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RESEARCH ARTICLE

Predicting fragment intensities and retention time of iTRAQ- and TMTPro-labeled peptides with Prosit-TMT

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Abstract

Isobaric labeling increases the throughput of proteomics by enabling the parallel identification and quantification of peptides and proteins. Over the past decades, a variety of isobaric tags have been developed allowing the multiplexed analysis of up to 18 samples. However, experiments utilizing such tags often exhibit reduced identification rates and thus show decreased analytical depth. Re-scoring has been shown to rescue otherwise missed identifications but was not yet systematically applied on isobarically labeled data. Because iTRAQ 4/8-plex and the recently released TMTpro 16/18-plex share similar characteristics with TMT 6/10/11-plex, we hypothesized that Prosit-TMT, trained exclusively on 6/10/11-plex labeled peptides, may be applicable to these isobaric labeling strategies as well. To investigate this, we re-analyzed nine publicly available datasets covering iTRAQ and TMTpro labeling for samples with human and mouse origin. We highlight that Prosit-TMT shows remarkably good performance when comparing experimentally acquired and predicted fragmentation spectra (R of 0.84 - 0.9) and retention times (ΔRT95% of 3% - 10% gradient time) of peptides. Furthermore, re-scoring substantially increases the number of confidently identified spectra, peptides, and proteins.

KEYWORDS
Fragment intensity prediction, iTRAQ, Prosit, Retention time prediction, TMTPro

1 | INTRODUCTION

Accurate peptide identification is an indispensable step in all proteomics experiments [1]. However, the most commonly used tools for peptide identification to date do not utilize all information captured in tandem mass spectra, as they typically match in silico generated unit-intensity fragmentation spectra to experimentally acquired tandem mass spectra [2]. Over the last years, different deep learning models were developed that can predict the expected fragment ion intensities of peptides with very high accuracy [3–9]. A particularly interesting application of such predictions is their incorporation into the process of peptide identification either by spectral library searching or, more commonly used to date, by re-scoring database search engine results.

Re-scoring has been shown to hold the potential to substantially increase the confidence and number of peptide identifications [10–12]. While initial research focused on the application on unlabeled peptides, the concept was recently shown to drastically increase the sensitivity and specificity also for data utilizing tandem mass tag (TMT) labeled

Abbreviations: iRT, indexed retention time; iTRAQ, isobaric tags for relative and absolute quantitation; iTRAQ4, iTRAQ 4-plex; iTRAQ8, iTRAQ 8-plex; RT, retention time; SA, normalized spectral contrast angle; TMT, tandem mass tag; TMTpro, proline-based reporter isobaric tandem mass tag structure; TMT16, TMTpro 16-plex; TMT18, TMTpro 18-plex.

peptides [13]. This type of multiplexing received a lot of attention over the last years because it parallelizes the analysis of multiple samples and thus increases the scalability of bottom-up proteomics [14, 15]. All commonly used isobaric labels [16–19] used to date utilize a similar mechanism and the tags share similar characteristics [20]. However, intensity prediction by machine learning models is only available for TMT 6/10/11-plex and isobaric tags for relative and absolute quantitation (iTRAQ) 4-plex accurate [4, 13].

Here, we report that Prosit-TMT, a model recently developed for TMT 6/10/11-plex labeled peptides, is applicable to peptides labeled with iTRAQ, (4/8-plex) or proline-based reporter isobaric tandem mass tag structure (TMTpro, 16/18-plex). To show this, we start by systematically comparing fragment ion intensity and RT predictions from Prosit-TMT to experimentally acquired data from nine datasets covering different iTRAQ and TMTpro labels, fragmentation methods, and sample complexities. Prosit-TMT shows remarkably good performance for spectra (Pearson correlation of 0.84 - 0.9) and RTs (ΔRT95% of 3 - 10% gradient time). Last, re-scoring of the nine datasets with Prosit-TMT increases the number of peptide spectrum matches, peptides, and proteins by on average 42%, 40%, and 31%, respectively.

Statement of the Significance

Re-scoring, utilizing fragment intensity and other peptide property predictions, has been successfully used to increase the confidence and number of identified peptides. Particularly for isobaric labeling strategies, increasing the number of confidently identified spectra is highly desirable as it provides new identification and quantification information with every spectrum. However, current prediction models are only available for a subset of commonly used labeling strategies. Here we investigated the generalization of Prosit-TMT to iTRAQ and TMTpro on nine publicly available datasets. The results show that Prosit-TMT is generally applicable to other isobaric labelling strategies and increases the number of peptide spectrum matches, peptides, and proteins by an average 42%, 40%, and 31%, respectively.

