

Specific screening of COVID-19 and Alzheimer's Disease biomarkers through sulphenic acids targeting by mass spectrometry coupled with Laser Induced Dissociation

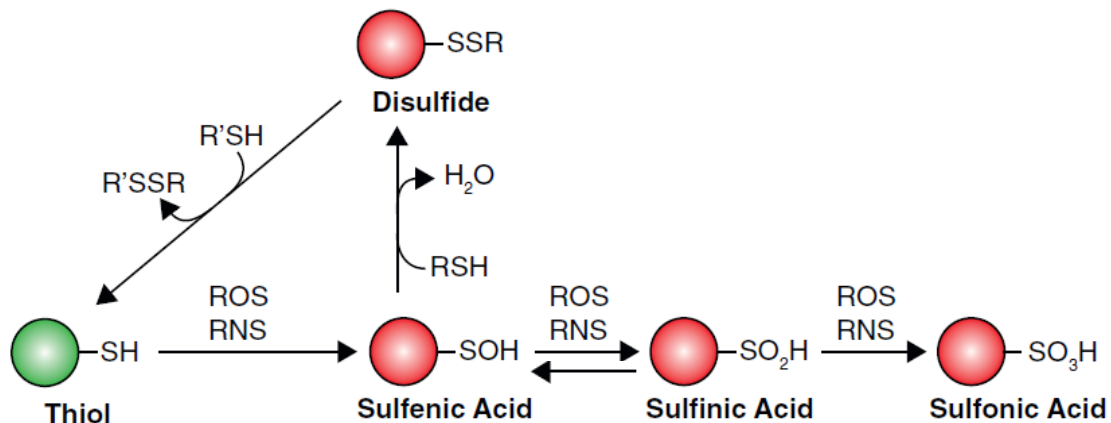
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Overview : Oxidative stress induced protein sulphenic acid (SOH) are grafted with a specific dione chromophore, then specifically fragmented, identified and quantified by Laser Induced Dissociation (LID) in the visible range. A SOH/SH oxidative ratio is determined for each samples, as proteins SH are simultaneously grafted with a second maleimide chromophore. This novel methodology allows increased sensitivity of SOH detection and relative quantification with limited inter-samples analysis bias for large oxidative biomarker screening.

Oxidative stress is the accumulation of Reactive Oxygen Species (ROS) within cells in toxic proportions to inner biomolecules. One of the modification induced is the oxidation of protein cysteine thiols (SH) into sulphenic acid (SOH).¹



Chemical 'omics' approaches for understanding protein cysteine oxidation in biology 10.1016/j.cbpa.2010.11.012

As transient intermediate in the SH/SS biological oxidative system, they are only present in relatively low concentrations, making their detection and quantification challenging.

Techniques of detection and quantification exists, and can be divided in two categories :

Global techniques

- Direct (UV/IR)
- Indirect (thiol tagging & SDS-page, IE, fluorescence)

• Do not provide SOH localization

MS/MS techniques

- Thiol blocking, SOH reduction-tagging (OxiCAT, iodoTMT ...)
- SOH derivatization (norborène, diones ...)

• Requires enrichment steps

➔ To improve SOH detection and quantification specificity, we propose an innovative experimental setup coupling mass spectrometry and laser induced dissociation (LID) in the visible range (473 nm) to add a stringent optical specificity to the mass selectivity. Since peptide do not naturally absorb in such range, this approach relies on the proper derivatization of SOH with a chromophore.

Plasma samples

- ♥ Alzheimer's Disease positive (34) and Control (15) samples were provided by CRB-CHUM from CHU de Montpellier (Pr Sylvain LEHMANN) (Identifiant BIOBANQUES - BB-0033-00031)
- ♥ COVID-19 positive (4) samples were provided by the Centre de Ressources Biologiques (CRB HCL), ref M3-P1-ST3.1 CRB-HCL
- ♥ Plasma sample were stored at -80°C. For sample preparation, plasma were defrost, pipetted and refrost as fast as possible.

