Supplementary Figures

Supplementary Figure 1

Characterization of the *S. pyogenes* proteome. Proteins identified by multidimensional peptide separation coupled to MS-identification divided into the cellular component (a), biological process (b) and molecular function (c) gene ontologies. The total number of proteins assigned to a particular ontology group is displayed together with the number of proteins identified demonstrating an even coverage of the proteome by our approach.

Supplementary Figure 2

Performance test of the utilized MS instruments. A dilution series of a β-galactosidase digest was analyzed with the MS instruments utilized in this study (Fig. 3-5). Both instruments yielded high quality spectra down to 3 fmol injected on column, the Q-TRAP even down to 0.3 fmol. This demonstrates that both instruments were operating at high performance.

Supplementary Figure 3

Quantification of additional proteins by MRM-analysis which were used for normalization. Data was acquired simultaneously with the data of the virulence factor proteins (Fig. 4) in the same LC-MS analysis runs.

A) Peptide averages and standard deviation of 6 (4) measurements. The replicate experiments at each concentration are distinguished by color (red and blue).

B) 95% confidence intervals for the mean relative protein abundance in dependence of plasma concentration (derived using linear mixed-effects models).

Supplemental Data

Supplemental Data 1

List of *S. pyogenes* proteins identified by the extensive proteome analysis. The last column contains a link to the respective PeptideAtlas entry where additional information is available including the corresponding MS/MS spectra.

Supplemental Data 2

Potential *S. pyogenes* virulence factor proteins targeted in this study. Based on the current literature we assembled a list of 25 potential virulence factors which we targeted by MRM. The last column indicates whether proteotypic transitions could be validated by MRM-triggered MS/MS spectra and used for MRM-based quantification. The majority of proteins not detected by MRM had not been identified by the multidimensional shotgun proteomics mapping experiment deposited in the PeptideAtlas. Presumably, these proteins are not amendable to mass spectrometric detection or they are not expressed under the applied growth conditions.

Supplemental Data 3

Table of 282 validated transitions used in this study for quantitative analysis.

Supplemental Data 4

Results of MRM-based quantification. Tables of quantification results depicted in Figure 4 and Supplementary Figure 3. Protein values were derived by averaging the peptide intensities.