2 | MATERIALS AND METHODS

2.1 | External data processing

The raw MS data from six studies were downloaded from PRIDE [21, 22] and grouped into nine datasets (Table 1). Each dataset was re-processed using MaxQuant 2.0.1 [2] using the parameters as mentioned in the original studies. However, for later re-scoring no false discovery rate (FDR) filtering on PSM, peptide, and proteins level was performed at this stage. For the datasets using TMT 16- and 18-plex labeling, TMTpro was added to the MaxQuant configuration and specified as a fixed modification.

2.2 | Fragment ion intensity and RT prediction with Prosit-TMT

For fragment ion intensity and RT prediction of iTRAQ and TMTpro labeled peptides, the Prosit-TMT intensity and RT model 2021 [13] was used. In both cases, the iTRAQ and TMTpro label was replaced by the TMT6 label for prediction. For fragment intensities, the resulting fragment masses were shifted to the correct m/z values based on the mass shifts induced by iTRAQ or TMTPro.

2.3 | Re-scoring workflow

The re-scoring was performed as described in Gabriel et al.[13]. Briefly, to utilize the RT predictions, the re-scoring pipeline described in Gessulat et al. [10] was extended. First, PSMs were filtered to 1% FDR using

TABLE 1 List of PRIDE datasets processed indicating the PXD identifiers, original publication, the isobaric label used, organism analyzed, sample type, acquisition method, and dataset name referred to in this study

ProteomeXchange identifier	Reference	Label	Organism	Sample	MS/MS acquisition method	Dataset name
PXD030340	Gabriel et al. [13]	TMT6	Human/Yeast	cell line	HCD/CID IT/OT	TMT6
PXD017472	Yang et al. [35]	iTRAQ4	mouse	serum	HCD/OT	D1
PXD017472	Yang et al. [35]	iTRAQ4	human	serum	HCD/OT	D2
PXD017621	Sánchez-González et al. [36]	iTRAQ4	mouse	skeletal muscle	HCD/OT	D3
PXD002214	Preil et al. [37]	iTRAQ8	human	artery	HCD/OT	D4
PXD021401	Kim et al. [38]	iTRAQ8	human	cervicovaginal fluid	HCD/OT	D5
PXD027089	Ramchandani et al. [39]	TMT16	mouse	breast and lung tissue	CID/IT	D6
PXD027089	Ramchandani et al. [39]	TMT16	human	breast and lung tissue	CID/IT	D7
PXD024275	Li et al. [19]	TMT16	human	cell line	CID/IT	D8
PXD024275	Li et al. [19]	TMT18	human	cell line	CID/IT	D9

the normalized spectral contrast angle (SA) calculated between the intensity prediction and the observed spectra. For this, a linear discrimination analysis was applied on PSMs level to estimate the FDR. For RT alignment, a lowess fit between predicted indexed retention time (iRT) and experimental RT was used. In order to stabilize the lowess fit, outliers were recursively filtered by computing the median absolute error between predictions and observations. When the median absolute error was > 0.02, outliers were removed. Initially, 0.1% of outliers are removed. The percentage is increased exponentially to a maximum of 50%. Finally, RT, predicted iRT, transformed iRT, and the absolute difference between transformed RT and experimental time are added as features to Percolator [23]. We used Percolator for PSM and peptide FDR estimation, followed by the picked FDR approach [24] for protein FDR estimation.

2.4 | Post processing

To calculate RT difference (ΔRT), we divide the absolute error observed between the predicted and observed RT by the gradient length. The resulting measure describes the related ΔRT in relation to the gradient time for any given experiment (ΔRT%).

For the quantitation analysis, we selected protein groups identified by both Andromeda and Prosit, that only represented a single gene. These were further filtered for groups with more than one PSM uniquely identified with Prosit. After filtering, we calculated the Pearson correlation of the sum of all reporter intensities of the shared (Prosit and Andromeda) PSMs for that protein group 1) to the PSMs uniquely identified by Prosit that are assigned to this protein group (target) and 2) to Prosit-unique PSMs from other protein groups (background).

3 | RESULTS AND DISCUSSION

3.1 | Prediction performance for iTRAQ 4/8-plex labeled peptides

TMT and iTRAQ both use amine-specific reactive groups (N-hydroxysuccinimide chemistry) that react with free N-terminal amino groups and lysine side chains of a polypeptide. The main difference between these isobaric labeling strategies is the molecular design and structure of the balancer and reporter group (Figure 1A, insets showing the structures for each isobaric label; flag symbol for iTRAQ 8-plex indicates proprietary structure of the balancer group). However, the differences fragmentation characteristics induced by these were reported to be minimal and thus allow the inter-conversion of spectra from one label to the other [25]. Prosit-TMT was trained to predict the fragment ion intensities (Prosit TMT intensity 2021) of up to triply charged b- and y-ions and the indexed retention time (Prosit TMT iRT 2021) for TMT 6/10/11-plex (TMT6) labeled peptides with a maximum length of 30 amino acids. Thus, we hypothesized that its predictions could be used to predict iTRAQ4 and iTRAQ8 labeled peptides as well.

