

**Integrated solid phase extraction – capillary liquid chromatography  
(speLC) interfaced to ESI-MS/MS for fast characterization and  
quantification of protein and proteomes.**

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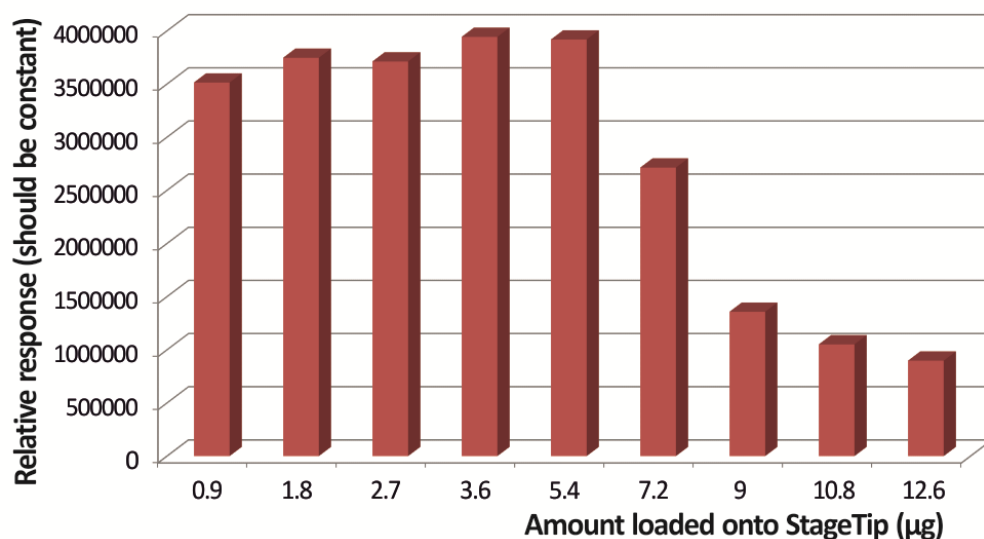
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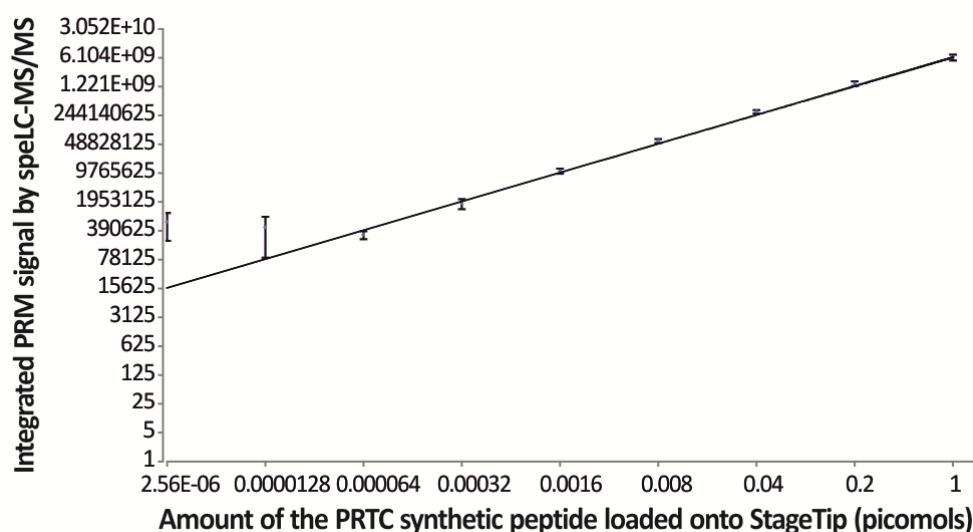
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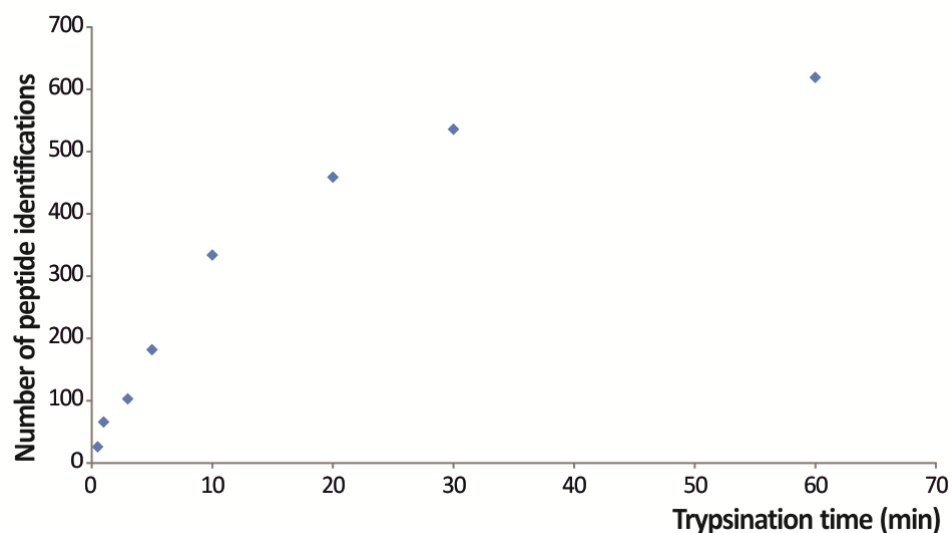
**Keywords:** Nano Liquid Chromatography, Solid Phase Extraction, StageTips, Mass Spectrometry,  
Protein Analysis



