Real-Time Search Improves Sensitivity of TMTpro Complementary Ion Quantification

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ABSTRACT

Purpose: to improve sensitivity while maintaining accuracy and precision in quantitative proteomics experiments at the MS2 level.

Methods: combine complementary ion quantification with Real-Time Search (TMTproC-RTS).

Results: TMTproC-RTS enables the quantification of low-abundant proteins of interest, like transcription factors and signaling molecules, in multiplexed experiments.

INTRODUCTION

Using the balancer-peptide conjugates (TMTproC complementary ions) in the MS2 spectra for quantification circumvents the ratio distortion problem of multiplexed proteomics. These TMTproC quantification scans require long transient and ion injection times for sufficient ion statistics and spectral resolution. Real-Time Search (RTS) algorithms have been shown to increase the sensitivity of SPS-MS3 methods. By informed selection of precursor peaks for quantification, analysis time can be prioritized to peptides useful for quantifying proteins. Nevertheless, the naive implementation of TMTproC still quantifies more proteins than SPS-MS3. Here, we combine complementary ion quantification with Real-Time Search (TMTproC-RTS) to improve sensitivity while maintaining accuracy and precision in quantitative proteomics experiments at the MS2 level.

Figure 1. TMT- and TMTpro-tags are comprised of a reporter region (red), a balancer region (blue) and an amine-reactive NHS-ester moiety (green rectangle). When analyzing complex samples via shotgun proteomics, in addition to the peptide of interest (orange), other peptides with similar m/z-ratio (interferents, green) will be co-isolated (grey box). During fragmentation of a TMTpro-modified peptide, the positively charged reporter ion is separated from the ion and a neutral CO-molecule is lost. This leads to an ion where the balancer part is still attached to the peptide, called the complementary ion.

