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## **Laser-Induced Dissociation combined with Data Independent acquisition (DIA-LID) : a proof of concept study covering human kinome expression**

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### ► **To cite this version:**

Lény Garcia, Marion Girod, Magali Rompais, Philippe Dugourd, Christine Carapito, et al.. Laser-Induced Dissociation combined with Data Independent acquisition (DIA-LID) : a proof of concept study covering human kinome expression. 65th American Society for Mass Spectrometry Conference, Jun 2022, Indianapolis, United States. hal-03922508

**HAL Id: hal-03922508**

**<https://hal.science/hal-03922508>**

Submitted on 4 Jan 2023

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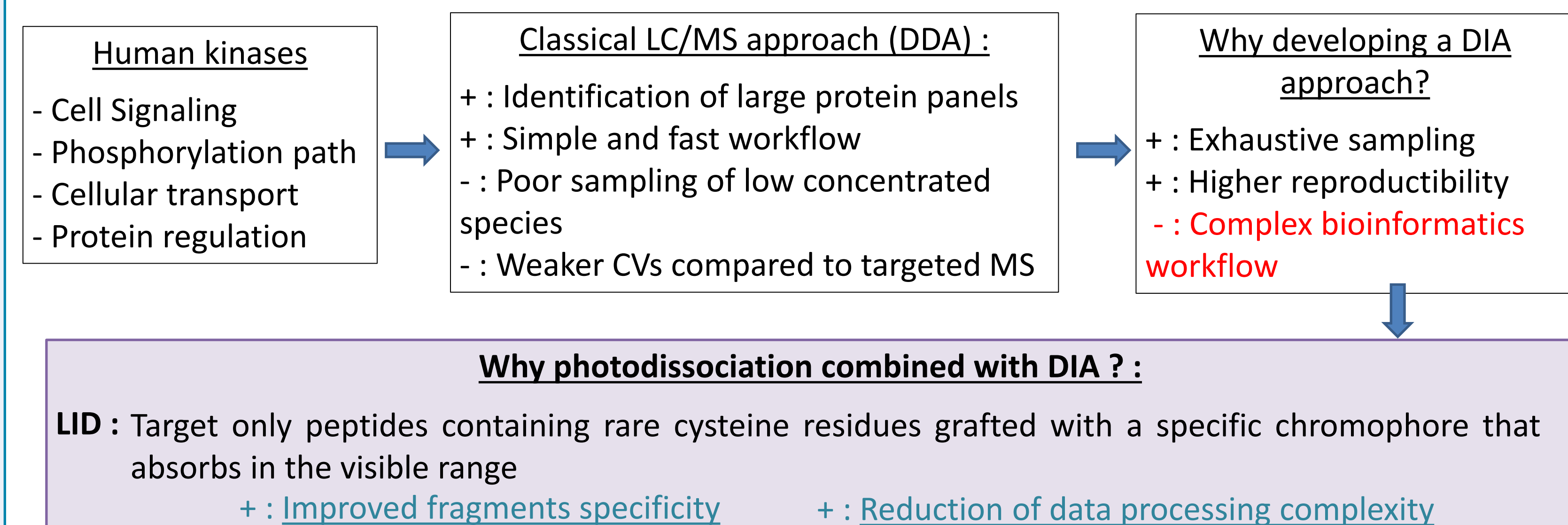
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## Overview