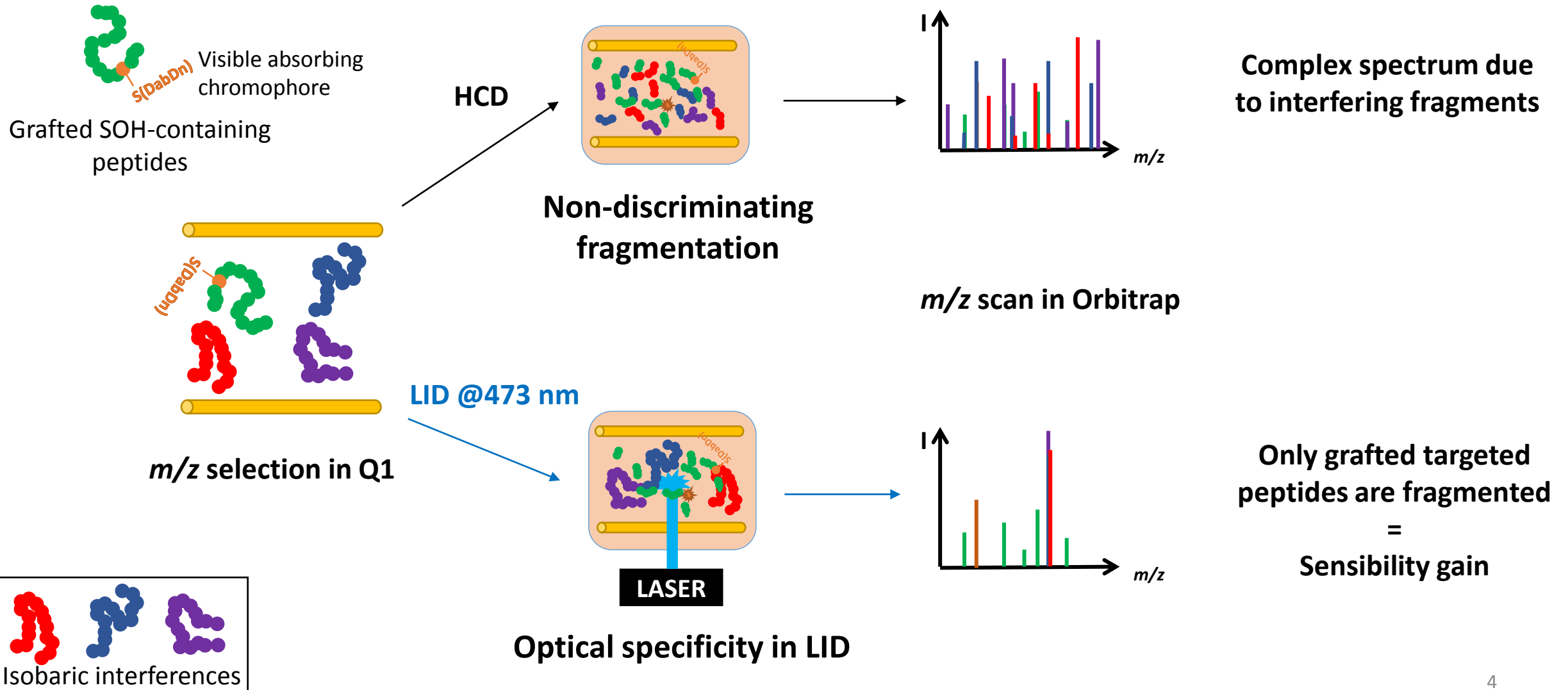
Instrumentation

- ◆ HPLC peptides separation on C18 column (\varnothing 2.1 mm, particles 2.5 μ m, L: 100mm) : 60 min run with a 5-45 % ACN gradient (300 mL/min)
- ◆ Parallel Reaction Monitoring (PRM) in LID@473nm (cw 1 W laser) on a modified QExactive (ThermoScientific) (R= 35,000 ; activation time 25 ms, activation energy 3 eV)

