Increasing specificity of tandem mass spectrometry by laser-induced dissociation

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Abstract

Mass spectrometry offers an arsenal of tools for diverse proteomic investigations. This perspective article reviews some of the recent developments in the field of coupling laser induced dissociation with mass spectrometry (LID-MS). Strategies involving labelling with a chromophore to induce specific photo-absorption properties are considered, with a focus on specific amino acid derivatization. Some of the opportunities and challenges of LID-MS after targeted labelling for increasing specificity in complex sample analysis are discussed.

Keywords

Laser induced dissociation, chromophore derivatization

Introduction

Nowadays, tandem mass spectrometry (MS/MS) coupled to liquid chromatography is the pivotal tool on the proteomics roadmap leading to protein identification and quantification. In particular, the bottom-up strategy relying on the analysis of peptides released by enzymatic digestion of a protein extract is still predominantly deployed[1]. Thus, the top n most intense peptide signals detected in the full MS spectrum sampling the chromatographic separation are then successively filtered in a Data Dependent Acquisition (DDA) mode for subsequent MS/MS experiment. Thanks to the continuous progress in acquisition speed and instrumentation sensitivity, deep and extensive proteome coverage is now routinely achieved. However, proteome tryptic lysates result in complex samples, not only in terms of molecule number (probably hundreds of thousands of peptides) but above all due to the wide dynamic range of concentration (up to 10^{12} in human plasma)[2,3]. Thus, stochasticity of DDA sampling may result in overlooking weakly concentrated peptides, masking potential biomarkers. Moreover, the huge complexity of the digested samples generates a huge amount of data that results in long search times with possibly difficult data interpretation and high false positive or negative identifications due to co-eluted interfering compounds. This co-fragmentation is also deleterious for accurate quantification. Extensive sample fractionation either at the protein or peptide level based on their physical properties or cellular localization can be performed in order to reduce sample complexity. Moreover, different strategies have been proposed to target a subset of a proteome, for example N-linked glycopeptides[4,5] or specific amino acid side chains[6,7], after selective enrichment. In turn, this compelling fractionation/enrichment step is deleterious to high throughput and precision of the analytical method.

Another way to decrease the sample complexity is to add specificity at the fragmentation level. Indeed, collision-induced dissociation (CID) and electron-transfer dissociation (ETD) are the most widespread techniques to dissociate peptides of relatively small size after trypsin digestion. However, these non-discriminant activation methods induce the fragmentation of any selected ion. On the contrary, many reports have illustrated the promising potential of laser-induced dissociation (LID) to introduce more specificity during the fragmentation step[8,9]. Compared to collisional activation, laser-induced dissociation (LID) enables specific excitation. As an illustration, infrared multiphoton photodissociation (IRMPD) at 10.6 μm has shown the interesting propensity to differentiate phospho- and non-phosphorylated peptides[10] due to the increased photon absorption of the phosphate group. Moreover, IRMPD shows specificity towards sulfonated peptides[11,12]. LID in the UV range (UVPD at 266 nm) also offers excellent versatility because the absorption of native biomolecules depends on their intrinsic chromophores such as disulfide bound or tyrosine, tryptophan and phenylalanine residues [13–15]. However, the most obvious strategy for incorporating a higher degree of fragmentation specificity into a proteomics workflow is to target only a subset of peptides after derivatization with specific chromophores in the visible range. When using wavelengths above 350 nm, where peptides and proteins do not absorb, only peptides that are tagged with the proper chromophore are activated and photo-dissociated. Highly selective and targeted approaches can be developed depending on how the chromophores are tagged, especially, via derivatization of a particular amino acid side-chain. We will see in the next section that chromophore derivatization of Tyr/His, Lys and Cys allows to target a subset of peptides when using near-UV or Visible wavelengths.

Chromophore-derivatized amino acid specific LID

Numerous strategies of derivatization combined to mass spectrometry analysis have been described. Adding ionizable sites or fixed charges allows to increase the ionization yield of molecules[16]. Moreover, hydrophobicity[17] and fragmentation pattern of ions[18,19] can be modified via derivatization strategies. Derivatization of proteins with chromophores is also used to probe conformational dynamics[20]. The popular iTRAQ[21,22] and ICAT[6] methods similarly exploit the chemical derivatization with isobaric tags in order to facilitate quantification. On the other hand, attachment of chromophores offers the opportunity to convert non-absorbing molecules into absorbing ones in the near-UV (>350 nm) and Visible ranges. In a pioneer study, N-terminus attachment of dinitrophenyl on amino acids and small peptides allows to produce fragments upon photoexcitation by 333-365 nm and 454-514 nm photons[23]. Other studies have been reported that illustrate the strategy of attaching chromophores to molecules at the N-terminal or C-terminal (and acidic residues) in order to increase their light absorption properties at 355[24,25] or 351 nm[26]. Fragmentation patterns were also simplified significantly, exhibiting only b ions, thus allowing to improve *de novo* peptide sequencing.

However, with the purpose of increasing the specificity of fragmentation in a complex mixture, derivatization of specific amino acids is more appropriate than all terminus peptide groups. Indeed, in this targeted approach, only the subset of derivatized peptides will specifically photo-fragment due to the high absorption cross section of the chromophore. This concept has been used to target Tyr/His-containing peptides after derivatization with a nitroazobenzene chromophore via a diazonium reaction[27] (Scheme 1). Diagnostic fragmentation patterns were produced only for the tagged peptides upon UVPD at 351 nm (Figure 1). This fragmentation specificity results in a streamlined LC-MS/MS data analysis of protein mixture by reducing the redundancy of database searches. Moreover, the exclusive fragmentation of chromophore derivatized peptides (containing His or Tyr) was exploited as a

means to eliminate the problem of congested MS/MS spectra from precursor ion overlap, allowing more confident protein identification.

The attachment of chromophores to specific amino acid residues can also be used to promote diagnostic fragmentation pathways. For example, iodination of tyrosine residues is used to localize sites of modification of proteins[28,29]. Indeed, site specific photodissociation at 266 nm generates radical at the iodinated tyrosine residues. Subsequent heating of these radicals by CID leads to radical directed dissociation primarily in the local environment of the iodinated tyrosine.

The specific detection of chemical probe-modified sites of proteins has been reported using UVPD at 351 nm[30].The amine-reactive NN probe incorporates a UV chromophore and a sulfo-NHS ester group, allowing to tag the Lys residues of native proteins. After enzymatic digestion, specific fragmentation of only the derivatized peptides allows to pinpoint them among complex mixtures of peptides. The NN probe-modified peptides undergo efficient photodissociation to produce b/y fragment ions as well as two characteristic reporter ions of the chromophore. The accessibility of lysine residues at the protein surface can be probed based on their relative reactivity with the NN probe by quantification of the modified vs. unmodified peptides, respectively by UVPD and CID.

