Universal Spectrum Explorer: A standalone (web-)application for cross-resource spectrum comparison

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Running Title: Web-based mirror spectrum viewer

Abbreviations

API - Application Programming Interface

CE - Collision Energy

DDA - Data-dependent Acquisition

DIA - Data-independent acquisition

EuBIC - European Bioinformatics Community

FDR - False Discovery Rate

HCD - Higher-energy Collision Dissociation

HLA - Human Leukocyte Antigen

HRAM - High-Resolution and Accurate Mass

IPSA - Interactive Peptide Spectral Annotator

MS - Mass Spectrum

MSMS - Tandem Mass Spectra

NPM - Node Package Manager

PCC - Pearson’s Correlation Coefficient

PRM - Parallel Reaction Monitoring

PSM - Peptide Spectrum Match

PTM - Post-Translational Modification

REST - Representational State Transfer

SA - Spectral Angle

USE - Universal Spectrum Explorer

USI - Universal Spectrum Identifier

Abstract

Here we present the Universal Spectrum Explorer (USE), a web-based tool based on IPSA for cross-resource (peptide) spectrum visualization and comparison (https://www.proteomicsdb.org/use/). Mass spectra under investigation can either be provided manually by the user (table format), or automatically retrieved from online repositories supporting access to spectral data via the universal spectrum identifier (USI), or requested from other resources and services implementing a newly designed REST interface. As a proof of principle we implemented such an interface in ProteomicsDB thereby allowing the retrieval of spectra acquired within the ProteomeTools project. In addition, USE can retrieve real-time prediction of tandem mass spectra from the deep learning framework Prosit. Comparison results like annotated mirror spectrum plots can be exported from USE as editable scalable high quality vector graphics. The USE was designed and implemented with minimal external dependencies allowing local usage and seamless integration into websites (https://github.com/kusterlab/universal_spectrum_explorer).

Introduction

In mass spectrometry (MS), peptide spectrum matches (PSMs) of tandem mass spectra (MSMS) play a fundamental role as they are prerequisites for various workflows. PSMs most commonly originate from data dependent acquisition (DDA) experiments, where MSMS spectra are matched against in silico generated fragmentation patterns(1). For targeted proteomics, such as parallel reaction monitoring (PRM)(2) previously generated PSMs are a prerequisite for the confident detection and quantification of peptides. In contrast to DDA experiments, the incorporation of fragment intensities plays a crucial role in order to increase the specificity of the detection. For data independent acquisition (DIA)(3) and sequential window acquisition of all theoretical mass spectra (SWATH-MS)(4) collections of PSMs in the form of spectral libraries are a prerequisite for data analysis.

Being the foundation for (almost) all proteomic experiments, the correctness of PSMs is of utmost importance. This is particularly the case when investigating e.g. potential single amino acid permutations(5), alternative reading frames(6), novel HLA peptides are used to decide on the presence of clinically relevant biomarkers or tumor (neo-) antigens(7). Although methods exist to control the false discovery rate(8), with the ever increasing speed of mass spectrometers the absolute number of false positive identifications will increase accordingly(9, 10). In addition, previous research has shown that false positives can accumulate and surpass the defined global FDR in certain peptide classes when not controlled for specifically(11).

One possible mechanism to verify and validate the correctness of certain peptide identification events is the manual verification by comparing acquired MS/MS spectra to reference spectra. Over the last years, the number of available (reference) spectra has increased drastically with the widespread adoption and use of public repositories (12-14) the implementation of a Universal Spectrum Identifier (USI) Deutsch et al. (submitted) to make these available, large resources systematically measuring synthetic peptides(15) and the possibility to predict spectra of any (largely unmodified) peptide at unprecedented accuracy(16-19). Unfortunately,

the retrieval of such reference data and comparison is still cumbersome as no publicly accessible tool for such a task exists.

