

Determination of the Time since Deposition (TsD) of Biological Crime Scene Traces by Mass-Spectrometry Based Proteomics

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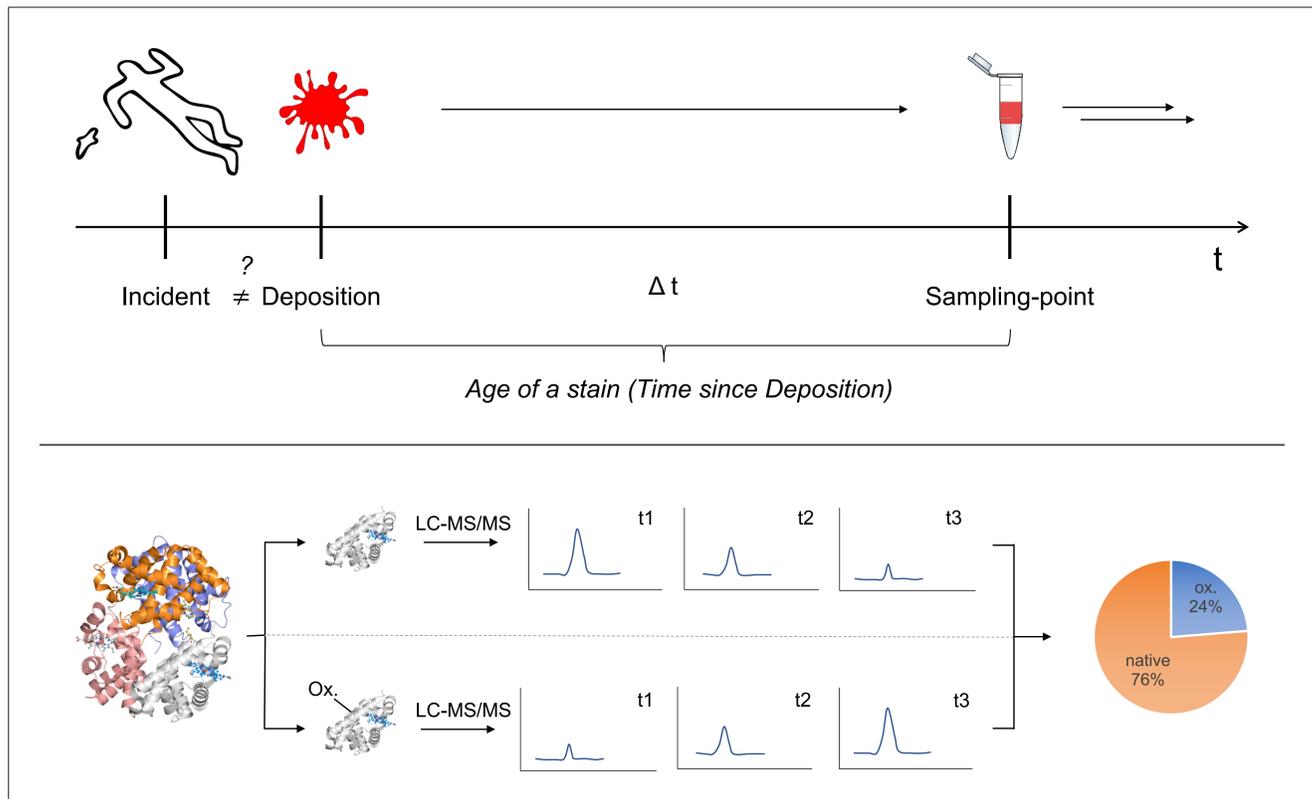


Fig.1: A schematic representation of the *Time since Deposition* and an example of how to approach its estimation by MS-based proteomics.

1. Introduction / Background

By comparing individual-specific DNA-profiles, a piece of evidence can be confidently assigned to a particular person. But evidence should not only be associated with a person, but with the crime itself as well. To date, there is no reliable method for (accurate) determination of the age of a trace.

The aim of this study was to investigate time-dependent changes in blood stains [1] by analyzing the proteome of a sample (global proteome profiling) and to determine the Time since Deposition (TsD) and to then draw conclusions about the age of forensically relevant, biological crime-scene traces.

