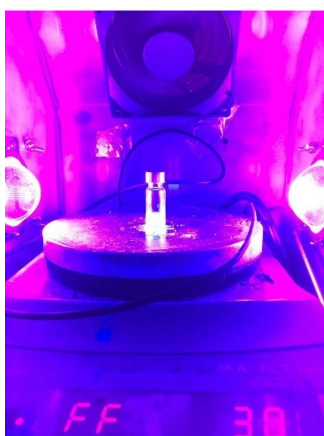
3. Supplementary information for desulfurization of peptides

3.1. General information

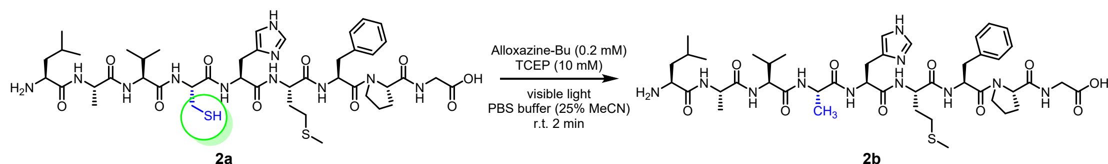
All chemical reagents are commercially available from *Energy Chemical* without purification. **High-Resolution Mass Spectrometry (HRMS)** and **MS/MS** analysis were measured on a Q_Exactive_Focus. **Reverse Phase High Performance Liquid Chromatography** (**HPLC**) was performed on SHIMAZU prominence LC-20AT instrument equipped with Kromasil 100-5-C18 column (4.6 × 250 mm, 5 μm). H_2O (containing 0.1% TFA) and pure CH_3CN were used as solvents in linear gradient mixtures. **Mass Spectrometry** (**MS**) to screen the molecular weight of HPLC fractions were carried out on SHIMAZU LC-MS 8030 in positive ion mode.

3.2. General procedure for the desulfurization reaction of peptide

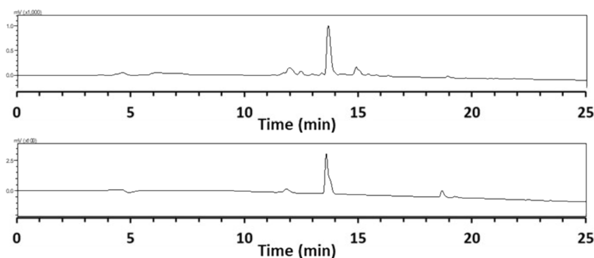
A 10 mM MeCN stock solution of alloxazine-Bu was made up of 3.4 mg alloxazine-Bu (0.01 mmol) and 1 mL MeCN, and a 100 mM water stock solution of TCEP was made up of 25 mg TCEP (0.1 mmol) and 1 mL deionized water. These stock solutions were stored at room temperature away from light. To a 2 mL vial was added specific peptide (0.2 μmol), 200 uL PBS buffer (pH 7.4, 25% MeCN) and 4 μL alloxazine-Bu (10 mM) and 20 μL TCEP (100 mM) stock solution. The vial was then caped and equipped with magnetic bar. The reaction was stirred and irradiated with 400 nm LED light (40 W) for 2 min, as shown in the following figure. The resulting solution was then analyzed directly *via* HPLC after filtration. Desired distillates were identified by MS and lyophilized to obtain target products.



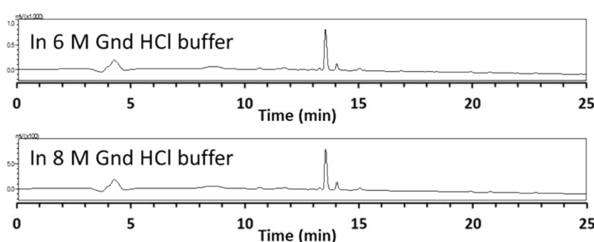
3.3. Characterization data for the reaction of peptide 2



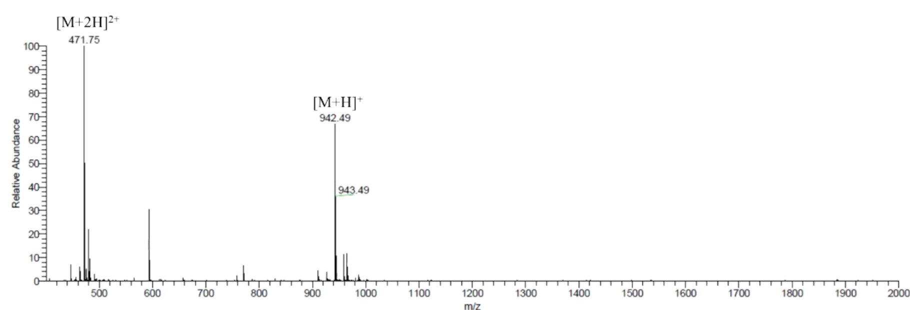
The reaction was followed General Procedure using peptide **2a**, sequence: NH2-LAV**C**HMFPG-OH.



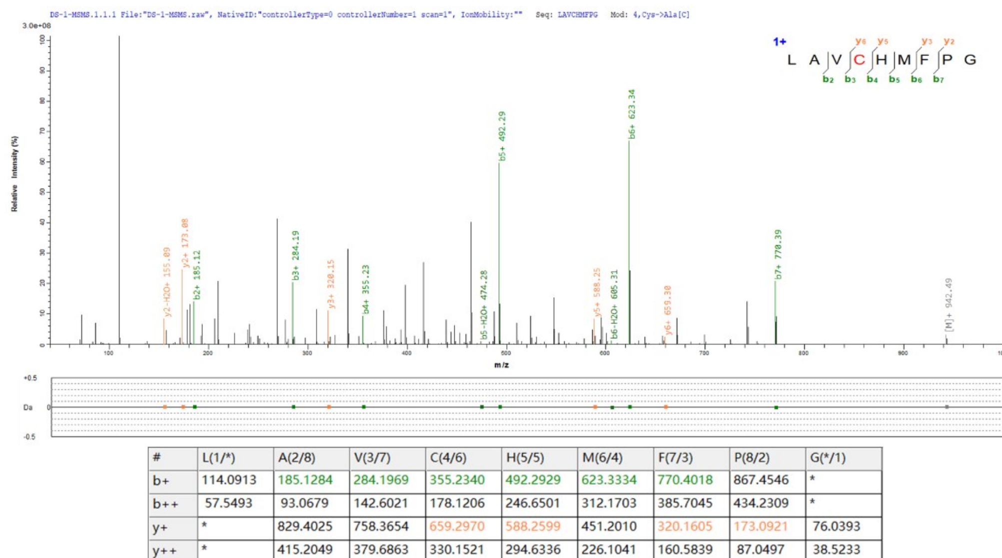
LC trace of the reaction of peptide **2a** and purified product **2b**.