Here, we present a versatile web-based tool, the Universal Spectrum Explorer (USE, https://www.proteomicsdb.org/use), originated from the EuBIC-MS Developer's Meeting 2020(20), that allows the visualization and comparison of any two spectra. Each spectrum can be entered in three different ways. First, originating from a local source (i.e. spectrum extracted from a raw file) by uploading a peak list (tuples of mass to charge ratio (position) and relative/absolute intensity. Secondly, by providing the USI of a spectrum available in a public repository allowing people to access more than 470 million spectra in PRIDE alone. Third, by retrieval of spectral information from a newly defined and developed REST(21) interface. An example implementation of this is provided on ProteomicsDB(22, 23) and allows access to either spectra acquired in the ProteomeTools(24) project (more than 5 million spectra) or real-time predicted spectra by accessing our deep-learning framework Prosit(16). The settings of the USE are open to the users to interact with the spectra, allowing adjusting and zooming into spectra as well as highlighting of matched peaks. If the user is satisfied with the visualization, we offer a download in tabular format for reprocessing or an export of the figures as editable and scalable high quality vector graphics. The USE is based on the Interactive Peptide Spectrum Annotator(25) and its source code is available on Github (github.com/kusterlab/universal_spectrum_explorer).

Experimental Procedures

Software implementation

The code base of USE is separated into three independent packages. The proteomicsJS package provides first basic algorithms and functionalities to work with spectral data in Javascript. It has no special requirements, can be used in any Javascript framework and is accessible via the Node Package Manager (NPM) or directly on Github (https://github.com/kusterlab/proteomicsJS).

The USE (https://github.com/kusterlab/universal_spectrum_explorer) is the client-facing interactive web visualizer using AngularJS based on IPSA. Its sole purpose is to provide a user interface and leverages proteomicsJS for all use cases.

The USE_embedded (https://github.com/kusterlab/use_embedded) is a static executable available for all major operating systems (Mac, Windows and Linux) and architectures (ARM and AMD64). It provides convenient wrappers for the API of PRIDE, jPOST and PeptideAtlas circumventing the need to proxy requests through ProteomicsDB.

Additionally, it combines proteomicsJS as well as the USE to a single file and allows the USE to be easily distributed either on local machines or any server. If professional hosting is available, there is no need for the USE_embedded and the USE can be used directly.

Peak matching

The peak matching algorithm applied by USE is designed towards high-resolution, accurate-mass (HRAM) data by exactly matching peaks between two spectra according to their mass to charge ratio (m/z). Matched peaks must adhere to relative or absolute matching tolerance defined in ppm or Dalton. The matching procedure is outlined below:

INPUT: two arrays, named S_1 and S_2, of (m/z, intensity, peak_id) triplets sorted by m/z

OUTPUT: one array $S_{matched}$ of elements of m/z_1, $intensity_1$, $peak_id_1$, m/z_2, $intensity_2$, $peak_id_2$. Peaks of S_1 that couldn't be matched to a peak of S_2 are included in the array as well, but the m/z_2, $intensity_2$, $peak_id_2$ is set to NA. The same is applied to peaks of S_2. A peak can just occur once.

1. For every peak in S_1, search for the closest peak in the S_2 according to matching tolerances
    a. If multiple peaks in a queried spectrum match to a reference spectrum, select the most intense one and unassign the rest
2. Repeat step 1 but search for every peak in S_2 a matching partner in S_1
3. Concatenate the results of step 1 and 2 and remove duplicate peak pairings. In case of contradictory assignments of peaks, select the most summed intensity.

REST interface specification

The USE can receive spectral data from any web endpoint adhering to a minimal schema. The USE sends a request as a JSON encoded list of sequence, collision energy (CE), charge and modification strings. If a peptide is modified at multiple residues, the modifications are appended to each other and separated by comma. The expected output is mirroring the input by also being a JSON encoded list of sequence, precursor charge, collision energy as well as a list of ions (m/z, intensity).

A complete description can be examined at https://github.com/kusterlab/universal_spectrum_explorer/wiki.

Results

Design of USE

The newly developed Universal Spectrum Explorer (USE) is based on the web-based spectral analysis tool IPSA(25). The main scope of the USE is the comparison of any two spectra while maintaining most of the functionality originally implemented. For this, we optimized all core features (spectrum comparison and annotation) to be easily handled by modern browsers and, as long as no spectra from external resources are requested, the USE can be used entirely offline (locally). To ensure this, some functionalities of IPSA were re-implemented to support local execution. In addition, all functionalities were developed without the use of additional external libraries, minimizing dependencies and overhead. This not only enables future contributors to easily and quickly understand and extend the current functionalities but also minimizes required changes in the codebase resulting from changes in external libraries. As a result, no special software is required to execute the USE and can be opened directly with any browser. During development, we followed the test-driven development principles and achieved close to 80% code coverage.

