# Data-Independent Acquisition Coupled to Visible Laser-Induced Dissociation at 473 nm (DIA-LID) for Peptide-Centric Specific Analysis of Cysteine-Containing Peptide Subset

Lény Garcia, Marion Girod, Magali Rompais, Philippe Dugourd, Christine Carapito, Jérôme Lemoine

# Data Independent Acquisition Coupled to Visible Laser-Induced Dissociation at 473 nm (DIA-LID) for Peptide-Centric Specific Analysis of Cysteine-Containing Peptide Subset.

Lény Garcia[a], Marion Girod[a], Magali Rompais[b], Philippe Dugourd[c], Christine Carapito[b] and Jérôme Lemoine[a*]

[a] Université de Lyon, CNRS, Université Claude Bernard Lyon 1, Ecole Normale Supérieure de Lyon, Institut des Sciences Analytiques, UMR 5280, 5 rue de la Doua, F-69100 Villeurbanne, France

[b] Laboratoire de Spectrométrie de Masse Bio-Organique (LSMBO), IPHC, Université de Strasbourg, CNRS, UMR 7178, 25 rue Becquerel, 67087 STRASBOURG, France

[c] Université de Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F-69622, VILLEURBANNE, France

[*]**To whom correspondence should be addressed:**
Jérôme Lemoine: jerome.lemoine@univ-lyon1.fr

**ABSTRACT**

Thanks to comprehensive and unbiased sampling of all precursor ions, the interest to move towards bottom-up proteomic with Data Independent Acquisition (DIA) is continuously growing. DIA offers precision and reproducibility performances comparable to true targeted methods, but has the advantage of enabling retrospective data testing with the hypothetical presence of new proteins of interest. Nonetheless the chimeric nature of DIA MS/MS spectra inherent to concomitant transmission of a multiplicity of precursor ions makes the confident identification of peptides often challenging, even with spectral library-based extraction strategy. The introduction of specificity at the fragmentation step upon ultraviolet or visible laser-induced dissociation (LID) range targeting only the subset of cysteine-containing peptides (Cys-peptide) has been proposed as an option to streamline and reduce the search space. Here, we describe the first coupling between DIA and visible LID at 473 nm to test for the presence of Cys-peptides with a peptide-centric approach. As a test run, a spectral library was built for a pool of Cys-synthetic peptides used as surrogates of human kinases (1 peptide per protein). By extracting ion chromatograms of query standard and kinase peptides spiked at different concentration levels in an *Escherichia coli* proteome lysate, DIA-LID demonstrates a dynamic range of detection of at least 3 decades and coefficients of precision better than 20 %. Finally, the spectral library was used to search for endogenous kinases in human cellular extract.

## Keywords

Laser-induced dissociation, data-independent acquisition, spectral library extraction, cysteine-containing peptides, chromophore derivatization

## Introduction

As an alternative to Data Dependent Acquisition (DDA), the Data Independent Acquisition (DIA) technique pioneered by Yates and coll.[1], then popularized by Aebersold and coll.[2] under the acronym SWATH (Sequential Window Acquisition of all Theoretical fragment-ion spectra), has gained a great interest in proteomics in the past few years. In DIA mode, a mass range of typically 400 to 1200 Th is segmented in small consecutive windows of 8 to 25 Th through which all simultaneously transmitted precursor ions are fragmented to form a composite MS/MS spectrum. When the duty cycle of concatenated windows is optimized to ensure at least 8 data points per peak, DIA combines comprehensive and unbiased peptide sampling with quantification metrics nearing those achieved with pure targeted modes[3,4]. By the use of peptide spectral libraries built either from DDA experiments or synthetic banks and indexed with their respective relative retention times, hundreds to few thousands of proteins[5] may thus be confidently and reproducibly quantified by DIA at a relatively high throughput level[6–9]. Moreover, DIA allows reanalysis of old datasets for testing new biological hypothesis.

Despite aforementioned recognized figures of merit, the composite nature of fragmentation spectra may also lead to false positive or negative identifications. This feature is particularly exacerbated when large spectral libraries, i.e. the whole human spectra library[5] are used to extract peptide/protein signals from data acquired on a particular biological compartment such as urine for instance[10]. With the aim of reducing both DIA data complexity and search space, laser-induced photodissociation (LID) could be an exquisite alternative to introduce the lacking dimension of specificity at the fragmentation level. Interestingly, LID implementation in commercial instruments is rather simple as it does not require any hardware modification other than endowing the collision cell with a window. As an illustration of LID specificity, infrared multiphoton photodissociation (IRMPD) at 10.6 μm

has shown interesting propensity to differentiate phospho- and non-phosphorylated peptides[11] due to increased photon absorption of the phosphate group. Moreover, IRMPD shows specificity towards sulfonated peptides[12,13]. The fragmentation specificity of peptides has been similarly illustrated in the ultraviolet range (UVPD) by leveraging natural absorption of intrinsic chromophores such as disulfide bonds or tyrosine residue[14–16]. Nonetheless, the most obvious rationale to introduce fragmentation specificity lies in targeting only the subset of peptides after grafting them with a proper chromophore[17,18]. Among the 20 natural amino-acids, cysteine is by far the best candidate for the development of such a targeted approach. Indeed, if cysteine is present in almost all human proteins (91 % contain at least one), its occurrence is very weak (3.3 %), which translates into low proportion (24%) of tryptic peptides containing one or more cysteine residues[19].

Furthermore, the sulfhydryl side chain group of cysteine is a highly reactive nucleophilic group that condenses with alkyl halide or maleimide reagents, usually in a stoichiometric proportion. Hence, many studies already reported site-selective peptide bond cleavages in the vicinity of cysteine derivatized with one of the two radical initiators, quinone through UVPD at 266 nm[20]. The same chemistry operates with the thiol alkylating reagent N-(phenylseleno)phtalimide, which initiates a characteristic neutral loss of 156 (benzeneselenol moiety) only from Cys-peptides under selective 266 nm excitation. Then, laser-induced neutral loss triggering CID MS/MS may afford selective sequencing of the cysteine peptide subset (UVPDnLossCID) for streamlining the search space in a proteomic database[21]. Another work from the same group describes the on-line LC-MS coupling with an excimer laser emitting at 351 nm to pinpoint in an antibody trypsin digest only the Cys-peptides selectively tagged with Alexa Fluor 350 on the basis of a reporter ion at *m/z* 296 and extensive series of b and y fragment ions[22]. We previously reported such an informative pattern of dissociation when irradiating Cys-peptides modified by a Dabcyl group in the visible range at 473 nm[23,24],

which shown promising applications in the context of Multiple Reaction Monitoring detection. Together, these studies illustrate the ability of carrying on UV or visible LID on a chromatographic time scale and to increase the detection specificity in targeted or in spectrum-centric approaches.