LC trace of the reaction of peptide **2a** in 6 M and 8 M guanidine hydrochloride buffer.



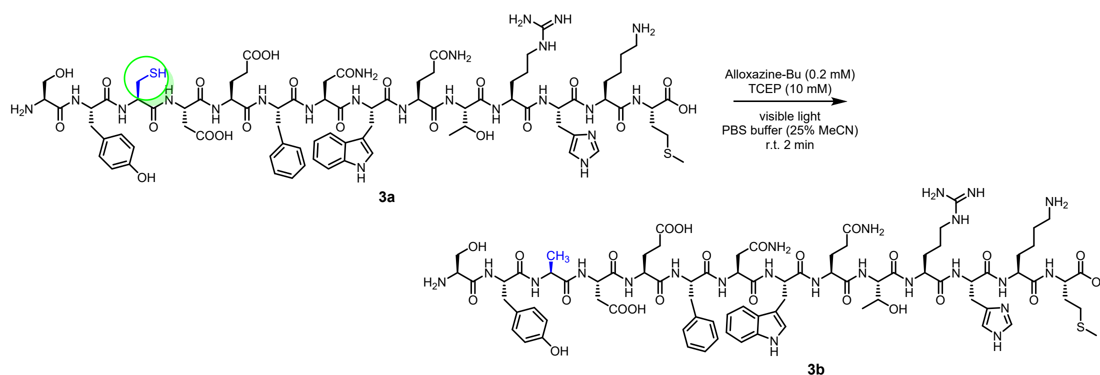
ESI Mass spectrum of purified product **2b**. Calculated Mass $[M+H]^+$: 942.49; $[M+2H]^{2+}$: 471.75; Mass Found (ESI+) $[M+H]^+$: 942.49; $[M+2H]^{2+}$: 471.75.



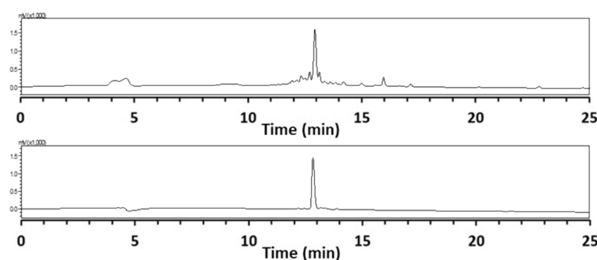
#	L(1/*)	A(2/8)	V(3/7)	C(4/6)	H(5/5)	M(6/4)	F(7/3)	P(8/2)	G(*/1)
b+	114.0913	185.1284	284.1969	355.2340	492.2929	623.3334	770.4018	867.4546	*
b++	57.5493	93.0679	142.6021	178.1206	246.6501	312.1703	385.7045	434.2309	*
y+	*	829.4025	758.3654	659.2970	588.2599	451.2010	320.1605	173.0921	76.0393
y++	*	415.2049	379.6863	330.1521	294.6336	226.1041	160.5839	87.0497	38.5233

De novo ms/ms analysis of purified product **2b**.

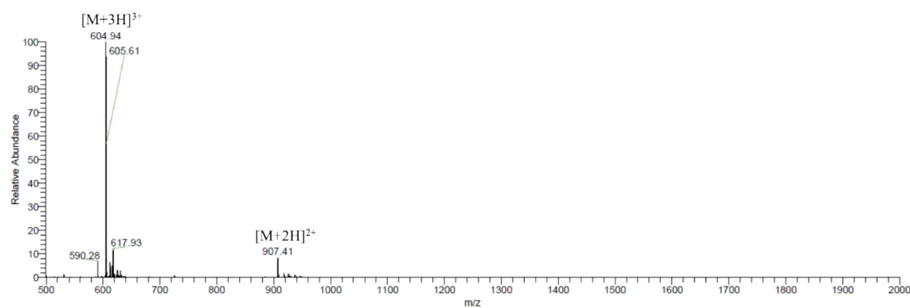
3.4. Characterization data for the reaction of peptide 3



The reaction was followed General Procedure using peptide **3a**, sequence: NH2-SY**C**DEFNWQTRHKM-OH.

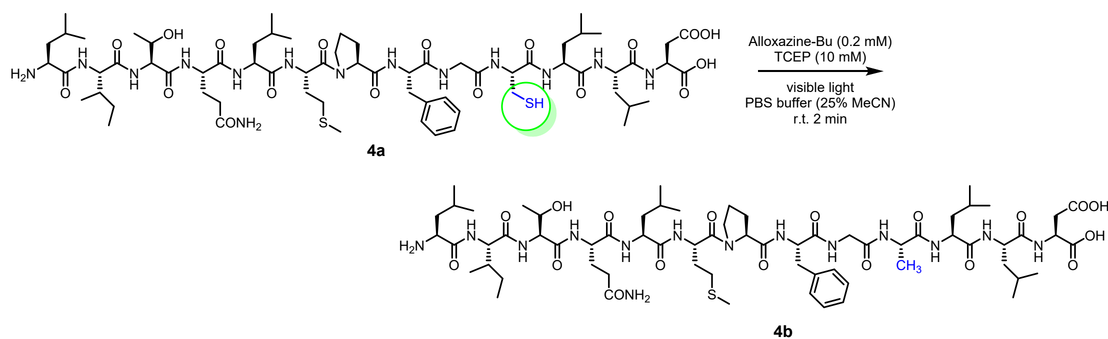


LC trace of the reaction of peptide **3a** and purified product **3b**.