To test this, we downloaded and re-analyzed five datasets (D1-5, see Methods) that used iTRAQ4 (D1, D2, D3) or iTRAQ8 (D4, D5) labeling. The confidently (< 1% FDR) identified peptide spectrum matches (PSMs) from the MaxQuant analysis were then compared to predictions generated by Prosit-TMT. This is possible because the fragment ion masses are added separately after Prosit's predictions (the neural network is unaware of the fragment masses), which enabled us to superimpose the TMT6 fragment intensities on the calculated fragment masses of iTRAQ labeled peptides (see Methods). In line with data acquisition, the higher-energy CID (HCD; also referred to as beam-type CID) fragmentation model was used for prediction. The normalized collision energy (NCE) used for prediction was calibrated for each dataset (see Methods). Figure 1A shows two exemplary mirror spectra where predictions by the Prosit-TMT model are compared to the experimental spectra of an iTRAQ4 (Figure 1A top mirror spectrum for APIRPDIVNFVHTNLR) or iTRAQ8 (Figure 1A bottom mirror spectrum for DIQMTQSPSSLSASVGDR) labeled peptide. In both cases, the spectral similarity reaches high levels of agreement indicated by the high SA of ∼0.71 and Pearson correlations (R) of ∼0.85. This is substantially higher than what was achieved when using Prosit models that were learned on unlabeled peptides (SA∼0.55 and R∼0.63, Supplementary Figure S1). Somewhat surprisingly, the prediction accuracy achieved with the base Prosit model appears to be different for y- and b-ions when using the Prosit model trained on unlabeled peptides. While the fragment intensities of y-ions are surprisingly well conserved, the b-ion intensities show a much more erratic characteristic. This may be caused by the fact that for both peptides, only the b-ion series contains the iTRAQ modification (no C-terminal lysine), and thus the y-ion intensities are largely unaffected, which is supported by a systematic investigation into the b- and y-ion similarities for peptides which contain no missed-cleavage ending in either lysine or arginine (Supplementary Figure S2A-E).

To systematically confirm the high level of agreement of the Prosit-TMT intensity model with acquired spectra of iTRAQ-labeled peptides, we compared 17,420 and 35,718 confident PSMs of datasets D1 and D4, respectively, against their predictions (Figure 1B, top panel for iTRAQ4, bottom panel for iTRAQ8). A median SA of 0.74 and 0.73 (R of 0.87 and 0.85) for iTRAQ4 and iTRAQ8, respectively, indicates that the exemplary mirror spectra represent the median performance that can be expected for any prediction. As expected, the overall performance of Prosit-TMT on iTRAQ is reduced when compared to TMT6-labeled peptides which achieves a median SA of 0.89 (R of 0.97) for HCD fragmentation. The results shown here for iTRAQ4 and iTRAQ8 are consistent across the five investigated datasets (Supplementary Figure S3A-C for D2, D3, and D5).

To evaluate the performance of Prosit TMT iRT 2021 model on iTRAQ-labeled peptides, we compared predicted and observed aligned RT, (see Methods) of labeled peptides, respectively. Figure 1C shows the distribution of the absolute retention time error (ΔRT%) in relation to the gradient time (percent of gradient; ΔRT95%) for iTRAQ4 (top)

