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Supporting Information

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MALDI in-source photo-oxidation reactions for on-line peptide tagging

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S1: Reagents and solution preparation

S1.1 Reagents:

Ethanol (≥99.7%) was purchased from Shanghai Zhenxing NO. 1 Chemical Company. Acetic acid (≥99.5%) was purchased from Shanghai NO.4 Reagent Company, Kunshan. Hydroquinone (HQ, 98%) was obtained from Shanghai Runjie Chemical Reagents Ltd. Titanium dioxide nanoparticle was obtained from Degussa (P25, Germany). Peptides SSDQFRPDDCT (C-pep), SSDQFRPDDGT, DNEAYEMPSEE, AEKTKEGVW, ACKCTCM (3C-pep), DRVYIHPF and RPVKVYPNGAEDESAEAFPLEF (90%, Mw 1269.5, 1223.5, 1312.5, 1046.5, 758.3, 1045.5, and 2464.2 g/mol respectively) were obtained from Shanghai HD Biosciences Corporation. Diammonium citrate (99%) was purchased from Amresco. Acetonitrile (ACN, 99.9%) and trifluoroacetic acid (TFA, 99.8%) were purchased from Merck (Darmstadt, Germany), while ammonium bicarbonate (99%), 2,5-Dihydroxybenzoic acid (DHB, 98%), 2-methoxyhydroquinone (MOHQ, 98%), α-Cyano-4-hydroxycinnamic acid (CHCA 99%), β-lactoglobulin A (from bovine milk, 90%), albumin (Bos Taurus, 95%) and trypsin (from bovine pancreas) were obtained from Sigma (St. Louis, MO). All these reagents were used as received without further purification. Deionized water (18.2 MΩ.cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

S1.2 Solutions:

CHCA matrix: mixture 1:1 (v/v) of 0.4 mg of diammonium citrate in 1ml of ACN/H_2O/TFA (50/49.9/0.1% (v/v)) and 8 mg of CHCA in 1mL of ACN/H_2O/TFA (50/49.9/0.1% (v/v)). Proteins, peptides, hydroquinone, DHB and MOHQ were diluted in the deionised water to the required concentrations.

S1.3 Protein digestion:

1mg β-lactoglobulin A or albumin was dissolved in 1 mL ammonium bicarbonate (25mM, pH ~8),

denatured at 100°C for 5 min and digested for 12 h at 37 °C with an enzyme to protein ratio of 1:30 (w/w). After proteolysis, 2mM DTT was added into each of the digests and the mixtures were incubated at 37 °C for 1 h to break the disulfide bonds.

S1.4 Preparation of suspension of TiO_2 nanoparticles:

The P25 TiO_2 nanoparticles were heated at 300°C for 2h and then separated in a mortar for another 2h. 1ml 10% acetic acid (in water) was added for every 1g TiO_2 particles dropwise during the separation procedure to keep wet. After separating, the nanoparticles were suspended in 89% ethanol (100mg/ml), followed by sonication for 1 h. Before use, this suspension was diluted in deionized water by 100 times.

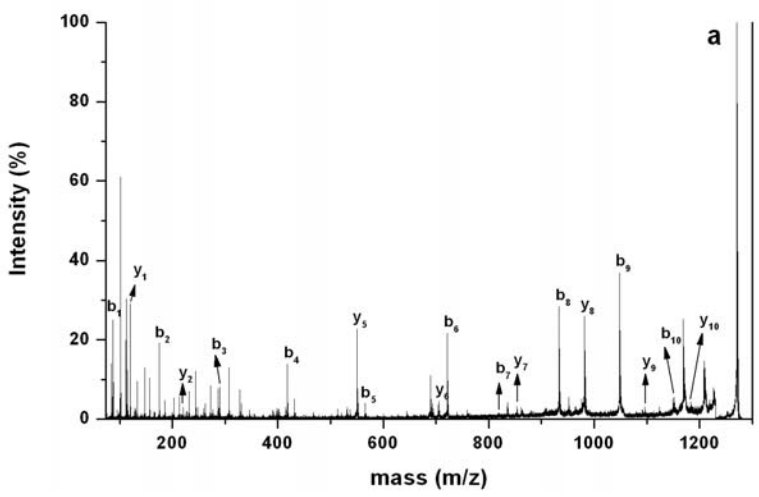
S1.5 TiO_2 photoelectrode plate preparation

The diluted suspension of TiO_2 nanoparticles was dropped on a stainless steel plate as an array of spots (~2μL) and dried at room atmosphere and temperature overnight. The Resulting modified plate is subsequently heated in an oven at 400°C for one hour (The oven temperature rises from room temperature to 400°C in 30 minutes) and naturally cooled-down to room temperature and stored at room temperature in a desiccator.