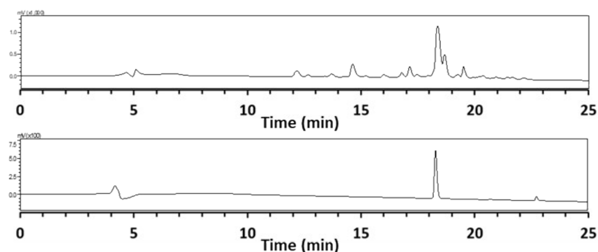


ESI Mass spectrum of purified product **3b**. Calculated Mass $[M+2H]^{2+}$: 907.42; $[M+3H]^{2+}$: 604.94; Mass Found (ESI+) $[M+2H]^{2+}$: 907.42; $[M+3H]^{2+}$: 604.94.

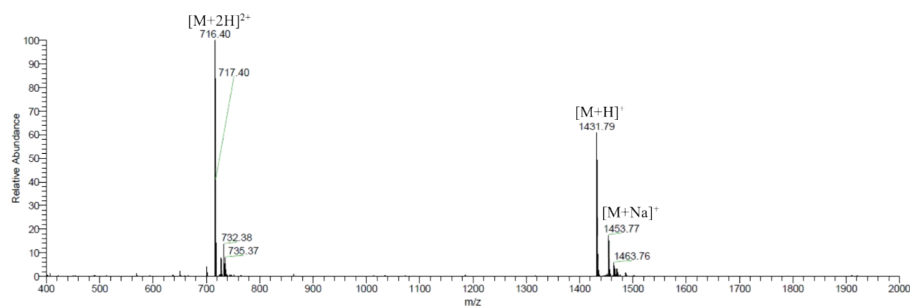
3.5. Characterization data for the reaction of peptide 4



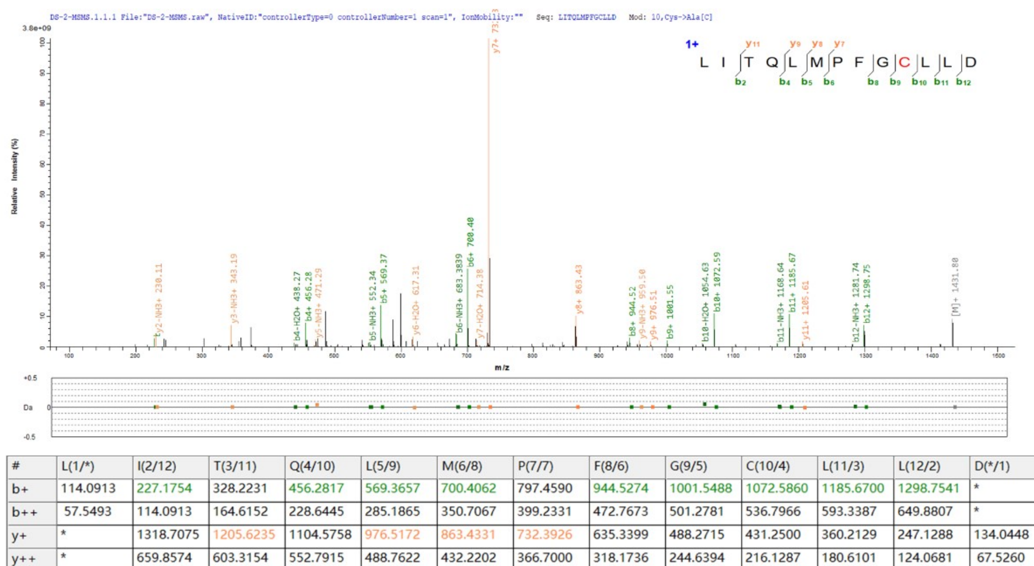
The reaction was followed General Procedure using peptide **4a**, sequence: NH2-LITQLMPFG**C**LLD-OH.



LC trace of the reaction of peptide **4a** and purified product **4b**.



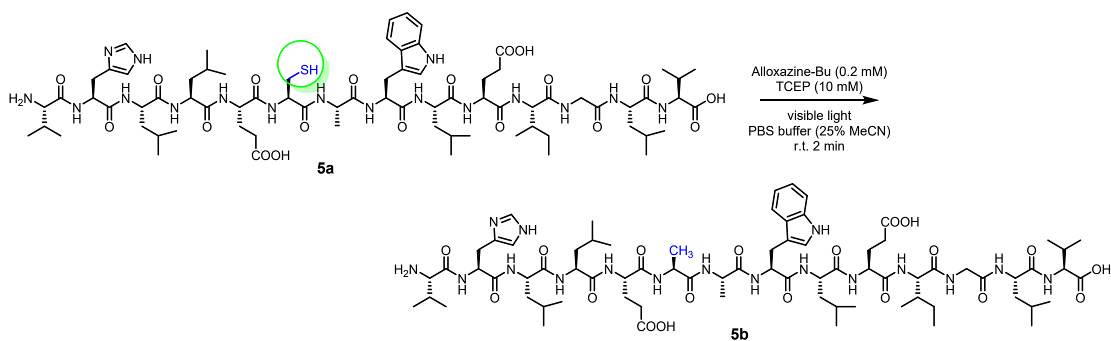
ESI Mass spectrum of purified product **4b**. Calculated Mass $[M+H]^{+}$: 1431.79; $[M+2H]^{2+}$: 716.40; Mass Found (ESI+) $[M+H]^{+}$: 1431.79; $[M+2H]^{2+}$: 716.40.



