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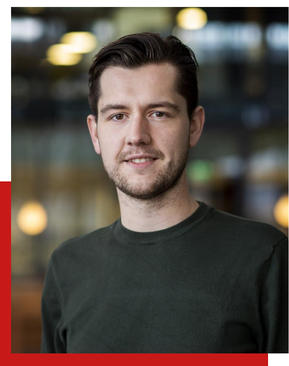


Immunoprecipitation Top-Down High-Resolution Mass Spectrometry for the Quantification of the Protein Tumor Biomarker Neuron Specific Enolase

Sebastian van den Wildenberg[1,2,4,*], Sylvia Roovers-Genet[1,2,4], Joost van Dongen[1,4], Daan van de Kerkhof[1,2,4], Maarten Broeren[1,3,4], Luc Brunsveld[1,4], Volkher Scharnhorst[1,2,4]

[1] Laboratory of Chemical Biology - Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands [2] Department of Clinical Chemistry, Catharina Hospital Eindhoven , The Netherlands [3] Department of Clinical Chemistry, Maxima Medical Centre Veldhoven , The Netherlands [4] Expert Center Clinical Chemistry Eindhoven, the Netherlands.

Introduction

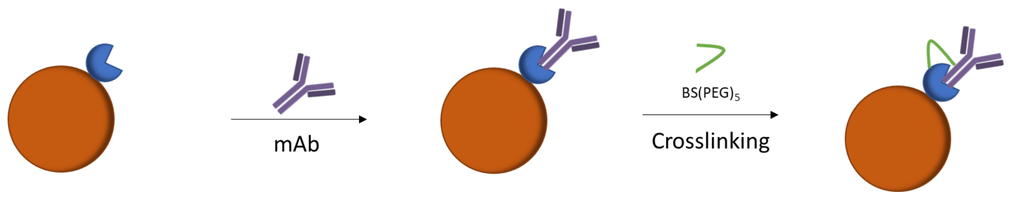
LC-MS immunoprecipitation (IP) methods using bottom-up based and/or middle-down have been popular methods for the quantification of proteins. However, these methods often come with extensive sample preparation that require thorough optimization. In addition to this using bottom-up proteomics sequence coverage is often limited and information about Post Translational Modification's (PTMs) is lost. Using top-down proteomics the intact protein is analyzed, without digestion, retaining the maximum amount of information with less extensive sample preparation. Top-down proteomic approaches have their own specific challenges, such as the availability of internal standards and sensitivity of the method.

Aim

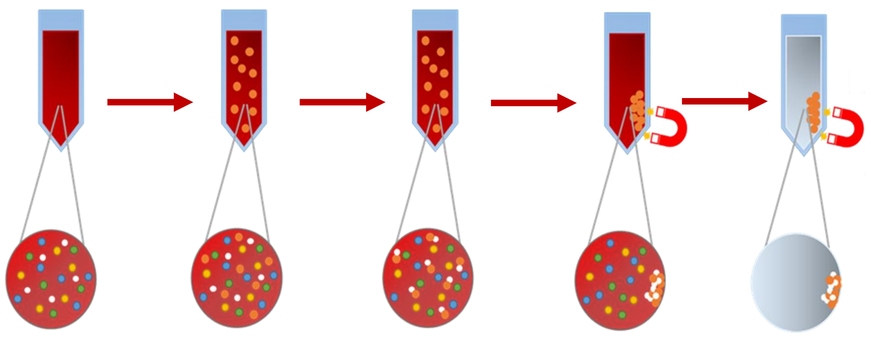
The aim of this study is to develop an immunoprecipitation assay combined with protein elution, followed by intact top-down protein quantification using LC-QToF-HRMS for the quantification of the lung cancer marker Neuron Specific Enolase Gamma (NSEγ).

Methods – Immunoprecipitation

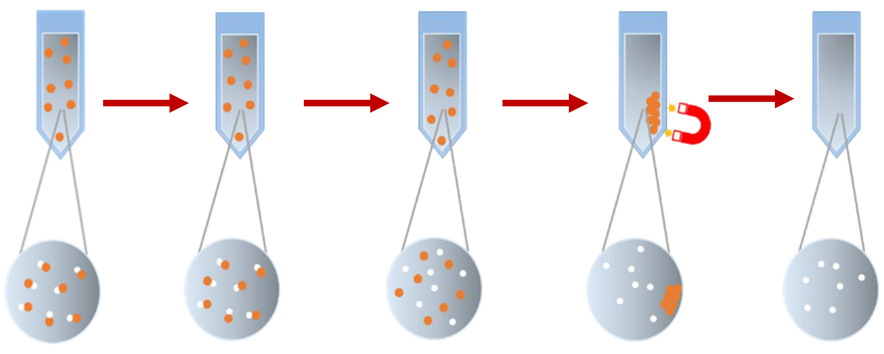
Step 1: Magnetic Protein G DynaBeads® are functionalized with NSEγ specific monoclonal antibodies (mAb) and crosslinked using $BS(PEG)_5$