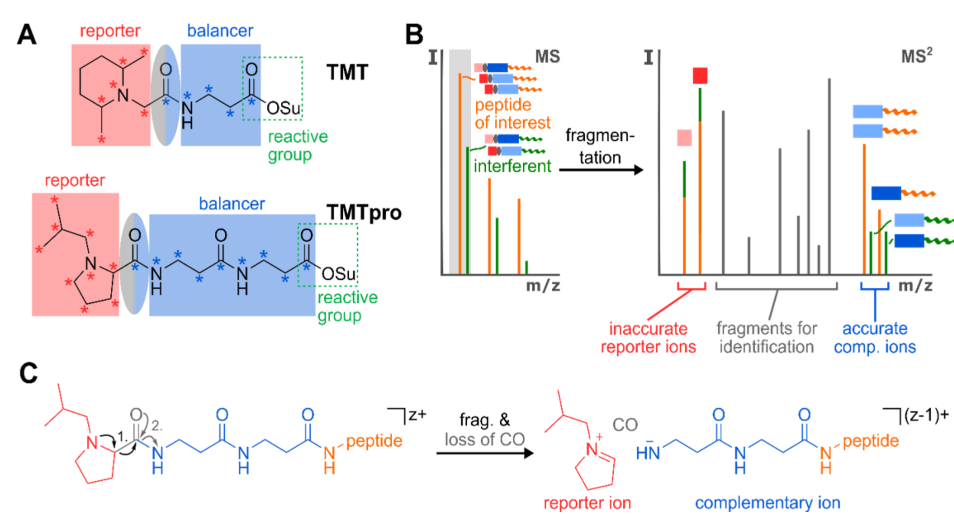
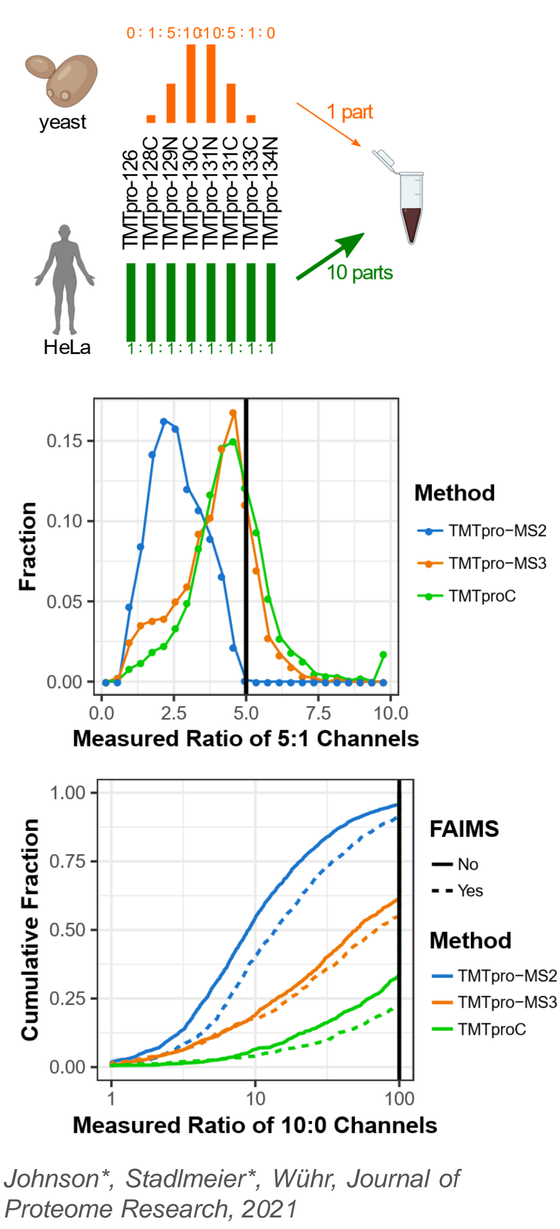
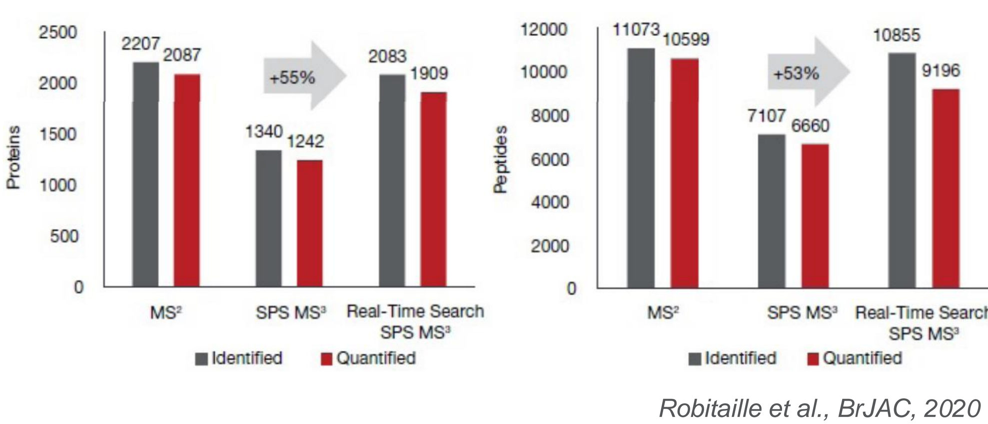


Figure 2. Yeast lysate labelled with TMTpro in ratios of 0:1:5:10:10:5:1:0 was mixed with HeLa lysate labelled with TMTpro in ratios of 1:1 at a mixing ratio of 1 (yeast):10 (HeLa). The mixed sample was analyzed using three different quantification methods with and without ion-mobility prefractionation (FAIMS).



Johnson, Stadlmeier*, Wühr, Journal of Proteome Research, 2021*

Figure 3. A 500-ng sample of Pierce TMT 11plex Yeast Digest Standard was analyzed on a 50 min gradient using MS2 , SPS MS3 , or Real-Time Search for SPS MS3 methods. With Real-Time Search for SPS MS3 , 53% more peptides and 55% more proteins were identified than in the classic SPS MS3 experiment, approaching the results of the MS2 experiment.