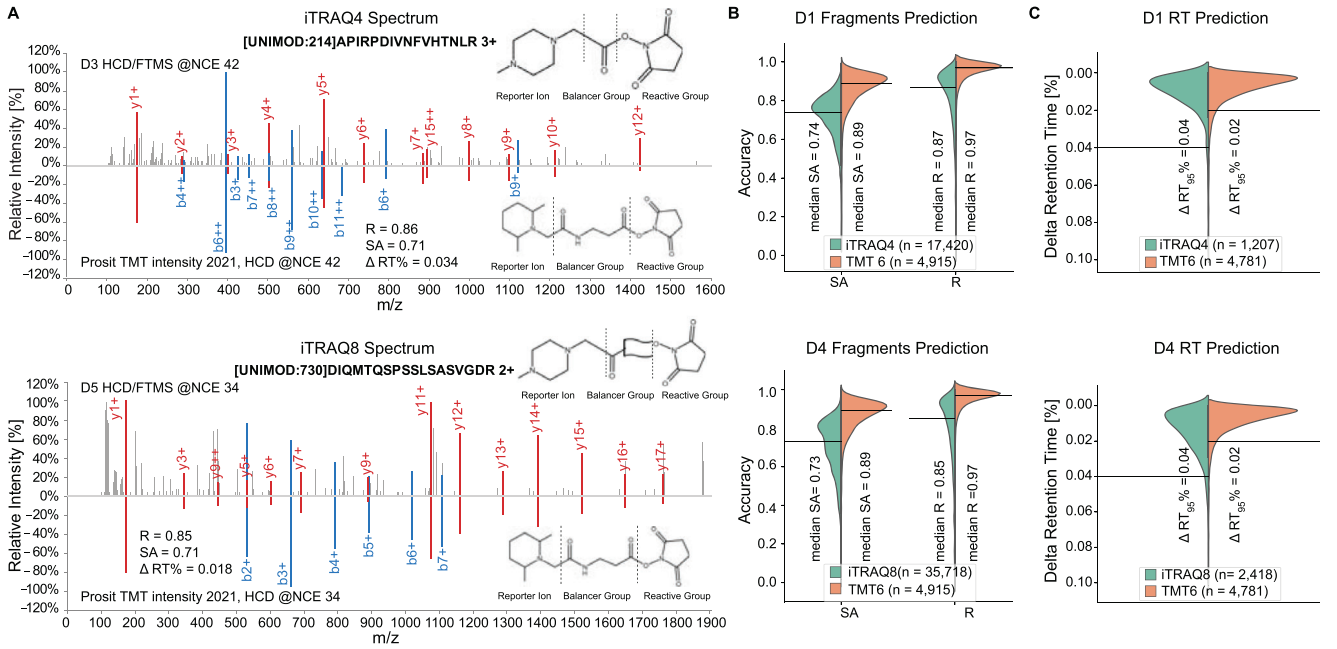

FIGURE 1 Prosit-TMT prediction performance for iTRAQ-labeled peptides. (A) Spectrum mirror plots of an iTRAQ4 labeled peptide (top) and iTRAQ8 labeled peptide (bottom) comparing an experimental spectrum (top spectrum in each) to the corresponding prediction performed by Prosit-TMT (bottom spectrum in each). The top mirror plot shows the triply charged peptide APIRPDIVNFVHTNLR from dataset D3 acquired at normalized collision energy (NCE) of 42 fragmented using HCD. The bottom mirror plots show the doubly charged peptide DIQMTQSPSSLSASVGDR acquired at NCE of 34 using HCD fragmentation in dataset D5. Fragment ions are blue and red for b- and y-ions, respectively, and unannotated peaks are grey. Shared y-ions and b-ion are labeled in the top and bottom spectrum, respectively, only. The spectral similarity was measured by Pearson correlation (R) and normalized spectral contrast angle (SA) calculated on the matching b- and y-ions only. The difference between observed and predicted retention time (RT) is expressed in relation to the gradient length. (B) Bean plots showing the prediction accuracy of the Prosit TMT intensity 2021 model for iTRAQ4 labeled (top, green distribution) and iTRAQ8 labeled (bottom, green distribution) peptides compared to TMT6 labeled peptides (red distribution in each). Prediction accuracies are measured using SA (left pair of beans) and R (right pair of beans) metrics. The number of underlying spectra (n) is indicated at the bottom. The horizontal lines correspond to the median SA/R observed indicated. (C) Bean plots showing the prediction accuracy of the Prosit TMT RT 2021 model on iTRAQ4 labeled (top, green distribution) and iTRAQ8 labeled (bottom, green distribution) peptides compared to TMT6 labeled (red distribution in each) peptides. The performance was measured by the difference between predicted and observed retention time relative to the gradient length. The number of underlying peptides (n) is indicated at the bottom. The horizontal lines correspond to the interval necessary to capture 95% of the peptides (ΔRT95%)

and iTRAQ8 (bottom) in comparison to a conservative upper bound estimated by the corresponding TMT6 ΔRT% distribution. For both iTRAQ labels, the difference between the prediction and observation was only 4% of the total gradient length for 95% of the peptides. In comparison, for TMT6 95% of the peptides were within 2% of the total gradient length. When using the base Prosit model, peptides that contain no missed-cleavage terminating on arginine show a marginally lower ΔRT95% than peptides terminating on lysine (Supplementary Figure S4).

As observed earlier [10], despite Prosit being trained on peptides with human origin only, no bias was visible when comparing the prediction performance for datasets with human or mouse samples, indicating that Prosit-TMT and its application on iTRAQ-labeled data may not be limited to a specific species. Although the analysis shows that Prosit-TMT performs slightly worse for iTRAQ than for TMT6, the predictions are much more accurate in comparison to using a Prosit model that was trained for unlabeled peptides.