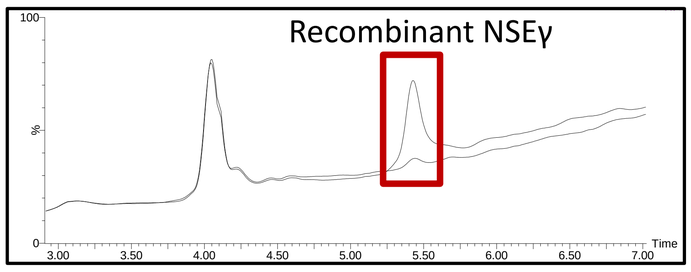
Step 2: The Functionalized Magnetic Beads with coupled antibodies are incubated with serum for isolation of NSE-γγ and -αγ dimers



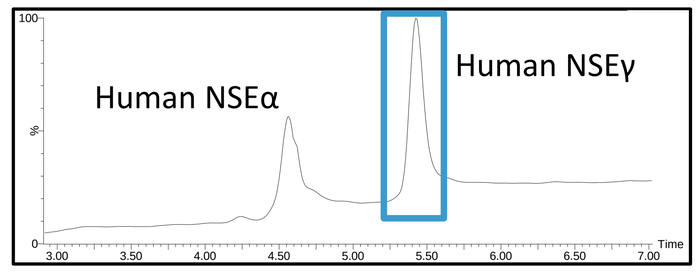
Step 3: The complex with beads and isolated NSEγ-dimers are washed followed by NSEγ elution from the complex and analysis using LC-MS



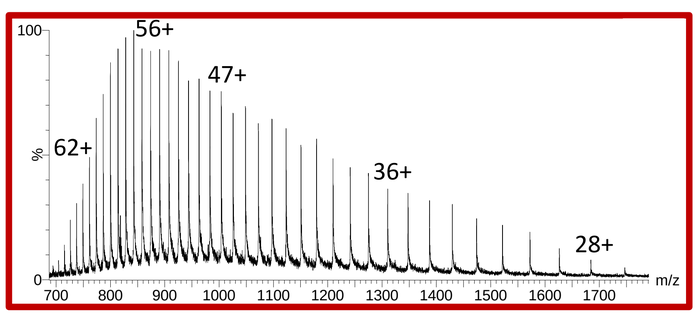
Results IP and LC-HRMS Analysis



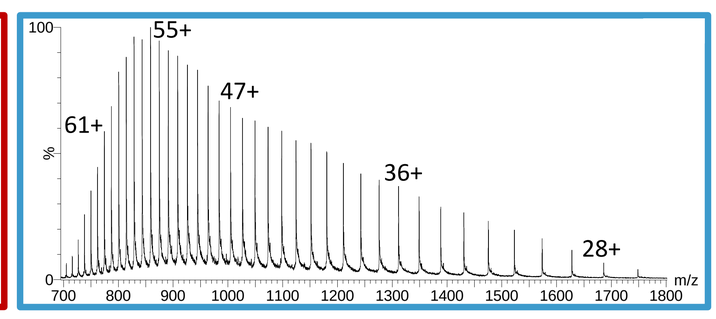
Chromatogram overlay of un-spiked and spiked (100 ng) human serum after IP assay, recombinant NSEγ peak indicated



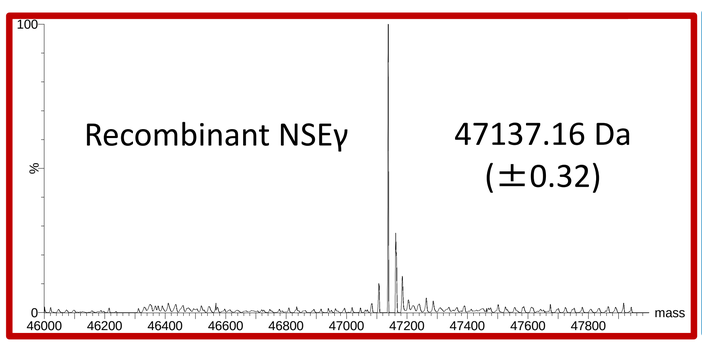
Chromatogram of human hemolysate after IP assay, human NSEγ indicated. Human NSEα is co-isolated