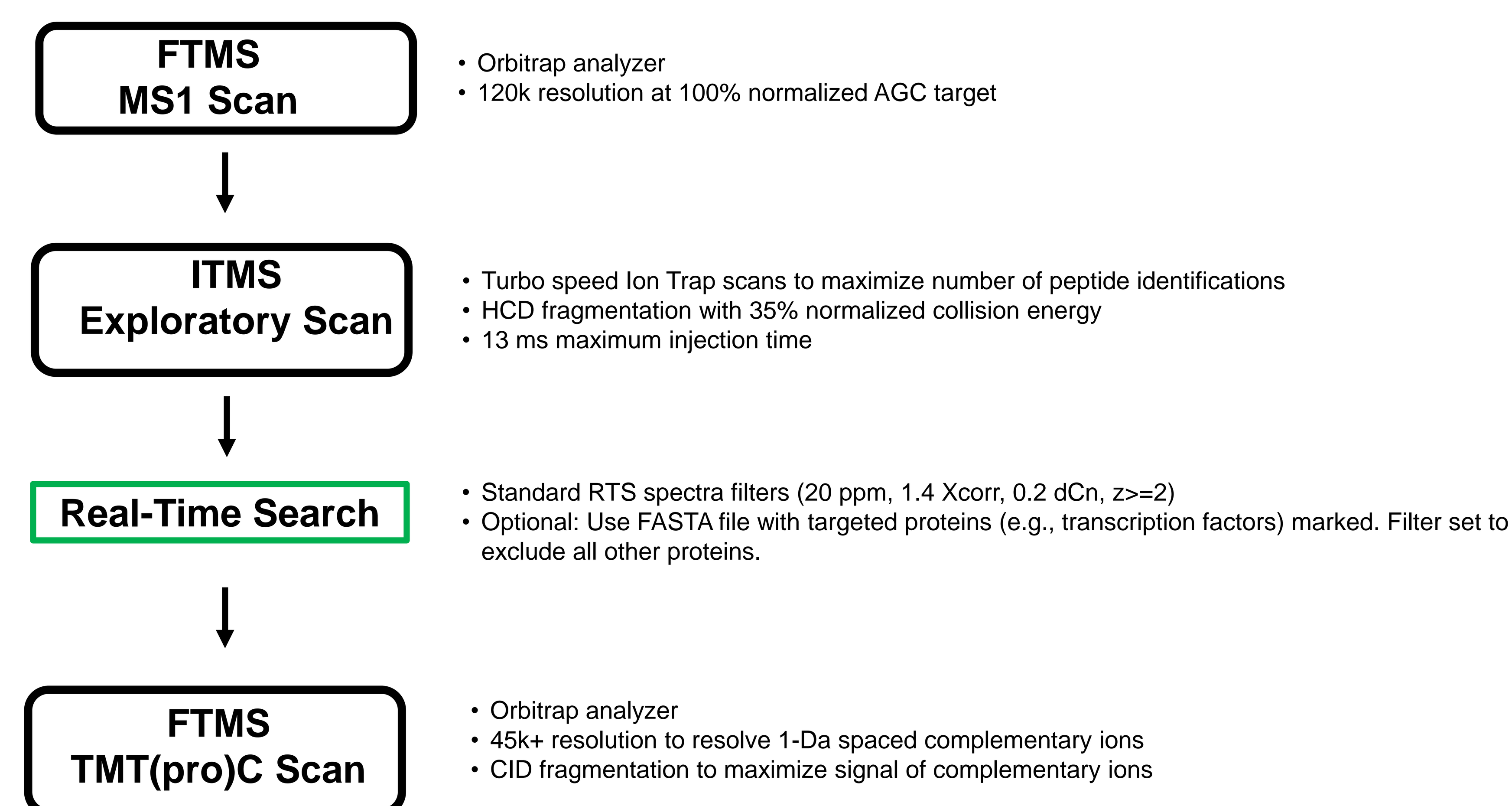
Robitaille et al., BrJAC, 2020

MATERIALS AND METHODS

We digested protein lysate from HeLa cells (human) and Saccharomyces cerevisiae (yeast) in a two-step protocol with trypsin/LysC. Human peptides were labeled with TMTpro at 1:1 ratios across the nine 1-Da spaced channels in the complementary ion region. Similarly, yeast peptides were labeled in ratios of 0:1:5:10:1:10:5:1:0 with TMTpro. Human and yeast peptides were mixed at a ratio of 10:1 in 0.1% formic acid before analysis. The mixed proteome was analyzed on an Orbitrap Ascend Tribrid coupled to a UltiMate 3000 HPLC system with a 90-minute acetonitrile gradient from 6% to 22%.