Samples preparation

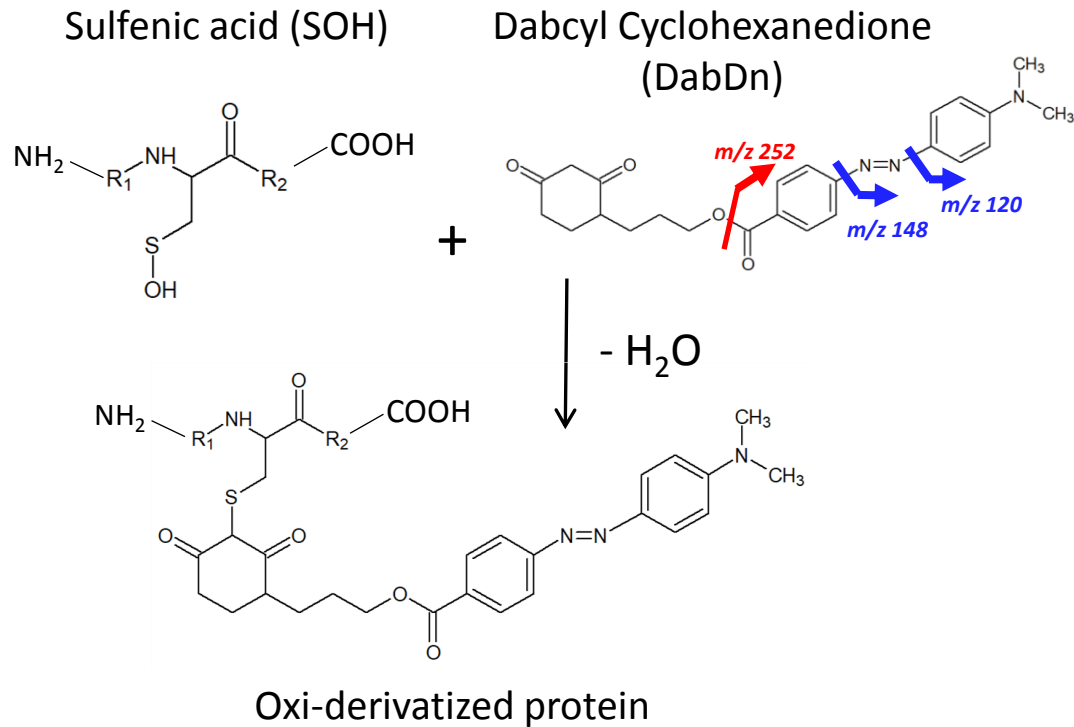
- ♣ DabcyI cyclohexanedione was synthesized on-demand by the Laboratoire de Chimie ENS Lyon and solubilized in DMSO as needed.
- ♣ LCTVATLR derivatization :
 - ♠ 100 μ L of LCTVATLR at 100 μ M in water and DabDn at 100 μ M in DMSO were mixed together.
 - ♠ H₂O₂ at 100 μ M was added to achieve a 1:1:1 Cys:DabDn:H₂O₂ ratio.
 - ♠ After 9h reaction, 10 μ L of solution was diluted in 90 μ L of a H₂O/ACN 70/30 + 0.1% formic acid (F.A.) before analysis
- ♣ Samples desalting and concentration : Solid Phase Extraction (Oasis™ HLB 3cc); Samples resuspension in 200 μ L of a H₂O/ACN 70/30 + 0.1% formic acid
- ♣ Full double derivatization protocol is available page 7.

LID vs. HCD PRM mode in a QExactive mass spectrometer



Chromophore derivatization of cysteine sulphenic acid

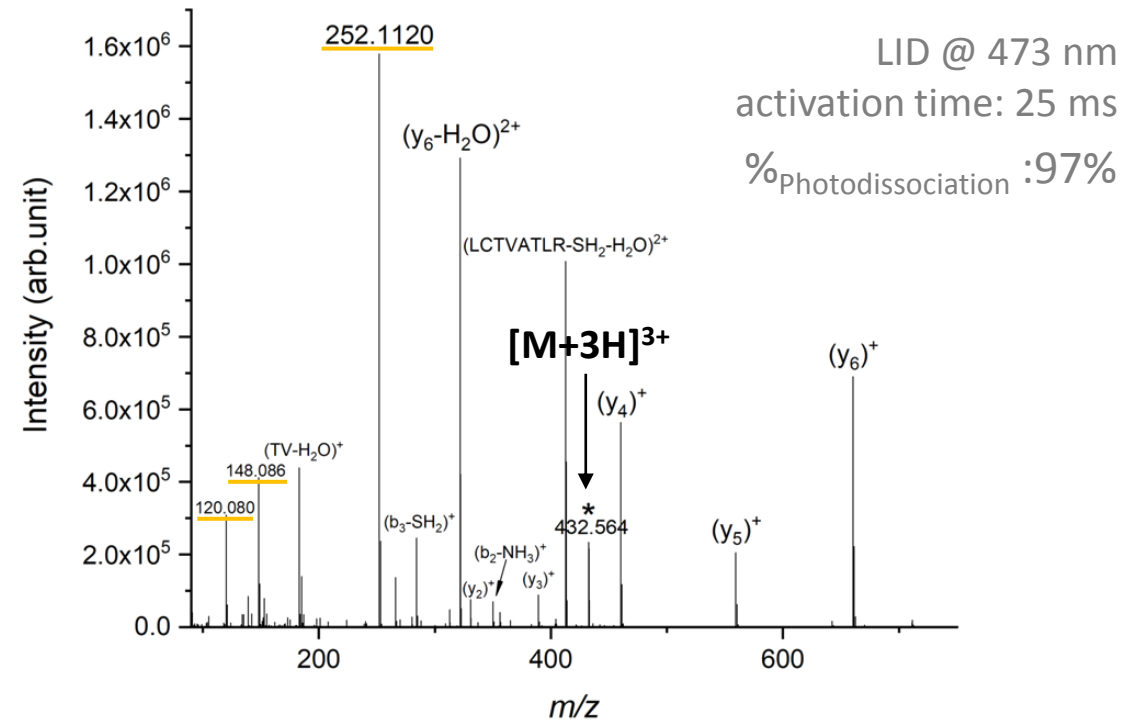
SOH derivatization reaction with specific chromophore




