

Can proteomics help determine the post-mortem interval?

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Summary

1 Introduction

2 Research project

3 Materials and methods

4 Results

5 Conclusions and perspectives

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The post-mortem interval (PMI)

■ What is PMI?

- Estimation of the **time elapsed** between the **discovery of the body** and **death**
- **Issue: determination of the forensic or historical nature** of a bone
→ role during judicial expertise



■ Methods used on bones?

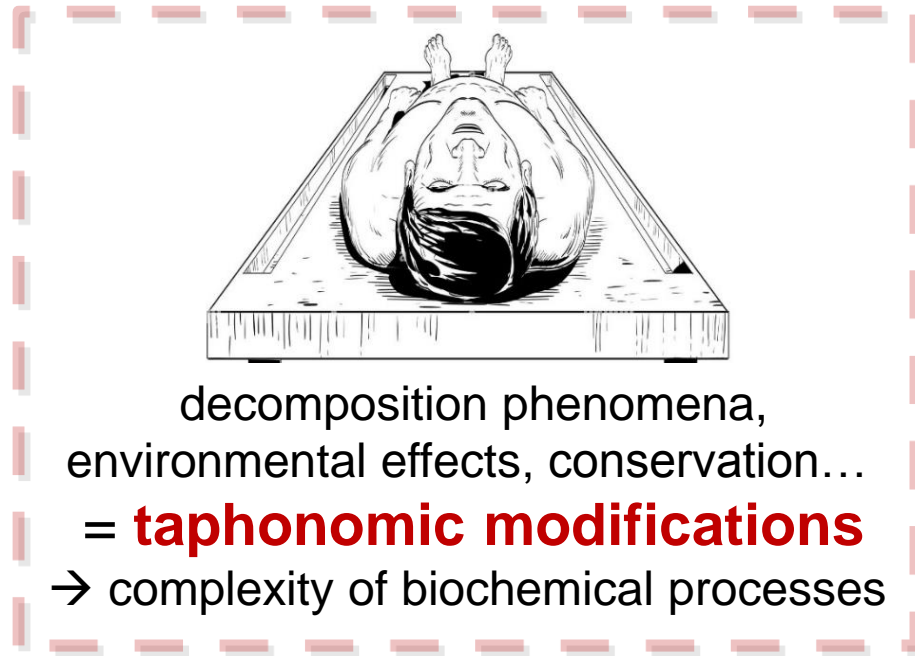
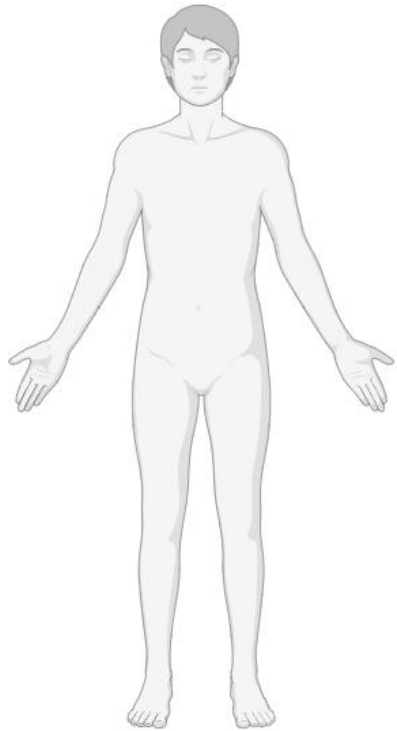
- ¹⁴C **radiocarbon** measurement
- **Chemiluminescence** and **UV fluorescence** tests
- **IR spectroscopies**...

} **Imprecision caused by what?**

¹⁴C : carbon 14, UV : ultraviolet, IR : infrared

New methodologies?

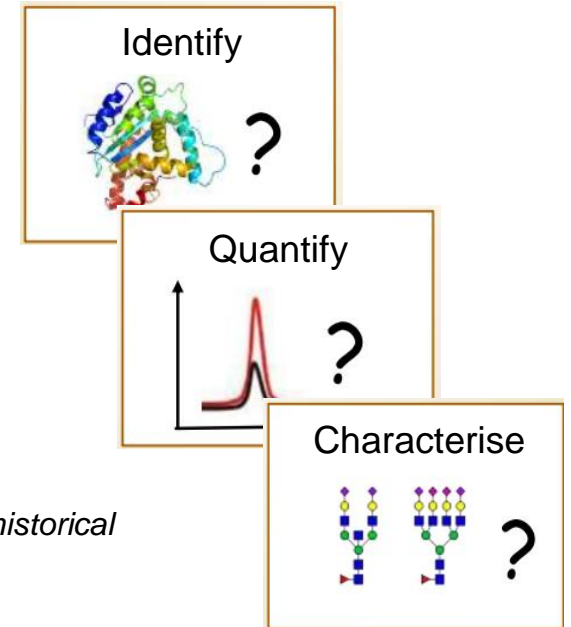
Imprecise determination of the post-mortem interval (PMI)



decomposition phenomena,
environmental effects, conservation...
= **taphonomic modifications**
→ complexity of biochemical processes



Forensic or historical dimension?



Few minutes

Few decades

Proteomics

Time markers?

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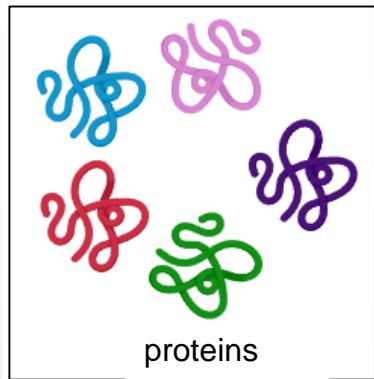
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Object of the study

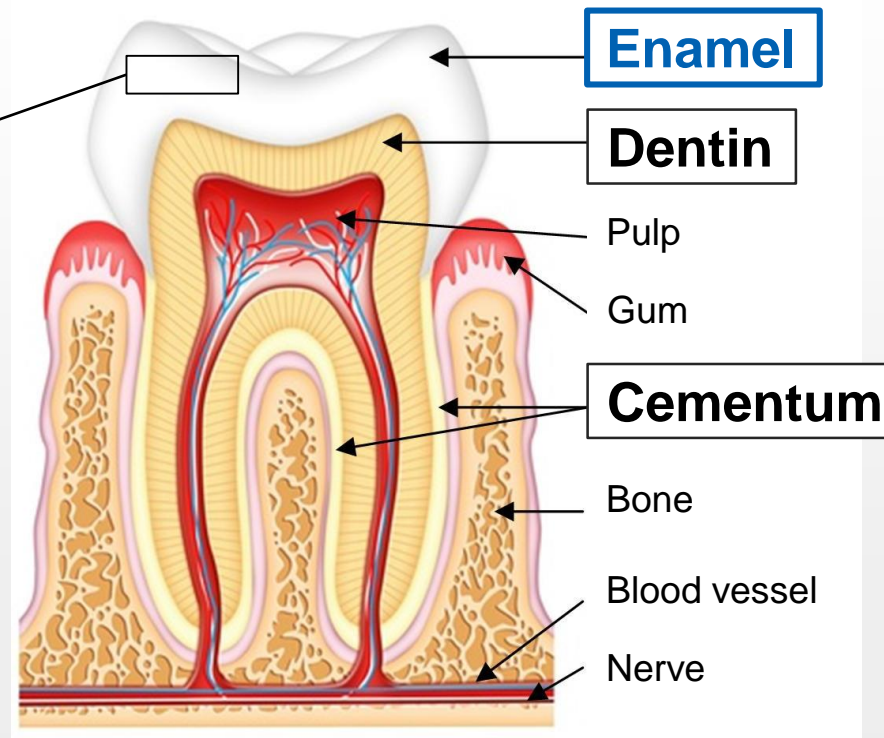
■ The tooth

Durable and very abundant post-mortem



**Accumulation of proteins
and post-translational
modifications (PTM)
= time biomarkers?**

zoom



Enamel:
hardest tissue of the
skeleton and
formed during childhood
**= perfect object for
time analysis**

Proteomics of the tooth

■ Identification, quantification and characterisation of...