S2: TiO_2 photoelectrode assisted in-source hydroquinone tagging

S2.1 MS/MS spectrum of the tagged and untagged peptides:

Special precursor ions were selected after the MS procedure, and these ions were analyzed by the MS/MS peptide sequencing on the Applied Biosystems 4700 proteomics analyzer, 3800 laser shots (355nm, 200Hz) at a laser intensity of 6500 instrument units.



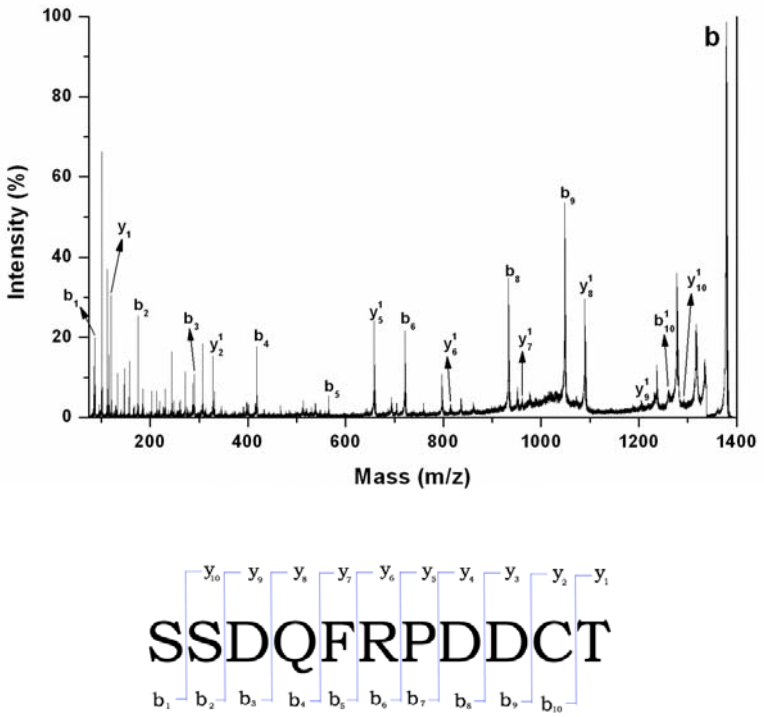


Figure SI-1. MS-MS spectra of untagged peptide peak m/z 1270.5 Th (a) and tagged peptide peak m/z 1378.6 Th (b).

Table SI-1. List of b- and y-type of fragments generated from parent ion peaks m/z 1270.5 Th and m/z 1378.6 Th.

Name of fragment	Mass(m/z)	Name of fragment	Mass(m/z)
b_1	88.1	b_2	175.1
b_3	290.1	b_4	418.2
b_5	565.3	b_6	721.4
b_7	818.5	b_8	933.5
b_9	1048.6	b_{10}	1151.6
y_1	120.1	y_2	220.1
y_5	550.2	y_6	706.4
y_7	853.5	y_8	981.6
y_9	1096.6	y_{10}	1183.7
b_{10}^1	1260.4	y_2^1	328.1
y_5^1	658.1	y_6^1	814.3
y_8^1	1089.4	y_9^1	1204.4
y_{10}^1	1292.5		

S2.2 Selectivity of the photo-induced cysteine tagging:

~0.4 μL mixture of pep/hydroquinone (1/1 (mol/mol) was deposited on the TiO_2 photoelectrode array immediately after mixing and dried at room temperature and atmosphere in dark for ~10 min.

After drying, ~0.4μL of CHCA was dropped and then dry in the same conditions and finally subjected to MALDI-TOF-MS (Applied Biosystems 4700 Proteomics Analyzer, 2000 laser shots (355nm, 200Hz) at a laser intensity of 5500 instrument units). As a comparison, ~0.4 μL of peptide was dropped on a normal target plate, dry in the same conditions and ~0.4 μL of the CHCA matrix was then dropped, dried (same conditions) and then subjected to MALDI-TOF-MS (Applied Biosystems 4700 Proteomics Analyzer, 2000 laser shots (355nm, 200Hz) at a laser intensity of 5500 instrument units). The concentrations of peptides and hydroquinone were fixed at ~4μM. All the peptides and hydroquinone were dissolved in deionized water.