Y. H. Seo, et al., *Angew. Chem. Int. Ed.* **2011**, 50, 1342–1345

Derivatization was first conducted on a single cysteine-containing model peptide, LCTVATLR, oxidized with hydrogen peroxide (H₂O₂) then grafted with DabDn at a Cys:DabDn:H₂O₂ 1:1:1 ratio.

LID spectrum of grafted LC*TVATLR peptide



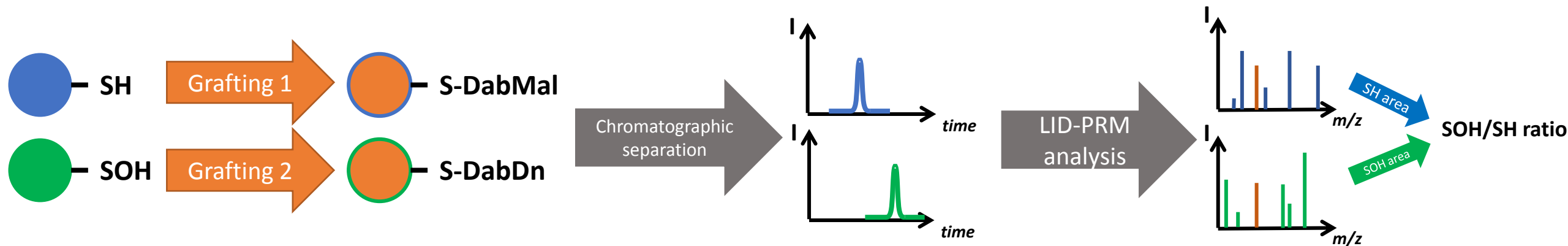

Chromophore grafted-oxidized LC*TVATLR precursor is the most intense ion detected, indicating a good reaction yield. Its fragmentation by LID generates b and y peptide backbone fragments, as well as reporter (m/z 252.1120) and LID-specific (m/z 120.080 & 148.086) chromophore fragment ions.

SOH quantification in biological samples

Grafting of SOH with DabDn chromophore could allow an inter-sample relative quantification of protein cysteine oxidation in plasma. However, as no standards of cysteine oxidation are available, the accuracy of this analysis may be affected by the daily variation of the instrument sensibility and laser alignment.

