A comparative study of synthetic winged peptides for absolute protein quantification

MUNI

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INTRODUCTION

- A choice of internal standard is critical for **accurate, precise, and reproducible** mass spectrometry-based proteomics assays
- Synthetic isotopically labeled (SIL) proteins and SIL peptides are currently used in proteomics workflow
- An alternative approach uses **SIL "winged" peptides** extended at C- or/and N-terminus with an amino acid sequence or a tag removed in enzymatic proteolysis
- However, **various designs** of winged peptides are used for absolute quantification and **a consensus** is missing
- In this study, we compared a “gold standard” SIL protein with four different designs of SIL winged peptides extended with:
  - i) proprietary trypsin cleavable tag at C-terminus (SIL-TCT, blue)
  - ii) five amino acid residues of a natural protein sequence at C-terminus (SIL-ExC5, orange)
  - iii) three amino acid residues of a natural protein sequence at both C- and N-termini (SIL-ExC3N3, green)
  - iv) five amino acid residues of a natural protein sequence at both C- and N-termini (SIL-ExC5N5, purple)

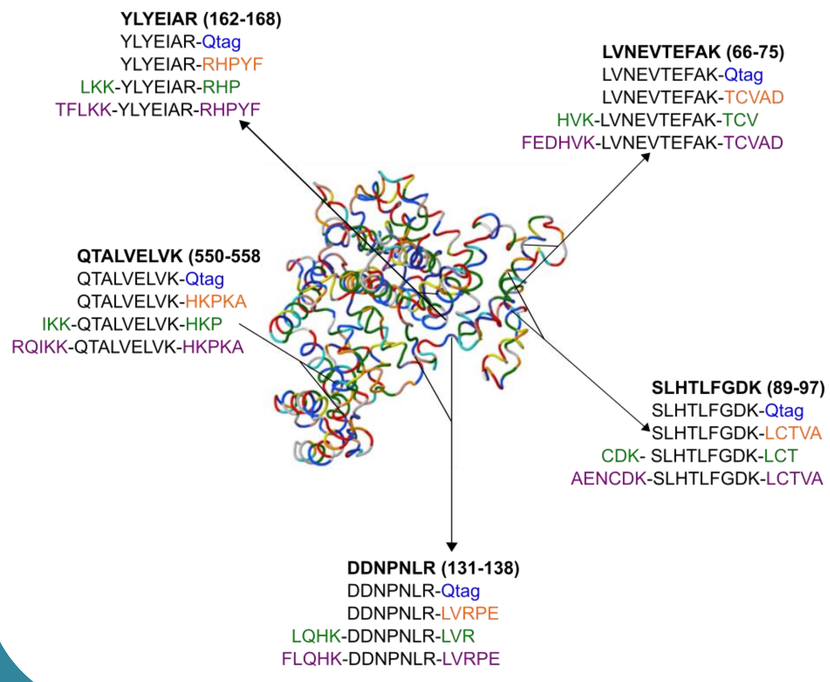


Fig. 1: Compared designs of SIL winged peptides. Proteotypic signature peptides are marked in bold with a peptide position in the protein sequence indicated in brackets. SIL-Ex designs are color-coded.