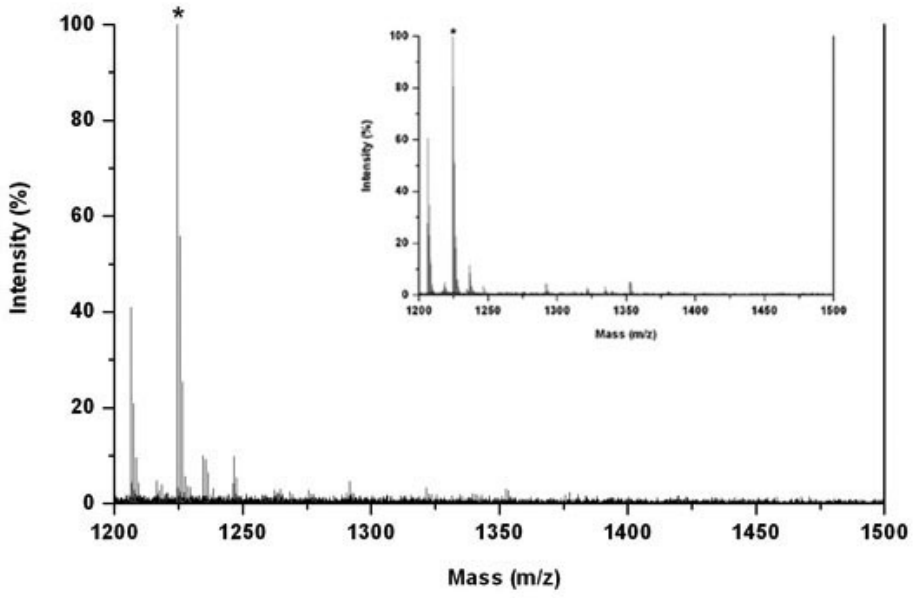


Figure SI-2a. MS spectrum of peptide (SSDQFRPDDGT)/hydroquinone (1/1 (mol/mol), peptide is ~1.5 pmol) performed on the TiO_2 modified target plate, and MS spectrum of peptide performed on the regular target plate (insert figure). Protonated form of * Native SSDQFRPDDGT (*m/z* 1224.5 Th)

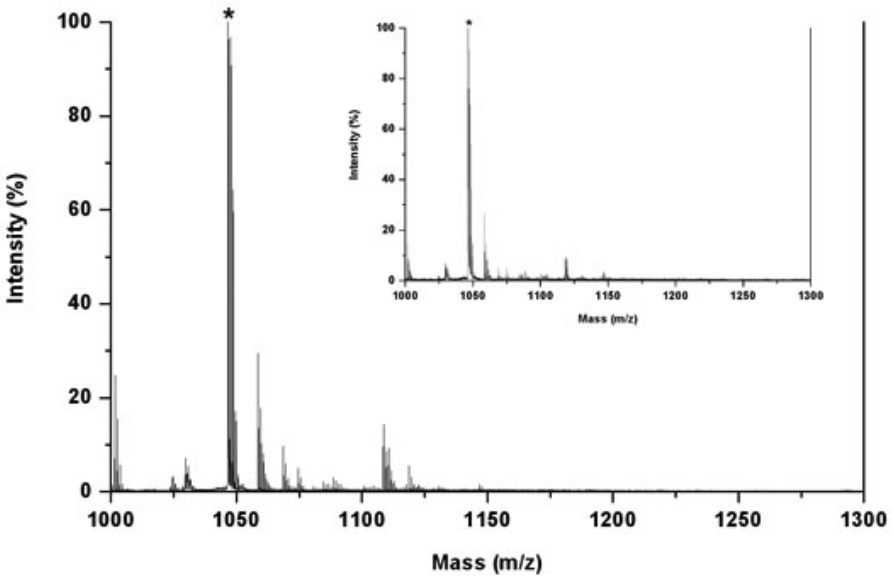


Figure SI-2b. MS spectrum of peptide (DRVYIHPF)/hydroquinone (1/1 (mol/mol), peptide is ~1.5 pmol) performed on the TiO_2 modified target plate, and MS spectrum of peptide performed on the regular target plate (insert figure). Protonated form of * Native peptide DRVYIHPF (*m/z* 1046.5 Th)