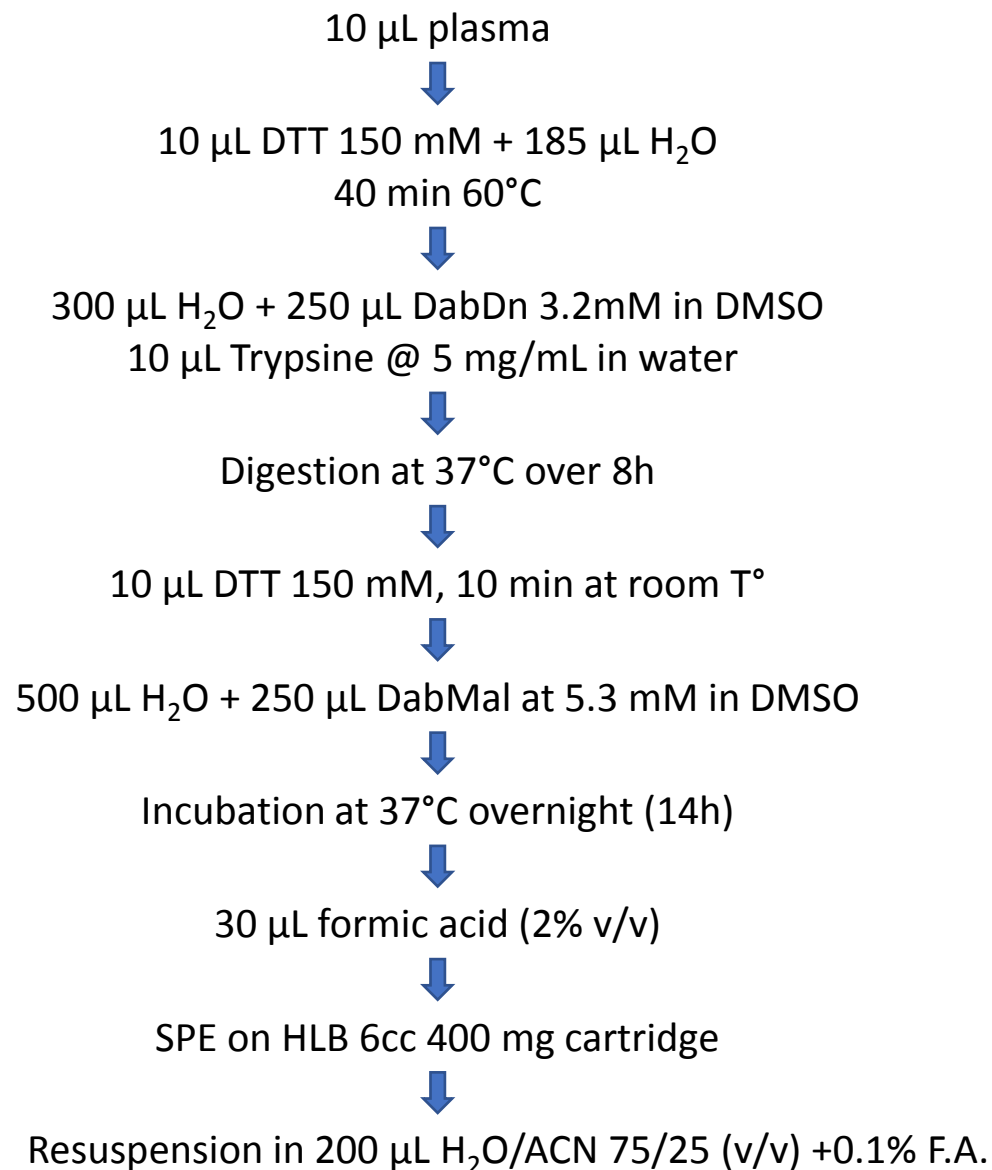
Internal normalization offers to overcome these drawbacks: The SOH amount for each peptide can be normalized by the corresponding SH peptide quantities.

To specifically quantify the non-oxidized peptides in LID, SH cysteine were grafted, in parallel, with a Dabcyl maleimide chromophore¹ (DabMal).




This double derivation allows to specifically fragment couples of SOH and SH derivatized peptides during the same LID analysis. SOH/SH normalization is performed for calculation of an oxidative ratio.

Double derivatization protocol



- ‡ Peptides were selected from 20 plasma proteins selected either from top10 analysis or literature researches.
- ‡ Protein list : HSA, TransfH, CO4A/B, FINC, CO3, A2MG, FIBB, APOB, FINC, FIBA, CERU, HEMO, HPT, IGCK, FIBG, CFAH, FETUA, AACT, APOE, CXCL7
- ‡ PRM list was built with 74 single-cysteine containing precursor ions of both SOH and SH-derivatized peptides for a total of 148 targets.
- ‡ Non-cysteine containing additional precursors (10) were added to assess tryptic digestion efficiency.

Data treatment was performed in 2 steps:

🏠 Skyline treatment for each precursor:

🏠 Transitions selection:

🏠 3 most intense, non-interfered pertinent peptide fragments

🏠 1-3 isotopes of precursor ion (depending on isobaric interferences).