Considering the above mentioned merits of DIA but also its inherent limitation arising from the composite nature of fragmentation spectra, we thus sought to implement and investigate the performances of the coupling between LID at 473 nm and DIA mode following a peptide-centric strategy for streamlining the detection of Cys-peptide surrogates in a spectral library. In order to test this new methodology, a LID spectral library was first built from a synthetic bank of 446 Cys-peptides derived from human kinases and that were previously identified as MS-friendly candidates (all peptides had already been identified in the PeptideAtlas repository). We next used this spectral library to identify the synthetic peptides spiked into an *Escherichia coli* background proteome in order to systematically evaluate DIA-LID precision and dynamic range of detection. Though our experimental set-up based on normal flow chromatography is not optimal from a sensitivity point of view with regard to the nano flow gold standard, we tentatively applied DIA-LID tool for targeting endogenous kinases in a total human cell extract.

## Materials and methods

Details about chemicals, samples, instrumental set-up and HPLC conditions are provided as Supplemental Information (SI).

### Sample preparation

The 446 synthetic Cys-peptides derived from human kinases were initially mixed to form 50 pools of stock solutions (9 peptides per pool on average) at a theoretical individual concentration of 400 $\mu$g/mL in $H_2O$/ACN (50/50) + 0.5 % of FA. For spectral library building, 2.5 $\mu$L of each stock solution pool were diluted with acidified water to reach an estimated final concentration around 1 $\mu$g/mL. These pools were then mixed in a single pool, reduced with a 3-fold molar excess solution of TCEP (500 $\mu$L of a 1 mg/mL in 60mM ammonium acetate), then derivatized with 5-fold molar excess of Dabcyl C2 maleimide chromophore (500 $\mu$L of a 1 mg/mL methanol solution). The mixture was stirred in a sonic bath, then stored in the dark for 4 h at room temperature for complete conversion. Subsequently, the peptides were desalted and concentrated using an Oasis™ HLB 3 cc (60 mg) reversed phase cartridge (Waters, Milford, MA, USA), as described in details elsewhere.[23]

To evaluate the detection and the identification of the cysteine containing peptides in DIA by photodissociation, the 354 synthetic peptides retained in the spectral library were spiked at theoretical amounts ranging from 17 ng to 14.15 $\mu$g in an *Escherichia coli* proteome extract (100 $\mu$g). The sample was reduced and derivatized with the DABCYL C2 maleimide chromophore as described above. The samples were diluted with 3 mL of a 60 mM AA solution prior to overnight digestion at 37 °C with trypsin using a 1:20 (w/w) enzyme to substrate ratio. Digestion was stopped by addition of formic acid to a final concentration of 0.5 %. All samples were desalted as mentioned previously and concentrated in 85 $\mu$L of water/MeOH (95:5, v/v) containing 0.5 % FA.

For a relative estimation of the detection dynamic range of DIA-LID mode, a mixture of 65 peptides selected among those showing the most intense signals and covering a large retention time distribution were spiked in an *Escherichia coli* protein extract with different dilution factors to get normalized concentrations of 1, 0.1, 0.01 and 0.001. For the precise

quantification of the 3 purified custom peptides, 6 different points of calibration were carried out for each peptide between 0.1 ng and 1000 ng in 100 $\mu$g of *Escherichia coli*. For the detection of peptides in DIA-LID directly in biological matrix, HS578T human cellular extracts were used. 60 $\mu$g of proteins were derivatized and analyzed. A preliminary Bradford test was performed to estimate the protein concentration and to adjust the derivatization protocol. Concentrations of 1177 $\mu$g/mL and 340 $\mu$g/mL were obtained for the *Escherichia coli* sample and the human cellular extract, respectively. All of these experiments were achieved following the aforementioned protocol (chromophore tagging, sample digestion and SPE) for the specific detection of Cys-peptides by photodissociation.

**Mass spectrometry operating condition**

Ionization was achieved using electrospray in positive ionization mode with an ion spray voltage of 4000 V. The sheath gas and auxiliary gas (nitrogen) flow rates were set at 35 and 10 (arbitrary unit), respectively, with a HESI vaporizer temperature of 300°C. The ion transfer capillary temperature was 300 °C with a sweep gas (nitrogen) flow rate at 5 (arbitrary unit). The S-lens RF was set at 50 (arbitrary unit). For building the spectral library, we used DDA (Top10) and PRM (Parallel Reaction Monitoring) modes for the pool of synthetic derivatized peptides. For DDA acquisitions, in full MS scan (FMS), the resolution was set to 35 000, the Automatic Gain Control (AGC) target was $5.10^6$ and the maximum injection time was set to 250 ms. In MS/MS mode, the resolution was set to 17 500, the AGC target was $5.10^6$, the maximum injection time was set to 120 ms and the quadrupole isolation width was 2 Th. In PRM acquisitions, the resolution was set to 17 500, the AGC target was $5.10^6$, the maximum injection time was set to 60 ms and the quadrupole isolation width was 2 Th. Synthetic Cys-peptides mixtures spiked in *Escherichia coli* background and total human cellular extracts were analyzed using the DIA mode. The full MS scan was performed over a *m/z* range of 400

to 848 Th with a resolution set to 35 000, an AGC target of $3.10^6$ and a maximum injection time of 55 ms. This *m/z* range was divided into 14 windows of 32 Th for LID scans with a resolution set to 35 000, an AGC target of $3.10^6$ and a maximum injection time of 95 ms. For LID experiments (PRM, DDA and DIA), the activation time was set to 25 ms. In order to avoid CID, the collision energy was set to 2 eV. For the comparison with DIA-LID, experiments were also done in DIA using the classical HCD activation mode. In this case, the same *m/z* range was divided into 14 windows of 32 Th with a resolution set to 17 500, an AGC target of $3.10^6$ and a maximum injection time of 75 ms. For HCD experiments, the activation time was set to 3 ms and the normalized collision energy (NCE) was set to 24.

**Spectral library generation and data processing**

MS/MS data acquired in DDA and PRM modes on the pools of synthetic Cys-containing peptides were searched using the Mascot database search algorithm (version 2.5.1 run on a local server, Matrix Science, London, UK) against a custom-built database containing the synthetic peptide sequences assembled into artificial protein sequences. Full trypsin enzyme specificity was used, no missed cleavage was allowed and the dabcyl maleimide modification (+391.164 Da) was set as a fixed modification on Cys residues. Mass tolerance for precursor ions was set at 5 ppm, and at 0.07 Da for fragment ions. Mascot .dat results files were loaded into Proline software (http://proline.profiproteomics.fr/[25]) and peptides were validated solely using spectrum quality criteria, namely ion scores higher than 30 and manual inspection of fragmentation spectra. The highest scored peptide spectrum match (PSM) for each peptide was retained and imported into Skyline software[26] to build the LID reference spectral library that will be further used to extract signals (user-selected specific transitions) of the selected Cys-containing peptides.

Details and validation criteria for targeted signal extraction from DIA data using the reference spectral library are provided in the results section.