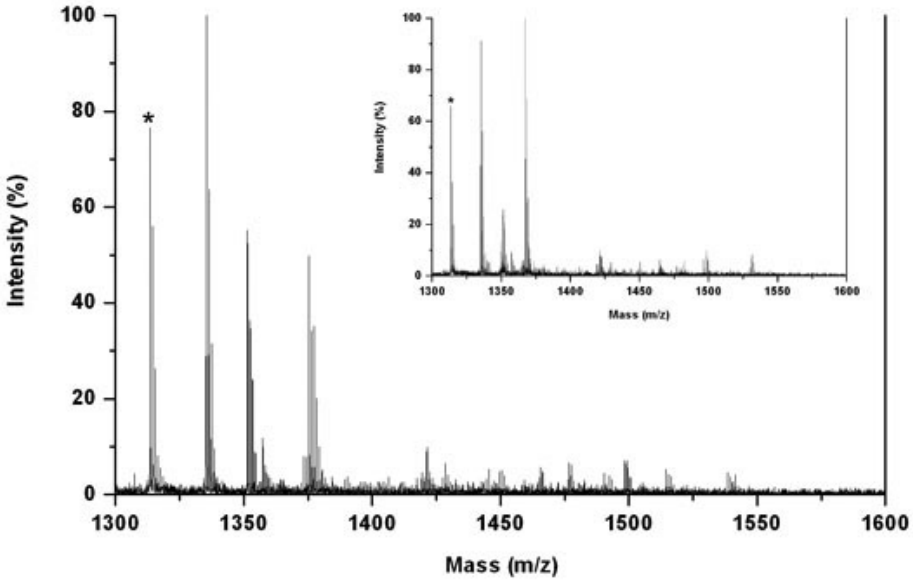


Figure SI-2c. MS spectrum of peptide (DNEAYEMPSEE)/hydroquinone (1/1 (mol/mol), peptide is ~1.5pmol) performed on the TiO_2 modified target plate and MS spectrum of peptide performed on the regular target plate (insert figure). Protonated form of * Native peptide DNEAYEMPSEE (*m/z* 1313.5 Th)

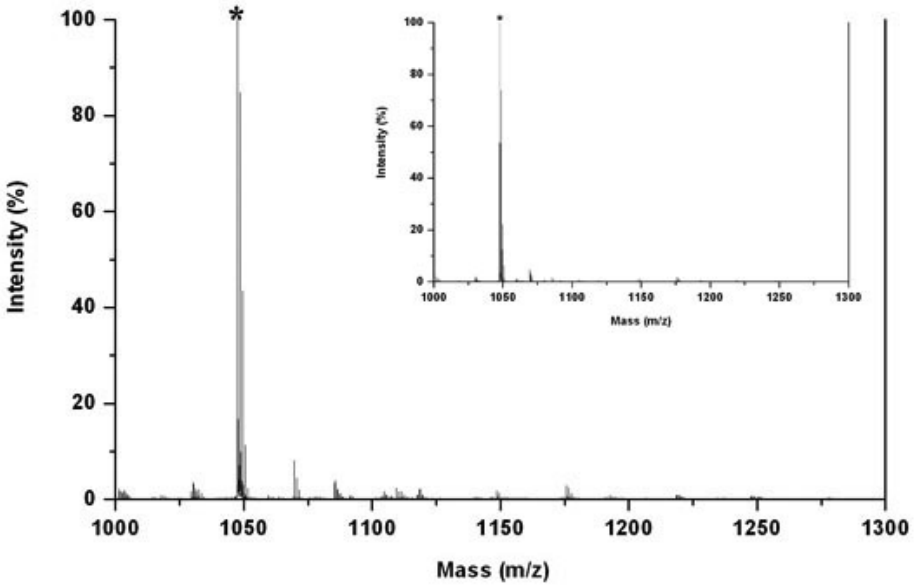


Figure SI-2d. MS spectrum of peptide (AEKTKEGVW)/hydroquinone (1/1 (mol/mol), peptide is ~1.5pmol) performed on the TiO_2 modified target plate and MS spectrum of peptide performed on the regular target plate (insert figure). Protonated form of * Native peptide AEKTKEGVW (*m/z* 1047.5 Th).

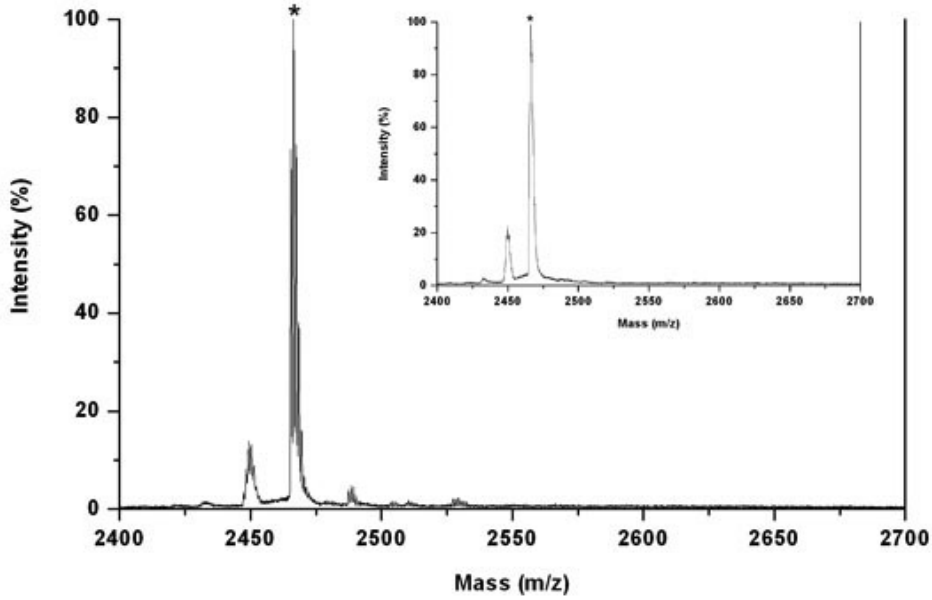


Figure SI-2e. MS spectrum of peptide (RPVKVYPNGAEDESAEAFPLEF)/hydroquinone (1/1 (mol/mol), peptide is ~1.5 pmol) performed on the TiO_2 modified target plate and MS spectrum of peptide performed on the regular target plate (insert figure). Protonated form of * Native peptide

RPVKVYPNGAEDESAEAFPLEF (*m/z* 2465.2 Th).