Sexing

- amelogenin Y (AMELY)
- amelogenin X (AMELX)

Global proteome of the tooth

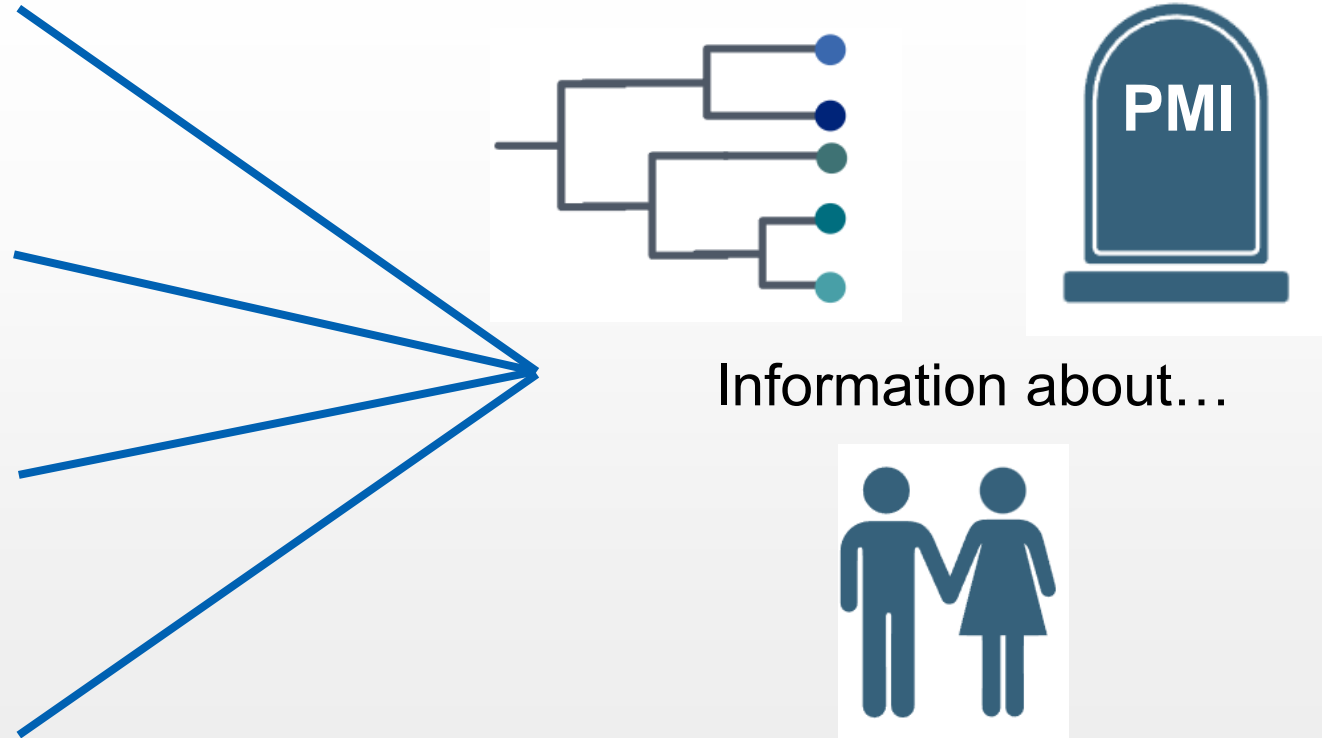
- collagens (COL1A1, COL1A2)

Enamel-specific proteins

- ameloblastin (AMBN)
- enamelin (ENAM)

PTM

- phosphorylation, oxidation, hydroxylation, glycosylation, deamidation



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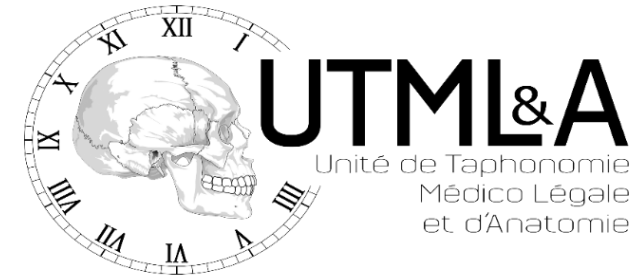
Reference samples

■ Collaboration with UTML&A in Lille (59), France

Cohort of 174 male and female canines

PMI for 11 sites of forensic or archaeological interest:

- **DEN:** 21st century (PMI 2020)
- **SAN:** 21st century (PMI between 1997 and 2009)
- **SCH:** 20th century (PMI 1930/1931)
- **CAR:** 20th century (PMI 18 March 1918)
- **CHA:** 19th-20th century (PMI 1870-1920)
- **STA:** 16th-18th century
- **STJ:** 16th-18th century
- **COS:** 11th century
- **ALL:** 6th-8th century
- **AUB:** 5th-7th century
- **MTH:** Neolithic 4250-3800 BC

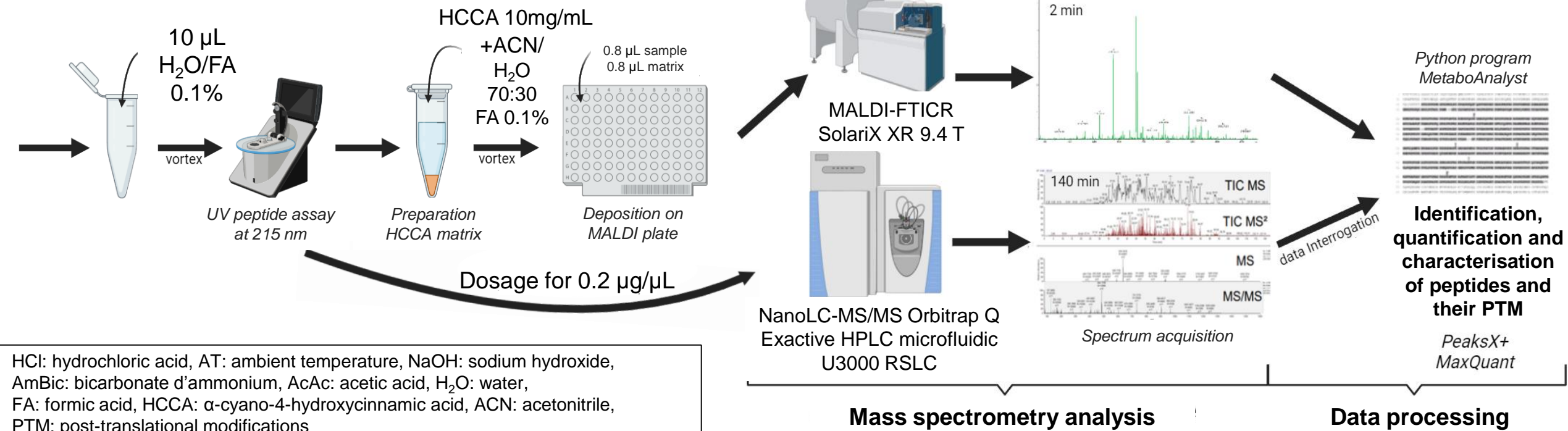
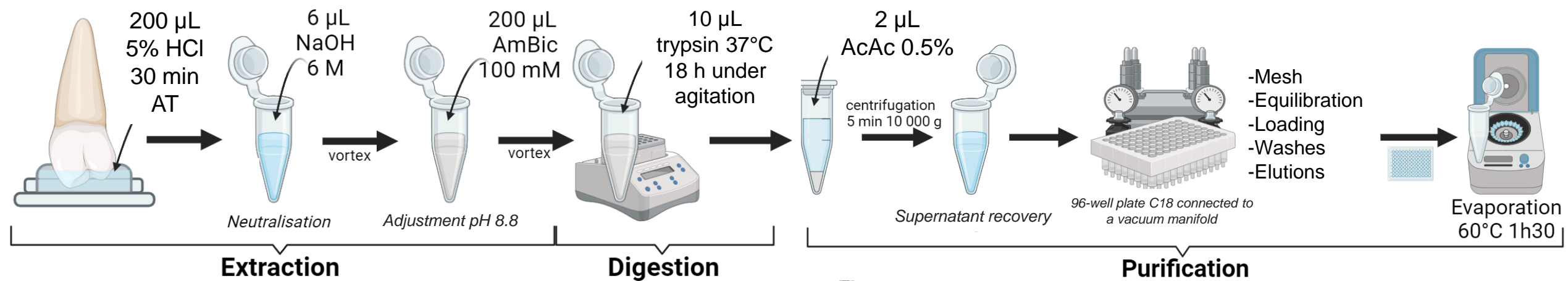