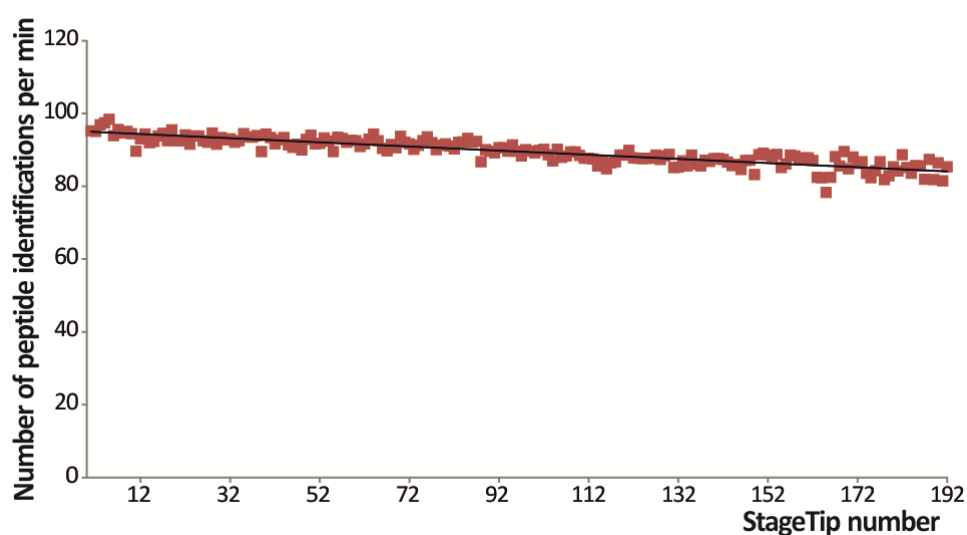
**Supplementary Figure S1. Maximum sample loading capacity for StageTips.** Increasing amounts of HeLa protein digests (x axis) were loaded and eluted from a StageTip, and a fraction corresponding to theoretical 0.1 µg were analysed by speLC-MS/MS. Normalised intensity of the total ion chromatogram (TIC) for each initial sample concentration is reported (y axis). The decrease observed at protein amounts above *ca.* 5 µg indicate StageTip saturation and sample loss.



**Supplementary Figure S2. Sensitivity and linearity of speLC-MS/MS analysis.** Correlation between different synthetic peptides (PRTC, commercial peptide mixture) (x axis) and the corresponding peptide peak area (y axis) from Parallel Reaction Monitoring (PRM) analysis by speLC-MS/MS. PRTC peptides were spiked into 0.2 µg of HeLa protein digests at increasing concentrations and directly analysed.



**Supplementary Figure S3. Limited trypsin digestion of *E. Coli* samples.** Limited trypsin digestion of *E. Coli* lysate proteins stopped at different data points (x axis) and correlated to the number of identified peptides after database searching process (y axis).



**Supplementary Figure S4. Robustness of speLC-MS/MS analysis.** Representation of number of peptide identifications per minute (y axis) obtained for the speLC-MS/MS analyses of 192 replicates (x axis) of partially digested *E. Coli* protein extracts.