S2.3 Rough calculation on the percentage of the radiation transmitted to the TiO2 surface:

The Nd:YAG laser (355 nm, Laser-Compact, Moscow, Russia) employed in this experiment was operated at the attenuator setting of 5500, corresponding to a laser intensity of ~665 μJ mm^{-2}, equal to ~1.2×10^{15} photons per unit area (mm^2).

The fabricated TiO_2 spot has an average diameter of 3mm, and the amount of CHCA matrix on one spot is 1.6μg, equal to 5.1×10^{15} molecules. Thus, we can calculate the number of matrix molecule on unit TiO_2 surface area and get a result of ~7.2×10^{14} mm^{-2}. Supposing a hard sphere model for matrix molecule with a diameter of ~1nm, the thickness of matrix overlayer is about 0.5 micron. Comparing with the density of photon provided in one laser pulse, even if every matrix molecule presented under the radiation is excited by capturing one photon, there is still about 40% of the radiation excess. Considering some matrix molecules may penetrate into the nanopores of the TiO_2 substrate, the thickness of matrix overlayer would be further decreased and more photons would reach the photoreactive TiO_2 nanoparticle surface.

S2.4 Tagging efficiency:

~0.4 μL mixture of C-pep/DHB (1/1 (mol/mol)), C-pep/MOHQ (1/1 (mol/mol)) or C-pep/HQ/DHB/MOHQ (1/1/1/1 in mol) was deposited on the TiO_2 photoelectrode array immediately after mixing respectively and dried at room temperature and atmosphere in dark for ~10 min. After drying, ~0.4μL of CHCA was dropped and then dry in the same conditions and finally subjected to MALDI-TOF-MS (Applied Biosystems 4700 Proteomics Analyzer, 2000 laser shots (355nm, 200Hz) at a laser intensity of 5500 instrument units). The C-pep and quinones were dissolved in deionised water at a final concentration of ~4 μM.

