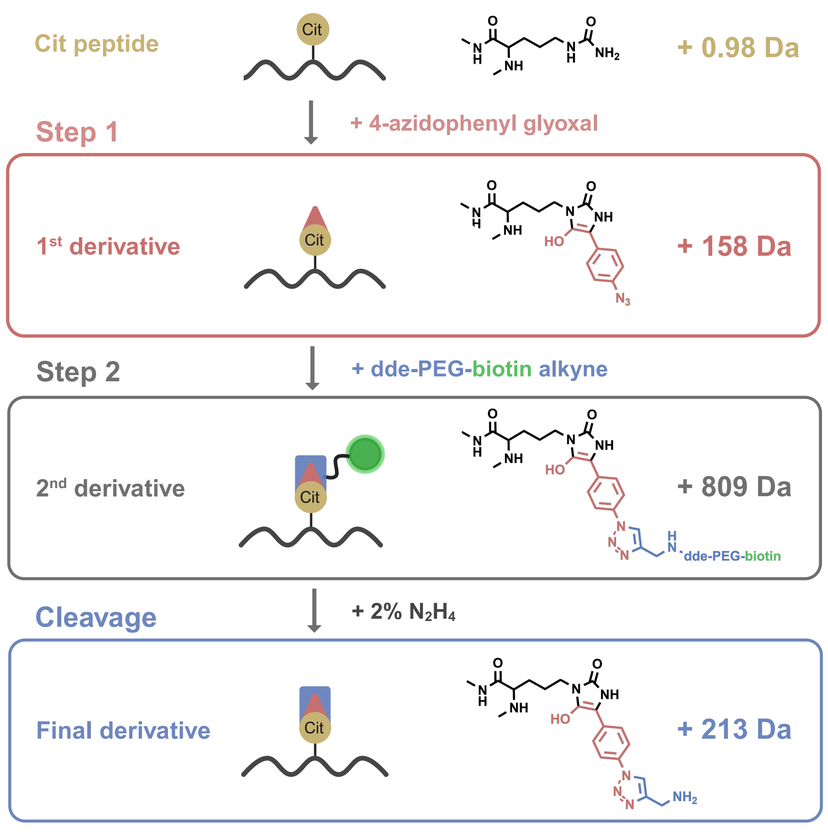
Development of a Mass Spectrometry-Compatible Chemical Probe for Protein Citrullination Enrichment

Rebecca Meelker Gonzalez [1,2], Erik Riedel [1,2], Sophia Laposchan [1,2], Chien-Yun Lee [1,2]

[1]Young Investigator Group: Mass Spectrometry in Systems Neurosciences, CLINSPECT-M consortium, Germany
[2]School of Life Sciences, Technical University of Munich (TUM), Germany

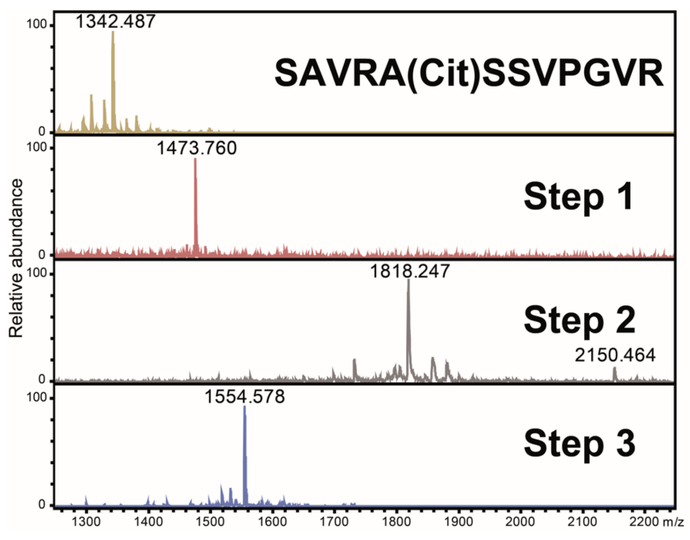
- Deregulation of citrullination (Cit) is found in : rheumatoid arthritis, multiple sclerosis and Alzheimer's disease
- Misinterpretation as deamidation (N&Q) due to +0.98 Da
- Low abundance and stoichoimetry call for **enrichment methods**
- Current probes lack specificity, sensitivity or have poor fragmentation
- **Cleavable chemical probe** to derivatize and enrich citrullinated peptides