In order to increase the specificity of the method, targeting of low frequency amino acids is preferable. The specific detection of cysteine-containing peptides by implementing LID in the Visible range at 473 nm[31], for which peptides do not naturally absorb, has then been introduced. Specific photodissociation is obtained for cysteine-containing peptides tagged with a Dabcyl maleimide chromophore (Scheme 2). Experiments were performed on protein mixture digests with and without laser. Due to the high absorption cross section of the chromophore at 473 nm, all tryptic peptides with at least one cysteine grafted with the Dabcyl

chromophore see their intensity decreasing under LID, while tryptic peptides that do not contain any cysteine residue are not affected by the laser (Figure 2). Cysteine derivatization by hydrophobic and basic tags such as the Dabcyl chromophore induces an improved ionization yield and a reduced matrix effect. Moreover, the presence of a specific reporter ion arising from the internal photofragmentation within the chromophore allows construction of extracted ion chromatogram highlighting the elution of peptides containing a derivatized cysteine within the complex chromatographic profile (Figure 3). This method was also used in order to reduce the complexity of Data Independent Acquisition (DIA) spectra[32]. A LID spectral library was first built from a synthetic bank of 446 Cys-peptides derived from human kinases. The increased specificity induced a dramatic decrease of interference peaks originating from isobaric peptides and product ions, compared to HCD (Figure 4). Overall, the targeted library signal extraction of the DIA-LID data allows to streamline identification of derivatized cysteine containing peptide in an Escherichia Coli background. Similarly, this strategy based on derivatization with the Dabcyl chromophore has been employed using selected reaction monitoring mode for targeted quantification of cysteine containing proteins. In this method, called photo-SRM, the traditional CID is substituted by visible LID at 473 nm[33,34]. Due to the increased specificity, linearity of photo-SRM signal was conserved even at the lowest concentrations (Figure 5). Photo-SRM analysis of a whole plasma hydrolysate has shown similar or even improved detection performance compared to conventional CID-SRM[34].

The identification of cysteine-containing peptides of antigen-binding region of IgG fragments was targeted after modification with Alexa Fluor 350 maleimide[35]. This chromophore is also thiol-reactive and exhibits strong absorption at 351 nm. Modified cysteine peptides shown photodissociation yielding the diagnostic sequence ions, while unmodified peptides are not photo-fragmented. Thus, this tagging method led to the facilitated identification of cysteine-

containing peptides by eliminating convoluting MS/MS data from other redundant peptide sequences.

Tagged cysteine selectivity using 266 nm UVPD has also been reported[36]. A selenium-based chromophore (NPSP) was used to derivatized cysteine peptides in protein mixture hydrolysate (Scheme 3). The formed S-Se bond in peptides is prone to cleave upon 266 nm excitation, releasing a benzeneselenol moiety corresponding to a neutral loss of 156 Da, only for cysteine peptides. A customized data dependent acquisition based on the UVPD specific neutral loss is used to trigger CID for selective characterization of those cysteine-containing peptides (Figure 6). This "UVPDnLossCID" mode, specific to the cysteine peptide subset, allows for reducing the search space in a proteomic database.

The use of excitation at 266 nm was also reported for the detection and location of reduced cysteine in peptides or proteins after derivatization with a quinone group[37]. Thiol functional group reacts with quinones via Michael type addition (Scheme 4). Photoexcitation of the quinone tagged peptide cleaves the C-S bond of the cysteine, yielding a radical located at the C_β position of the cysteine. Site specific photodissociation of the backbone is then observed, pinpointing the location of the cysteine residues in the proteins.

Challenges and perspectives

This field of analysis using LID specificity after labelling with chromophore is relatively new and some improvements are highly desirable. For instance, the derivatization reaction specificity and stoichiometry is very crucial. Some aspects of the MS acquisition and data processing are also important to develop in order to take the best of LID specificity, and will also be discussed.

Derivatization reaction

Even if labelling of molecules is used in many applications[38], one can argue that the derivatization step of amino acids maybe the limiting step in these targeted strategies. Indeed, to profit from photofragmentation for reducing sample complexity, the derivatization reaction needs to be highly specific. It has to be checked that only the targeted peptides are derivatized with the chromophore tag. Next, the specificity of the method will be even better if few molecules are derivatized. Targeting tagging of NH_2 anime groups is not appropriated in this case because all peptides carry such a group. The derivatized chemical moiety has to be specific to one amino acid, which is not exactly the case for the OH or aromatic groups. The magnitude of reduction in the number of photo-dissociable targeted peptides also depends on the frequency of the derivatized amino acid. Among the 20 natural amino-acids, cysteine is by far the best candidate for increasing specificity in such targeted approaches. Indeed, if cysteine is present in almost all human proteins (97 % contain at least one), its occurrence is very weak (3.3 %), which translates into low proportion (15 %) of tryptic peptides containing one or more cysteine residues[38,39]. Non-covalent binding of chromophores could be envisaged to induce specific photofragmentation onto peptides. This has been reported for peptide-crown ether complexes[40,41]. The fragmentation yield is increased but this coupling does not seem really specific to a subset of peptides. Efforts could be made in this way to increase specificity of non-covalent binding for photodissociation analysis.

Another issue to take care with is the derivatization yield. Indeed, for accurate quantitative analysis, the tagging reaction of particular amino acid has to be stoichiometric. Incomplete derivatization could be deleterious for detection sensitivity and quantitative analysis, and thus has to be checked by classical CID method in parallel to specific LID analysis. For the Tyr modification with diazonium, the average reaction efficiency was estimated to 96 %[27], while

around 97 % of efficiency is obtained for the Cys derivatization with maleimide groups[35,31,34]. The rate of derivatization at a given amino acid also depends on its degree of accessibility to solvent and reactive agent. This raises the question of protein derivatization before or after the enzymatic digestion. One could argue that, after digestion, the targeted amino acid would be more accessible to reactive agent [27] but it is not always the case and derivatization of cysteine containing protein after denaturation but before digestion is usually reported [35,31,34]. Moreover, in order to allow identification of targeted derivatized peptides, laser induced dissociation should yield sequence informative b/y fragment ions. Indeed, consistent backbone fragmentation is required to yield good sequence coverages. This is the case for diazonium-tyrosine modified peptides[27], the NN-modified peptides[30] and Alexa Fluor 350[35] and Dabcyl maleimide[31,34] derivatized cysteine-containing peptides. After the initial absorption at the chromophore site, energy is redistributed all along the peptide backbone. The fragmentation efficiency principally depends on the peptide length and the location of the modification[42]. The LID spectra of NN-, Alexa Fluor 350- and Dabcyl-derivatized peptides also present a reporter ion arising from internal fragmentation of the chromophores. The signal of these reporter ions provided a facile means to track derivatized peptide elution in the whole LID-MS/MS data set. Unfortunately, when radical directed dissociation at 266 nm [28,29,36,37] is used, the formation of a radical species induces fragmentation only in the vicinity of the initial radical in subsequent CID, which is valuable to easily identify the site of modification but does not provide substantial sequence information for identification of unknown whole proteins or the peptides. In case of photo-induced specificity after chromophore derivatization, it would be better if LID could always allow complete structural identification instead of requiring additional CID steps.