## Results and discussion

Despite the use of spectral libraries including high quality reference fragmentation spectra and precise relative retention time coordinates, the unambiguous assignment of peptides following data independent acquisition (DIA) experiments remains challenging due to the composite nature of fragmentation spectra. Hence, DIA coupled to laser-induced dissociation (DIA-LID) is proposed as a complementary strategy to collisional-activation in the HCD cell (DIA-HCD) by leveraging the high specificity of fragmentation of Cys-peptides grafted with a Dabcyl chromophore in the visible range. As a first proof of concept, a LID spectral library was built from a panel of synthetic Cys-peptide used as surrogates of human kinases and ATP-binding proteins. The linearity, repeatability and detection dynamic range of the LID-DIA method were evaluated using spiking experiments in an *Escherichia coli* background proteome, while the transferability of the method was evaluated with the detection of endogenous proteins in a total human cell lysate. The library generation and data processing workflow is illustrated by the synopsis of Figure 1.

### Building of LID reference spectra library

Thanks to mining of public proteomics data repositories (ProteomeXchange[27], SRMAtlas[28]), 446 peptide surrogates of human-related protein kinases (one per protein) containing a single cysteine residue and between 9 and 25 amino-acids were selected for parallel chemical synthesis. After grafting them with a Dabcyl C2 maleimide group, pools of peptides in pure solvent condition were first submitted to standard Top10 DDA experiments without any constrain on the charge state of selected precursor ions. The generated MS/MS data were

searched against a custom-built database containing the synthetic peptide sequences assembled into artificial protein sequences. Peptides identified during this first database search round with high quality fragmentation spectra and reasonable sequence coverage were added to the reference LID spectra library built using Skyline software. DDA experiments allowed validating 209 peptides. Then, to increase the chance of detecting the 237 remaining non-detected peptides, a specific monitoring using targeted PRM acquisitions was carried out to complete the LID reference spectra collection. LID reference spectra and relative retention time coordinates were finally recovered for 354 peptides while 92 remained undetected, mostly due to synthesis failure as it is often observed with low-grade synthetic libraries. Note that this partial recovery was considered not detrimental for subsequent evaluation of LID-DIA figure of merit. LID spectra were recorded after a constant irradiation time of 25 ms to yield, in average, to 85 % depletion of precursor ion signal. All spectra exhibit a characteristic reporter ion at *m/z* 252.113 arising from internal fragmentation of Dabcyl chromophore as well as informative backbone fragmentations[29] (examples are shown in Figure S1A and B in Supporting Information). The relative intensity of the reporter ion at *m/z* at 252.113 corresponds in average to 20 % of the total fragment ions intensity while the sum of singly and doubly charged $y_n$, and $b_n$ ion signals contribute to 51 and 29 %, respectively (Figure S2) (these percentages were calculated from a random selection of 40 peptides). Importantly, the combination of $y_n$ and $b_n$ fragments overall provide consistent peptide sequence coverage of 60 and 30 % for the entire library, which beside the fragmentation specificity is clearly another useful feature for reliable peptide assignment at a given retention time.

**DIA-LID method development and evaluation**

In DIA mode, the acquisition process usually combines a MS scan followed by n concatenated MS/MS isolation windows, which duty cycle completion needs to ensure the

246 record of at least 8 data points per chromatographic peak.[30] For DIA-LID experiments, 14
247 windows of 32 Th were recorded to cover the 400 to 848 *m/z* range of all precursor ions
248 present in the spectral library. For comparison of the relative merit of DIA-LID towards
249 conventional DIA-HCD condition, the 400 to 848 *m/z* range was similarly divided in 14
250 windows of 32 Th. In order to validate our spectral library-based signal extraction strategy,
251 the 354 Cys-peptides were spiked in a whole *Escherichia coli* background proteome prior to
252 chromophore grafting and trypsin digestion. Based on previous experiments and peptide
253 signal levels measured during the spectral library generation, the 354 peptides concentrations
254 were adjusted before being spiked into 100 $\mu$g of *Escherichia coli* proteins background.
255 Dilutions of peptide stock solutions were made in order to normalize individual peptide
256 signals around $10^7$ cps for which 6 fragment ions were at least detected in the pure solvent
257 condition. Figures 2 and S3 (Supporting Information) exemplify archetypes of LID and HCD
258 fragmentation patterns recorded for equivalent DIA transmission windows at two given
259 retention times. For instance, Figure 2A and B displays the extracted fragmentation spectra of
260 *m/z* 432-464 DIA window acquired at retention time 6.69 min in LID and HCD regime,
261 respectively. In LID, though at least four precursor ions (isotope clusters labeled with stars on
262 the LID spectrum) have been simultaneously transmitted, they remain intact since no
263 noticeable fragments are detected on both sides of them (Figure 2A), which features the sole
264 transmission of cysteine-free peptides. On the contrary, its analog recorded in HCD mode
265 exhibits a complete depletion in the precursor ion window leading to a complex pattern of
266 fragment ions both in the high and low *m/z* regions (Figure 2B). Such alignment comparisons
267 are straightforward provided reproducible chromatographic behavior and thus retention times
268 are achieved, which was the case thanks to the use of a conventional flow coupling enabling
269 highly stable retention time with coefficients of variation (CV) <1.5 %. Figure 2C and D
270 provides the same comparison between LID and HCD but at a higher retention time (36.40

min). Most of the Cys-peptides are eluted later in the gradient (between 25 and 40 % of ACN) due to the increased retention factor caused by the Dabcyl group[23]. This chromatographic behavior also contributes to extract them from the bulk of their cysteine-free congeners. At this retention time, the characteristic signal of reporter ion at *m/z* 252.113 is detected both in LID (Figure 2C) and HCD (Figure 2D) modes, indicating that at least one derivatized Cys-peptide ion felt within the *m/z* 592-624 DIA window. The DIA-LID data processing using our spectral library extraction approach identified peptide (NILWSAENECFK). It is noteworthy that upon photoirradiation (Figure 2C), the reporter ion and specific backbone fragments ($y_{10}^{++}$, $y_9^{++}$, $b_2^+$, $b_3^+$, $b_4^+$and $a_2^+$ ions labeled with black triangles) related to the targeted peptide sequence are the major species, while the concurrently transmitted cysteine-free peptides remain intact (* labeled ions). In HCD mode (Figure 2D), the spectrum is more complex due to co-fragmentation. Indeed, in contrast to LID for which only derivatized Cys-peptides are specifically photo-fragmented, in HCD all cysteine-free peptides present in the DIA window are also fragmented (yielding ions labeled with empty squares). The spectrum is dominated by the intense reporter ion at *m/z* 252.113. This could be explained by preferential protonation on the chromophore moiety which drives the fragmentation mechanism in HCD (charge-driven fragmentation), while the energetic redistribution seems more efficient in LID.[29] As for backbone fragments, due to intense contributions from other cysteine-free ions in the DIA window, only $a_2^+$, $b_2^+$ and $b_3^+$ fragment ions can be clearly assigned to the aforementioned kinase related peptide. Together, all DIA-LID spectra validate the concept of streamlined spectral library-based signal extraction using Skyline by leveraging the specific fragmentation of the subset of Cys-peptides upon visible LID.