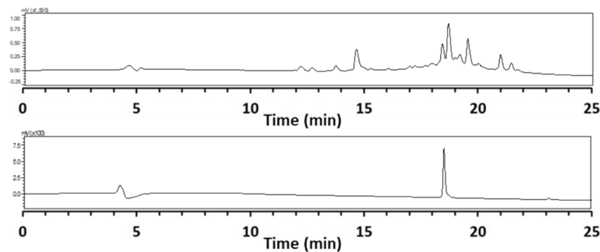
#	L(1/*)	I(2/12)	T(3/11)	Q(4/10)	L(5/9)	M(6/8)	P(7/7)	F(8/6)	G(9/5)	C(10/4)	L(11/3)	L(12/2)	D(*/1)
b+	114.0913	227.1754	328.2231	456.2817	569.3657	700.4062	797.4590	944.5274	1001.5488	1072.5860	1185.6700	1298.7541	*
b++	57.5493	114.0913	164.6152	228.6445	285.1865	350.7067	399.2331	472.7673	501.2781	536.7966	593.3387	649.8807	*
y+	*	1318.7075	1205.6235	1104.5758	976.5172	863.4331	732.3926	635.3399	488.2715	431.2500	360.2129	247.1288	134.0448
y++	*	659.8574	603.3154	552.7915	488.7622	432.2202	366.7000	318.1736	244.6394	216.1287	180.6101	124.0681	67.5260

De novo ms/ms analysis of purified product **4b**.

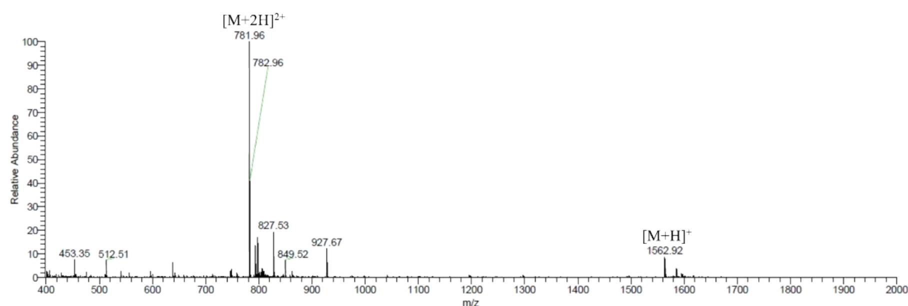
3.6. Characterization data for the reaction of peptide 5



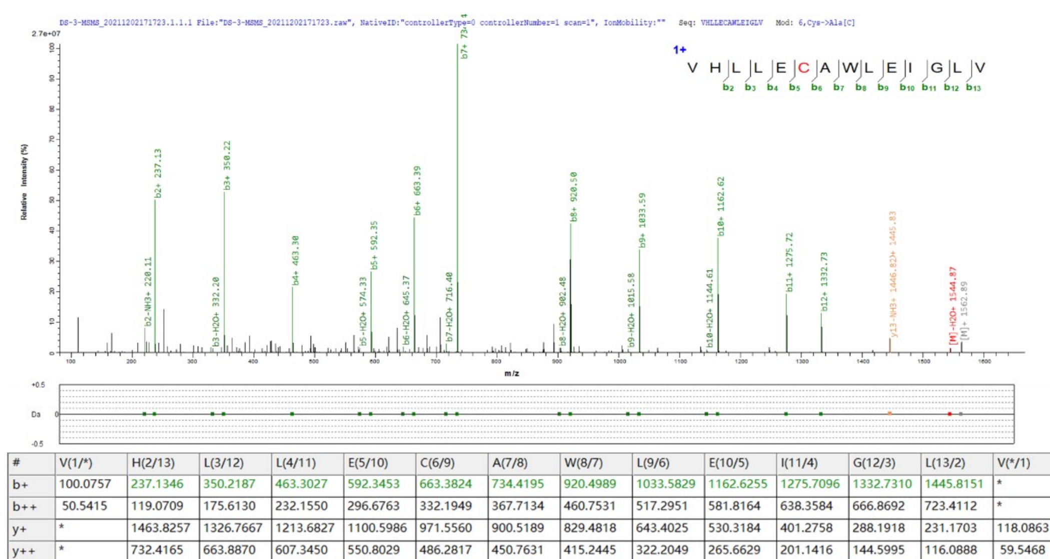
The reaction was followed General Procedure using peptide **5a**, sequence: NH2-VHLLE**C**AWLEIGLV-OH.



LC trace of the reaction of peptide **5a** and purified product **5b**.



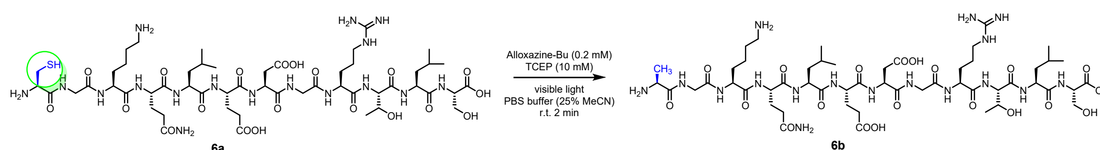
ESI Mass spectrum of purified product **5b**. Calculated Mass $[M+H]^{+}$: 1562.92; $[M+2H]^{2+}$: 781.96; Mass Found (ESI+) $[M+H]^{+}$: 1562.92; $[M+2H]^{2+}$: 781.96.



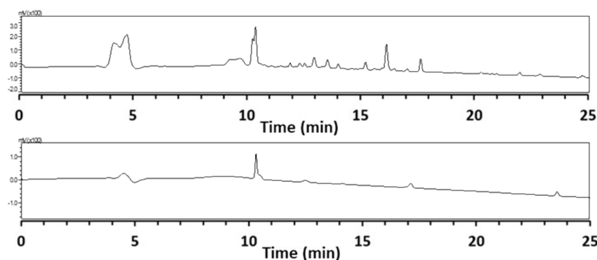
#	V(1/*)	H(2/13)	L(3/12)	L(4/11)	E(5/10)	C(6/9)	A(7/8)	W(8/7)	L(9/6)	E(10/5)	I(11/4)	G(12/3)	L(13/2)	V(*/1)
b+	100.0757	237.1346	350.2187	463.3027	592.3453	663.3824	734.4195	920.4989	1033.5829	1162.6255	1275.7096	1332.7310	1445.8151	*
b++	50.5415	119.0709	175.6130	232.1550	296.6763	332.1949	367.7134	460.7531	517.2951	581.8164	638.3584	666.8692	723.4112	*
y+	*	1463.8257	1326.7667	1213.6827	1100.5986	971.5560	900.5189	829.4818	643.4025	530.3184	401.2758	288.1918	231.1703	118.0863
y++	*	732.4165	663.8870	607.3450	550.8029	486.2817	450.7631	415.2445	322.2049	265.6629	201.1416	144.5995	116.0888	59.5468

De novo ms/ms analysis of purified product **5b**.