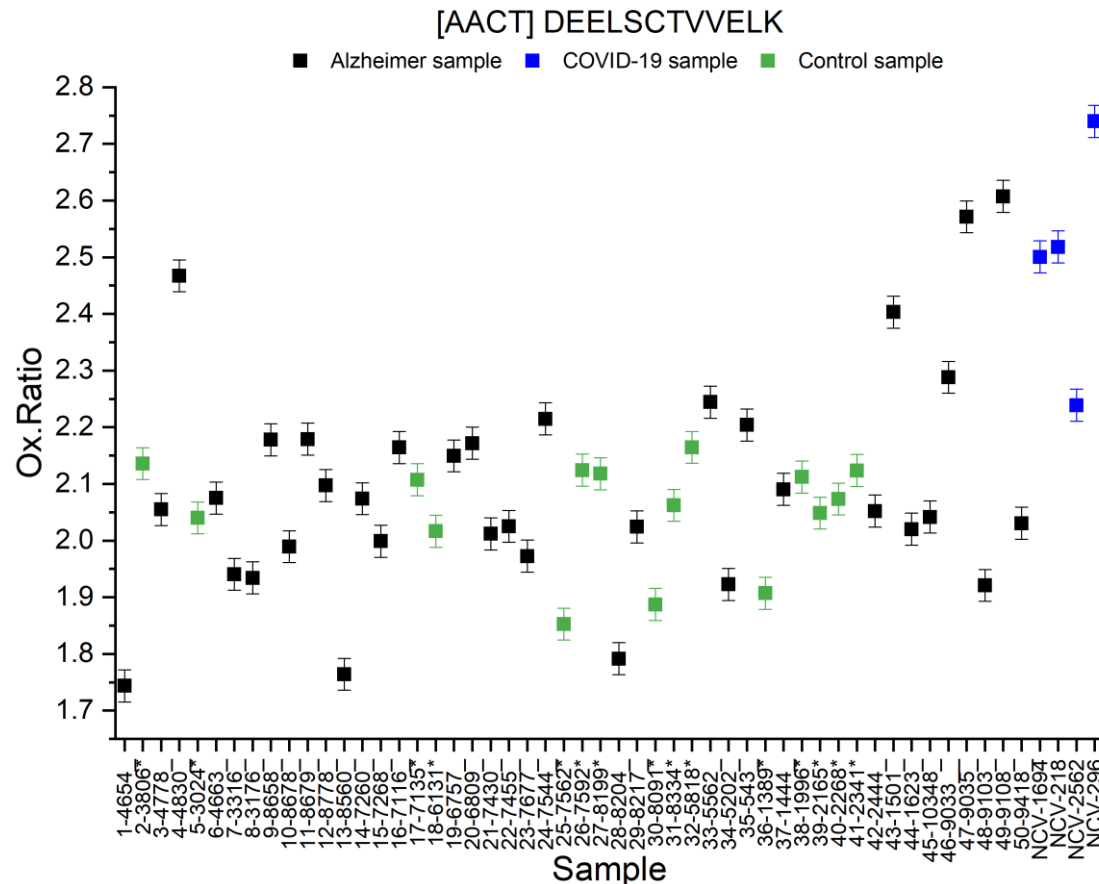
🏠 Integration of chromatographic peak for each selected transitions

🏠 Manual calculation of oxidative ratio: $\frac{(\text{derivatized_SOH})\text{peak area}}{(\text{derivatized_SH})\text{peak area}} * 100$

Oxidation ratio of plasma samples

A pool of 53 plasmas including 34 Alzheimer's disease (AD) positive patients, 4 COVID-19 positive, and 15 controls was analyzed using double derivation protocol. For each sample, the oxidation ratio was calculated.

74 SOH/SH couples amongst 15 plasma protein were screened. No evidence of possible SOH biomarker for AD was found yet, but COVID-19 positive samples provide putative biomarkers.



As an example, oxidative ratio of AACT (α -antichymotrypsin) protein (which is upregulated during pulmonary infection) is increased in COVID-19 positive samples compared to control samples.

This suggests that the uptake in SOH content of this protein is not due to a global population increase but to oxidative damage during infection.

Further investigation on more samples is required for confirmation, but this results consists in an interesting potential target for biomarker research.

- SOH grafting with chromophore
 - ✓ Specific reaction
 - ✓ Specific fragmentation
 - ✓ Simple protocol
- Quantification of derivatized endogenous Cys-SOH peptides
 - ✓ Successful parallel derivatization of SOH and SH peptides
 - ✓ Oxidative ratio : Normalization of SOH and SH peak areas
 - ✓ Analysis of AD and COVID samples
 - ✓ Robust quantification for biomarker research
- ❖ Perspectives on biomarker screening
 - AACT SOH increase confirmation
 - Alzheimer's disease target from extensive literature research
 - Diabetes, cancer, cardiovascular and neurodegenerative disease markers

Acknowledgments :



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Cohort : BB-0033-00031



Cohort : M3-P1-ST3.1 CRB-HCL