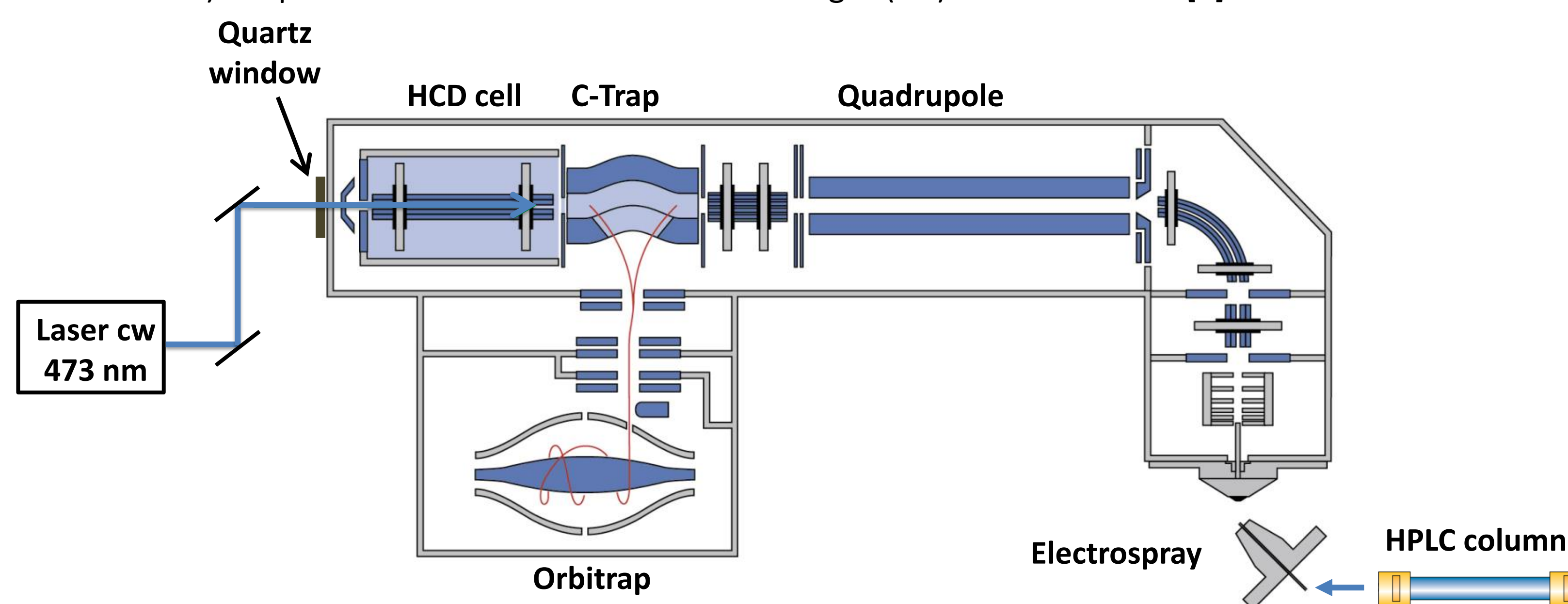
First coupling between Data Independent Analysis (DIA) and visible Laser Induced Dissociation (LID) at 473 nm to test for the presence of cysteine-containing peptides. A spectral library in LID was built for synthetic peptide surrogates of human kinases grafted with a dabcyll chromophore. By extracting ion chromatograms of query standard and kinase peptides spiked at different concentration levels in a 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 354 kinases in human cellular extract [1].

## Introduction



## Experimental Setup

The experimental setup consists of a hybrid quadrupole-orbitrap mass spectrometer (Q-exactive, Thermo Fisher Scientific) coupled to a 473 nm continuous wavelength (cw) excitation laser [2].



## Methods

### Spectral library generation :

- Derivatization of 354 proteotypic synthetic cysteine-containing peptides in water with Dabcyll C2 maleimide chromophore.
- HPLC separation (C<sub>18</sub> column: 74 min 15-50% ACN) and LID fragmentation spectra acquisition in DDA (Top 10) and PRM modes.
- Data interpretation: Mascot database searches in a home made database containing concatenated peptides.

