New methodology to monitor the oxidation of MET due to LC separation

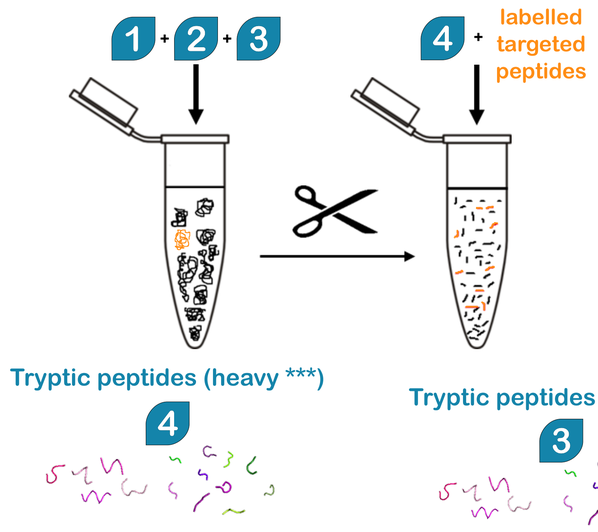
France Baumans[1], Dominique Baiwir[1,2], Maria Colombo[3], Camille Allain[4], Vincent Tavernier[4], Baptiste Leroy[4], Ruddy Wattiez[4], Edwin De Pauw[1], Gauthier Eppe[1], Gabriel Mazzucchelli[1,2]

[1]Mass Spectrometry Laboratory, MolSys RU, University of Liège, Belgium
[2]GIGA Proteomics Facility, University of Liège, Belgium
[3]Kaneka Eurogentec S.A., Seraing, Belgium
[4]Proteomics and Microbiology Laboratory, University of Mons, Belgium

france.baumans@uliege.be

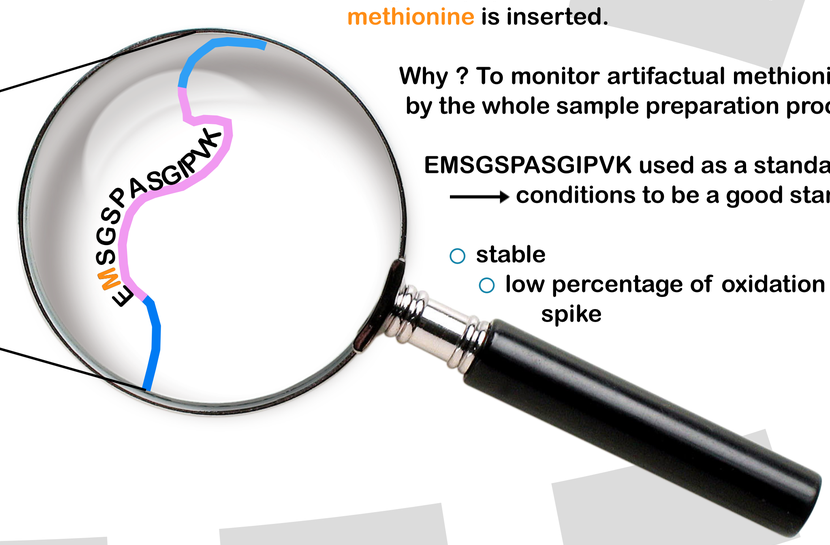
Framework

In the context of biomarker discovery and their absolute quantification in complex samples, a standardization strategy aiming to control the entire sample preparation process before LC-MS analysis would be extremely valuable. Our approach involves the design of a kit containing a chimeric protein and different levels of its heavy peptides spiked at opportune moment during sample processing.



Oxidation sensor

Among the peptides included in the protein, one containing a methionine is inserted.