Forensic
interest

Archaeological
interest



Protocol



HCl: hydrochloric acid, AT: ambient temperature, NaOH: sodium hydroxide, AmBic: bicarbonate d'ammonium, AcAc: acetic acid, H₂O: water, FA: formic acid, HCCA: α -cyano-4-hydroxycinnamic acid, ACN: acetonitrile, PTM: post-translational modifications

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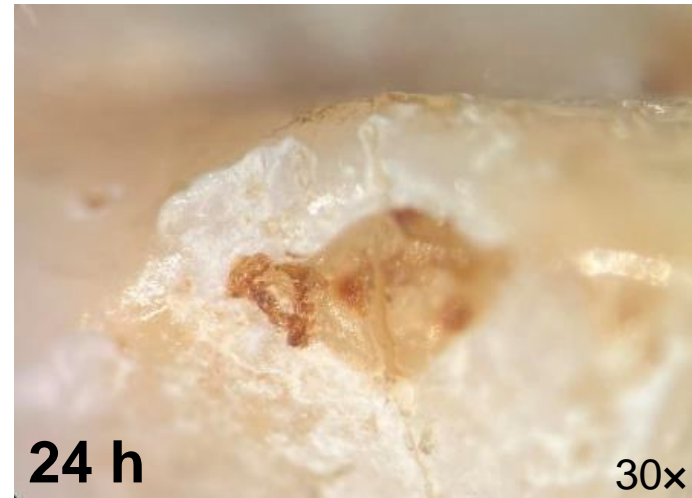
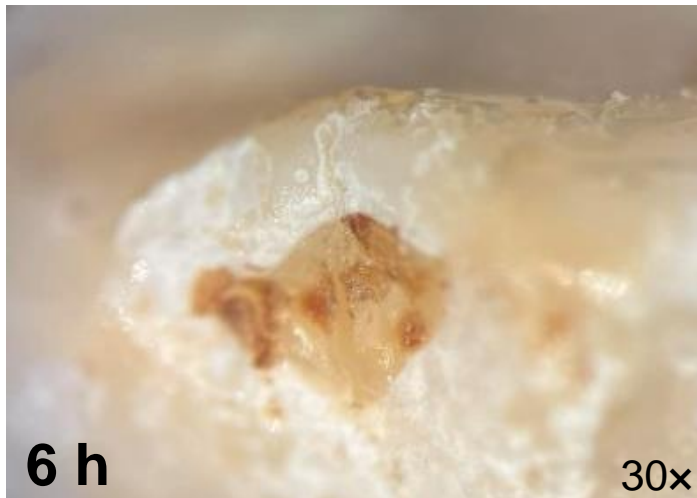
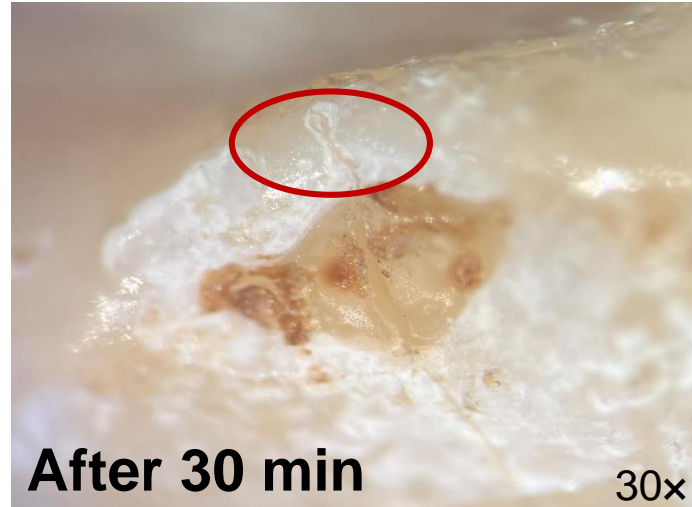
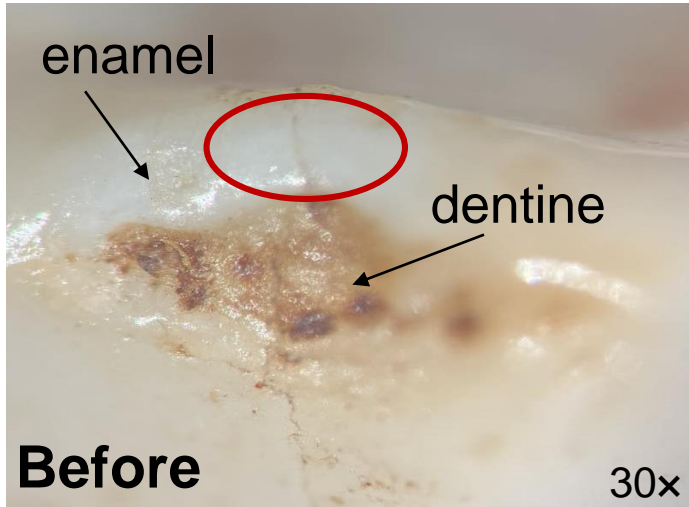
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Impact of using hydrochloric acid

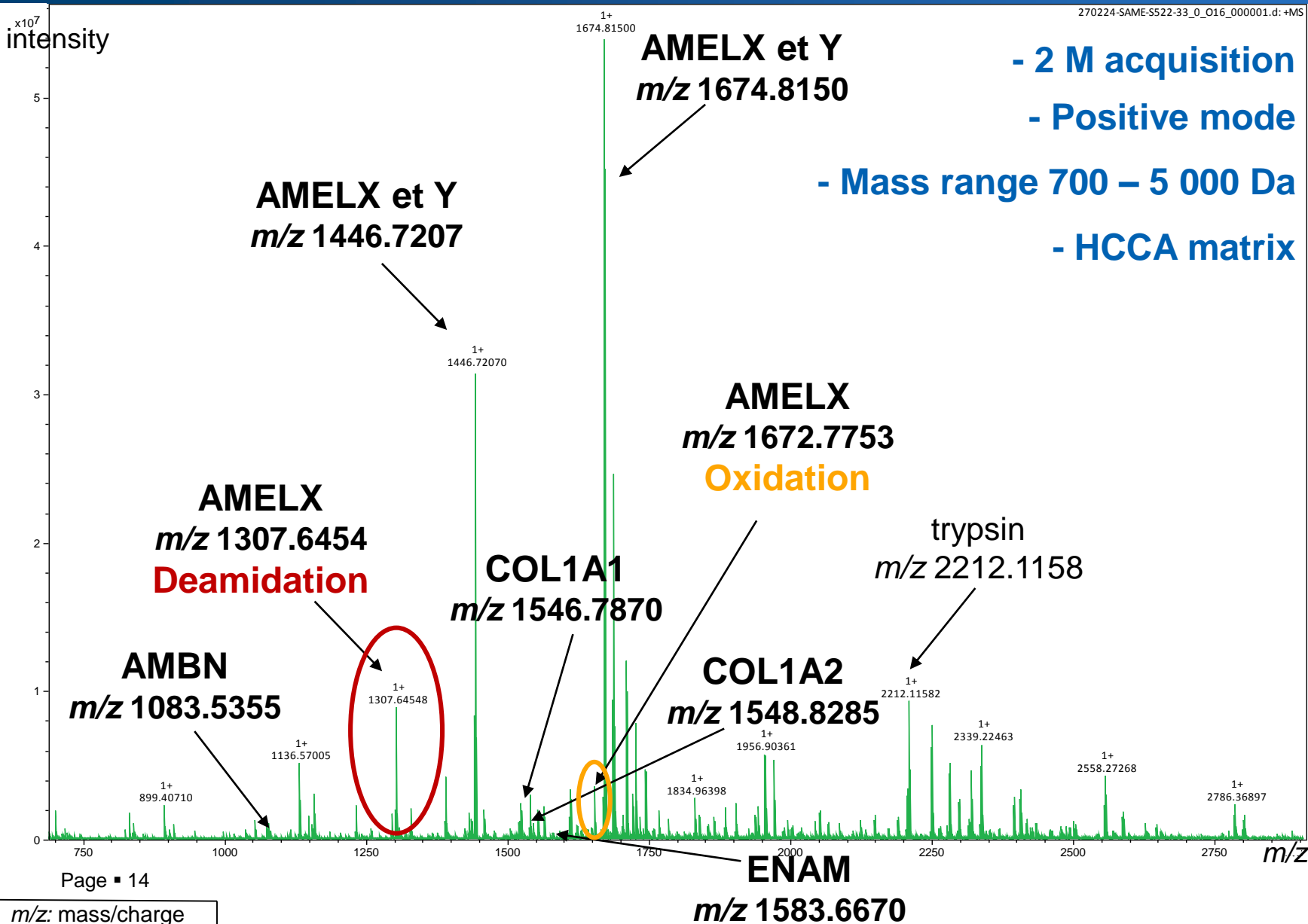
- Enamel appearance of tooth SCH 743 – 43 before and after HCL