The current developments in labelling chemistry offer the perspective of direct specific detection of low abundance PTM modifications for biomarker analysis. Indeed, instead of classical amino acid, PTM modifications could be derivatized and specifically detected by LID in complex matrices. Julian *et al.* reported the facile identification of phosphorylation sites in peptides by radical directed dissociation[43]. Phosphorylation sites are selectively modified through β-elimination followed by Michael addition of a naphtalenethiol chromophore (Scheme 5). Photodissociation at 266 nm induces homolytic cleavage at the modification site, generating a β radical which yields primarily d-type ions at the previously phosphorylated residue (Figure 7). Other labelling could be envisaged but keeping in mind that complex sample preparation could be deleterious for quantitative analysis due to incomplete reaction yields and risks of losing compounds.

MS acquisition modes and data analysis

Acquisition mode in specific LID experiments is a crucial issue. For quantification, use of the LID specificity in SRM targeted mode (photo-SRM)[34] is a really efficient way to reduce interfering signal from co-eluted isobaric compounds and increase detection sensibility. However, in discovery "non-targeted" analysis, it is difficult to take the most of the specificity of the LID. Indeed, in classical data dependent topN analysis, only the most intense ions are selected for fragmentation. The selection process is linked to precursor abundance rather than specific derivatization. Then, even if LID is used for fragmentation, if the most intense ions do not contain the chromophore tag, data set will enclose a lot of uninformative non-fragmented spectra. Moreover, even if large exclusion windows are used, low intensity derivatized ions can be missed. The "UVPDnLossCID" seems a good alternative to acquire only CID spectra of targeted derivatized peptides but as the first UVPD analysis is done in top5 mode, some low

abundance targeted peptides are probably not selected for fragmentation, explaining the non-exhaustive identification of all cysteine-containing peptides in the protein mixture[36]. The optimal mode would be to select all ions for fragmentation and search in a restricted database or using the unique and specific photo-fragmentation capability as a DDA criterion for further LID sequencing. Such recent developments have been proposed using LID with DIA mode[32], allowing a complete sampling of compounds. But a lot of efforts still have to be made in this direction in collaboration with mass spectrometer constructors.

Moreover, even if protein search engines (for example OMSSA, SEQUEST or MassMatrix) allow the introduction of specific modifications for the database interrogation[27,31], most cannot take into account specific fragmentation. This can lead to missed information from the LID spectra. Indeed, the mass increment of the chromophore tag can be integrated to a specific amino acid but then fragments which do not contain the intact chromophore (i.e. with the initial mass modification) are not considered during the data search. Furthermore, many search engines do not propose easy search in sub-database representative of the targeted sub-proteome. Thus, custom versions of the algorithm have to be implemented to allow only targeted subset of peptides to be searched in order to reduce the search space[27,35]. The development of specific software for result analysis and interpretation is really needed to match with the specificity of LID.

In summary, the developments in mass spectrometry coupled to laser induced dissociation that have occurred over the last 10 years have opened a vast array of applications in analysis of complex mixtures. Continuous developments in these areas keep on pushing the limits of proteomic workflows and detection sensitivity. Indeed, using chromophore derivatization of specific amino acids allows increasing the fragmentation specificity when using near-UV or

Visible wavelengths. In order to further validate and broaden the fields of application of those approaches, the performances of LID analytical methods will have to be systematically compared to classical CID. Overall, LID-based methods are not intended to replace conventional CID methods but to provide a complementary tool to streamline the detection of a subset of proteins or peptides, facing specificity issues.

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References

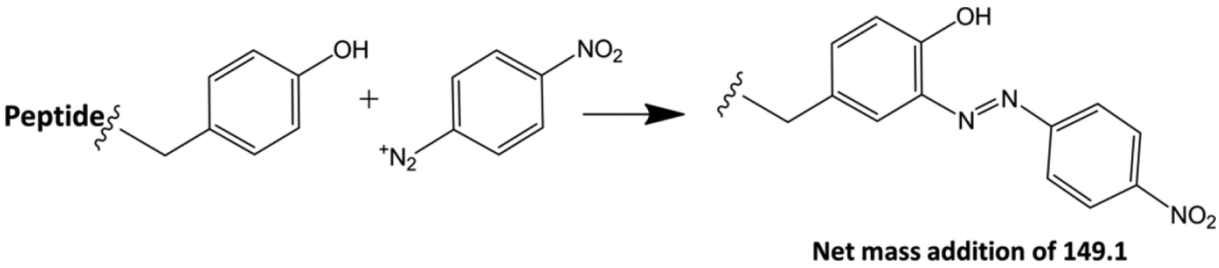
1. Aebersold, R. & Mann, M. Mass spectrometry-based proteomics. *Nature* **422,** 198–207 (2003).
2. Zeiler, M., Moser, M. & Mann, M. Copy Number Analysis of the Murine Platelet Proteome Spanning the Complete Abundance Range. *Mol. Cell. Proteomics* **13,** 3435–3445 (2014).
3. Michalski, A., Cox, J. & Mann, M. More than 100,000 Detectable Peptide Species Elute in Single Shotgun Proteomics Runs but the Majority is Inaccessible to Data-Dependent LC−MS/MS. *J. Proteome Res.* **10,** 1785–1793 (2011).
4. Zhou, Y., Aebersold, R. & Zhang, H. Isolation of N-linked glycopeptides from plasma. *Anal. Chem.* **79,** 5826–5837 (2007).
5. Tian, Y., Zhou, Y., Elliott, S., Aebersold, R. & Zhang, H. Solid-phase extraction of N-linked glycopeptides. *Nat. Protoc.* **2,** 334–339 (2007).
6. Gygi, S. P. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat. Biotechnol.* **17,** 994–999 (1999).
7. Rose, C. M. *et al.* Neutron encoded labeling for peptide identification. *Anal. Chem.* **85,** 5129–5137 (2013).
8. Reilly, J. P. ULTRAVIOLET PHOTOFRAGMENTATION OF BIOMOLECULAR IONS. *Mass Spectrom. Rev.* **28,** 425–447 (2009).
9. Larraillet, V., Antoine, R., Dugourd, P. & Lemoine, J. Activated-electron photodetachment dissociation for the structural characterization of protein polyanions. *Anal. Chem.* **81,** 8410–8416 (2009).
10. Crowe, M. C. & Brodbelt, J. S. Differentiation of phosphorylated and unphosphorylated peptides by high-performance liquid chromatography-electrospray ionization-infrared multiphoton dissociation in a quadrupole ion trap. *Anal. Chem.* **77,** 5726–5734 (2005).
11. Borotto, N. B., McClory, P. J., Martin, B. R. & Hakansson, K. Targeted Annotation of S-Sulfonylated Peptides by Selective Infrared Multiphoton Dissociation Mass Spectrometry. *Anal. Chem.* **89,** 8304–8310 (2017).
12. Wilson, J. J. & Brodbelt, J. S. Infrared multiphoton dissociation for enhanced de novo sequence interpretation of N-terminal sulfonated peptides in a quadrupole ion trap. *Anal. Chem.* **78,** 6855–6862 (2006).
13. Joly, L., Antoine, R., Broyer, M., Dugourd, P. & Lemoine, J. Specific UV photodissociation of tyrosyl-containing peptides in multistage mass spectrometry. *J. Mass Spectrom.* **42,** 818–824 (2007).
14. Agarwal, A., Diedrich, J. K. & Julian, R. R. Direct elucidation of disulfide bond partners using ultraviolet photodissociation mass spectrometry. *Anal. Chem.* **83,** 6455–6458 (2011).
15. Antoine, R. & Dugourd, P. Visible and ultraviolet spectroscopy of gas phase protein ions. *Phys. Chem. Chem. Phys.* **13,** 16494 (2011).
16. Krusemark, C. J., Frey, B. L., Belshaw, P. J. & Smith, L. M. Modifying the charge state distribution of proteins in electrospray ionization mass spectrometry by chemical derivatization. *J. Am. Soc. Mass Spectrom.* **20,** 1617–1625 (2009).
17. Xu, Y. W., Zhang, L. J., Lu, H. J. & Yang, P. Y. Mass spectrometry analysis of phosphopeptides after peptide carboxy group derivatization. *Anal. Chem.* **80,** 8324–8328 (2008).