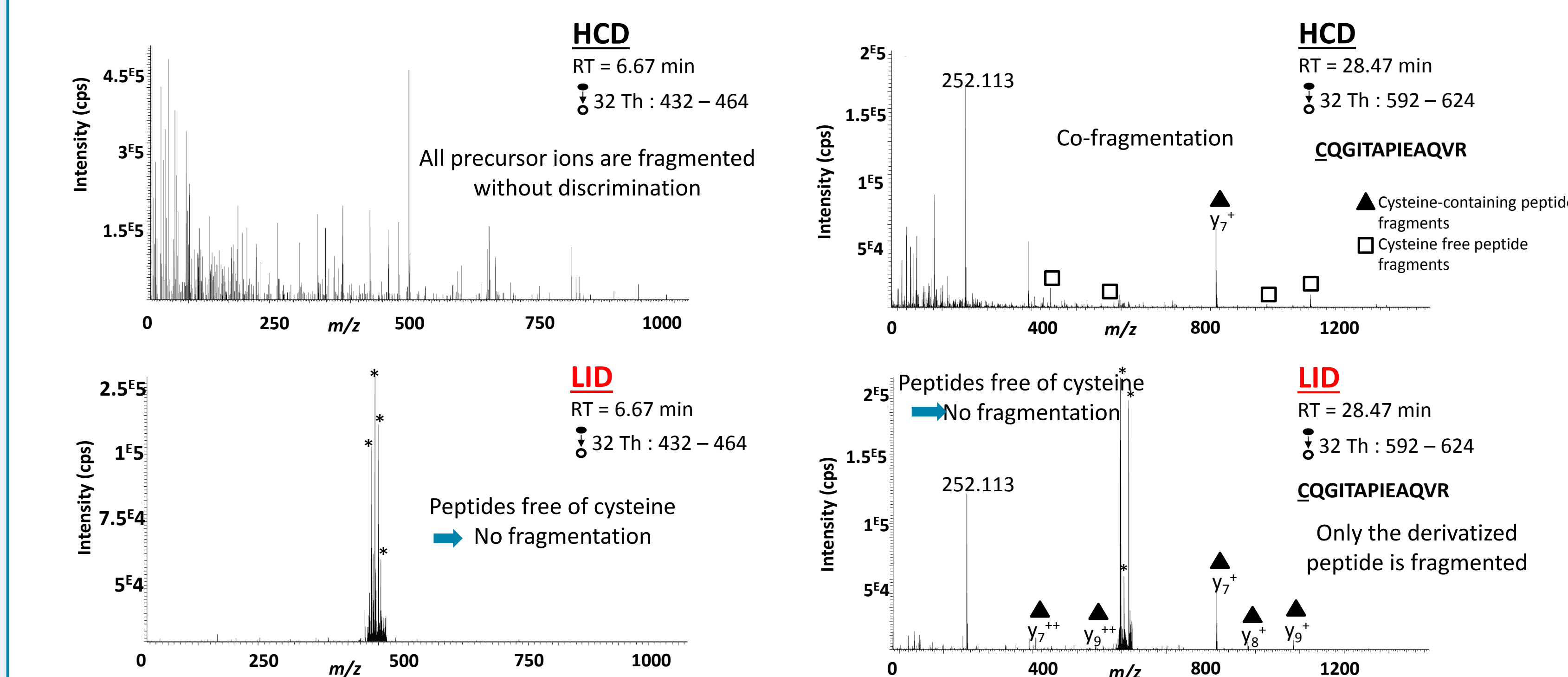
### Standard applications :

- Derivatization with Dabcyll maleimide chromophore of :
  - 354 cysteine-containing peptides spiked (1 µg/mL) in E.Coli lysate.
  - 65 cysteine-containing peptides spiked with different dilution ratio: 1, 0.1, 0.01 and 0.001 in E.Coli lysate.
  - 3 query standard cysteine-containing peptides spiked in E.Coli at different concentrations from 1 ng to 1000 ng / 100 µL.
  - 60 µg of human cellular extract (cancerous mammalian cells).
- HPLC separation (C<sub>18</sub> column: 74min 15-50% ACN) and Data Independent Acquisition : Full MS (m/z 400-848), 14 isolation windows of 32 Th, resolution 35 000, LID activation time: 25 ms.