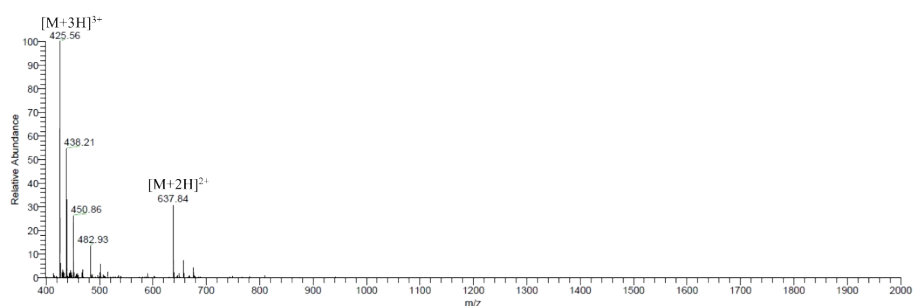
3.7. Characterization data for the reaction of peptide 6



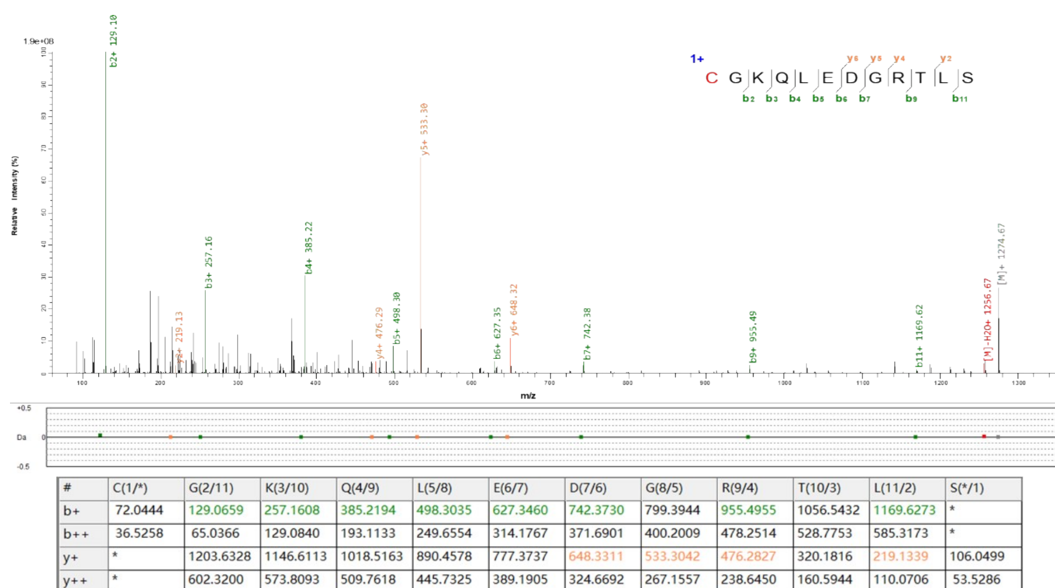
The reaction was followed General Procedure using peptide **6a**, sequence: NH2-**C**GKQLEDGRTLS-OH.



LC trace of the reaction of peptide **6a** and purified product **6b**.



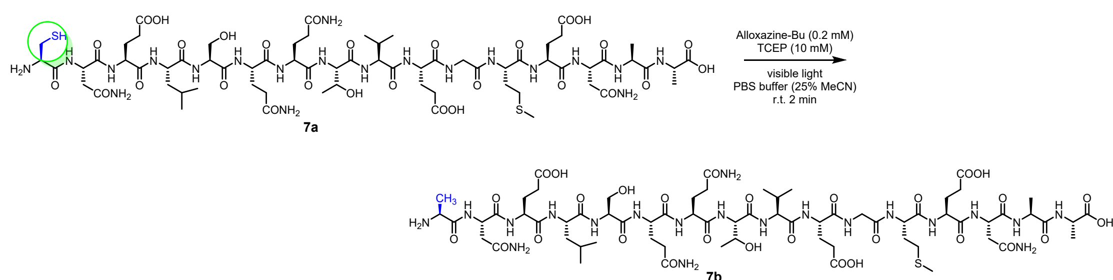
ESI Mass spectrum of purified product **6b**. Calculated Mass $[M+2H]^{2+}$: 637.84; $[M+3H]^{3+}$: 524.56; Mass Found (ESI+) $[M+H]^{+}$: $[M+2H]^{2+}$: 637.84; $[M+3H]^{3+}$: 524.56.



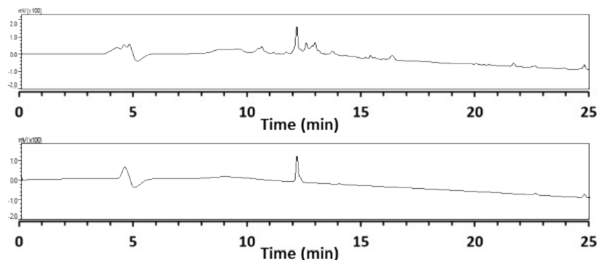
#	C(1/*)	G(2/11)	K(3/10)	Q(4/9)	L(5/8)	E(6/7)	D(7/6)	G(8/5)	R(9/4)	T(10/3)	L(11/2)	S(*/1)
b+	72.0444	129.0659	257.1608	385.2194	498.3035	627.3460	742.3730	799.3944	955.4955	1056.5432	1169.6273	*
b++	36.5258	65.0366	129.0840	193.1133	249.6554	314.1767	371.6901	400.2009	478.2514	528.7753	585.3173	*
y+	*	1203.6328	1146.6113	1018.5163	890.4578	777.3737	648.3311	533.3042	476.2827	320.1816	219.1339	106.0499
y++	*	602.3200	573.8093	509.7618	445.7325	389.1905	324.6692	267.1557	238.6450	160.5944	110.0706	53.5286

De novo ms/ms analysis of purified product **6b**.