18. Madsen, J. A. & Brodbelt, J. S. Simplifying fragmentation patterns of multiply charged peptides by N-terminal derivatization and electron transfer collision activated dissociation. *Anal. Chem.* **81,** 3645–3653 (2009).
19. Zhang, L. J., Xu, Y. ., Lu, H. J. & Yang, P. Y. Carboxy group derivatization for enhanced electron-transfer dissociation mass spectrometric analysis of phosphopeptides. *Proteomics* **9,** 4093–4097 (2009).
20. Daly, S. *et al.* Action-FRET: Probing the molecular conformation of mass-selected gas-phase peptides with förster resonance energy transfer detected by acceptor-specific fragmentation. *Anal. Chem.* **86,** 8798–8804 (2014).
21. DeSouza, L. *et al.* Search for cancer markers from endometrial tissues using differentially labeled tags iTRAQ and cICAT with multidimensional liquid chromatography and tandem mass spectrometry. *J. Proteome Res.* **4,** 377–386 (2005).
22. Ross, P. L. *et al.* Multiplexed Protein Quantitation in *Saccharomyces cerevisiae* Using Amine-reactive Isobaric Tagging Reagents. *Mol. Cell. Proteomics* **3,** 1154–1169 (2004).
23. Tecklenburg, R. E., Miller, M. N. & Russell, D. H. Laser Ion Beam Photodissociation Studies of Model Amino Acids and Peptides. *J. Am. Chem. Soc.* **111,** 1161–1171 (1989).
24. Wilson, J. J. & Brodbelt, J. S. MS/MS simplification by 355 nm ultraviolet photodissociation of chromophore-derivatized peptides in a quadrupole ion trap. *Anal. Chem.* **79,** 7883–7892 (2007).
25. Ko, B. J. & Brodbelt, J. S. Ultraviolet photodissociation of carboxylate-derivatized peptides in a quadrupole ion trap. *J. Am. Soc. Mass Spectrom.* **22,** 49–56 (2011).
26. Robotham, S. A., Kluwe, C., Cannon, J. R., Ellington, A. & Brodbelt, J. S. De novo sequencing of peptides using selective 351 nm ultraviolet photodissociation mass spectrometry. *Anal. Chem.* **85,** 9832–9838 (2013).
27. Aponte, J. R. *et al.* Streamlining bottom-up protein identification based on selective ultraviolet photodissociation (UVPD) of chromophore-tagged histidine-and tyrosine-containing peptides. *Anal. Chem.* **86,** 6237–6244 (2014).
28. Ly, T. & Julian, R. R. Residue-specific radical-directed dissociation of whole proteins in the gas phase. *J. Am. Chem. Soc.* **130,** 351–358 (2008).
29. Sun, Q., Yin, S., Loo, J. A. & Julian, R. R. Radical directed dissociation for facile identification of iodotyrosine residues using electrospray ionization mass spectrometry. *Anal. Chem.* **82,** 3826–3833 (2010).
30. O'Brien, J. P., Pruet, J. M. & Brodbelt, J. S. Chromogenic chemical probe for protein structural characterization via ultraviolet photodissociation mass spectrometry. *Anal. Chem.* **85,** 7391–7397 (2013).
31. Girod, M. *et al.* Implementing visible 473 nm photodissociation in a Q-Exactive mass spectrometer: towards specific detection of cysteine-containing peptides. *Analyst* **139,** 5523–5530 (2014).
32. Garcia, L. *et al.* Data Independent Acquisition Coupled to Visible Laser-Induced Dissociation at 473 nm (DIA-LID) for Peptide-Centric Specific Analysis of Cysteine-Containing Peptide Subset. *Anal. Chem.* **90,** 3928−3935 (2018).
33. Enjalbert, Q. *et al.* Photo-SRM: Laser-induced dissociation improves detection selectivity of selected reaction monitoring mode. *Rapid Commun. Mass Spectrom.* **25,** 3375–3381 (2011).
34. Enjalbert, Q. *et al.* Improved detection specificity for plasma proteins by targeting cysteine-containing peptides with photo-SRM. *Anal. Bioanal. Chem.* **405,** 2321–2331 (2013).