Low-destructive protocol

Observation with the Nikon SMZ1000 Japan binocular loupe at 30x magnification

Identification of peptides by MALDI-FTICR

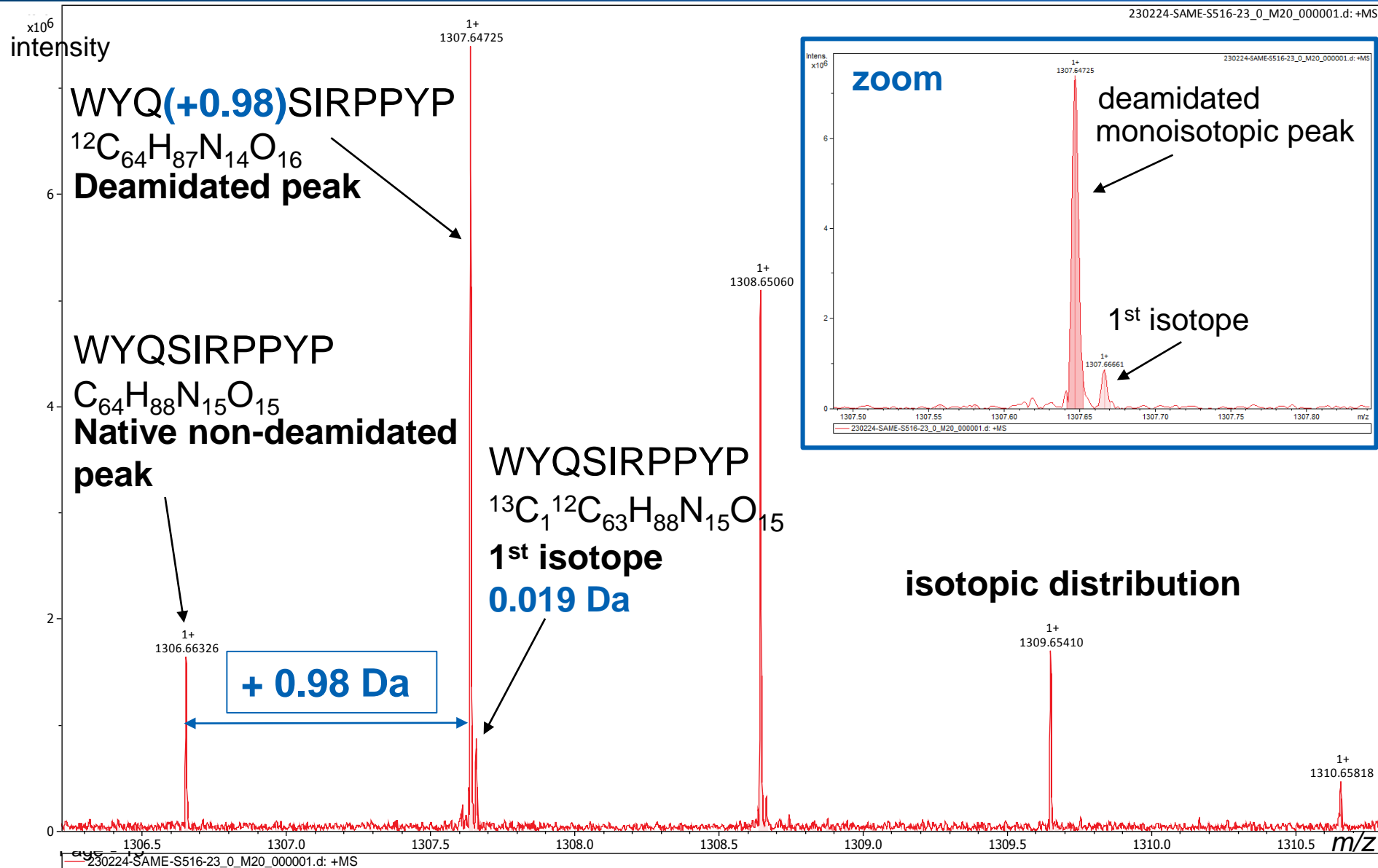


■ Example of a MALDI-FTICR spectrum of sample STA S.522 - 33

Observation of peaks corresponding to the peptides of the proteins of interest:

- AMELX, AMELY
- COL1A1, COL1A2
- AMBN
- ENAM

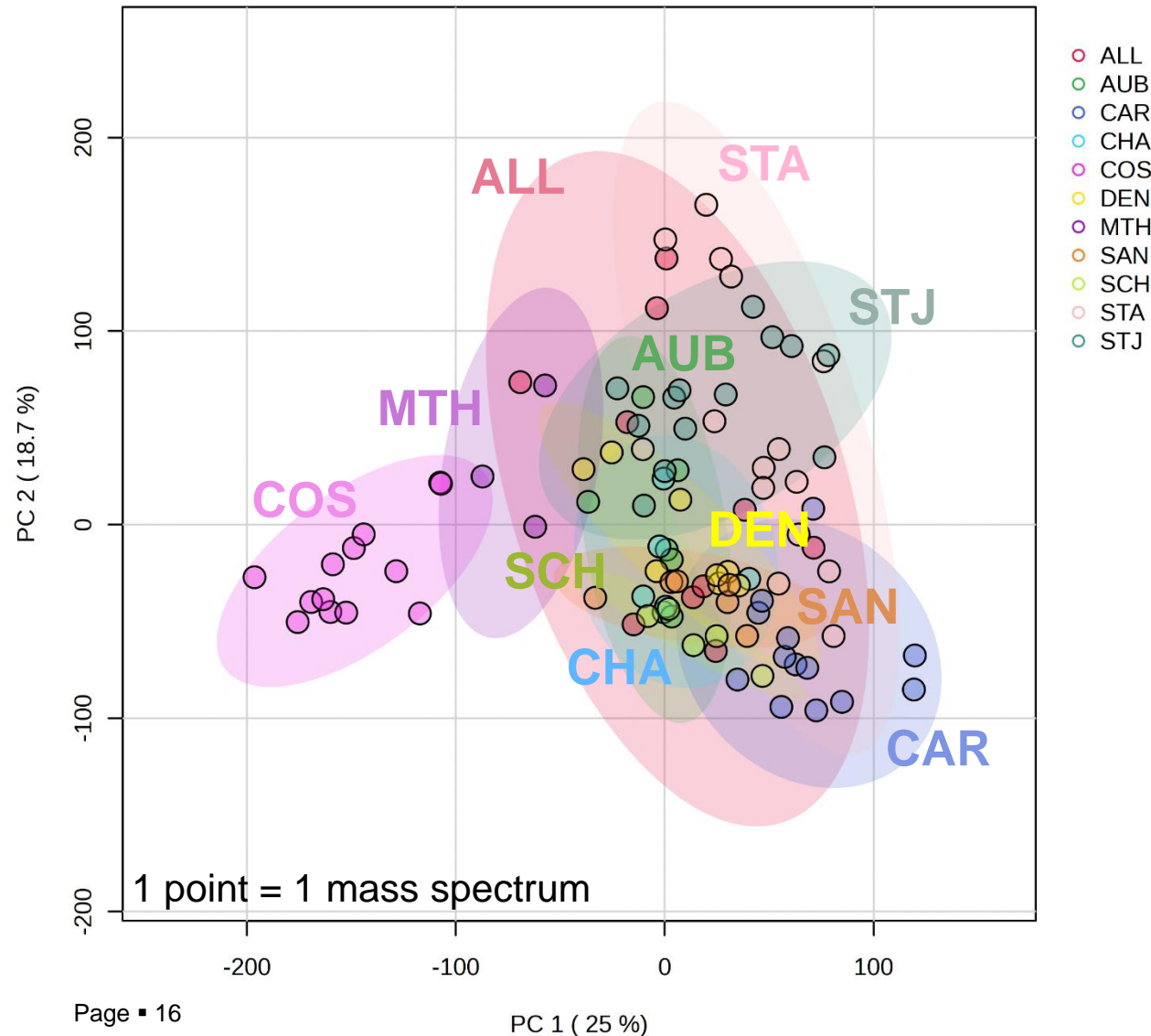
Distinction between deamidated and non-deamidated peptides