## Specific detection of labeled peptides by DIA-LID

### Simplification of DIA-MS/MS spectra

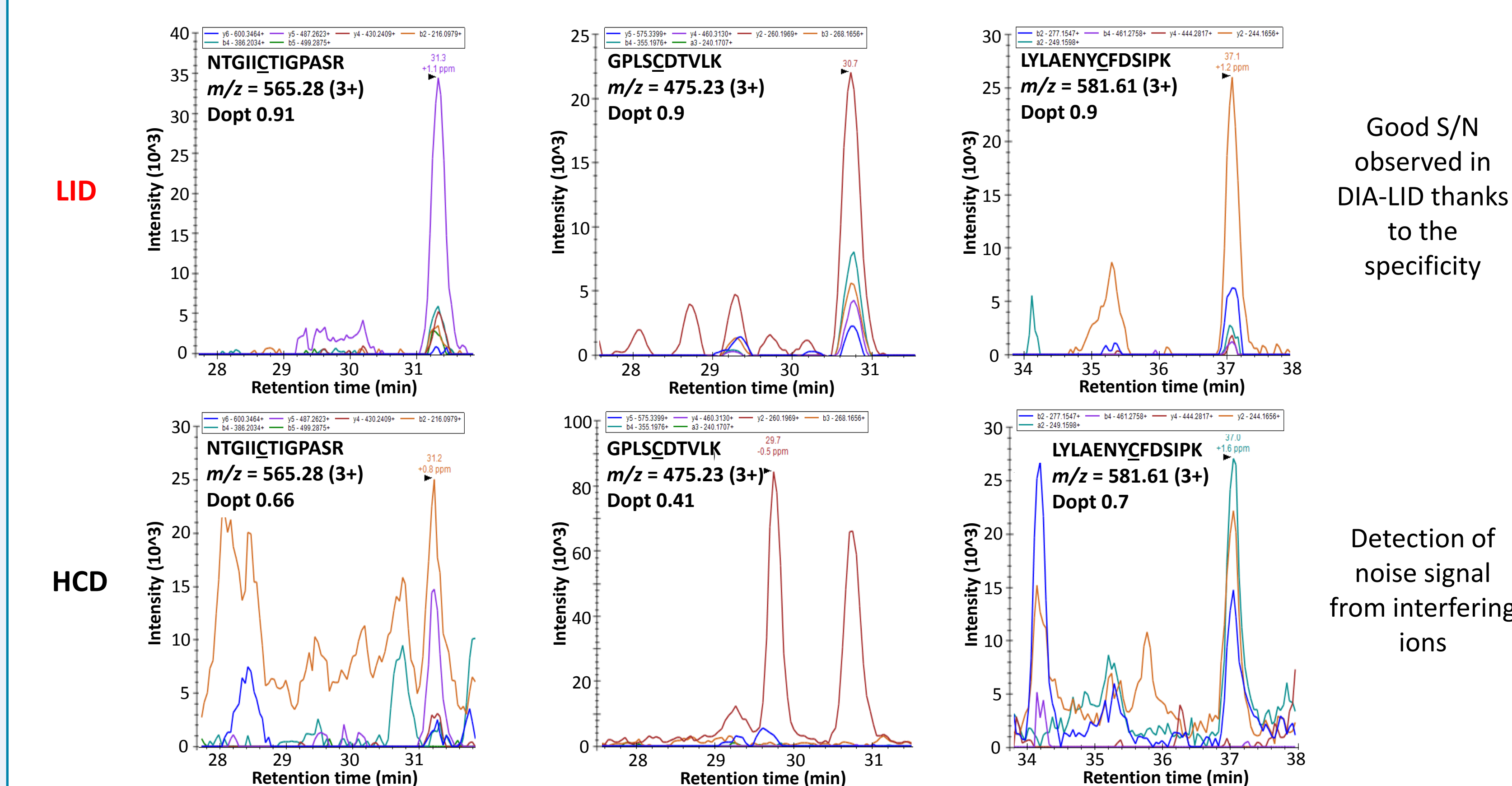
DIA-MS/MS spectra from the 354 derivatized cysteine-containing peptides spiked in an E.Coli lysate.



Due to the LID specificity, DIA-MS/MS spectra are less complex (compared to HCD where co-fragmentation results in crowded spectra), allowing to streamline the data processing.

### Identification of derivatized cysteine-containing peptides with the spectral library

Experimental DIA-LID data from the 354 derivatized cysteine-containing peptides spiked in an E.Coli lysate were processed for peptide identification using Skyline with the established spectral LID library.



### Data processing (automatic)

- Extraction of 3 and 6 transitions per peptide
- Filters : Relative intensities (Dopt > 0.80)
- Aligned retention time (RT)
- Peak shape

Identification of 275 peptides out of the 354 derivatized cysteine-containing peptides (78%) in DIA-LID.