The TMTproC-RTS method collects fast exploratory ion trap MS2 scans at the turbo scan rate with quadrupole isolation and HCD fragmentation. MS2 spectra are searched immediately against a concatenated human-yeast fasta file. Successfully identified peptides were then re-isolated, and high-resolution MS2 spectra were collected in the Orbitrap with quadrupole isolation and CID fragmentation. In addition to ppm, XCorr, and dCn filters, in separate runs the RTS filter was set to trigger a high-res MS2 scan on either peptides originating from yeast proteins or peptides originating from transcription factors.

Figure 4. Overview of the TMT(pro)C-RTS workflow



RESULTS

Figure 5. A 500-ng sample of Pierce TMT 11-plex yeast TKO standard was analyzed on a 60 min gradient using SPS-MS3, SPS-MS3-RTS, TMTc, or TMTc-RTS. With RTS, TMTc peptide identifications were increased by 5% and protein identifications increased by 7%.

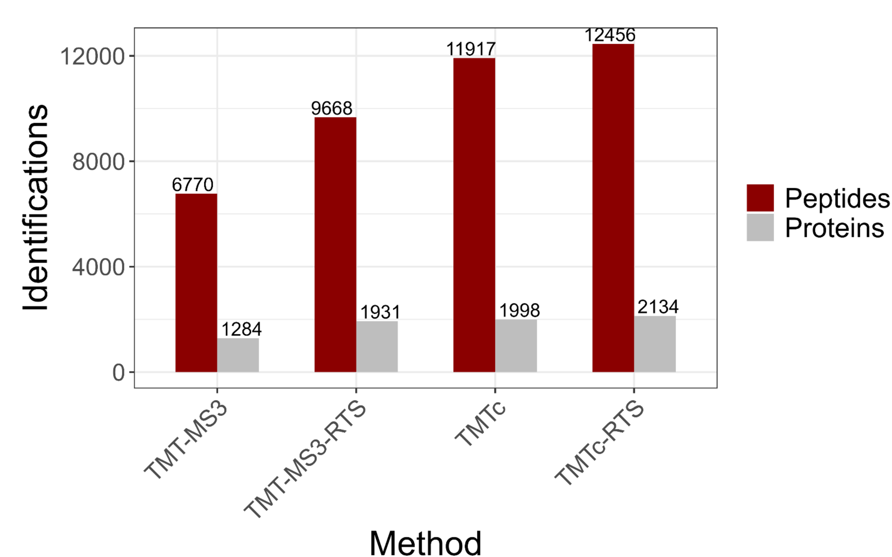


Figure 6. A 500-ng sample of Pierce TMT 11-plex yeast TKO standard was analyzed on a 60 min gradient using TMTc or TMTc-RTS while varying the maximum ion injection time of TMTc FTMS2 scans. With RTS, the number of TMTc scans and peptide identifications decreased by less than without RTS.