For the comparison of any two spectra, the user is asked to define the source or spectral data of the top and bottom spectrum by either providing the spectral information directly, a USI, or information necessary to retrieve a spectrum from any other source implementing the newly developed interface. By this, the USE offers the functionality to allow the comparison of, for example, a locally acquired spectrum to a spectrum stored in PRIDE, a modified to an unmodified peptide spectrum, two spectra of the same peptide acquired at different collision energies or two in silico generated spectra from two different sources.

Data import and annotation

The simplest way to import data into the USE is the manual input tab where a user specifies general metadata such as the unmodified sequence and the precursor charge. The webform for the spectral data can be filled directly by pasting data from any spreadsheet program (Google sheets/excel), a csv-file or the output of Xcalibur Software of Thermo Fisher Scientific. Post-translational or other peptide modification can be either selected from a predefined dropdown menu or added manually by the user. Manually added modifications are stored locally and will neither be persistent nor available to other users (Figure 1a).

A second option is the retrieval of spectra from public resources by supplying a USI and resource of origin. The USE will then attempt to retrieve the spectrum and transfer the meta data and spectral data into the manual input (Figure 1b). This procedure allows the user to manually edit or extend the spectral data (Figure 1a) and ensures a consistent processing of the data.

Lastly, a new interface was defined allowing access to other resources not adhering to the USI standard (yet). This interface was implemented in ProteomicsDB allowing access to spectra acquired in the ProteomeTools project and direct access to Prosit for real-time spectrum prediction (limited currently to the monoisotopic y/b ions generated by HCD fragmentation). Similar to the manual user input tab, the peptide sequence, precursor charge and modifications are specified. In addition, the collision energy must be provided. Since the ProteomeTools resource might not (necessarily) contain the supplied CE, the spectrum with the closest matching collision energy is automatically selected (Figure 1c). If the data is requested from Prosit or ProteomeTools, similarly to the USI tab (Figure 1d), the metadata and spectral information is stored in the manual input for consistent spectral processing.

The original IPSA viewer focused on the comprehensive annotation of fragment peaks in a spectrum by utilizing a remote server for computation. We re-implemented the core annotation functions of the IPSA backend in JavaScript in order to minimize external dependencies. The annotation allows the highlighting of peaks in the spectrum viewer given a selection of

fragment types and annotation thresholds. All necessary parameters for annotation and visualization can be set in the metadata section of the viewer.

Spectrum similarity calculation

In order to assess the similarity of two spectra, peaks occurring in either spectrum need to be matched to the other. The process of matching peaks for two spectra does not require the annotation of the peaks and the result of this process is referred to as a “matched spectra” in this manuscript (see Experimental Section).

With matched spectra at hand, the similarity of two spectra can be scored in two ways: First, the similarity can refer to the question “How identical are these two spectra?”. This metric will take all peaks (matched or unmatched) of both spectra into account. Peaks missing in one or the other spectrum will penalize the similarity score. However, this can lead to low similarity scores in case of insufficient fragmentation, or co-isolation events (frequent in current shotgun data) only present in one spectrum. Second, spectral similarity can also be addressed by answering the question: “How well is one spectrum contained in another?”. Here, all peaks of one spectrum whether they are matched or not are used for similarity calculation. This ensures that unmatched peaks from the other spectrum do not penalize the similarity artificially, e.g. excluding peaks from co-isolated peptides. In the USE, all possible spectral similarities are shown allowing the user to quickly identify whether two spectra are identical or whether one spectrum is contained in the other.

Pearson’s correlation coefficient (PCC) and (normalized) spectral (contrast) angle (SA) are used as similarity measures in the USE. Although both measures correlate well, we(16) and others(26) have previously observed that the spectral angle offers higher sensitivity to differentiate similar spectra, while most users will be more familiar with PCC.

The USE generates an interactive view of the spectrum comparison (Figure 2), consisting of two spectrum visualizations (Figure 2a and c) showing both selected spectral data and a peak-assignment/intensity difference plot in the middle (Figure 2b). All three plots are coupled to each other, allowing zooming on the m/z axis as well as hovering over peaks or error circles highlighting the matched peak in the other spectrum. The spectrum plots show the peptide sequence, detected fragments and precursor information as originally implemented in the IPSA viewer. In addition, we show the similarity measures PCC and SA. The similarity based on the contains-strategy is shown in brackets. The peak-assignment/intensity difference plot in between both spectra visualizes the difference of the selected spectra by showing matched peaks in black and unmatched peaks in grey dots. The size of the dots correlates with the difference in intensity whereas the height of the dots represents the observed mass difference in part per million (0 for unmatched peaks). Note, as both spectra might be experimentally acquired, the observed mass difference is calculated with respect to the average mass of the matched peaks and thus does not (necessarily) reflect the actual mass accuracy in the spectrum.