■ MALDI-FTICR spectrum of sample STA S.516 - 23

Identification of the deamidated peptide AMELX m/z 1307.6472 in high intensity

Statistical analysis without « preconceptions »



Blind principal components analysis (PCA)

MALDI-FTICR data of monoisotopic peaks treated with Snap 0.8

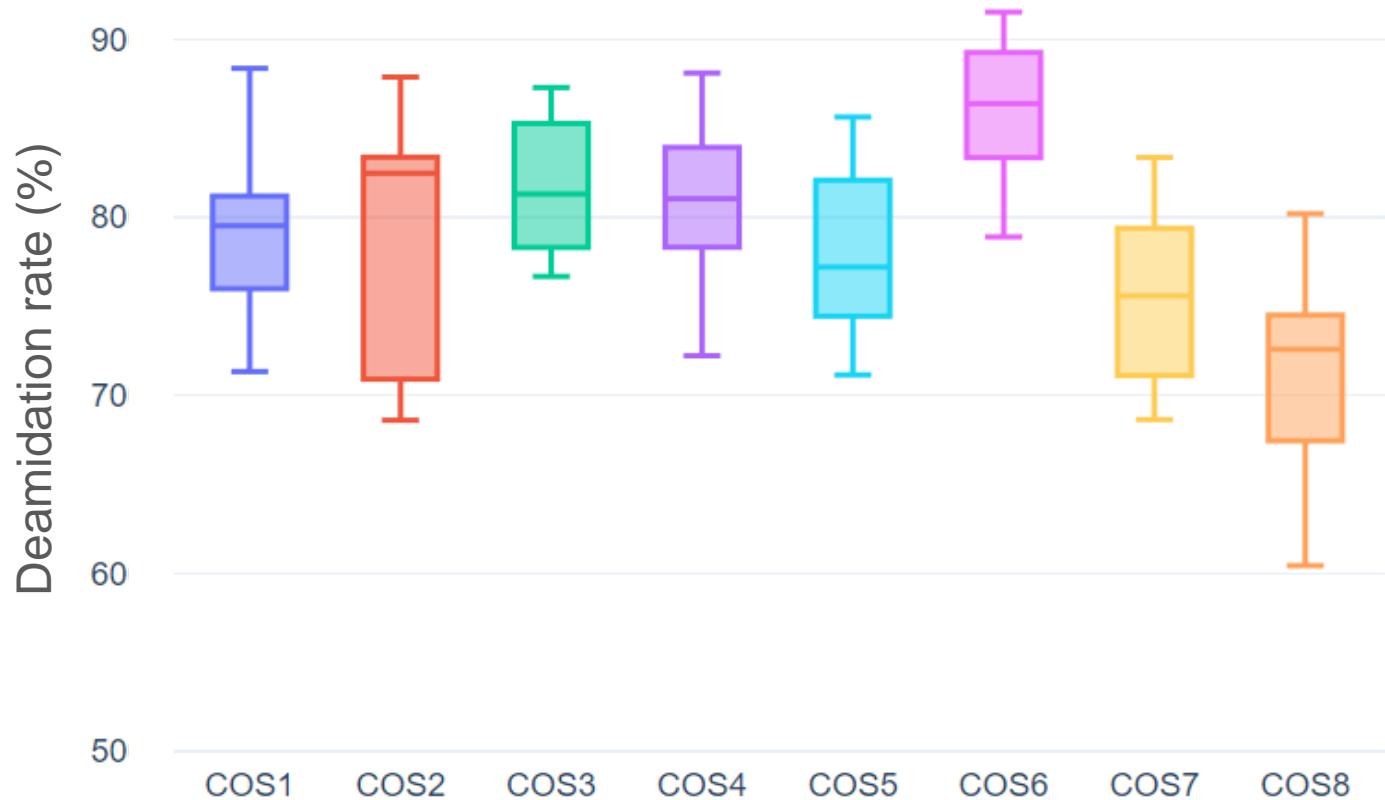
- MTH, COS separate
- ALL, AUB similar
- STJ, STA, CAR detached
- CHA, SCH, SAN, DEN same cluster



Observable differences and similarities but « due to what »?

Deamidation of several peptides at the same site

Deamidation rates of 8 AMELX peptides within the COS site (11th century) obtained by MALDI-FTICR



- Some peptides **deamidate more rapidly** than others within the same site

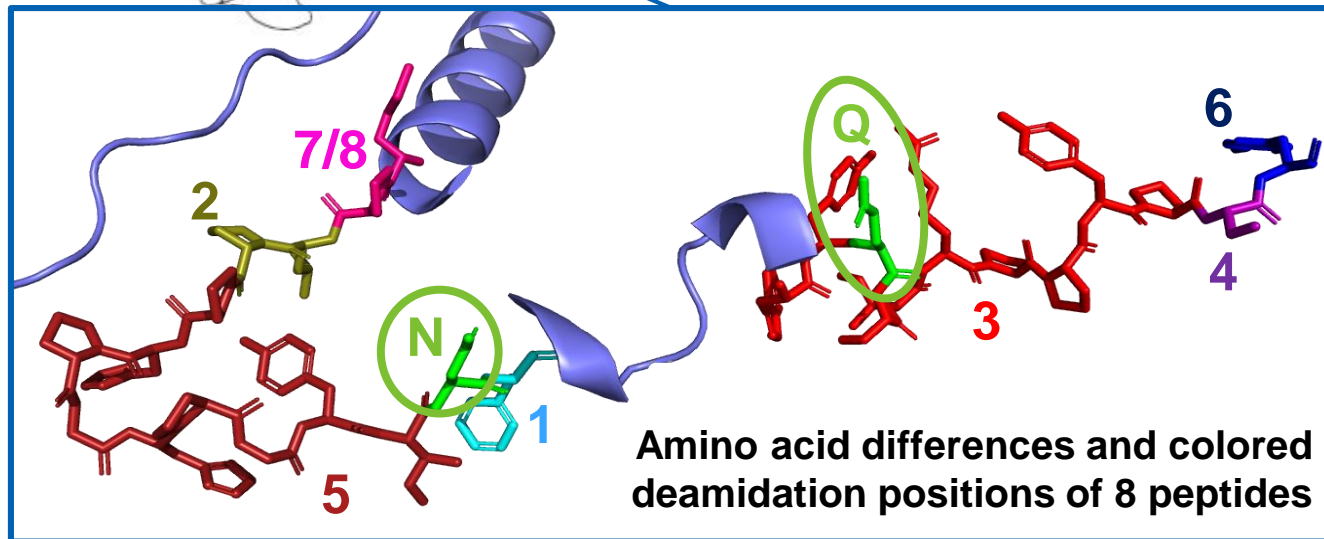
<i>m/z</i>	peptide number	sequence	protein
1306.66	3	WYQSIRPPYP	AMELX
1393.69	4	WYQSIRPPYPS	AMELX
1556.76	6	WYQSIRPPYPSY	AMELX
1235.6	1	PHPGHPGYINF	AMELX
1298.67	2	LPPHPGHPGYIN	AMELX
1445.74	5	LPPHPGHPGYINF	AMELX
1673.83	7	MPLPHPGHPGYINF	AMELX
1689.83	8	M(ox)PLPHPGHPGYINF	AMELX

$$\% \text{ deamidation} = \frac{\text{intensity of deamidated peak}}{\text{intensity of deamidated peak} + \text{intensity of non-deamidated peak}} \times 100$$