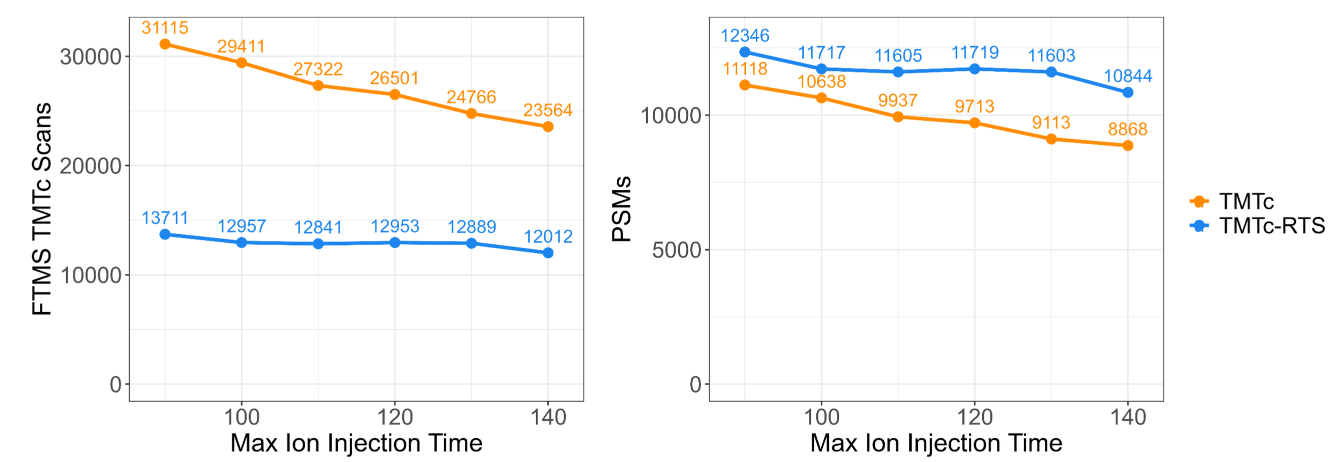


Figure 7. A 1-µg sample of Drosophila melanogaster peptides was labeled with TMTpro0 and analyzed using TMTproC or TMTproC-RTS. The RTS filter was set to only perform FTMS TMTproC scans when the peptide could be matched to a D. melanogaster transcription factor. These low abundant proteins are extremely difficult to quantify. TMTproC-RTS was able to increase peptide identifications by 65% over TMTproC.

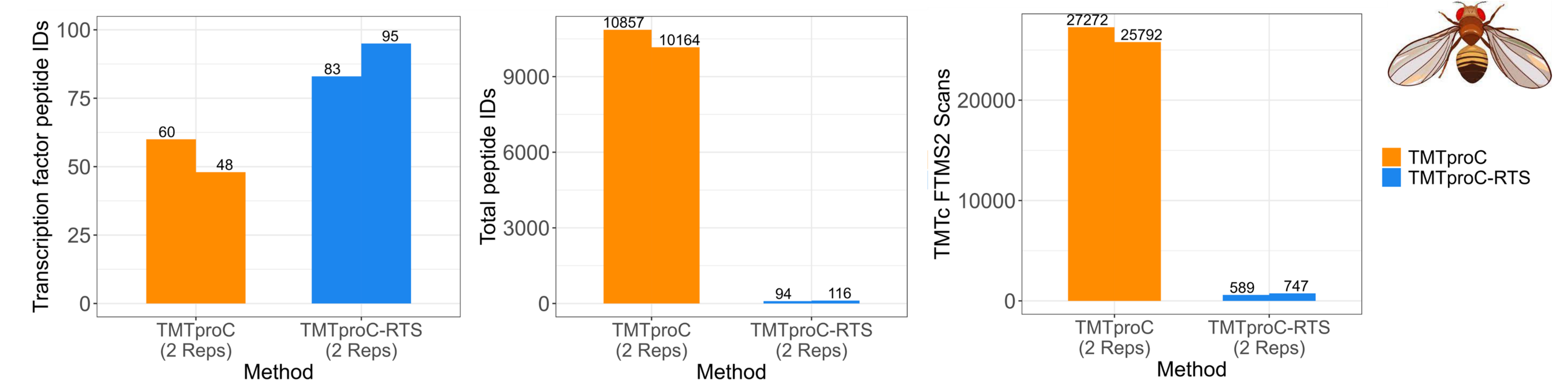


Figure 8. Yeast lysate labelled with TMTpro in ratios of 0:1:5:10:10:5:1:0 was mixed with HeLa lysate labelled with TMTpro in ratios of 1:1 at a mixing ratio of 1 (yeast):10 (HeLa). The mixed sample was analyzed at three different resolutions with and without RTS. RTS was set to only isolate peptides originating from yeast proteins. Quantification accuracy and precision can be improved while using TMTproC-RTS while maintain the number of peptide identifications