Why ? To monitor artifactual methionine oxidation induced by the whole sample preparation process

EMSGSPASGIPVK used as a standard ⟶ conditions to be a good standard:

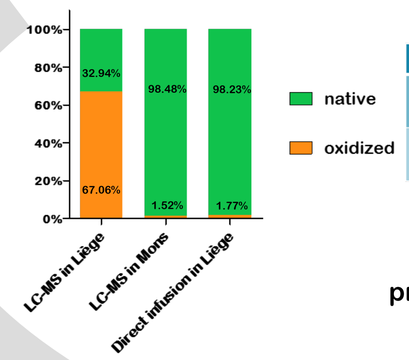
- stable
- low percentage of oxidation at the moment of the spike

Is this a good standard ?

Injection on M-Class system coupled with QExactive™ Hybrid Quadrupole-Orbitrap™

First results

Comparison of percentage of oxidation of EMSGSPASGIPVK between LC-MS in Liège (stainless steel system), LC-MS in Mons (biocompatible system) and direct infusion in Liège



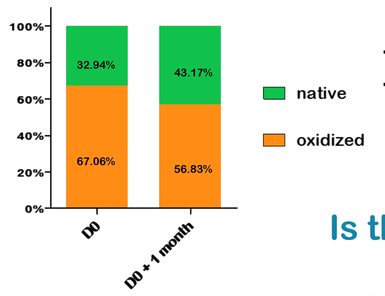
	LC-MS
Biocompatible system	Low percentage of oxidation
Stainless steel system (silica-based phase)	High percentage of oxidation

Direct infusion
Low percentage of oxidation

⟶ oxidation due to the LC

probably promoted by the metal ions present in the LC[1]

Percentage of methionine oxidation in EMSGSPASGIPVK



⟶ not stable in time
⟶ high percentage of oxidation already at D0

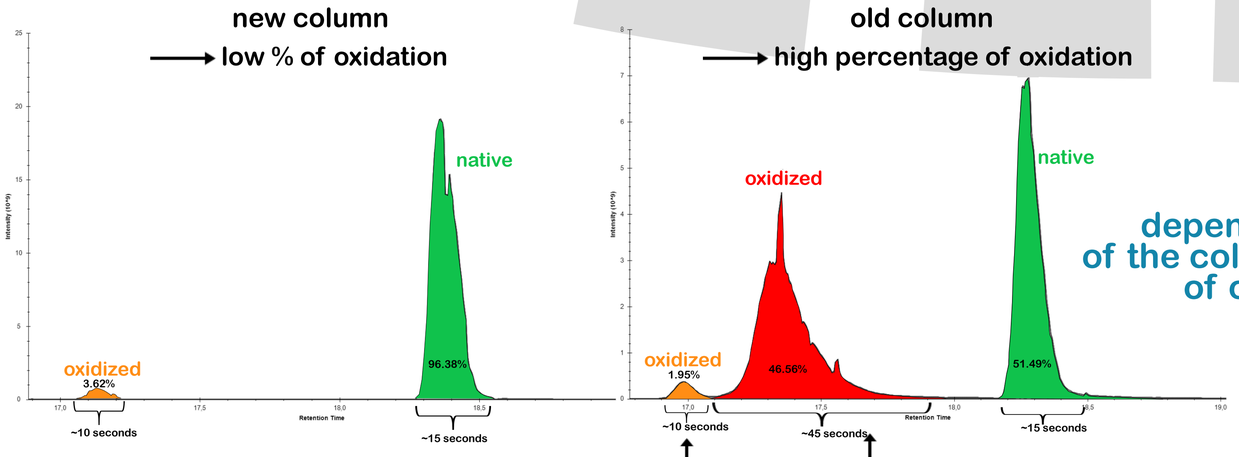
Is this the real oxidation level ?