Chemical derivatization of Cit peptides



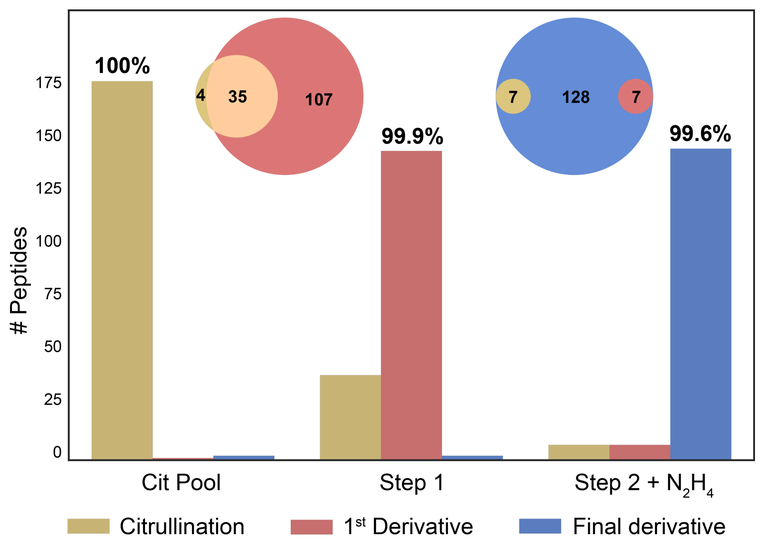
Optimization on synthetic peptides

MALDI MS characterization



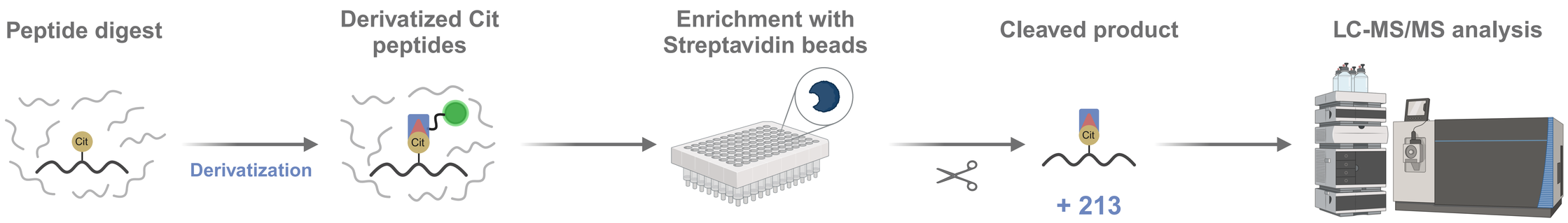
Reaction conditions were optimized until full conversion was reached using single synthetic peptides and MALDI MS monitoring.

CitPool of synthetic peptides (n = 174)

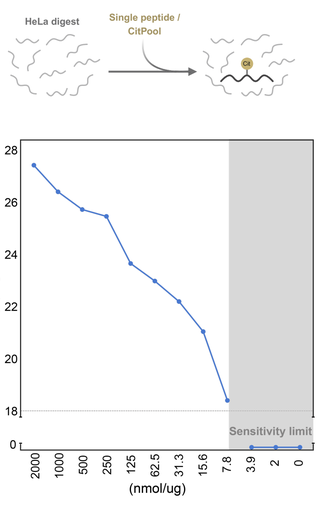


Derivatization of a pool of 174 citrullinated synthetic peptides. More than 80% of the peptides were efficiently derivatized with 99.6% of intensity.