METHODS

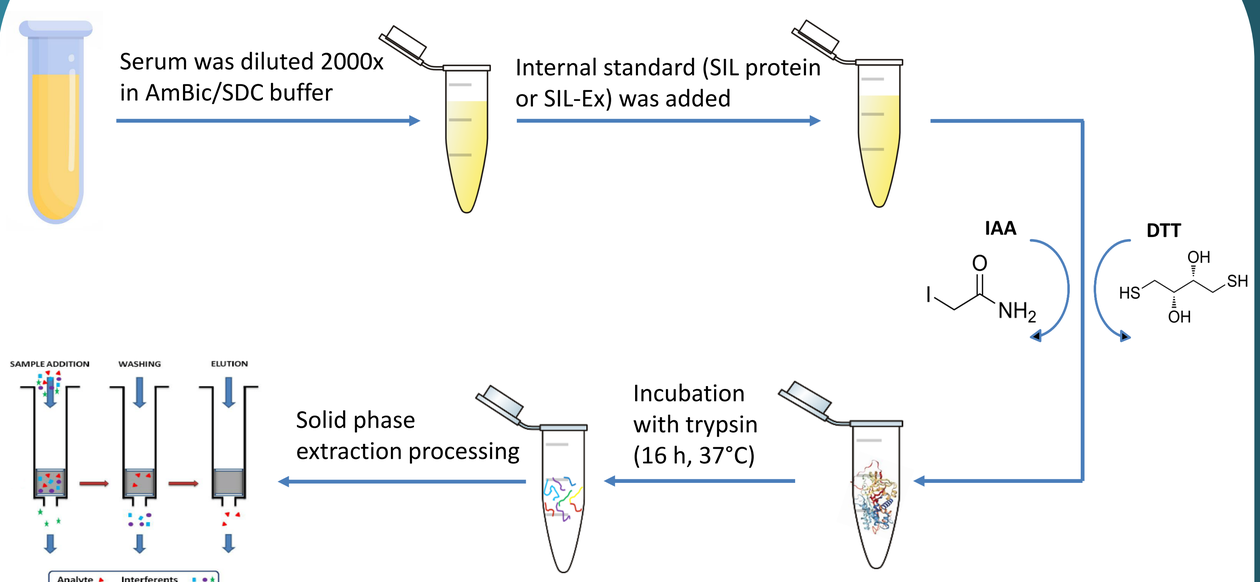


Fig. 2: Sample processing workflow. A pool of serum samples collected from 14 healthy volunteers was diluted 2000-fold using 50 mM ammonium bicarbonate with 5 mg/ml sodium deoxycholate (AmBic/SDC buffer) to albumin concentration of approx. 500 nM. SIL-Ex internal standard was added, proteins were reduced and alkylated and enzymatically digested over night. Samples were desalted using HLB-SPE and analyzed by UHPLC/MS-SRM.

RESULTS

- In this study, we tested the influence of the sequence extension design on enzymatic digestion efficiency, solubility, and quantitative performance

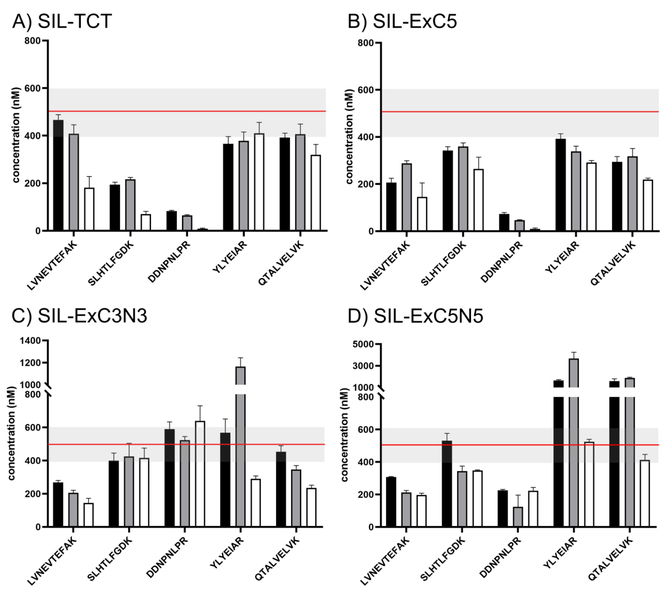
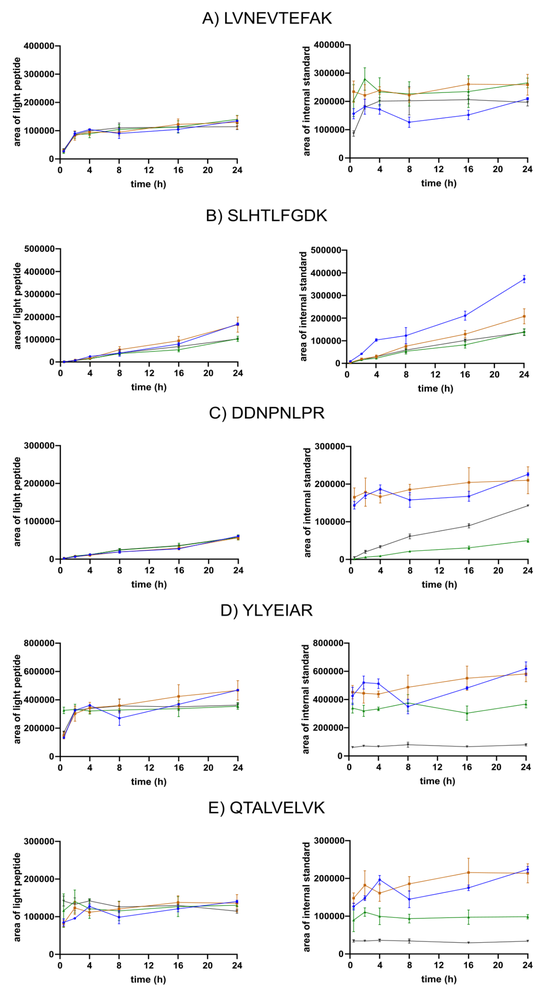