Aliquots of the solubilized peptide, freezed at 80°C.
Injection with the same parameters at 1 month of interval

Unexpected results

new column
⟶ low % of oxidation

old column
⟶ high percentage of oxidation

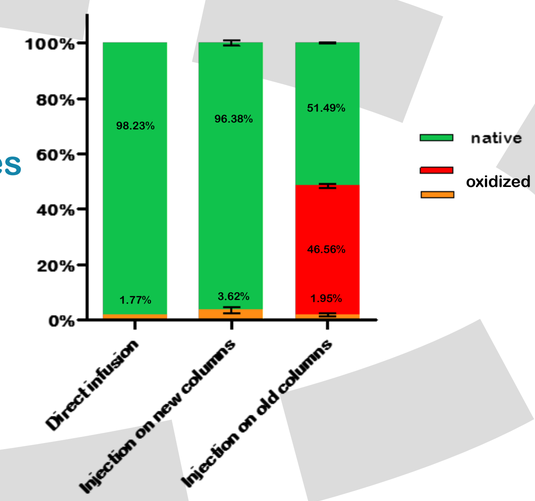


oxidation already present in the sample
--> similar to the oxidation obtained with the new column

broad peak
--> oxidation occuring during the LC separation

depending on the previous use of the columns, different percentages of oxidation are observed

Percentage of methionine oxidation in EMSGSPASGIPVK



In complex background

Conclusions/perspectives

Difference in percentage of oxidation between old and new columns (lifetime ? nature of the previous injection ?)

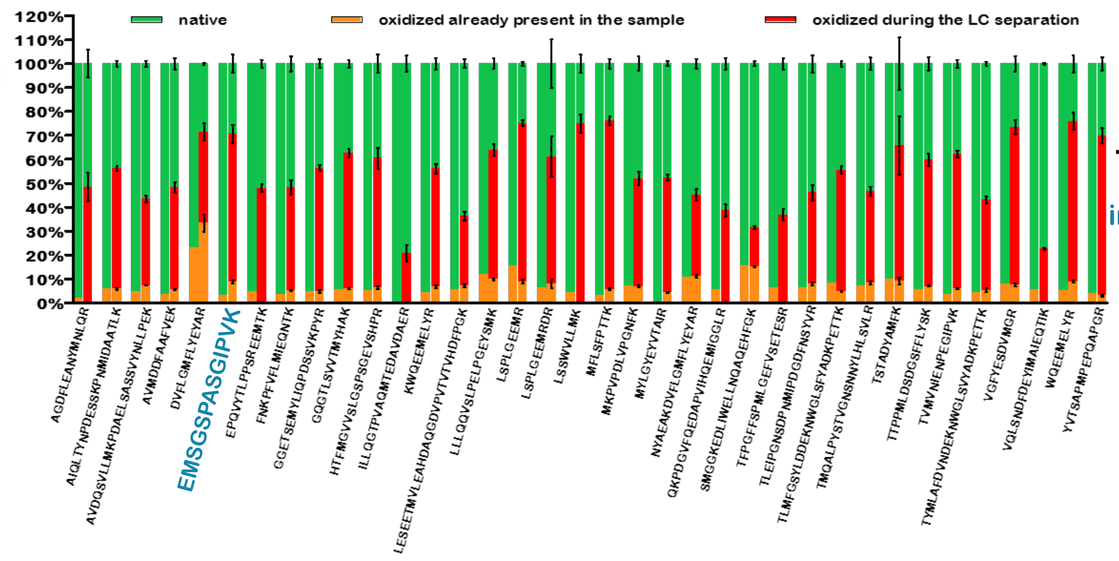
Peptide with methionine introduced in the kit:
- will allow to monitor the degree of oxidation of peptides present in the sample studied
- will allow to evaluate the LC system

Strategy to reduce the oxidation within the columns:
- chelating agents in the recommended pH range of the LC columns
- washing
- competitivity between our peptides and another compound more easily oxidized than the peptides

Research on how metal ions can catalyze the oxidation of methionine

Percentage of methionine oxidation in plasma peptides: set of oxidized and non-oxidized peptides including our target peptide

left: new column; right: old column



%ox new column = 6.53%
%ox old column = 54.33%

⟶ all methionine containing peptides in plasma are affected by oxidation in our LC system

Acknowledgement

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[1] L. Zang et al., "Residual metals cause variability in methionine oxidation measurements in protein pharmaceuticals using LC-UV/MS peptide mapping," Journal of Chromatography B, vol. 895–896, pp. 71–76, May 2012.