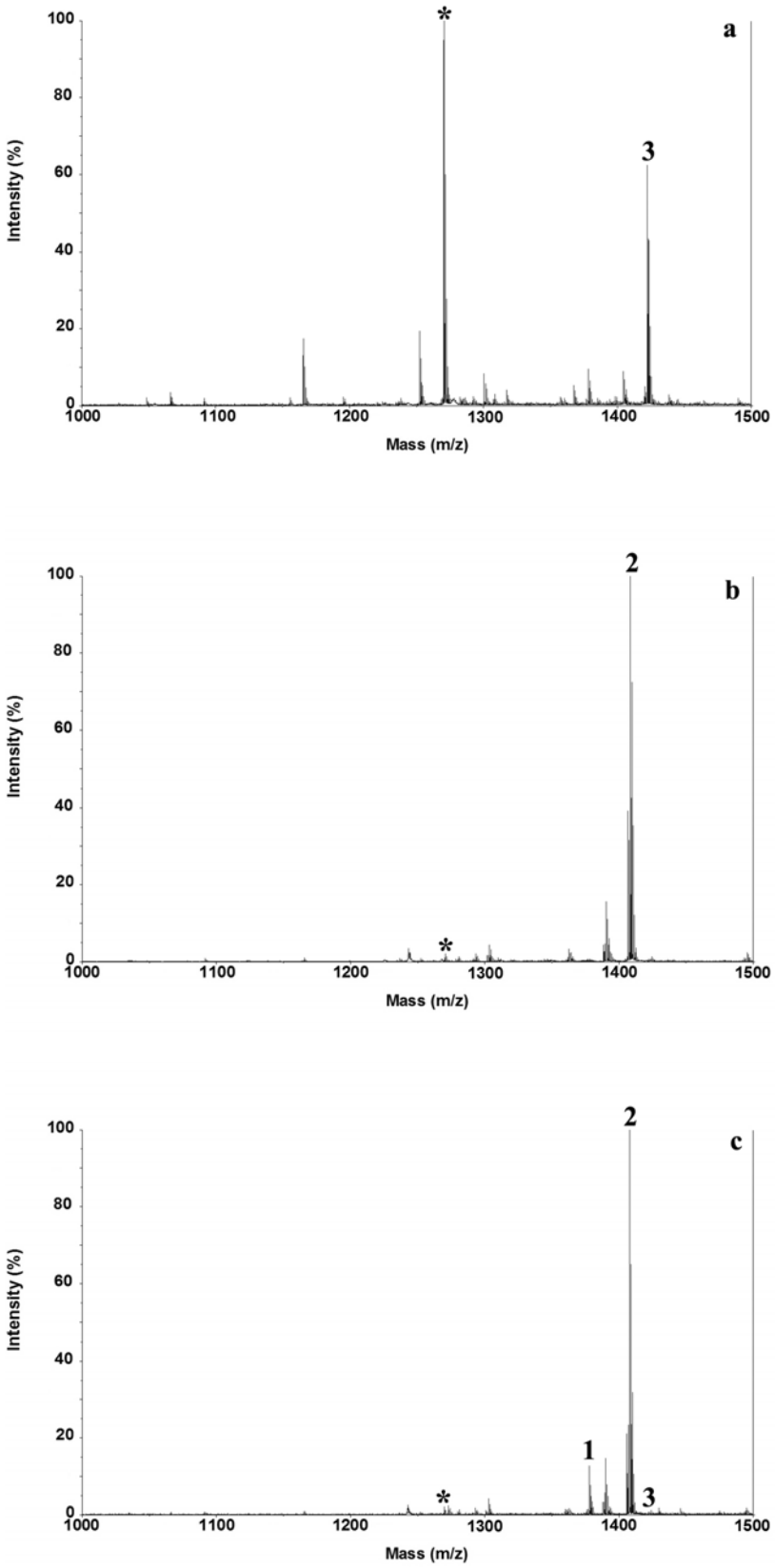


Figure SI-3. MS spectrum of C-pep/DHB (a, 1/1 (mol/mol), C-pep is 1.5pmol), C-pep/MOHQ (b, 1/1 (mol/mol), C-pep is 1.5pmol) and C-pep/HQ/DHB/MOHQ (c, 1/1/1/1 in mol, C-pep is 1.5 pmol) performed on the TiO_2 modified target plate. Protonated form of *native C-peptide (m/z 1270.5 Th),

protonated form of 1, HQ tagged C-peptide (*m/z* 1378.6 Th), 2, MOHQ tagged C-peptide (*m/z* 1408.6 Th), 3, DHB tagged C-peptide (*m/z* 1422.6 Th).

S2.5 Peptides mass fingerprinting (PMF) of β-lactoglobulin A and albumin using the in-source tagging:

Two cysteine-containing proteins β-lactoglobulin A (~1.84×10^4 Da, 5 cysteine residues) and albumin (Bos taurus, ~6.93×10^4 Da, 35 cysteine residues) were employed to illuminate the usability of this in-source photo-induced tagging strategy in protein profiling. Protein digests were mixed with excess amount of hydroquinone respectively. ~0.4μL of each mixture was deposited on the TiO_2 photoelectrode array immediately after mixing and dried at room temperature and atmosphere in dark for ~10 min. After drying, ~0.4μL of CHCA was dropped and dried in the same conditions and finally subjected to MALDI-TOF-MS (Applied Biosystems 4700 Proteomics Analyzer, 2000 laser shots (355nm, 200Hz) at a laser intensity of 5500 instrument units). Cysteine containing peptides were read out through finding a pair of peaks with an m/z shift of 108.1 Th directly from one MS spectrum without separation.

The entire list of observed peaks (S/N＞10) of each protein digests was submitted to Mascot sequence query for a search in the NCBInr database with or without giving information on the cysteine content. The number of cysteines is specified for cysteinyl-containing peptides by "comp(number[C])" entered after the peptide *m/z*.

Table SI-2. Identified cysteine containing peptides from β-lactoglobulin digest

m/z of the observed precursor and tagged peptides[#]	number of cysteine	sequence of precursor peptides
1122.45, 1230.56[#]	1	WENDECAQK
1250.55, 1358.66[#]	1	WENDECAQKK
1658.78, 1766.89[#]	1	LSFNPTLQEEQCHI
2675.23, 2783.34[#], 2891.45[#], 2999.56[#]	3	YLLFCMENSAEPEQSLVCQCLVR

Table SI-3. Identified cysteine containing peptides from albumin digest

m/z of the observed precursor and tagged peptides[#]	number of cysteine	sequence of precursor peptides
841.46, 949.57[#]	1	LCVLHEK
1011.42, 1119.53[#]	1	QNCDQFEK
1024.45, 1132.56[#], 1240.66[#]	2	CCTESLVNR
1052.45, 1160.56[#]. 1268.66[#]	2	CCTKPESER
1362.67, 1470.78[#]	1	SLHTLFGDELCK
1386.62, 1494.73[#]	1	YICDNQDTISSK