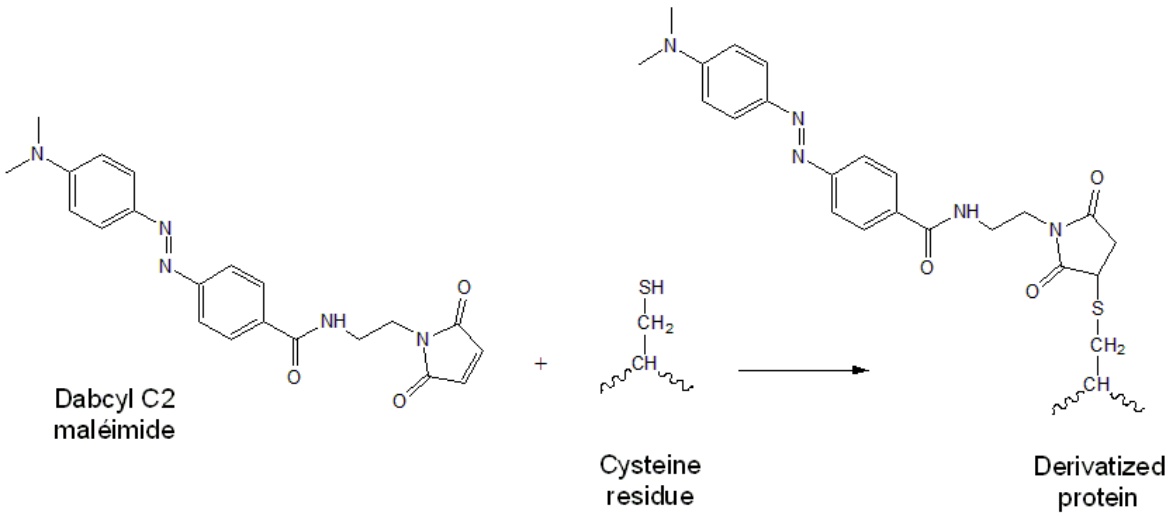
35. Cotham, V. C., Wine, Y. & Brodbelt, J. S. Selective 351 nm photodissociation of cysteine-containing peptides for discrimination of antigen-binding regions of IgG fragments in bottom-Up liquid chromatography-tandem mass spectrometry workflows. *Anal. Chem.* **85,** 5577–5585 (2013).
36. Parker, W. R., Holden, D. D., Cotham, V. C., Xu, H. & Brodbelt, J. S. Cysteine-Selective Peptide Identification: Selenium-Based Chromophore for Selective S-Se Bond Cleavage with 266 nm Ultraviolet Photodissociation. *Anal. Chem.* **88,** 7222–7229 (2016).
37. Diedrich, J. K. & Julian, R. R. Site selective fragmentation of peptides and proteins at quinone modified cysteine residues investigated by ESI-MS. *Anal Chem.* **82,** 4006–4014 (2011).
38. Giron, P., Dayon, L. & Sanchez, J.-C. Cysteine tagging for MS-Based proteomics. *Mass Spectrom. Rev.* **30,** 366–395 (2011).
39. Lin, D., Li, J., Slebos, R. J. C. & Liebler, D. C. Cysteinyl peptide capture for shotgun proteomics: Global assessment of chemoselective fractionation. *J. Proteome Res.* **9,** 5461–5472 (2010).
40. Yeh, G. K., Sun, Q., Meneses, C. & Julian, R. R. Rapid Peptide Fragmentation without Electrons, Collisions, Infrared Radiation, or Native Chromophores. *J. Am. Soc. Mass Spectrom.* **20,** 385–393 (2009).
41. Wilson, J. J., Kirkovits, G. J., Sessler, J. L. & Brodbelt, J. S. Photodissociation of Non-Covalent Peptide-Crown Ether Complexes. *J. Am. Soc. Mass Spectrom.* **19,** 257–260 (2008).
42. Garcia, L., Lemoine, J., Dugourd, P. & Girod, M. Fragmentation patterns of chromophore-tagged peptides in visible laser induced dissociation. *Rapid Commun. Mass Spectrom.* **31,** 1985–1992 (2017).
43. Diedrich, J. K. & Julian, R. R. Facile identification of phosphorylation sites in peptides by radical directed dissociation. *Anal. Chem.* **83,** 6818–6826 (2011).

Figures and Schemes

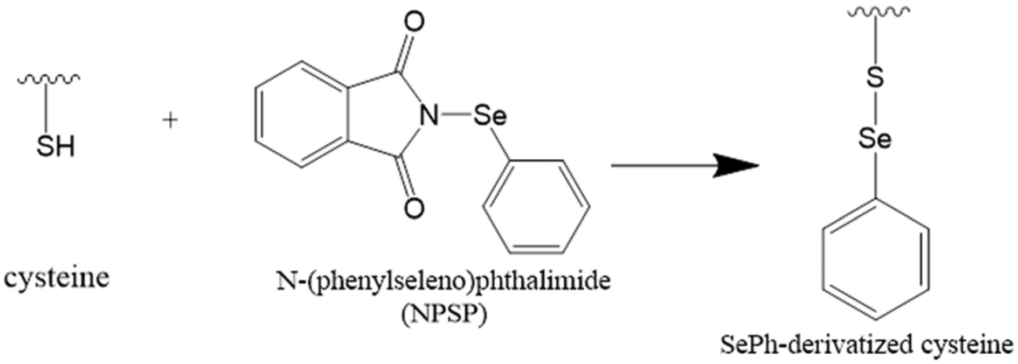
Scheme 1. Reaction Schematic of Diazonium Coupling with Tyrosine. Reproduced with permission from Ref[27] Copyright 2014 American Chemical Society



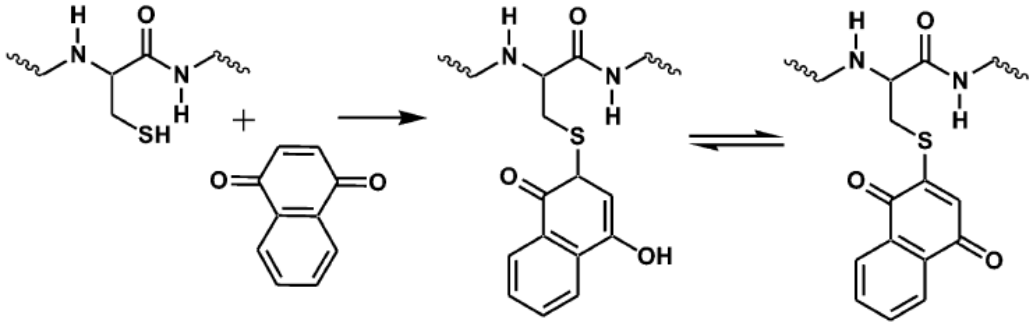
Scheme 2. Reaction of cysteine with Dabcyl C2 maleimide.



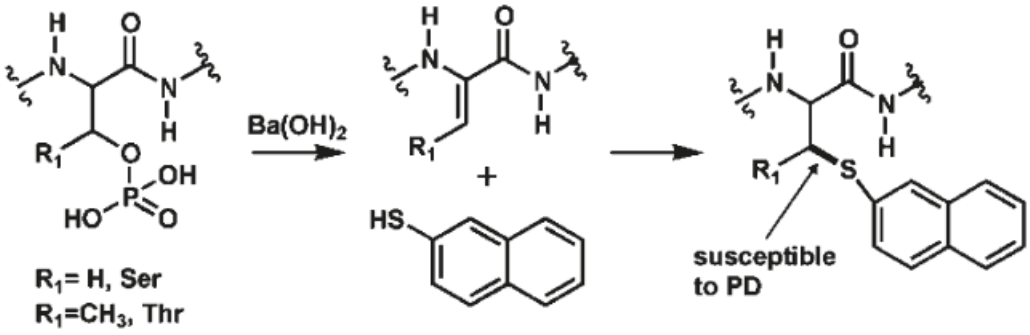
Scheme 3. Reaction of Free Thiols with N-(Phenylseleno)phthalimide. Reproduced with permission from Ref[36] Copyright 2016 American Chemical Society



Scheme 4. Modification of cysteine by quinone. Reproduced with permission from Ref[37] Copyright 2010 American Chemical Society



Scheme 5. Dephosphorylation and derivatization by Naphthalenethiol. Reproduced with permission from Ref[43] Copyright 2011 American Chemical Society