3.2 | Prediction performance for TMTpro 16/18-plex labeled peptides

Supported by the results obtained from the analysis on iTRAQ, we hypothesized that similar results may be observed when comparing Prosit-TMT predictions to TMTpro 16- (TMT16) or 18-plex (TMT18) labeled peptides. Similar to iTRAQ, TMTpro largely only differs in the specifics of the balancer and reporter group from the TMT6 (Figure 2A). We downloaded, processed and filtered four datasets (D6-D9, see Methods) that used TMT16 (D6, D7, D8) and TMT18 (D9) labeling. The confidently (< 1% FDR) identified PSMs from the MaxQuant analysis were then compared to predictions where Prosit-TMT fragment intensities were superimposed on TMT16 or TMT18 calculated fragment masses (see Methods). All four datasets were acquired using CID fragmentation and thus NCE calibration was not necessary. The SA of ~0.76 and R of ~0.89 for the two exemplary mirror spectra for TMT16 (Figure 2A top mirror spectrum for

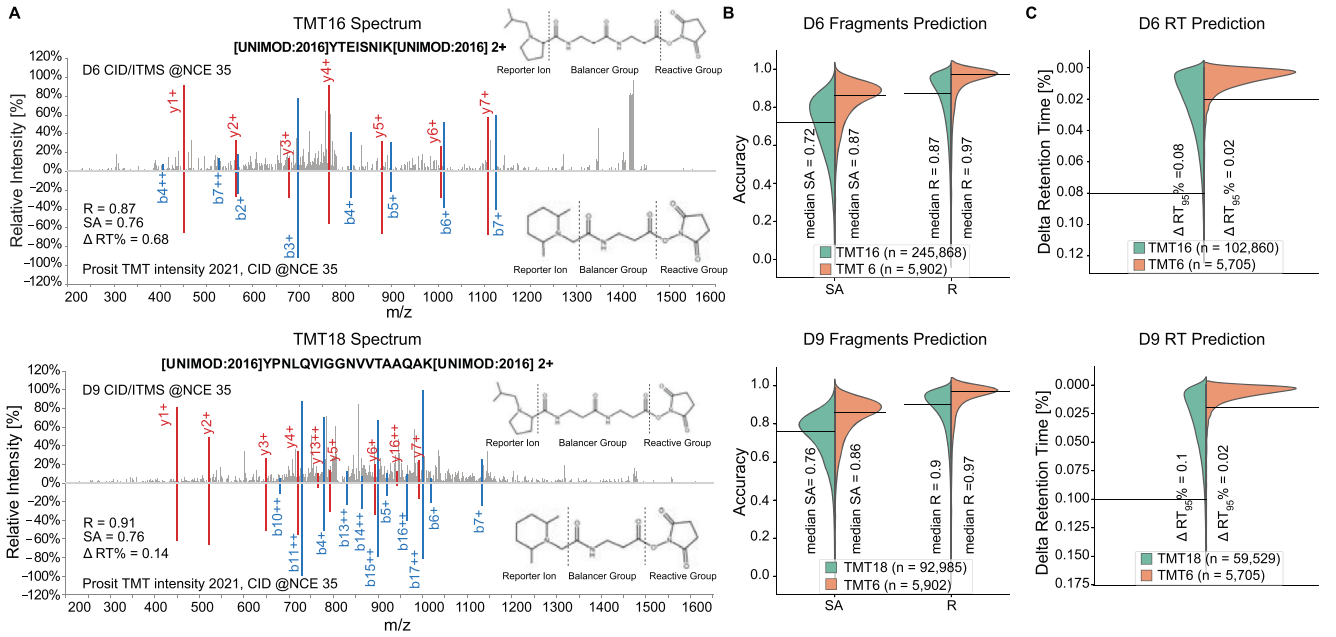

FIGURE 2 Prosit-TMT prediction performance for TMTpro-labeled peptides. (A) Spectrum mirror plots of an TMT16 labeled peptide (top) and TMT18 labeled peptide (bottom) comparing an experimental spectrum (top spectrum in each) to the corresponding prediction performed by Prosit-TMT (bottom spectrum in each). The top mirror plot shows the triply charged peptide YTEISNIK from dataset D6 acquired at collision energy (CE) of 35 fragmented using CID. The bottom mirror plots show the doubly charged peptide YPNLQVIGGNVVTAAQAK acquired at CE of 35 using CID fragmentation in dataset D9. Fragment ions are blue and red for b- and y-ions, respectively, and unannotated peaks are grey. Shared y-ions and b-ion are labeled in the top and bottom spectrum, respectively, only. The spectral similarity was measured by Pearson correlation (R) and normalized spectral contrast angle (SA) calculated on the matching b- and y-ions only. The difference between observed and predicted retention time (RT) is expressed in relation to the gradient length. (B) Bean plots showing the prediction accuracy of the Prosit TMT intensity 2021 model for TMT16 labeled (top, green distribution) and TMT18 labeled (bottom, green distribution) peptides compared to TMT6 labeled peptides (red distribution in each). Prediction accuracies are measured using SA (left pair of beans) and R (right pair of beans) metrics. The number of underlying spectra (n) is indicated at the bottom. The horizontal lines correspond to the median SA/R observed indicated. (C) Bean plots showing the prediction accuracy of the Prosit TMT RT 2021 model on TMT16 labeled (top, green distribution) and TMT18 labeled (bottom, green distribution) peptides compared to TMT6 labeled (red distribution in each) peptides. The performance was measured by the difference between predicted and observed retention time relative to the gradient length. The number of underlying peptides (n) is indicated at the bottom. The horizontal lines correspond to the interval necessary t capture 95% of the peptides (ΔRT95%)