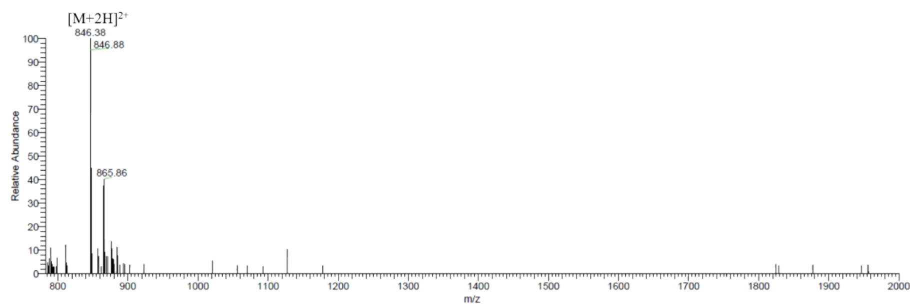
3.8. Characterization data for the reaction of peptide 7



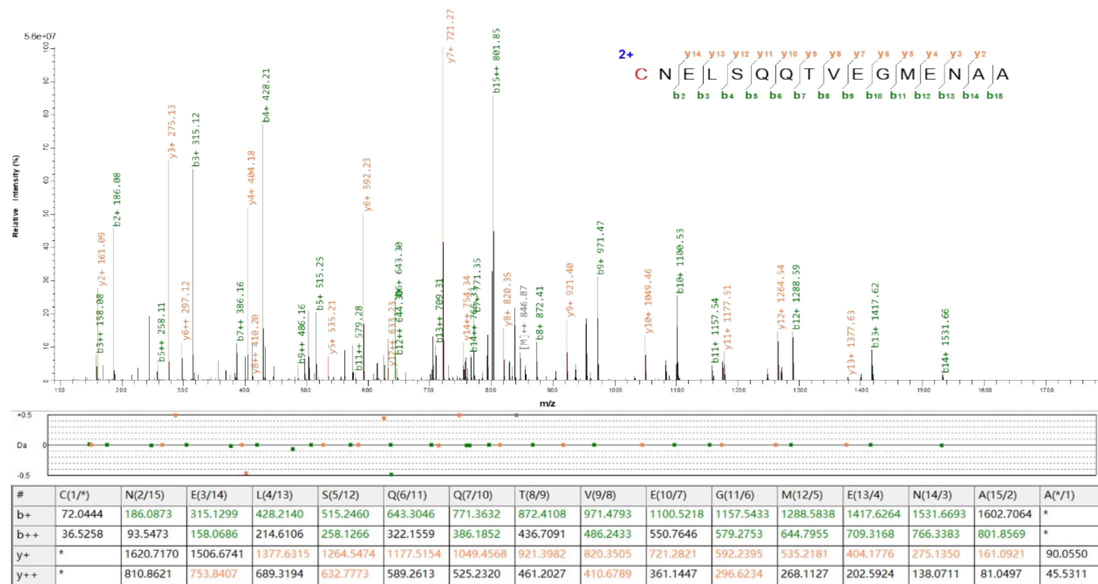
The reaction was followed General Procedure using peptide **7a**, sequence: NH2-**C**NELSQQTVEGMENAA-OH.



LC trace of the reaction of peptide **7a** and purified product **7b**.



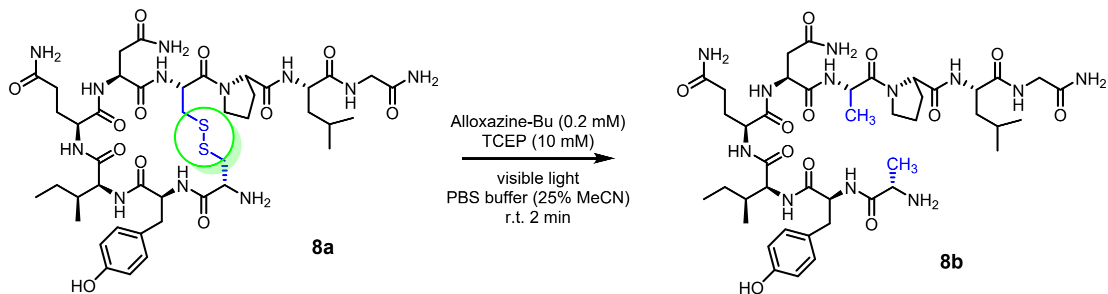
ESI Mass spectrum of purified product **7b**. Calculated Mass $[M+2H]^{2+}$: 846.38; Mass Found (ESI+) $[M+2H]^{2+}$: 846.38.