Overall, the spectral library-based extraction of DIA-LID data lead to the successful identification of 272 peptides out of 354 (76 %) by applying the following validation filters: a minimum of 3 detected transitions, a Dotp value (attesting for the correlation of relative

fragment intensities with those measured on the reference LID spectrum) equal or above 0.8 and a retention time tolerance of +/- 2.5 min (centered on the reference retention time recorded in the spectral library). In fact, 96 % of the 272 peptides were detected with at least 4 of the most intense fragments contained in the spectral library. Applying these stringent validation criteria for a control experiment consisting in the DIA-LID analysis of a non-spiked *Escherichia coli* lysate derivatized with Dabcyl but free of human kinase-related peptides led to no identification. This tight specificity of LID (Figures 3A, B and C) compared to indiscriminant HCD mode (Figures 3D, E and F) is similarly highlighted by the extracted ion chromatograms of fragment ions corresponding to three identified peptides. LID allows significant depletion of interfering signals in the chromatograms over different DIA windows. In HCD mode, the detection of noise signal resulting from co-fragmentation of cysteine-free peptides leads to poor Dopt scores and difficult identification of peptides. After thorough manual inspection, 14 peptides out of the 82 missing candidates were putatively retrieved after enlarging the retention time tolerance to +/- 6 min. This result is likely the consequence of a retention time shift caused by a matrix effect, which strengthens the proposal of DIA workflows based on sample type-specific spectral libraries[10]. No signal could be assigned for any of the remaining lacking 68 peptides that may be attributed to lower concentration than estimated for some peptides in the synthetic library or signal suppression caused by the matrix.

**Response curve, linearity and sensitivity of Cys-peptides detection with DIA-LID**

In order to assess the dynamic range of detection and precision of our DIA-LID method, we selected a subset of 65 peptides based on their signal intensity and retention time distribution and set up a new concentration-balanced mixture that was spiked into 100 $\mu$g of *Escherichia coli* total lysate. Since the true peptide concentrations were not accessible for

those crude peptides, the dilution curves were normalized to 1, 0.1, 0.01 and 0.001 where the value of 1 corresponds to adjusted concentrations of the stock solutions as described above. By analyzing the results from injection triplicates, both slopes and coefficients of determination $R^2$ values of the response curves demonstrated a linearity over 3 decades with all $R^2$ better than 0.95 (Table S1). Imprecision values of peak areas expressed by the coefficient of variation (CV) were better than 20 % for 94 % of the concentration points (Figure 4 and Table S1). In order to better evaluate the intrinsic sensitivity of our DIA-LID experimental set-up, here based on a first generation of Q-Exactive Orbitrap mass spectrometer, response curves were acquired in triplicate for 3 custom peptides (purity of 70 %) with concentrations spanning 0.1 to 1000 ng spiked in 100 $\mu$g of *Escherichia coli* proteome. Linearity was observed between 1 ng and 1000 ng ($R^2$ = 0.99) both for VMCDSNDLPK and DICNDVLSLLEK peptides, and between 5 ng and 1000 ng ($R^2$ = 0.9824) for YMPQNPCIIATK peptide (Figure S4). Again, the CVs were all better than 20 % (Table S2). Assuming that the limit of quantification (LOQ) corresponds to the lowest concentration of the linearity range for which a CV better than 20 % is achieved, we could thus express these LOQ at 210, 170 and 840 femtomoles injected on column. By extrapolation, the LOQ that could be then reasonably expected by shifting from the present normal flow (300 µL/min) to a more sensitive nano flow format (200 nL/min) should attain the low femtomole on column range, in line with the recently published multisite comparison of SWATH-MS performances[9].

**DIA-LID applicability**

Overall, the DIA-LID metrics assessed by response curves reveals the validity of DIA-LID approach for selective, precise and sensitive detection of Cys-peptides in a complex proteome. DIA-LID reliability was ultimately investigated by tracking endogenous kinases in

the HS578T human cell line. From triplicate analyses, 5 kinase-related peptides were confidently identified (Table S3 and Figure 5) with acceptable precision as coefficients of variation of peak area calculated on the most intense fragment were all better than 25 %. In the first instance, this identification rate may appear relatively disappointing but could have been in part anticipated due to the adverse normal flow chromatographic configuration used in the present study. Indeed, the increased sensitivity of the nano flow standard, suitable for extensive detection of weakly abundant proteins, is the unique format compatible with all miniaturized kinase enrichment tools required for human kinome assay[31–33]. Nonetheless, a focus on the extracted ion chromatogram of the reporter ion at *m/z* 252.113 between RT 27 and 35 min. (Figure S5) and randomly selected DIA MS-MS spectra of different *m/z* transmission windows give us a glimpse on the potential of DIA-LID to massively and specifically detect Cys-peptides. Interestingly, DIA-LID could also insert into a proteomic workflow integrating prior chemoselective capture of Cys-peptides, which was already proved highly efficient in enhancing the detection of low abundance proteins, hence in expanding proteome coverage[34,35]. To reach that goal, the next step will be focused on spectral library expansion towards two peptides for all human proteins bearing at least two cysteine residues.

## Conclusion

We have herein documented a streamlined, peptide-centric detection of Cys-peptides by combining visible laser induced dissociation with Data Independent Acquisition (DIA-LID). Collectively, the response curve experiments advance the robustness of the entire DIA-LID workflow, as highlighted by good CVs metrics over a dynamic range of 3 orders of magnitude. The present DIA-LID configuration foreshadows a range of detection sensitivity in line with more specifically dedicated instrumental set-ups mostly running in nanoLC

configuration, deployed today for DIA studies in proteomics. In this regard, further work needs to be done to expand the spectral library to a second Cys-peptide per kinase-related protein and ultimately to all human protein sequence harboring cysteine residues and to endow the present mass spectrometer with a chromatographic format compatible with kinase enrichment tools. Such a configuration is indeed mandatory to attain the detection sensitivity level required for proteomics, especially when the sample amounts are limited. Overall, DIA-LID is at this stage not intended to replace conventional DIA-CID but could provide a complementary tool to streamline the detection of a subset of proteins or peptides whose detection faces specificity issues.

## Supporting Information

The Supporting Information is available free of charge on the ACS Publications website:

Additional example of MS/MS spectra, analytical performance levels (repeatability and linearity) and endogenous kinases detected in a tryptic digest of HS578T cell whole proteome.

## Acknowledgment

The authors would like to thank Diane Cala and Ruth Rimokh for providing the *Escherichia coli* and human HS578T cells, as well as Joelle Saulnier for her assistance in protein concentration measurements. This work was supported by the French Proteomic Infrastructure ProFI (ANR-10-INBS-08-03).

### Conflict of interests

The authors declare no conflict of interest

**Data access**

DIA-LID data are available via PeptideAtlas (http://www.peptideatlas.org/PASS/PASS01120)

**Author ORCID number**

Jérôme Lemoine: 0000-0002-5497-6880

Marion Girod: 0000-0003-0728-2111

## References


(1) Venable, J. D.; Dong, M.-Q.; Wohlschlegel, J.; Dillin, A.; Yates, J. R. *Nat. Methods* **2004**, *1* (1), 39–45.