YTEISNIK) and TMT18 (Figure 2A bottom mirror spectrum for YPNLQVIGGNVVTAAQAK) indicate high levels of agreement. Similar to iTRAQ, the position and overall presence/absence of peaks is matched between predictions and observations and is substantially higher than what was achieved when using a Prosit model that was trained on unlabeled peptides (Supplementary Figure S5). However, contrary to what was observed for iTRAQ, the SA of b- and y-ions for peptides containing no missed-cleavage when predicted with the base Prosit model shows that y-ion prediction correlates very poorly irrespective of the terminal amino acid (Supplementary Figure S2F-I). This suggests that the presence of a TMTpro tag has a more long-range effect on fragmentation in comparison to iTRAQ, which may be due to the use of a proline-based reporter.

The spectra similarity distribution on the 245,868 and 92,985 confident PSMs from the TMT16 datasets D6 and TMT18 dataset D9 showed a similar pattern as for iTRAQ labeled peptides (Figure 2B). The median SA reaches 0.72 (R of 0.87) and 0.76 (R of 0.9) for TMT16 and TMT18, respectively, but shows decreased performance in comparison to the conservative upper bound estimated by the existing TMT6 model, which surpasses SAs of ~0.86 (R of 0.97) in half of the cases. The RT prediction performance for both TMT16 and TMT18 appears to be worse in comparison to iTRAQ as an RT tolerance of about 8% for TMT16 and 10% for TMT 18 of the gradient is necessary to capture 95% of the peptides (Figure 2C). This may be the result of TMTpro inducing a stronger RT shift, which is supported by the expectation that the reporter and longer balancer group lead to more hydrophobic peptides in comparison to TMT6. The results for fragment ion intensity and RT prediction are consistent across the four investigated datasets that used TMTpro labeling (Supplementary Figure S3D-E for D7-8).

3.3 | Re-scoring drastically increases identified peptide spectrum matches, peptides and proteins for iTRAQ and TMTpro labeled data

We and others have shown that re-scoring, an approach where predicted fragment ion intensities and RTs of peptides are used for scoring peptide spectrum matches, can drastically increase the number of