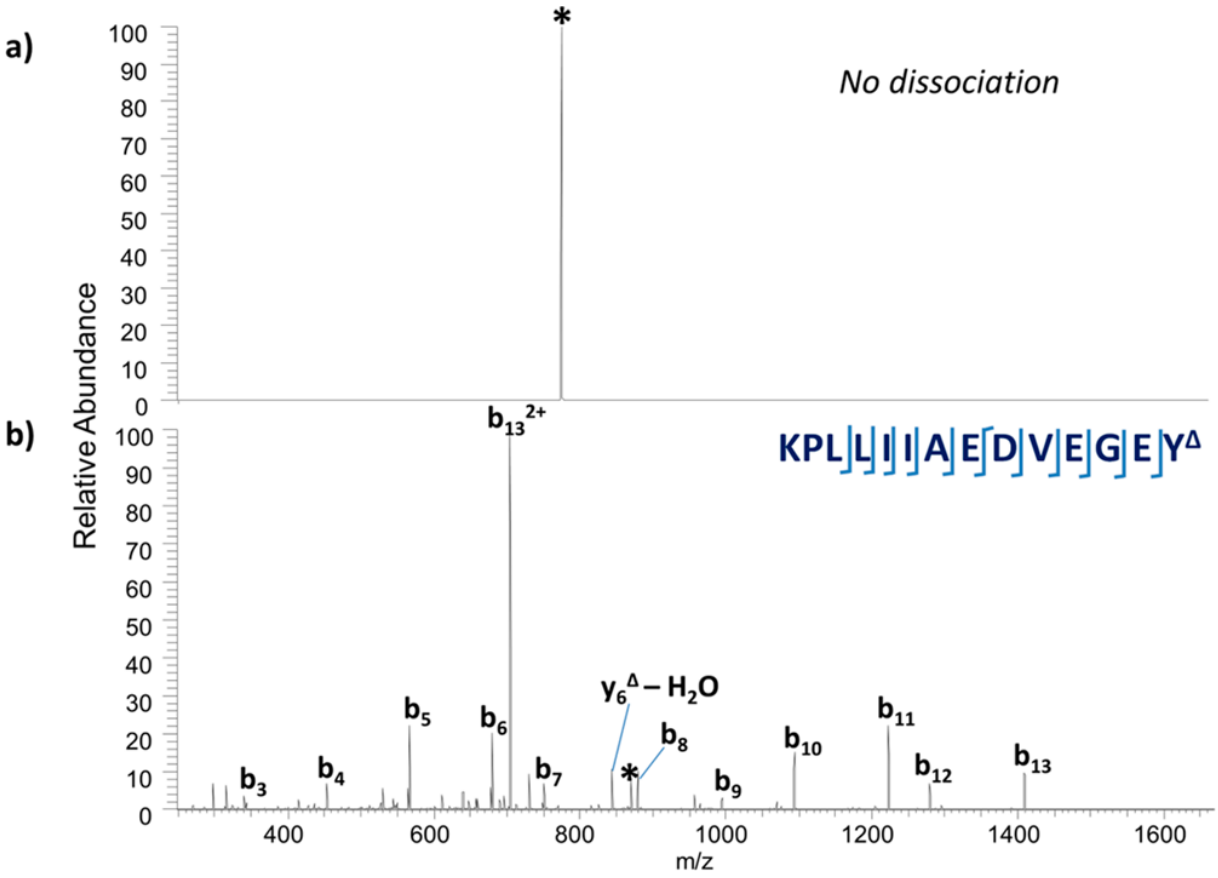


Figure 1: Comparison of 351 nm UVPD (10 5-ns pulses) of (a) unmodified and (b) diazonium-modified peptide. The selected precursor ion is indicated by an asterisk (*), and the addition of diazonium is indicated by an uppercase delta symbol (Δ). Reproduced with permission from Ref[27] Copyright 2014 American Chemical Society

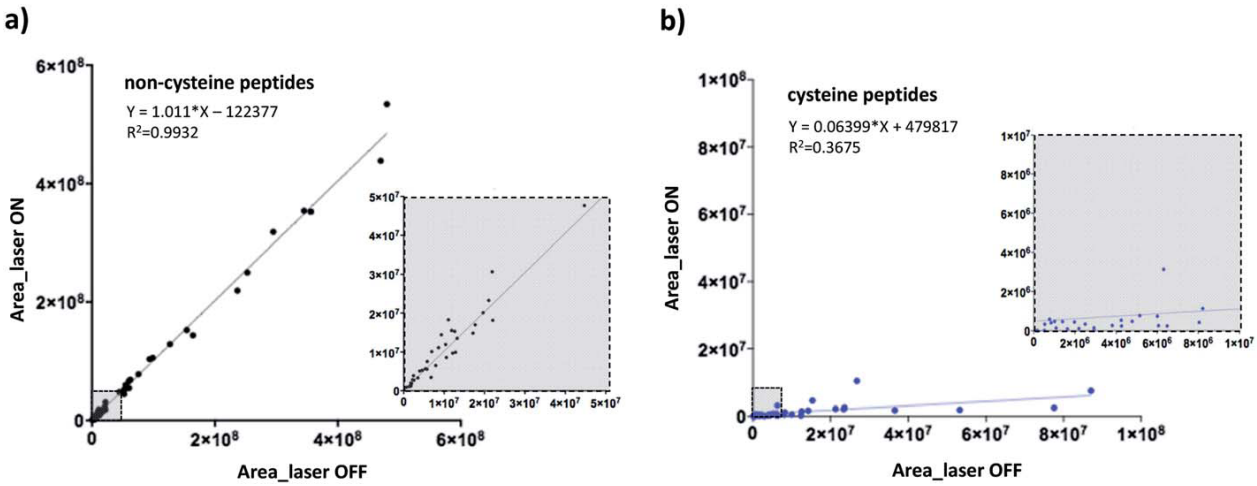


Figure 2: Areas of all non-cysteine peptides (a) and cysteine peptides (b) with laser vs. without laser. Reproduced with permission from Ref[31] Copyright 2014 Royal Chemical Society