Three-dimensional structure of AMELX

3D structure of AMEL, isoform X (Homo Sapiens)

via Uniprot (Q99217) and Pymol



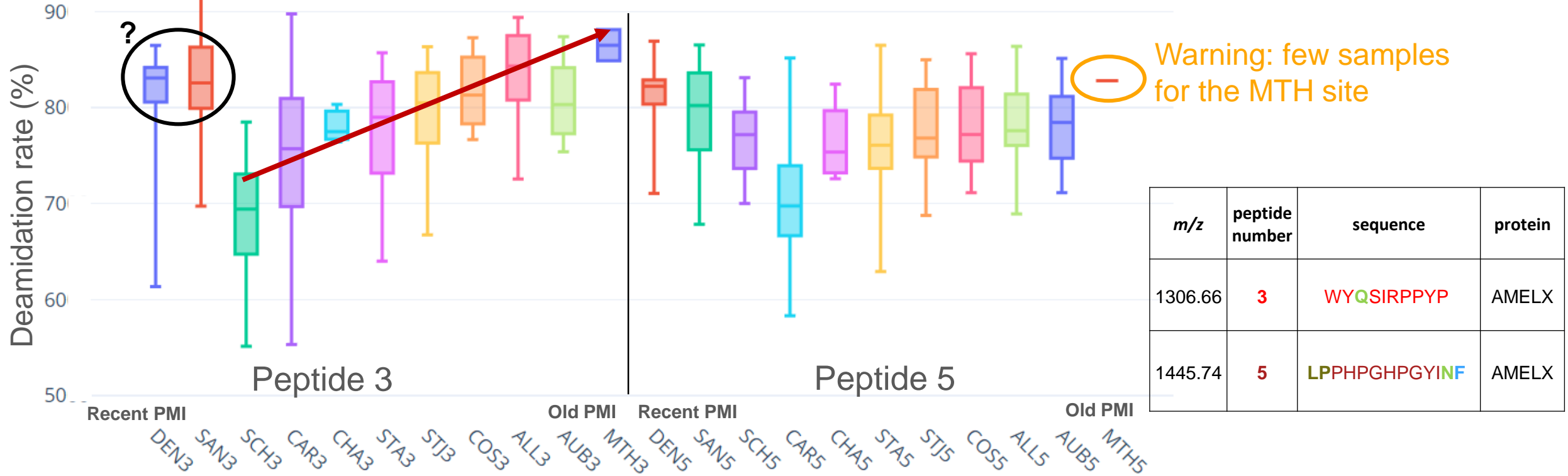
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1445.74	5	LPPHPGHPGYINF	AMELX
1673.83	7	MPLPHPGHPGYINF	AMELX
1689.83	8	M(ox)PLPHPGHPGYINF	AMELX

- Effect of amino acid **sequence** and **position** of N,Q or **3D conformation**?

(ox) = oxidation N = asparagine Q = glutamine } deamidation position

Deamidation of two peptides at different sites

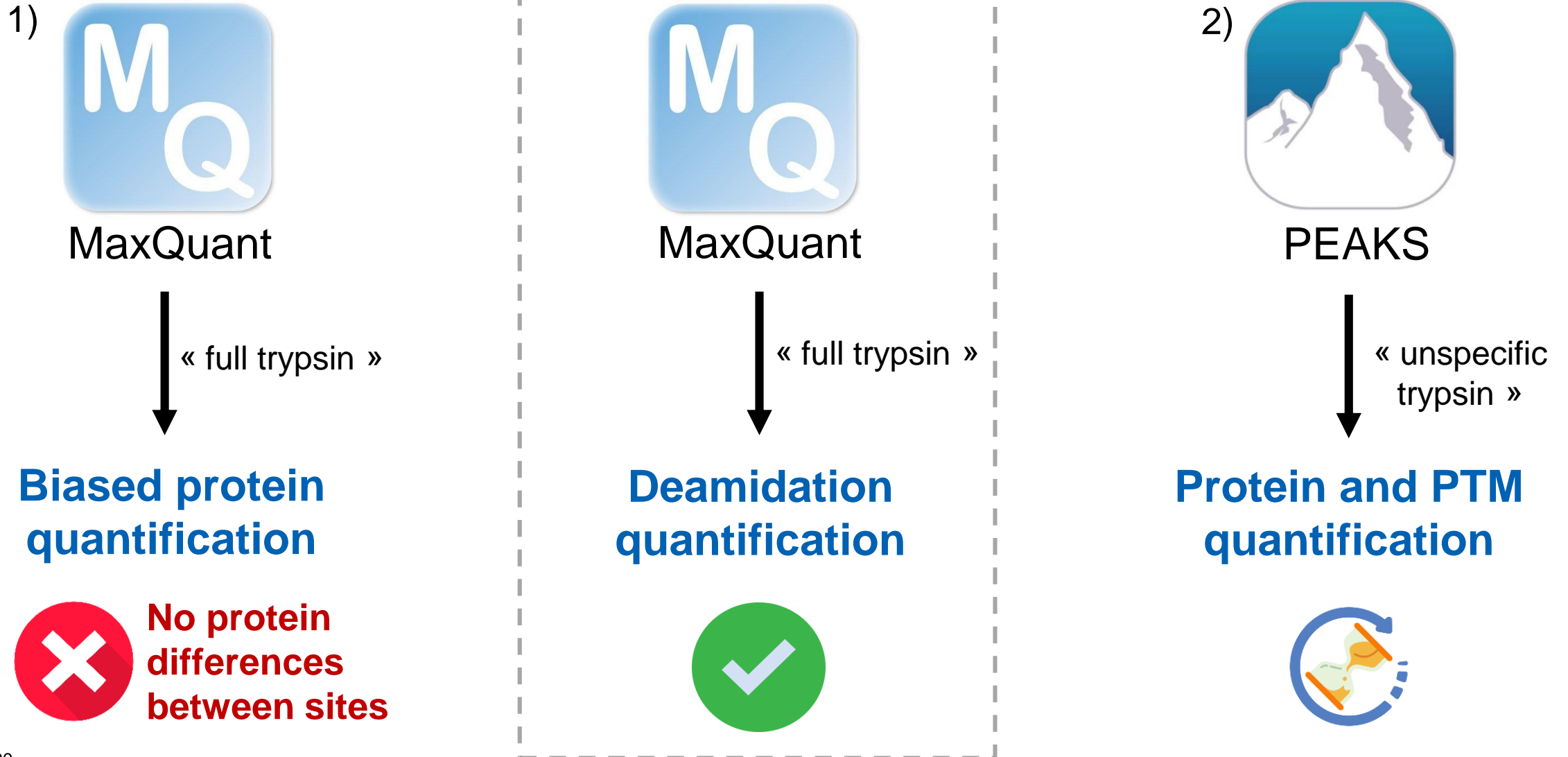
Deamidation rate of AMELX peptides 3 and 5 in 11 sites obtained by MALDI-FTICR



- Site-dependent variation in peptide deamidation
- Deamidation increases with site age