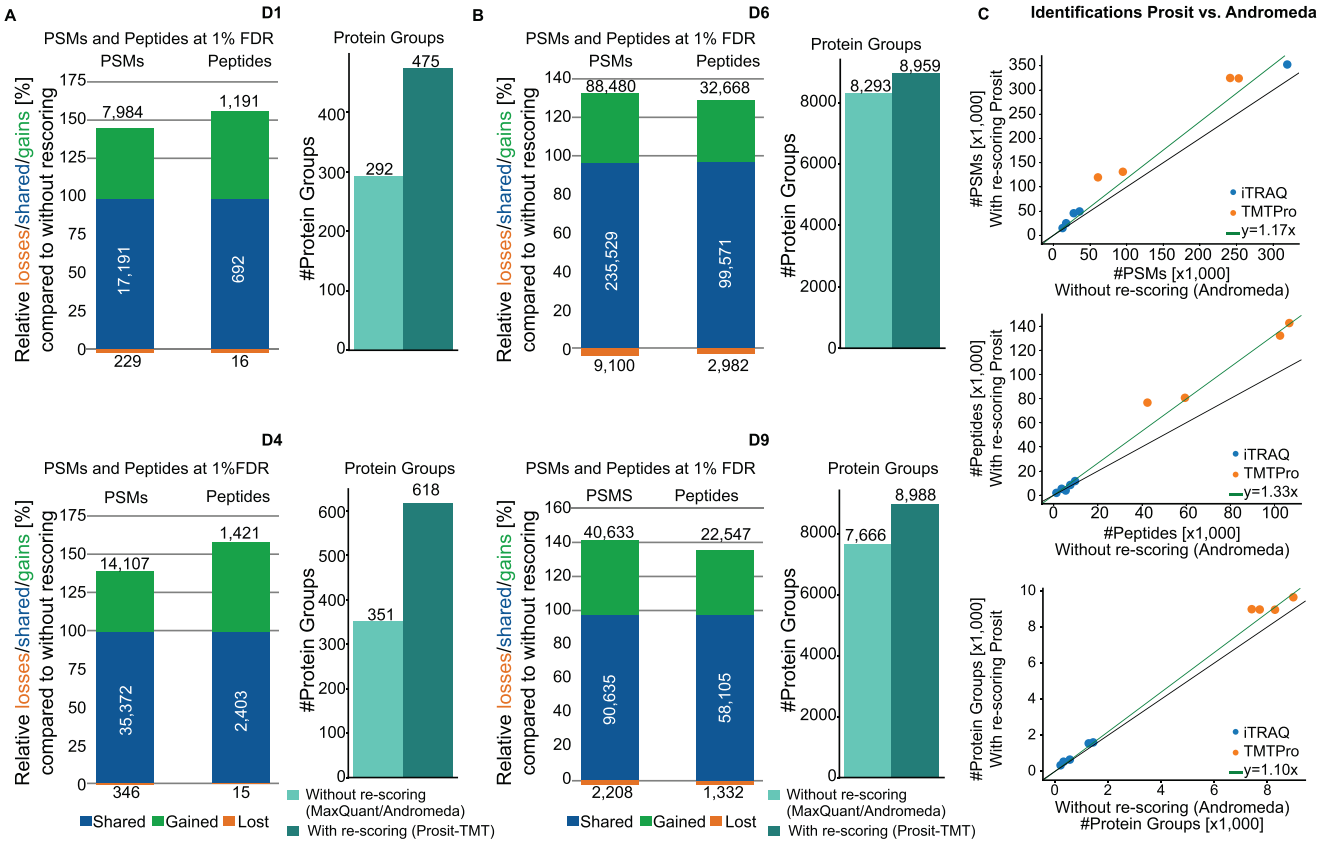

FIGURE 3 Rescoring iTRAQ and TMTPro datasets using Prosit-TMT. (A) Simplification of a Venn diagram (Vennbar, first column) showing the number of confident PSMs (left) and peptides (right) below 1% FDR lost (orange), shared (blue), and gained (green) when using re-scoring (Prosit-TMT) compared to without re-scoring (MaxQuant/Andromeda) results. Barplot (second column) showing the number of confidently identified protein groups either with (Prosit-TMT) or without rescoring (MaxQuant/Andromeda). First row shows the results when re-scoring the iTRAQ4 dataset D1 and the last row for the iTRAQ8 dataset D4. (B) Same as A but for the TMT16 dataset D6 (first row) and the TMT18 dataset D9 (second row). (C) Scatter plot showing the number of identifications with rescoring (Prosit-TMT, y-axis) as a function of the number of identifications without rescoring (MaxQuant/Andromeda, x-axis) for PSMs, peptides, and proteins (top to bottom). Points are colored blue for iTRAQ and orange for TMTPro datasets. The solid black line indicates the diagonal (y = x), whereas the green line shows a regression fit to all datasets without a variable intercept

confidently identified spectra, peptides, and proteins [4, 10, 11, 26, 27]. We have recently shown that this is particularly the case for TMT6 labeled peptides. However, prior research also indicates that very high prediction accuracies - as achieved by, for example, Prosit when trained and applied on the same peptide class reaching a median SA of 0.9 (R~0.99) - might not be necessary for re-scoring [12]. Taken together, we hypothesized that we should observe gains in PSMs, peptides, and proteins for iTRAQ and TMTpro data when using Prosit-TMT, even though this model was not trained to yield optimal predictions. To investigate this, we systematically re-scored the nine investigated datasets (see Methods), covering CID and HCD fragmentation, ion trap (IT) and Orbitrap (FTMS) readout, iTRAQ and TMTpro labeling, and samples from human and mouse origin.

The results obtained from re-scoring for four representative datasets (D1, D4, D6, D9) are visualized in Figure 3. Starting with iTRAQ4 (Figure 3A, top panels, D1), we observed a substantial gain in confidently identified PSMs (17,420 to 25,175), peptides (708 to 1,883) and proteins (292 to 475) of ~ 40%, ~ 50%, and ~ 60%, respectively. The 183 rescued proteins are on average supported by 3 peptides suggesting that these are not the result of random (false positive) matches. For iTRAQ8 (Figure 3A, bottom panels, D4), a similar increase in PSMs (35,718 to 49,479), peptides (2,418 to 3,824) and proteins (351 to 618) of ~ 35%, ~ 50%, and ~ 75%, respectively, was observed. Also here, the rescued protein identifications are supported by on average of 3.6 peptides. This trend is conserved across the five investigated iTRAQ datasets (Supplementary Figure S6A-C) and in single cases gains of up to 70% on PSM, 50% on peptide level, and 75% protein level were observed for iTRAQ. The results for TMT16 (Figure 3B, top panels, D6) and TMT18 (Figure 3B, bottom panels, D9) exhibited similar gains of ~ 35% on PSM, ~ 30% on peptide, and ~ 10% protein level.

