In-StageTip digestion improves sample preparation prior to LC-MS/MS analysis for clinical research

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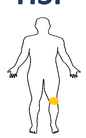
Introduction

The instrumental improvements made over the last decade allowed mass spectrometry to produce useful proteomics data for clinical research. However, clinical proteomics still faces challenges such as an increasing number of samples to analyze and working with a low amount of starting material while achieving in-depth analyses of complex samples. Tackling these issues requires a fast, robust and reproducible sample preparation with minimal sample loss. To this end, we evaluated the in-StageTip[1] (iST) complete solution developed by PreOmics, including a peptide fractionation add-on step, on three different samples:
murine brain tissue extracts (MBT), murine brain isolated cell extracts (MBC) and human synovial fluid (HSF).

Samples

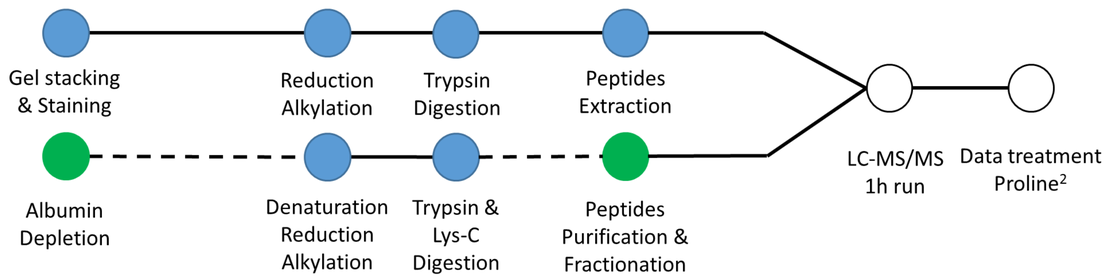


	MBT	MBC	HSF
Starting material	80 µg	<10 µg (≅200.000 cells)	80 µg (1.2 µL) - 1 mg
Protocol	Gel stacking/iST	iST	Depletion Gel stacking/iST Fractions (x3)
Instrument	QEx plus	TimsTOF Pro/QEx HF-X	TimsTOF Pro

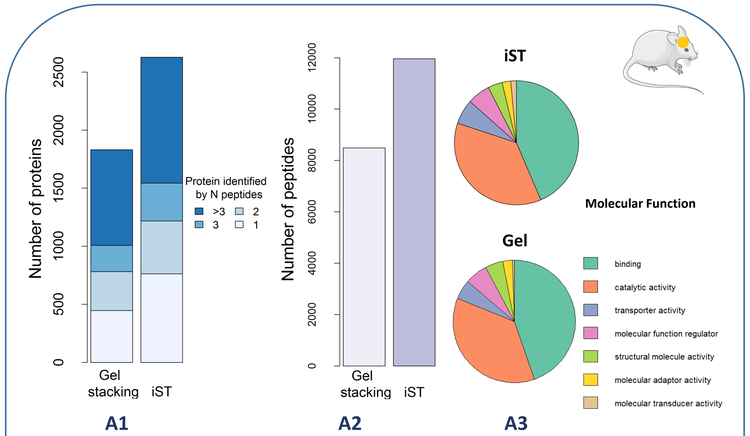
Gel stacking
Within 2 days

In-StageTip
Within 4 hours
(1 day with depletion)

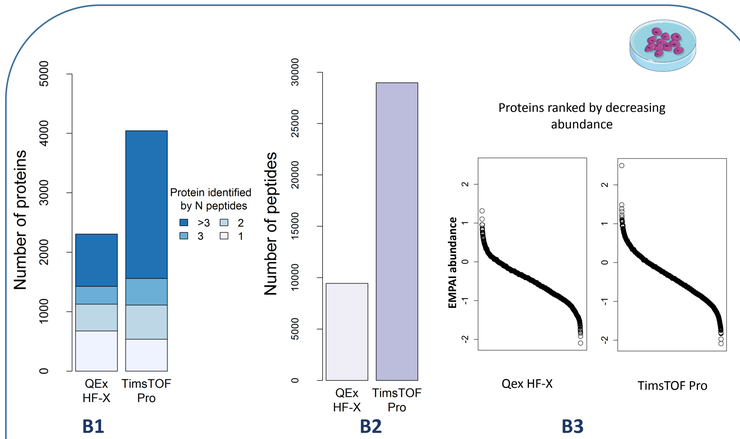
Workflow



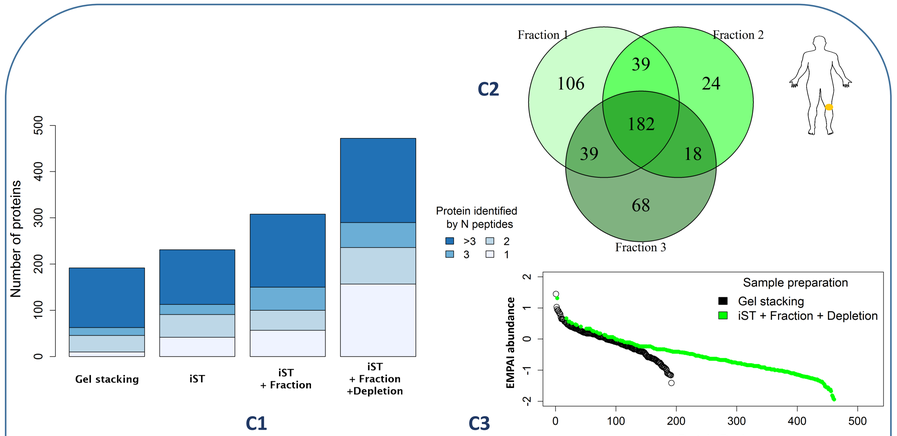
Results



Number of proteins (A1) and peptides (A2) identified for **MBT samples**, comparing gel stacking and iST preparation. Main categories obtained by functional analysis (A3).



Number of proteins (B1) and peptides (B2) identified for **MBC samples**, comparing QEx HF-X and timsTOF Pro instruments. Log-abundances covered by both instruments (B3).



Number of proteins (C1) identified for **HSF samples** with optional protocol steps. Venn diagram of proteins identified in the 3 fractions (C2). Log-abundance gain by depletion and fractionation steps (C3).

Conclusions

The iST sample preparation is compatible with various samples containing high to low protein amounts. It is faster than a classical gel stacking protocol and provides better results in terms of protein and peptide identification. No functional bias for the identified proteins was observed. The iST protocol coupled to high performing nanoLC-MS/MS instruments allows the detection of proteins over a 3 log dynamic range. Moreover iST can be improved by a depletion step or by a provided add-on for peptide fractionation. This sample preparation should improve the detection and identification of proteins of interest eg from pathogens or biomarkers in the context of diagnosis process.

Contact

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References

[1]Rappsilber *et al.,* Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. *Nat. Protoc.* **2**, 1896–1906 (2007)

[2] Bouyssie *et al.,* Proline: an efficient and user-friendly software suite for large-scale proteomics. *Bioinformatics* ***36***, 3148–3155 (2020)