Fig. 3. The quantitative performance of different types of SIL-Ex peptides depending on organic modifier content in the reconstitution solvent. HSA protein concentration was determined using SIL-Ex peptides reconstituted in AmBic/SDC buffer with 5% (black), 20% (grey), and 50% ACN (white). HSA concentration determined by reference methods (500 nM) indicated as a red line; the grey area represents +/-20% tolerance.

Fig. 4. The trypsin digestion kinetics of signature peptides. SIL-Ex peptide types are color-coded: SIL-TCT (blue), SIL-ExC5 (orange), SIL-ExC3N3 (green), and SIL-ExC5N5 (black). For each signature peptide, the signal of light serum peptide (left panel) and SIL-Ex peptide (middle panel) is shown.

For accurate quantification, the rate of signature peptide formation from HSA protein has to be equal to SIL-Ex peptide. However, the signal of SIL-Ex signature peptides varied markedly depending on the type. For instance, a signature peptide DDNP (C) was formed quickly from a SIL peptide with a single C-terminal extension. In contrast, the formation of DDNP from SIL peptides elongated at both C- and N-termini was slower, more reliably mimicking the digestion kinetics of light HSA protein. It results in more accurate quantification using an adequately designed SIL-Ex peptide.



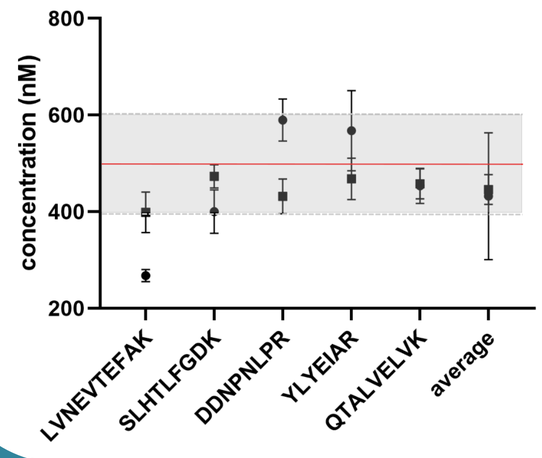


Fig. 5. The comparison of the quantitative performance of SIL-ExC3N3 winged peptide (●) and SIL-HAS protein (■). SIL-ExC3N3 peptides determined average HSA concentration at 432.2 nM, nearly identical to SIL-HSA protein (445.9 nM).

CONCLUSIONS

- The HSA concentration determined using SIL peptides elongated only at C-terminus was highly inaccurate.
- The quantitative accuracy improved with the use of SIL peptides extended at both C- and N-termini.
- We recommend using SIL-ExC3N3 peptides as an equally accurate but more available alternative to SIL protein internal standard in quantitative proteomics.

Acknowledgements

This work supported by the Grant Agency of the Czech Republic (project No. 17-24592Y), the Grant Agency of Masaryk University (GAMU project No. MUNI/G/1131/2017), the Czech Health Research Council (AZV project No. NV19-08-00472), the RECETOX research infrastructure (the Czech Ministry of Education, Youth, and Sports–MEYS, LM2018121), CETOCOEN EXCELLENCE Teaming (Horizon2020, 857560 and MEYS, 02.1.01/0.0/0.0/18_046/0015975).