(2) Gillet, L. C.; Navarro, P.; Tate, S.; Rost, H.; Selevsek, N.; Reiter, L.; Bonner, R.; Aebersold, R. *Mol. Cell. Proteomics* **2012**, *11* (6), O111.016717.

(3) Liu, Y.; Huttenhain, R.; Collins, B.; Aebersold, R. *Expert Rev. Mol. Diagn.* **2013**, *13* (8), 811–825.

(4) Sajic, T.; Liu, Y.; Aebersold, R. *Proteomics. Clin. Appl.* **2015**, *9* (3–4), 307–321.

(5) Rosenberger, G.; Koh, C. C.; Guo, T.; Rost, H. L.; Kouvonen, P.; Collins, B. C.; Heusel, M.; Liu, Y.; Caron, E.; Vichalkovski, A.; Faini, M.; Schubert, O. T.; Faridi, P.; Ebhardt, H. A.; Matondo, M.; Lam, H.; Bader, S. L.; Campbell, D. S.; Deutsch, E. W.; Moritz, R. L.; Tate, S.; Aebersold, R. *Sci. data* **2014**, *1*, 140031.

(6) Lambert, J.-P.; Ivosev, G.; Couzens, A. L.; Larsen, B.; Taipale, M.; Lin, Z.-Y.; Zhong, Q.; Lindquist, S.; Vidal, M.; Aebersold, R.; Pawson, T.; Bonner, R.; Tate, S.; Gingras, A.-C. *Nat. Methods* **2013**, *10* (12), 1239–1245.

(7) Guo, T.; Kouvonen, P.; Koh, C. C.; Gillet, L. C.; Wolski, W. E.; Rost, H. L.; Rosenberger, G.; Collins, B. C.; Blum, L. C.; Gillessen, S.; Joerger, M.; Jochum, W.; Aebersold, R. *Nat Med* **2015**, *21* (4), 407–413.

(8) Liu, Y.; Buil, A.; Collins, B. C.; Gillet, L. C. J.; Blum, L. C.; Cheng, L.-Y.; Vitek, O.; Mouritsen, J.; Lachance, G.; Spector, T. D.; Dermitzakis, E. T.; Aebersold, R. *Mol. Syst. Biol.* **2015**, *11* (1), 786.

(9) Collins, B. C.; Hunter, C. L.; Liu, Y.; Schilling, B.; Rosenberger, G.; Bader, S. L.; Chan, D. W.; Gibson, B. W.; Gingras, A.-C.; Held, J. M.; Hirayama-Kurogi, M.; Hou, G.; Krisp, C.; Larsen, B.; Lin, L.; Liu, S.; Molloy, M. P.; Moritz, R. L.; Ohtsuki, S.; Schlapbach, R.; Selevsek, N.; Thomas, S. N.; Tzeng, S.-C.; Zhang, H.; Aebersold, R. *Nat. Commun.* **2017**, *8* (1), 291.

(10) Muntel, J.; Xuan, Y.; Berger, S. T.; Reiter, L.; Bachur, R.; Kentsis, A.; Steen, H. *J. Proteome Res.* **2015**, *14* (11), 4752–4762.

(11) Crowe, M. C.; Brodbelt, J. S. *Anal. Chem.* **2005**, *77* (17), 5726–5734.

(12) Borotto, N. B.; McClory, P. J.; Martin, B. R.; Hakansson, K. *Anal. Chem.* **2017**, *89* (16), 8304–8310.

(13) Wilson, J. J.; Brodbelt, J. S. *Anal. Chem.* **2006**, *78* (19), 6855–6862.

(14) Agarwal, A.; Diedrich, J. K.; Julian, R. R. *Anal. Chem.* **2011**, *83* (17), 6455–6458.

(15) Antoine, R.; Dugourd, P. *Phys. Chem. Chem. Phys.* **2011**, *13* (37), 16494–16509.
(16) Joly, L.; Antoine, R.; Broyer, M.; Dugourd, P.; Lemoine, J. *J. Mass Spectrom.* **2007**, *42* (6), 818–824.
(17) Aponte, J. R.; Vasicek, L.; Swaminathan, J.; Xu, H.; Koag, M. C.; Lee, S.; Brodbelt, J. S. *Anal. Chem.* **2014**, *86* (13), 6237–6244.
(18) O'Brien, J. P.; Pruet, J. M.; Brodbelt, J. S. *Anal. Chem.* **2013**, *85* (15), 7391–7397.
(19) Lin, D.; Li, J.; Slebos, R. J. C.; Liebler, D. C. *J. Proteome Res.* **2010**, *9* (10), 5461–5472.
(20) Diedrich, J. K.; Julian, R. R. *Anal. Chem.* **2010**, *82* (10), 4006–4014.
(21) Parker, W. R.; Holden, D. D.; Cotham, V. C.; Xu, H.; Brodbelt, J. S. *Anal. Chem.* **2016**, *88* (14), 7222–7229.
(22) Cotham, V. C.; Wine, Y.; Brodbelt, J. S. *Anal. Chem.* **2013**, *85* (11), 5577–5585.
(23) Girod, M.; Biarc, J.; Enjalbert, Q.; Salvador, A.; Antoine, R.; Dugourd, P.; Lemoine, J. *Analyst* **2014**, *139* (21), 5523–5530.
(24) Enjalbert, Q.; Girod, M.; Simon, R.; Jeudy, J.; Chirot, F.; Salvador, A.; Antoine, R.; Dugourd, P.; Lemoine, J. *Anal. Bioanal. Chem.* **2013**, *405* (7), 2321–2331.
(25) Carapito, C.; Lane, L.; Benama, M.; Opsomer, A.; Mouton-Barbosa, E.; Garrigues, L.; Gonzalez de Peredo, A.; Burel, A.; Bruley, C.; Gateau, A.; Bouyssie, D.; Jaquinod, M.; Cianferani, S.; Burlet-Schiltz, O.; Van Dorsselaer, A.; Garin, J.; Vandenbrouck, Y. *J. Proteome Res.* **2015**, *14* (9), 3621–3634.
(26) MacLean, B.; Tomazela, D. M.; Shulman, N.; Chambers, M.; Finney, G. L.; Frewen, B.; Kern, R.; Tabb, D. L.; Liebler, D. C.; MacCoss, M. J. *Bioinformatics* **2010**, *26* (7), 966–968.
(27) Deutsch, E. W.; Csordas, A.; Sun, Z.; Jarnuczak, A.; Perez-Riverol, Y.; Ternent, T.; Campbell, D. S.; Bernal-Llinares, M.; Okuda, S.; Kawano, S.; Moritz, R. L.; Carver, J. J.; Wang, M.; Ishihama, Y.; Bandeira, N.; Hermjakob, H.; Vizcaino, J. A. *Nucleic Acids Res.* **2017**, *45* (D1), D1100–D1106.
(28) Kusebauch, U.; Campbell, D. S.; Deutsch, E. W.; Chu, C. S.; Spicer, D. A.; Brusniak, M.-Y.; Slagel, J.; Sun, Z.; Stevens, J.; Grimes, B.; Shteynberg, D.; Hoopmann, M. R.; Blattmann, P.; Ratushny, A. V; Rinner, O.; Picotti, P.; Carapito, C.; Huang, C.-Y.; Kapousouz, M.; Lam, H.; Tran, T.; Demir, E.; Aitchison, J. D.; Sander, C.; Hood, L.; Aebersold, R.; Moritz, R. L. *Cell* **2016**, *166* (3), 766–778.
(29) Garcia, L.; Lemoine, J.; Dugourd, P.; Girod, M. *Rapid Commun. Mass Spectrom.* **2017**,

*31* (23), 1985-1992.