Discussion

Here we presented the Universal Spectrum Explorer (USE), an open-source and web-based tool for the comparison of any two. It allows the quick and easy cross-resource comparison of two spectra. We strongly believe that the USE will become an important tool for researchers and reviewers to be able to directly and quickly investigate, compare and validate spectra and PSMs of claimed peptide identifications circumventing the necessity to download large amounts of raw data or opening long lists of annotated spectra. With the ability to directly link to spectra stored in public repositories by using the USI, we envision that shareable links can be provided in e.g. excel sheets (https://www.proteomicsdb.org/use/?usi=mzspec:PXD015890:18May18_Olson_WT2.raw%20(F001551).mzid_18May18_Olson_WT2.raw_(F001551).MGF:index:6913:AEAEAQAEELSFPR/2&usi_origin=pride).

Furthermore, the modular layout of the USE allows complete or partial integration into third party websites. Due to its minimal dependencies, the USE requires no complex built system and can also be run locally. In order to allow researchers to benefit from recent advances in spectrum predictions using e.g. deep learning, we developed a new REST interface allowing the retrieval of spectral data from other sources. We showcase this in ProteomicsDB which enables the USE to retrieve predicted spectra from Prosit in real-time. Any other prediction service can in principle be integrated by this mechanism and would allow scientists to pick and evaluate which prediction engine best fits their data.

Assessing the global probability or likelihood of a correct match by comparing an experimental (or query) spectrum to a reference spectrum still remains difficult. Although spectral similarity measures such as PCC and SA have a clearly defined value range, the definition of a threshold indicative of spectral identify requires the estimation of a similarity distribution(27) or scoring of all possible spectral combinations(28). We implemented and tested an approach to estimate E-values(29) based on scoring a query spectrum to multiple (predicted) spectra with essentially equal precursor mass and precursor charge but differing amino acid composition

to estimate the similarity distribution. Although this approach seems to work reasonably well when knowing the biological background of the sample and thus possible amino acid combinations, the method unfortunately lacks specificity when generating peptides with random composition of amino acids (data not shown). Depending on the sample size, many alternative amino acid combinations can be found whose spectra only vary to a very minor degree to the query spectrum, resulting in significantly reduced E-values, in turn suggesting that the achieved similarity could have happened by chance. The USE and the implemented interfaces to public repositories and prediction servers might play an important role in the development of more robust E-value estimation methods in the future.

However, with the ever increasing amount of publicly available data, we strongly believe that the USE will become a useful tool in many areas of research. For example, the comparison of spectra of modified and unmodified peptides helps to identify modification specific diagnostic ions(24). With the increase of open modification searches, the USE provides the opportunity to investigate and localize the spectral difference between spectra containing novel modifications to its unmodified counterparts from public repositories or predictions. Similarly, the USE can be used to investigate (and if necessary even manually adjust) peptide identification results retrieved from de novo search engines. Since reference spectra of peptide sequences suggested by de novo search engines are likely not present in public repositories, the integration of fragment intensity predictions in the USE offers a unique way to investigate and potentially validate such PSMs.

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Competing Financial Interests Statement

B.K. and M.W. are founders and shareholders of OmicScouts GmbH (Freising, Germany), which operates in the field of proteomics. They have no operational role in the company. T.S., B.K. and M.W. are founders and shareholders of MSAID GmbH (Freising, Germany), which operates in the field of proteomics. T.S., B.K. and M.W. have no operational role in the company.

Code availability

All source code has been made available on GitHub

- https://github.com/kusterlab/universal_spectrum_explorer
- https://github.com/kusterlab/use_embedded
- https://github.com/kusterlab/proteomicsJS

DOIs will be provided upon acceptance.