3. Results

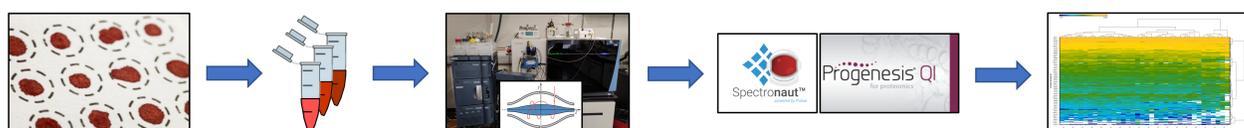
- 1082 (environmental) and 1433 (controlled) peptides could be measured and quantified across all samples
- these peptides infer to a total of 172 (environmental) and 143 (controlled) proteins
- post-translational modifications (PTMs) show discriminant time-dependent changes
- log-space regression models from multiple peptides and their modified species can be built to estimate the Time since Deposition
- storage conditions display great impact on the dynamics of human blood proteome

2. Methods

Samples

- dried-blood spots (DBS) from 11 healthy subjects (6 female / 5 male) were created and exposed to two different storage conditions:
 - controlled:** lab-drawer, ambient temperature, 45-60% relative humidity
 - environmental:** outside, exposed to weather-effects and climate, but sheltered from direct precipitation
- blood-samples were harvested after **aging them for 0, 3, 7, 14, 28 and 56 days**

Sample-preparation and analysis by LC-FTMS/MS



- **Sample preparation** by extraction (50 mM ammonium bicarbonate), followed by sonication, centrifugation and transfer of supernatant into fresh vessels. Reduction, alkylation and proteolytic digestion with trypsin. Sample clean-up with C18 μ -columns and vacuum-drying prior to analysis.
- **Separation** with Waters nanoAcquity M-Class nano-HPLC, HSS T3, 1.8 μ m particle size, 0.075 i.d. and 250 mm length with a gradient of 105 minutes and a flow-rate of 300 nL/min.
- **Data collection** via FT-MS/MS (Orbitrap Fusion Lumos, Thermo Scientific) in data-independent acquisition (DIA) mode [2, 3].
- **Data-analysis** and post-processing with Spectronaut (Biognosys) and Progenesis Qi for Proteomics (Nonlinear Dynamics) for deconvolution, statistical analysis, cross-run alignment, identification and quantification of proteins, peptides and post-translational modifications (PTMs).

Conclusion

These initial investigations show that the analysis of the proteome at both peptide and PTM-level seems to be a suitable technique to understand and elucidate time-dependent changes of a body-fluid derived trace, such as blood. Further investigations will be necessary to show robustness of the analytical technique as well as to improve the quality of the model used for the determination of Time since Deposition. Dynamic environmental conditions must be considered when research is conducted to understand the Time since Deposition, since they will interfere with regression-modelling and may require to change the estimation approach – or at least the group of analytes considered for modelling. Other forensically relevant biological matrices (saliva, semen, vaginal secretion and menstrual blood) need to be investigated, too.

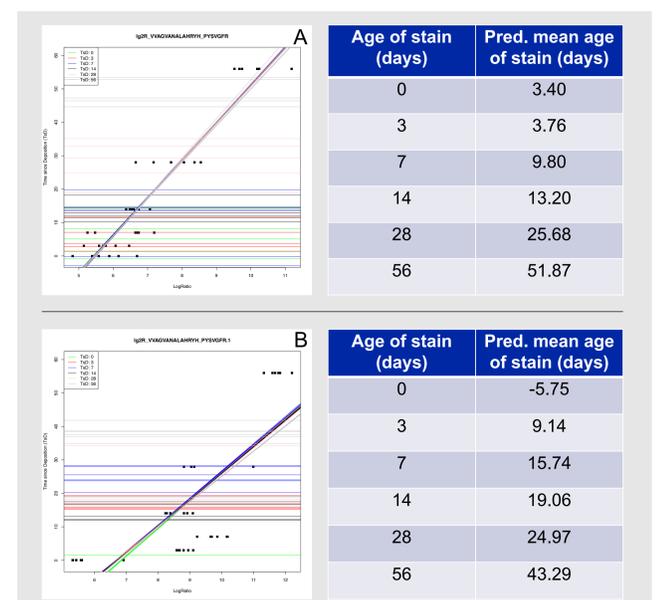


Fig.2: Log-regression modelling of peptide-ratios for TsD-estimation. Prediction based on statistical re-sampling (bootstrapping) of data. Differences shown between samples aged under controlled (Fig. 2A) and environmental (Fig. 2B) conditions.

4. Discussion

- the proteome of a human blood sample shows complex dynamics over time, especially when aged under non-controlled conditions – and adaptive modelling might be required in order to consider these environmental effects when trying to determine the Time since Deposition
- aged traces provide numerous possibilities (and challenges) to tackle the question of the determination of the Time since Deposition
- the presented (initial) results are promising in terms of feasibility for TsD-estimation of crime scene blood-traces

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References

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