Workflow

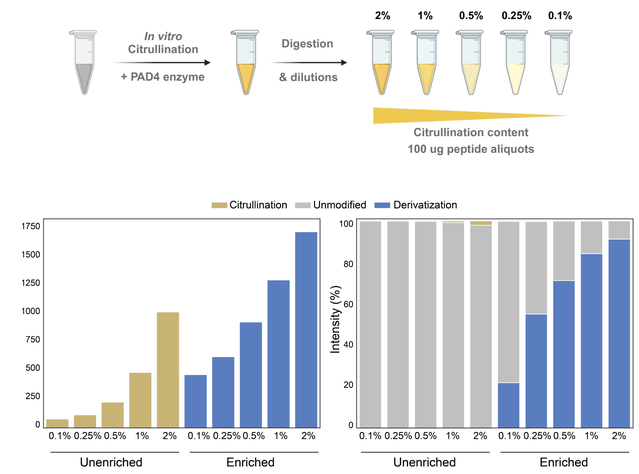


Sensitivity



Spiked-in peptide could be quantified down to 7.8 nmol/ug.

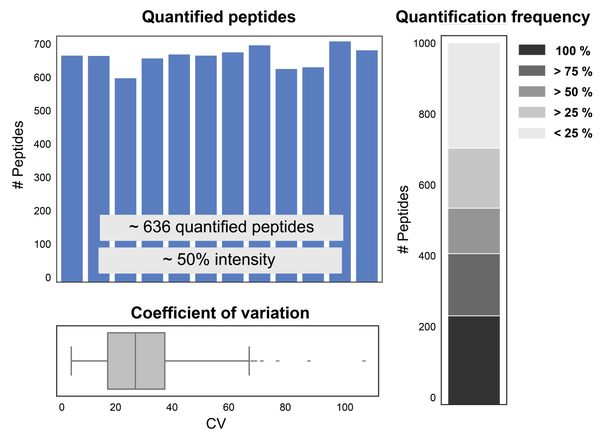
Quantification



Biological citrullination content is expected around 0.1%. At this dilution we see a 6 fold increase in peptide identifications with 20% intensity based enrichment.

Reproducibility

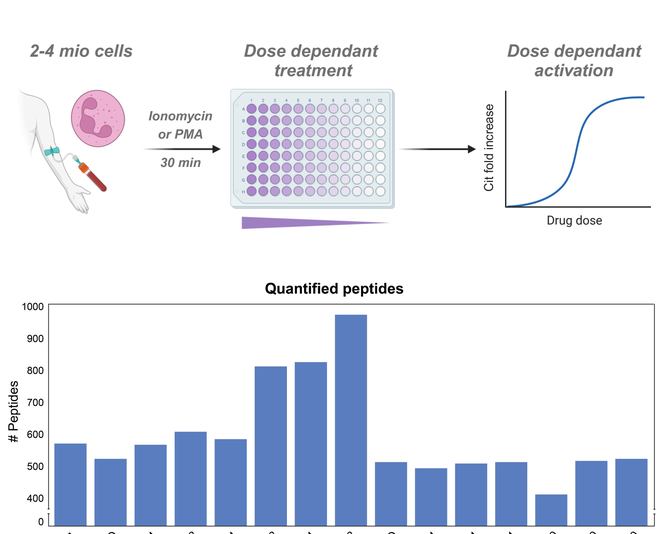
12 x 0.25% In vitro Cit Hela



Reproducible quantification was observed. The median of the coefficient of variation (CV) was under 30 and 400 peptides were consistently identified in 75% of the replicates.

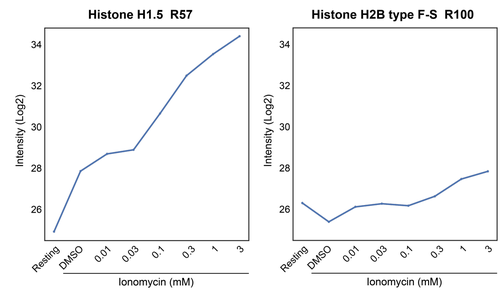
Application

Neutrophil isolation and treatment



Summary

- Workflow to enrich citrullinated peptides from complex samples on peptide level and suitable to any sample type
- High sensitivity and high throughput on 96-well plate with reproducible quantification
- Commercially available azide and alkyne compounds



Acknowledgements : The authors wish to acknowledge BMBF (161L0215) for financial support towards the project and attending this conference.

SPONSORED BY THE Federal Ministry of Education and Research

https://mspec-sysns.de/ @msleemslab

Created with BioRender Poster Builder