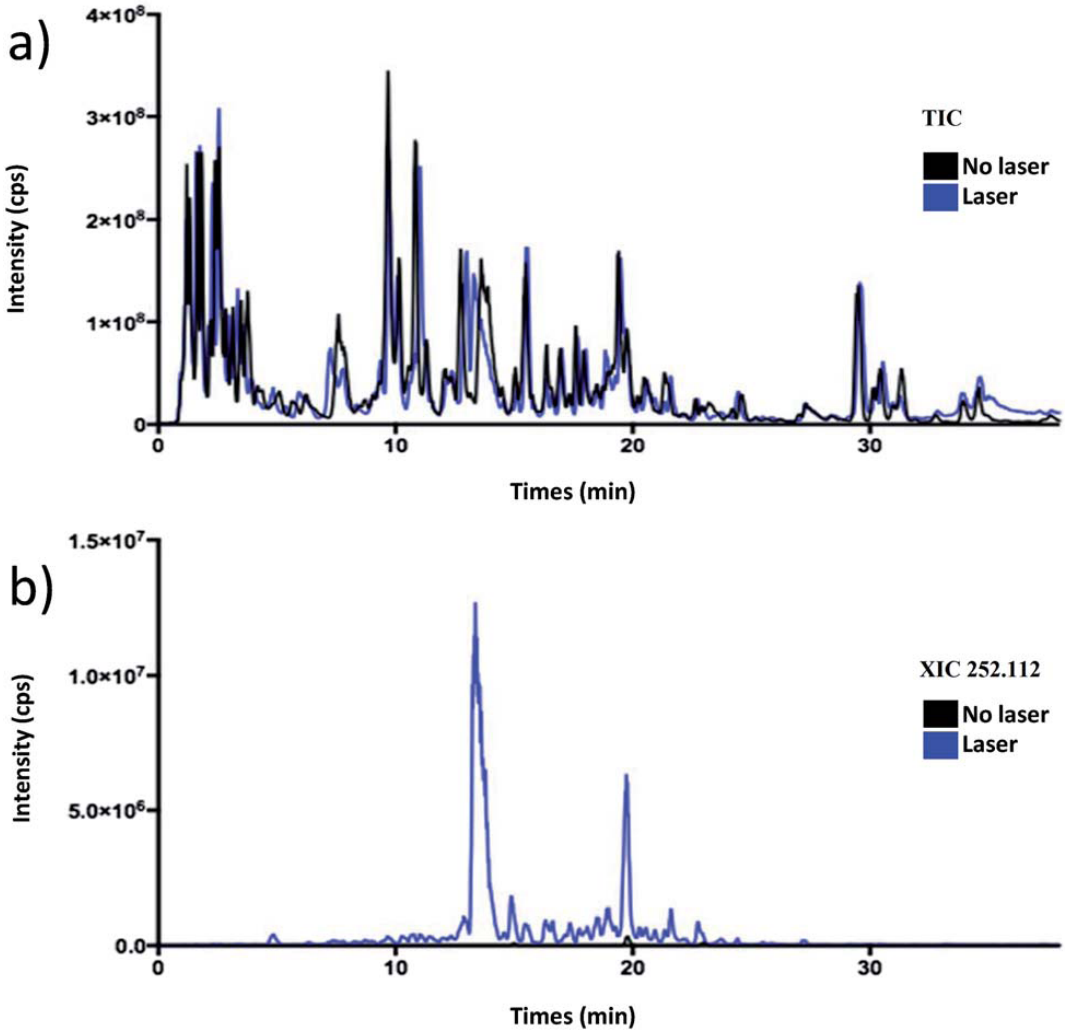


Figure 3: a) Total ion chromatogram of the AIF-MS/MS of a digest of 3 proteins during 50 min with a gradient of acetonitrile 20–45 % and (b) extracted chromatogram of the reporter ion *m/z* 252, with (blue) or without (black) laser. Reproduced with permission from Ref[31] Copyright 2014 Royal Chemical Society

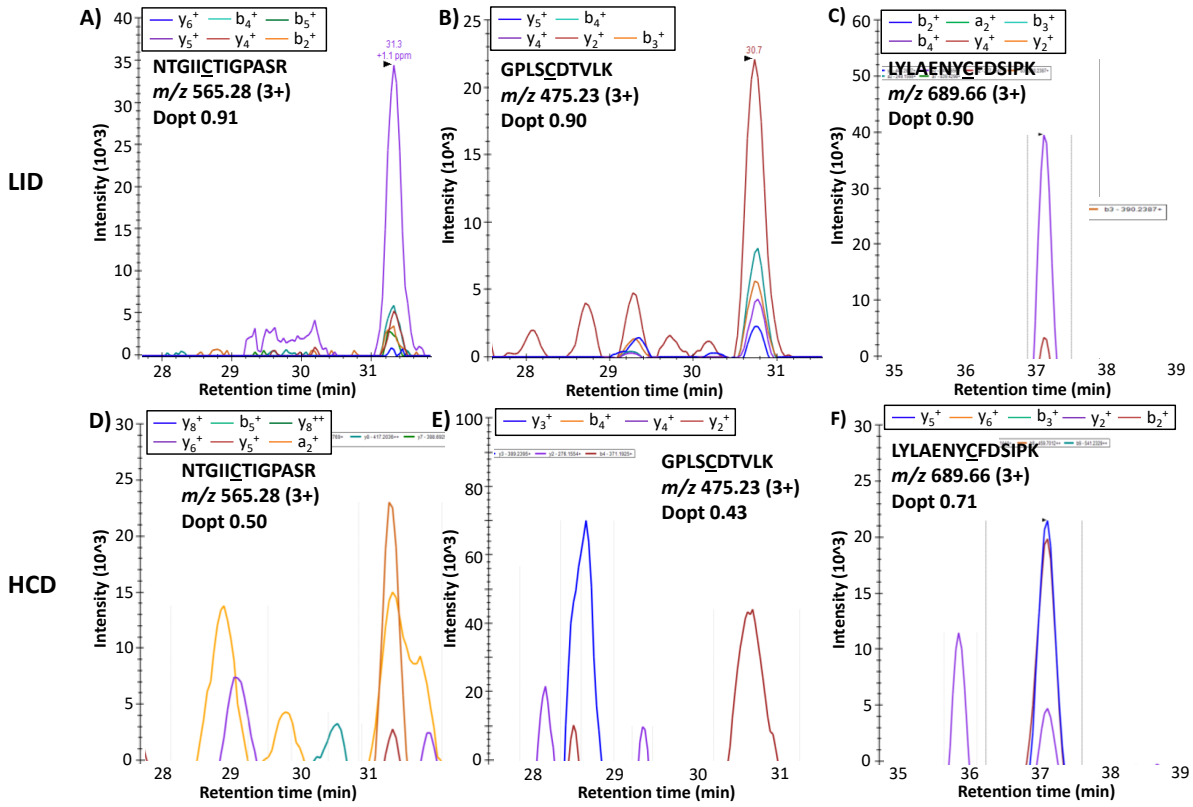


Figure 4. Extracted ion chromatograms of fragment ions from 3 derivatized Cys-peptides spiked in an *Escherichia coli* lysate monitored in DIA-LID (A, B, C) and DIA-HCD (D, E, F). Reproduced with permission from Ref[32] Copyright 2018 American Chemical Society