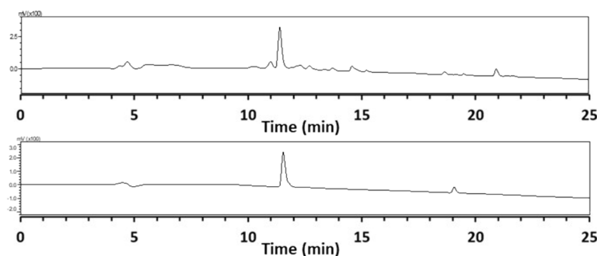
#	C(1/*)	N(2/15)	E(3/14)	L(4/13)	S(5/12)	Q(6/11)	Q(7/10)	T(8/9)	V(9/8)	E(10/7)	G(11/6)	M(12/5)	E(13/4)	N(14/3)	A(15/2)	A(*/1)
b+	72.0444	186.0873	315.1299	428.2140	515.2460	643.3046	771.3632	872.4108	971.4793	1100.5218	1157.5433	1288.5838	1417.6264	1531.6693	1602.7064	*
b++	36.5258	93.5473	158.0686	214.6106	258.1266	322.1559	386.1852	436.7091	486.2433	550.7646	579.2753	644.7955	709.3168	766.3383	801.8569	*
y+	*	1620.7170	1506.6741	1377.6315	1264.5474	1177.5154	1049.4568	921.3982	820.3505	721.2821	592.2395	535.2181	404.1776	275.1350	161.0921	90.0550
y++	*	810.8621	753.8407	689.3194	632.7773	589.2613	525.2320	461.2027	410.6789	361.1447	296.6234	268.1127	202.5924	138.0711	81.0497	45.5311

De novo ms/ms analysis of purified product **7b**.

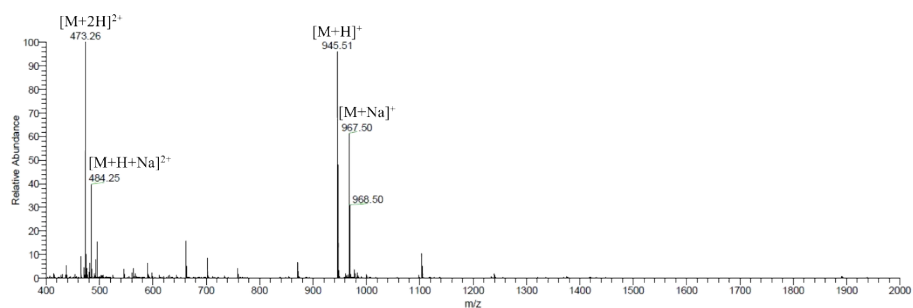
3.9. Characterization data for the reaction of peptide 8



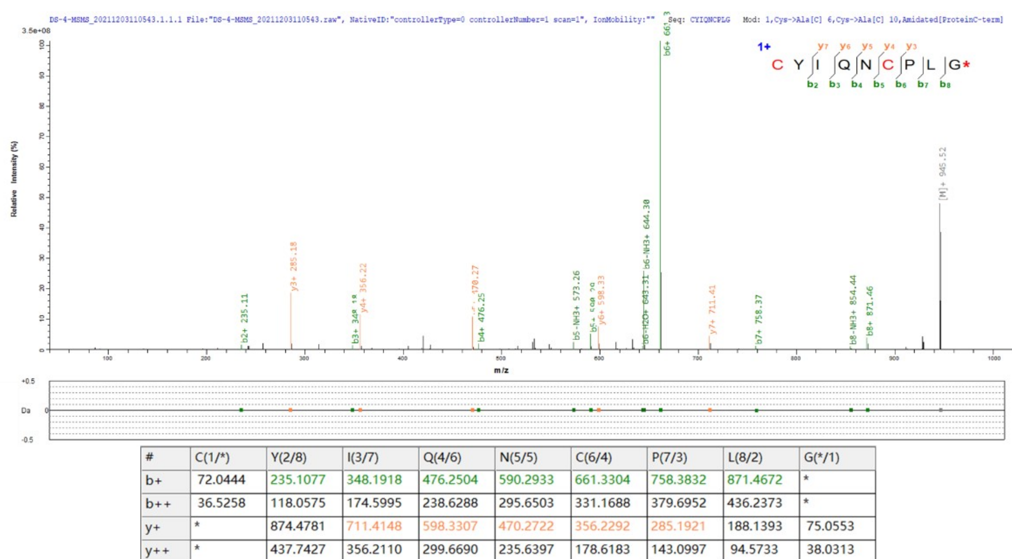
The reaction was followed General Procedure using peptide **8a**, sequence: NH_2-**C**YIQN**C**PLG-OH.



LC trace of the reaction of peptide **8a** and purified product **8b**.



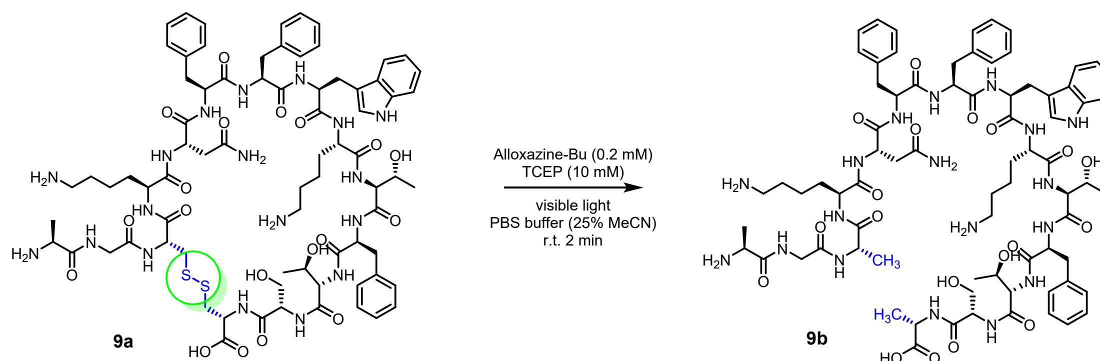
ESI Mass spectrum of purified product **8b**. Calculated Mass $[M+H]^+$: 945.51; $[M+2H]^{2+}$: 473.26; Mass Found (ESI+) $[M+H]^+$: 945.51; $[M+2H]^{2+}$: 473.26.