1418.74, 1526.85#, 1634.96#	2	LKECCDKPLLEK
1519.75, 1627.86#	1	LKPDPNTLCDEFK
1616.75, 1724.86#	1	QEPERNECFLSHK
1637.80, 1745.91#	1	MPCAEDYLSLILNR
1667.81, 1775.92#	1	MPCTEDYLSLILNR
1738.81, 1846.92#	1	DDPHACYSTVFDKLK
1823.90, 1932.01#	1	RPCFSALTPDETYVPK
1850.92, 1959.03#	2	SLGKVGTRCCTKPESER
1962.95, 2071.06#	1	LKPDPNTLCDEFKADEK
2076.88, 2184.99#, 2293.10#, 2401.21#	3	ECCHGDLLECADDRADLAK
2091.04, 2199.15#	1	LKPDPNTLCDEFKADEKK
2441.17, 2549.28#	1	AFDEKLFTFHADICTLPDTEK
2484.15, 2592.26#	1	QEPERNECFLSHKDDSPDLPK

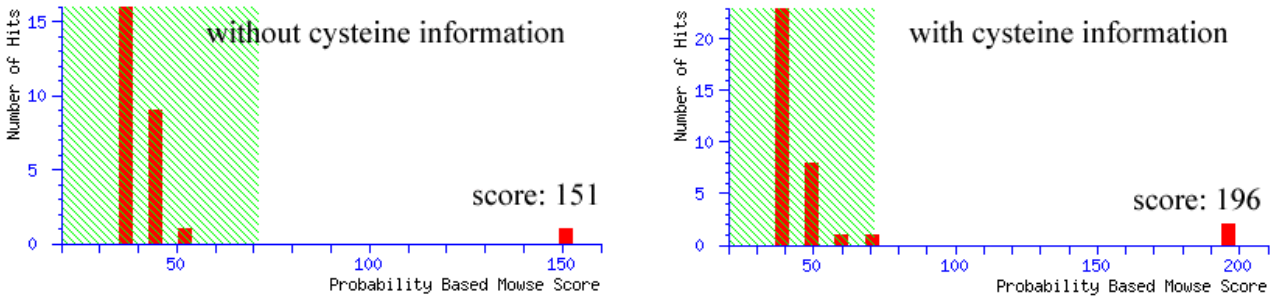


Figure SI-4a. Database searching results got without and with the cysteine content information for β-lactoglobulin using Mascot.

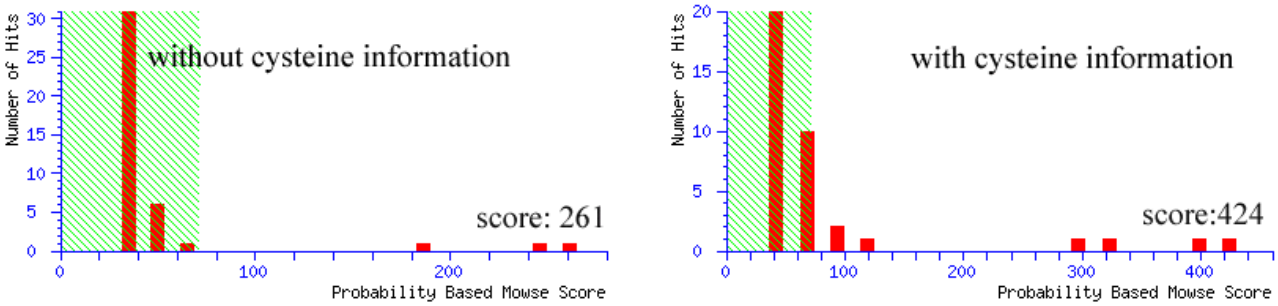
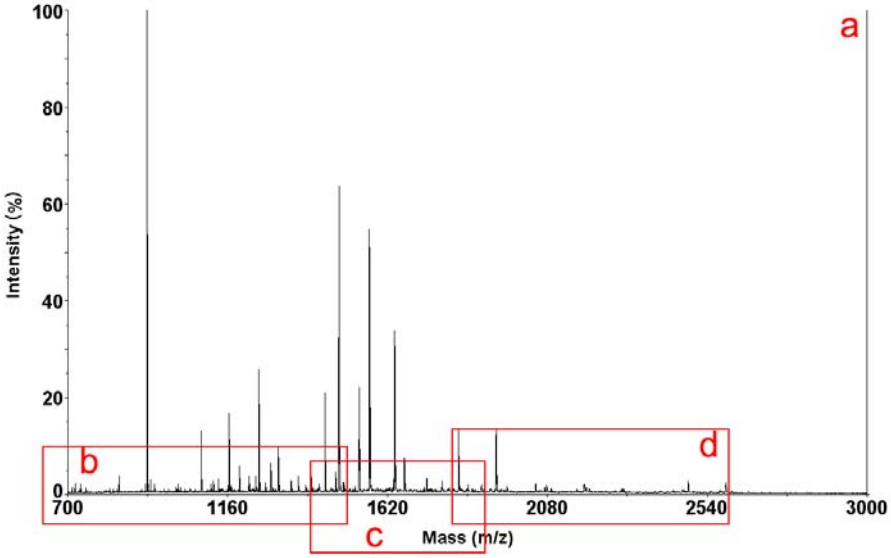


Figure SI-4b. Database searching results got without and with the cysteine content information for albumin using Mascot.