In line with earlier observations, re-scoring particularly boosts the confidence of low abundant proteins and peptides. All studies using iTRAQ investigated either body fluids (very large dynamic range of protein expression) or specimens with limited sample amounts (see Methods, Supplementary Figure S6). In these cases, a larger number

of low signal-to-noise spectra is acquired. Because re-scoring does not solely rely on the number of matched fragment ions but includes metrics that score the relative intensity patterns, many of such spectra can still result in high scores and thus survive FDR filtering, ultimately leading to the confident identification of more peptides and often more proteins. However, even the TMTpro datasets where the increase on protein levels was not as substantial as for iTRAQ benefit from re-scoring as every confidently identified spectrum and peptide adds additional data points for quantification. To investigate the validity of the added PSMs further, we compared the reporter intensity pattern of a protein group to PSMs uniquely identified by re-scoring (see Methods). We observed that the reporter intensities of a protein group correlates very well with Prosit-unique PSMs assigned to the same protein group, whereas this correlation drops substantially for PSMs uniquely identified by re-scoring assigned to other protein groups (Supplementary Figure S7). This indicates that the PSMs uniquely identified by re-scoring are very likely valid hits.

On average, 42%, 40%, and 31% more PSMs, peptide and proteins where identified by re-scoring for the nine datasets. When fitting a linear model on the number of identification with re-scoring as a function of identifications without re-scoring, the slopes highlight an expected increase of 17% in PSMs, 33% for peptides, and 10% for proteins (Figure 3C). No bias for the increase on PSM, peptide and protein level was observed with respect to fragmentation, the mass analyzer, labeling, and species, indicating that the re-scoring approach by Prosit-TMT is generally applicable to datasets using the here investigated isobaric tags.

3.4 | Concluding remarks

Prosit-TMT (for TMT6) is applicable to iTRAQ, and TMTpro labeled peptides due to the similar design of different isobaric tags. While the overall achieved prediction accuracy is slightly worse than expected when training specific models for these labels, it does not appear to impair re-scoring when compared to the TMT6 labeled peptides as evident by the substantial gains in PSMs, peptide and protein groups. However, particularly for RT prediction, models supporting different modifications may assist the discrimination between correct and incorrect matches even further [9]. The extension of Prosit-TMT to iTRAQ and particularly TMTpro enables a large community to benefit from recent advances in machine learning and data processing and we expect to see more studies utilizing the re-scoring approach in the future. To this day, Prosit has been used >6 billion times to predict the fragment ion intensities or RT of peptides, indicating that there is great interest in accurate predictions. Additional opportunities for such predictions are its integration into real-time searches that may further increase the efficiency of data acquisition or result in improved quantification due to the accurate selection of interference-free fragments for quantification [28].

To the best of our knowledge, Prosit-TMT is the only model that offers the prediction of fragment intensities or RT of iTRAQ8 and TMTpro labeled peptides. For iTRAQ4, MS2PIP provides a pre-trained model that achieves a median R of ~0.9 [29]. However, this model only predicts the intensities of singly charged fragment ions, while Prosit-TMT is capable of predicting fragment ion intensities of up to triply charged ions with a median R of 0.87. Comparing the prediction accuracies for singly charged fragments only, Prosit-TMT does seem to perform marginally worse (median R of 0.89 for Prosit-TMT) than MS2PIP.

We observed that the increase a user may expect when using re-scoring is mostly dependent on the experimental design, for example, the number of batches, the extent of offline fractionation, and - most importantly - the biological material used (e.g., body fluids vs. cell lines). The functionality to re-score iTRAQ and TMTpro labeled datasets, generate spectral libraries or perform NCE calibration is available online (https://www.proteomicsdb.org/prosit) and our previously developed universal spectrum viewer (https://www.proteomicsdb.org/use) supports the prediction of iTRAQ and TMTpro labeled peptides by the Prosit-TMT model as well.

Previous research has shown that traditional search engines may yield fewer identifications for iTRAQ8 and TMT6 than for iTRAQ4, likely as a result of a number of tag-less ions being generated during fragmentation which are not accounted for by the search engine scores [30]. Our implementation of re-scoring only considers matched fragments during score calculation and does not penalize for non-annotated peaks. This was a conscious decision due to the fact that Prosit only predicts the intensities of singly to triply charged b- and y-ion fragments and the potentially large number of additional ions which may be generated during fragmentation (e.g., neutral losses, immonium ions and remnants of the precursor) or co-isolation would otherwise distort the similarity calculation. To avoid this, extending Prosit by additional fragment types is a promising future avenue which is likely particularly interesting for confident site localization of modifications [31–33]. For isobaric labels, another interesting avenue is the prediction of the summed reporter ion intensity which could be used in combination with Prosit's unique ability to predict intensities in a NCE-dependent manner to optimize fragmentation for either identification or quantification for targeted assays and real-time peptide identification [15, 34].

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CONFLICT OF INTEREST

Mathias Wilhelm is a founder and shareholder of MSAID GmbH and OmicScouts GmbH, with no operational role in either companies.

DATA AVAILABILITY STATEMENT

The mass spectrometric raw and search data including intermediate results underlying the presented analysis have been deposited with the ProteomeXchange Consortium via the PRIDE repository with the dataset identifier PXD031575.

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SUPPORTING INFORMATION

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