(30) Bruderer, R.; Bernhardt, O. M.; Gandhi, T.; Xuan, Y.; Sondermann, J.; Schmidt, M.; Gomez-varela, D.; Reiter, L. *Mol. Cell. Proteomics* **2017**, No. 34, 2296–2309.

(31) Worboys, J. D.; Sinclair, J.; Yuan, Y.; Jorgensen, C. *Nat Meth* **2014**, *11* (10), 1041–1044.

(32) Xiao, Y.; Wang, Y. *Mass Spectrom. Rev.* **2016**, *35* (5), 601–619.

(33) McAllister, F. E.; Niepel, M.; Haas, W.; Huttlin, E.; Sorger, P. K.; Gygi, S. P. *Anal. Chem.* **2013**, *85* (9), 4666–4674.

(34) Wang, S.; Zhang, X.; Regnier, F. E. *J. Chromatogr. A* **2002**, *949* (1–2), 153–162.

(35) Guo, J.; Gaffrey, M. J.; Su, D.; Liu, T.; Camp, D. G.; Smith, R. D.; Qian, W.-J. *Nat. Protoc.* **2014**, *9* (1), 64–75.

# Figures

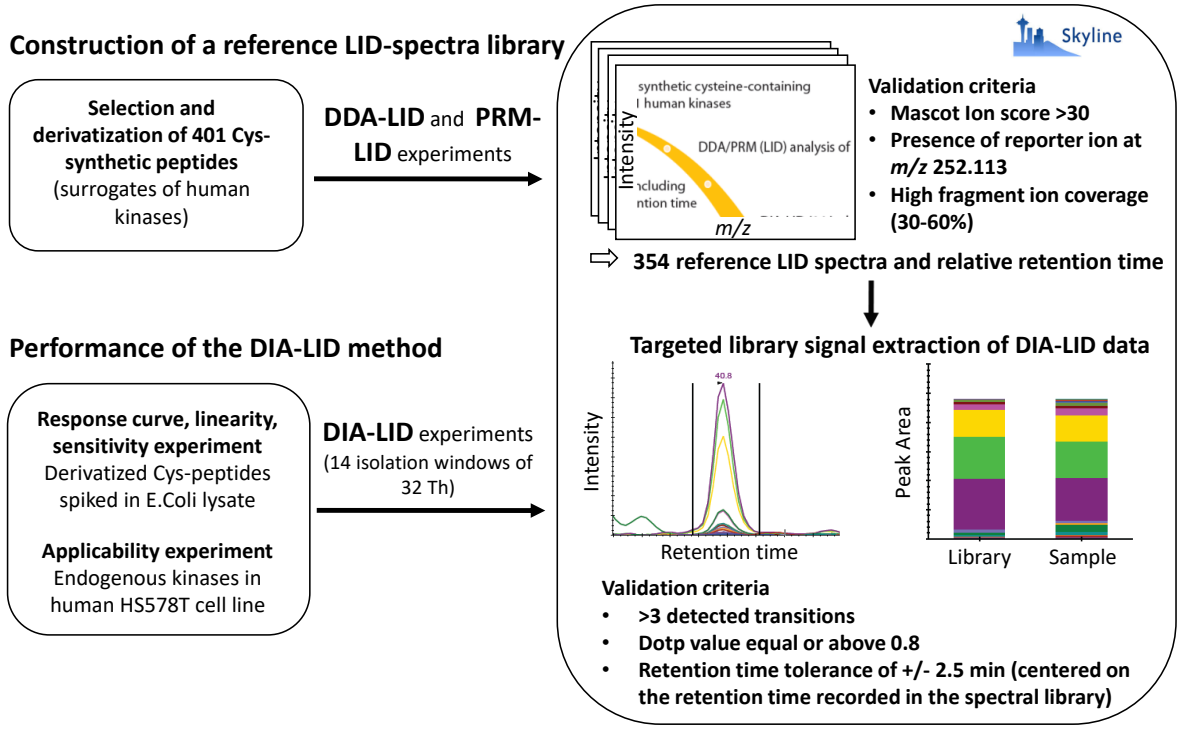
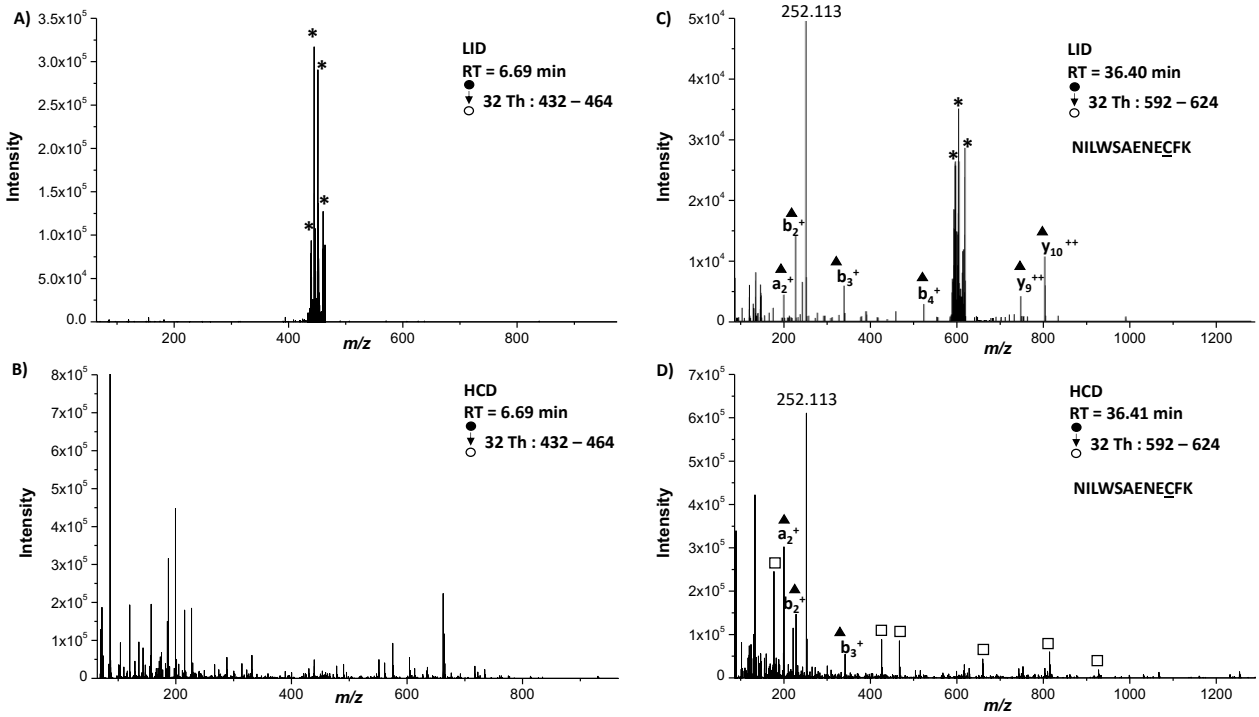



**Figure 1.** Workflow for LID reference spectra library generation and evaluation of the DIA-LID method based on targeted signal extraction of Cys-peptides grafted with a Dabcyl maleimide group.




