Targeted analysis in PRM mode towards clinical application:
Optimization of experimental setup and data processing for accurate amyloid diagnosis.

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Amyloidosis is a disease whose prevalence is rare and which suffers from a lack of accurate diagnosis tools. This disease results from a wrong folding of specific amyloidogenic proteins and their identification is essential for proper medical care. Today most patients' cases are identified thanks to immunohistochemistry analysis after surgery or biopsy on the defective tissues. However they can be inconclusive on certain cases, leading to a lack of information about the underlying ethiology.
We showed that ultrasonic treatment could help for the completion of enzymatic proteolysis (60s instead of 15h incubation [1]) of either fixed or raw biopsy samples and to get closer to the clinical routine application for amyloidosis subtyping [2].

In discovery phase, abundance species were evaluated according to the Top 3 Protein Quantification [3] and data were manually classified. Protein quantification has also been assessed with label-free MaxQuant software and classified with their statistical Perseus Tool [4].
In order to implement our approach in the French clinical departments, a targeted proteomics method has been evaluated. Parallel reaction monitoring (PRM) based on high resolution and accurate mass (HR/AM) measurement in MS/MS mode was performed with a hybrid quadrupole-Orbitrap mass spectrometer [5].
Up to 25 amyloidogenic proteins with a maximum of 8 peptides per proteins have been simultaneously targeted. When less than 8 peptides were experimentally identified in the discovery phase, the list was completed using the Peptide Atlas repository [6].

We propose a specific protocol that allows to blindly discriminate healthy and pathological biopsies first and to classify the pathological samples according to the nature of the amyloidogenic protein. The number of peptides and transitions should be then minimized and optimized for a rapid and accurate amyloid subtyping with a clinical benchtop mass spectrometry (Triple-Quadrupole). Statistical processing will be also validated to ascertain the diagnostic.

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