a
b
c
d
Intensity (%)
Mass (m/z)
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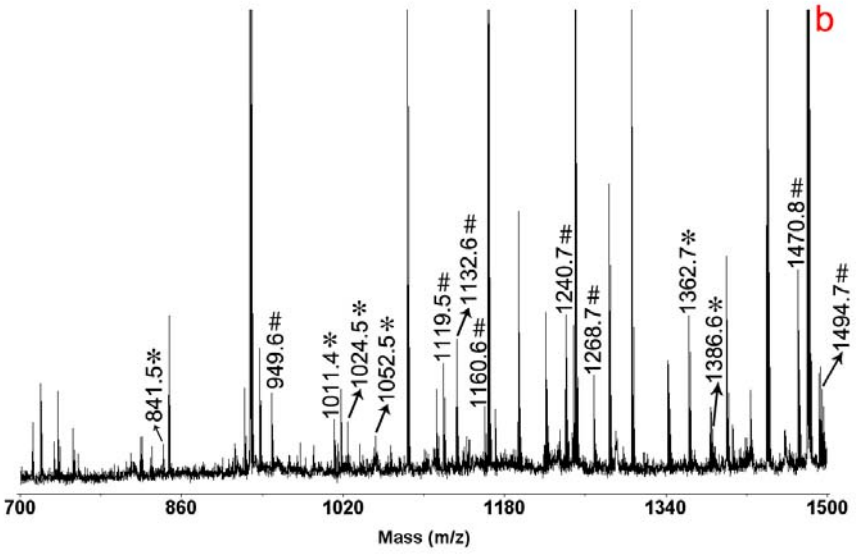
60
40
20
0
700
1160
1620
2080
2540
3000


b
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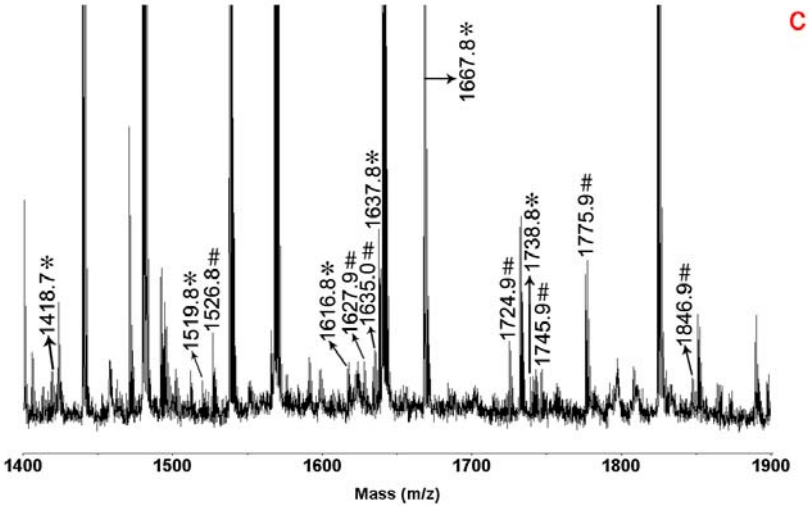
Mass (m/z)


c
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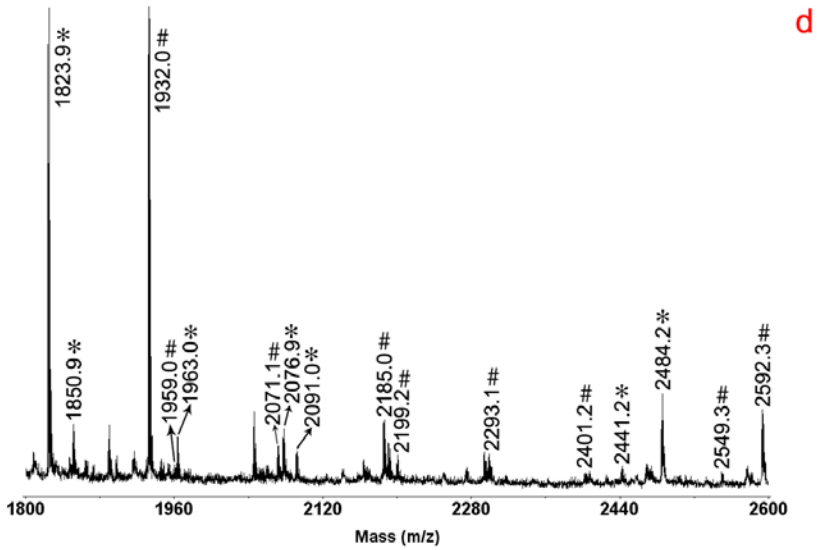

Figure SI-5. a) MS spectrum of albumin digest (~0.3 pmol) in the presence of hydroquinone on the TiO_2 modified target plate; b, c and d) Amplified figures of corresponding regions.