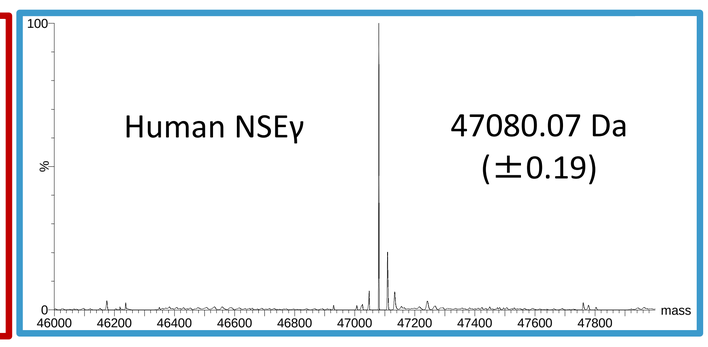
MS Spectrum of the indicated isolated recombinant NSEγ peak



MS Spectrum of the indicated isolated human NSEγ peak



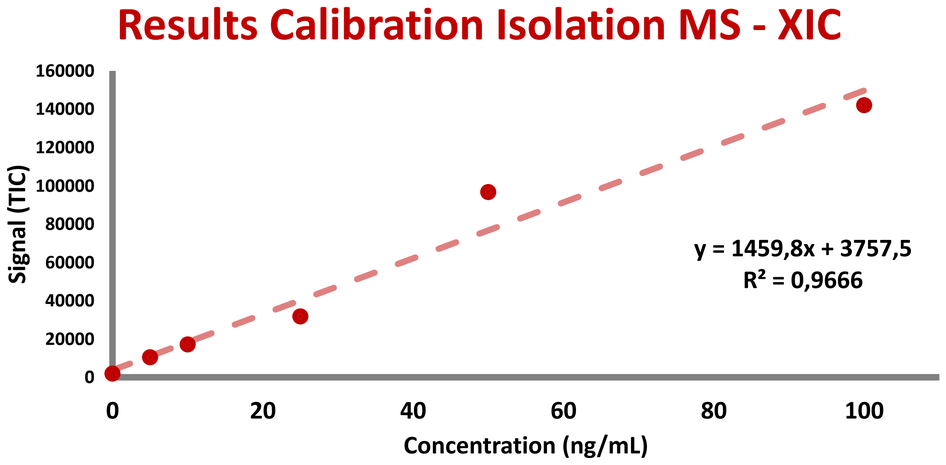
Deconvoluted Mass of the indicated peak confirming recombinant NSEγ



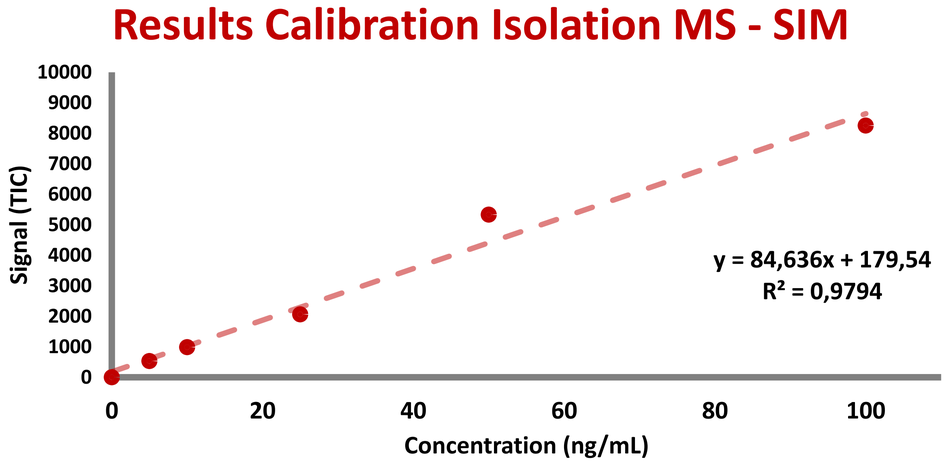
Deconvoluted Mass of the indicated peak confirming human acetylated NSEγ

Analysis Performed using a Waters Xevo G2-XS QToF using an Agilent Polaris 3 C18-A 100 x 2.0 mm Column

Results Calibration Isolation MS - XIC



Results Calibration Isolation MS - SIM



Calibration experiments are performed by spiking human serum with recombinant NSEγ at various concentration levels. LC-HRMS data integration using Extracted Ion Chromatograms (XIC) (left) and Single Ion Monitoring (SIM) (right) were used. Adequate linearity and sensitivity was achieved, additional experiments are required for further method validation.

Conclusion

- Using the magnetic Dynabeads functionalized with NSEγ-antibodies the NSEγ protein biomarker could be successfully isolated from spiked human serum.
- Different types of MS modes and analysis made it possible to analyze the spiked protein in a relevant concentration range.

Advantages Top-Down Approach

- Simplified Sample preparation compared to bottom-up LC-MS
- More protein information is retained compared to bottom-up LC-MS
- Information about Post Translational Modifications (PTM) might offer extra information about disease

Laboratory of Chemical Biology - Department of Biomedical Engineering - Eindhoven University of Technology – *Contact: s.v.d.wildenberg@tue.nl