Figures

Figure 1

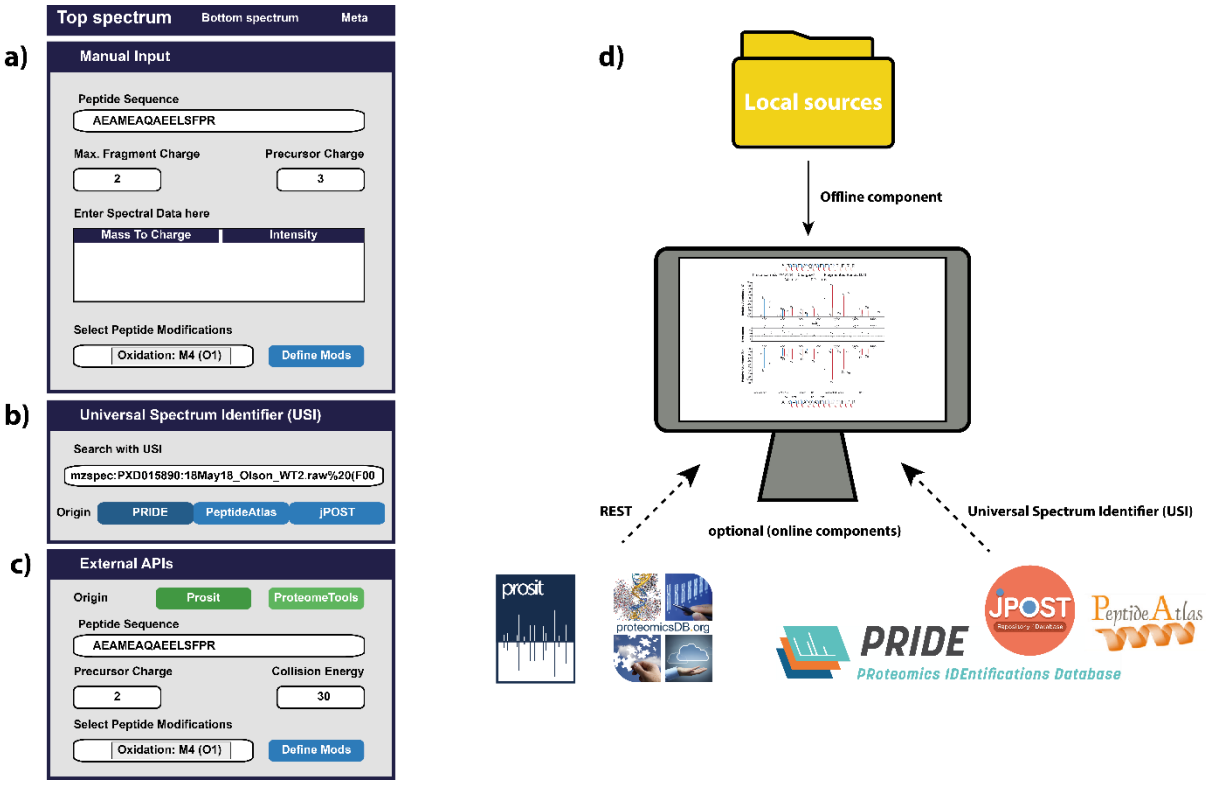

Figure 1. The USE interface. a) The “manual input” section allows the definition of every setting by hand up to the manual generation of modifications. b) The “USI” section allows pasting of an USI and automated retrieval from three different data repositories implementing the USI standard. c) The “external APIs” section is an extension of the USI and allows a retrieval of spectral data from any resource implementing our defined REST endpoint. ProteomicsDB offers additional endpoints and provides data from the ProteomeTools project and the peptide fragmentation prediction tool Prosit. d) The input of spectral data for the ISV can originate from various sources, such as local mass spectrometer files, public data repositories and prediction services.