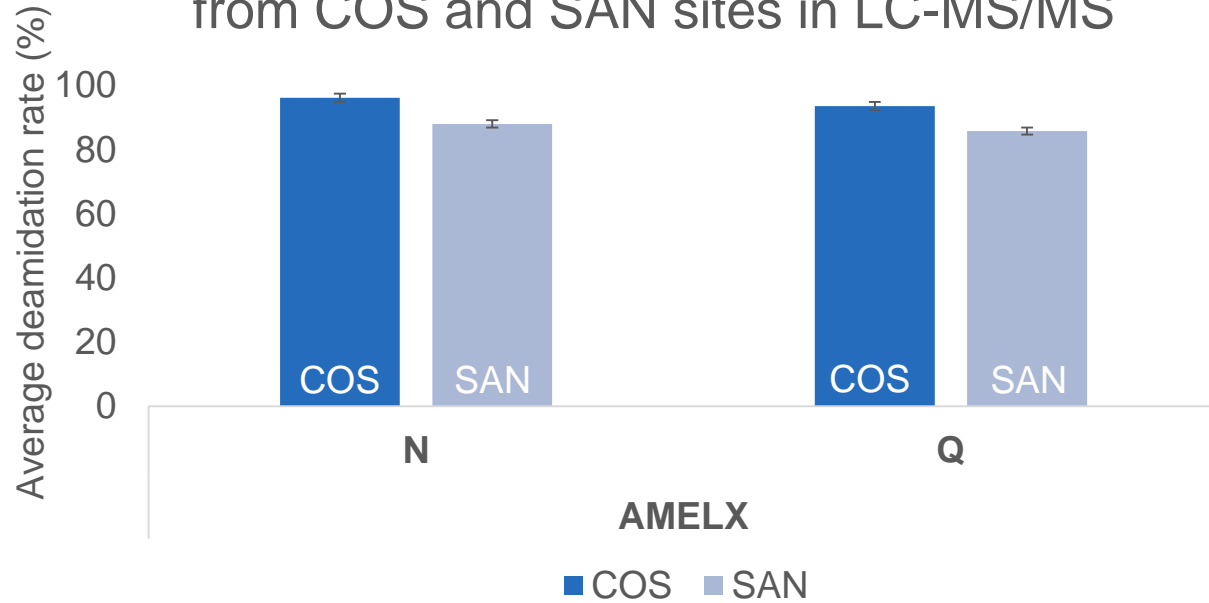
Peptide 3 is more deamidated as a function of time
 → Sequence effect

Comparison of two sites: COS and SAN in LC-MS/MS



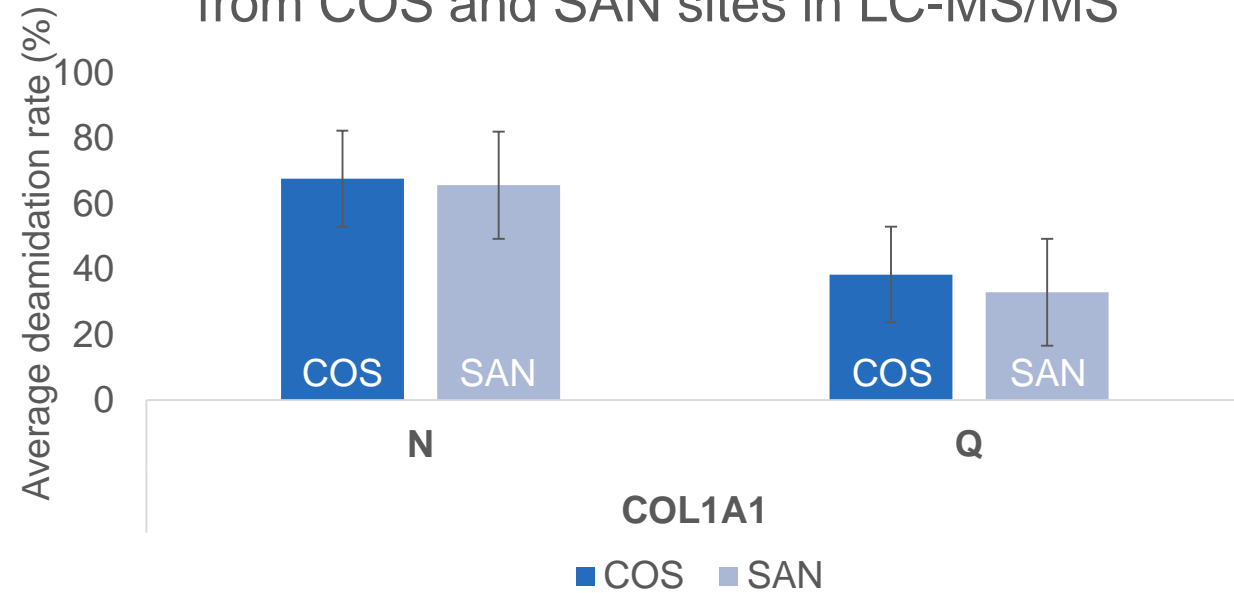
Quantification of AMELX and COL1A1 deamidation

Average N and Q deamidation rate of all AMELX peptides from COS and SAN sites in LC-MS/MS



- Few differences between COS (11th century) and SAN (21st century)
- **No differences** in N and Q deamidation

Average N and Q deamidation rate of all COL1A1 peptides from COS and SAN sites in LC-MS/MS