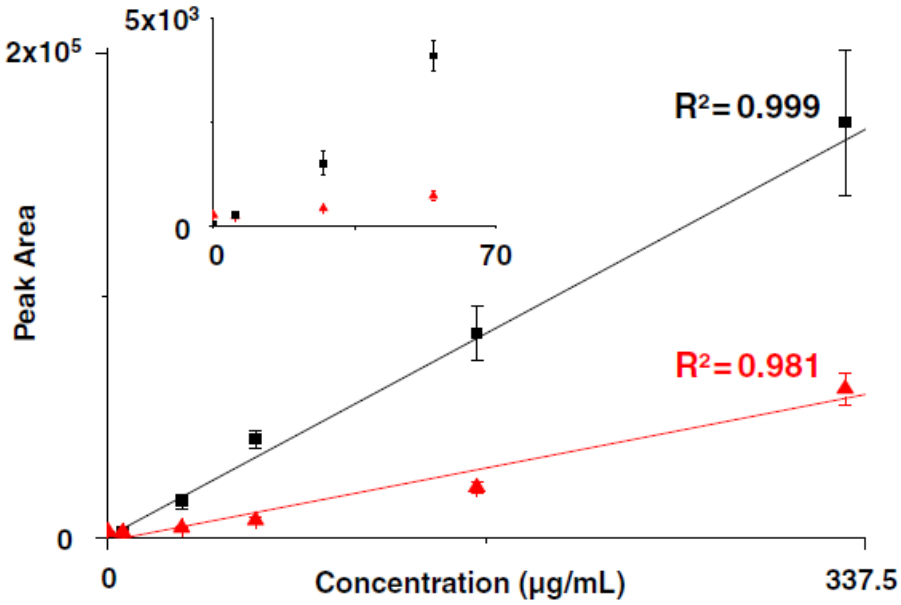


Figure 5: Calibration curves for the most intense specific transition for chromophore-derivatized proteotypic C4b binding protein alpha chain peptide monitored by use of photo-SRM (squares) and SRM (triangles). Inset shows low concentration data (0, 6.75, 33.75, and 67.5 $\mu g mL^{-1}$). Reproduced with permission from Springer Nature Ref[34] Copyright 2013 American Chemical Society

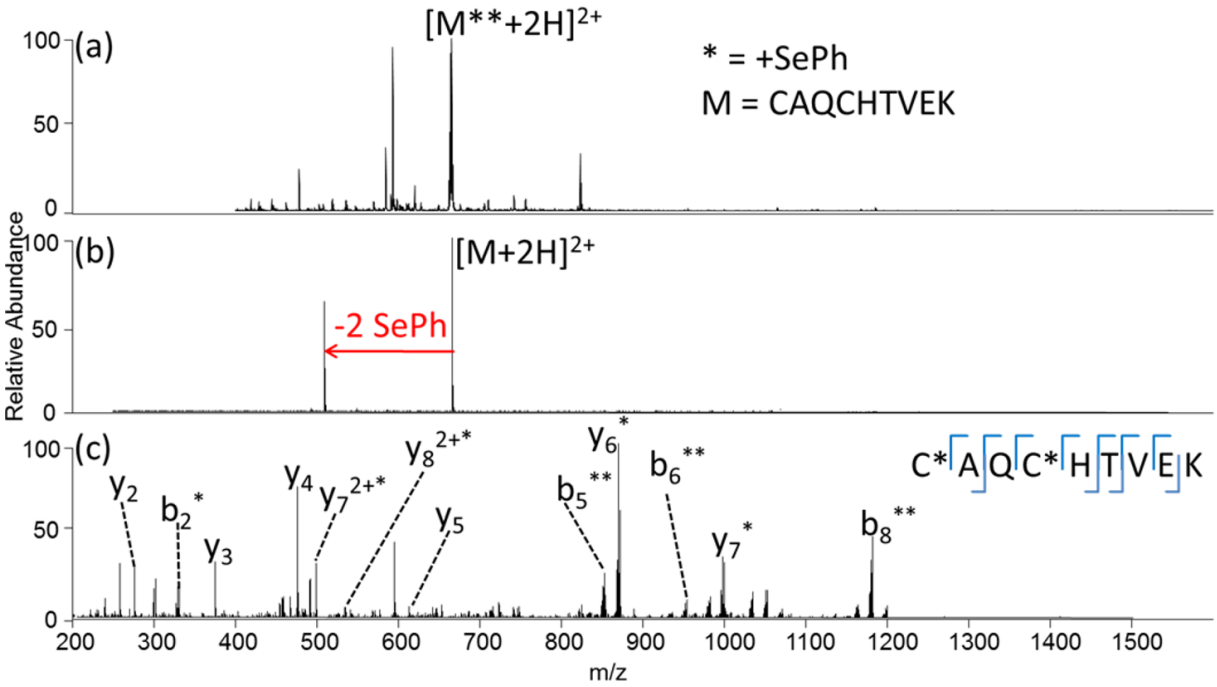


Figure 6: (a) ESI mass spectrum of the tryptic peptide CAQCHTVEK eluting at 61.13 min in the LC trace, (b) UVPD mass spectrum of C*AQC*HTVEK (2+), and (c) UVPDnLossCID spectrum. Reproduced with permission from Ref[36] Copyright 2016 American Chemical Society

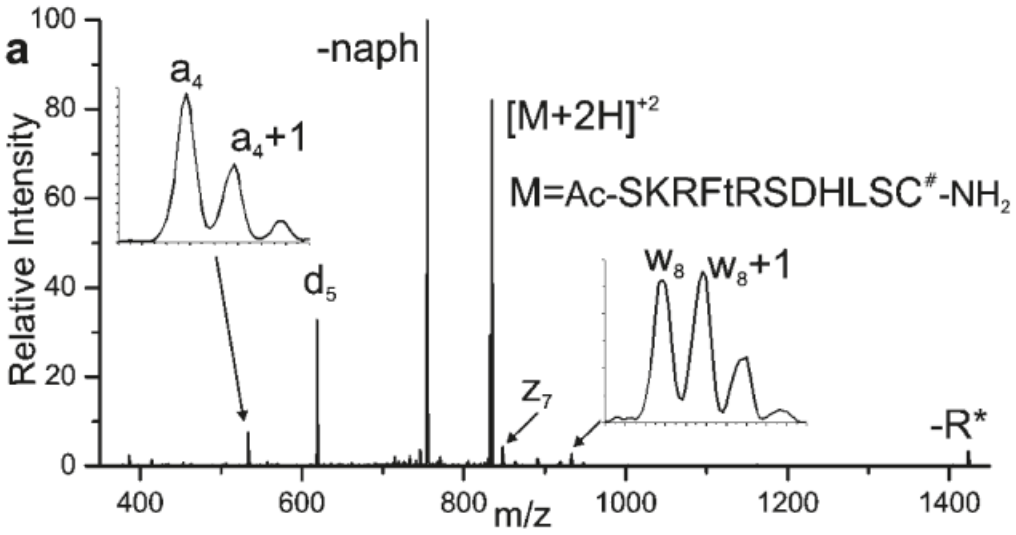


Figure 7: PD of 2+ charge state of naphthalenethiol derivative of Ac-SKRFtRSDHLSC$^{\#}$-NH_2. Loss of naphthalenethiol due to C_β-S cleavage is observed (labeled -naph). d_5, a_4 and z_7 fragments are observed at the modified pThr. Side chain loss of arginine is labeled -R*. Reproduced with permission from Ref[43] Copyright 2011 American Chemical Society