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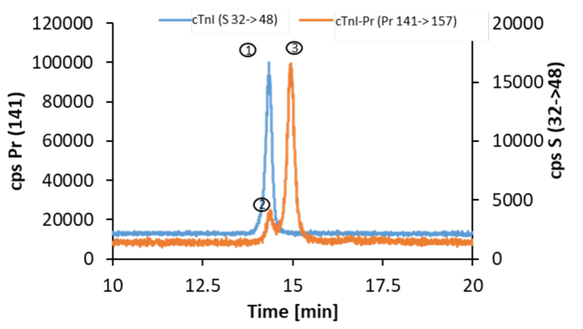
Quantification of cardiac troponin via lanthanide-labeled peptides using ICP-MS

Introduction

Cardiac troponin (cTn) is considered the preferred biomarker used in the diagnosis of acute myocardial infarction (MI) and other cardiovascular diseases (CVDs). cTn levels of patients are usually in the low µg/L range and below, which makes quantification challenging. Currently in clinical practice, cTn is measured with high sensitivity ELISAs, which have limits of quantification (LOQ) as low as 1 ng/L. These tests often show discordant results. To ensure reliable diagnosis, accurate measurement of cTn concentrations is crucial. For that, reference measurement procedures are required to provide “anchor points” for routine methods and to enable standardization.
In the CardioMet project, measurement methods using inductively coupled plasma mass spectrometry (ICP-MS) are investigated to improve the sensitivity of MS methods. With the developed method, comparability of cTnI measurements should be enabled, which finally leads to a reference measurement procedure.

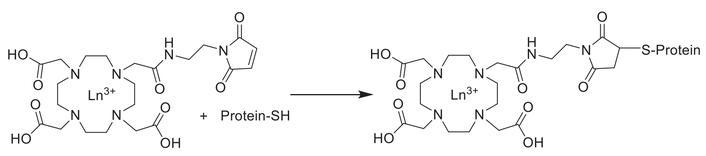
Results



SEC-ICP-MS chromatogram of labeled and unlabelel cTnI

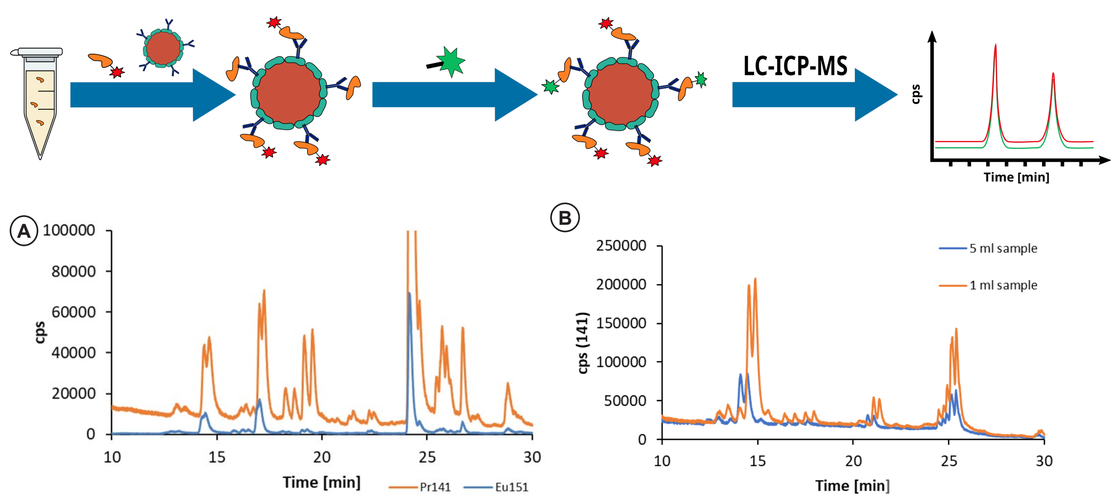
For the highest possible sensitivity, recombinant cardiac troponin I (cTnI) was labeled via its sulfhydryl-groups with a praseodymium containing label. Recombinant cTnI (1 µg) (blue) and cTnI-Pr (0.1 µg) (orange) were analyzed on a SEC column. Peak 1 and 2 indicate the presence of cTnI and cTnI-Pr and Peak 3 of unreacted Pr-Label.

ESI-ToF-Data identified Peak 1 as cTnI with a MW of around 24 kDa.