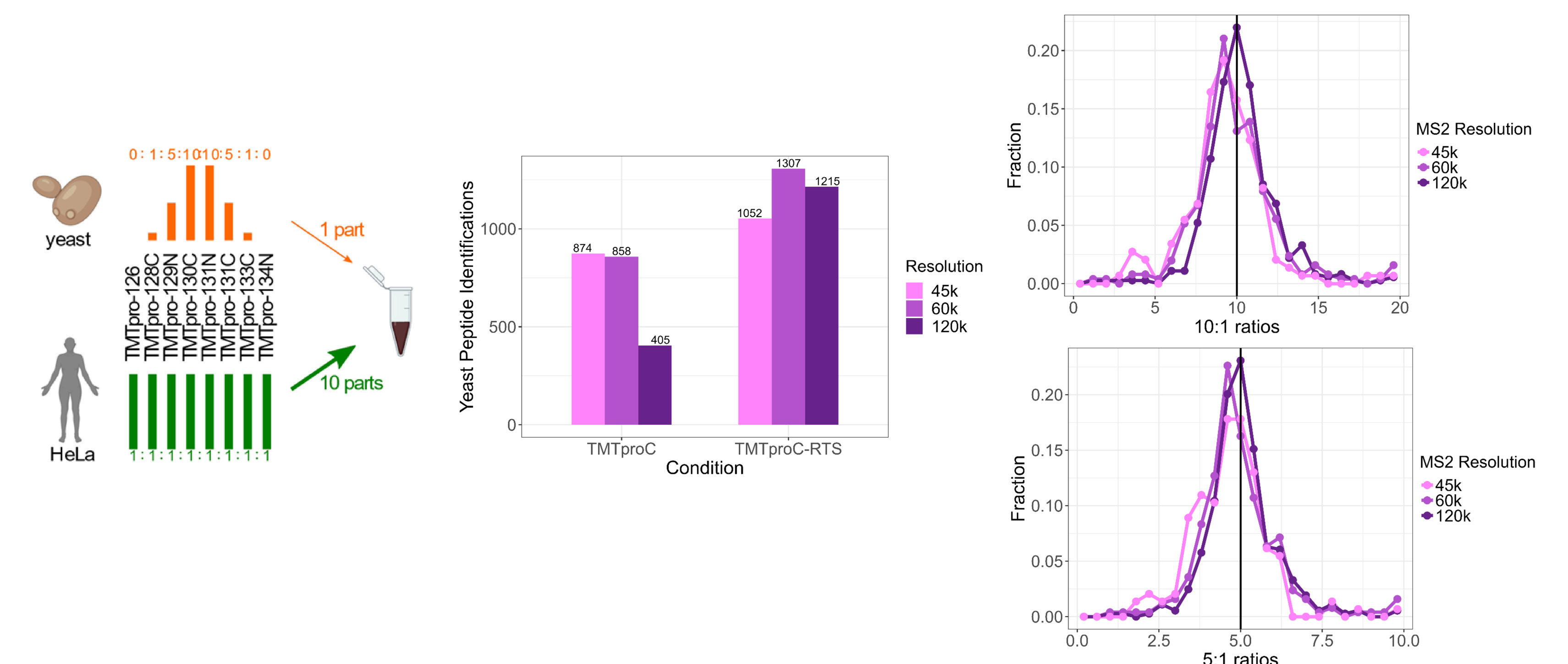
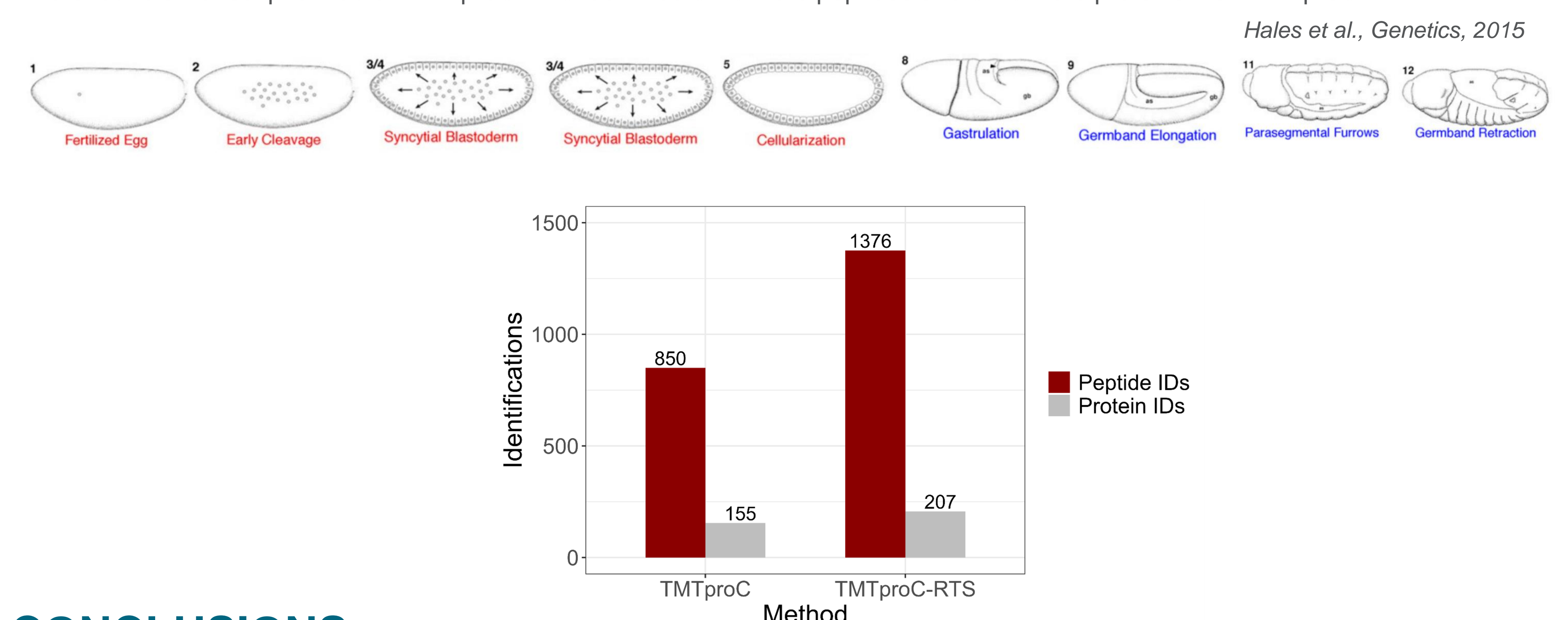