489

490 **Figure 2.** Example of DIA-MS/MS spectra at retention time (RT) 6.69 and 36.40 min
491 recorded either in LID (A and C) or HCD mode (B and D) during the analysis of 354 Cys-
492 peptides derived from human kinase-related proteins spiked in an *Escherichia coli* proteome
493 prior to trypsin digestion and dabcyl chromophore derivatization. (*) indicates peptide ions
494 free of cysteine residue, (▲) highlights specific Cys-peptide fragment ions and (□)
495 highlights fragment ions from cysteine-free peptides.

496

497

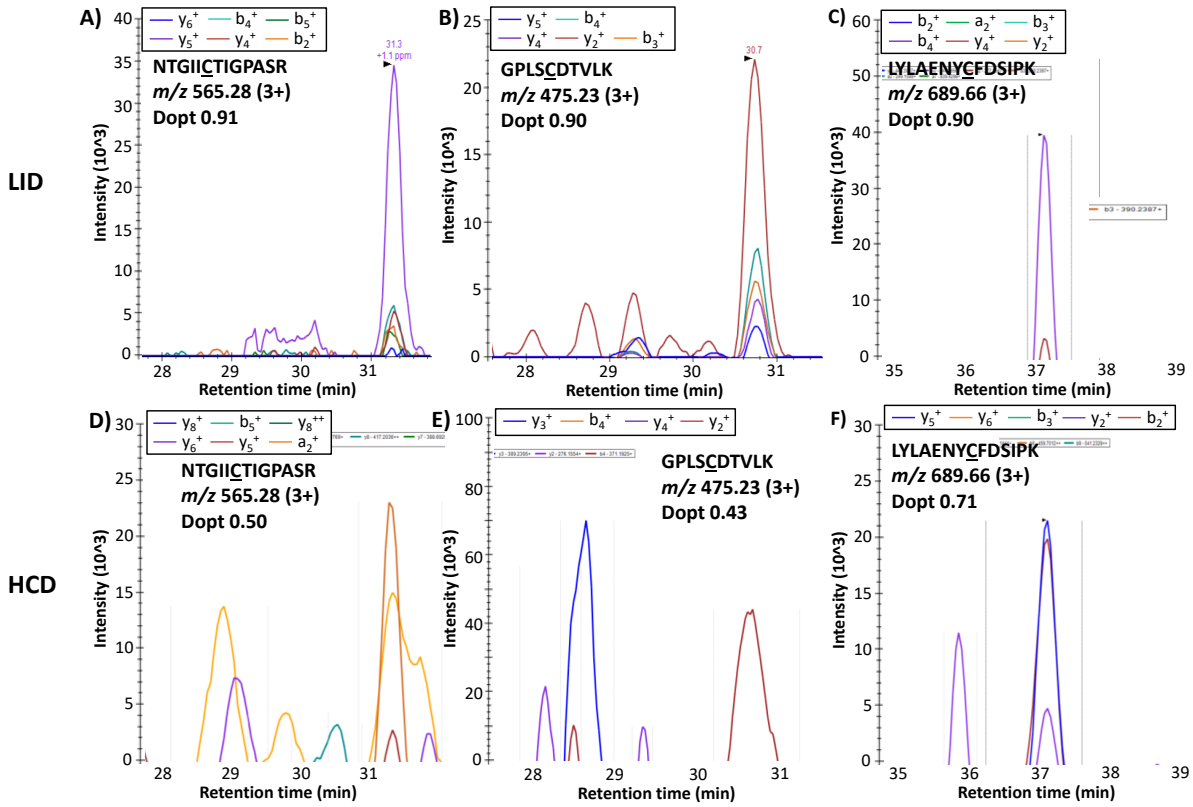
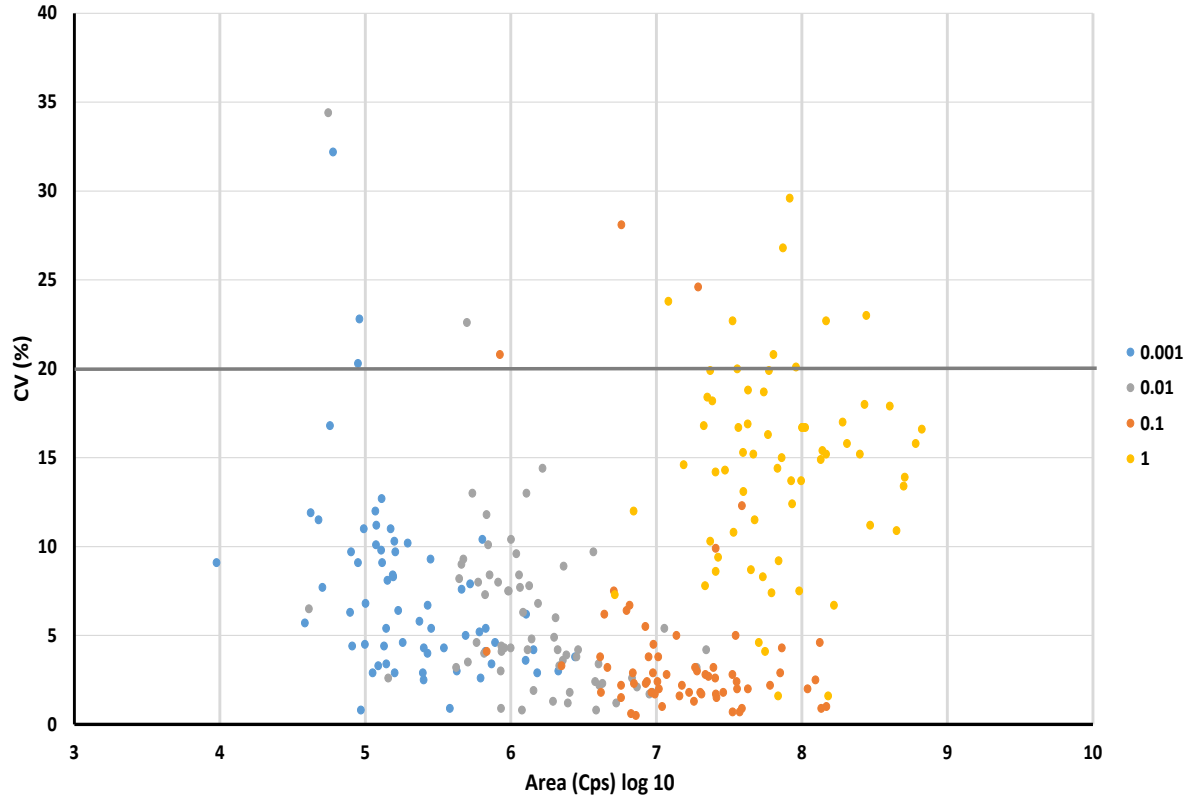



**Figure 3.** Extracted ion chromatograms of fragment ions from 3 derivatized Cys-peptides spiked in an *Escherichia coli* lysate monitored in DIA-LID (A, B, C) and DIA-HCD (D, E, F).