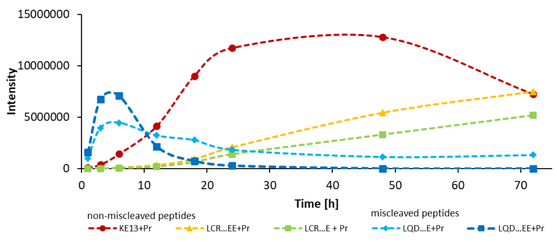
DOTA-Maleimide binds to cysteine residues in proteins

Due to the lack of sensitivity, an enrichment method was required to reach the desired low LOQ. Magnetic Protein G beads were functionalized with an anti-cTnI-antibody (anti-TnI-Ab), which binds labeled and unlabeled cTnI from a larger sample volume. Additionally, interferences e.g. from serum proteins were removed during the enrichment step.

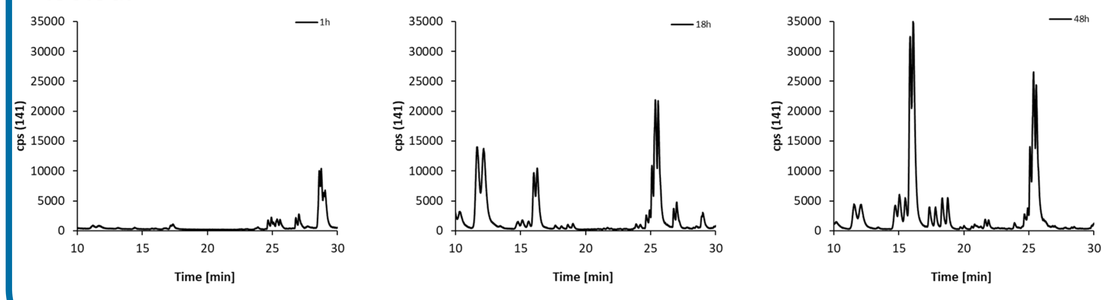


(A) LC-ICP-MS measurement of a sample prepared as shown in the figure above
(B) Same amount of cTnI enriched from 1 ml and 5 ml serum sample

Quantification of cTnI should be achieved via proteolytic peptides. GluC was chosen as a suitable protease to get two label containing specific peptides. A digestion time of more than 24 h was necessary to fully release the proteotypic peptides. . In TIMS-ToF measurement no unlabeled peptides could be detected.

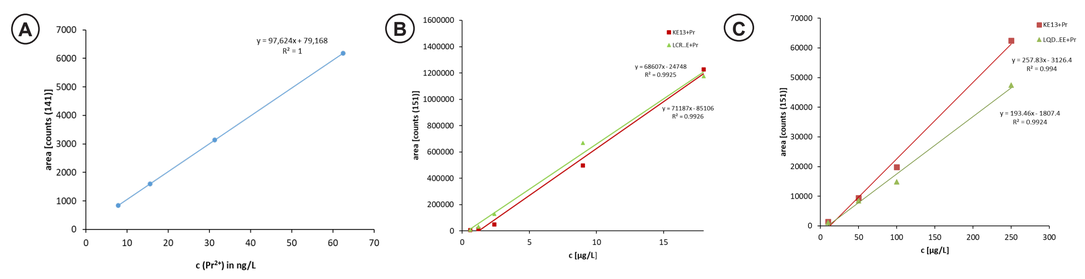


Timeline of GluC digest measured with TIMS-ToF



Digestion of cTnI at three different time points measured with ICP-MS

Measured limit of detection (LOD) for praseodymium is 7 ng/L. For the praseodymium-labeled synthetic peptides the LOD is found to be 25 pmol/L which corresponds to a cTnI concentration of 600 ng/L. For magnetic bead enriched and GluC digested labeled recombinant protein the LOD is at 10 µg/L, wich is still higher than expected from the results for the synthetic peptides.



Calibration curves of Praseodynium (A), Peptides (B) and recombinant cTnI (C)

Outlook

The results show that lanthanide labeled cTn makes quantification via specific peptides measured by ICP-MS possible. Enrichment with magnetic beads enables us to get closer to the desired concentration range of low ng/l. However, several parameters are still unknown, and have to be further investigated. For example, the behavior of the natural form versus the recombinant form used in this work during their enrichment from serum samples has to be investigated. The next steps will be to modify instrumental and sample preparation parameters to realize an isotope dilution mass spectrometry method that is traceability to the SI.

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