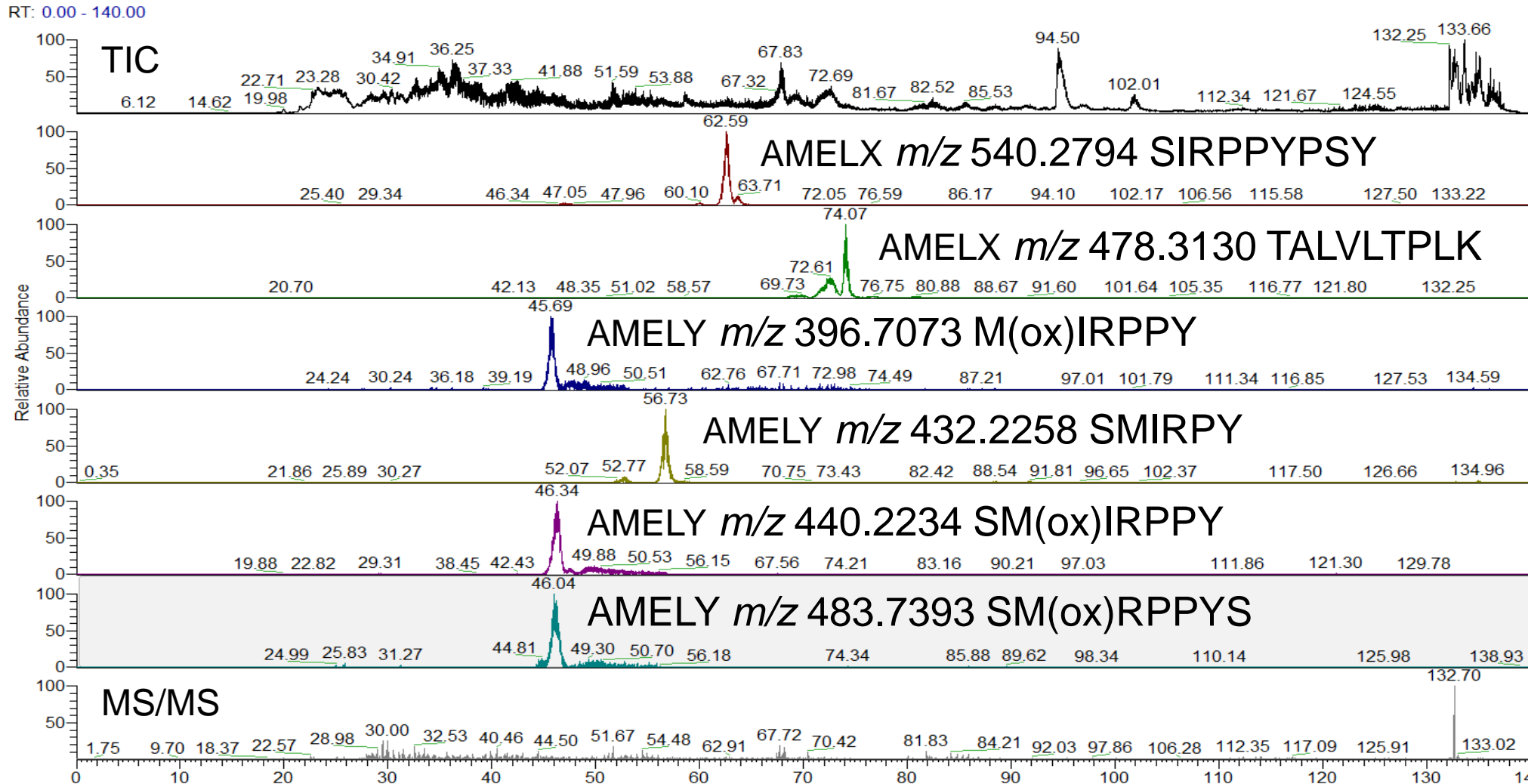


- Few differences between sites
- **Difference in deamidation: Q < N**

- **COL1A1** deamidates **less than AMELX** in enamel → structure?
- **No significant increase** in deamidation over time between sites

Detection of sex marker peptides by LC-MS/MS

Tracking of the 6 AMELX and AMELY peptides by ion calling

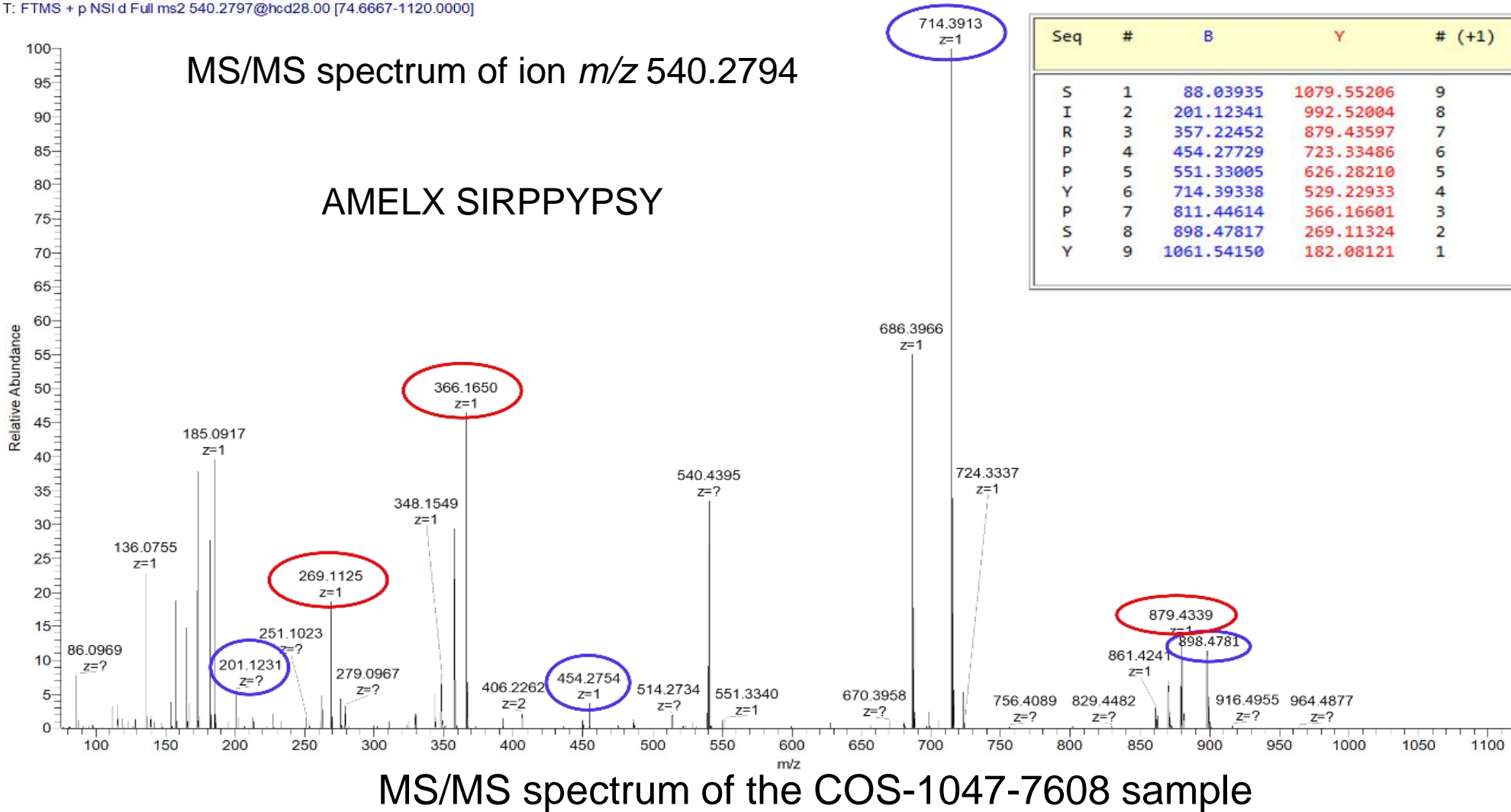


Chromatogram of the COS-1047-7608 sample

Identification of AMELX and AMELY peptides

■ Obtaining amino acid sequences from MS/MS spectra

PSC-1047-7608 #17311 RT: 62.34 AV: 1 NL: 1.98E6
 T: FTMS + p NSI d Full ms2 540.2797@hcd28.00 [74.6667-1120.0000]



Determination of the sex of each individual at the COS and SAN sites

- Sexing table for individuals at COS and SAN sites

Example for 5 samples from COS site

Samples	AMELX		AMELY		Sex (W ou M)
	Intensity m/z 540.2794; $z = 2$	Intensity m/z 568.7903; $z = 2$	Intensity m/z 440.2234; $z = 2$	Intensity m/z 483.7393; $z = 2$	
COS-1005-7619	8305244.5	9356547	14716486	3759956.20	M
COS-1044-7545-2	25766896	30442490	28261150	5371245	M
COS-1044-7545	12571382	15394729	13343898	2429948	M
COS-1046-7603	17951502	17404620	14251777.00	2970574.50	M
COS-1047-7608	35537280	37483172	17250306	3916913.20	M

AMELX/AMELY intensity ratio comparison:

Intensity AMELX and AMELY (XY) = man

Intensity AMELX (XX) = woman

Note: AMELX is represented 4 times more than AMELY

- All **48 individuals** at the COS and SAN sites **are men**

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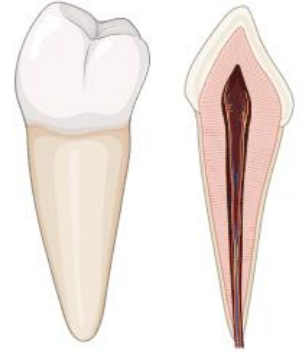
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Conclusions and perspectives

- **Minimally invasive** protocol
 - Observation of **peptides from proteins of interest in enamel**
 - Identification of PTM: **oxidations** and **deamidations**
 - **Different deamidation** rate between peptides for the **same site**
 - **Deamidation** rate **increases** with the **post-mortem interval**
 - **Sex determination** by mass spectrometry
-
- **LC-MS/MS analysis** of the **cohort** using independent data acquisition (DIA)
 - Absolute **quantification** of **proteins of interest** and validation of **sexing**
 - **Optimisation** of **data processing** time

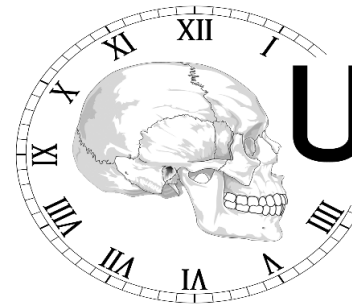


Acknowledgements



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ProFI
PROTEOMICS