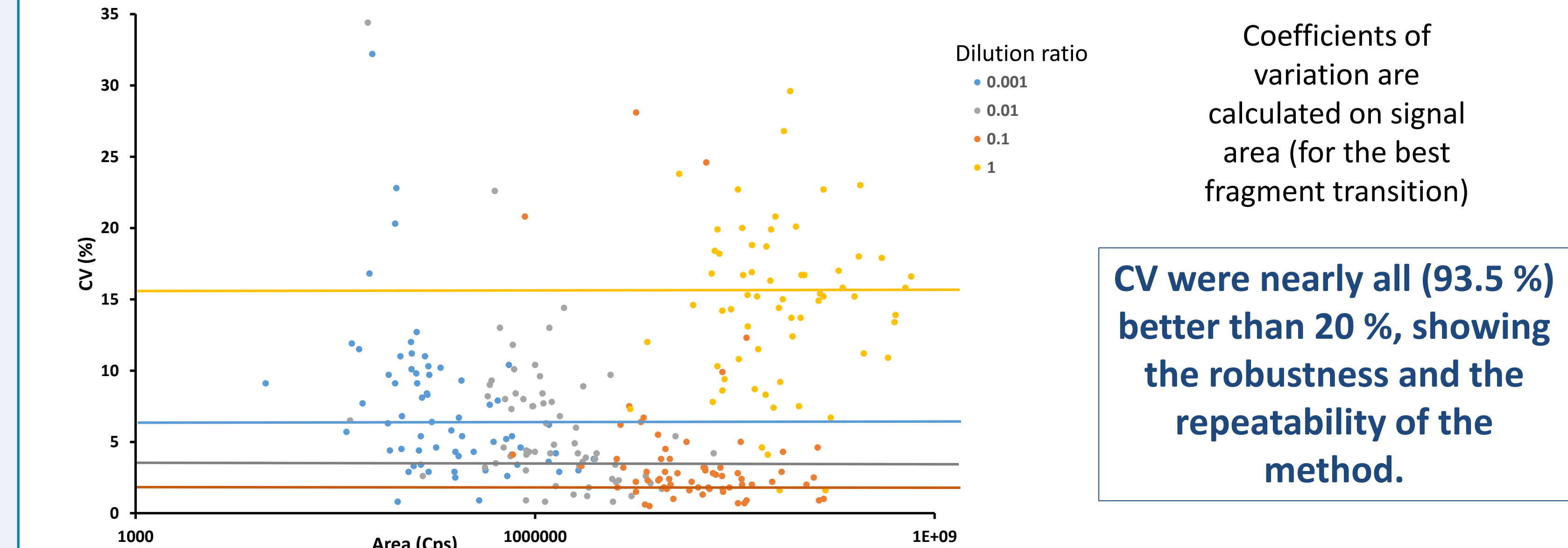
## References

- [1] Garcia L, Girod M, Rompais M, Dugourd P, Carapito C, Lemoine J. *Anal. Chem.* (2017), submitted  
 [2] Girod M, Biarc J, Enjalbert Q, Salvador A, Antoine R, Dugourd P, Lemoine J. *Analyst* (2014), 139(21), 5523-5530