Figure 2

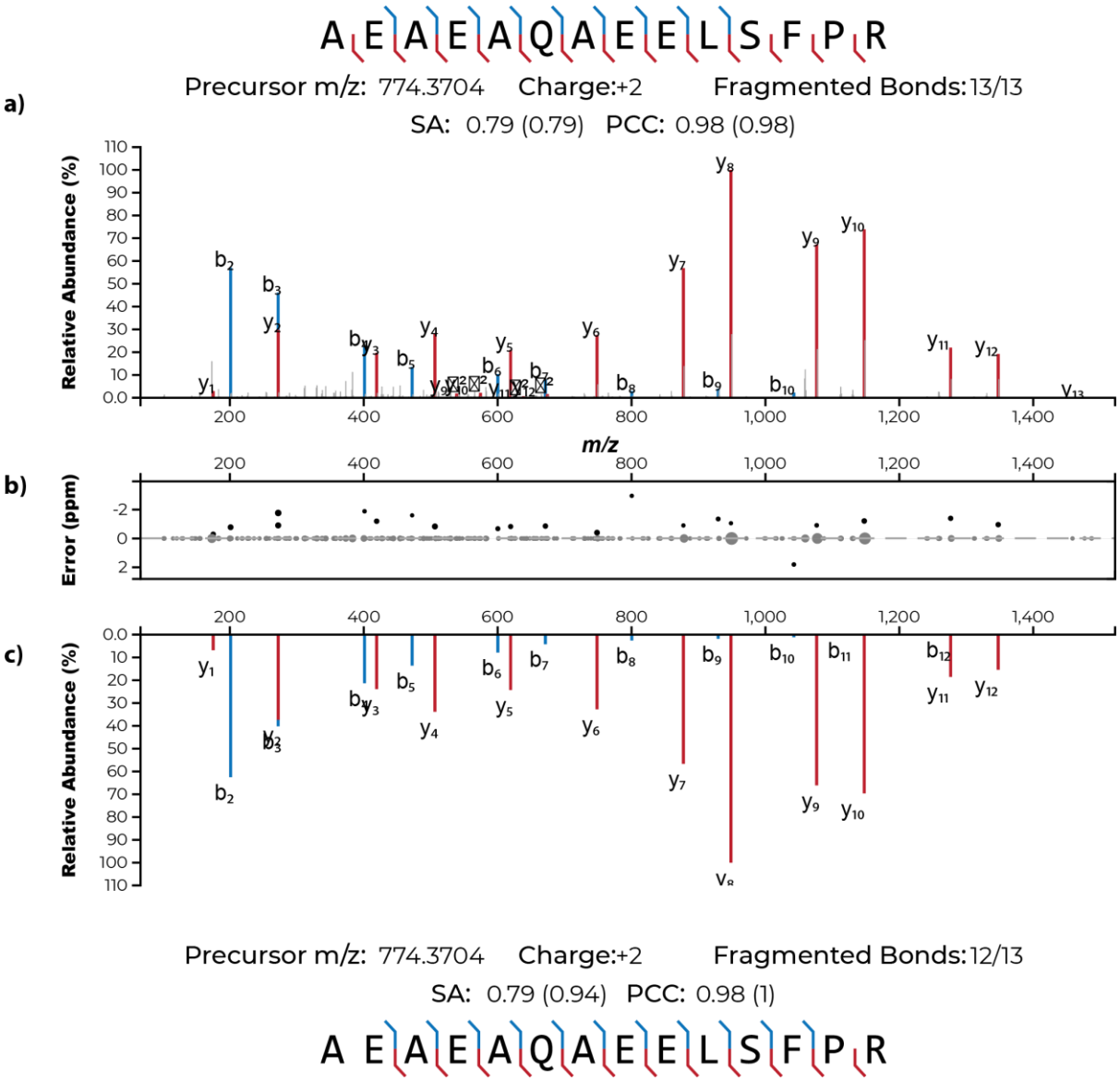

Figure 2. Mirror spectrum visualization of the USE. The top spectrum representing the doubly charged peptide spectrum match AEAEAQAEELSFPR (PXD015890) (USI: mzspec:PXD015890:18May18_Olson_WT2.raw%20(F001551).mzid_18May18_Olson_WT2.raw_(F001551).MGF:index:6913:AEAEAQAEELSFPR/2 stored at PRIDE), the bottom spectrum a predicted spectra by Prosit of the same sequence and precursor charge. a+c) MSMS spectrum visualization. Annotated peaks are coloured according to their inferred ion

type (blue = b series, red = y series; colouring can be adjusted via the settings tab). Underneath the bottom spectrum as well as above the top spectrum meta information is shown. Spectral angle (SA) and Pearson's correlation coefficient (PCC) describe the similarity of the two spectra. Similarity measures in brackets over the top spectrum describe how similar both spectra are based solely on the peaks of the top spectrum. The same is shown for the bottom spectrum based solely on the peaks of the bottom spectrum. b) Visualization of the mass and intensity discrepancy between peaks in both spectra. Black dots represent matched peaks and their mass error. Grey dots depict unmatched peaks. The size of the dots is used to indicate the intensity discrepancy of individual peaks.

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