

Overview

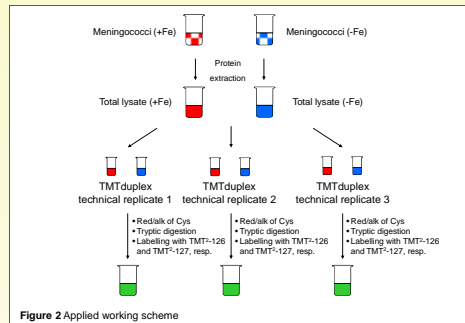
- TMT isobaric mass labeling offers integrated workflows for multiplex gel-free proteomics analysis of up to six samples^[1].
- We have applied TMTduplex labeling to the analysis of the response of the opportunistic pathogen *Neisseria meningitidis* to iron depletion.
- Regulation of several iron-sequestering proteins reported by TMT were confirmed by Western blot experiments.

Introduction

- The Gram-negative bacterium *Neisseria meningitidis* can cause inflammation of the meninges and/or infection of the bloodstream making it a serious health problem worldwide^[2].
- In human infection *N. meningitidis* responds to an iron limited environment by increasing expression of iron-sequestration proteins, several of which are under clinical evaluation as promising vaccine candidates.
- Two sets of isobaric label reagents have been developed, namely TMTsixplex and TMTduplex, that label peptides and proteins at amino groups allowing the simultaneous, combined analysis of two and six samples respectively. This delivers improved performance by drastically reducing analytical variability.
- TMTduplex was used for the investigation of total lysates of cells grown under conditions of either iron-restriction or iron-excess.

Methods

- Proteins were extracted from ultrasonicated *N. meningitidis* strain MC58 grown in TSB medium (+Fe, -Fe), reduced, alkylated and tryptically digested using standard methods and labelled with TMT²-126 (+Fe samples) and TMT²-127 (-Fe samples) respectively.
- To demonstrate the performance and the technical reproducibility of the TMTduplex workflow, protein extracts were processed in triplicate (Figure 2).

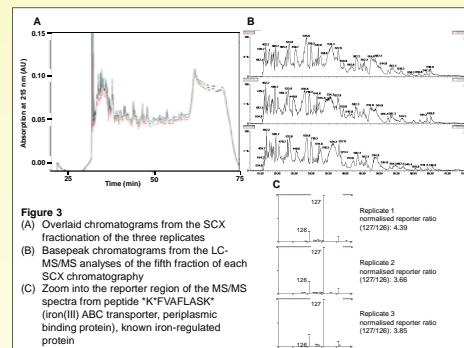


- SCX chromatography was performed on a Vision BioCAD HPLC workstation using a Polysulfoethyl A column (PolyLC).
- All MS analyses were performed on a Qtof-2 instrument coupled to a Capillary-LC (Waters).
- Peptide and protein identification was performed using SEQUEST followed by the PeptideProphet and ProteinProphet algorithms.
- Quantification by log-scale based averaging of processed intensities of TMTduplex reporter ions (m/z 126 and 127) were performed by proprietary software.
- Western blot analyses were performed using antibodies and semi-quantitative signal intensity recorded with a commercial CCD-camera system (Versadoc5000, Biorad).

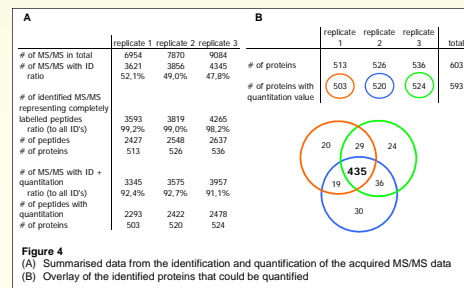
Results

Reproducibility

- Each TMT-labelled mixture was applied to SCX chromatography to obtain 22 fractions which were then subjected to LC-MS/MS analysis with intensity-dependent MS/MS acquisition.
- Chromatographic separations and reporter ratios were found to be highly reproducible across the three experiments (Figure 3).



- The total number of peptide and protein ID's and their overlap in the three experiments were highly reproducible (Figure 4) with a high efficiency of the labelling reaction.



Performance

- Out of 593 identified proteins, 43 showed significant regulation (≥ 1.25 up or ≤ 0.80 down) in at least two of the three replicates.
- Several known iron-modulated proteins were quantified by TMT, including current vaccine candidates, whose regulations were confirmed independently by Western blot analyses. Other previously unreported iron-modulated proteins were also discovered that may represent alternate vaccine candidates

Acc	Name	replicate 1 MS/MS mean	replicate 2 MS/MS mean	replicate 3 MS/MS mean	Regulation by Western blot analysis		
NMB1729	lipopolymer transport protein ExbB (exbB) (<i>Neisseria meningitidis</i> MC58)	2	2.8	1	2.8	1	3.2
NMB1412	FrpC operon protein (<i>Neisseria meningitidis</i> MC58)	3	1.6	5	1.7	4	1.9
NMB1668	hemoglobin receptor (hmbR) (<i>Neisseria meningitidis</i> MC58)	2	3.6	1	3.4	1	3.3
NMB0634	non(III) ABC transporter, periplasmic binding protein (BpA) (<i>Neisseria meningitidis</i> MC58)	38	4.3	29	3.9	42	4.4
NMB1988	iron-regulated outer membrane protein FrpB (frpB) (<i>Neisseria meningitidis</i> MC58)	only ID	...	2	9.7	2	13.5
NMB1540	lactoferrin-binding protein A (LbpA) (<i>Neisseria meningitidis</i> MC58)	1	2.1	1	2.5		12.0
NMB0035	lipoprotein, putative (<i>Neisseria meningitidis</i> MC58)	5	6.6	7	4.1	6	7.1
NMB1730	TadB protein (tadB) (<i>Neisseria meningitidis</i> MC58)	1	2.8	1	2.5	1	2.8
NMB0461	transferrin-binding protein 1 (Tbp1) (<i>Neisseria meningitidis</i> MC58)	11	5.5	11	4.3	12	5.8
NMB0460	transferrin-binding protein 2 (Tbp2) (<i>Neisseria meningitidis</i> MC58)	2	22.7	4	9.2	4	13.7
NMB1429	outer membrane protein PorA (porA) (<i>Neisseria meningitidis</i> MC58)	73	0.9	57	1.0	71	1.0
NMB0390	outer membrane protein class 4 (mpM) (<i>Neisseria meningitidis</i> MC58)	30	1.1	45	1.1	42	1.1

Figure 5 Observed regulation values (mean) for a selected set of proteins in comparison with Western blot data if available (MS/MS = number of MS/MS spectra quantified from named protein)
 (A) Known iron-modulated proteins
 (B) House-keeping proteins

Conclusions

- The TMT workflow provides rapid quantitative analysis of a complex proteome with high reproducibility and reliability.
- Using TMT we identified both well-known and previously unreported regulations of iron-modulated proteins in an important human pathogen, providing potential new vaccine candidates and therapeutic targets.

References

- Dayon, L., Hainard, A., Licker, V., Turck, N., Kuhn, K., et al., Relative Quantification of Proteins in Human Cerebrospinal Fluids by MS/MS Using 6-Plex Isobaric Tags. *Analytical Chemistry* 2008, 2921.
- van Deuren, M., Brandtzaeg, P., van der Meer, J. W. M., Update on meningococcal disease with emphasis on pathogenesis and clinical management. *Clinical Microbiology Reviews* 2000, 13, 144.