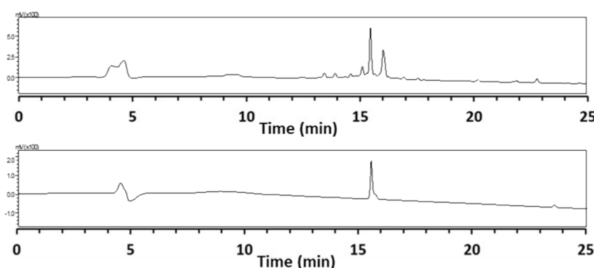
#	C(1/*)	Y(2/8)	I(3/7)	Q(4/6)	N(5/5)	C(6/4)	P(7/3)	L(8/2)	G(*/1)
b+	72.0444	235.1077	348.1918	476.2504	590.2933	661.3304	758.3832	871.4672	*
b++	36.5258	118.0575	174.5995	238.6288	295.6503	331.1688	379.6952	436.2373	*
y+	*	874.4781	711.4148	598.3307	470.2722	356.2292	285.1921	188.1393	75.0553
y++	*	437.7427	356.2110	299.6690	235.6397	178.6183	143.0997	94.5733	38.0313

De novo ms/ms analysis of purified product **8b**.

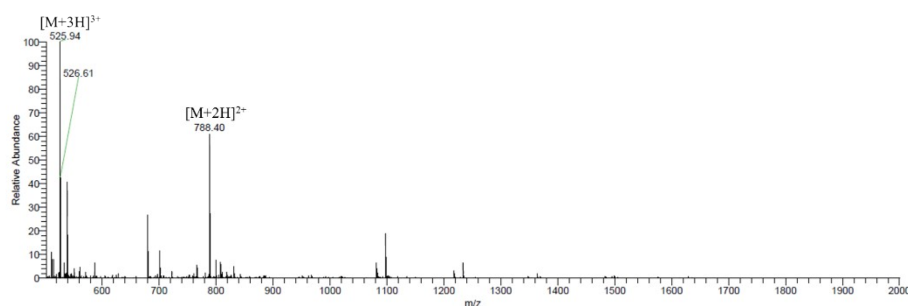
3.10. Characterization data for the reaction of peptide 9



The reaction was followed General Procedure using peptide **9a**, sequence: NH_2-AG**C**KNFFWKTFTS**C**-OH.



LC trace of the reaction of peptide **9a** and purified product **9b**.



ESI Mass spectrum of purified product **9b**. Calculated Mass $[M+2H]^{2+}$: 788.40; $[M+3H]^{3+}$: 525.94; Mass Found (ESI+) $[M+2H]^{2+}$: 788.40; $[M+3H]^{3+}$: 525.94.

4. Supplementary information for desulfurization of proteins

4.1. General information

All chemicals were purchased from *Sigma-Aldrich* or *Energy Chemical*, unless otherwise stated. All solutions were made with ultrapure Milli-Q water (Millipore, Bedford, MA). **ESI-MS** analysis was measured on a Q_Exactive_Focus.

4.2. General procedure for the desulfurization reaction of protein

A 10 mM MeCN stock solution of alloxazine-Bu was made up of 3.4 mg alloxazine-Bu (0.01 mmol) and 1 mL MeCN, a 100 mM water stock solution of TCEP was made up of 25 mg TCEP (0.1 mmol) and 1 mL deionized water, and a 100 umol stock solution of protein was made up with 1 mL PBS buffer (pH 7.4). These stock solutions were stored at room temperature away from light. To a 2 mL vial was added 200 µL protein solution, 0.4 µL alloxazine-Bu (10 mM) and 10 µL TCEP (100 mM) stock solution. The vial was then caped and equipped with magnetic bar. The mixture was then degassed with nitrogen. The reaction was stirred and irradiated with 400 nm LED light (40 W) for 20 s. The resulting solution was then analyzed by ESI-MS and LC-MS/MS.

The full sequence of BSA:
DTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELTEFAKTCVADESHAGCE
KSLHTLFGDELCKVASLRETYGDMADCCEKQEPERNECFLSHKDDSPDLPKLKPDPNTLCDEF
KADEKKFWGKYLYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETMREK
VLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKAEFVEVTKLVTDLTKVHKECCHGDL
LECADDRADLAKYICDNQDTISSKLKECCDKPLLEKSHCIAEVEKDAIPENLPPLTADFAEDKD
VCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRLAKEYEATLEECCAKDDPHACYSTVFDK
LKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCT
KPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTESLVNRRPCFSALTPDETYVPKAFD
EKLFTFHADICTLPDTEKQIKKQTALVELLKHKPKATEEQLKTVMENFVAFVDKCCAADDKE
ACFAVEGPKLVVSTQTALA

The full sequence of UBE2D2 with His-tag:
MNHKHHHHHHSSGENLYFQGAMDPMALKRIHKELNDLARDPPAQCSAGPVGDDMFHWQAT
IMGPNDSPYQGGVFFLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTISKV
LLSICSLLCDPNPDDPLVPEIARIYKTDREKYNRIAREWTQKYAM

4.3. ESI-MS analysis the reaction of protein

Mixture was subjected to centrifuge (5000 rpm) by using ultrafiltration centrifuge tube (with 10K molecular weight cutoff), which was pretreated with water. The protein substrates were washed (3 × 200 µl water) to remove the remaining substrates and the mixture concentration was determined by

Nano drop for ESI-MS analysis.

4.4. LC-MS/MS analysis the reaction of protein

After dry in the speed VAC, obtained samples were loaded onto a Thermo analytical column (75 μm i.d. × 25 cm) C18 column with an Easy-nLC 1200 chromatography pump coupled with Orbitrap Exploris^{TM}480. For each analysis, we reconstituted peptides in 10 μl 0.1% FA and loaded 8 μl onto the column for running. Peptides in each running were separated on a 110 min (8-40% ACN) gradient. Parameters are as follows in Full MS/ data dependent -MS2 TopN mode: mass analyzer over m/z range of 350−1500 with a mass resolution of 60000 (at m/z=200) in a data-dependent mode, 1.6 m/z isolation window. 20 most intense ions are selected for MS/MS analysis at a resolution of 15000 using collision mode of HCD. Peptides labeled in vitro were analyzed by Orbitrap Exploris^{TM}480 and peptides labeled in vivo were analyzed by Lumos, under the same setting parameters.

5. Reference

1. X. F. Gao, J. J. Du, Z. Liu and J. Guo, *Org Lett*, 2016, **18**, 1166-1169.