504

505 **Figure 4.** Coefficient of variation (CV) of peak areas (most intense transition) calculated for

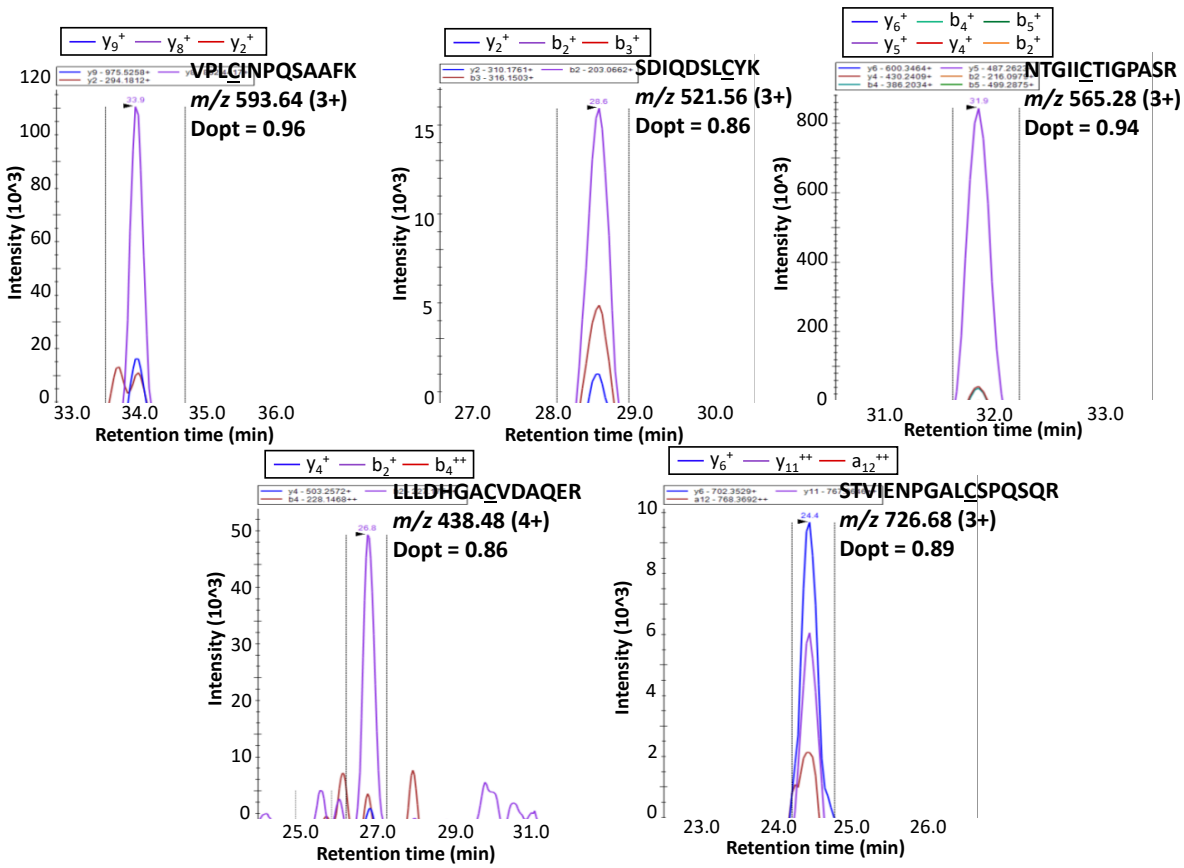
506 triplicate dilution points (1, 0.1, 0.01 and 0.001) for 65 derivatized Cys-peptides spiked into

507 an *Escherichia coli* lysate.

508

509

510




511

512 **Figure 5.** Extracted ion chromatograms of all fragment ions detected for 5 Cys-peptide

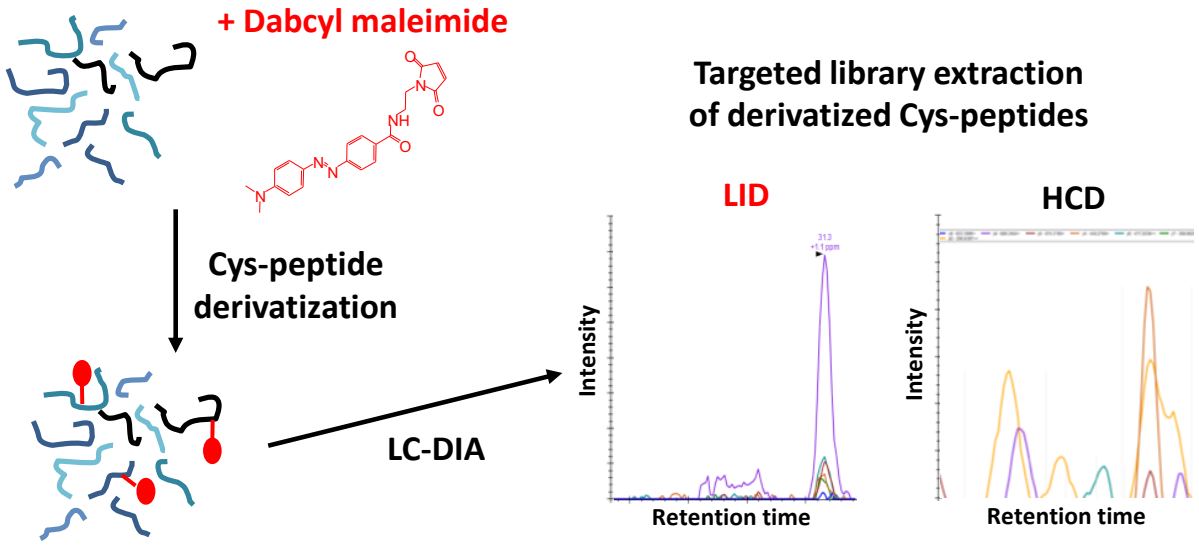
513 surrogates of endogenous kinases detected in a tryptic digest of HS578T cell whole proteome

514 in DIA-LID.

515

516 **Table of Contents**

517




518