Figure 9. Application: quantifying transcription factors while a fruit fly egg develops into an embryo: Transcription factors are important in specifying cell fates during development. Drosophila melanogaster embryos were sampled throughout embryonic development. Peptides were labeled with a 9-plex of TMTproC and prefractionated into 24 fractions with medium pH reverse-phase HPLC. Each fraction was shot with TMTproC and TMTproC-RTS, with RTS filters set to only perform FTMS TMTproC scans if spectra could be matched to a transcription factor. TMTproC-RTS identified 62% more peptides and 34% more proteins than TMTproC.

Hales et al., Genetics, 2015



CONCLUSIONS

- ❑ TMTproC circumvents the ratio distortion problem of multiplexed proteomics
- ❑ Real-Time Search (RTS) algorithms increase the sensitivity of SPS-MS3 methods.
- ❑ Combining Real-Time Search with TMTc increases peptide and protein identifications.
- ❑ Maximum ion injection time can be increased using TMTc-RTS with little loss of sensitivity.
- ❑ Sensitivity gains from TMTproC-RTS are more pronounced when targeting a subset of the proteome for FTMS2 scans.
- ❑ Quantification accuracy and precision can be improved without significant loss of sensitivity

REFERENCES

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ACKNOWLEDGEMENTS

G.M., W.B., J.C., J.H., and V.Z. are employees of Thermo Fisher Scientific. Thermo Fisher provides limited support to M.W. laboratory under a non disclosure agreement with Princeton University. M.W. acknowledges funding from NIH grant R35GM128813, DOE grant DE-SC0018260, and Eric and Wendy Schmidt Transformative Technology Fund.

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