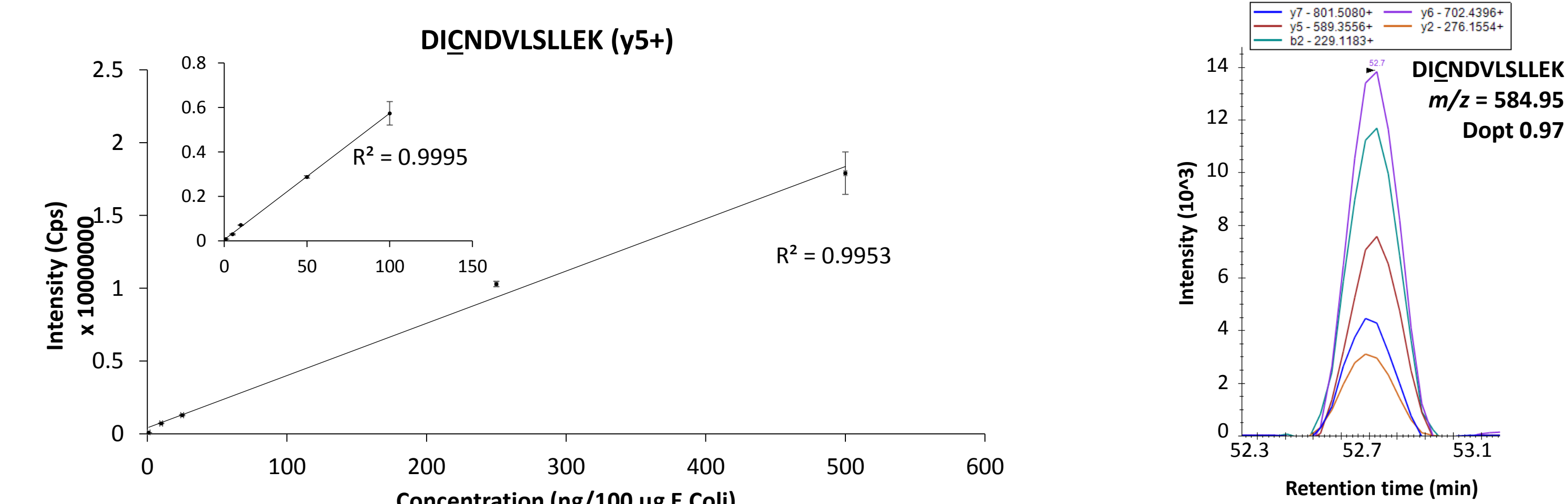
## Analytical performances of DIA-LID

### Repeatability and response linearity

65 derivatized cysteine-containing peptides were spiked at different concentrations in E. coli lysate (dilution ratio: 1, 0.1, 0.01, 0.001) and analyzed by DIA-LID in triplicate.



Calibration curve in triplicates with custom derivatized cysteine-containing peptide



Linearity over 3 decades was obtained between 1 ng and 1000 ng / 100 µg of E.Coli with R<sup>2</sup> > 0.99 (greatly preserved for the lowest concentrations with R<sup>2</sup> > 0.999).

## Analysis of endogenous peptides in biological matrix

60 µg of human cellular extract were derivatized and analyzed in DIA-LID in triplicates. From these DIA-LID analyses, 12 human kinase peptides were detected using the established spectral library.

Protein	Peptide	CV (%)
Pyruvate kinase PKM	NTGICTIGPASR	0.17
MAP/microtubule affinity-regulating kinase 4	ASCSTAGSGSR	1.57
Ankyrin repeat and protein kinase domain-containing protein 1	LLLDHGACVDAQER	3.26
Tyrosine-protein kinase 6	NILVGENTLCK	3.71
Serine/threonine-protein kinase	FLLFCVHGDAR	6.14
NUAK family SNF1-like kinase 1	LENILDDNCKNIK	27.60
Hormonally up-regulated neu tumor-associated kinase	SDIQDSLCKYK	25.42
Tyrosine-protein kinase FRK	STVIENPGALCSPQSOR	16.45
Serine/threonine protein kinase NLK	VPLCINPQSAAFK	8.27
Tyrosine-protein kinase BLK	CLDEGGYISPR	6.19
Mitogen-activated protein kinase 14	EPGLGQLCFK	8.29
Transcription initiation factor TFIID 210 kDa subunit	VGTTVHCDYLNIPHK	2.22

NTGICTIGPASR m/z = 565.28 (3+) Dopt = 0.94

## Conclusions

- Building of a LID peptide spectral library covering the human kinome .
- DIA-LID method able to identify and quantify derivatized cysteine-containing peptides in a repeatable way on 3 decades and at low concentrations (down to 1 ng/100 µg) in biological matrix.
- Capability of DIA-LID to target and detect endogenous kinases in human cellular extract.
- Nano-chromatography and kinase-specific enrichment step required in order to improve the detection specificity in a biomarker discovery approach.